AUTISM SPECTRUM DISORDERS (ASD) - SEARCHING FOR THE BIOLOGICAL BASIS FOR BEHAVIORAL SYMPTOMS AND NEW THERAPEUTIC TARGETS

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AUTISM SPECTRUM DISORDERS (ASD) - SEARCHING FOR THE BIOLOGICAL BASIS FOR BEHAVIORAL SYMPTOMS AND NEW THERAPEUTIC TARGETS

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Autism Spectrum Disorder (ASD) is currently diagnosed based on a series of behavioral tests. The challenge for researchers is to try to uncover the biological basis for these typical behaviors in order to improve diagnosis and identify potential targets for treatment. A multidisciplinary approach is necessary in order to move forward. This includes analysis of the current animal models for ASD and their suitability, reviewing immunological, immunogenetic and epigenetic research, reassessing clinical diagnostic tools, and surveying radiological, pathological, and serological records for clues. This volume includes research from some of the leading researchers on ASD. We are hopeful that it will stimulate further dialogue and research in this challenging field.

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EDITORIAL
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Editorial: Autism Spectrum Disorders (ASD)-Searching for the Biological Basis for Behavioral Symptoms and New Therapeutic Targets

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Keywords: Autism Spectrum Disorders (ASD), HLA antigens, behavior, intelligence, sensory thresholds, genetic syndromes, autoimmune diseases

Editorial on the Research Topic

Autism Spectrum Disorders (ASD)-Searching for the Biological Basis for Behavioral Symptoms and New Therapeutic Targets

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The frequency of Autism Spectrum Disorders (ASDs) is increasing with a 30% reported increase in pediatric prevalence from 2012 to 2014 in the U.S. until present rates of about 1 in 68 children or 1.5% of children in the U.S. (Corcoran et al., 2015). Yet, little is known about the etiology of this spectrum. As of now, ASD is diagnosed based on a series of behavioral tests. The challenge for researchers is to try to uncover the biological basis for these typical behaviors in order to improve diagnosis and identify potential targets for treatment. A multidisciplinary approach to understanding the biological basis for the behavioral symptoms is necessary in order to move forward. This includes analysis of the current animal models for ASD and their suitability, reviewing behavioral, immunological, immunogenetic, and epigenetic research, reassessing clinical diagnostic tools, and surveying radiological, pathological, and serological records for clues.

With over 500 animal models available with varying construct validity and face validity, and a variety of behavioral tests for animals (Kazdoba et al., 2016; three chamber, T maze, elevated plus maze) and for humans (ADOS, ADIR, CARS, ABC) and still no FDA approved effective treatments for the core symptoms of autism, much more needs to be done to understand the behavioral features of autism and their underlying etiology. Thillay et al. used EEG to record 12 adults diagnosed with ASD and age matched controls performing a visual target detection task. Their data suggests that patients diagnosed with autism overreact to stimuli coming from an unclear context, which matches their sense of being overwhelmed by incoming data, and that they are unable to control cortical activity according to varying levels of uncertainty. Parallel to the response to an uncertain context, Corbett et al. investigated the difference in stress response to interaction with peers as well as the role of sensory sensitivity. They found that children with ASD showed significantly higher cortisol levels than their typically developing control group.

These results indicated that increased cortisol was associated with increased sensory sensitivity and enhanced stress. Schauder and Bennetto integrated the empirical literature on sensory processing in ASD with those papers that investigate neural response to sensory stimuli. Sensory symptoms start demonstrating relationships with adaptive functioning and language proficiency in the early years. Therefore, in order to generate a multidisciplinary approach to sensory processing in ASD, it is critical to integrate the sensory symptoms and neuroscience perspectives. Internal and external stimuli can elicit two different categories of responses, an excitatory response, and an inhibitory response. Frye et al. reviewed biological abnormalities shared by ASD and epilepsy and found that autism and epilepsy are associated with comparable aberrations that may alter the excitatory to inhibitory balance of the cortex. They suggested that these parallels may explain the high prevalence of epilepsy in ASD and the elevated prevalence of ASD features in individuals with epilepsy. Instead of looking at other disorders that are similar to autism such as epilepsy, Zachor and Ben-Itzchak set out to investigate whether specific medical conditions in ASD are associated with unique behavior profiles. They found two unique medical behavioral subtypes in ASD that affect inherited traits of cognition and/or autism severity. Crespi analyzed the innovative hypothesis that autism is actually a disorder of high intelligence. They propose that looking at both intelligence and autism studies together could provide unique insights into the neurological and genetic causes of high mental abilities.

Efforts at twin studies, identifying HLA associations, specific genes, single nucleotide variants (SNVs) or single nucleotide polymorphisms (SNPs), and hotspots for copy number variations (CNVs) in autism have yielded limited but promising results so far. In light of the fact that Rett Syndrome, Fragile X, and other genetic syndromes comorbid with ASD have been shown to be associated with epigenetic modifications the theory that epigenetic mechanisms might potentially be associated with the etiology of ASD deserves more attention. Due to mounting evidence indicating immune involvement in the etiology of autism, Torres et al. looked at common genetic variants found in HLA and KIR immune genes, particularly HLA genes on chromosome 6 and KIR genes on chromosome 9. They show that for HLA class I alleles, frequencies are significantly increased by more than 5% over control populations. They also found that three activating KIR genes have increased frequencies of 15, 22, and 14% in the autism populations, and that there is a 6% increase in total activating KIR genes in autism when compared to controls. Similarly, Gamliel et al. performed a study comparing the KIR:HLA frequencies in ASD children with those of their healthy parents. They found a higher frequency of HLA-C2 allotypes among the fathers, while its corresponding ligand 2DS1, was higher in the maternal group. Francis et al. also looked at genetic variants, yet they focused on the receptor genes of oxytocin and vasopressin, since studies have reported significant associations between these genes and ASD diagnosis and ASD-related phenotypes. They found associations between vasopressin receptor single nucleotide polymorphisms (SNPs) and specific oxytocin receptor SNPs and diagnosis and behavioral profile. Instead of looking at specific genetic variants, Ansel et al.) analyzed gene expression studies from the past decade and came up with a comprehensive list of genes that were found to be dysregulated in ASD children as compared to typically developing controls.

Ornoy et al. emphasized that an ASD diagnosis is often an important clinical presentation of some well-known genetic syndromes in men. They reviewed these syndromes and also looked at the role of the most important prenatal factors affecting the fetus throughout pregnancy, which may be associated with ASD as well as maternal autoimmune diseases, and infections, which are associated with ASD. Similarly, Nardone and Elliott reviewed the growing evidence for a complex interaction between immune system activation in the mother during pregnancy and epigenetic control in the brain of the fetus that may help generate an autistic phenotype. They looked at this particularly because of molecular studies that have highlighted the role of epigenetics in brain development as a process susceptible to environmental influences and potentially causative of ASD. Looking at both the immune state of the mother and the fetus, Grether et al. found that in both maternal and newborn there was a significantly lower risk of ASD associated with higher levels of Toxo IgG. These results support previous studies indicating that immune factors during early development may be relevant to the etiology of ASD. Many serological studies aimed at identifying any abnormalities in the blood of children with ASD have yielded conflicting results (Kalra et al., 2015). Nevertheless, various inflammatory cytokines and immunological markers reflecting immune dysfunction have been documented in ASD. Preliminary studies even suggest a correlation between certain antibodies and clinical severity (Ashwood et al., 2011). In order to counteract the heterogeneity of ASD, larger studies with broader screening of immune factors are necessary.

Though there is still much work to do in uncovering the biological basis for ASD, patterns are beginning to emerge. The combination of serological data with genetic data enables researchers to isolate pathways that demonstrate particular association with ASD. The precise mechanisms between these networks and the behavioral symptoms have yet to be fully elucidated. However, larger studies with more unified diagnostic inclusion criteria and multidisciplinary testing will hopefully yield further hints toward identifying the underlying mechanism of ASD.

AUTHOR CONTRIBUTIONS

BG contributed substantially to the conception of the work and revised it critically. JR contributed substantially to the conception, design, and analysis of the work. JR also drafted the work.

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Atypical Brain Mechanisms of Prediction According to Uncertainty in Autism

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Resistance to change is often reported in autism and may arise from an inability to predict events in uncertain contexts. Using EEG recorded in 12 adults with autism and age-matched controls performing a visual target detection task, we characterized the influence of a certain context (targets preceded by a predictive sequence of three distinct stimuli) or an uncertain context (random targets) on behavior and electrophysiological markers of predictive processing. During an uncertain context, adults with autism were faster than controls to detect targets. They also had an enhancement in CNV amplitude preceding all random stimuli—indexing enhanced preparatory mechanisms, and an earlier N2 to targets—reflecting faster information processing—compared to controls. During a certain context, both controls and adults with autism presented an increase in P3 amplitude to predictive stimuli—indexing information encoding of the predictive sequence, an enhancement in CNV amplitude preceding predictable targets—corresponding to the deployment of preparatory mechanisms, and an earlier P3 to predictable targets—reflecting efficient prediction building and implementation. These results suggest an efficient extraction of predictive information to generate predictions in both controls and adults with autism during a certain context. However, adults with autism displayed a failure to decrease mu power during motor preparation accompanied by a reduced benefit in reaction times to predictable targets. The data reveal that patients with autism over-anticipate stimuli occurring in an uncertain context, in accord with their sense of being overwhelmed by incoming information. These results suggest that adults with autism cannot flexibly modulate cortical activity according to changing levels of uncertainty.

Keywords: autism, prediction, uncertainty, ERP, mu oscillations

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INTRODUCTION

Autism Spectrum Disorder (ASD) is a pervasive neurodevelopmental disorder characterized by difficulties in social communication and interaction, associated with restricted, repetitive patterns of behavior, interests, or activities (American Psychiatric Association, 2013). The insistence on sameness is a fundamental feature of ASD and is incorporated into diagnostic criteria. Clinical

reports of individuals with ASD show that they react in an unusual way (they may feel stressed and anxious) to unpredictable change occurring in their environment. Such a crucial need for stability in individuals with ASD might arise from a dysfunction in the ability to predict events especially in an ever-changing world (Gomot and Wicker, 2012; Pellicano and Burr, 2012; Palmer et al., 2013; Lawson et al., 2014; Van de Cruys et al., 2014). Pathological restricted and repetitive behaviors and interests, rituals, and routines could represent attempts to regulate uncertainty by imposing sameness and order (Gomot and Wicker, 2012; Pellicano and Burr, 2012; Lawson et al., 2014). In addition, social-communication impairments in ASD could be the consequence of difficulties in adapting quickly to the unpredictable social world (Gomot and Wicker, 2012; Lawson et al., 2014; Van de Cruys et al., 2014; Robic et al., 2015). However, to our knowledge, no study has investigated the brain mechanisms of predictive processing in adults with ASD.

Predictive coding formulations of perception propose that expectations in higher brain areas generate top-down predictions that meet bottom-up stimulus signals in lower hierarchical areas (e.g., Friston, 2005). This prediction capacity is essential to efficiently adapt behaviors in an ever-changing world (Bubic et al., 2010). Predictive processing comprises several processes such as the generation of prediction based on encoding of predictive information, and the implementation of prediction via the deployment of both attentional and motor preparatory mechanisms, resulting in facilitated processing of upcoming events, and optimized behaviors indexed by reduced reaction times. In a previous study using a detection task manipulating target predictability (Bidet-Caulet et al., 2012), we defined electrophysiological (EEG) markers of these different stages in typically developing adults. The P3 amplitude to predictive stimuli was found to index predictive information encoding, increase in the Contingent Negative Variation (CNV; pre-stimulus slow ERP) amplitude to reflect the deployment of preparatory mechanisms, decrease in mu power to reflect motor cortex activation, and the P3 latency to predicted target to serve as a measure of the prediction building and implementation (Bidet-Caulet et al., 2012).

While the encoding of explicit predictive non-social cues has not been examined in ASD, some electrophysiological studies have found inconsistent findings, with evidence for atypical preparation in 8–13 year old children (indexed by an increase in CNV amplitude; Tye et al., 2014) or for a preserved preparation in adults (no significant difference in CNV amplitude compared to controls; Strandburg et al., 1993) with ASD without intellectual disability. Reduced motor anticipation has been clinically reported in ASD since the Kanner initial case reports (Kanner, 1943) and recently from a retrospective study (Brisson et al., 2012). Electromyographic studies found substantial anticipation difficulties, reinforcing these clinical observations (Schmitz et al., 2003; Cattaneo et al., 2007). More precisely, an electrophysiological study investigating the theta frequency band in children (which corresponds to the classic mu rhythm recorded in adults) using a bimanual load-lifting task revealed a lack of increased cortical activity of the motor areas before voluntary unloading in the ASD group (Martineau et al., 2004).

Using EEG recorded in 12 adults with autism and age-matched controls performing a visual target detection task, we characterized the influence of a certain context (targets preceded by a 100% predictive sequence of three distinct stimuli), or an uncertain context (random targets) on behavior and electrophysiological markers of predictive processing. Thus, based on this paradigm, we wanted to answer the following questions: (1) do adults with ASD benefit from the predictive information behaviorally (indexed by reduced reaction times)? (2) do the brain mechanisms involved in predictive processing in a certain context are atypical in adults with ASD? (3) do the brain mechanisms involved in predictive processing in an uncertain context are atypical in adults with ASD (4) what steps involved in prediction, such as extraction of predictive information (indexed by an increase in P3 amplitude) required to generate prediction, attentional, and motor preparation mechanisms (reflected by an increase in CNV amplitude and a decrease in mu power) corresponding to the implementation of prediction (indexed by a reduced target-P3 latency) are specifically affected in adults with ASD?

MATERIALS AND METHODS

Subjects

Twelve adults with ASD without intellectual disability (10 males and 2 females, 1 left-handed), aged from 18 to 27 years (mean ± Standard Error of the Mean = 21 years, 4 ± 10 months) were recruited from the Child Psychiatry Department specialized in autism, University Hospital of Tours, France. They were diagnosed by expert clinicians according to DSM-IV-TR criteria (American Psychiatric Association, 2000) and using the Autism Diagnostic Observation Schedule-Generic (ADOS-G; Lord et al., 2000) and/or the Autism Diagnostic Interview-Revised (ADI-R; Lord et al., 1994). ASD participants did not present any comorbidity at the time of the study. Intelligence quotients (IQ) were assessed by the Wechsler intelligence scales according to the subjects' ages and developmental levels (Wechsler, 1997, 2005). Intelligence scales provided overall intellectual (mean ± SEM = 101 ± 5), verbal (mean ± SEM = 100 ± 3), and performance quotients (mean ± SEM = 104 ± 7).

Twelve healthy volunteers (mean ± SEM = 21 years, 7 ± 11 months; 10 males and 2 females, 1 left-handed) also participated in the study as control (CTRL) subjects. None of these healthy adults had a previous history of psychiatric or neurological problems and they were not taking any drug. The two groups were matched in age, gender, and handedness. While a full Wechsler was administered to the adults with ASD, two non-verbal subtests (block design and matrix reasoning) of Wechsler intelligence scales were used in the CTRL group. Block design standard scores ranged from 1 to 16 (ASD: 10.8 ± 1.2; CTRL: 11.8 ± 0.8), and matrix reasoning standard scores ranged from 6 to 14 (ASD: 10.1 ± 0.8; CTRL: 10.7 ± 0.4). No significant difference between groups was found on the standard scores obtained from these 2 subtests using randomization tests ($p > 0.45$).

All participants had normal or corrected-to-normal vision. The local ethical committee board (Comité de Protection des Personnes de Tours Ouest-1, France) approved the protocol. Written informed consent was obtained from all participants.

Stimuli and Tasks

Subjects sat in a chair in a sound-attenuated room, 94 cm in front of a 19-inch PC screen. The experimenters and computers delivering the visual stimuli and recording the EEG were located in a separate room. We used a paradigm designed to investigate predictive context processing adopted from Fogelson et al. (2009). Stimuli were presented centrally on a computer screen and subtended 3° of visual angle (**Figure 1**).

Stimuli consisted of 15% of targets (downward-facing triangle) and 85% of equal amounts of three types of standards: Triangles facing left, upward, or right. A target could be a random target (randT) preceded by an uncertain context (random sequence of stimuli) or a predictable target (predT) preceded by a certain context, i.e., a three-stimulus predictive sequence (leftward-, upward-, and rightward-facing triangles). Triangles of the predictive sequence are labeled as predS1, predS2, and predS3 stimuli, whereas the corresponding triangles outside the predictive sequence are labeled as randS1, randS2, and randS3, for leftward-, upward-, and rightward-facing triangles, respectively. Participants were instructed to press a button with the dominant-hand index finger in response to target stimuli (downward-facing triangles) and to look for the predictive sequence. Before the recording began, subjects performed a first training session to ensure they were able to detect the target accurately. In a second training session, subjects were introduced to the predictive sequence and were aware that it would be 100% predictive of a target, but that targets would also appear randomly throughout the block. Especially for the adults with ASD, training sessions were repeated as many times as necessary to ensure full understanding of the instructions.

In each block (∼2.3 min long), a total of 127 stimuli (11 randTs, 28 randS1, 28 randS2, 28 randS3, 8 predTs, 8 predS1, 8 predS2, and 8 predS3) were presented each for 150 ms with an inter-stimulus interval of 1 s. 17 subjects performed 15 blocks, one subject performed 12 blocks, 2 subjects performed 10 blocks, and 4 subjects performed 4–8 blocks due to fatigue. The stimulus presentation and response recordings were controlled using Presentation software (Neurobehavioral Systems, Albany, CA, USA).

Electroencephalography Recording and Analysis

EEG was recorded from 64 electrodes using Active Two system (Biosemi, The Netherlands). Vertical eye movements were monitored using electrodes placed above and below the left eye. The signal was recorded with a sampling frequency of 512 Hz and filtered at 0–104 Hz. Data were re-referenced offline to the average potential of the two earlobe electrodes.

EEG analyses were based on results from a previous study (Bidet-Caulet et al., 2012). They were performed on standard and target visual stimuli embedded or not embedded in the predictive sequence. We excluded from further analysis: Trials corresponding to standards after a target, standards before or after a button press, a randS2 standard preceded by a randS1 standard but not followed by a randS3 standard (as it is a potential predS2 standard), missed targets, and targets preceded by less than three standards. Eye-movement artifacts were detected using independent component analysis (ICA) and were selectively removed via the inverse ICA transformation. Only 1 or 2 independent components were removed in each subject to clean the data. In five subjects, the flat or excessively noisy signals at one or two electrodes were replaced by their values interpolated from the remaining electrodes using spherical spline interpolation (Perrin et al., 1989). Trials contaminated with excessive muscular activity in the (−700; 700 ms) time-window relative to stimulus onset were also excluded.

As the number of trials for stimuli embedded in the predictive sequence was lower than for the other stimuli, we equalized the number of trials within each pair of to-be-compared stimuli by random selection, for each participant. On average across participants, we obtained mean ± SEM: 68 ± 4, 83 ± 5, 83 ± 5, and 72 ± 5 clean trials for randS1/predS1, randS2/predS2, randS3/predS3, and randT/predT pairs, respectively, for each participant.

Event-Related Potential (ERP) Analysis

We averaged single trials, locked to stimulus onset, separately for each of the eight stimulus categories (randS1, randS2, randS3, randT, predS1, predS2, predS3, predT). The resulting

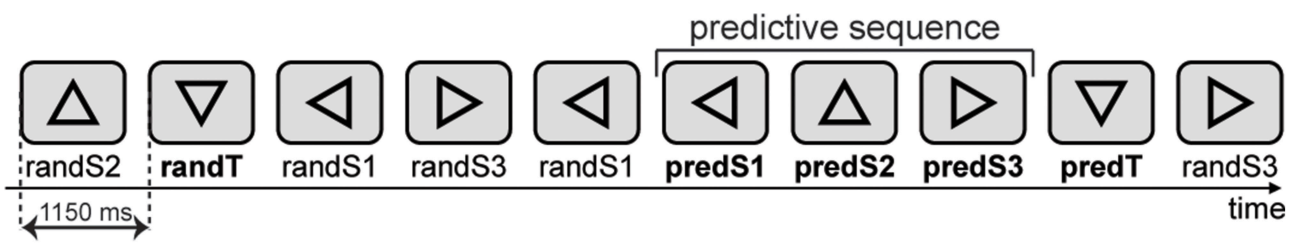

FIGURE 1 | Stimuli. A sequence of triangles was centrally presented on a screen. A target could be a random target (randT) preceded by a non-informative context (random sequence of stimuli) or a predictable target (predT) preceded by an informative context, i.e., a three-stimulus predictive sequence (leftward-, upward-, and rightward-facing triangles). Triangles of the predictive sequence are labeled as predS1, predS2, and predS3 stimuli, whereas the corresponding triangles outside the predictive sequence are labeled as randS1, randS2, and randS3, for leftward-, upward-, and rightward-facing triangles, respectively.

event-related potentials (ERPs) were digitally band-pass filtered between 0.5 and 30 Hz to analyze slower components, or between 4 and 30 Hz to extract early and transient responses by filtering out slow and large components (such as the Continent Negative Variation or CNV and P3) that can overlap fast and small responses (Bidet-Caulet et al., 2012). For post-stimulus analysis, ERPs were corrected with a −100 to 0 ms baseline before stimulus onset. For pre-stimulus analysis, ERPs were not baseline corrected. ERP scalp topographies were computed using spherical spline interpolation (Perrin et al., 1989).

Time-Frequency (TF) Analysis

We analyzed oscillatory activities by means of a Gaussian Morlet's wavelet decomposition (for details, see Tallon-Baudry and Bertrand, 1999). This method led to a power estimate of both evoked (phase-locked to stimulus onset) and induced (jittering in latency) activities in the TF domain. To distinguish induced from evoked activities (reflecting the frequency content of ERPs), we computed, at each point of the TF domain, the stimulus phase-locking factor from the single-trial TF analysis (Tallon-Baudry et al., 1996). This factor ranges from 0 (uniform phase distribution, i.e., high-latency jitter) to 1 (strict phase-locking to the stimulus). The Rayleigh statistic was used to test for the non-uniformity of phase distribution (Jervis et al., 1983), with a threshold of 0.25 to test non-uniformity with $\alpha = 0.05$: A phase-locking factor superior to 0.25 indicated a non-uniform phase distribution and the underlying oscillations were considered to be phase-locked to the stimulus. To assess the deployment of oscillatory activities around the stimuli, we analyzed the oscillation on a large time-window (−500; 500 ms) around each type of stimulus. In each group, we applied the same baseline correction to all stimuli by subtracting the mean power between −500 and −250 ms before all S1 onset, in each frequency band. We focused our analysis on the alpha frequency band (8–14 Hz). Since mu rhythm is recorded over the sensorimotor cortex (central electrodes) at the same frequency range than alpha rhythm (Pineda, 2005), we deliberately distinguished mu and alpha oscillations based on the topography. Importantly, no difference was observed between CTRL and ASD on the mean power in the 8–14 Hz band in the −500 to 500 ms time-window around S1.

Statistical Analysis

To assess statistical differences between groups and conditions, we used a repeated-measure analysis of variance (rmANOVA) with group (ASD vs. CTRL) as the between-subject factor and predictability (predictable vs. random) as the within-subject factor.

Post-hoc analyses were performed with statistical tests based on permutation or randomization for intra- or inter-group comparisons, respectively (Edgington, 1995). Permutation tests consisted of (1) the random permutation of the 12 pairs (corresponding to the 12 subjects) of values, (2) the sum of squared sums of values in the two obtained samples, and (3) the computation of the difference between these two statistic values. We performed all possible permutations (4096) to obtain an estimate of the distribution of this difference under the null hypothesis. This distribution was then compared to the actual difference between the values in the two conditions. Randomization tests consisted of (1) the random constitution of the two samples to compare, (2) the sum of squared sums of values in the two obtained samples, and (3) the computation of the difference between these two statistic values. We performed 10,000 such randomizations to obtain an estimate of the distribution of this difference under the null hypothesis. This distribution was then compared to the actual difference between the values in the two conditions.

Statistical Analysis of Behavioral Data

A button press within the interval of 100–1100 ms after a target onset was considered as a correct response, and a press after a standard was counted as a false alarm (FA). Reaction times (RTs) were computed for correct trials, only. We investigated the benefit in RTs with the predictive context independently of RT to randTs by calculating a RT prediction index [(RT randT-RT predT)/RT randT].

The effect of predictability on the % of hits and RTs was assessed using rmANOVAs. The differences between groups on the % of FAs and the RT prediction index were assessed using randomization tests.

Statistical Analysis of Event-Related Potentials and Oscillatory Activities

To investigate predictive processing in adults with ASD, we compared ERPs and oscillatory activities to the same physical stimuli embedded (predictive stimuli) or not embedded (non-predictive stimuli) in the predictive sequence. No difference was predicted and none was observed between predS1 and randS1 as participants did not know at that time if the stimulus was part of the predictive sequence or not.

For statistical analysis, we computed the rmANOVA on the latencies of N1 (105; 230 ms) and P2 (205; 310 ms) peaks at PO4, and on the latencies of P2 (155; 255 ms) and N2 (215; 350 ms) peaks at FCz. For the P3 to targets, we also analyzed the latency and amplitude of the P3 maximum peak at Pz in the (250; 750 ms) time-window.

To go further and beyond peaks and components, we also performed rmANOVAs for each of the 64 electrodes on specific time-windows based on results in previous EEG studies (Fogelson et al., 2009; Bidet-Caulet et al., 2012). To correct for multiple tests, we first calculated a corrected *p*-value across time (e.g., 0.05 divided by the number of tested time-windows) and then an effect was deemed significant if a *p*-value inferior to this threshold was found on at least 4 adjacent electrodes.

To analyze early and transient ERPs, we computed the rmANOVA on the 4–30 Hz band-pass-filtered ERP (pre-stimulus baseline-corrected) amplitude within successive 10 ms time-windows of the (0; 400 ms) time-window relative to stimulus onset. The *p*-value threshold for significance was set to 0.00125.

To analyze pre-stimulus activity, we computed the rmANOVA on the 0.5–30 Hz band-pass-filtered ERP (not baseline-corrected) mean amplitude in the (−150; 0 ms) time-window, corresponding to the CNV (pre-stimulus slow ERP) latencies. We also computed a randomization test on the pre-stimulus activity before all standards (randS) on the mean amplitude in the (−50; 0 ms) time-window to analyze predictive processing within the uncertain context. For ease of reading, we will refer to the CNV component.

We also computed the rmANOVA on the 0.5–30 Hz band-pass-filtered ERP (pre-stimulus baseline-corrected) mean amplitude in the (200; 600 ms) analysis window, corresponding to the P3 latencies. For ease of reading, we will refer to the P3 component.

For oscillatory activities, the rmANOVA was applied to the mean TF energy values within successive 200 ms time-windows regularly shifted by 100 ms to cover the entire analysis time-window (−500; 500 ms). To correct for multiple tests in the time dimension, the p-value threshold for significance was set to 0.005. To avoid a possible confound due to inclusion of left-handed participants, time-frequency analysis was run with a sample of right-handed participants only ($n = 11$ for CTRL and ASD). Relation between the RT prediction index and electrophysiological values was assessed using the Spearman rank correlation coefficient.

Results of the rmANOVAs are illustrated on topographical views at a typical latency (usually at the maximum of the difference between conditions). As examples, corresponding ERP or TF time-courses are depicted for a typical electrode showing a significant effect.

The ELAN software package was used for visualization and analysis of EEG, ERP, and TF (Aguera et al., 2011). Custom MATLAB R2010b (MathWorks, Inc) programs were used for rmANOVAs on ERP and TF measures and for the randomization tests. STATISTICA v10 (StatSoft, Inc) software was used for rmANOVAs.

RESULTS

Behavioral Results

All subjects correctly performed the task (CTRL: 97.0 ± 1.3 and 95.6 ± 1.1%, ASD: 93.3 ± 3.1 and 95.3 ± 1.8%, to randTs and predTs, respectively). No effect of group [$F_{(1, 22)} = 0.76$, $p = 0.391$], nor effect of predictability [$F_{(1, 22)} = 0.04$, $p = 0.848$], nor predictability × group interaction [$F_{(1, 22)} = 1.13$, $p = 0.300$] were found significant for the % of hits. Controls made less FAs (0.24 ± 0.05%) than adults with ASD (0.70 ± 0.19%; $p = 0.003$).

Reaction times (RTs) to targets displayed a significant main effect of predictability [$F_{(1, 22)} = 42.15$, $p < 0.001$], a significant predictability × group interaction [$F_{(1, 22)} = 7.58$, $p = 0.012$], but no effect of group [$F_{(1, 22)} = 2.15$, $p = 0.156$; **Figure 2**]. *Post-hoc* tests showed that, in both groups, RTs to predTs were shorter than those to randTs ($p \leq 0.001$). Importantly, RTs to randTs were longer in CTRL than in ASD ($p = 0.012$) while no difference was found to predTs ($p = 0.748$). The RT prediction index was also larger in CTRL compared to ASD ($p = 0.020$; **Figure 2**). In summary, controls present a larger benefit in RTs with the predictive context but are slower to detect randTs than adults with ASD.

Event-Related Potential Results

Early and Transient ERPs

No effect was significant on the amplitude of early and transient ERPs in response to targets, S3 or S2, nor on the N1 and P2 latencies at PO4, the P2, and N2 latencies at FCz to S3 or S2 ($p > 0.061$), the target-N1 latency at PO4 ($p > 0.050$), the target-P2 latency at FCz ($p > 0.068$).

A main effect of predictability was found on the target-P2 latency at PO4 [$F_{(1, 22)} = 6.91$, $p = 0.015$], but no predictability ×

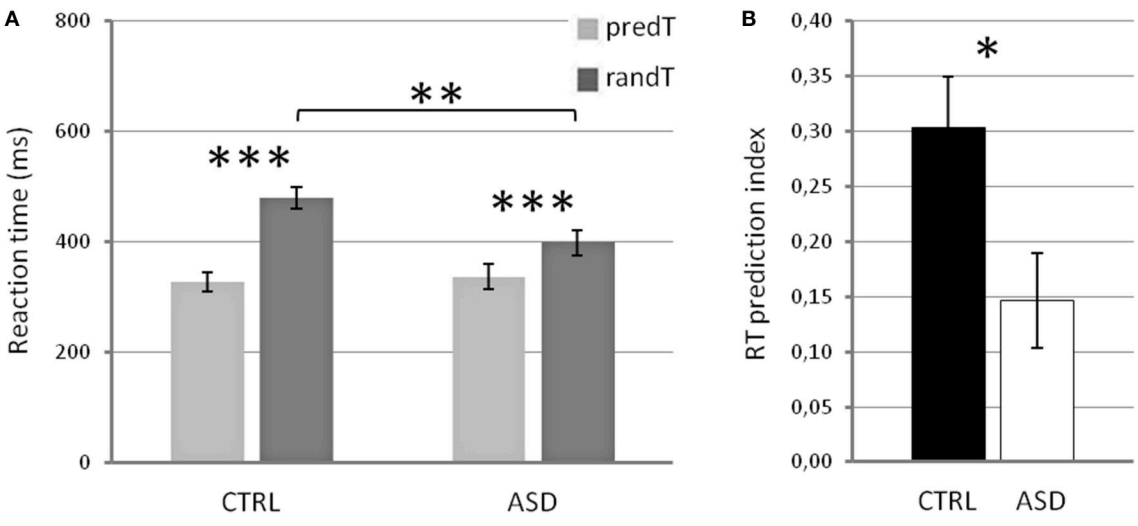

FIGURE 2 | Reaction times in ms (A) for predictable and random targets, and RT prediction index (B) in controls (CTRL) and adults with ASD. Error bars: Standard errors of the mean. Significant differences are indicated by asterisks: *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$.

group interaction [$F_{(1, 22)} = 0.07$, $p = 0.789$], nor group effect [$F_{(1, 22)} = 3.44$, $p = 0.077$], with earlier target-P2 latency to randTs than to predTs.

A predictability × group interaction was found on the target-N2 latency at FCz [$F_{(1, 22)} = 7.40$, $p = 0.012$], but no predictability effect [$F_{(1, 22)} = 2.52$, $p = 0.127$], nor group effect [$F_{(1, 22)} = 1.21$, $p = 0.283$; **Figure 3**]. The N2 to predTs was earlier in latency than to randTs in controls only (CTRL: $p < 0.001$; ASD: $p = 0.440$). A reduced target-N2 latency to randTs ($p = 0.010$) but not to predTs ($p = 0.333$; **Figure 3C**) was found in ASD compared to CTRL.

Only controls displayed a reduction of the target-N2 latency to predTs; whereas adults with ASD showed a reduced target-N2 latency to randTs compared to controls.

CNV

No effect was significant on CNV amplitude preceding S2. A predictability effect, only, was found on CNV amplitude preceding S3, with larger amplitude at parietal electrodes to predS3 [e.g., Pz: $F_{(1, 22)} = 8.34$, $p = 0.008$; **Figure 4**]. CNV amplitude preceding targets (−150 and 0 ms) displayed a predictability × group interaction at left centro-parietal electrodes [e.g., PO3: $F_{(1, 22)} = 6.98$, $p = 0.015$], an effect of predictability on a large fronto-centro-parietal group of electrodes [e.g., Fz: $F_{(1, 22)} = 30.05$, $p < 0.001$], but no effect of group.

At left centro-parietal electrodes, *post-hoc* tests showed an increased CNV before predTs in comparison to randTs in controls only (e.g., at PO3, CTRL: $p = 0.022$; ASD: $p = 0.639$). Moreover, CNV amplitude to randTs was found larger in ASD than in CTRL (e.g., at PO3, randTs: $p = 0.014$; predTs: $p = 0.482$). Furthermore, randomization test showed an increased CNV before randS at parietal electrodes in ASD compared to CTRL (e.g., at PO3, randS: $p = 0.018$).

In summary, at frontal electrodes, both groups displayed an enhancement of the CNV amplitude before targets with increased predictability. At left centro-parietal electrodes, only controls displayed an enhancement of the CNV amplitude before predTs;

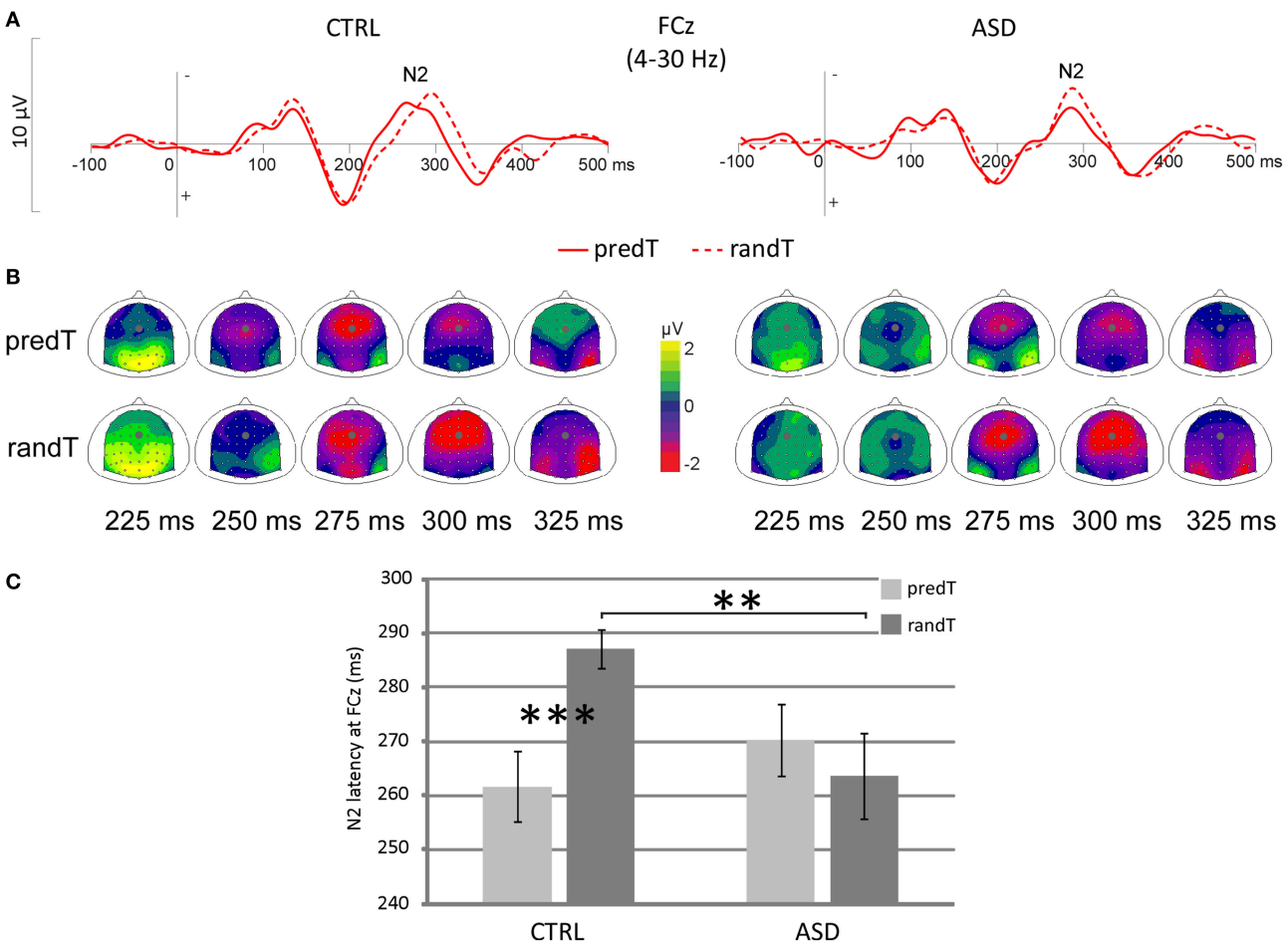

FIGURE 3 | Effect of predictive context on N2 in controls (CTRL) and adults with ASD. (A) Grand-average ERP waveforms band-pass filtered between 4 and 30 Hz to predTs and randTs (solid and dashed red lines, respectively), at the FCz electrode. **(B)** Scalp topographies (top views) of the N2 to predTs and randTs for controls (CTRL) and adults with ASD. The dots indicate the position of FCz electrode. **(C)** Target-N2 latency at FCz in ms for predTs and randTs in controls (CTRL) and adults with ASD. Error bars = standard errors of the mean. Significant differences are indicated by asterisks: $^{**}p \leq 0.01$ $^{***}p \leq 0.001$.

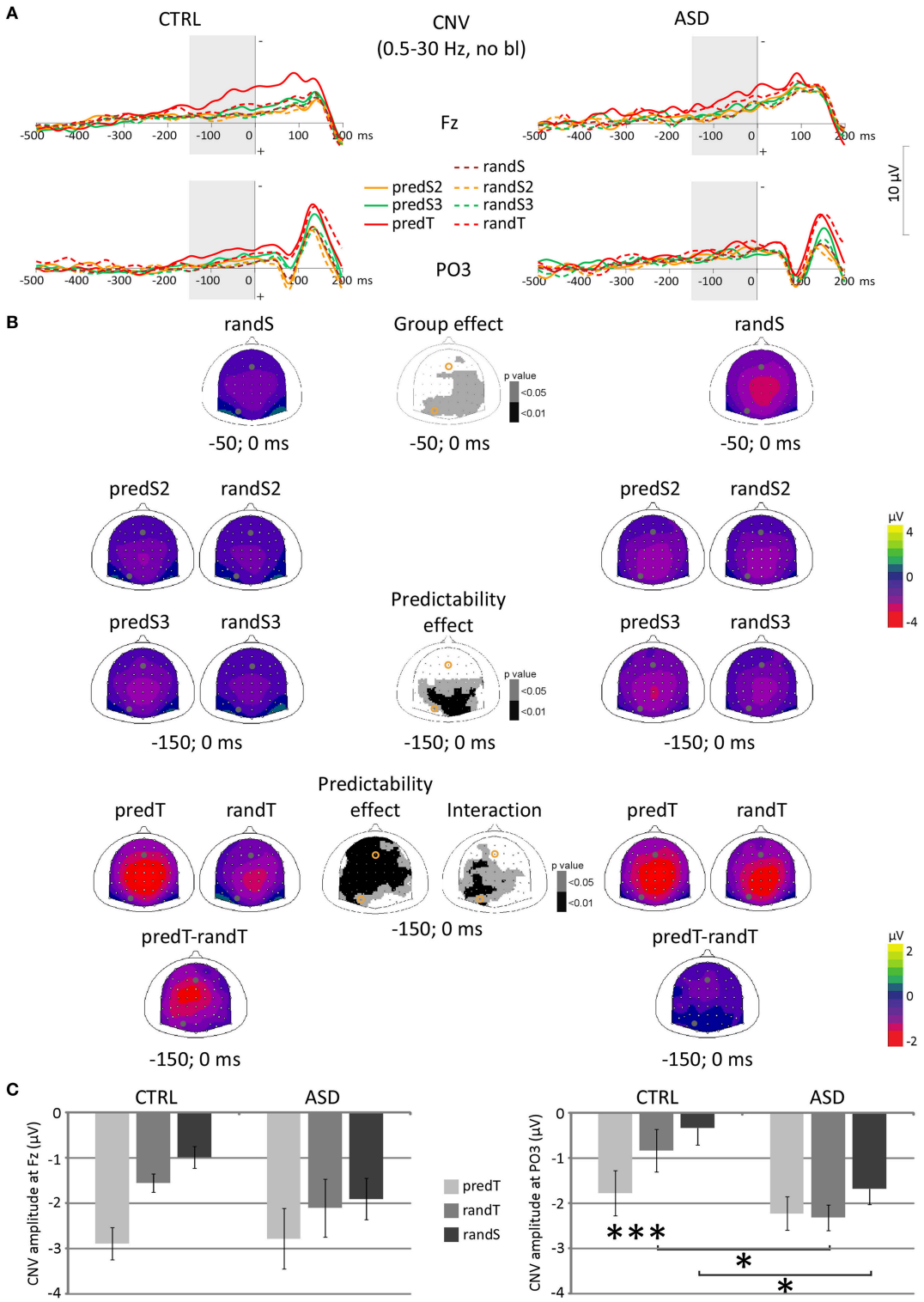

FIGURE 4 | Effect of predictive context on CNV amplitudes in controls (CTRL) and adults with ASD. (A) Grand-average non-baseline-corrected ERP waveforms band-pass filtered between 0.5 and 30 Hz, at the Fz and PO3 electrodes. **(B)** Scalp topographies (top views) of the mean CNV amplitude for each pair of predictive stimulus and its non-predictive analog, and for the difference between predTs and randTs in the −150–0 ms time-window, and for randS in the −50–0 ms time-window for controls (CTRL) and adults with ASD, and scalp topographies of the *p*-value resulting from the ANOVA. The dots and circles indicate the position of Fz and PO3 electrodes. **(C)** CNV mean amplitude between −150 and 0 ms at Fz and PO3 in µV for predTs, randTs, and between −50 and 0 ms for randS in controls (CTRL) and adults with ASD. Error bars = standard errors of the mean. Significant differences are indicated by asterisks: $^{*}p \leq 0.05$, $^{***}p \leq 0.001$.

whereas adults with ASD showed an increased CNV preceding randTs and randS compared to controls.

P3

P3 amplitude to S2 and S3 displayed a predictability effect, only, at centro-parietal electrodes [e.g., Pz: $F_{(1, 22)} = 10.28$, $p = 0.004$; and $F_{(1, 22)} = 25.73$, $p < 0.001$, respectively; **Figure 5**]. These effects corresponded to an enhancement of the P3 amplitude to standard stimuli with predictive value in both groups.

No effect was found significant on the maximum P3 amplitude at Pz to targets.

A predictability effect was found on the target-P3 latency [$F_{(1, 22)} = 13.37$, $p = 0.001$]. The P3 to predTs was found earlier than to randTs at Pz.

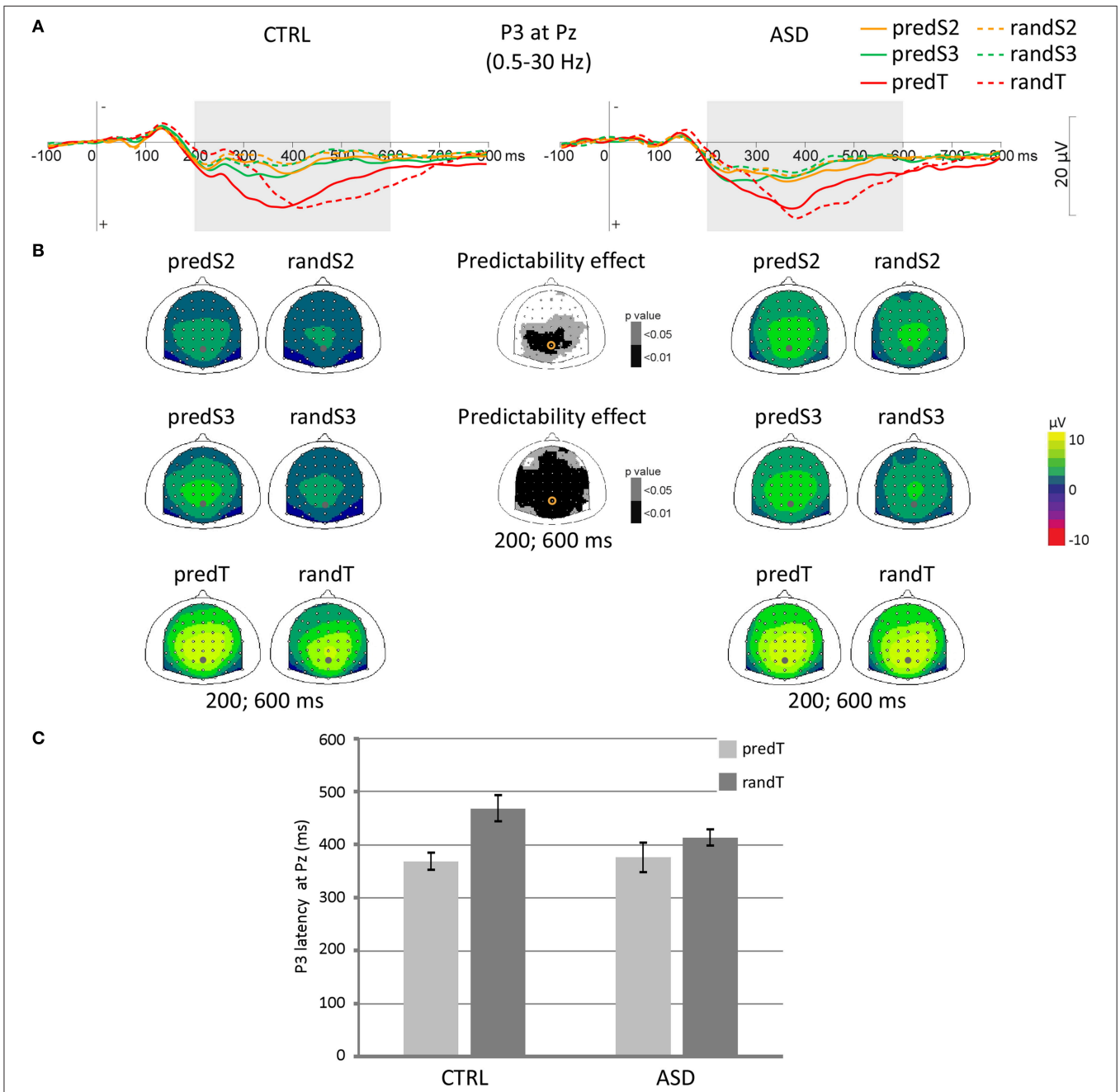

FIGURE 5 | Effect of predictive context on P3 in controls (CTRL) and adults with ASD. (A) Grand-average ERP waveforms band-pass filtered between 0.5 and 30 Hz, at the Pz electrode. **(B)** Scalp topographies (top views) of the mean P3 ERP for each pair of predictive stimulus and its non-predictive analog in the 200–600 ms time-window for controls (CTRL) and adults with ASD, and scalp topographies of the *p*-value resulting from the ANOVA. The dots and circles indicate the position of the Pz electrode. **(C)** Latency of the P3 maximum in ms at Pz for predicted and random targets in controls (CTRL) and adults with ASD. Error bars = standard errors of the mean.

In both groups, P3 amplitude increased throughout the predictive sequence and P3 latency was shortened to predTs.

Time-Frequency Results

No effect was found on the 8–14 Hz power before S3 or S2.

Left Central Electrodes

A predictability × group interaction was found on the mu power preceding targets between −400 and −200 ms at left central electrodes [e.g., C5: $F_{(1, 20)} = 10.68$, $p = 0.004$; **Figure 6**]. *Post-hoc* tests showed that only controls displayed a decrease in mu power between −400 and −200 ms before predTs compared to randTs (e.g., C5, CTRL: $p = 0.015$; ASD: $p = 0.256$). Moreover, the decrease in mu power was found larger in CTRL than in ASD between −400 and −200 ms before predTs (e.g., C5: $p = 0.002$), but not before randTs (e.g., C5: $p = 0.247$).

Predictability effect on mu power to targets (difference in mu power between randTs and predTs) at C5 was found correlated with the RT prediction index ($r = 0.627$, $p = 0.002$; **Figure 6D**). The larger the power reduction, the larger the benefit in reaction time.

Fronto-Central Electrodes

A predictability × group interaction was found on the alpha power preceding targets between −400 and −100 ms at fronto-central electrodes [e.g., FCz: $F_{(1, 20)} = 11.90$, $p = 0.002$; **Figure 6**]. Controls displayed a decrease in alpha power between −400 and −100 ms before predTs compared to randTs (e.g., FCz: $p = 0.002$); whereas adults with ASD showed a trend for a pre-stimulus alpha increase before predTs compared to randTs (e.g., FCz: $p = 0.054$). The decrease in alpha power was larger in CTRL than in ASD between −400 and −200 ms before predTs (e.g., FCz: ASD > CTRL, $p = 0.047$), but not before randTs (e.g., FCz: $p = 0.394$).

Moreover, a group effect was found on the alpha power between 0 and 300 ms [e.g., FCz: $F_{(1, 20)} = 14.39$, $p = 0.001$]. Controls presented a decrease in alpha power after target onset; whereas adults with ASD showed an increase in alpha power. Analysis of the phase-locking factor indicated an increase in phase-locking to target onset in the alpha band in the same latency range at frontal electrodes in both groups (**Figure 7**). This increase in phase-locking factor corresponds to the alpha content of the P2 and N2 frontal ERP components.

In summary, adults with ASD did not display a decrease in mu power at left central electrodes before predTs, nor a decrease in alpha power at frontal electrodes after all targets.

DISCUSSION

During an uncertain context, adults with ASD were faster to detect the target, presented an increased CNV amplitude indexing enhanced preparatory mechanisms, and a shortened N2 latency reflecting faster information processing.

During a certain context, both controls and adults with ASD presented an increased P3 amplitude indexing information encoding of the predictive sequence, an enhanced CNV amplitude corresponding to the deployment of preparatory mechanisms and a reduced target-P3 latency reflecting efficient prediction building and implementation. However, adults with ASD displayed a failure to decrease mu power during motor preparation. This physiological deficit was accompanied by a reduced benefit in reaction times to predictable targets in patients with ASD.

Taken together, the present results provide novel evidence indicating an atypical detection and processing of targets in an uncertain context, coupled with an atypical motor preparation to predictable targets despite a preserved extraction of predictive information.

CNV and N2: Target Over-Anticipation within an Uncertain Context

Studies of visual target detection in ASD have reported inconsistent findings, with evidence for equivalent (Tsai et al., 2011) or shorter (Dichter et al., 2009; Maekawa et al., 2011) RTs in response to non-cued target compared to controls. In the present study, adults with ASD were faster than controls to detect the target preceded by a non-informative context with similar overall accuracy. No group differences were found on the visual ERP components, suggesting that targets receive similar degrees of sensory processing in adults with ASD and in controls. Critically, adults with ASD displayed an enhanced CNV before the random standards and targets compared to controls, providing evidence of deployment of atypical increased preparatory mechanisms. In addition, adults with ASD displayed a shortened N2 latency to the random target, suggesting shorter stimulus evaluation (Donchin et al., 1986; Hillyard and Picton, 2011), and response activation time (Smid et al., 1990), supporting faster visual information processing in adults with ASD. The enhanced CNV, the earlier N2 and the shorter reaction times suggest an over-anticipation of stimuli in an uncertain context in adults with ASD. This excessive processing may be counterproductive in daily life and may lead to feelings of sensory overload often reported by individuals with ASD.

CNV and P3: Preserved Extraction and Use of Predictive Contextual Information

P3 amplitude progressively increased throughout the predictive sequence, i.e., as a function of task relevance and confidence (Sawaki and Katayama, 2006) comparably in controls and ASD subjects. In agreement with a role of the P3 in context-updating (Donchin and Coles, 1988), the present results support the notion that adults with ASD are able, as well as controls, to extract predictive information from the stimulus train.

Adults with ASD displayed a benefit in reaction time with predictive context suggesting that they generate prediction and use it in order to anticipate the predictable target. Moreover, target predictability shortens P3 latency (indicating a shortened duration of stimulus evaluation processing; Kutas et al., 1977; Duncan-Johnson and Kopell, 1981) and enhances CNV amplitude before the predictable targets (reflecting the enhanced recruitment of preparatory mechanisms; Brunia and van Boxtel, 2001) in both groups, confirming that prediction has been implemented.

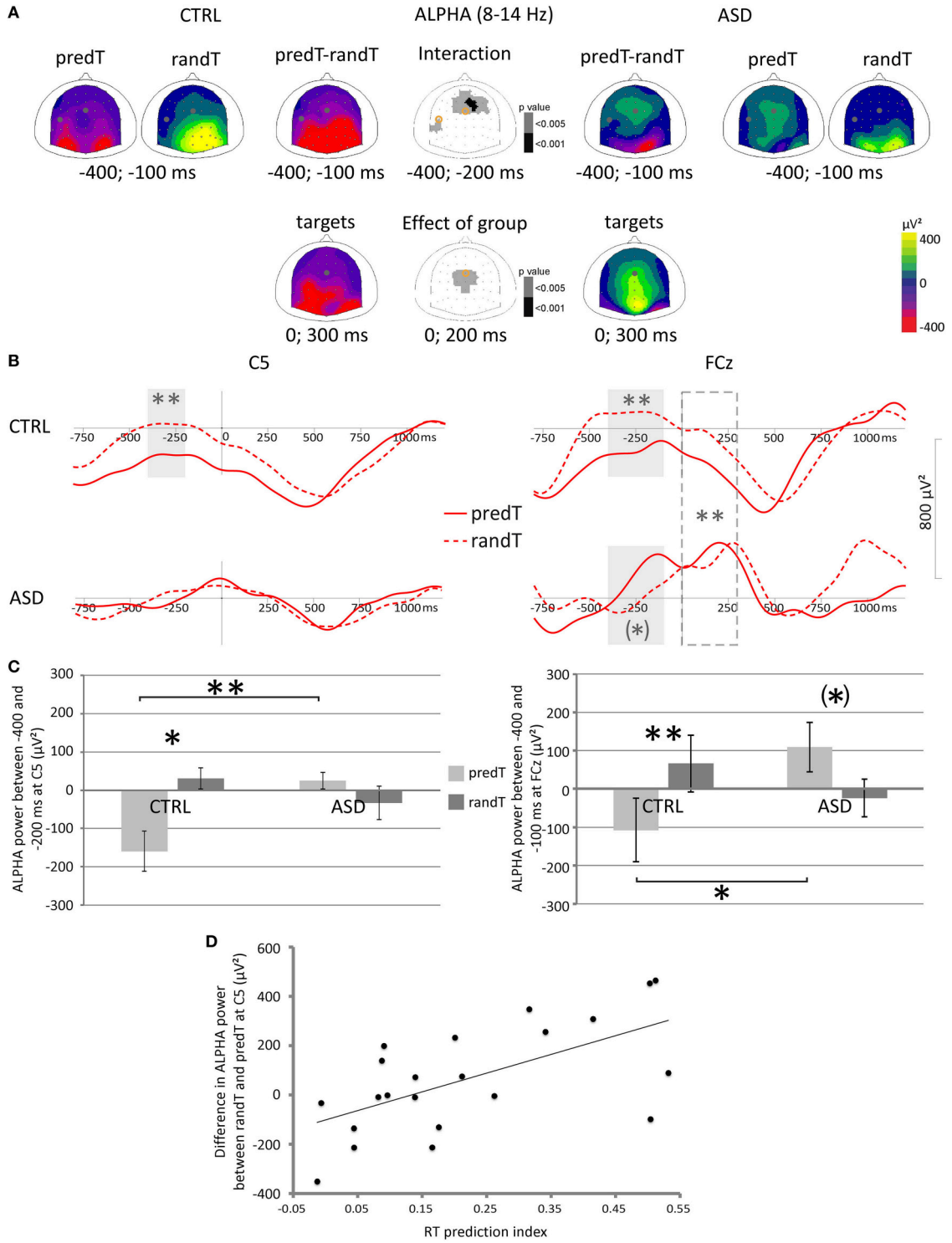

FIGURE 6 | Effect of predictive context on motor and frontal oscillatory activities in the 8–14 Hz frequency band in controls (CTRL) and adults with ASD. (A) Scalp topographies (top views) of the mean TF power value between −400 and −100 ms and 0 and 300 ms, and of the *p*-value resulting from the ANOVA. The dots and circles indicate the position of the C5 and FCz electrodes. **(B)** Alpha frequency band profiles of TF power at the Fz and C5 electrodes to predTs and randTs (solid and dashed red lines, respectively) in controls (CTRL) and adults with ASD. Time-windows showing a significant difference between the two conditions are indicated by gray bars. Time-window showing a significant difference between the two groups is indicated by dashed gray bar. **(C)** Mean alpha power (μV^2) between −400 and −200 ms at C5 electrode for predTs and randTs in controls (CTRL) and adults with ASD. Mean alpha power (μV^2) between −400 and −100 ms at FCz electrode for predTs and randTs in controls (CTRL) and adults with ASD. Error bars = standard errors of the mean. Significant differences are indicated by asterisks: (*) $p = 0.054$, *$p < 0.05$, **$p \leq 0.01$ **(D)** Difference in mean alpha power (μV^2) between randTs and predTs at C5 plotted against the RT prediction index.

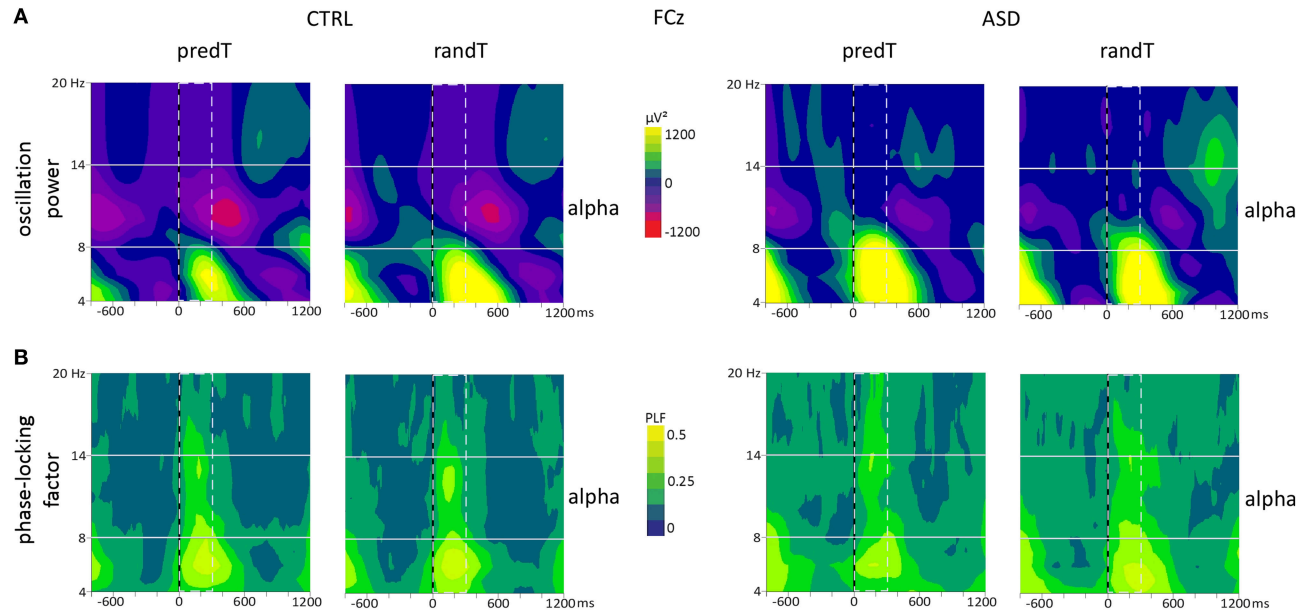

FIGURE 7 | Effect of predictive context on frontal oscillatory activities in controls (CTRL) and adults with ASD. (A) Grand-average TF plots of the oscillation power and **(B)** phase-locking factor (PLF) at the FCz electrode for predTs and randTs. Alpha (8–14 Hz) frequency band is depicted. Time-window showing a significant difference between the two groups is indicated by dashed bar.

Mu Oscillations: Motor Anticipation Failure

In accordance with a previous study (Bidet-Caulet et al., 2012), we observed in controls a decrease in mu power before the predictable target onset at left central electrodes, reflecting motor cortex activation prior to execution of the button press (Pfurtscheller and Lopes da Silva, 1999). However, adults with ASD failed to display this mu decrease before the predictable target, suggesting reduced motor preparation. This motor anticipation failure explain why adults with ASD took less advantage from the predictive information compared to controls (smaller RT prediction index).

This result is in accordance with Kanner's first description (Kanner, 1943), and studies on motor anticipatory functions (Schmitz et al., 2003; Martineau et al., 2004) showing major anticipation difficulties.

Frontal Alpha Oscillations: Atypical Frontal Mechanisms

Electrophysiological results revealed an increased alpha activity in adults with ASD before the predictable targets over fronto-central regions. This alpha increase may reflect frontal compensation strategies to counteract the lack of motor cortex pre-activation for response execution, or an impairment in integrating prediction with behavior, i.e., an executive dysfunction (Luna et al., 2002).

Moreover, after target onset, adults with ASD presented a phase-locked increase in alpha power at fronto-central electrodes (corresponding to the alpha content of the P2 and N2 frontal ERP components); whereas controls showed a large decrease in alpha power overlapping the phase-locked alpha response. Greater alpha power after both random and predictable targets over the fronto-central regions in adults with ASD may reflect an abnormal inhibition of potential frontal processes needed for executive control during predictive processing, which is consistent with previous findings of atypical executive functions associated with frontal hypo-activation in adults with ASD (Luna et al., 2002).

Link with Predictive Coding Model

Predictive models of ASD agree about an imbalance of the weight ascribed to bottom-up sensory signals relative to top-down influence of prior information (Brock, 2012; Pellicano and Burr, 2012; Friston et al., 2013; Lawson et al., 2014; Van de Cruys et al., 2014; Skewes et al., 2015) with ASD perception dominated by sensory input. This would result in a tendency to perceive the world in a more veridical way rather than modulated by prior experience (Gomot and Wicker, 2012; Pellicano and Burr, 2012; Lawson et al., 2014; Skewes et al., 2015; Van de Cruys et al., 2014).

According to predictive models, in typically developing individuals, the changing levels of environmental uncertainty determine the assigned weight to prediction errors (Feldman and Friston, 2010; Van de Cruys et al., 2014). In an optimal system, precision in prediction errors (i.e., brain's degree of confidence in the sensory signal) decreases in contexts with higher uncertainty (i.e., when there are no learnable regularities in the environment). The CNV component has been proposed as a proxy for the precision of prediction errors (Feldman and Friston, 2010; Hesselmann et al., 2010) and its amplitude is enhanced with increasing certainty in normal populations. In agreement with this model, the CNV amplitude increased with

enhanced predictability of the upcoming stimulus in typically developing participants in the present and previous studies (Bidet-Caulet et al., 2012). Interestingly, we found that adults with ASD generate a larger CNV, compared to controls, in an uncertain context before all random standards and targets. This result suggests that, in the random context, patients with ASD give a high precision to prediction errors as if they were still looking for learnable regularities; whereas typically developing individuals reduce their precision in prediction errors—they sense that there are no learnable regularities. Adults with ASD resist uncertainty and tend to generate higher levels of sensory precision (Van de Cruys et al., 2014). This finding is in line with an inability to flexibly process prediction errors (Palmer et al., 2013; Van de Cruys et al., 2014), and with a failure to attenuate sensory precision.

A limitation of this study is the relatively small sample size. Further, investigations on bigger sample size are needed in order to confirm our results.

We demonstrate that adults with ASD over-anticipate stimuli occurring in an uncertain context. In a certain context, ASD subjects are able to extract predictive information and to use it in order to anticipate the predictable targets. However, the present results may reflect frontal compensation strategies to counteract the lack of automatic motor cortex pre-activation for execution of the motor response. There is a cost to this excessive processing that may be counterproductive in unpredictable and fluctuating situations, such as the social world, leading to stressful reactions, and a sense of overwhelming. Taken together, these results provide evidence that adults with ASD cannot flexibly modulate cortical activity according to changing levels of uncertainty. Moreover, these findings could ultimately contribute to the treatment of adults with ASD without intellectual disability. Further, research is needed in order to build cognitive remediation programs to provide strategies to patients with ASD so that they overcome prediction weaknesses.

AUTHOR CONTRIBUTIONS

AT has made a substantial contribution to the conception and design, to the acquisition, analysis and interpretation of the data; ML, ED, and CB have made a substantial contribution to the interpretation of the data; SR has made a substantial contribution to the conception and design, and to the acquisition and analysis of data; RK, AC, and FB have made a substantial contribution to the conception and design, and to the analysis and interpretation of the data. All authors have made a substantial contribution to drafting the article or reviewing it critically, have given final approval of the version of the article to be published and have agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy of integrity of any part of the work are appropriately investigated and resolved.

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Impact of Sensory Sensitivity on Physiological Stress Response and Novel Peer Interaction in Children with and without Autism Spectrum Disorder

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Background: For many children with Autism Spectrum Disorder (ASD), social interactions can be stressful. Previous research shows that youth with ASD exhibit greater physiological stress response during peer interaction, compared to typically developing (TD) peers. Heightened sensory sensitivity may contribute to maladaptive patterns of stress and anxiety. The current study investigated between-group differences in stress response to peer interaction, as well as the role of sensory sensitivity.

Methods: Participants included 80 children (40 ASD) between 8 and 12 years. Children participated in the peer interaction paradigm (PIP), an ecologically valid protocol that simulates real-world social interaction. Salivary cortisol was collected before and after the 20 min PIP. Parents completed questionnaires pertaining to child stress (*Stress Survey Schedule*) and sensory sensitivity (*Short Sensory Profile*). Statistical analyses included *t*-tests and ANCOVA models to examine between-group differences in cortisol and play; Pearson correlations to determine relations between cortisol, play, and questionnaire scores; and moderation analyses to investigate interactions among variables.

Results: Controlling for baseline cortisol values, children with ASD showed significantly higher cortisol levels than TD peers, in response to the PIP [$F_{(1,\ 77)} = 5.77$, $p = 0.02$]. Cortisol during play was negatively correlated with scores on the SSP ($r = -0.242$, $p = 0.03$), and positively correlated with SSS ($r = 0.273$, $p = 0.02$) indicating that higher cortisol was associated with greater sensory sensitivity (lower SSP reflects more impairment) and enhanced stress in various contexts (higher SSS reflects more stress). Furthermore, diagnosis was a significant moderator of the relation between cortisol and SSP, at multiple time points during the PIP ($p < 0.05$).

Conclusions: The current study extends previous findings by showing that higher physiological arousal during play is associated with heightened sensory sensitivity and a pattern of increased stress in various contexts. Results are discussed in a broader context, emphasizing the need to examine relationships between social, behavioral, and physiological profiles in ASD to enhance understanding and improve treatments aimed at ameliorating stress and sensory dysfunction, while enhancing social skills.

Keywords: autism, stress, cortisol, sensory, peer interaction, social, auditory

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INTRODUCTION

Impaired social communication is a primary characteristic of Autism Spectrum Disorder (ASD) (American Psychiatric Association, 2013) and may be the result of limitations in social cognition (e.g., Baron-Cohen, 1995) or social motivation (Chevallier et al., 2012). Underlying physiological factors may also impact the extent to which individuals with ASD engage with others in social situations. For example, there is considerable evidence that social interactions are highly stressful for children with ASD (Lopata et al., 2008; Corbett et al., 2010). It may be this exaggerated stress response that drives, in part, reductions in social engagement. There is also significant prevalence of sensory dysfunction in ASD (Rogers et al., 2003; Kern et al., 2006; Tomchek and Dunn, 2007; Ben-Sasson et al., 2009), and these hyper- and/or hypo- sensory sensitivities may further contribute to atypical patterns of social interaction.

Compared to typically developing (TD) peers, children with ASD tend to interact less with other children (Corbett et al., 2010, 2012) and engage in less cooperative play (Corbett et al., 2014b), instead showing a preference to engage in self-play (Humphrey and Symes, 2011). There is also evidence that individuals with ASD have abnormal preferences for interpersonal distance (IPD), including both standing too close or preferring more distance relative to TD peers (Kennedy and Adolphs, 2014; Lough et al., 2015; Perry et al., 2015). As children with ASD become more aware of their limitations in social skills with age (Knott et al., 2006), later peer interactions may be characterized by exacerbated social stress (Schupp et al., 2013). As a result of this stress, these children may display increased avoidance behaviors characterized by decreased social motivation to interact with peers. This notion is supported by research showing that higher cortisol response to a novel social interaction was associated with reduced social communication among children with ASD (Corbett et al., 2014b).

The hypothalamic-pituitary-adrenal (HPA) axis is a highly regulated system that is responsible for maintaining homeostasis via maintenance of a diurnal rhythm, activation in response to stress or threat, and restoration of basal activity via negative feedback mechanisms. Cortisol, an important stress hormone, serves as a measurable indicator of HPA regulation, increasing in concentration after exposure to stressors (Herman and Cullinan, 1997). Cortisol also shows a diurnal rhythm of fluctuating concentration, throughout the day. Typically, diurnal cortisol values are at peak levels in the morning and slowly decline to lowest levels in the evening. In ASD, the diurnal rhythm appears to be altered, such that children with ASD have lower morning cortisol and higher evening cortisol values relative to TD children (Corbett et al., 2008, 2009). Further, many children with ASD show an increased response to stress, as indicated by higher cortisol levels, when interacting with unfamiliar peers (Lopata et al., 2008; Corbett et al., 2010). This stress response appears to be tied to level of social cognition, as those with highest cortisol values tend to show less social motivation (Corbett et al., 2014b). It is important to note, however, that this response is considerably variable within the ASD population (Lopata et al., 2008; Schupp et al., 2013). This suggests the possibility of interactions with other characteristics, which may explain part of the high variability in psychosocial stress response as seen in ASD.

Sensory processing abnormalities are now considered a core symptom of ASD under the Restrictive and Stereotypic Behaviors criteria of the DSM-5 (American Psychiatric Association, 2013). Sensory behaviors have historically been divided into four patterns, including sensory hypo-reactivity, hyper-reactivity, sensory seeking, and sensory avoidance (Dunn, 2001; Ben-Sasson et al., 2009). Complexity arises from the fact that many individuals with ASD will show several of these patterns at one time, with mixed patterns being displayed across sensory domains (Baranek et al., 2006; Tomchek and Dunn, 2007; Lidstone et al., 2014). Recent conceptualizations, therefore, have attempted to classify sensory processing abnormalities in ASD into specific subtypes (Lane et al., 2011, 2014; Uljarević et al., 2016), such as sensory adaptive, sensory moderate, and sensory severe (Uljarević et al., 2016).

Emerging research demonstrates a relationship between sensory sensitivities and arousal/anxiety in ASD. In one study, children with ASD showed lower morning cortisol relative to TD peers, which was associated with higher parent-reported stress as measured by the Stress Survey Schedule (SSS) (Corbett et al., 2009). Similarly, morning cortisol was associated with various domains of sensory functioning derived from the Short Sensory Profile—a parent-reported indicator of sensory sensitivities—(SSP; Dunn, 1999) painting a complex picture based on individual profiles (Corbett et al., 2009). Other studies investigating sensory profiles in ASD have also found relationships between sensory responsivity and anxiety (Green et al., 2012; Lidstone et al., 2014; Wigham et al., 2015; Uljarević et al., 2016), repetitive behaviors and stereotyped movements (Gabriels et al., 2008; Gal et al., 2010; Lidstone et al., 2014), and intolerance of uncertainty (Chamberlain et al., 2013; Wigham et al., 2015). Furthermore, a sample of TD young adults showed a correlation between sensory sensitivity and increased interpersonal distance (Perry et al., 2016), suggesting some role of sensory processing in social behavior. Currently, however, there is limited research that attempts to elucidate the impact of sensory dysfunction on social motivation and communication, especially in a naturalistic play setting.

The current study sought to investigate between-group differences in biobehavioral profiles of psychosocial stress following play with unfamiliar peers, in conjunction with sensory profiles. In order to elucidate the impact of sensory dysfunction on stress and social interaction, the current study sought to measure social engagement during a Peer Interaction Paradigm (PIP) (Corbett et al., 2010) and then to compare social behavior to stress and sensory profiles, as indicated by measures of cortisol and parent reports of stress and sensory sensitivity. It was hypothesized that children with ASD would have greater cortisol in response to play, relative to TD peers. Furthermore, it was hypothesized that cortisol response would be positively

correlated with both increased parent-reported stress and sensory sensitivity.

MATERIALS AND METHODS

Participants

The sample included 80 un-medicated, pre-pubertal, children between the ages of 8-to 12 years, including 40 with ASD (mean = 9.65 years) and 40 TD (mean = 9.79 years). The gender composition included 14 females (6 = ASD, 8 = TD) and 66 males (34 = ASD, 32 = TD). ASD diagnosis was based on the Diagnostic and Statistical Manual (DSM-5) criteria (American Psychiatric Association, 2013) and established by all of the following: (1) a previous diagnosis by a psychologist, psychiatrist, or behavioral pediatrician with ASD expertise; (2) current clinical judgment; and (3) corroborated by the ADOS (Lord et al., 2000), administered by research-reliable personnel. For inclusion in the study all participants were required to have an estimated IQ of 80 or above, as measured by the Wechsler Abbreviated Scale of Intelligence (Wechsler, 1999). In addition, pubertal status was determined by the Pubertal Development Scale (Petersen et al., 1988) to confirm that the child had not yet entered puberty.

The Vanderbilt University Institutional Review Board approved the study. The investigation was performed in accordance with the Helsinki Declaration for research involving human subjects. Prior to inclusion in the study, informed written consent from parents and verbal assent from research participants were obtained. Participants were recruited by IRB approved flyers and several recruitment systems via clinics, subject tracking systems, resource centers, support groups, schools, and recreational facilities.

As described below, the study also included children who served as confederates (actor that participates in the study), who were of the same age and gender as the ASD and TD children. Parents of the confederates provided informed consent for them to train for and participate in the study. Confederates were selected based on demonstrated strong social skills, genuine desire to play and interact with children with and without disabilities, and an ability to follow research personnel instructions and translate them into age appropriate play behaviors. All confederates underwent several training procedures, including reading an instruction manual, direct skills modeling, and playground practice. The majority of the confederates had previously participated in research or served as a peer helper for children with disabilities.

Peer Interaction Playground Paradigm

The Peer Interaction Paradigm examines social exchanges between children with and without ASD within a naturalistic playground environment (Corbett et al., 2010). The playground is a 130 by 120 ft. fenced in play area that is part of the Susan Gray Preschool. The 20-min paradigm consisted of periods of free play and opportunities for cooperative play, which was facilitated by an age- and gender-match TD confederate. The trained confederate followed cues provided by research personnel through an earpiece with a remote transmitter, in order to provide structure to play by simultaneously soliciting play with both research participants. Use of a confederate permitted each interactive sequence of free or solicited play to occur within an otherwise natural setting, while also maintaining an even level of play to prevent increased aerobic activity, which could affect cortisol levels. In addition to the confederate, the paradigm involved one child with ASD and one TD child, all three of whom were unfamiliar with each other prior to the interaction. Each participant only took part in one 20-min session, such that the exposure represented a novel social experience. During the protocol, research personnel remained in the building in order to facilitate more natural play behavior.

The paradigm was divided into four 5-min time (T) periods of intermittent free play and solicited play (see **Figure 1**). The first period (T1) consisted of unsolicited free play. During the second period (T2), the confederate was instructed to solicit interaction for cooperative play on the playground equipment. During the third period (T3), the confederate returned to unsolicited free play. During the fourth period (T4), the confederate was instructed to once again solicit the two participants to engage in a cooperative game involving toys.

Interactions were recorded using state-of-the-art video equipment, including four professional Sony EVI D70 (Sony, New York, NY, USA) remotely operated cameras housed in glass cases, which were affixed to the four corners of the playground's fence. The cameras contain pan, tilt, and zoom features allowing full capture of the playground. To record remote audio communication, Motorola MC22OR GMRS-FRS (Motorola, Libertyville, IL, USA) and Audio-Technica (Audio-Technica, Stow, Ohio, USA) transmitters and receivers functioning as battery-operated microphones were clipped to each child's shirt. Audio was recorded using an eight-channel mixing board.

Behavior Coding

The Observer XT Version 8.0 software was used for the collection and analysis of the observational social interaction data (Noldus, 2008). Data were analyzed based on a predefined list of operationalized behaviors (Corbett et al., 2010; Schupp et al., 2013) by research-reliable raters who were unaware of the current study aims. Behaviors were analyzed using a transactional approach (i.e., *who does what to whom*) based on predefined operationalized behaviors (Mendoza and Mason, 1989; Lyons et al., 1990, 1992; Mason et al., 1993). Inter-rater reliability was calculated using Cohen's Kappa at $K = 0.80$, while test-retest reliability was $K = 0.89$. Cooperative play interactions were calculated as percentage of time engaged (verbal 90% and $K = 0.85$; group play 91% and $K = 0.89$). Variables such as cooperative play and verbal interaction were operationalized based upon previously described definitions and as part of a larger study (Corbett et al., 2014b). For the purpose of this study, cooperative play was defined as the percentage of time engaged in a reciprocal activity for enjoyment that involved and relied on the participation of two or more

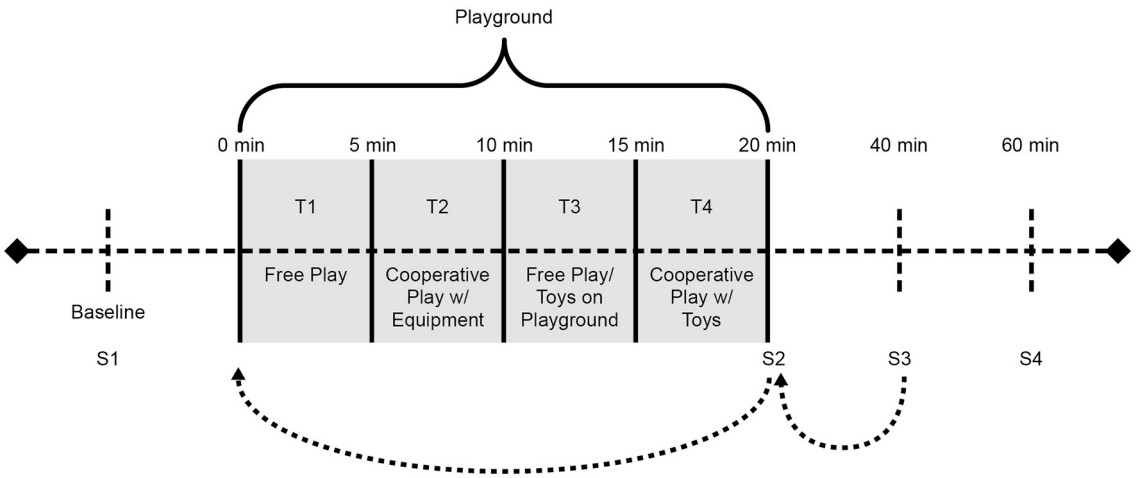

FIGURE 1 | Peer interaction paradigm timeline of events. The 20-min paradigm consists of four 5-min periods of free play (T1), solicited play with equipment (T2), free, unsolicited play (T3), and solicited cooperative play with toys (T4). Salivary cortisol measures were taken at baseline (S1), immediately after the paradigm (S2), 20-min after the paradigm (S3), and 40-min after (S4).

children (e.g., hide and seek). For an expanded description of the behavior coding protocol and operationalized variables, see previous studies (Corbett et al., 2010, 2014b; Schupp et al., 2013).

Salivary Cortisol Sampling

Basal levels of salivary cortisol were collected from home to ascertain the child's afternoon baseline over 3 days, using established methods, as part of a larger study (Corbett et al., 2008). For the purpose of this study, only playground cortisol levels were analyzed.

It is important to note that there is an approximate 20-min time lag between when an event occurs and when a related change in cortisol can be detected in saliva (Kirschbaum and Hellhammer, 1989). The peer interaction included four salivary cortisol samples taken 20 min apart for each subject: S1–(baseline), S2–(immediately post-play), S3–(20 min post-play), and S4–(40 min post play). The S2 measurement taken immediately after the peer interaction represents circulating cortisol levels at the start of the paradigm, while the S3 measurement is representative of levels at the end of the peer interaction (see **Figure 1**). All peer interactions were held in the afternoon between 13:00 and 16:00, for comparison to afternoon baseline values.

Immediately before and following the playground paradigm, the ASD and TD participants were each assigned to an individual room and sat with a research assistant for cortisol sampling. A similar bag of toys and activities was provided to each child in order to maintain consistency in experience across all participants. Samples were collected following the standardized drool procedures outlined in previous studies (Corbett et al., 2008, 2010).

Cortisol Assay

The salivary cortisol assay was performed using a Coat-A-Count® radioimmunoassay kit (Siemens Medical Solutions Diagnostics, Los Angeles, CA) modified to accommodate lower levels of cortisol in human saliva relative to plasma. Saliva samples, which had been stored at −20°C, were thawed and centrifuged at 3460 rpm for 15 min to separate the aqueous component from mucins and other suspended particles. The coated tube from the kit was substituted with a glass tube into which 100 μl of saliva, 100 μl of cortisol antibody (courtesy of Wendell Nicholson, Vanderbilt University, Nashville, TN), and 100 μl of ^{125}I-cortisol were mixed. After incubation at 4°C for 24 h 100 μl of normal rat serum in 0.1% PO4/EDTA buffer (1:50) and precipitating reagent (PR81) were added. The mixture was centrifuged at 3460 rpm for 30 min, decanted, and counted. Serial dilution of samples indicated a linearity of 0.99. Interassay coefficient of variation was 10.4%.

Dependent Measures

Short Sensory Profile (SSP; Dunn, 1999)

The SSP is a parent-report questionnaire designed to assess sensory sensitivity across seven sensory domains, such as auditory sensitivity, tactile sensitivity, and underresponsive/seeks sensation. Parents are asked to rate the frequency with which their child engages in behaviors related to sensory sensitivity in each domain. Possible scores range from 5 points to 1 point, ranging from "never responds in this manner" (5 points) to "always responds in this manner" (1 points). High raw scores indicate typical performance, while lower scores on the SSP are indicative of greater sensory dysfunction. The primary variable of interest for analysis was total raw score.

Stress Survey Schedule (SSS; Groden et al., 2001)

The SSS is a 60-item, parent-report survey designed to measure the daily stress of individuals with ASD and other developmental disabilities. The survey addresses eight domains of stress: Anticipation/Uncertainty, Changes and Threats, Unpleasant Events, Pleasant Events, Sensory/Personal Contact, Food Related Activity, Social/Environmental Interactions, and Ritual Related Stress. Parents are asked to rate intensity of stress for each item on a five-point Likert scale, ranging from none to mild stress (1) to severe stress (5). Internal consistency correlations range from 0.70 to 0.87. Higher total scores are indicative of enhanced stress, and total raw score was used for analysis.

Salivary Cortisol

The primary outcome variable of interest for characterizing the stress response was salivary cortisol. Samples were analyzed for S1–S4, as described above. Because salivary cortisol measurements are positive and skewed toward large values, a log transformation was performed to achieve approximate normality. Log-transformed values were used in all analyses.

Social Behavior

The primary outcome variable for social behavior was percent time spent in cooperative play during solicited play at T4 of the PIP.

Statistical Analysis

Descriptive statistics were calculated for demographic information, as well as parent-report measures of sensory functioning (SSP) and child stress (SSS); possible group differences in these variables were examined using independent samples *t*-tests. Analysis of covariance (ANCOVA) models were utilized to examine group differences in salivary cortisol response, controlling for baseline cortisol values. Pearson product moment correlations were conducted to examine associations between the sensory and stress measures. Moderation analyses were conducted to determine whether diagnosis was a moderator of the relation between SSP and cortisol response.

All statistical analyses were conducted using SPSS Version 22.0 (IBM Corp, 2013). The PROCESS application for SPSS was used to conduct moderation analyses (Hayes, 2013).

RESULTS

Demographic information for each group is presented in **Table 1**, including age, cognitive, and diagnostic information. Descriptive statistics for SSP, SSS, and cooperative play (separated by group) are listed in **Table 2**. Using independent samples *t*-tests, there were significant between-group differences on the SSP [$t_{(78)} = 140.44$, $p < 0.0001$] and the SSS [$t_{(77)} = 109.32$, $p < 0.0001$], showing greater parent-reported stress and sensory sensitivity in the ASD group, compared to the TD group.

In order to assess group differences in social behavior, the percent duration of cooperative play during the solicited play period T4 of the PIP was compared between groups, using independent samples *t*-tests. Results revealed a significant difference between groups in percent duration of cooperative play [$t_{(83)} = 8.349$, $p = 0.005$], showing that children with ASD engage in significantly less cooperative play than TD peers.

TABLE 1 | Demographics.

Variable	ASD mean (SD)	TD mean (SD)	*P*-value
Age	9.65 (1.485)	9.79 (1.631)	0.699
SCQ total	20.72 (7.229)	2.37 (2.123)	<0.0001
Performance IQ	111.03 (53.394)	112.45 (12.831)	0.973
Verbal IQ	108.72 (62.858)	112.16 (13.997)	0.240
WASI estimated IQ	101.22 (22.505)	119.47 (13.123)	<0.001

ASD, Autism spectrum disorder; IQ, intelligence quotient; TD, typical development; SCQ, social communication questionnaire; WASI, Weschler abbreviated scale of intelligence.

TABLE 2 | Descriptive statistics for parent-report measures and play.

Variable	ASD mean (SD)	TD mean (SD)	*P*-value
TOTAL SCORES			
Short sensory profile	122.28 (21.393)	172.82 (16.629)	<0.0001
Stress survey schedule	122.02 (30.243)	62.59 (18.823)	<0.0001
PERCENT TIME INTERACTING			
Cooperative play	55.15 (36.08)	74.54 (24.13)	0.005

TABLE 3 | Descriptive statistics for log cortisol at baseline, in response to the PIP, and after the PIP.

Cortisol value	ASD mean (SD)	TD mean (SD)
Baseline cortisol (S1)	−0.1515 (0.333)	−0.1034 (0.267)
Cortisol during play (S3)	−0.1284 (0.303)	−0.2730 (0.373)
Cortisol after play (S4)	−0.2190 (0.293)	−0.3148 (0.266)

Descriptive statistics for cortisol at baseline, during play, and after play are shown in **Table 3**. Analysis of covariance (ANCOVA) was conducted to examine the effect of group on stress (cortisol levels during cooperative play with peers), while controlling for baseline values. Results indicate a significant group difference in cortisol during play (S3) with peers [$F_{(1, 77)} = 5.77$, $p = 0.02$]. There was also a significant group difference in cortisol after play (S4) [$F_{(1, 77)} = 4.78$, $p = 0.03$].

In consideration of these group differences, Pearson product correlations were calculated to assess the relations between physiological stress (cortisol), sensory functioning (SSP), and parent-report stress (SSS). Cortisol during play was negatively correlated with scores on the SSP ($r = -0.24$, $p = 0.03$) and positively correlated with scores on the SSS ($r = 0.27$, $p = 0.02$). Therefore, as cortisol levels increased during the PIP, SSP scores decreased (indicating greater sensory dysfunction) and global stress as measured by the SSS increased.

Moderation analysis was conducted to examine the impact of diagnosis on the relation between stress (cortisol) and sensory (SSP) functioning. Results show that diagnosis was a significant moderator of the association between SSP and cortisol at baseline (S1) [$\Delta R^2 = 0.01$, $F_{(1, 76)} = 8.46$, $p = 0.0047$], the beginning

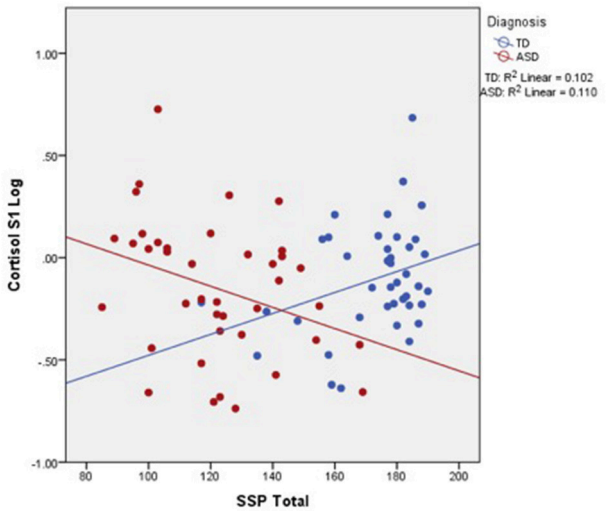

FIGURE 2 | Relationship between cortisol and sensory profile in children with ASD and TD peers.

of play (S2) [$\Delta R^2 = 0.06$, $F_{(1, 76)} = 4.85$, $p = 0.03$], during play (S3) [$\Delta R^2 = 0.02$, $F_{(1, 76)} = 3.94$, $p = 0.05$], and after play (S4) [$\Delta R^2 = 0.05$, $F_{(1, 76)} = 3.95$, $p = 0.05$]. **Figure 2** shows the moderating effect of diagnosis on the relation between SSP and cortisol, indicating that the negative correlation observed between cortisol and sensory functioning in the total sample is driven by the ASD group, whereas the opposite trend appears to hold true for the TD group.

In order to examine the impact of sensory sensitivity on play, we examined the association between SSP score and percent duration spent in cooperative play during the solicited play period, T4. There was a trend level positive association between cooperative play and total SSP ($r = 0.197$, $p = 0.07$). This association suggests that more sensory impairment was associated with less time spent in cooperative play (lower scores on the SSP are indicative of greater sensory dysfunction).

In an effort to further assess the impact of sensory sensitivity on play, *post-hoc* correlational analyses were conducted for individual domains of the SSP along with time spent in cooperative play. Scores on the Auditory domain were significantly positively correlated with cooperative play ($r = 0.218$, $p = 0.04$), while Underresponsive/Seeks Sensation correlated with play at a trend level ($r = 0.196$, $p = 0.07$).

DISCUSSION

While challenges in reciprocal social interaction are synonymous with ASD, sensory dysfunction and heightened stress are often found as well. Thus, the current study sought to examine the impact of sensory dysfunction on stress and social engagement with unfamiliar peers in children with and without ASD. The study utilized measures of social interaction (cooperative play) and physiological arousal (cortisol) during peer play using the PIP (Corbett et al., 2010) to assess the associations of social behavior and physiological stress with parent reports of stress and sensory sensitivity. It was hypothesized that children with ASD would show greater levels of salivary cortisol in response to play, relative to TD peers. Furthermore, it was hypothesized that cortisol response would be positively correlated with both increased parent-reported stress and sensory sensitivity. Therefore, the current study sought to further investigate biobehavioral profiles following play with unfamiliar peers with an emphasis on the impact of sensory sensitivity.

Autism is characterized by prevalence of sensory dysfunction (Rogers et al., 2003; Kern et al., 2006; Tomchek and Dunn, 2007; Ben-Sasson et al., 2009) and increased stress, especially during social interactions (Lopata et al., 2008; Corbett et al., 2010). In the current study, children with ASD showed both increased sensory dysfunction and parent-reported stress, relative to their TD peers. Research has shown that both altered sensory sensitivity and stress have deleterious effects on the overall functioning of children with ASD, especially given their potential associations with a variety of challenges, such as poor response to change (e.g., Chamberlain et al., 2013; Wigham et al., 2015) and anxiety (Green et al., 2012; Lidstone et al., 2014; Wigham et al., 2015; Uljarević et al., 2016).

In addition to group differences in sensory sensitivity and parent-reported stress, differences in salivary cortisol response were observed. Consistent with previous reports (Corbett et al., 2010, 2012, 2014b; Schupp et al., 2013), the ASD group had significantly greater physiological arousal in response to social interaction relative to the TD group, as indicated by higher cortisol levels both during and after the PIP. The increased arousal suggests that children with ASD may perceive social interaction with peers to be more stressful, compared to TD peers. Moreover, cortisol response was significantly correlated with parent-reported stress suggesting that heightened physiological reactions to peer interaction are also associated with a global pattern of elevated stress. Findings add to a growing body of work showing dysregulated patterns of stress in youth with ASD, both in terms of global and diurnal stress (Corbett et al., 2008, 2009), as well as stress in response to social interaction (e.g., Corbett et al., 2014b).

In light of observing group differences for both physiological stress and sensory sensitivity, we explored correlations between these variables. Significant associations were found between cortisol and SSP, such that higher cortisol in response to play was associated with greater sensory impairment. Moderation analyses were conducted to determine whether the correlation was driven by group status. Results showed that ASD diagnosis was a significant moderator of this relation, such that sensory dysfunction was associated with increased salivary cortisol response to peer interaction in the ASD group only. Moreover, this relationship held for cortisol levels at each time point of collection, including baseline cortisol as well as before, during, and after play. Interestingly, the TD group appeared to have the opposite relationship between cortisol and sensory functioning (**Figure 2**), implying that the negative impact of sensory sensitivity on stress reactivity during play is unique to ASD. In other words, greater levels of sensory dysfunction in ASD may lead to increased physiological stress. Thus, it appears

that the symptom profile of ASD ostensibly contributes to enhanced reactivity to the environment, which can be manifested in both sensory and physiological functioning. The question still remains whether or not these relationships between stress and sensory sensitivity are bi-directional or whether a uni-directional model exists, such that one symptom precedes and drives the other.

When assessing group differences in cooperative play, the ASD group engaged in significantly less cooperative play, relative to the TD group. This is in line with previous findings that children with ASD tend to engage less than their TD peers during the PIP (e.g., Corbett et al., 2014b). While factors such as social cognition (Baron-Cohen, 1995; Corbett et al., 2014a) and social motivation (Chevallier et al., 2012; Corbett et al., 2014b) appear to play a significant role in explaining group differences in social engagement, sensory dysfunction may also have an impact on social behavior. For example, sensory sensitivity was recently shown to associate with interpersonal distance in TD individuals (Perry et al., 2016). In order to better understand the potential impact of sensory sensitivity on play behavior in ASD, duration spent in cooperative play was analyzed along with sensory processing scores. While overall sensory dysfunction was not associated with play behavior, auditory sensitivity was significantly correlated with play.

Adults with ASD have shown impairments in auditory localization through an inability to use prior perceptual experience to locate sounds (Skewes and Gebauer, 2016). Difficulties in auditory processing and orienting to a stimulus may be linked to emergence of impairments in social and communication behaviors early in development (Osterling et al., 2002). As such, children with ASD fail to orient to stimuli, especially critical social stimuli such as response to their own name (Dawson et al., 1998). It is possible that the inability to attend to stimuli is due, in part, to altered auditory processing, resulting in reduced early social interactions through a lack of joint attention with the caregiver. According to the Enactive Mind hypothesis (Klin et al., 2003), limited early social experiences may contribute to the social communication deficits that characterize ASD. Therefore, if sensory sensitivity alters one's experience in the physical world, interactions in the social world may be absent, incomplete, or unpredictable among children with ASD, thus leading to difficulties in social communication and cognition. While this is an intriguing theory for connecting the sensory and social symptoms of ASD, a great deal of empirical evidence is needed to test for direct links between sensory and social functioning in ASD.

Limitations and Future Directions

The study had several strengths, including a relatively large sample size and use of a validated protocol for observing play behavior in a naturalistic playground setting. Some limitations do exist. Although parent-reports are the most commonly used measure of sensory profile in ASD and provide a useful representation of sensory dysfunction, future studies would benefit from more objective tests of sensory sensitivity such as assessment of various sensory domains (e.g., auditory, tactile) or through clinician-based observations. Furthermore, this study assessed only one novel exposure, representing everyday experiences of interacting with new people. A limitation of the current study is the lack of repeated exposure, which would allow for assessing stress response across several repeat social encounters. The study was also limited to one measure of physiological arousal (i.e., cortisol). Use of other measures such as respiratory sinus arrhythmia (e.g., Benevides and Lane, 2013) could provide a more detailed, continuous assessment of regulation and arousal throughout the protocol.

Novel social experiences are only one aspect of daily social interactions. The current study focused on these novel experiences. Results were consistent with previous reports (Corbett et al., 2010, 2012, 2014b; Schupp et al., 2013) suggesting that when exposed to new peers for the first time, children with ASD experience greater physiological stress and tend to engage in less cooperative play with those new people, relative to their TD peers. However, social interactions are not solely reliant on single interactions with new people. Children interact with the same peers on a daily basis at school, and physiological stress or sensory sensitivity could have significant effects on how children with ASD interact with familiar peers in a setting such as the classroom. Therefore, future studies should investigate the role of sensory sensitivity and physiological stress during repeat exposure.

The findings that sensory dysfunction may negatively impact social behavior is notable and warrants further investigation. It would be helpful to discern whether targeting sensory symptoms via treatment could indirectly improve social motivation in individuals with ASD. While results show several interactions are present between physiological stress, sensory sensitivity, and parent-report stress, the directionality among these variables remains unclear. Future studies are needed to determine the extent to which change in one variable leads to change in another. The findings that the association between cortisol and sensory sensitivity was driven by the ASD group requires further investigation to enhance understanding of the mechanisms behind the purported link, which appears specific to ASD. Finally, while auditory sensitivity and cooperative play were correlated, further research is needed to determine any possible causal impact of sensory sensitivity on social motivation.

Summary

The primary objective of the current study was to examine the impact of sensory sensitivity on stress response and peer interaction in children with and without ASD. Results show that cortisol response to play is elevated in children with ASD and that higher physiological arousal during play is associated with greater sensory sensitivity and parent-reported stress. Additionally, ASD diagnosis confers risk for atypical sensory and stress reactivity, which may further contribute to the core social deficits. Taken together, results provide evidence for important interactions among social, sensory, and physiological profiles in ASD. Characterizing and assessing such profiles may better predict global outcomes, while also serving to inform more effective methods for intervention.

AUTHOR CONTRIBUTIONS

The named authors made significant contributions to the investigation and manuscript. Specifically, BC conceptualized the study design, outlined the organization of the manuscript, guided the literature search, ran data analysis, provided interpretation to the findings, and made significant contributions to the final manuscript. RM read the extant literature, synthesized the results, contributed to interpretation of the findings, and co-wrote the initial draft of the manuscript. SB participated in the acquisition, analysis, and interpretation of the physiological and behavioral data, and contributed to the writing of the manuscript. All authors read and approved the content of the work.

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REVIEW
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Toward an Interdisciplinary Understanding of Sensory Dysfunction in Autism Spectrum Disorder: An Integration of the Neural and Symptom Literatures

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Sensory processing differences have long been associated with autism spectrum disorder (ASD), and they have recently been added to the diagnostic criteria for the disorder. The focus on sensory processing in ASD research has increased substantially in the last decade. This research has been approached from two different perspectives: the first focuses on characterizing the symptoms that manifest in response to real world sensory stimulation, and the second focuses on the neural pathways and mechanisms underlying sensory processing. The purpose of this paper is to integrate the empirical literature on sensory processing in ASD from the last decade, including both studies characterizing sensory symptoms and those that investigate neural response to sensory stimuli. We begin with a discussion of definitions to clarify some of the inconsistencies in terminology that currently exist in the field. Next, the sensory symptoms literature is reviewed with a particular focus on developmental considerations and the relationship of sensory symptoms to other core features of the disorder. Then, the neuroscience literature is reviewed with a focus on methodological approaches and specific sensory modalities. Currently, these sensory symptoms and neuroscience perspectives are largely developing independently from each other leading to multiple, but separate, theories and methods, thus creating a *multidisciplinary* approach to sensory processing in ASD. In order to progress our understanding of sensory processing in ASD, it is now critical to integrate these two research perspectives and move toward an *interdisciplinary* approach. This will inevitably aid in a better understanding of the underlying biological basis of these symptoms and help realize the translational value through its application to early identification and treatment. The review ends with specific recommendations for future research to help bridge these two research perspectives in order to advance our understanding of sensory processing in ASD.

Keywords: sensory processing, autism spectrum disorder, interdisciplinary approaches, sensory symptoms, hyper-responsiveness, hypo-responsiveness

INTRODUCTION

Autism spectrum disorder (ASD) is characterized by deficits in social communication and the presence of restricted and repetitive behaviors, including sensory atypicalities. Sensory processing abnormalities have been reported in ASD since the earliest firsthand and clinical accounts (Kanner, 1943; Cesaroni and Garber, 1991). Recent estimates suggest a high prevalence of sensory symptoms, with reports ranging from 60 to 96% of children with ASD exhibiting some degree of atypical responses to sensory stimuli (Dunn et al., 2002; Baranek et al., 2006; Billstedt et al., 2007; Leekam et al., 2007; Klintwall et al., 2011; Lane et al., 2011; Marco et al., 2011). The updated diagnostic criteria for ASD in DSM-5 includes abnormal sensory behaviors (American Psychological Association, 2013). Sensory processing differences likely contribute to many of the higher-order cognitive and social deficits associated with ASD (Cascio et al., 2016), highlighting the broad potential impact of atypical sensory processing. Understanding the mechanisms through which these sensory symptoms manifest could help parents, educators, clinicians, and individuals themselves attend to the sensory environment and make adjustments accordingly in the hopes of normalizing one's sensory experiences and alleviating any downstream effects of atypical responding to sensory input. Over the last decade, there has been a significant increase in research related to sensory processing in ASD, which builds upon the initial accounts and research studies in this domain (see Rogers and Ozonoff, 2005, for a review). This accumulating research has been approached from two major perspectives. The first focuses on the symptoms that manifest in response to real world sensory stimulation. The second focuses on the neural pathways and mechanisms underlying sensory processing.

Currently, the symptom literature largely utilizes self- or parent- report questionnaires and/or observational, lab-based paradigms, in an attempt to characterize the observable reactions that impact an individual on a daily basis. Current neuroscience approaches measure the degree and timing of neural response. Despite each field lending itself to the study of unique aspects of sensory processing in ASD, there is a gap in our understanding of how neural response to sensory input is related to symptoms. Nonetheless, neural reactivity and processing patterns underlie and give rise to the presence of sensory symptoms (Marco et al., 2011), making it essential to understand *how* neural processing contributes to sensory symptoms. Each field has unique strengths, which have led to important contributions in our understanding of sensory processing in ASD; however, they each have a lot to learn from the other as we begin to bridge these two fields to gain a more comprehensive picture of this newly recognized diagnostic feature of ASD.

The purpose of this paper is to integrate the current research perspectives and methodological approaches related to sensory processing in ASD. Specifically, we will first clarify important terminology that is currently used in the study of sensory processing in ASD. Then, empirical studies from the last decade that focus on non-social, unisensory experiences, including those from both the sensory symptoms and neuroscience perspectives, will be reviewed with the goal of increased understanding of each respective field in order to move toward an interdisciplinary approach to sensory processing in ASD. Some recent studies have begun to integrate these perspectives (Green et al., 2013, 2015; Cascio et al., 2015) and provide a basis to further build upon. Furthermore, both of these perspectives investigate various domains of ASD (e.g., sensory, cognitive, social, language), and ASD research as a whole would benefit from integration of these perspectives. However, given that sensory processing differences potentially have broad downstream effects in higher-order domains (e.g., cognitive, social, language), it is essential to first bridge these perspectives at the sensory level. Thus, although a systematic review of all pieces of sensory processing in ASD is beyond the scope of this paper, its goal is to provide a foundation for shared understanding among disciplines investigating sensory processing in ASD.

Terminology

In order to integrate the literatures on sensory processing in ASD, it is important to clarify the current terminology used across fields. Recently, Cascio et al. (2016) highlighted the inconsistent conceptualizations and definitions across fields related to sensory processing in ASD and how this poses a challenge to cross-discipline communication and collaboration. The definitions that follow are an attempt to summarize and organize the existing terms used to describe sensory processing in ASD (**Table 1**). Our goal is to highlight the many components of sensory processing that might exist and to highlight the nuanced differences between these possible components of sensory processing.

Terminology confusion exists for two main reasons. First, both the neural and symptoms literatures adopt some of the same terms, but these terms oftentimes refer to very different concepts. For example, *behavior* is a problematic term because it is conceptualized differently across fields. Namely, neuroscientists discuss behavior in terms of perceptual decisions (e.g., detection or discrimination abilities; Weigelt et al., 2012), whereas clinicians tend to focus on observable reactions (e.g., a child covering his/her ears at the sound of a blender; McCormick et al., 2016). The second point of confusion regarding terminology surrounds the large range of terms within the sensory symptom literature that are oftentimes used interchangeably, but are arguably different constructs. The discussion that follows highlights both aspects of this confusion.

Sensory processing is the umbrella term that refers to the entire process from the brain registering sensory input from the outside world to the individual generating a response based on that input. Atypical neural responding to sensory input is thought to impact responses at multiple levels, including perceptual, physiological, and observable differences (Marco et al., 2011; **Figure 1**). Clinically, atypical sensory processing is manifested in inappropriate responses to sensory input that involve emotional and behavioral disruptions, and interfere with an individual's daily functioning (Miller et al., 2007). More generally, these disruptions can be considered *sensory symptoms*. Although it is understood that

sensory symptoms are a result of how sensory information is processed in the brain and the body (Cascio et al., 2016), the translation from neural firing to sensory symptoms is a complex process.

Early models of sensory processing, developed to explain behavior and plan clinical interventions, emphasized both neurological thresholds and response patterns in the generation of sensory reactions, or symptoms (Ayres, 1972; Dunn, 1997). Neurological thresholds refer to the amount of sensory input needed for the brain to register that input. Clinically, *low threshold to stimulation* is thus conceptualized as an individual requiring less sensory input to generate the typical response, whereas *high threshold to stimulation* is conceptualized as an individual requiring more sensory input to generate a typical response. These thresholds are important because it follows that typical levels of sensory input might then generate an atypical response—over-reaction for low threshold to stimulation and under-reaction for high threshold to stimulation. Researchers who have adopted these models generally rely on questionnaires to measure these constructs (e.g., Joosten and Bundy, 2010; Reynolds et al., 2011). However, questionnaires are unable to accurately measure an individual's threshold to stimulation, and thus rely on an assumption that an observable reaction accurately captures the complexity of processing sensory input (see Sensory Symptoms section for more information).

Neuroscientists measure this construct of neurological threshold more directly, but have adopted different terminology. At the neural level, *hyper-* and *hypo-responsiveness*[1] refer to increased or decreased neural firing, respectively (e.g., Gomot et al., 2008). Additionally, thresholds are determined by the process of sensory gating and habituation. *Sensory gating* refers to the brain's inhibitory ability to filter out redundant or unnecessary neural responses to irrelevant environmental stimuli (Orekhova et al., 2008). *Habituation* refers to decreased neural response to repetitive sensory stimulation (e.g., Guiraud et al., 2011). At the perceptual level, *sensitivity* is determined by the smallest stimulus intensity that is detectable, and is defined as the inverse of a perceptual threshold (Engen, 1988). Adding to the confusion, sensory symptom researchers define *sensitivity* as a negative reaction in response to low threshold to stimulation (Dunn, 1997). In addition, they refer to *hyper-responsiveness* as the presence of an atypical response, such as covering one's ears in response to everyday noises, and *hypo-responsiveness* as the absence of a typical response, such as failure to orient to salient sounds (e.g., Ben-Sasson et al., 2009; McCormick et al., 2016). Based on the theoretical conceptualization that these observable components are a result of neurological thresholds, hyper- and hypo-responsiveness have thus each become more of a single construct than is theoretically warranted. Furthermore, because of this parallel terminology, connections between neural firing patterns and symptoms are often portrayed as overly simplistic.

The complexity of sensory processing is further highlighted when incorporating the response pattern component, which suggests a wide range of possible symptoms that could result from atypical neural registration and perception of sensory stimuli. *Sensory defensiveness* is observed as a negative reaction to a sensory stimulus that is not typically considered to be aversive, such as repeatedly itching or screaming in reaction to a tag in clothing (Baranek et al., 1997). *Sensory avoidance* is observed as a resistance or unwillingness to interact with the stimulus (Dunn, 1997). *Poor registration* is defined as decreased ability to register sensory input and is oftentimes measured by a lack of response (Dunn, 1997). Conversely, *sensory orienting* is the direction of attention to a sensory stimulus, which is assumed to be in response to successful registration of that stimulus (Liss et al., 2006). Similar to the neuroscience definition, *habituation* is defined as decreased response to repetitive sensory stimulation; however, in this literature, it is measured by orienting responses, often to repeated sounds (Baranek et al., 2007). *Sensory filtering*, which has been predominately defined in the auditory modality, refers to the ability to selectively attend to relevant sensory information and exclude irrelevant or distracting sensory information (Tomchek and Dunn, 2007). *Sensory seeking* is defined as excessively seeking out sensory input (Dunn, 1997; Liss et al., 2006).

Although these symptoms are uniquely defined, these terms are sometimes being operationalized more broadly (e.g., defensiveness as high sensitivity or having a low threshold to stimulation; Kern et al., 2006), rather than focusing exclusively on the observable reaction. Furthermore, in some cases it is unclear as to how these symptoms originate from neural responding patterns. For example, sensory seeking was originally conceptualized as a compensatory response in an individual with high threshold to stimulation (Dunn, 1997), but has also been conceptualized as a compensatory response to overarousal (Liss et al., 2006), which would theoretically occur in individuals with low threshold to stimulation. This example underscores the importance of measuring both the neural response and the symptom presentation and cautions against assuming that the observable reaction accurately captures the complexity of sensory processing.

In sum, the conceptualization of sensory processing as a unitary construct is challenged by the broad array of existing terms that each define specific components of this complicated process. The inconsistent definitions across disciplines, highlighted above, have contributed to confusion within the study of sensory processing in ASD. At the neural level, thresholds determine which sensory information is registered in the brain. This then influences perceptual sensitivity and bodily response, and leads to observable reactions and symptoms (**Figure 1**). The literature defines and differentiates between several possible symptom presentations (defensiveness, avoidance, poor registration, poor habituation, poor filtering, seeking), measured mainly at the level of observable reactions. Organizing these terms and reconciling the manner in which they are used in both the neural and symptom literatures provides an important step toward clarifying the often overly simplified connections between sensory symptoms and their underlying neural patterns and promoting more collaborative future research.

[1]Hyper-responsive and over-responsive are synonymous terms, as are hypo-responsive and under-responsive. The term hyper-responsive and hypo-responsive will be used exclusively in this paper.

TABLE 1 | Terminology in sensory processing in ASD research.

Term	Definition in symptom research	Definition in neuroscience research
Sensory processing	The process of the brain registering sensory input from the outside world and the individual generating a response based on that input	
Symptom	Atypical responses to sensory input that interfere with an individual's daily functioning	
Behavior	Observable reactions	Ability to detect or discriminate, measured by perceptual decisions
Low threshold	Requires less sensory input to generate a typical response (referred to as low threshold to stimulation)	Requires less sensory input to generate a neural response
High threshold	Requires more sensory input to generate a typical response (referred to as high threshold to stimulation)	Requires more sensory input to generate a neural response
Hyper-responsiveness	Presence of an atypical response, or over-reaction	Increased neural responding
Hypo-responsiveness	Absence of a typical response, or under-reaction	Decreased neural responding
Sensory gating		Inhibitory functioning at the neural level, which filters out redundant or unnecessary neural responses to all other environmental stimuli
Sensitivity	Negative reaction to sensory input	Degree to which one is susceptible to perceiving small changes in stimulus intensity; inverse of perceptual threshold
Habituation	Decreased response of the individual to repetitive sensory stimulation	Decreased neural response to repetitive sensory stimulation
Defensiveness	Negative reaction to sensory input	
Avoidance	Resistance or unwillingness to interact with sensory stimuli	
Poor registration	Decreased ability to register sensory input (typically measured by lack of response)	
Sensory orienting	Directed attention to a sensory stimulus	
Sensory Filtering	Ability to process relevant sensory information and exclude irrelevant/distracting sensory information	
Sensory Seeking	Excessively seeking out sensory input	

These definitions are adopted from the literature, but they are fine-tuned to emphasize important differences between terms that are oftentimes used interchangeably.

SENSORY SYMPTOMS IN ASD

In the past 10 years, sensory experiences of individuals with ASD have been assessed via three methods: self-, parent-, and teacher-report questionnaires; behavioral observations; and autonomic measurement. Questionnaire measures are by far the most commonly used, and have largely been successful in clinical settings to generally discriminate typical from atypical sensory processing. However, their ability to more sensitively capture individual differences in sensory processing is less clear for three main reasons. First, the inherent nature of questionnaires limits their utility to assessing components of sensory processing to those that can be observed. However, many of the sensory questionnaire measures yield scores that imply information about other components of sensory processing. For example, Poor Registration, a factor score on the commonly used Sensory Profile (Dunn, 1999), includes items such as "decreased awareness of pain and temperature," "doesn't notice when people come into the room," and "does not seem to smell strong odors." Thus, all of these infer poor registration of the stimulus from an observation.

Secondly, a common practice in sensory questionnaire measures is to combine items that target different levels of sensory processing. For example, the Hyperresponsiveness score of the Sensory Experience Questionnaire (Baranek et al., 2006) includes items such as "does your child notice sounds in the environment before other people do?," "is your child disturbed by too much light inside or brightness outside?," "does your child react negatively or pull away when touched by a person?" Thus, this score collapses the perceptual, emotional, and observable responses across multiple sensory modalities, and possibly misses important differences that may occur within more precisely defined sensory processing levels and modalities. Finally, many sensory questionnaires include some items evaluating social-sensory experiences (e.g., does your child respond to his/her name?; Dunn, 1999; Baranek et al., 2006), making it impossible to disentangle the independent contributions of sensory and social components. Given these limitations of questionnaire measures in the assessment of sensory processing in ASD, the literature that follows mostly captures a coarse picture of the observable reactions component of sensory processing. To parallel this level of inquiry, we will use the general terms of hyper- and hypo-responsiveness in our discussions of findings and will clarify specific aspects of those responding patterns when appropriate.

In the past few years, there have been some positive advancements in the development and analysis of questionnaire measures that provide promising avenues for future research. Specifically, more focused questionnaires have been developed to address some of the limitations outlined above. An example is the Sensory Perception Quotient (Tavassoli et al., 2014a). This questionnaire focuses on basic detection and discrimination abilities and includes items that target specific sensory receptors across a variety of sensory modalities (e.g., "I find it difficult to see individual stars on a clear night" and "I would be the first

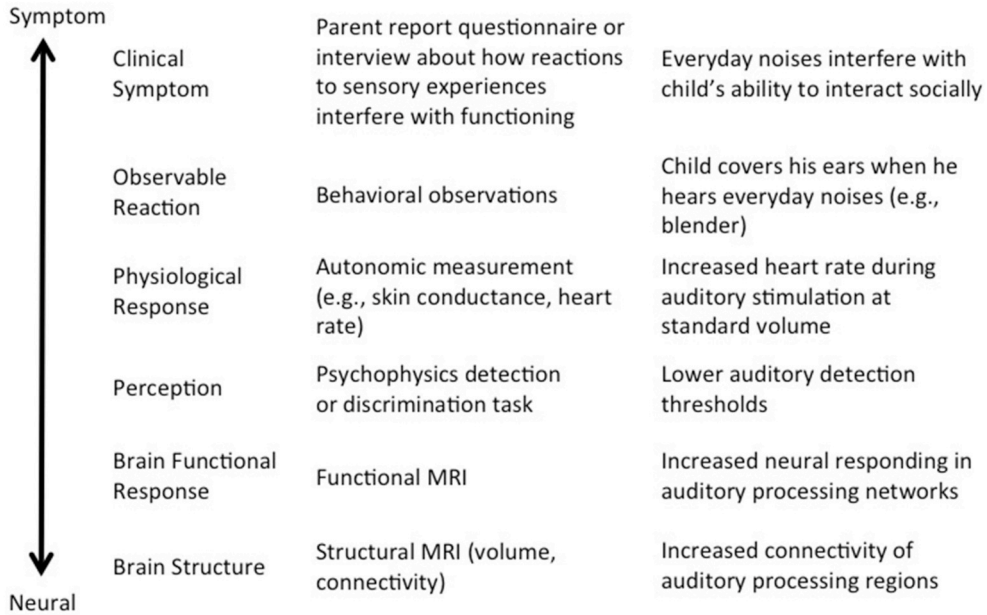

FIGURE 1 | Conceptual map of sensory processing using a common example of hyper-responsiveness to auditory stimulation. The first column outlines various levels of analysis from brain to symptom. For the sake of simplicity, these are organized using a bottom-up conceptualization in a single pathway from brain to symptom; however, in reality, there are likely several different pathways from brain to symptom, including bidirectional relationships between certain levels. The second column provides an example of an appropriate method for each level of analysis. The third column provides one example of a possible finding related to auditory hyper-responsiveness using each particular method.

to hear if there was a fly in the room"). Thus, this questionnaire is still limited to observable responses, but begins to selectively target the perceptual level of analysis. Another advancement that has recently been applied to sensory questionnaires is the use of cluster-based statistical analysis to identify sensory-based subtypes within ASD. Subtype identification is motivated by the known heterogeneity within ASD and has implications for neurobiological studies that aim to link sensory features with specific underlying mechanisms. Such studies have yielded somewhat inconsistent subtypes (Lane et al., 2010, 2011, 2014; Ausderau et al., 2014), which is possibly due to imprecise measurement tools. Nonetheless, this subtype identification approach is a positive step in our understanding of underlying mechanisms.

Other methods that assess sensory symptoms include lab-based observational paradigms and autonomic measurements to characterize physiological responses. Lab-based observational paradigms are similar to questionnaire measures in that they largely focus on observable reactions to real world sensory input (e.g., toys, objects with certain sensory features). However, they differ from questionnaire methods in that they rely on behavioral coding in the lab to obtain a more controlled and objective measure of these symptoms. Psychophysiological studies focus on the body's response to sensory stimulation, specifically looking at functioning of the autonomic nervous system (ANS). Thus, these studies provide objective measures of bodily states that have been linked with emotional states. The following sections review the sensory symptom literature that uses questionnaires, lab-based observational coding paradigms, and psychophysiological methodologies.

Questionnaire-Based Studies

Questionnaire-based studies on sensory processing in ASD, detailed below, have culminated in two major conclusions: (1). Individuals with ASD respond to sensory input in ways different from the typical population, across a variety of modalities and across the entire lifespan, and (2). Sensory processing differences are related to a variety of the core and associated symptoms of ASD and affect the quality of life in these individuals.

Sensory Symptoms Across Development

Some studies examined developmental trends in samples spanning large age ranges. These studies showed atypicalities in ASD across all sensory modalities that decrease with age (Kern et al., 2006; Leekam et al., 2007). Additionally, symptoms in different modalities (e.g., visual, auditory, tactile) were found to be moderately correlated with each other across the lifespan, but only correlated with general autism symptom severity in childhood. (Kern et al., 2007). These findings support a general disruption in sensory processing, with responses to stimuli in each modality being related to each other, at least at the symptom level. Additionally, these findings suggest a maturational process

that leads to a decrease in sensory symptoms with increasing age that is independent from change in autism severity more globally. Given these general developmental trends, studies investigating narrower age ranges provide more detailed information at important developmental stages.

Four studies have specifically looked at the profile of sensory symptoms in infants and toddlers. Patterns of hyper-responsiveness best differentiated those with ASD from those with typical development (TD); this was seen both within the modalities of touch, audition, and taste/smell (Wiggins et al., 2009), and across low threshold patterns more globally including both sensitivity and avoidance (Ben-Sasson et al., 2007). These sensory symptoms presented irrespective of cognitive ability and expressive language level (Ben-Sasson et al., 2007; Klintwall et al., 2011). However, hypo-responsiveness best differentiated toddlers with ASD from those with developmental delay and TD, suggesting that a pattern of decreased responding may be the most unique pattern in ASD (Baranek et al., 2006), a finding confirmed by a meta-analytic review (Ben-Sasson et al., 2009). A subgroup of toddlers with ASD had atypical scores across multiple patterns, highlighting the comorbidity of these symptoms even at this early developmental stage (Baranek et al., 2006; Ben-Sasson et al., 2007). A study using high-risk infants found that those who developed ASD had more auditory symptoms and hypo-responsive patterns compared to high-risk infants that do not develop ASD and low-risk infants (Germani et al., 2014), suggesting that these may be risk factors for developing ASD and making these symptoms potentially important for early identification of the disorder.

In slightly older (3–6 years old) children, Tomchek and Dunn (2007) reported similar findings of global hypo-responsiveness as well as hyper-responsiveness in the auditory, tactile, and taste/smell modalities. Also in line with the findings in infants and toddlers, sensory symptoms in children do not seem to be related to cognitive abilities (O'Donnell et al., 2012). However, these sensory symptoms do seem to be related to language skills and adaptive functioning in children (Tomchek et al., 2015). McCormick et al. (2016) examined developmental trajectories of sensory symptoms in children with ASD compared to those with other developmental disorders and TD, and found that children with ASD have elevated sensory symptoms from an early age (2 years) that remain stable through the age of 8, and that hyper-responsiveness in the taste/smell modality and poor auditory filtering best differentiated ASD from other developmental disorders. In a large study of 3–10 year old children, sensory symptoms across modalities were higher in children with ASD and ADHD compared to those with TD (Cheung and Siu, 2009). Although the ASD and ADHD groups were not distinguishable overall, as age increased, the ASD group showed decreases in symptoms while the ADHD group showed stable or increased symptoms. Finally, two studies investigated sensory symptoms in the home vs. school environments, and demonstrated both shared and unique expression of sensory symptoms across contexts (Brown and Dunn, 2010; Fernandez-Andres et al., 2015).

In adolescents, two studies found less sensory seeking in ASD (De La Marche et al., 2012; Stewart et al., 2016) with De La Marche et al. (2012) also showing more hyper-responsiveness (specifically in sensory avoidance) and Stewart et al. (2016) also showing hypo-responsiveness (Low Registration). Using a small sample of adults, Crane et al. (2009) found similar self-reported symptoms in adults with ASD compared to TD adults, with the most obvious differences in sensory avoidance. These sensory scores were all correlated with IQ, suggesting that higher IQ in adults may serve as a protective factor against persistent sensory symptoms. Increased levels of self-reported hyper-responsiveness in a large sample of adults with ASD have been shown using the SensOR (Tavassoli et al., 2014b) and the Sensory Processing Quotient (Tavassoli et al., 2014a), measures specifically targeting hyper-responsive sensory symptoms.

Developmentally, the literature paints a picture of early hypo-responsiveness, as well as hyper-responsiveness particularly in the auditory, taste/smell, and touch modalities; these symptoms pervasively affect individuals with ASD and remain stable through at least 8 years of age. The pattern of sensory seeking remains unclear, and may be due in part to the varying conceptualizations of these symptoms; additional work is needed to characterize this pattern of symptoms. Although sensory symptoms appear to be unrelated to more global functioning in toddlers, sensory symptoms begin to show relationships with adaptive functioning and language skills in early childhood. As individuals with ASD mature, hyper-responsive symptoms best characterize adults. This could indicate that early hypo-responsiveness leads to later global hyper-responsiveness or alternatively, could be due to differences in parent- vs. self-reporting strategies that are typically used in younger children and adults, respectively. If the latter is the case, it is possible that subjective and observed experiences differ, raising the importance of collecting multiple types of data in the study of sensory symptoms in ASD.

Sensory Symptoms and Their Relation to Other ASD Symptoms and Challenges

Several studies have examined relationships between sensory differences and a variety of the core and associated symptoms of ASD, revealing important information about the functional impact of sensory processing and its interference in day-to-day life. Three studies have looked at the relationship between sensory symptoms and general autism symptoms. In school-aged children, general sensory symptoms measured by the Sensory Processing Measure were related to autism severity measured by the Gilliam Autism Rating Scale, 2nd Edition in both the home and school environment (Sanz-Cervera et al., 2015). In a similarly aged sample, Hilton et al. (2010) used the Sensory Profile and identified that more proximal senses (touch, taste) may be more related to social difficulties, measured by the Social Responsiveness Scale in children with ASD. In adults both with and without ASD, Tavassoli et al. (2014b) showed a relationship between self-reported sensory hyper-responsiveness and autism symptoms.

Other studies have looked at sensory processing in relation to more specific types of functioning, such as school-related difficulties and activity participation. In school-aged children with ASD, Ashburner et al. (2008) found empirical support

for the theoretical links between atypical sensory responding and difficulties in classroom settings. Specifically, they found hypo-responsiveness and difficulties with auditory filtering to explain about half of the variance in academic performance, above and beyond IQ and general ASD symptoms. Additionally, tactile hyper-responsiveness and auditory filtering deficits explained 36% of the variance in inattention problems. Together, these findings show the impact of sensory symptoms on children's ability to pay attention and perform successfully in school. Different sensory responding profiles have also been linked to different preferred activities; children with sensory hyper-responsiveness participate less in social, school, and extracurricular activities (Reynolds et al., 2011; Little et al., 2015), while children with sensory hypo-responsiveness participate more in activities outside the home, which the authors speculate may be because they are more passive (Little et al., 2015). These sensory responding and functional impairment relationships also exist in younger children with ASD; preschoolers with more significant sensory abnormalities also have more behavior problems (O'Donnell et al., 2012).

Ben-Sasson et al. (2008) used cluster profiles in toddlers and found that those with high hyper- and hypo-responsiveness also had more negative emotions and higher levels of anxiety and depression symptoms. These authors speculated that internalizing disorders in ASD may develop from the accumulated negative experiences with sensory input throughout development. Sensory hyper-responsiveness, specifically, was moderately correlated with anxiety in a very large sample of children with ASD (Mazurek et al., 2013). Green et al. (2012) longitudinally tested the relationship between sensory hyper-responsiveness and anxiety in 149 toddlers with ASD. This study confirmed the correlation between sensory hyper-responsiveness and anxiety in ASD, and importantly established a directional link from sensory hyper-responsiveness to anxiety. While sensory hyper-responsiveness remained stable and predicted change in anxiety over time, anxiety levels increased over time and did not predict change in sensory hyper-responsiveness. These findings suggest that sensory hyper-responsiveness may emerge earlier than and exacerbate the presentation of anxiety, or that these symptoms may have a common cause with different developmental manifestations.

Sensory hyper-responsiveness has also been linked to gastrointestinal (GI) problems (Mazurek et al., 2013), picky eating (Cermak et al., 2010; Nadon et al., 2011), sleep problems (Mazurek and Petroski, 2015), more externalizing behaviors, and increased parenting stress and family impairment (Ben-Sasson et al., 2013). Mazurek et al. (2013) proposed that sensory hyper-responsiveness, anxiety, and GI problems may be explained by shared neural mechanisms through which heightened stress leads to physiological and cognitive symptoms of anxiety in addition to experiencing particular stimuli as aversive. Individuals exhibiting this trio of symptoms may represent a unique subgroup of ASD. Furthermore, sensory hyper-responsiveness has been linked with increased sleep problems through similar hypothesized mechanisms (Mazurek and Petroski, 2015). Overall, this body of work focusing specifically on sensory hyper-responsiveness represents theoretically relevant and convincing evidence for the link between sensory hyper-responsiveness and anxiety, GI, sleep, and other problems in ASD. The observable nature of hyper-responsive reactions likely contributes to the comprehensiveness and replicability of this finding.

Several studies have examined the relationship between sensory symptoms and restricted and repetitive behaviors. Among children 5–18 years with intellectual disability, hyper-responsiveness (sensory sensitivity and sensory avoidance) distinguished those with ASD from those without ASD (Joosten and Bundy, 2010). The authors suggest a mechanism whereby hyper-responsiveness leads to increased anxiety, which subsequently leads to engagement in low-level repetitive behaviors to cope with that anxiety. Boyd et al. (2009) investigated relationships between sensory responding (measured by the Sensory Questionnaire), repetitive behaviors (measured by the Repetitive Behavior Scale—Revised), and executive functioning (measured by the Behavior Rating Inventory of Executive Function) in 6–17 year olds with and without ASD. No hypothesized relationships were found between sensory responding and executive functioning; however, sensory responding was related to two specific types of repetitive behaviors: stereotypy and compulsions. This data did not support the theoretical claim that executive dysfunction is the shared mechanism for sensory symptoms and repetitive behaviors in ASD, and the authors suggest that neurobiological, rather than neurocognitive, mechanisms might better explain this link. In other words, it is possible that the questionnaire measure designed to target neurocognitive processes does not accurately capture the neurobiological underpinnings that give rise to these neurocognitive processes. Wigham et al. (2015) investigated the relationship between sensory symptoms, repetitive behaviors, and intolerance of uncertainty, finding that the relationship between sensory responding and repetitive behaviors (particularly sameness behaviors) was mediated by intolerance of uncertainty, providing a specific cognitive explanation for the relationship. These studies provide some initial empirical evidence to support the conceptual relationship between sensory processing and repetitive behaviors in ASD, highlighting intolerance of uncertainty, but not executive functioning, as a possible mediating factor.

In sum, questionnaire-based studies have repeatedly confirmed that sensory processing is atypical in ASD at the level of reported observable reactions. Specific aspects of sensory processing difficulties, including hyper-responsiveness in the modalities of touch, audition, and taste/smell and patterns of general hypo-responsiveness, have most consistently emerged in the questionnaire-based literature with other differences being less consistently reported. Thus, patterns of both hyper- and hypo- responsiveness in ASD exist at the group level, with hyper-responsiveness further broken down at the level of sensory modality. This may reflect greater differentiation of sensory processing abilities in the hyper-responsiveness, vs. hypo-responsiveness, pattern, but it likely reflects the better precision of characterizing these symptoms given that they are defined by the *presence* of an atypical reaction and thus easier to observe and report. Additionally, the literature that specifically focuses on hyper-responsiveness has provided a convincing link between

these sensory symptoms and other challenging symptoms (e.g., anxiety, GI problems) that occur in ASD at a disproportional rate. Nonetheless, improved measurement development and analysis techniques will be necessary to enhance the sensitivity of these questionnaires in order to understand individual differences in sensory processing. In addition, the current literature provides some evidence for differences in subjective and observed sensory experiences based on self- and parent- report, respectively. Self-report in ASD has been criticized because verbal and cognitive deficits (e.g., insight) may limit accurate reporting of symptoms (Ozsivadjian et al., 2012); however, these types of questionnaires may be the only way to capture subjective experiences. Parent-report questionnaires in ASD have been criticized for relying on retrospective report, which can lead to inaccurate reporting and recollection biases (Hoyle et al., 2001) and can additionally be influenced by individual factors such as parenting stress (Ooi et al., 2016). However, questionnaires allow for observations across time and contexts and thus may be one of the best ways to evaluate more generalizable observable reactions to sensory input and their impact on day-to-day functioning. Future measure development would benefit from focus on this component rather than attempting to use questionnaires for aspects of sensory processing that cannot be readily observed (e.g., high threshold to stimulation).

Lab-Based Observational Coding Studies

Several lab-based observational coding paradigms have been developed to assess sensory symptoms, including the Sensory Processing Assessment (SPA), Tactile Defensiveness and Discrimination Test—Revised (TDDT-R), Sensory Processing Scales, and Sensory Challenge Protocol. The SPA Baranek et al. (2007) and the TDDT-R were designed as play-based assessments to observe sensory patterns in the lab. The SPA has been validated in children 9 months to 6 years and the TDDT-R has been validated in children 2–14 years; however, these tools are still in development and thus clinical norms have not been published. Additionally, the play-based nature of these assessment tools limits their use to younger and/or lower functioning individuals. The Sensory Processing Scales (Schoen et al., 2014) consists of structured games that involve sensory components (e.g., observe a spinning sparkle wheel; paint your arm with a feather, brush, and rough sponge). Although not developed for individuals with ASD specifically, it has now been applied to high functioning children ages 4–16 years with and without ASD (note: high functioning ASD typically refers to individuals with at least low average cognitive ability). The play-based assessment may also be appropriate for lower functioning individuals, but remains to be tested in this population. The Sensory Challenge Protocol (McIntosh et al., 1999; discussed in the Psychophysiological Studies section) was designed to assess physiological responses to the presentation of items with sensory features (e.g., strobe light for visual, feather touching the face for tactile). This protocol has been used in children ages 4–15 years, but has been modified for use in younger children (McCormick et al., 2014).

Baranek et al. (2007) utilized the SPA, which involves presenting infants and young children with a variety of toys with different sensory features and coding the resulting behaviors. They found increased hyper-responsiveness (sensory aversion) in children with ASD and developmental delay compared to those with TD, and that across all three groups, these symptoms decrease as both chronological and mental age increase. They also found a deficit in sensory orienting in ASD at a mental age of 6 months that normalized by a mental age of 5.5 years. Baranek et al. (2013) specifically looked at sensory orienting using the SPA, confirming the early orienting deficit in ASD and extending this to be true for both social and non-social stimuli, and to be related to joint attention. Foss-Feig et al. (2012) utilized the TDDT-R, a structured behavioral observation paradigm, and tactile specific scores on parent report questionnaires revealing specific associations between tactile hypo-responsiveness and both core features of autism: social communication impairments and restricted/repetitive behaviors. Tavassoli et al. (2016) utilized the Sensory Processing Scales in high functioning children with and without ASD ages 4–15. Children with ASD showed significantly greater sensory reactivity compared to controls, and this reactivity was correlated with parent-reported sensory symptoms across the entire sample.

In these studies, questionnaire measures and lab-based observational coding measures were inconsistently correlated with each other, suggesting that unique information can be gleaned from each measure. This is likely due in part to the limitations of each type of measure: observations from lab-based paradigms may not be generalizable to real life settings and questionnaire measures are prone to reporter biases that decrease internal validity. Although additional paradigm development work is needed, observational coding paradigms represent a more structured and objective way to understand observable reactions to the presentation of sensory input.

Several studies have combined questionnaire-based measures with observational coding paradigms to create composite scores for different sensory response patterns. This approach adheres to the multi-method model and allows for a more comprehensive assessment. One study using composite scores found an association between hyper-responsiveness and repetitive behaviors in both ASD and developmental delay (Boyd et al., 2010) and another found an association between hypo-responsiveness and sensory seeking symptoms with various measures of social and communication deficits (Watson et al., 2011), confirming the findings previously highlighted in studies utilizing only questionnaires. Furthermore, in a study looking at associations between sensory responding patterns and temperament, Brock et al. (2012) found that increased sensory symptoms across all three patterns (hyper-responsiveness, hypo-responsiveness, sensory seeking) were associated with increased withdrawal and negative mood. This multi-method approach is commendable, but future studies should expand on this approach to investigate both shared and unique contributions of symptoms assessed using different methods. Furthermore, hypo-responsiveness may be better understood by methods that do not rely on observation and report given that they are defined by the *absence* of a typical response and may never be able to be accurately assessed by questionnaire or other observational methods.

Psychophysiological Studies

Psychophysiological studies focus on the body's response to sensory stimulation, specifically looking at functioning of the ANS. The ANS functions through the sympathetic and parasympathetic branches, which are responsible for fight or flight responses and the regulation of those responses and maintenance of homeostasis, respectively. This method provides objective measures of the individual's bodily responses to sensory input that have been systematically linked to emotional states.

Three studies have used the Sensory Challenge Protocol while measuring ANS responding and have yielded conflicting results. Schoen et al. (2009) assessed children ages 4–15 years with ASD, Sensory Modulation Disorder, and TD, measuring arousal levels with electrodermal activity, a measure of sympathetic activity. Children with ASD had typical habituation patterns, but lower baseline arousal levels and lower reactivity, especially to the first two stimuli within each modality, suggesting that children with ASD may have a reduced ability to initially attend to, and thus process, environmental stimuli. Interestingly, they also found that parent-reported sensory symptoms were not related to the physiological arousal levels, again pointing to a divergence between lab- and questionnaire-based measures. Schaaf et al. (2015) collected cardiac measures associated with sympathetic and parasympathetic activity in children with and without ASD ages 6–9 years. Respiratory sinus arrhythmia, the measure of parasympathetic activity, was lower in ASD in response to sensory stimulation. However, pre-ejection period, the measure of sympathetic activity, did not differ between groups. These findings suggest that parasympathetic regulatory functions are specifically faulty in children with ASD. Finally, Lane et al. (2012) tested the mediating role of reactivity to sensory stimuli in the path from baseline reactivity to anxiety symptoms in children ages 6–10 years with ASD, attention-deficit hyperactivity disorder, or TD. Findings suggest a direct relationship between parent-reported sensory hyper-responsiveness and child-reported anxiety. Additionally, they found a relationship between baseline reactivity and anxiety fully mediated by reactivity to sensory stimuli, and a relationship between baseline reactivity and habituation partially mediated by reactivity to sensory stimuli. Together, these findings suggest that the initial state of arousal influences the degree of physiological response to sensation, which then determines both the nervous system's recovery ability and symptoms of anxiety. One additional study used a modified version of the Sensory Challenge Protocol in children 2–5 years with and without ASD and found no group differences in psychophysiological responses to sensory stimuli nor relationships between these responses and parent-reported sensory symptoms (McCormick et al., 2014). In sum, the Sensory Challenge Protocol represents an ecologically valid paradigm (presenting real world objects with sensory features), and when combined with psychophysiological measurement, provides objective information about an individual's emotional response beyond the level of behavioral observations. Additional studies using this paradigm are necessary to better understand the currently conflicting results of sympathetic and parasympathetic differences in response to sensory input in ASD.

Two additional studies have tried to relate ANS responses to reported and/or coded observable responses to sensory input. Woodard et al. (2012) presented sensory stimuli to a small group of children ages 2–3 years with and without ASD. They recorded heart rate during the presentations and coded observable reactions. Although heart rate and behavioral codes were only weakly correlated, both measures showed that toddlers with ASD were more hyper-responsive compared to toddlers with TD. Additionally, there was only one significant relationship between scores on the Infant-Toddler Sensory Profile (parent report questionnaire) and heart rate: hypo-responsiveness was negatively associated with heart rate. These findings suggest that questionnaire measures and behavioral ratings are at best a weak indicator of autonomic activity and highlight the importance of including multiple measures to examine sensory processing in ASD. In a study of 5–19 year olds looking at ANS function in ASD and its relation to sensory symptoms, Daluwatte et al. (2015) found that lower pupil constriction amplitude, a measure associated with parasympathetic activity, was related to increased overall sensory symptoms in ASD. The authors conclude that certain atypical sensory symptoms in ASD seem to be related to reduced parasympathetic modulation.

Overall, these studies provide mixed evidence at younger ages and consistent evidence at older ages of ANS dysfunction in ASD, particularly in response to sensory stimulation, with some studies pointing to deficits in sympathetic activity and others pointing to specific deficits in the regulatory role of the parasympathetic system. These studies begin to characterize the body's response to sensory input by using measures that tap the peripheral nervous system. In this way, this collection of studies begins to bridge the neural and symptom literatures, while including an objective measure of the affective component associated with symptoms.

Summary of Sensory Symptoms Literature

In sum, our understanding of sensory symptoms in ASD has improved somewhat in the past decade. Specifically, larger, better-characterized, and narrower age range samples have provided opportunities to use more advanced analytic approaches and to characterize the development of sensory symptoms. Additionally, the specific focus on hyper-responsiveness taken by several studies has provided the start to a more comprehensive understanding in that pattern of sensory processing. Similarly, development of more specific questionnaires and lab-based paradigms has advanced the measurement tools available and begun to allow for a multi-method approach (e.g., ability to create composite scores using questionnaire *and* observational methods). Finally, an increased focus on psychophysiological responses provides a new level of understanding about the body's underlying responses to sensory stimulation.

NEURAL RESPONSE TO SENSORY INPUT

The sensory symptoms literature presented above focuses on the individual's response to sensory input and highlights the significant impact of these symptoms on individuals with ASD and their families. The neuroscience literature that follows

focuses on the brain's *functional* response to sensory input. Thus, studies of brain structure and connectivity patterns, both of which are impacted in ASD and likely contribute to the functional responding differences, are not evaluated here. However, the relevant functional consequences of these differences will still likely be captured.

By in large, neuroscience studies focus on a specific sensory modality and employ a specific methodology based on which characteristics of neural processing are of interest. The two most commonly used methods in ASD research are electroencephalography (EEG) and functional magnetic resonance imaging (fMRI), with EEG studies focusing primarily on the timing of neural responses and fMRI studies focusing primarily on the location of neural responses. Magnetoencephalography (MEG), which captures both timing and location, has also more recently been utilized in ASD. These studies will be reviewed by methodology used and by sensory modality studied.

Electroencephalography (EEG)

Several studies use EEG to measure cortical reactivity to sensory stimulation, focusing largely on individual components that correspond to specific sensory events (event related potentials; ERP). EEG is a relatively non-invasive approach that can be used successfully across all ages and functioning levels. Task demands are typically minimal and paradigms can be short. Finally, EEG provides high temporal resolution at the expense of spatial resolution. Given this strength of temporal resolution, the following EEG studies highlight both bottom-up and top-down influences on sensory processing, which typically occur at earlier and later temporal stages of processing, respectively. Bottom-up refers to the influence of purely sensory information on processing, while top-down refers to higher-order influences (e.g., attention) that interact with the incoming sensory information.

Auditory

The vast majority of EEG studies have targeted the auditory modality. Generally, two paradigms have been used in conjunction with EEG recordings: paired-clicks and auditory oddball tasks. In paired-clicks paradigms, two auditory stimuli are presented in succession allowing for a comparison in cortical response to the first and second (repetitive) input. Typically, there is a decrease in amplitude of these early components of the auditory ERP to a repeated stimulus, reflecting inhibition of the repetitive input, known as sensory gating. Oddball tasks typically utilize three stimuli: a standard stimulus that is the most commonly presented stimulus, a deviant stimulus that is presented less frequently and varies in one dimension (e.g., frequency) from the standard stimulus, and a novel stimulus that is also presented less frequently but varies greatly from the standard stimulus. Typically, there are distinct cortical responses to the deviant and novel stimuli reflecting appropriate change and novelty detection.

Orekhova and Stroganova (2014) recently reviewed the auditory ERP studies in ASD, which include participants across a range of cognitive ability. Early auditory components include the P50 and P100. Studies of sensory gating in ASD collectively show intact P50 responses, but reduced reactivity of the P100 in the right hemisphere. The later auditory ERP components, MMN and P3a, are both involved in processing change events, with MMN being critical for initial deviancy detection and P3a being important for involuntary attention orienting and evaluation preceding a response. The results for these later ERP components in ASD are not entirely consistent, but overall, suggest intact cortical change detection and evaluation when stimuli are within the focus of attention, but show cortical hypo-responsiveness when stimuli are outside the focus of attention, highlighting the importance of top-down modulation of sensory processing in ASD. Additionally, the authors suggest that age and cognitive ability of participant samples may explain some of the discrepant findings across studies.

Donkers et al. (2015) sought to relate auditory ERPs to sensory symptom patterns in children with ASD ages 4–12 across a range of cognitive ability. Utilizing an auditory oddball paradigm, they found marginally smaller amplitudes of the early P1 and N2 ERPs to standard tones, smaller P3a amplitude to novel tones, and longer P1 latency to deviant tones in ASD compared to TD. Although no single ERP component predicted any sensory symptom pattern, complex and conditional associations between auditory ERPs and sensory symptom patterns were revealed, highlighting both bottom-up (early sensory) and top-down (attentional) influences on the severity of sensory symptoms in ASD. Sensory symptoms were assessed broadly (i.e., across all sensory modalities) and included both parent report and behavioral observation measures to calculate composite scores of sensory hyper-responsiveness, hypo-responsiveness, and seeking. This attempt to relate neural responsiveness to symptoms is commendable; however, the neural measure was exclusively in the auditory modality while the symptoms were more broadly assessed across multiple sensory modalities.

Visual

Whereas the majority of EEG studies have been in the auditory modality, an increasing number of studies have used visual paradigms. Findings regarding early visual processing are mixed. Individuals with ASD across a range of cognitive ability showed hyper-responsiveness to flashes of light, demonstrated by stronger and quicker initial visual evoked responses and a slower recovery (Isler et al., 2010). In a study investigating cortical representation of the visual periphery in ASD, individuals with high functioning ASD had larger early responses (increased P1 amplitude) to checkerboard pattern stimuli presented in the periphery (Frey et al., 2013), suggesting early hyper-responsiveness and increased cortical representation of the visual periphery in ASD. Visual evoked responses to grating stimuli suggest a dissociation between hyper- and hypo-responsiveness based on spatial frequency, with high and low spatial frequency of a stimulus yielding increased and decreased responding, respectively (Vlamings et al., 2010; Pei et al., 2014). Hypo-responsiveness may be further restricted to the right hemisphere (Pei et al., 2014). Two studies investigated later components of visual processing by using visual oddball tasks in children with and without ASD. Both found cortical hyper-responsiveness

to visual change, regardless of whether it was an active (Baruth et al., 2010) task with high functioning ASD or a passive (Clery et al., 2013b) task with individuals with ASD across a range of cognitive ability. These results suggest that attention to visual input does not differentiate hyper- and hypo-responsiveness in the same way that it seems to in the auditory modality.

Milne et al. (2009) studied visual processing by examining changes in EEG power, which provides an index of neural synchronization. Participants with and without ASD across a range of cognitive ability viewed grating stimuli of varying spatial frequencies and were asked to press a button each time they saw a zebra on a screen. Overall, they found earlier peak latencies for the early C1 and P1 ERPs in ASD supporting faster reactions to basic visual stimuli. After localizing the clusters that accounted for the greatest amount of variance in EEG activity, the authors found stronger power in ASD in cingulate gyrus (reflecting greater attentional control), reduced effect of spatial frequency in ASD in striate and extrastriate regions (reflecting less neural specialization in networks recruited for basic visual perception), and no differences in the parietal region. Together, these findings suggest that early visual areas (e.g., primary visual cortex) are not hyper-responsive in ASD, but rather that there is reduced modulation of the networks involved in basic visual perception that likely contributes to the disruption of perceptual binding in ASD. There is also evidence of reduced synchrony of visual areas between the right and left hemisphere (Isler et al., 2010).

Tactile

Only one study has used ERP to investigate the brain's functional response to non-social tactile stimulation in high functioning children and adolescents with ASD, delivering air puffs to participants' finger tips while they were attending to this stimulation (Cascio et al., 2015). Although no group differences were seen in the ERP response to the stimulation, ERP responses at different time points post-stimulus were related to tactile hyper- and hypo- responsiveness as measured by parent report. These findings suggest that earlier neural responses to tactile stimulation are related to tactile hyper-responsiveness, while slightly later neural responses are related to tactile hypo-responsiveness and may involve higher-level processes such as attention allocation and assignment of emotional valence.

In sum, the EEG literature has revealed important differences in the timing of response to auditory, visual, and tactile input in ASD. Although many studies suggest atypicalities in temporally later stages of sensory processing, earlier stages have also been implicated. Additionally, both bottom-up and top-down processes seem to be affected, with top-down processes (e.g., attention) possibly differentiating impairment, at least in the auditory modality. Importantly, each sensory modality provides slightly different conclusions, providing merit to the single sensory modality approach in the study of neural mechanisms of sensory processing. Finally, existing attempts to relate EEG measures to questionnaire measures serve as models for integrating neural and symptom perspectives.

Functional Magnetic Resonance Imaging (fMRI)

Rather than measuring the electrical activity of neurons like in EEG methods, fMRI is based on the indirect measurement of brain activity through changes in blood flow. Predicated on the assumption that increased blood flow (i.e., activation) to a neural region is indicative of increased neural activity, fMRI studies provide exceptional spatial resolution at the expense of temporal resolution. Unlike EEG, which has been utilized across a variety of ages and functioning levels, fMRI is typically used in older and higher functioning ASD participants, who can remain still for the duration of the paradigm and tolerate the MRI environment. The studies described below use high functioning samples and often include participants that span a broad age range. Although the majority of the fMRI studies in ASD investigate higher order cognitions (e.g., theory of mind, face processing, language processing), several studies, outlined below, assess basic sensory processing. These studies provide information about where in the brain sensory processing differences exist in ASD. These findings largely parallel the EEG findings, in that regions involved in purely sensory processing and those involved in attentional control both show atypical responding patterns in ASD.

Auditory

Gomot et al. (2006, 2008) demonstrated the modulating role of attention across two auditory oddball fMRI studies, one with a passive listening task and one with an active task. Children and adolescents with ASD showed lower brain activation in the passive task and higher brain activation in an active task, in both parietal and frontal areas in response to deviant and novel stimuli. These results show both neural hypo-responsiveness (Gomot et al., 2006) and hyper-responsiveness (Gomot et al., 2008) to auditory change dependent on attention, consistent with the ERP data of the MMN and P3a components (Orekhova and Stroganova, 2014). Thus, the role of attention seems to be critical in auditory change perception in ASD, with consistent findings across EEG and fMRI studies. In a study of adolescents and adults with ASD investigating non-social auditory complexity, temporal but not spatial stimulus complexity was associated with increased and decreased fMRI activity in ASD in primary and other (anterolateral and posterior superior temporal gyrus) auditory cortical regions, respectively (Samson et al., 2011).

Visual

In a small fMRI visual attention study looking at children with ASD, their unaffected siblings, and controls (Belmonte et al., 2010), children with ASD had decreased activation in attention networks during the trials, but they and their unaffected siblings showed delayed activation of these networks (immediately following each trial). The unaffected siblings showed greater delayed attention network activation suggesting a stronger compensatory process that may be protective against developing symptoms of ASD. Clery et al. (2013a) looked at visual change detection using fMRI in a small group of adults with and without ASD. Adults with ASD showed increased activation in visual areas and decreased activation in frontal areas to deviant and novel stimuli, consistent with the idea of increased sensory

processing and reduced top-down modulation of sensory areas. The anterior cingulate cortex, a region important for attention switching and allocation of attentional resources, was also shown to be hyper-responsive in ASD while processing visual change events, offering an explanation of attention switching deficits as a mechanism for altered visual change detection in ASD. Thus, auditory change detection seems to be dependent on attention engagement, and visual change detection may be dependent on attention switching.

Ohta et al. (2012) investigated sensory (visual) filtering by implementing an fMRI paradigm manipulating perceptual load and presence of a distractor stimulus in adults with and without ASD. In typical individuals, the degree of processing of unattended stimuli is dependent on task load (e.g., greater processing when task load is low), which reflects efficient processing abilities. Results from this study showed no group differences in activation in the fronto-parietal attention network across conditions, but found that distractor-evoked activity in visual cortex was modulated less by perceptual load in ASD. These findings suggest a lack of flexible top-down regulation of sensory processing.

Tactile

Two fMRI studies have looked at responses to tactile stimulation in ASD. Kaiser et al. (2016) investigated neural responses to touch on the palm in children and adolescents with and without ASD, and found increased response in primary somatosensory cortex and insula in ASD, suggesting hyper-responsiveness to non-social touch. Cascio et al. (2012) demonstrated increased fMRI activation in attention areas in adults with ASD when presented with aversive, but not pleasant, tactile stimulation. This study also collected subjective ratings of roughness and pleasantness for the same stimuli and found no correlations between these ratings and the fMRI response, highlighting a disconnect between neural responding and subjective experiences. Together, these studies suggest neural hyper-responsiveness to basic tactile stimulation, but there is inconsistency as to whether this hyper-responsiveness is localized in sensory (Kaiser et al., 2016) or attention (Cascio et al., 2012) areas.

Multiple Modalities

Two fMRI studies have made notable attempts to link neural responding to sensory symptoms by investigating sensory and limbic responses to mildly aversive sensory stimuli (Green et al., 2013: auditory, visual, and audiovisual combined stimuli; Green et al., 2015: auditory, tactile, and audiotactile combined stimuli) in children and adolescents with and without ASD. Across both studies, they found neural hyper-responsiveness in ASD across the unisensory and multisensory conditions in both sensory and limbic areas, including frontal regions, with the strongest increases in neural responding during the multisensory condition. In the multisensory conditions, signal increase in several areas, including sensory, limbic, and frontal regions, was positively associated with sensory hyper-responsiveness scores on a composite variable generated from two parent report questionnaires (Short Sensory Profile and SensOR) above and beyond anxiety levels. Unfortunately, a similar analysis was not reported in the unisensory conditions. Green et al. (2015) conducted additional analyses looking at the specific role of sensory hyper-responsiveness symptoms, finding that those with ASD *and* high sensory hyper-responsiveness symptoms were driving the effects of neural hyper-responsiveness, and that those with ASD only (and low sensory hyper-responsiveness symptoms) were similar to controls. Connectivity analyses suggest that the hyper-responsiveness in primary sensory areas observed in those with ASD and high hyper-responsive symptoms may lead to hyper-responsiveness in limbic areas, which may then over-engage frontal areas in an attempt to regulate the response. Together, these two studies highlight the value of investigating differences in neural responding patterns based on sensory subgroups within ASD, the role of top-down modulation, and the value of combining neural and symptom measures.

In sum, there are many fewer fMRI studies investigating neural response patterns to basic sensory stimuli compared to the EEG literature. However, the few studies outlined above allow for preliminary understanding of how the brain responds when processing basic auditory, visual, and tactile stimuli. In higher order regions, atypical responding patterns are consistently reported in ASD; however, the evidence is mixed in that some reports show hyper-responsiveness and other reports show hypo-responsiveness. In purely sensory regions, atypicalities in ASD are not always observed; however, when these atypicalities are observed, evidence is overwhelmingly in the direction of neural hyper-responsiveness, especially in the most simplistic tasks (e.g., presenting sensory stimuli with no requirement of task engagement). Recent advancements in this literature highlight the value of integrating neural and symptom perspectives.

Variability in Responding to Sensory Input in EEG and fMRI Studies

The studies reviewed above focus on the amount of responding to a particular sensory stimulus collapsed across several stimulus presentations. Another approach is to look at the variability in responding across the different stimulus presentations to determine the consistency or amount of "noise" in the neural response. In fact, increased (or decreased; Davis and Plaisted-Grant, 2015) neural noise as a heuristic theory of ASD has generated a lot of attention recently (Dinstein et al., 2015). Four neural studies provide empirical evidence for increased variability in neural responding to basic sensory input, consistent with such an account. Milne (2011) reanalyzed a subset of the data from Milne et al. (2009) to investigate intra-participant variability of the P1 (early sensory) cortical response (amplitude and latency) and inter-trial phase coherence. Inter-trial phase coherence measures the degree to which EEG activity is phase-locked to a specific stimulus presentation across trials. They found increased intra-participant variability in the children with ASD across all three measures, pointing to evidence for increased neural noise in ASD. Weinger et al. (2014) also reported increased neural noise in a sample of children with ASD, as measured by EEG in the visual modality. They presented checkerboard pattern stimuli and found no group differences in amount of

neural responding; however, they found decreased signal to noise ratio and increased neural noise (greater variability in amplitude across trials) in ASD.

Similar findings of increased neural noise exist across two fMRI studies (Dinstein et al., 2012; Haigh et al., 2015) in adults with ASD. Visual, auditory, or tactile stimuli were presented while participants completed an unrelated task to divert attention away from sensory input. There were no differences in amount or location of neural activation to these stimuli; however, the variance of responses was larger in ASD compared to controls. This increased variance was specific to sensory regions during stimulus presentation; it was not seen in other neural areas during stimulus presentation or in sensory areas during rest. Overall, these studies of variability in neural response do not support theories of neural hyper- or hypo-responsiveness to simple sensory input (at least when attention is diverted away), but offer increased intra-participant neural variability as a consistent marker of sensory processing dysfunction in ASD.

Magnetoencephalography (MEG)

Three studies of sensory processing in ASD have used MEG, two in the auditory and one in the tactile modality. MEG provides similar temporal resolution to EEG but with better spatial resolution. Roberts et al. (2010) conducted an MEG study on auditory processing in high functioning children with and without ASD and found delayed M100 latency in ASD across all four tone frequencies presented. There were no group differences in the M50 response. Typical maturational processes of the M100 response becoming earlier with age was observed in the control group, but absent in the ASD group, suggesting atypical maturation of the auditory cortex in ASD. Orekhova et al. (2012) utilized a paired clicks paradigm (detailed above in the EEG section) with MEG in children with and without ASD, and found atypical P100 lateralization in children with ASD. Children with ASD showed less right lateralization in response to the auditory stimulus, and this was correlated with total sensory problems measured by a parent questionnaire. The child P100 is thought to be involved in arousal, spatial orienting, and attention processing, all of which are typically right lateralized functions. Atypical P100 lateralization seen in ASD may reflect disrupted preattentive arousal, in which children with ASD rely on non-optimal left hemisphere processing.

In a tactile MEG study with high functioning children with and without ASD, Marco et al. (2012) applied a finger tapping paradigm to investigate the timing and amplitude of responses in primary somatosensory cortex. Children with ASD had reduced responses in the slow, but not fast, rate version of the paradigm, specifically in the left hemisphere. Additionally, tactile sensitivity scores on the Sensory Profile correlated with amount of neural response in primary somatosensory cortex across the combined sample. An additional analysis separating the groups by tactile sensitivity scores revealed more robust neural differences between these groups in both the right and left hemisphere, suggesting that these neural differences are more closely related to individual differences in tactile functioning than to ASD specifically. Together, these three studies point to maturational and lateralization differences related to sensory processing that may be present in ASD, and indicates the need for further work using this approach.

Summary of Neuroscience Literature

In sum, the neural processing of sensory input in ASD literature points to atypical neural processing of even the most basic sensory stimuli that can be observed across a variety of methodologies. EEG studies provide evidence for atypicalities in ASD during both earlier and later sensory processing stages. fMRI studies provide evidence for different spatial activation patterns across areas of the brain responsible for these earlier and later sensory processing stages. Emerging MEG evidence points to maturational and lateralization differences related to sensory processing that may be present in ASD. Higher-order behaviors at the symptom level are complex and arise from complicated interactions of these simpler processes. Although we are far from understanding these complicated interactions, this neural literature highlights the basic nature of some of the differences associated with ASD. Additional research building upon existing efforts to combine neural and symptom perspectives is necessary to sort through the broad array of findings outlined above.

PSYCHOPHYSICS: AN INTERMEDIATE APPROACH

Psychophysics is an approach that has been recently applied to the study of sensory processing in ASD by both neuroscientists and sensory symptom researchers. Psychophysical studies rely on a decision related to a perceptual experience, and are designed to closely model neural responding patterns. Thus, this approach capitalizes on an intuitive intermediary between the neural response to sensory input and the individual's observable reaction. Additionally, psychophysical studies allow for the study of isolated features of real world stimuli that can be conceptualized as the building blocks of higher-level perception. For example, if studying motion perception within the visual modality, one would present the most basic motion stimulus (i.e., a moving grating pattern) and determine an individual's ability to perceive that stimulus in either a detection task (e.g., press a button when you see the moving stimulus on the screen) or a discrimination task (e.g., decide if the stimulus is moving to the right or the left). Although a review of this literature is beyond the scope of this paper, examples of this approach applied to ASD include multiple sensory modalities, including visual (e.g., Bertone et al., 2005), auditory (e.g., Jones et al., 2009), and tactile (e.g., Cascio et al., 2008).

Although these types of measurements lie in between neural response and observable reactions, this method on its own has yet to provide an integrative framework for understanding sensory processing in ASD. This is likely because important links are missing between neural responding, detection/discrimination decisions, and observable reactions. In fact, a large research area in the field of basic neuroscience seeks to understand how neural firing translates to these types of decisions in humans generally

(and primates more globally). Additionally, much remains to be known about the link between these more basic perceptual decisions and higher-order observable reactions. In particular, these measurements largely ignore the affective component and real-life impact that characterize sensory symptoms of ASD. However, these tools may allow us to characterize hypo-responsive symptoms in a way that questionnaires and observational coding paradigms miss, because this category of symptoms is defined by the absence of typical reactions.

RECOMMENDATIONS FOR FUTURE RESEARCH

Until 2005, sensory processing research in ASD was largely focused on whether individuals with ASD exhibited atypical sensory responses and if these sensory responding patterns could be used to differentiate ASD from other developmental disorders (e.g., intellectual disability, Fragile X syndrome). Because of these motivations, sensory processing research was mostly descriptive and one-dimensional. Additionally, this early research suffered from studies with small, and often poorly characterized samples resulting in uncertainty about the presence and uniqueness of sensory processing difficulties in ASD. In the last decade, sensory processing research in ASD has expanded significantly—in the number of studies published, increased methodological rigor of these studies, and diversification of approaches used. This has led to a *multidisciplinary* understanding of sensory processing in ASD. Currently, the field has a vast amount of descriptive and emerging mechanistic information about how individuals with ASD (and possible subgroups within ASD) perceive and respond to sensory information differently. However, this information has been generated from two very different perspectives: clinical science and neuroscience. In order to integrate this information into a cohesive picture of *how* and *why* sensory processing differences manifest in ASD and to be able to see the translational value in its application to early identification and treatment, it is essential for these two perspectives to communicate more effectively and move toward an *interdisciplinary* understanding. Specific recommendations are outlined below that will hopefully allow us to embark on the next phase of sensory processing research in ASD.

What Can Sensory Symptom Researchers Learn from Neuroscientists?

Greater Use of Modality-Specific Measurement

One of the difficulties with the current approach to studying sensory symptoms in ASD is the use of measures that collapse across auditory, visual, tactile, and other sensory modalities. The neuroscience research has revealed important differences between sensory processing modalities in ASD and has highlighted the importance of precision and specificity in conceptualizing atypical responses. The sensory symptom literature has begun to address this by using subscale or factor scores that isolate specific modalities. However, the use of more modality-specific measures, both questionnaire and lab-based, will improve sensitivity of these measures and hopefully help begin to bridge knowledge from the neuroscience and clinical perspectives.

Appropriate Selection of Measurement Based on Pattern of Responding

In line with the above recommendation, the most appropriate measurement tool may depend on the pattern of responding of interest. For example, hyper-responsiveness may be best investigated with questionnaires and lab-based observational coding paradigms, if these measurement tools are enhanced. However, hypo-responsiveness is difficult to capture with observation in the lab or via parent questionnaires, given that these symptoms are defined as the *absence* of typical responding. Such symptoms may be more directly and accurately captured using psychophysical approaches that rely on detection and/or discrimination thresholds (i.e., the minimum amount of sensory input needed for reliable perception).

What Can Neuroscientists Learn from Sensory Symptoms Researchers?

Increased Focus on Developmental Change Over Time

The sensory symptoms literature has moved toward investigating specific developmental periods, with a few longitudinal studies that directly explore developmental effects, but the neuroscience research lags behind in this manner. Although the symptoms literature suggests little developmental change at the symptom level in childhood, developmental differences do seem apparent when one looks across the lifespan. Future neuroscience studies should consider narrower age ranges and specific consideration of developmental changes occurring at those ages, as developmental changes may be more pronounced at the neural level. Although there are some longitudinal studies investigating structural neural changes in ASD, longitudinal studies examining functional responding to sensory input are lacking.

Recognition and Investigation of the Heterogeneity within Sensory Processing in ASD

The sensory symptom literature provides emerging evidence for sensory-based subgroups in ASD. It will be beneficial for neuroscientists to appreciate the heterogeneity within ASD, rather than conceptualizing ASD as a single, homogenous disorder. Given the sensitivity and precision of neuroscience approaches, it is possible that these approaches will aid our understanding of this heterogeneity and reveal meaningful subgroups within the disorder.

Greater Emphasis on Top-Down Modulation of Sensory Processing

From the sensory symptom literature, the modulation of sensory input is most related to clinical problems. The current neural literature also points to the importance of the modulatory role of cognitive processes, such as attention, on sensory processing. Thus, future studies should continue to examine these top-down effects in an effort to better map the mechanisms involved in the presentation of sensory symptoms in ASD.

General Recommendations

Exploration of Sensory Processing in Lower Functioning Individuals with ASD

The sensory symptoms literature suggests that sensory difficulties are present across functioning levels in ASD. However, the presentation of these difficulties likely differs across various levels of analysis. Current measures, particularly those with the greatest sensitivity, are largely limited to high functioning individuals with ASD, thus over-representing this population in research. Although measure development for lower functioning individuals is challenging, adaptation of measures for this population presents an opportunity for creative collaboration between fields.

Increased Understanding That the Relationship Between Neural Hyper- and Hypo-Responsiveness and Symptoms of Hyper- and Hypo-Responsiveness is Highly Complex

It is tempting to assume that the relationship between neural responding and observable reactions is simplistic, and many of the current theories about sensory functioning in ASD adopt that framework. This is especially true given the shared terminology used to describe both responses. However, this assumption leads to oversimplification, which hinders progress toward unraveling the complex reality of these relationships. Each field is able to provide information on specific aspects of sensory processing, but researchers should be mindful of the limitations of each method.

Utilization of a Multi-Method Approach to Assess Different Aspects of Sensory Symptoms

Several studies that have used multi-method approaches suggest divergence among different aspects of sensory processing. In fact, review of the literature suggests that each method contributes to our understanding of sensory processing in a unique way. Questionnaires and lab-based observational coding paradigms can be best used to characterize observable reactions to sensory input, most notably in the hyper-responsive pattern, and the day-to-day impact on functioning; psychophysiological measurements add additional information about the underlying bodily response, including an emotional reaction component; psychophysical methods characterize the perceptual responses (detection/discrimination) that result from atypical neural processing; and direct neural measures (EEG, fMRI, MEG) provide the best information about underlying timing, degree, and location of neural response to sensory input. An important caveat to consider is that each of these methods is only as useful as the measurement tool selected, and measurement work, particularly for questionnaires, continues to be necessary. However, by improving upon and then combining these methodologies, we will protect against over-interpretation of any single-method. Specifically, each method can contribute knowledge about a specific and appropriate *aspect* of sensory processing rather than attempt to make claims about sensory processing as a whole. Then, upon combination of these different methods, a more accurate understanding of sensory processing in ASD can be achieved.

CONCLUSIONS

The interest in sensory processing in ASD has expanded substantially in the last decade, as evidenced by an increased number of studies using well-characterized samples with sufficient sample sizes, the development of new measures and paradigms, and the adoption of neuroscience approaches. At this point, sensory processing should no longer be conceptualized as a single construct that can be measured similarly by different tools. Instead, each approach offers a unique contribution to a piece of sensory processing, and if applied appropriately, the understanding of sensory processing in ASD as a whole can progress. It is our hope that this paper highlighted the importance of sensory processing in ASD, explained the two major research perspectives related to sensory processing in ASD, and provided a framework for conceptualization of sensory processing moving forward. Finally, by first understanding the link between brain and symptoms *within* the sensory domain, we can more successfully understand the relationship between brain and symptoms in ASD more broadly.

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KS conceived the idea and drafted the first version of the paper. LB supervised the manuscript and reviewed the paper for intellectual content. KS and LB revised the manuscript. Both authors approved the final version.

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Neuropathological Mechanisms of Seizures in Autism Spectrum Disorder

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This manuscript reviews biological abnormalities shared by autism spectrum disorder (ASD) and epilepsy. Two neuropathological findings are shared by ASD and epilepsy: abnormalities in minicolumn architecture and γ-aminobutyric acid (GABA) neurotransmission. The peripheral neuropil, which is the region that contains the inhibition circuits of the minicolumns, has been found to be decreased in the post-mortem ASD brain. ASD and epilepsy are associated with inhibitory GABA neurotransmission abnormalities including reduced $GABA_A$ and $GABA_B$ subunit expression. These abnormalities can elevate the excitation-to-inhibition balance, resulting in hyperexcitablity of the cortex and, in turn, increase the risk of seizures. Medical abnormalities associated with both epilepsy and ASD are discussed. These include specific genetic syndromes, specific metabolic disorders including disorders of energy metabolism and GABA and glutamate neurotransmission, mineral and vitamin deficiencies, heavy metal exposures and immune dysfunction. Many of these medical abnormalities can result in an elevation of the excitatory-to-inhibitory balance. Fragile X is linked to dysfunction of the mGluR5 receptor and Fragile X, Angelman and Rett syndromes are linked to a reduction in $GABA_A$ receptor expression. Defects in energy metabolism can reduce GABA interneuron function. Both pyridoxine dependent seizures and succinic semialdehyde dehydrogenase deficiency cause GABA deficiencies while urea cycle defects and phenylketonuria cause abnormalities in glutamate neurotransmission. Mineral deficiencies can cause glutamate and GABA neurotransmission abnormalities and heavy metals can cause mitochondrial dysfunction which disrupts GABA metabolism. Thus, both ASD and epilepsy are associated with similar abnormalities that may alter the excitatory-to-inhibitory balance of the cortex. These parallels may explain the high prevalence of epilepsy in ASD and the elevated prevalence of ASD features in individuals with epilepsy.

Keywords: autism spectrum disorder, seizures, epilepsy, genetic syndrome, metabolic disorders, excitatory-to-inhibitory cortical balance, gamma-aminobutyric acid

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INTRODUCTION

Autism spectrum disorders (ASD) is a behaviorally defined disorder that has recently been estimated to affect as many as 1 out of 45 individuals (Zablotsky et al., 2015). Although, ASD is defined by behavioral features, it is associated with co-occurring medical conditions. For example, epilepsy is more prevalent in ASD than in the typically developing children with a prevalence ranging from 5 to 38% (Deykin and Macmahon, 1979; Volkmar and Nelson, 1990; Tuchman and Rapin, 2002; Danielsson et al., 2005; Hara, 2007). Data from surveys performed by the Autism Research Institute on over 1200 participants suggests that the prevalence is between 15 and 19%. Epilepsy is one of the most disabling ASD co-morbidities as children with ASD and epilepsy are more likely to have intellectual disability (Tuchman, 2013) and increased mortality (Shavelle et al., 2001; Pickett et al., 2011) as compared to children with ASD without epilepsy. In addition, epilepsy in ASD tends to be more treatment-resistant as compared to epilepsy in typically developing children (Sansa et al., 2011).

One of the major questions in ASD research is its etiology. Much ASD research concentrates on genetic causes (Rossignol and Frye, 2012b) even though inherited single gene and chromosomal defects only account for a minority of ASD cases (Schaefer et al., 2013). However, genetic etiologies may be overrepresented in children with ASD and epilepsy as many genetic syndromes and gene mutations associated with ASD include epilepsy as a common feature (Murdoch and State, 2013; Tuchman et al., 2013).

Although some have suggested that clinical seizures do not have any special causative significance in ASD (Tuchman and Rapin, 1997), ASD coexists with epilepsy in several disorders (see Section Specific Medical Disorders Associated with Both ASD and Epilepsy) suggesting that the same neuropathology may result in both ASD and epilepsy. Thus, this manuscript reviews the shared biological abnormalities in ASD and epilepsy in two sections. The section called Basic Neuropathological Mechanisms of Seizures in ASD discusses two neuropathological mechanisms that have been described in ASD that can also cause epilepsy. Both mechanisms involve an abnormal reduction in inhibitory mechanisms of the brain, thereby resulting in an increase in the excitatory-to-inhibitory balance. The section called Specific Medical Disorders Associated with Both ASD and Epilepsy will review specific clinical disorders that have been described in both ASD and epilepsy with special reference to underlying neuropathological mechanisms that can cause seizures.

Overall, our review finds that many disorders associated with ASD increase the excitatory-to-inhibitory balance by either (1) reducing inhibitory circuits in the brain through a decrease in the inhibitory neurotransmitter γ-aminobutyric acid (GABA), or (2) increasing excitatory circuits in the brain through an increase in glutamate neurotransmission. Elevation in the excitatory-to-inhibitory balance in the brain can lead to seizures. By carefully outlining these disorders, insight into the etiologies that underlie ASD may be better understood.

Abbreviations: AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; NMDA, N-methyl-D-aspartate; ASD, autism spectrum disorder; ATP, adenosine-5′-triphosphate; CNS, central nervous system; GABA, γ-aminobutyric acid.

BASIC NEUROPATHOLOGICAL MECHANISMS OF SEIZURES IN ASD

Several neuropathological processes associated with ASD are also associated with epilepsy. Here we review two such neuropathological processes: (1) minicolumn architecture and (2) GABA neurotransmission.

Minicolumn Architecture

The minicolumn is a radially-oriented assembly of neurons and cellular elements considered to be an elemental modular microcircuit of the neocortex (Buxhoeveden and Casanova, 2002; Casanova et al., 2006). The minicolumn core contains pyramidal cell arrays surrounded by a peripheral neuropil space that contains GABAergic inhibitory interneurons and other cells such as the double-bouquet cell (Mountcastle, 1997; Buxhoeveden and Casanova, 2002; Defelipe, 2005). Double-bouquet cells feature axonal bundles which provide a vertical stream of inhibition (Mountcastle, 1997). This inhibitory stream insulates the minicolumn core from the excitation from other surrounding minicolumns (Defelipe et al., 1990; Favorov and Kelly, 1994; Defelipe, 1999).

The peripheral neuropil space has been shown to be reduced in post-mortem brain tissue from ASD individuals (Buxhoeveden and Casanova, 2002), with this reduction most prominent over the prefrontal cortex (Casanova et al., 2006). The neuropil space is reduced within the region that contains the inhibition circuits of minicolumns (Defelipe et al., 1990; Favorov and Kelly, 1994; Defelipe, 1999). These architectural changes should, theoretically, disrupt the normal balance between excitation and inhibition influences within the columnar organization of the cortex (Casanova et al., 2003). A reduction of GABAergic inhibitory activity has been proposed to result in hyperexcitability of minicolumn circuits and can explain some of the symptomatology observed in ASD, including the high incidence of seizures and auditory-tactile hypersensitivity (Rubenstein and Merzenich, 2003). Networks of inhibitory interneurons acting as GABA gated pacemakers are also critically involved in gamma oscillations (Grothe and Klump, 2000). Abnormalities in gamma oscillations are associated with problems with binding and the coactivation of neural assemblies. A deficit in binding and gamma oscillations has been proposed to explain many of the symptoms related to ASD (e.g., visuoperceptual defects, understanding and using context; Grice et al., 2001; Brock et al., 2002; Brown et al., 2005; Rippon et al., 2007; Tommerdahl et al., 2007).

GABA Transmission

GABA is the major inhibitory neurotransmitter of the central nervous system (CNS). Abnormalities in GABA neurotransmission have been associated with epilepsy. GABBR1A, GABBR1B, and GABBR2 receptor subunits are reduced in the hippocampi of patients with temporal lobe epilepsy (Princivalle et al., 2003), and animal models have also

shown a link between GABA receptor expression and epilepsy (Schuler et al., 2001; Han et al., 2006). Individuals with ASD have been shown to have abnormalities in GABAergic brain systems (Blatt et al., 2001; Dhossche et al., 2002; Fatemi, 2008), as well as a reduction in $GABA_A$ (Fatemi et al., 2009) and $GABA_B$ (Fatemi et al., 2009) receptor subunits in both the frontal and parietal cortices, as compared to controls, with the ASD group also demonstrating a markedly higher rate of epilepsy than the controls. In addition, the GABA subunits found to be reduced in individuals with ASD (i.e., GABRα1 and GABBR1) have been associated with childhood absence epilepsy, juvenile myoclonic epilepsy, and atypical absence seizures (Delgado-Escueta, 2007; Kang et al., 2009).

SPECIFIC MEDICAL DISORDERS ASSOCIATED WITH BOTH ASD AND EPILEPSY

Genetic Disorders

The neurobiological mechanisms leading to seizures in genetic syndromes that are associated with ASD are diverse and complex. Imbalances in GABA and glutamate have been suggested to underlie CNS dysfunction in several of these genetic syndromes. Defects in $GABA_A$ function has been implicated in Fragile X (D'hulst and Kooy, 2007) and recent studies on Fragile X suggest that mGluR5 dysfunction results in heightened excitability and secondary alterations in GABA function (Frye, 2014). Dysfunction in $GABA_A$ receptor function has also been implicated in Angelman syndrome (Pelc et al., 2008). Indeed, a cluster of genes coding for three $GABA_A$ receptor subunits lie adjacent to the critical Angelman region (i.e., UBE3A). Mutations within the Rett syndrome gene (i.e., MECP2) decreases expression of GABRB3, a gene responsible for encoding the $beta_3$ subunit of the $GABA_A$ receptor, and DLX5, a gene which regulates the production of enzymes responsible for GABA production.

Some genetic syndromes associated with epilepsy and ASD are associated with metabolic abnormalities. For example, mouse models of both Angelman and Rett syndromes demonstrate mitochondrial dysfunction (Kriaucionis et al., 2006; Su et al., 2011) and mitochondrial dysfunction is reported in a Rett syndrome case (Condie et al., 2010). Phelan-McDermid Syndrome (PMS) and duplication of the 22q13 region are both associated with ASD and mitochondrial dysfunction (Frye, 2012b; Frye et al., 2016a). As mentioned below, disruption of mitochondrial metabolism can result in changes to the excitatory-to-inhibitory balance.

Single gene disorders associated with both ASD and epilepsy have been associated with abnormalities in the excitatory- to-inhibitory balance (Srivastava and Schwartz, 2014). Mutations in CNTNAP2 (Peñagarikano et al., 2011) or CNTNAP4 (Karayannis et al., 2014) result in reduced GABAergic neurotransmission. The SYNGAP1 haploinsufficiency animal model shows an increase in neuronal excitability and an increase in seizure susceptibility (Clement et al., 2012). Other genes are associated with a relative decrease in the excitatory-to-inhibitory balance. Animal model with NLGN3 mutations demonstrates increased inhibitory neurotransmission (Tabuchi et al., 2007). Cellular (Shcheglovitov et al., 2013) and animal models (Bangash et al., 2011; Wang et al., 2011b) demonstrate a reduction in excitatory neurotransmission when SHANK3 is disrupted. Animal models with decreased synapsin I (SYN1) demonstrate reduced glutamate release (Li et al., 1995).

Metabolic Disorders

Disorders of Energy Metabolism

Disorders of energy metabolism have been associated with ASD (Giulivi et al., 2010; Frye and Naviaux, 2011; Frye, 2012c; Rose et al., 2014a,b) and epilepsy (Frye, 2015). Some children with ASD have mitochondrial dysfunction that is different than classic mitochondrial disease (Frye and Rossignol, 2011; Rossignol and Frye, 2012a; Frye, 2012a). Of children with mitochondrial disease and ASD, 41% have seizures (Rossignol and Frye, 2012a).

Other disorders of energy metabolism are associated with ASD and epilepsy, including disorders of creatine metabolism (Póo-Argüelles et al., 2006; Longo et al., 2011) and adenylosuccinate lyase deficiency (Spiegel et al., 2006; Jurecka et al., 2008). Creatine and phosphocreatine play important roles in energy storage and transmission of high-energy phosphates. Adenylosuccinate lyase deficiency is a rare autosomal disorder of *de novo* purine synthesis (Spiegel et al., 2006; Jurecka et al., 2008). The purine nucleotide cycle regulates cellular metabolism by controlling levels of fumarate, a citric acid cycle intermediate, and adenosine, the precursor to adenosine-5′-triphosphate (ATP) (Spiegel et al., 2006).

An energy deficiency can result in seizures. Neurons with high firing rates, such as inhibitory GABA interneurons (Anderson et al., 2008), are disproportionally affected by an energy deficit. In addition, processes critically involved in the release and reuptake of neurotransmitters and maintenance of the neuronal resting potential, such as calcium homeostasis, are critically dependent on mitochondrial function (Li et al., 2004; Quiroz et al., 2008; Chen and Chan, 2009).

Disorders of GABA Neurotransmission

Several metabolic disorders directly lead to GABA metabolism abnormalities. Pyridoxine and its primary biologically active form, pyridoxal-5-phosphate, are essential cofactors for over 110 enzymes, including glutamic acid decarboxylase (GAD), the enzyme that produces GABA from glutamate. Pyridoxal-5-phosphate depletion reduces GAD activity which, in turn, increases glutamate, decreases GABA synthesis and decreases cortical inhibition (Gospe et al., 1994; Gospe, 2002; Mills et al., 2006). This occurs in pyridoxine dependent seizures.

Succinic semialdehyde dehydrogenase deficiency is an autosomal recessive disorder of GABA metabolism. It results from a defect in the aldehyde dehydrogenase gene (ALDH5A1; Jakobs et al., 1981). Aldehyde dehydrogenase is partially responsible for the degradation of GABA and when this enzyme is deficient GABA is degraded through an alternative pathway, resulting in the formation of gamma-hydroxybutyric acid and GABA elevations in the brain. Positron emission tomography studies suggest that chronic elevation in GABA down-regulates

$GABA_A$ receptors, leading to a deficit in cortical inhibition and an elevation in the excitatory-to-inhibitory balance (Pearl et al., 2009a,b).

Disorders of Glutamate Neurotransmission

Two types of metabolic disorders (urea cycle defects and phenylketonuria) may result in dysfunction of glutamate neurotransmission. Glutamate is the major excitatory cortical neurotransmitter and excess glutamate results in an elevation in the excitatory-to-inhibitory balance, leading to seizures.

Urea cycle defects result in ammonia elevations. Astrocytes exposed to ammonia do not express glutamate reuptake transporters that normally reduce extracellular glutamate (Rose, 2006). Thus, increased ammonia levels in the brain can result in elevated extracellular glutamate.

Neurological consequences of phenylketonuria are usually avoided by dietary treatment starting at birth (Williams et al., 2008). However, epilepsy and ASD may develop in untreated children and in those noncompliant to the prescribed diet (Baieli et al., 2003). Such children demonstrate high levels of phenylalanine in the brain. Phenylalanine antagonizes both N-methyl-D-aspartate (NMDA) and non-NMDA glutamate receptors (i.e., α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors; Glushakov et al., 2003). Chronic elevation in phenylalanine leads to an upregulation of several NMDA and AMPA receptor subunits (Glushakov et al., 2005), increased glutamate receptor density and increased glutamate release (Martynyuk et al., 2005). Such changes in glutamate neurotransmission predispose the brain to heightened excitability and seizures, especially if the phenylalanine level is transiently lowered (Martynyuk et al., 2005).

Mineral Deficiencies

Magnesium is essential in neurotransmitter metabolism and in modulating neurotransmitter receptor function. Ionized magnesium is important in seizure control. Ionized magnesium is a NMDA antagonist (Ault et al., 1980; Hallak, 1998) and may be a factor in some epilepsies (Hallak et al., 1992; Mathern et al., 1998; Mikuni et al., 1999). NMDA receptor activation by glutamate results in calcium influx (Macdermott et al., 1986; Delorenzo and Limbrick, 1996), which is pro-epileptogenic (Delorenzo, 1986; Heinemann and Hamon, 1986). Low ionized magnesium or altered balance between ionized magnesium and ionized calcium may precipitate seizures (Chaistitwanich et al., 1987). Patients with epilepsy have been shown to have significantly lower mean ionized magnesium levels and an increase in the ionized calcium to ionized magnesium ratio in spite of normal total serum magnesium levels (Sinert et al., 2007).

The role of zinc in epilepsy is not clear. Low zinc levels has been associated with seizures in children (Ganesh and Janakiraman, 2008; Mollah et al., 2008) and in the EL epileptic mouse (Fukahori and Itoh, 1990). Zinc acts as an anticonvulsant (Williamson and Spencer, 1995; Cole et al., 2000) and decreases seizure susceptibility (Fukahori and Itoh, 1990). However, zinc has been shown to be proconvulsant in a mouse model (Pei et al., 1983). Zinc co-localizes with glutamate where it inhibits the reuptake of synaptic GABA, thereby increasing the cortical inhibitory tone (Cohen-Kfir et al., 2005). Thus, a zinc deficiency could increase the relative excitatory-to-inhibitory balance.

Vitamin Deficiencies

Children with ASD have been shown to have abnormalities in cobalamin dependent pathways (Frye and James, 2014), and cobalamin supplementation improves metabolites in these pathways (James et al., 2009a; Adams et al., 2011; Frye et al., 2013a; Hendren et al., 2016) and behavior (Adams et al., 2011; Frye et al., 2013a,b; Hendren et al., 2016). The exact mechanism in which cobalamin deficiency causes seizures is unclear but infants with cobalamin deficiency manifest seizures (Benbir et al., 2007; Erol et al., 2007). Cobalamin is essential for myelin synthesis and methylation (Kumar, 2004). Neurons with damaged myelin sheaths are more susceptible to the excitatory effects of glutamate (Akaike et al., 1993).

Cerebral folate deficiency (CFD) is characterized by low 5-methyltetrahydrofolate in the CNS and is associated with ASD and seizures (Ramaekers et al., 2002; Ramaekers and Blau, 2004). Children with idiopathic ASD have a high prevalence of folate receptor alpha autoantibodies that causes CFD (Frye et al., 2013c, 2016b). Folate is essential in a wide range of metabolic processes, including redox and homocysteine metabolism and gene methylation (Obeid et al., 2007). Disruption in these processes could disrupted redox metabolism, thereby depleting glutathione which, in turn, can decreased glutamate degradation, leading to increased cortical excitability (Deepmala et al., 2015).

Heavy Metals

Several epidemiologic studies support a relationship between ASD and exposure to mercury or other heavy metals (Rossignol et al., 2014). Epilepsy has been associated with exposure to toxic levels of heavy metals including lead (Silbergeld et al., 1979; Swartzwelder, 1985; Lockitch et al., 1991; Arrieta et al., 2005) and mercury (Torres et al., 2000). Heavy metals may have toxic effects on the brain by reducing mitochondrial function (James et al., 2009b; Belyaeva et al., 2011; Wang et al., 2011a; Rose et al., 2015), causing apoptosis (Wang et al., 2011a; Pal et al., 2012), and increasing levels of reactive oxygen species (James et al., 2009b; Furieri et al., 2011; Wang et al., 2011a). Although the mechanism(s) by which heavy metals cause epilepsy are not clear, both mitochondrial dysfunction (Rossignol and Frye, 2012a) and high levels of reactive oxygen species (Riazi et al., 2010; Specchio et al., 2010; Waldbaum and Patel, 2010), have been linked to epilepsy.

Immune Dysregulation

Multiple studies have demonstrated evidence of abnormal immune system activation in individuals with ASD. Unusually high levels of proinflammatory cytokines have been found in the cerebrospinal fluid of individuals with ASD (Vargas et al., 2005). Abnormal activation of the intrinsic immune system in the cerebral cortex, white matter, and cerebellum has been demonstrated in individuals with ASD at autopsy (Vargas et al., 2005).

Children with ASD manifest autoantibodies implicated in childhood epilepsy syndromes associated with language regression (Connolly et al., 2006) and cognitive and behavioral changes (Ganor et al., 2004; Vincent et al., 2004) and drug-resistant epilepsy (Majoie et al., 2006) as well as autoantibodies to critical brain elements, such as myelin basic protein, brain derived neurotrophic factor and endothelial cells (Connolly et al., 1999). GAD65 autoantibodies are associated with several neurological disorders including drug-resistant epilepsy (Blanc et al., 2009). One study found GAD65 autoantibodies in 15% of children with ASD (Rout et al., 2012) but other studies have failed to find these autoantibodies in ASD children (Kalra et al., 2015).

Certain autoantibodies, such as the folate receptor alpha autoantibody, could result in specific syndromes like CFD and a recent study suggests that the folate receptor alpha autoantibody may also interfere with cobalamin metabolism (Frye et al., 2016b). Autoantibodies associated with specific seizure syndromes could also result in the dysfunction of specific neural elements. Autoantibodies can also be an epiphenomenon of underlying immune dysregulation.

SUMMARY

Many of these disorders associated with both seizures and ASD increase the excitatory-to-inhibitory balance. Some disorders reduce brain inhibition by reducing the inhibitory neurotransmitter GABA by a reduction in GABA production, metabolic failure of inhibitory GABA neurons or dysfunction of GABA receptors. Other disorders increase brain excitation by increasing the excitatory neurotransmitter glutamate through increased production, alterations in degradation, or altering glutamate receptors. Independent of these disorders, neuropathological research on ASD points to abnormalities in inhibitory GABA pathways.

A few studies suggest that some gene mutations are associated with a reduction in the excitatory-to-inhibitory balance. This appears to contradict the classic association of seizures with cortical excitability. It may be that these changes cause instability in neuronal networks or compensatory changes at the neuronal level that may create abnormalities in neural excitability. For example, although brain GABA is increased in patients with succinic semialdehyde dehydrogenase deficiency, GABA receptors are down regulated, leading to an elevation in the excitatory-to-inhibitory balance (Pearl et al., 2009a,b).

Clearly further research examining these pathways in more detail could help guide the development of targeted treatments and improve our understanding of the clinical implication of these changes. This review suggests that neurological dysfunction in at least a subset of children with ASD is based on alterations in the excitatory-to inhibitory balance in the brain.

AUTHOR CONTRIBUTIONS

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Specific Medical Conditions Are Associated with Unique Behavioral Profiles in Autism Spectrum Disorders

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Autism spectrum disorder (ASD) is a heterogeneous group of disorders which occurs with numerous medical conditions. In previous research, subtyping in ASD has been based mostly on cognitive ability and ASD symptom severity. The aim of the current study was to investigate whether specific medical conditions in ASD are associated with unique behavioral profiles. The medical conditions included in the study were macrocephaly, microcephaly, developmental regression, food selectivity, and sleep problems. The behavioral profile was composed of cognitive ability, adaptive skills, and autism severity, and was examined in each of the aforementioned medical conditions. The study population included 1224 participants, 1043 males and 181 females (M:F ratio = 5.8:1) with a mean age of 49.9 m (SD = 29.4) diagnosed with ASD using standardized tests. Groups with and without the specific medical conditions were compared on the behavioral measures. Developmental regression was present in 19% of the population and showed a more severe clinical presentation, with lower cognitive abilities, more severe ASD symptoms, and more impaired adaptive functioning. Microcephaly was observed in 6.3% of the population and was characterized by a lower cognitive ability and more impaired adaptive functioning in comparison to the normative head circumference (HC) group. Severe food selectivity was found in 9.8% and severe sleep problems in 5.1% of the ASD population. The food selectivity and sleep problem subgroups, both showed more severe autism symptoms only as described by the parents, but not per the professional assessment, and more impaired adaptive skills. Macrocephaly was observed in 7.9% of the ASD population and did not differ from the normative HC group in any of the examined behavioral measures. Based on these findings, two unique medical-behavioral subtypes in ASD that affect inherited traits of cognition and/or autism severity were suggested. The microcephaly phenotype occurred with more impaired cognition and the developmental regression phenotype with widespread, more severe impairments in cognition and autism severity. In contrast, severe food selectivity and sleep problems represent only comorbidities to ASD that affect functioning. Defining specific subgroups in ASD with a unique biological signature and specific behavioral phenotypes may help future genetic and neuroscience research.

Keywords: autism spectrum disorders (ASD), microcephaly, macrocephaly, developmental regression, food selectivity, sleep problems, cognition, autism severity

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INTRODUCTION

Autism spectrum disorder (ASD) is a heterogeneous group of disorders which, in addition to its core symptoms, occurs with numerous medical and behavioral comorbidities and conditions. Therefore, there is great variability in the clinical manifestations, which may suggest different neurobiological and genetic etiologies.

In previous research, subtyping in ASD has been based on a variety of factors. Several studies have used autism symptom severity as a basis for subtyping, including severity of social communication deficit and level of restricted and repetitive behaviors (RRB) (Ingram et al., 2008; Wiggins et al., 2012; Georgiades et al., 2013). Others have used verbal and non-verbal cognitive ability for subgrouping. Individuals with ASD with and without intellectual disabilities differed in symptom severity and later outcomes (Sheinkopf and Siegel, 1998; Lord et al., 2006; Ben-Itzchak et al., 2008, 2014; Grzadzinski et al., 2013). In addition, cognitive profiles based on the discrepancy between verbal and non-verbal skills have been identified (Joseph et al., 2002; Munson et al., 2008).

Several medical conditions have frequently been described as co-occurring with ASD, including: macro—and microcephaly, developmental regression, feeding and sleep problems (Coury et al., 2012; Ben-Itzchak et al., 2013a). Research so far has not addressed the question of whether these medical conditions occur in specific ASD subtypes. Medical phenotypes can potentially be used as biological variables to define specific endophenotypes in ASD.

Accelerated head growth in early childhood, resulting in a relatively enlarged head circumference, has been reported in numerous studies. An increased prevalence (14–34%) of macrocephaly, head circumference ≥97%, has been described in children with ASD (Sacco et al., 2007, 2010; Ben-Itzchak et al., 2013a; Grandgeorge et al., 2013). It is hypothesized that in children with ASD, the brain undergoes an abnormal growth trajectory that includes a period of early overgrowth. This theory is supported by neuroimaging and neuropathological findings (Courchesne et al., 2003, 2007; Mraz et al., 2007; Webb et al., 2007). Whether macrocephaly is associated with specific clinical presentation in ASD has been inconsistently reported in previous studies. Some have described a higher level of functioning in children with ASD and macrocephaly in comparison to those with normative head circumference (Aylward et al., 2002; Courchesne and Pierce, 2005; Sacco et al., 2007). Larger head circumference percentiles were found in children with ASD and special abilities, as compared to those without special abilities (Ben-Itzchak et al., 2013b). Other studies have found no correlation between head circumference and cognitive abilities (Gillberg and deSouza, 2002; Deutsch and Joseph, 2003; Ben-Itzchak and Zachor, 2007).

Although macrocephaly has been widely explored, microcephaly is less well researched in individuals with ASD. Previous studies have reported an increased prevalence of microcephaly between 5.9 and 15.1% (Fombonne et al., 1999; Miles et al., 2005; Ben-Itzchak et al., 2013a) in comparison to the 3% reported in the general population. This condition was more frequent among girls (Miles et al., 2005; Ben-Itzchak et al., 2013a) and among participants with intellectual disabilities (Fombonne et al., 1999).

A unique aspect of the developmental trajectory in ASD has been the occurrence of developmental regression, characterized by the loss of previously acquired skills. Studies have described an estimated prevalence of developmental regression of 15–30% in individuals with ASD (Baird et al., 2008; Parr et al., 2011; Ben-Itzchak et al., 2013a). Several studies reported that children with a history of developmental regression demonstrated more severe autism symptoms and more impaired cognition in comparison to population with ASD without a history of regression (Meilleur and Fombonne, 2009; Xi et al., 2010; Parr et al., 2011).

Food selectivity is one of the major eating problems described in ASD (Cermak et al., 2010). Previous studies on the prevalence of food selectivity in individuals with ASD have reported highly variable rates, ranging from 13 to 87% (Cornish, 1998; Klein and Nowak, 1999; Whiteley et al., 2000). Several studies have compared food selectivity in ASD populations to typically developing children (Schreck et al., 2004; Schmitt et al., 2008) and to children with developmental disabilities (Field et al., 2003; Williams et al., 2005; Dominick et al., 2007). Overall these studies found that children with ASD refused more foods and had less varied diets than did the other populations, both clinical and typically developing. This lack of variation in diet is often associated with an inadequate nutrition intake (Postorino et al., 2015). Food preference was affected by food texture (Dominick et al., 2007; Schmitt et al., 2008) and food presentation (Schreck and Williams, 2006). It has been suggested that food selectivity is affected by sensory processing problems, which are common in individuals with ASD (Williams et al., 2000; Kern et al., 2006). Food selectivity may also stem from tactile and oral hypersensitivities resulting from a more general problem in sensory modulation (reviewed in Marí-Bauset et al., 2014). The presence of food selectivity was associated with more severe autism symptoms based only on parental report, but not as observed by clinicians' assessments. ASD with food selectivity was associated with poorer non-verbal cognitive abilities, but no difference was found in adaptive skills in comparison to ASD subjects without food selectivity (Postorino et al., 2015).

Sleep problems are highly prevalent in ASD and are considered one of the most common co-morbid disorders (Couturier et al., 2005; Cotton and Richdale, 2006; Ming et al., 2008). Prevalence rates of sleep problems in ASD vary widely, ranging from 40 to 80% (Johnson et al., 2009) and are markedly higher than the 25–40% expected rate in the general population (Meltzer and Mindell, 2008; Reynolds and Malow, 2011). Chronic circadian rhythm sleep-wake cycle disturbances are common in ASD; however, when using a grading system to define the severity of the sleep problems, a lower rate of 13% was found for the ASD group, as compared with 5% for the typically developing populations (Krakowiak et al., 2008). Sleep serves many functions during early development, including brain growth, memory consolidation, and cognition (Stores and Wiggs, 1998). In the general population, sleep disturbances may impact the child's behavior, attention, cognition, and school performance (Gregory and Sadeh, 2012). Previous research has

reported that sleep problems are associated with more severe core autism symptoms in social communication and repetitive behaviors, and in maladaptive behaviors like self-injury tantrums and aggression (reviewed in: Cohen et al., 2014; Herrmann, 2015). Parent-reported autism severity has been found to be the strongest predictor of sleep problems in children with ASD (Mayes and Calhoun, 2009). Certain core features of ASD have been associated with specific sleep problems, suggesting that sleep problems are inherently a part of the ASD diagnosis (Hollway et al., 2013). Children with ASD and sleep problems have lower adaptive functioning (Sikora et al., 2012), and lower performance scores on cognitive memory tasks as compared to young adults with ASD who do not experience sleep problems (Limoges et al., 2013). However, other studies that have looked at the association between sleep disorders and cognitive and adaptive skills have found that the subjects' cognitive level and adaptive functioning did not predict the severity of their sleep problems (Richdale and Prior, 1995; Krakowiak et al., 2008).

The aim of the current study was to investigate whether specific medical conditions in ASD are associated with unique behavioral profiles. Medical conditions included in the study were macrocephaly, microcephaly, developmental regression, food selectivity, and sleep problems. The behavioral profile was composed of cognitive ability, adaptive skills, and autism severity, and was examined in each of the aforementioned medical conditions.

MATERIALS AND METHODS

Participants

Participants who underwent a comprehensive assessment and were diagnosed with ASD at a tertiary autism center between the years 2001–2016 were included. Inclusion criteria were: having a diagnosis of ASD, falling in the age range of 15 month–12:0 year, and not having any known genetic syndromes. The final population included 1224 participants, 1043 males and 181 females (M:F ratio = 5.8:1) with a mean age of 49.9 m (SD = 29.4).

Measures

The outcome measures for this study are described briefly below.

Autism Diagnostic Interview-Revised (ADI–R): A semi-structured interview administered to parents, designed to diagnose autism according to DSM-IV criteria (Rutter et al., 2003). For assessment of autism severity we used the ADI algorithm scores in social interaction, communication, and repetitive restricted behavior (RRB) domains. In the ADI-R scoring system, higher scores reflect more severe autism symptoms.

Autism Diagnostic Observation Scales (ADOS)–A semi-structured, interactive schedule designed to assess social and communicative functioning in individuals who may have an ASD. Only one of the modules was administered, depending on the examinee's age and/or expressive language (Lord et al., 1999). The ADOS total algorithm score was used for calculating the total severity score using the ADOS calibrated severity scales (CSS) (Gotham et al., 2009). The scores of each of the ADOS sub-domains, social affect (SA) and RRB, were used to calculate each sub-domain severity score using the new SA-CSS and RRB-CSS (Hus et al., 2014). In the ADOS scoring system, higher scores reflect more severe autism symptoms.

Vineland Adaptive Behavior Scales (VABS; Sparrow et al., 1984, 2005)–a standardized caregiver interview designed to assess adaptive behaviors in children from birth through 18 years of age. The VABS is organized into four sub-domains: Communication, Daily Living Skills, Socialization, and Motor Skills, each of which yield a standard score (mean of 100, SD of 15). In addition, the measure yields a total score, the Adaptive Behavior Composite (mean of 100, SD of 15). In the VABS, higher scores reflect better functioning.

Head circumference (HC): HC measurements were performed using standard methods (Deutsch and Farkas, 1994; Deutsch and Joseph, 2003) by a senior child neurologist and were plotted on normative head circumference growth charts and converted to percentile values (Nellhaus, 1968).

Developmental regression was based on the definition of loss within the ADI-R. The ADI-R requires that any loss be coded only if the skill was established initially for at least 3 months, and the loss of skill must have continued for at least 3 months. In this study, the occurrence of regression was explored using the coding of definite loss (score = 2) of specified skills in language, social engagement, constructive or imaginary play, or motor skills in the ADI-R.

Food selectivity: was defined according to parental reports during the medical evaluation and was graded as follows: 0 = eats normally, or has a few specific foods that he won't eat (especially fruits/vegetables); 1 = has more restrictions (will only eat certain food groups), fairly limited options; 2 = very limited options, and/or will not eat certain textures or certain colors. (For example, only eats white foods; only eats dry foods).

Sleep problems: were defined according to parental reports during the medical evaluation and were graded as follows: 0 = normal sleep behaviors, or occasional sleep disturbances; 1 = either wakes up regularly in the middle of the night (falls asleep again independently), or has difficulty falling asleep on a regular basis (at least 3 times per week); 2 = significant induction and sleep maintenance problems which interfere with family life and/or require medical treatment.

Procedure

The study was conducted at a specialized autism center within a tertiary medical center that provides diagnosis and treatment services and is involved in research in the field of ASD. The evaluation included a neurological assessment and behavioral and cognitive evaluations. Assessments were conducted by a skilled interdisciplinary team. Pediatric neurologists obtained medical, developmental and familial histories from the parents and conducted a comprehensive neurological examination of all the participants.

The diagnosis of ASD was obtained by using two standardized tests, the Autism Diagnosis Interview-Revised (ADI-R) (Rutter et al., 2003) and the Autism Diagnosis Observation Schedule (ADOS) (Lord et al., 1999), and by meeting criteria for ASD based

on DSM-IV (American Psychiatric Association, 2000) or DSM 5 (American Psychiatric Association, 2013) criteria, depending on the date of the evaluation. Data on ADI scores were available for 1194 participants and on ADOS severity scale scores for 1174 participants. All the professionals involved in the diagnostic process established reliability as required.

Cognitive and developmental abilities (IQ/DQ) were assessed using standardized cognitive assessments according to the child's age and language level. The following tests were used: The Mullen Scales of Early Learning (Mullen, 1995), Bayley Scales of Infant Development (Bayley, 1993); Wechsler Preschool and Primary Scale of Intelligence (Wechsler, 1989); Stanford-Binet Intelligence Scales (Thorndike et al., 1986); Kaufman Assessment Battery for Children-II (Kaufman and Kaufman, 1983); and Wechsler Intelligence Scale for Children IV (Wechsler, 2003). DQ/IQ scores were available for 758 participants.

Adaptive skills were assessed using the Vineland Adaptive Behavior Scales (VABS) (Sparrow et al., 1984) and were available for 937 participants.

The entire study population was classified according to the following medical phenotypes:

1. Head circumference (HC): HC data was available on 1194 participants. Three groups were defined based on the HC measurements: microcephaly HC≤3 percentile, macrocephaly HC≥97 percentile, and a normative HC group between the 25 and 75th percentiles.
2. Developmental regression: the group included participants with a history of definite loss of specified skills (ADI-R items 11 and/or 20 score = 2).
3. Food selectivity: the group included participants with major food selectivity (score = 2). Data was available on 1188 participants.
4. Sleep problems: the group included participants with significant sleep problems (score = 2). Data was available on 1224 participants.

This research was approved by the IRB at the Medical Center as required.

Data Analysis

The dependent variables in this study included the following behavioral measures: autism severity measures (ADI-R social interaction, communication and RRB subdomains scores, ADOS-CSS in SA and RRB subdomain scores); cognitive ability (DQ/IQ scores); adaptive behavior (VABS communication, DLS, socialization and motor skills subdomain scores). The independent variables included the specific medical phenotypes: microcephaly, macrocephaly, developmental regression, eating problems and sleeping problems. To investigate the differences between the groups with and without the examined medical phenotype, a series of one way MANOVAs (for the ADOS-CSS, ADI-R and VABS subdomains scores) and ANOVAs (for DQ/IQ scores) were performed. The microcephaly group and the macrocephaly group were compared to the normative HC group (measures between 25 and 75th percentiles). The macrocephaly group differed in age from the normative HC group, and therefore age was controlled for when using ANOVA and MANCOVAs for this independent variable. Head circumference is expressed in percentiles based on normative data in large populations. Therefore, it was possible to compare the frequencies of microcephaly and macrocephaly percentile measures in the study population to the expected percentage in the general population (3%), Chi Square goodness-of-fit tests were used.

RESULTS

For each of the medical conditions, the frequency of occurrence in the ASD population was examined. Then, the groups with and without the specific medical conditions were compared for their clinical presentation in autism severity, cognitive ability and adaptive skills.

The Macrocephaly condition: of the ASD population that had data on HC, 89 (7.9%) had macrocephaly, 77 males and 12 females (6.4:1). This rate is significantly higher than the expected 3% in the general population [$\chi^2_{(1)} = 126.8$, $p < 0.001$]. The macrocephaly group was significantly older than the normative HC group (**Table 1**) but did not differ significantly in their cognitive level (**Table 1**). The MANOVAs for the VABS scores [$F_{(4,\ 679)} = 1.6, p = 0.182, h^2 = 0.009$] ADOS-CSS [$F_{(2,\ 831)} = 0.4$, $p = 0.658$, $h^2 = 0.001$], and the ADI-R [$F_{(3,\ 857)} = 0.8$, $p = 0.764$, $h^2 = 0.001$] while controlling for age, did not yield a significant group effect.

Since the group with macrocephaly was significantly older than the group without macrocephaly, we examined the frequency of macrocephaly in 3 age ranges (15 month–2 year; 2:1–5 year; 5:1–12 year). A nonparametric analysis showed that the older the group, the higher the frequency of macrocephaly. The frequency of macrocephaly in the youngest age range was 6.7% (6 out of 218 participants), 7.3% in the middle age range (42 of 578 participants), and 12.2% in the oldest age range (41 of 337 participants) [$\chi^2_{(2)} = 16.8, p < 0.001$].

The microcephaly condition: in the research population, 77 participants (6.3%) had microcephaly, among them 64 males and 13 females (4.9:1). This rate was significantly higher than the expected 3% in the general population [$\chi^2_{(1)} = 46.0$, $p < 0.001$]. The MANOVA for the VABS scores [$F_{(4,\ 667)} = 4.6$, $p = 0.001$, $h^2 = 0.027$] yielded a significant group effect. The autism severity scores in both the ADOS-CSS [$F_{(2,\ 811)} = 0.3$, $p = 0.732$, $h^2 = 0.001$] and the ADI-R [$F_{(3,\ 836)} = 0.7$, $p = 0.519$, $h^2 = 0.003$] did not yield a significant group effect. As shown in **Table 2**, the microcephaly group had lower DQ/IQ scores and lower VABS subdomains scores (communication, DLS, socialization, and motor skills) than the normative HC group.

The developmental regression condition: in the research population, 230 participants (19.0%), including 186 males and 44 females (4.2:1), had a history of developmental regression. The MANOVAs for the VABS scores [$F_{(4,\ 924)} = 9.4$, $p < 0.001$, $h^2 = 0.014$], the ADOS-CSS [$F_{(2,\ 1160)} = 4.4$, $p = 0.012$, $h^2 = 0.007$] and the ADI-R [$F_{(3,\ 1189)} = 17.5$, $p < 0.000$, $h^2 = 0.042$] yielded significant group effects. As shown in **Table 3**, the group with a history of developmental regression had lower DQ/IQ and VABS scores in all examined subdomains

TABLE 1 | Mean and SD of cognitive scores, VABS scores and autism severity measures for the normative and macrocephaly groups.

	Normative HC		Macrocephaly		*F*	*p*	η^2
	n	*M(SD)*	*n*	*M(SD)*			
Age	781	49.1 (28.4)	89	65.2 (36.8)	24.0*	0.000	0.027
DQ/IQ	507	79.3 (21.7)	58	81.8 (24.9)	0.1	0.737	0.000
VABS	621		64				
Communication		80.3 (15.7)		78.9 (20.6)	1.1	0.282	0.002
DLS		76.6 (12.2)		73.8 (14.4)	3.1	0.078	0.005
Socialization		74.2 (10.9)		71.1 (13.2)	3.5	0.061	0.005
Motor skills		85.6 (13.8)		80.4 (15.1)	5.3**	0.022	0.008
ADOS-CSS	747		88				
SA		6.9 (2.2)		7.2 (2.2)	0.7	0.409	0.001
RRB		8.1 (1.7)		7.9 (2.2)	0.0	0.932	0.000
ADI-R	773		89				
Social interaction		14.2 (6.6)		14.2 (6.7)	0.2	0.659	0.000
Communication		10.4 (4.1)		11.1 (3.9)	1.1	0.303	0.001
RRB		4.9 (2.6)		5.3 (2.4)	0.0	0.871	0.000

$^{**}p < 0.01$, $^{*}p < 0.05$.

TABLE 2 | Mean and SD of cognitive scores, VABS scores and autism severity measures for the normative and the microcephaly groups.

	Normative HC		Microcephaly		*F*	*p*	η^2
	n	*M(SD)*	*n*	*M(SD)*			
Age	772	49.1 (28.8)	78	44.5 (24.9)	1.8	0.176	0.002
DQ/IQ	507	79.6 (22.0)	47	67.7 (17.1)	13.0***	0.000	0.023
VABS	612		61				
Communication		80.3 (15.8)		73.8 (16.2)	9.5**	0.002	0.014
DLS		76.8 (12.0)		71.4 (13.6)	10.8**	0.001	0.016
Socialization		74.0 (10.7)		70.6 (10.8)	5.6*	0.019	0.008
Motor skills		85.8 (13.8)		78.5 (13.8)	15.3***	0.000	0.022
ADOS-CSS	739		76				
SA		6.9 (2.2)		6.9 (2.1)	0.1	0.759	0.000
RRB		8.1 (1.8)		8.0 (1.4)	0.3	0.559	0.000
ADI-R	765		76				
Social interaction		14.5 (10.0)		15.5 (7.2)	0.7	0.402	0.001
Communication		10.5 (4.1)		10.3 (3.5)	0.2	0.667	0.000
RRB		4.9 (2.5)		5.1 (2.8)	0.6	0.420	0.001

$^{***}p < 0.001$, $^{**}p < 0.01$, $^{*}p < 0.05$.

(communication, DLS, socialization, motor skills). In addition the group had higher autism severity scores in both of the autism severity measures. The group with developmental regression had higher ADOS-CSS-SA scores and higher ADI-R scores in all the examined subdomains (social interaction, communication and RRB), compared to the group without a history of developmental regression.

The food selectivity condition: in the research population, 33.5% had food selectivity problems (score = 1 or 2). Of the ASD population, 116 participants (9.8%) had severe food selectivity (score = 2), including 98 males and 18 females (5.4:1). The MANOVAs for the VABS scores [$F_{(4,\ 912)} = 4.7$, $p = 0.001$, $h^2 = 0.020$] and the ADI-R [$F_{(3,\ 1162)} = 6.8$, $p < 0.000$, $h^2 = 0.017$] yielded significant group effects. The ADOS-CSS [$F_{(2,\ 1133)} = 1.1$, $p = 0.324$, $h^2 = 0.002$] did not yield a significant group effect. As shown in **Table 4**, the group with food selectivity had lower VABS scores in all the examined subdomains (communication, DLS, socialization, motor skills) as compared to the group without food selectivity. In addition, the food selectivity group had higher autism severity scores in all the ADI-R subdomains (social interaction, communication, RRB) than the group with no food selectivity. The DQ/IQ scores did not differ between these two groups.

The sleep problems condition: in the research population, 35% had sleep problems (scores = 1 or 2). Of the ASD population, 60 participants (5.1%) had severe sleep problems (score = 2),

TABLE 3 | Mean and SD of cognitive scores, VABS scores and autism severity measures for the groups with and without a history of developmental regression.

	Regression−		Regression+		*F*	*p*	η^2
	n	*M(SD)*	*n*	*M(SD)*			
Age							
DQ/IQ	624	80.0 (22.1)	128	73.2 (19.9)	10.6**	0.001	0.014
VABS	757		173				
Communication		80.0 (15.8)		73.5 (16.7)	23.9***	0.000	0.025
DLS		76.2 (12.3)		72.6 (13.8)	11.6**	0.001	0.012
Socialization		74.1 (10.7)		69.6 (12.0)	24.4***	0.000	0.026
Motor skills		84.9 (13.9)		84.3 (15.5)	0.3	0.595	0.000
ADOS-CSS	943		219				
SA		6.9 (2.2)		7.3 (2.1)	8.1**	0.004	0.007
RRB		8.1 (1.7)		8.3 (1.8)	2.9	0.89	0.002
ADI-R	967		227				
Social interaction		13.9 (6.5)		18.4 (15.4)	47.1***	0.000	0.038
Communication		10.3 (4.0)		11.6 (3.9)	20.9***	0.000	0.017
RRB		4.8 (2.5)		5.4 (2.4)	9.0**	0.003	0.007

****p < 0.001, **p < 0.01.*

TABLE 4 | Mean and SD of cognitive scores, VABS scores and autism severity measures for the groups with and without food selectivity.

	Food selectivity−		Food selectivity+		*F*	*p*	η^2
	n	*M(SD)*	*n*	*M(SD)*			
Age	1072	49.4 (29.0)	116	53.3 (33.5)	1.8	0.181	0.002
DQ/IQ	691	78.9 (21.8)	58	76.9 (21.8)	0.5	0.491	0.001
VABS	842		75				
Communication		79.6 (16.0)		73.4 (15.7)	10.4**	0.001	0.011
DLS		76.2 (12.7)		70.0 (11.6)	16.4***	0.000	0.018
Socialization		73.8 (11.1)		68.7 (9.8)	15.1***	0.000	0.016
Motor skills		85.2 (14.1)		80.3 (15.0)	8.1**	0.005	0.009
ADOS-CSS	1024		113				
SA		6.9 (2.2)		7.2 (2.2)	2.2	0.138	0.002
RRB		8.1 (1.7)		8.2 (1.7)	0.5	0.488	0.000
ADI-R	1052		115				
Social interaction		14.3 (9.3)		16.7 (6.5)	7.3**	0.007	0.006
Communication		10.3 (4.0)		11.7 (3.5)	12.6***	0.000	0.011
RRB		4.8 (2.5)		5.8 (2.5)	13.9***	0.000	0.012

****p < 0.001, **p < 0.01.*

including 49 males and 11 females (4.4:1). The MANOVAs for the VABS scores [$F_{(4,\ 908)} = 6.0$, $p < 0.001$, $h^2 = 0.026$] and the ADI-R [$F_{(3,\ 1153)} = 9.2$, $p < 0.000$, $h^2 = 0.023$] yielded significant group effects. The ADOS-CSS [$F_{(2,\ 1121)} = 0.8$, $p = 0.455$, $h^2 = 0.001$] did not yield a significant group effect. As shown in **Table 5**, the group with sleep problems had lower VABS scores in all the examined domains (communication, DLS, socialization, motor skills). In addition, the group with sleep problems had higher autism severity scores in all ADI-R subdomains (social interaction, communication, RRB) as compared to the group with no sleep problems. The DQ/IQ scores did not differ between the groups.

DISCUSSION

The current study investigated whether specific medical conditions in ASD are associated with unique behavioral profiles. Developmental regression was present in 19% of the population. The developmental regression subgroup showed a more severe clinical presentation in all the examined domains, with lower cognitive abilities, more severe autism symptoms in both the professional assessment (ADOS) and the parental descriptions (ADI-R), and more impaired adaptive functioning. Microcephaly (head circumference $\leq$3%) was observed in 6.3% of the study population. The subgroup with microcephaly was characterized

TABLE 5 | Mean and SD of cognitive scores, VABS scores and autism severity measures for the groups with and without sleep problems.

	Sleep problems−		Sleep problems+		*F*	*p*	η^2
	n	*M(SD)*	*n*	*M(SD)*			
Age	1116	49.9 (29.6)	60	49.6 (30.7)	0.0	0.941	0.000
DQ/IQ	709	78.9 (21.8)	36	76.6 (24.1)	0.4	0.545	0.000
VABS	862		52		6.0***	0.000	0.026
Communication		79.4 (16.0)		72.1 (15.2)	10.3**	0.001	0.011
DLS		76.1 (12.5)		69.4 (13.7)	13.6***	0.000	0.015
Socialization		73.8 (11.0)		66.6 (11.0)	21.2***	0.000	0.023
Motor skills		85.2 (13.9)		77.7 (16.6)	14.0***	0.000	0.015
ADOS-CSS	1067		58		0.8	0.455	0.001
SA		6.9 (2.2)		6.7 (2.2)	0.8	0.373	0.001
RRB		8.1 (1.7)		7.9 (1.7)	1.3	0.262	0.001
ADI-R	1099		59		9.2***	0.000	0.023
Social interaction		14.4 (9.1)		18.8 (6.8)	13.3***	0.000	0.011
Communication		10.4 (4.0)		11.6 (4.7)	5.2*	0.022	0.005
RRB		4.8 (2.5)		6.4 (2.8)	20.5***	0.000	0.017

$^{***}p < 0.001$, $^{**}p < 0.01$, $^{*}p < 0.05$.

by a lower cognitive ability and more impaired adaptive functioning. Food selectivity was found in 9.8% and severe sleep problems in 5.1% of the ASD population. The food selectivity and sleep problem subgroups showed more severe autism symptoms only as described by the parents (ADI-R), but not by the professional assessment (ADOS). In addition, the subgroup with severe sleep problems showed more impaired adaptive skills than the group without severe sleep problems. Macrocephaly was observed in 7.9% of the ASD population, which was significantly more than expected. A higher percentage of macrocephaly was found with increasing age. The macrocephaly subgroup did not differ from the normative head circumference group in any of the examined behavioral measures (cognition, autism severity and adaptive behavior).

These findings suggest the existence of three unique clinical subgroups (microcephaly, developmental regression, and sleep problems/food selectivity). All three subgroups showed poorer adaptive skills in comparison to the research population without these specific medical characteristics. However, the source of these adaptive impairments seems to stem from a different origin in each subgroup. The microcephaly and developmental regression subgroups seem to represent different medical-behavioral phenotypes, with a potential neurobiological origin that affects inherited traits. In the microcephaly phenotype, cognitive abilities were impaired, and seemed to be the basis for poorer adaptive skills. The developmental regression phenotype may stems from extensive neurodevelopmental insult, which results in global impairments in cognition and more severe autism symptoms. These insults may be the basis for the poor adaptive skills in this subgroup.

The food selectivity and sleep problems subgroup did not show different cognitive abilities, nor did it differ in observed ASD symptom severity in comparison to populations without these problems. In this subgroup, it seems that the medical problems directly affected the severity of the adaptive skills, as well as the parental perception of autism severity. Severe food selectivity and sleep problems seem to be comorbid symptoms in ASD that adversely affect the child's functioning, and lead to a harsher parental perception of adaptive skills and autism severity.

Macrocephaly is associated with an enlarged head circumference and has been well documented in many studies in ASD. Here, we found an overall high frequency of macrocephaly in the examined population, which increased with the age of the participants. These findings support the relationship between abnormal head growth and the occurrence of ASD, but macrocephaly is probably not related to a unique behavioral phenotype.

Regarding developmental regression, the 19% prevalence found in this study is within the range previously reported (15–30%) (Baird et al., 2008; Parr et al., 2011; Ben-Itzchak et al., 2013a). In agreement with our results, several studies have noted that poor cognitive and language outcomes are more likely in individuals with ASD who regress (Parr et al., 2011). Several reports have noted that ASD symptomatology is more severe in individuals who have regressed (Meilleur and Fombonne, 2009; Parr et al., 2011). The current study also identified more severe autism symptoms on standardized observation results (ADOS) and in parental reports (ADI-R). In contrast, several studies found no significant differences in ASD symptom severity between groups with and without a history of developmental regression (Malhi and Singhi, 2012; Kern et al., 2014).

A greater prevalence of microcephaly, a head circumference below the third percentile, has been documented in ASD (Miles et al., 2000, 2005; Ben-Itzchak et al., 2013a). The current study confirmed these findings by reporting twice the expected frequency of microcephaly, as well as a correlation between microcephaly and more pronounced cognitive impairment. Only one previous study has described similar results. Miles et al.

(2005) reported that a subgroup in ASD defined as 'complex autism' had microcephaly and/or significant dysmorphology, and showed a more impaired cognitive level than the 'essential autism' group. In general, the association between microcephaly and intellectual impairments has been well documented in the literature (Von der Hagen et al., 2014).

A second medical condition related to head circumference is macrocephaly. In agreement with previous studies, the current study found a higher overall prevalence of macrocephaly in ASD (7.9%), which is, however, lower than was previously reported (14–34%) (Sacco et al., 2007, 2010; Grandgeorge et al., 2013.) The current study's finding that the prevalence of macrocephaly increases with age provides a more nuanced perspective of this condition. This finding may be related to accelerated head growth in ASD during childhood, as previously described in the literature (Courchesne et al., 2003, 2007). In this study, macrocephaly was not associated with a specific behavioral phenotype, a finding which differs from some previous studies that described a higher level of functioning in children with ASD and macrocephaly in comparison to those with a normative head circumference (Aylward et al., 2002; Courchesne and Pierce, 2005; Sacco et al., 2007).

Food selectivity has been commonly described in ASD (13–87%). In this study, the prevalence of food selectivity was found in a third of the research population. Only one previous study has explored an association between food selectivity and distinctive clinical features in ASD (Postorino et al., 2015). In accordance with this study, we found that the presence of severe food selectivity was associated with more severe reported autism symptoms (ADI-R). However, the clinical assessments (ADOS) did not support differences in autism severity in populations with and without food selectivity. In the current research, adaptive skills were significantly impaired among children with severe food selectivity, unlike the findings in Postorino et al. (2015), which did not report on differences in adaptive skills.

Prevalence rates of sleep problems in ASD vary widely, ranging from 13 to 80% depending on the definition of the severity of sleep problems (Krakowiak et al., 2008; Johnson et al., 2009). In the current study, more than a third of the research population had sleep problems. Our findings confirm the results of several previous studies, which noted that sleep problems in ASD are associated with more severe autism symptoms as reported by parents (Mayes and Calhoun, 2009; Goldman et al., 2012; Park et al., 2012; Tudor et al., 2012). One study reported that ASD severity, as assessed by the ADOS, predicted sleep disturbances (Hollway and Aman, 2011). This was not confirmed in our study. In addition, several previous studies found that lower adaptive skills were more pronounced among children with ASD and sleep problems, which corresponds with the findings of the current study (Sikora et al., 2012; Taylor et al., 2012). Only a few studies have reported a positive association between intellectual disability and sleep problems (Hollway and Aman, 2011; Taylor et al., 2012). However, other studies have looked at the association between sleep disorders and cognitive and adaptive skills, and have found that the subjects' cognitive levels did not predict the severity of their sleep problems (Richdale and Prior, 1995; Krakowiak et al., 2008), which is similar to our findings. Several studies have reported findings similar to ours that subjects with ASD and sleep problems were associated with more impaired adaptive functioning (Hollway and Aman, 2011; Sikora et al., 2012; Taylor et al., 2012). Others did not find such an association (Richdale and Prior, 1995; Krakowiak et al., 2008).

Our findings suggest that the existence of severe food selectivity or severe sleep problems has a negative impact on the family life, and therefore parents perceive their children as having more severe autism symptoms and poorer functioning. It is worth noting that the direct and objective examination of cognitive ability and ASD severity by professional assessments is not affected by the presence of food selectivity or sleep problems. The difficulties encountered with feeding the child and a lack of sleep at night imposes a great burden on the family. The findings of this study strengthen the notion that the parental perception of their child's functioning is influenced by negative day-to-day experiences.

To summarize, the findings of this study point to the existence of two subtypes with specific biological markers that occur with inherited impairments. One subtype includes the microcephaly phenotype, which is accompanied by more impaired cognitive ability. The second subtype includes the developmental regression phenotype, which is accompanied by widespread, more severe impairments in cognition and autism severity. The medical food selectivity and sleep problems seem to be comorbid to ASD, and are associated with a more severe parental perception of the child's adaptive functioning and severity of autism symptoms. Macrocephaly was not associated with a specific phenotype and seems to be a feature of a subgroup with idiopathic ASD.

This study is innovative in its use of a comprehensive examination of different developmental domains in relation to specific medical problems to identify specific medical-behavioral subtypes. The study has several strengths. The ASD study population was large and well-characterized, with comprehensive medical backgrounds for the participants. Direct assessments of the participant and parental interview enabled us to receive different perspectives of the participants' characteristics. In addition, the definition of the medical problems was based on very stringent criteria.

One of the study's limitations was that not all the participants had all the examined variables. In addition, the background information provided and the severity of the food selectivity and sleep problems were based on parental reports and not on standardized measures.

The two medical-behavioral subtypes described in this study, the microcephaly and the developmental regression phenotypes, should be further investigated using advanced genetic, imaging, and neurobiological research. Future studies should explore other medical variables in relation to developmental and behavioral features of ASD. Defining specific subgroups with a unique biological signature that are associated with specific medical-behavioral phenotypes may help identify more homogenous groups within the heterogeneous ASD population.

AUTHOR CONTRIBUTIONS

The authors DZ and EB tasks include substantial contributions to the conception or design of the work; the acquisition, analysis, and interpretation of data for the work; drafting the work and revising it critically for important intellectual content; final approval of the version to be published, and agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Autism As a Disorder of High Intelligence

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A suite of recent studies has reported positive genetic correlations between autism risk and measures of mental ability. These findings indicate that alleles for autism overlap broadly with alleles for high intelligence, which appears paradoxical given that autism is characterized, overall, by below-average IQ. This paradox can be resolved under the hypothesis that autism etiology commonly involves enhanced, but imbalanced, components of intelligence. This hypothesis is supported by convergent evidence showing that autism and high IQ share a diverse set of convergent correlates, including large brain size, fast brain growth, increased sensory and visual-spatial abilities, enhanced synaptic functions, increased attentional focus, high socioeconomic status, more deliberative decision-making, profession and occupational interests in engineering and physical sciences, and high levels of positive assortative mating. These findings help to provide an evolutionary basis to understanding autism risk as underlain in part by dysregulation of intelligence, a core human-specific adaptation. In turn, integration of studies on intelligence with studies of autism should provide novel insights into the neurological and genetic causes of high mental abilities, with important implications for cognitive enhancement, artificial intelligence, the relationship of autism with schizophrenia, and the treatment of both autism and intellectual disability.

Keywords: intelligence, autism, schizophrenia, genetic correlation, pleiotropy, evolution

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INTRODUCTION

'How wonderful that we have met with a paradox. Now we have some hope of making progress.'
Niels Bohr

Autism is conventionally regarded as a neurodevelopmental disorder that involves deficits in social interaction and social communication, combined with restricted or repetitive patterns of behavior and interests. However useful this definition may be for practical purposes, it also represents a reified, more or less arbitrary, historical and societal construction that fits neither with Kanner's (1943) original description (Evans, 2013) nor with the standard medical model of disease whereby maladaptive phenotypes must be understood in terms of alteration to specific adaptive systems (Nesse and Stein, 2012; Crespi, 2016).

Autism may, alternatively, be regarded as a syndrome, a constellation of phenotypes, sets of which tend to be found together relatively often, or sets of which when found together cause particular problems for children, families, and communities (Happé et al., 2006). Any given individual "with" autism will exhibit some more or less unique collection of such phenotypes (e.g., van Os, 2009), which is due to their more or less unique genomic makeup and early developmental environment. By this simple logic, any diagnoses of autism should be regarded

not as any sort of endpoint, but as a rough, initial signpost toward eventual determination of the genetic, developmental, hormonal, neurological, psychological, and/or environmental causes of each individual's altered autism-related cognition, affect and behavior.

The finding that autism has many causes (Happé and Ronald, 2008) should direct attention to improved means of differentially diagnosing its personalized bases. This process, in turn, centers on determining what adaptive neural and psychological systems has been altered, and how, to result in some set of autistic traits in some individual. Autistic phenotypes have been linked, for example, to increased protein synthesis at synapses (Bourgeron, 2009), higher excitatory to inhibitory neurotransmission (Rubenstein and Merzenich, 2003), enhanced local compared to global processing and connectivity (Happé and Frith, 2006), a bias toward systemizing over empathizing (Baron-Cohen, 2009), and enhanced perceptual functioning (Mottron et al., 2006). These patterns and theories are not mutually exclusive, but none of them includes an explicit evolutionary dimension, such that their proximate causes remain ungrounded in the one discipline that unites across all biological-psychological levels in the context of ultimate, long-term determinants of psychological adaptation and maladaptation (Crespi, 2016).

Risks for autism, like risks of cancer, diabetes, or arthritis, have evolved along the human lineage (Crespi and Leach, 2016). As such, evolutionary biology becomes a valuable conceptual and analytic tool for connecting adaptive brain systems with the ways in which they can become altered and maladaptive in psychiatric disorders (Fjell et al., 2015). So what adaptive, evolved human brain systems are changed, in what different ways, to produce the phenotypes characteristic of autism? The most prominent neurological change along the human lineage is, of course, the tripling of brain size and associated changes in brain organization and functions, and our concomitant tremendous increase in intelligence compared to other great apes (Roth and Dicke, 2005). A notable set of "brain size genes" has been demonstrated to have been subject to natural selection in humans and other mammals (Montgomery and Mundy, 2014), and variation among extant humans in intelligence is now known to be highly polygenic, underlain by hundreds to thousands of alleles each of small effect (e. g., Davies et al., 2011; Benyamin et al., 2014; Plomin and Deary, 2015).

The genetical evolution of high intelligence in humans has increased scope for two main forms of dysregulation. First, the developmental and neural systems that connect genetic variation and environments with intelligence may be subject to maladaptive alterations by purely deleterious mutations, maladapted genotypes, or harmful environments, that degrade the "intelligence development" system. This route generally leads to what we call intellectual disability (Vissers et al., 2016), with overall reductions in intelligence and the functioning of its physiological and neural subsystems. Second, the development of intelligence can be affected, by genes or environments, in the opposite direction, toward higher levels of functioning. If this change results in balanced enhancements in all of the components of high intellect, general intelligence will be increased. However, if some, or most, but not all inter-dependent general cognitive-intellectual functions are enhanced, what would we observe?

Autism has long been characterized by relatively low intelligence as measured by most standard tests (e. g., Hoekstra et al., 2009). However, a suite of recent studies, described in more detail below, has demonstrated that alleles "for" autism, that is, common alleles that each contributes slightly to its risk, overlap substantially and significantly with alleles "for" high intelligence (Bulik-Sullivan et al., 2015; Clarke et al., 2015; Hill et al., 2015; Hagenaars et al., 2016). To a notable, and well-replicated, degree, then, many "autism" alleles are "high intelligence" alleles. How can these paradoxical observations be reconciled?

In this article I describe and evaluate the hypothesis that a substantial proportion of "autism risk" is underlain by high, but more or less imbalanced, components of intelligence. First, I provide a brief overview of the genetic, developmental and neurological bases and correlates of human intelligence, from research within this particular domain, and relate the structure of intelligence in neurotypical individuals to the differences between autistic individuals and neurotypical individuals. Second, I compare the best-validated correlates of variation among humans in intelligence with established characteristics of autistic individuals, compared to controls. These two areas of study, intelligence and autism, have thus far developed virtually independently from one another; I thus integrate and synthesize them in the context of testing the hypothesis addressed here. In doing so, I also compare results for autism with those for schizophrenia, the other primary human neurodevelopmental disorder, in light of theories for how these two conditions are related to one another (Crespi and Badcock, 2008; Crespi, 2016). Finally, I develop a framework for consilience of these findings with previous theory on autism, and describe the implications of the results, with regard to the causes, treatments, and understanding of autism, and the structure and study of human intelligence. The primary novelty and usefulness of this synthesis is that it provides the first comprehensive connections of the causes and symptoms of autism with alterations to a specific human-elaborated adaptive system, intelligence, and thereby generates new insights and research questions into the natures and inter-relationships of intelligence, autism, and schizophrenia.

THE ARCHITECTURE AND CORRELATES OF HUMAN INTELLIGENCE

Human intelligence has been studied predominantly from psychometric, genetic, neurological, and psychological perspectives. Psychometric studies tracing back to Spearman (1904) have demonstrated that virtually all measures of human mental abilities are moderately to highly positively correlated with one another, such that a common factor, typically called "*g*," underlies their joint co-variation. Between the general, primary *g* factor, and the diverse, specific measures of mental abilities, is a small set of secondary factors that each statistically accounts for covariation among a larger set of functionally-similar

abilities. The Verbal-Perceptual-Rotational (VPR) model of Johnson and Bouchard (2005a,b, 2007; **Figure 1**), with three such secondary factors, represents the currently best-supported psychometric description of human intelligence structure. Under this model, "Verbal" refers to verbal fluency and knowledge, "Perceptual" refers to perceptual speed and mechanical and spatial abilities other than mental rotation, and "Rotational" refers to mental rotation, which involves mental movements of imagined objects or persons, as in the classic Mental Rotation test from Vandenberg and Kruse (1978; Johnson and Bouchard, 2005b; Major et al., 2012). The components of the VPR model correspond on a broad scale with brain structural organization in that verbal skills are relatively left-hemispheric, spatial and non-verbal abilities are relatively right-hemispheric, and mental rotation depends, in part, on strongly bihemispheric functions associated with corpus callosum size (Johnson and Bouchard, 2005b; Karadi et al., 2006; Schoenemann, 2006).

In addition to its success in describing patterns of co-variation among aspects of human intelligence, the VPR model also demonstrates evidence of tradeoffs between sets of cognitive abilities: when controlling for variation in *g*, image rotation ability is inversely associated with verbal ability, and scores on tests indicative of a strong focus of attention are inversely associated with scores indicating diffuse focus (Johnson and Bouchard, 2007; **Figure 2**). These two tradeoffs also exhibit sex differences, with males over-represented at the high rotational-strong focus pole, and more females at the pole with higher verbal abilities and more-diffuse focus (Johnson and Bouchard, 2007). Such sex differences and negative correlations are important given the strong male biases found in autism (Fombonne, 2009; Baron-Cohen et al., 2011), the extensive data showing that autism involves reductions in verbal skills but (1) increases in focus of attention (e. g., Ploog, 2010; Sabatos-DeVito et al., 2016), (2) enhanced perceptual and spatial abilities [as reflected in prowess, for example, in Block Design and the Embedded Figures test (EFT); Mottron et al., 2006; Muth et al., 2014], and (3) superior ability in non-rotational (though not rotational) aspects of the mental rotation task (Zapf et al., 2015). Considered together, these findings provide evidence that the cognitive structure of autism dovetails with the structure of the independently derived best-supported model for the psychometric architecture of human intelligence, but that it is characterized by increases in some, specific, components of intelligence and decreases in others, leading to a profile that is imbalanced and reflects extremes of typical variation. As such, autism-related differences in VPR-model structured intelligence appear to reflect its evolutionary bases in altered expression of adaptive cognitive variation (Crespi, 2016), with autistic cognition mediated by

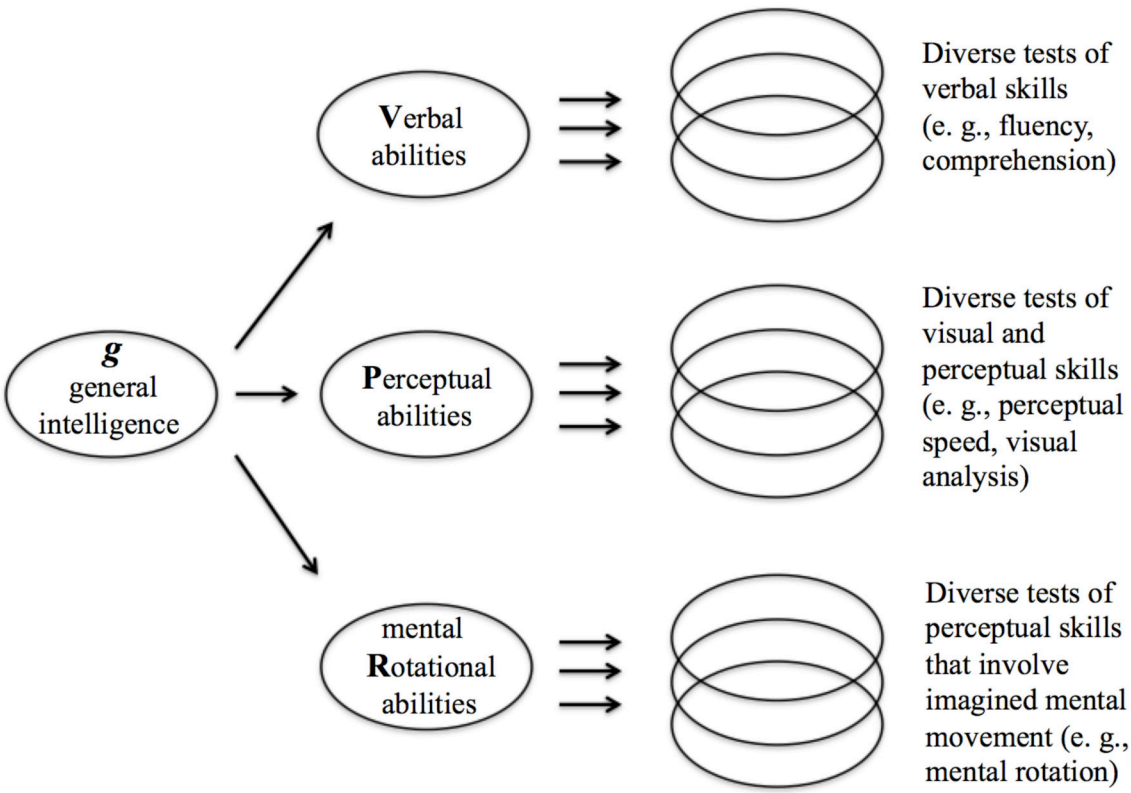

FIGURE 1 | The VPR model of intelligence. Under this model, the higher-level architecture of human intelligence, as indicated by diverse mental-ability tasks, involves one general factor, *g*, and three mid-level factors, Verbal, Perceptual, and image Rotation, that reflect variation among individuals in large-scale neural structure and processing. Adapted from Johnson et al. (2007).

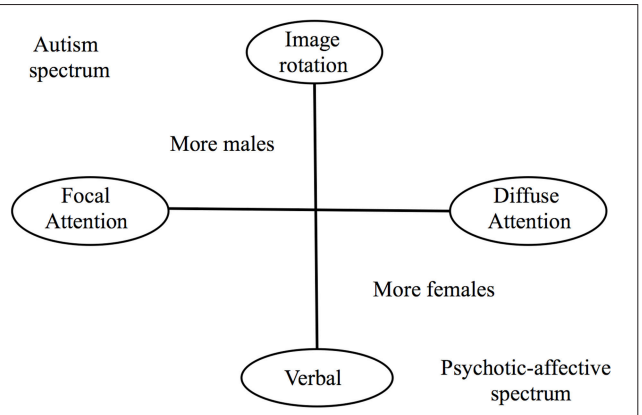

FIGURE 2 | Two orthogonal dimensions of intelligence, which emerge after the general factor *g* is statistically removed. Poles of each of the two dimensions demonstrate inverse associations with one another, indicative of neurally-based cognitive trade-offs. The psychotic-affective spectrum includes mainly schizophrenia, bipolar disorder, depression, and borderline personality disorder. Adapted from Johnson et al. (2007).

relative extremes of tradeoffs (Johnson and Bouchard, 2007, 2008). In contrast to autism, schizophrenia is characterized by a focus of attention decreased relative to controls (e.g., Morris et al., 2013), notably-poor visual-spatial relative to verbal abilities (Kravariti et al., 2006), and reduced ability in non-rotational (though not in rotational) aspects of mental rotation tasks (Thakkar and Park, 2010; Benson and Park, 2013). These patterns of findings derive directly from the VPR model, and indicate that its further application and extension will help to clarify the psychometric structure of autistic, neurotypical, and schizophrenia-associated cognition.

A second model for the higher-level psychometric structure of intelligence is its division into a "fluid" component, reflecting ability to solve novel problems, use logic, and identify patterns, in ways that are independent of acquired and cultural knowledge, and a "crystallized" component, indicative of ability to utilize acquired and learned knowledge and experience (e. g., Nisbett et al., 2012). This subdivision of intelligence fits less well than the VPR model to patterns of covariation across mental ability tests (Johnson and Bouchard, 2005a,b), but its validity and usefulness are supported by the underpinning of fluid vs. crystallized intelligence by distinct sets of genes (Christoforou et al., 2014), their different patterns of change with age (with fluid changing like physical traits, but crystallized showing little age-related decline; Deary et al., 2010), and the primacy of social learning in human cultural adaptation (Henrich, 2015). As for the VPR model, the structure of intelligence in autism reflects the fluid-crystallized dichotomy, in that fluid intelligence is relatively or absolutely enhanced in autism, but crystallized intelligence is reduced, compared to controls (Dawson et al., 2007; Hayashi et al., 2008; see also Nader et al., 2016). This pattern again indicates imbalance, and elevation, in components of intelligence in autism that correspond to its evolved and psychometrically characterized structure.

Intelligence, usually measured by strong correlates of *g*, has a clear polygenic basis, as well as established connections with neurological variation. Recent GWA studies have provided evidence that many hundreds or thousands of alleles, each of very small effect, underlie variation among individuals in *g*, although only a fraction of its high heritability can be accounted for at this point (Plomin and Deary, 2015). By contrast, data from studies of the genetic basis of intellectual disability show that it is due mainly to moderate or large effect *de novo* deleterious alleles, rather than a concentration of weakly-deleterious, small-effect, segregating alleles; these findings indicate that "high intelligence requires that everything work right, including most of the positive alleles and few of the negative alleles associated with intelligence" (Plomin and Deary, 2015, p. 103; Franić et al., 2015; Hill et al., 2015). Plomin and Deary (2015, p. 103) also point out an important question regarding such "positive genetics" of intelligence, for psychiatric disorders: if individuals at the positive end of the polygenic distribution of "risk" simply have low risk, or if they "have special powers." In this article, I am evaluating the hypothesis that such "special powers" indeed exist, in the contexts of autism and intelligence, and in comparison to schizophrenia. Finally, Plomin and Deary (2015) point out that assortative mating is notably stronger (∼0.40) for intelligence than for most other human traits, which maintains additive genetic variation for this trait as well as generating more "extreme" intelligence phenotypes than otherwise expected. Increased autism risk has been attributed by Baron-Cohen et al. (2006) to assortative mating between two individuals high in "systemizing," and assortative mating is much high among individuals diagnosed with ASD than other disorders (Nordsletten et al., 2016); how might intelligence variation play a role in this process and its sequelae?

As described in detail below, the neurological basis of intelligence has been well established for a suite of phenotypes, including large brain size and high numbers of neurons, large hippocampus size, high efficacy of working memory, fast neuronal processing speed, neural efficiency, fast rates of brain growth and pruning, and specific patterns of gray matter and white matter distributions. Most broadly, high intelligence appears to reflect high functionality, speed, and integration of a fronto-parietal brain network that subserves the sensory acquisition, abstraction, alternative model-testing, and deployment of information (the Parieto-Frontal Integration Theory; Jung and Haier, 2007; Colom et al., 2010; **Figure 3**). This "intelligence network" overlaps substantially with the "task-positive network," whose activation is inversely associated with that of the "default mode" or "task-negative" network (e.g., Uddin et al., 2009), as might be expected given that mental abilities and intelligence are measured in the context of particular tasks. The general and specific functioning of this distributed, fronto-parietal network are highly compatible with the VPR psychometric model described above (Deary et al., 2010), with genetic bases to VPR abilities and fronto-parietal structure and function (Johnson et al., 2007).

In addition to its substantial psychometric and genetic basis, and neurological underpinnings, intelligence is also notably associated with two additional factors that are relevant to the autism spectrum: sensory abilities and socioeconomic status. Positive correlations of *g* with sensory abilities in the auditory,

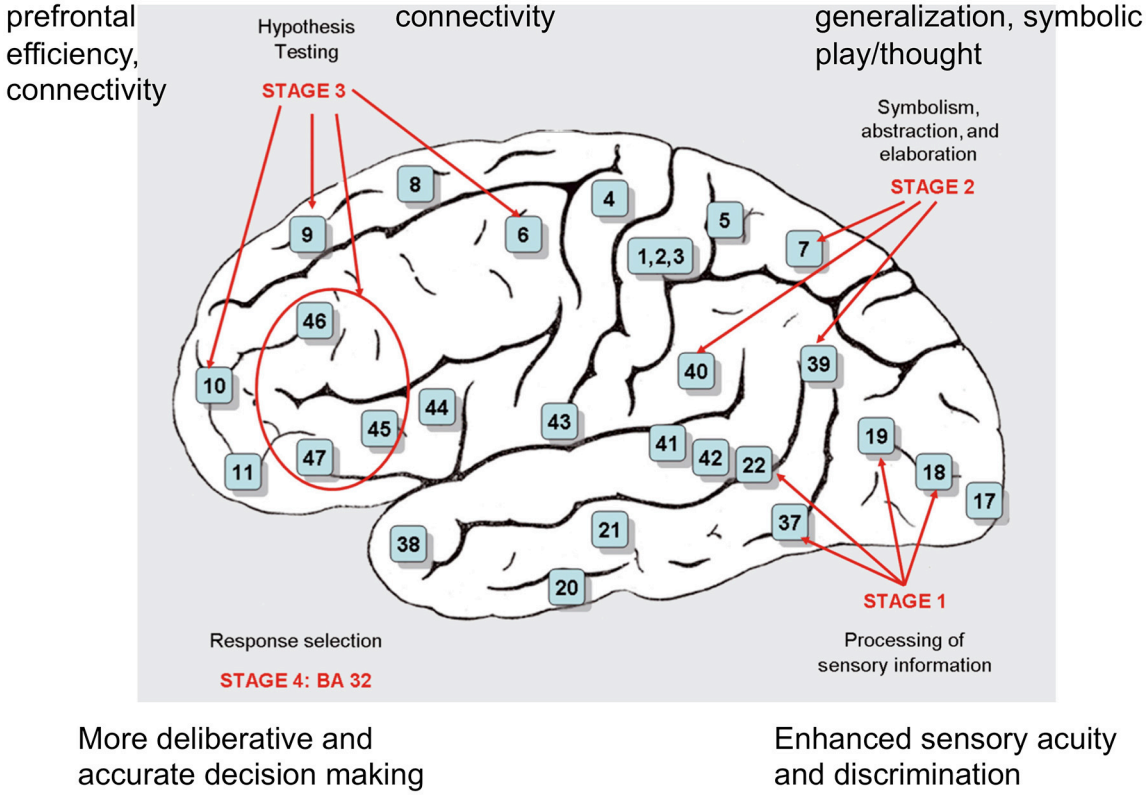

FIGURE 3 | Brain regions, and temporal stages, representing the P-FIT (Parietal Frontal Integration Theory) model of intelligence. Postulated alterations in autism, compared to control individuals, are shown along the periphery, and described in the text. Adapted from Colom et al. (2010).

visual, and tactile domains have been well documented and replicated, across over a century of testing (Deary et al., 2004). Such sensory tests include, for example, ability to discriminate closely-similar stimuli, temporal information processing skill, or sensitivity in stimulus detection, that apparently reflect aspects of processing speed, focal-attentional abilities, and ability to suppress irrelevant stimuli. As described further below, Galton (1883) and Spearman (1904, pp. 268–272) indeed virtually equated higher cognitive abilities with more-accurate sensory discrimination, and postulated "general discrimination" skills that underpinned sensory abilities across different modalities and correlated near unity with general intelligence. Genetically-based positive associations of intelligence with socioeconomic status, as well as with education levels and occupational status, have also been well documented (Marioni et al., 2014; Trzaskowski et al., 2014; Krapohl and Plomin, 2016; review in Plomin and Deary, 2015). These findings indicate that the same alleles pleiotropically mediate high intelligence and high socioeconomic status (vs. low levels of both), again through many alleles of small effect that account for a small, though statistically significant, proportion of the variance in both traits.

The upshot of these considerations from psychometrics, genetics, neuroscience, psychology, and sociology is that high intelligence involves many beneficial alleles of small effect, an absence of *de novo* deleterious mutations, high performance with balanced integration of neural subsystems (as well as large brain size and more neurons), enhanced sensory abilities, high socioeconomic status, and a notable degree of assortative mating. How, then, do these genetic and phenotypic correlates of intelligence, and associated ones, relate to the genetic basis and phenotypic correlates of autism?

GENETIC OVERLAP OF AUTISM WITH INTELLIGENCE

Recent increases in sample sizes, extensions of target phenotypes analyzed, and developments in analytic methods for genome-wide association studies, have allowed the first robust tests of the sign and magnitude of genetic correlations, due to pleiotropy and linkage disequilibrium, between intelligence and other traits including risk of psychiatric conditions. Four studies have used data from the Psychiatric Genetics Consortium (PGC) on polygenic risk for autism (Cross-Disorder Group of the Psychiatric Genomics Consortium, 2013) to assess overlap of autism risk alleles (identified from over 5000 cases) with alleles for aspects of cognitive ability and intelligence. All four of these studies, which used diverse, independent populations and tests

(or correlates) of cognitive abilities, have reported significant, substantial genetically-based positive associations of autism risk with intelligence, notably including full-scale IQ and a PCA-based measure of *g* (Clarke et al., 2015), childhood IQ, college attendance, and years of education (Bulik-Sullivan et al., 2015), cognitive function in childhood and educational attainment (Hill et al., 2015), and verbal-numerical reasoning and educational level reached (Hagenaars et al., 2016). These studies indicate that polygenic, small-effect size alleles that increased risk of autism are also associated with increased intelligence (and strong correlates of intelligence, such as education level; Davies et al., 2016) among neurotypical individuals.

In direct contrast to these findings for autism, genetic risk for schizophrenia has been demonstrated to be negatively associated with measures of cognitive ability and intelligence, across a broad suite of studies (McIntosh et al., 2013; Lencz et al., 2014; Bulik-Sullivan et al., 2015; Hill et al., 2015; Hagenaars et al., 2016; Hubbard et al., 2016). These results indicate that a substantial proportion of schizophrenia risk alleles also represent alleles "for" lower intelligence. Such findings for schizophrenia accord well with large bodies of research on cognitive deficits in first-degree relatives of individuals with schizophrenia (Snitz et al., 2006), and among children who are premorbid for schizophrenia (and thus will develop it at or after adolescence; Woodberry et al., 2008).

The question of whether intelligence, or any of its components, are elevated, compared to controls, among parents, siblings, or higher-order relatives of individuals with autism has yet to be investigated systematically, although this pattern is predicted by the positive genetic correlations of autism risk with measures of IQ. Tests conducted thus far have yielded diverse results, but individuals with autism clearly exhibit a similar cognitive profile to their sibs across WAIS subtests, with high scores on Block Design and Object Assembly, and low scores on Comprehension and Coding, relative to controls (Gizzonio et al., 2014). Similarly, siblings of individuals with autism have shown better working memory (for non-social targets) than control individuals (Noland et al., 2010) and higher visual-motion sensitivity (McCleery et al., 2007), and parents of individuals with autism are faster than controls at the EFT (Baron-Cohen and Hammer, 1997). Kanner (1943) and Rimland (1964, pp. 29–30) believed that parents of individuals with autism were of higher intelligence than control parents (see Levine and Olson, 1968), but their hypotheses have apparently not been subject to robust empirical testing, with most such studies of parents focusing on social deficits and alterations. By contrast, as described above, there is clear evidence for relatively low IQ, on average, among individuals with autism, at least as measured by most standardized tests.

How can this paradox of low IQ, but positive genetic correlations of autism risk with intelligence, be resolved? None of the papers on genetic correlations of autism with intelligence discuss possible explanations, or ways to investigate the conundrum further. I have proposed here the hypothesis that autism involves high but imbalanced intelligence, such that some or many genetically-based components of intelligence are enhanced, but imbalance across components increases risk and patterns of expression for autistic phenotypes, and for diagnoses. By this hypothesis, higher intelligence may co-occur with higher risk for imbalance and cognitive and affective consequences from it, given that the components of cognitive ability are expected to interact strongly and may do so to a greater degree at the higher end of ability. The "high intelligence imbalance" hypothesis is useful because it makes clear predictions, and thus directs attention toward specific forms of existing data and new, informative future data to collect.

AUTISM AND THE CORRELATES OF INTELLIGENCE

The "high intelligence imbalance" hypothesis predicts that autism should be associated, at a phenotypic level, with substantiated correlates of intelligence. I elaborate here on the most-notable joint correlates of intelligence and autism, focusing on phenotypes that are associated with intelligence and that are over-developed or over-expressed in autism.

Brain Size and Growth

Large brain size and head circumference, especially in childhood but also adulthood, represent some of the best-substantiated phenotypic correlates of autism (e.g., Fukumoto et al., 2011; Foster et al., 2015; meta-analysis in Sacco et al., 2015). Autism-linked increases in brain size have been shown to involve higher numbers of neurons (Courchesne et al., 2013), a thicker cortex (Hardan et al., 2006; Karama et al., 2011; Ecker et al., 2013; Smith et al., 2016), increased hippocampus volume (Barnea-Goraly et al., 2014; Maier et al., 2015), increased brain growth rates in early childhood (Campbell et al., 2014), increased rate of cortical thinning in adolescence (Hardan et al., 2009; Mak-Fan et al., 2012), a combination of "accelerated expansion in early childhood" with "accelerated thinning in in later childhood and adolescence" (Zielinski et al., 2014), and increased processing of more-local, detailed information (White et al., 2009).

Faster increase in cortical thickness between ages 6 and 12, followed by faster cortical thickness deceleration between ages 12 and 18 (indicative of neuronal and synaptic pruning), has been linked with higher intelligence in typically-developing children (Shaw et al., 2006). These findings provide evidence that trajectories of brain growth rate during middle childhood to adolescence are notably associated with IQ, with an overall pattern of accelerated growth and accelerated pruning that matches trajectories reported in autism, though with different timings of growth in early childhood. Within humans (e.g., Ivanovic et al., 2004; Witelson et al., 2006; Menary et al., 2013), and among non-human primates (Deaner et al., 2007) species, brain size (and cortical thickness, for humans) are also positively correlated with measures of intelligence, an effect that appears to be mediated predominantly by numbers of neurons (Roth and Dicke, 2005; Dicke and Roth, 2016).

In contrast to these patterns in autism, brain size, hippocampus size, and cortical thickness are reduced among individuals with schizophrenia, including at first episode (e.g., Steen et al., 2006; Rais et al., 2012; Rimol et al., 2012;

Oertel-Knöchel et al., 2013). These reductions appear to be associated with reduced brain growth rates in late childhood and early adolescence (Gogtay et al., 2008), followed by increased gray matter loss in adolescence and early adulthood (e.g., Pantelis et al., 2005; Rapoport and Gogtay, 2011).

The genetic bases of brain size and growth, and intelligence, in relation to autism and schizophrenia, remain largely unknown. However, as one example of such inter-relations, numbers of repeat units of a protein domain referred to as *DUF1220* have been positively associated with a suite of characters including: (1) brain size (within humans, and across species of anthropoid primates; Dumas et al., 2012; Keeney et al., 2014), (2) IQ and mathematical aptitude (Davis et al., 2015), and (3) the severity of autism (Davis et al., 2014); by contrast, *DUF1220* repeat numbers are inversely associated with positive-symptom (though not negative-symptom) severity in schizophrenia (Quick et al., 2015). These findings indicate strongly pleiotropic effects of this molecular domain on brain size, intelligence, autism, and schizophrenia, whereby autism is linked with large brain size and high IQ, but positive symptom schizophrenia shows a diametric result.

Brain Connectivity

Low global, relative to local, structural and functional connectivity of the brain has been demonstrated in autism by a large suite of studies (reviews in Courchesne and Pierce, 2005; Maximo et al., 2014), and it follows in part, presumably, from increased brain size itself. Under the P-FIT model of intelligence (**Figure 3**), effective long-range connectivity is required for the integration of parietal and frontal brain regions that underlies IQ (Jung and Haier, 2007); "efficient" patterns of brain connectivity involving small-world network-level organization (with an optimal mix of short and long-rage connections) have thus, for example, been positively associated with measures of intelligence (van den Heuvel et al., 2009; Koenis et al., 2015; Kim et al., 2016). These findings suggest that relative reductions in long-range connectivity may represent an important constraint on general intelligence among individuals with autism, contributing to imbalances between its constituent parts. Testing of this hypothesis will, however, require analyses of local and global brain connectivity patterns in relation to both autism (compared to controls) and variation in intelligence (in each group) using the same methodology, to determine the degree to which the long-range connectivity pathways central to the P-FIT model (e.g., the arcuate fasciculus, and connections to the lateral prefrontal cortex; Jung and Haier, 2007; Cole et al., 2012) are differentially reduced in efficiency among individuals with autism.

In contrast to reduced long-range brain connectivity, increased local connectivity has been linked with enhanced ability in some domains, such as auditory pitch perception (Loui et al., 2011), which shows a notable association with the autism spectrum (Stanutz et al., 2014). Increased local connectivity, especially of the prefrontal cortex, also characterizes the valproic acid animal model of autism (Rinaldi et al., 2008) and the "intense world" theory of autism etiology (Markram and Markram, 2010), which involves perception, attention, and memory that are enhanced to levels that interfere with social functioning.

Higher local connectivity in sensory regions of the brain has been suggested as the basis for sensory hyper-sensitivities in autism (Belmonte et al., 2004) as well as hyper-developed attention to detail and systemizing (Baron-Cohen et al., 2009). Considered together, these findings suggest that increased local brain connectivity in autism is linked with specific enhanced abilities or interests, such that components or facets of general intelligence are increased whereas *g* itself is reduced. These brain network alterations in autism can be described most simply as involving increased brain modularity and parallel processing, which may enhance region-specific functions (such as sensory abilities and visual-spatial skills) but also lead to reduced general intelligence due to under-developed long-range connectivities.

Decreased local and increased long-range connectivity have been described in childhood-onset schizophrenia (Baribeau and Anagnostou, 2013), and increased connectivity has also been found within the default mode in schizophrenia across multiple studies (Whitfield-Gabrieli et al., 2009; Tang et al., 2013), with several reviews pointing out the opposite nature of this pattern compared to that found in autism (Broyd et al., 2009; Karbasforoushan and Woodward, 2012). However, robust tests of this hypothesis require joint connectivity analysis of individuals with autism and schizophrenia using the same protocols.

Neuronal Function

Synaptic plasticity represents a core component of brain function, and, in principle, it underlies on a neuronal scale the long-term macroscopic changes in cortical thickness across childhood and adolescence that have been linked with intelligence (e.g., Shaw et al., 2006). Protein synthesis in dendritic spines mediates synaptic plasticity, and has been associated with diverse aspects of cognition, learning and memory (Sutton and Schuman, 2006; Kasai et al., 2010). Evidence of exaggerated protein synthesis at dendrites has been reported in human syndromic autism and in multiple animal models of autism (Kelleher and Bear, 2008; Bourgeron, 2009; Gkogkas et al., 2013; Santini et al., 2013; Santini and Klann, 2014; review in Mottron et al., 2014), indicating that gains of function or expression in key molecular mediators of cognition may characterize the autism spectrum (e.g., Figure 1 in Kulkarni and Firestein, 2012). Increased levels of neuronal plasticity and synaptic remodeling likewise characterize some theory for autism and animal models (Markram and Markram, 2010; Isshiki et al., 2014; Oberman and Pascual-Leone, 2014).

The degree to which neuronal functions such as synaptic plasticity, dendritic spine protein synthesis levels, and dendrite dynamics including flexibility and stability influence variation in general intelligence remains unclear. However, a recent GWA study found that the strongest functional enrichment for genes linked with fluid intelligence was synaptic "efficiency," whereas for crystallized intelligence it was synaptic depression and LTD (long-term depression; Christoforou et al., 2014). These findings suggest strong associations of neuronal and synaptic function with intelligence, such that autism may commonly involve dysregulation of intelligence-associated neuronal processes

toward hyper-functional dynamics. The gene *CYFIP1* represents an apparent example of a locus that mediates such effects: high expression of this gene, which coordinates mRNA translation at dendrites, has been associated with autism (Oguro-Ando et al., 2015; Wang et al., 2015); by contrast, deletions that reduce its expression have been strongly linked with risk of schizophrenia, as well as with aspects of impaired cognition (especially dyslexia and dyscalculia) among otherwise-neurotypical individuals (Stefansson et al., 2008, 2014; Tam et al., 2010).

Sensory Functions, Attention, and Special Abilities

As noted above, Galton (1883) and Spearman (1904) first described hypotheses and psychometric evidence that sensory abilities and sensory discrimination skills were strongly positively associated with high intelligence. A resurgence of interest in this phenomenon has led to consistent and diverse evidence for small to moderate links of specific sensory discrimination abilities with intelligence, but strong correlations (e.g., 0.68 and 0.92 in Deary et al., 2004) of intelligence with latent factors that integrate sensory ability variation across domains (Meyer et al., 2010). The causes of correlations between general intelligence and sensory discrimination abilities remain largely unknown, but they appear to be related to: (a) ability to focus intensely while ignoring irrelevant stimuli (Melnick et al., 2013); (b) a strong positive genetic correlation between intelligence and sensory-neural processing speed (Lee et al., 2012); (c) speed of neural oscillations, which may underlie both sensory discrimination skills and intelligence (Troche and Rammsayer, 2009a,b; Troche et al., 2009); (d) white matter structure and integrity, which are positively associated with neural processing speed (e.g., Turken et al., 2008; Kerchner et al., 2012); (e) regional or global increases in gray matter (e.g., Deary et al., 2010; Hyde et al., 2010), and (f) the role of sensory input as a limiting step in general cognitive ability, upon which all further neurological components of intelligence depend. Further evaluation of these hypotheses, through GWAS-based tests for genetic correlation and neurological studies that jointly address sensory discrimination and intelligence, are required to evaluate their robustness and generality.

A large body of evidence has shown that sensory discrimination and sensory acuity abilities are commonly enhanced in autism compared to controls, across auditory (O'Riordan and Passetti, 2006; Heaton et al., 2008; Eigsti and Fein, 2013; Stanutz et al., 2014), visual (Ashwin et al., 2009; Brosnan et al., 2012; Falter et al., 2012), and tactile (Blakemore et al., 2006; Cascio et al., 2008; Nakano et al., 2012) domains. As for the links with IQ, causation remains largely obscure. However, Blaser et al. (2014) demonstrate that the autism-associated advantage in visual search is associated with stronger phasic pupillary response, which is indicative of stronger attentional focus and implicates the locus coeruleus-norepinephrine system in (at least) visual discrimination proficiency. This finding is of especially notable interest given that autism is characterized, on a general diagnostic basis, by increased attention to detail, difficulties in switching of attention, and attentional stimulus "overselectivity" on specific aspects of the physical environment (Murray et al., 2005; Ploog, 2010). High attention to detail on the Autism Quotient test, high intelligence, and high rates of autism in family members have also been reported among child prodigies (children who display highly-advanced abilities in fields such as music, mathematics, chess, or art; Ruthsatz and Urbach, 2012). Baron-Cohen et al. (2009) describe evidence that such high attention to detail in autism is a consequence of enhanced sensory abilities, and also leads to high levels of an analytical, "systemizing" cognition. Finally, Sabatos-DeVito et al. (2016) describe experiments that link atypical sensory processing in autism to attentional engagement, suggesting that these two facets of autism share neurological and psychological links.

One visual-spatial test, the embedded-figures test (EFT), represents a paradigmatic task showing superiority in autism for speed, accuracy, or both (Happé and Frith, 2006; Muth et al., 2014; Horlin et al., 2016). This test has traditionally been considered as indicative of a local cognitive "style," but three sets of findings: (a) high positive correlations of EFT performance with measures of fluid intelligence (e, g., McKenna et al., 1986; McKenna, 1990), (b) demonstration by Khodadady and Tafaghodi (2013) that EFT performance is strongly, positively linked with intelligence, and (c) the visual-spatial search and discrimination nature of the task, suggest that it can also be interpreted as a metric of visual sensory ability and discrimination that is, like other such metrics, highly associated with intelligence, especially fluid intelligence that is often not matched in autism-control comparison studies. In contrast to the patterns of EFT enhancement found for autism, meta-analysis indicates that EFT performance is significantly and substantially reduced in schizophrenia (Panton et al., 2016). Indeed, more generally, sensory discrimination and abilities are consistently reduced in schizophrenia (Bates, 2005; Force et al., 2008; Javitt, 2009a,b), as expected under the hypothesis that they represent psychologically-diametric conditions (Crespi and Badcock, 2008; Crespi, 2016).

Considered together, these findings suggest that increased sensory discrimination ability in autism represents a component or strong correlate of intelligence that is frequently enhanced to the point of imbalance with other aspects of IQ. Indeed, under the P-FIT model (**Figure 3**), sensory abilities are represented by the first, sensory processing stage of intelligence circuitry: data acquisition and coding mainly via occipital and parietal regions of the brain. Hyper-functioning of these regions may thus result in imbalanced intelligence, whereby efficient integration with downstream regions, especially parietal regions that subserve symbolism, abstraction and categorization of sensory information, becomes dysregulated (e.g., Froehlich et al., 2012; Church et al., 2015; Eduardo Mercado et al., 2015; **Figure 3**). The gene *GABRB3*, which codes for a GABA receptor, represents a possible example of a locus that is pleiotropically linked to sensory sensitivity, autism risk, and components of intelligence, given that SNPs in this gene have been linked with tactile sensitivity, risk of Asperger syndrome, and scores on the embedded figures and mental rotation tests (Tavassoli et al., 2012; Warrier et al., 2013).

Finally, autism is the only psychiatric condition characterized by notable rates of savant skills, which in this context represent highly-structured, rule-based abilities largely restricted to a few spheres of mental ability: calendar calculating, rote memory, mathematical computation, musical memory, and realistic drawing (Howlin et al., 2009; Snyder, 2009; Treffert, 2014; Meilleur et al., 2015). Savantism appears to represent an extreme of imbalanced components of mental ability in autism, given its highly limited range of enhancements and apparent negative associations of special skills with verbal and social abilities (Crespi and Leach, 2016).

Decision-Making

Decision making, or "response selection," mediated by the anterior cingulate cortex, represents the fourth stage in the P-FIT model of intelligence (Jung and Haier, 2007; **Figure 3**). Autism has been characterized, in a recent suite of studies, by more "deliberative" decision making (compared to controls), that tends to reduce biases and errors associated with fast and intuitive, but often "irrational," decision-making (De Martino et al., 2008; Brosnan et al., 2014, 2016; South et al., 2014). Given that susceptibilities to cognitive biases are negatively associated with measures of intelligence, weakly though significantly (e.g., Teovanović et al., 2015), these findings suggest that this component of intelligence is enhanced in autism, at least in some contexts. More-deliberative decision making in autism may be associated, and underpinned, by enhanced explanatory drive to seek information in ambiguous circumstances, with regard to physical (rather than social) problems (Rutherford and Subiaul, 2015).

In contrast to these results for autism, some cognitive biases, such as "jumping to conclusions," and "bias against disconfirmatory evidence" are increased in schizophrenia compared to controls (Woodward et al., 2006; Dudley et al., 2016). Similarly, performance on the Iowa Gambling task of decision-making ability is reduced in schizophrenia (Sevy et al., 2007; Adida et al., 2011) but increased in autism (South et al., 2014), in each case compared to controls. Despite such findings, the degree to which more-deliberative or enhanced decision-making is more intelligent *per se*, and does not itself entail costs, remains unclear; for example, faster, more-intuitive decision-making may be favored in many social situations (South et al., 2014), and rationality appears to be at least partially dissociable from intelligence (Stanovich and West, 2014).

Socioeconomic Status

Socioeconomic status, intelligence, and education level achieved have been demonstrated to exhibit strong positive correlations amongst themselves, although the reasons for these associations have remained unspecified (Deary and Johnson, 2010). A recent suite of studies has shown that such links are substantially genetically based, indicating that a large number of alleles pleiotropically affect socioeconomic status, intelligence, and educational achievement (Marioni et al., 2014; Trzaskowski et al., 2014; Krapohl and Plomin, 2016). These findings are of interest in the context of autism because autism also shows significant, positive genetic correlations with educational attainment, which is strongly positively associated with socioeconomic status as well (Bulik-Sullivan et al., 2015; Hill et al., 2015; Hagenaars et al., 2016).

The hypothesis that autism risk in offspring is positively associated with high parental intelligence, and high socioeconomic status, traces to Kanner (1943, p. 248), who stated, referring to autistic children, that "they all come of highly intelligent families," at high levels of educational, socioeconomic and occupational achievement (Kanner and Lesser, 1958; Rimland, 1964). King (1975) reviewed a set of demographic studies motivated by these findings, and reported strong support for the pattern of high socioeconomic status linked with autism, including support from studies (e.g., Lotter, 1966, 1967) that checked all young children (of 8–10 years) in a given geographic area for infantile autism, and thus should be largely independent of confounding ascertainment or help-seeking biases, variation in access to relevant health care, or variation in parental awareness.

Recent studies of socioeconomic correlates of autism have generated variable results, most of which, however, find that autism is positively related to indicators or strong correlates of high socioeconomic status (Durkin et al., 2010; Van Meter et al., 2010; King and Bearman, 2011; Leonard et al., 2011; Thomas et al., 2012; Bakian et al., 2015); other studies report links with low socioeconomic conditions, or no associations (Rai et al., 2012; Sun et al., 2014). The degree to which biases and confounding factors mediate the positive associations remains largely unknown, although it is noteworthy that both mild to moderate intellectual disability, and schizophrenia, show notable patterns of association with measures of low socioeconomic status (Werner et al., 2007; Leonard et al., 2011; Emerson, 2012; Zheng et al., 2012).

Given that autism risk shows strong genetic correlations with intelligence and years of education, and that these two variables are strongly linked with higher socioeconomic status in a demographic context, the findings described above suggest that autism risk and high socioeconomic status are also expected to show a basis in pleiotropy as demonstrated by positive genetic correlation. However, this hypothesis requires direct genetic tests that take account of known confounding factors and inter-correlated traits (King and Bearman, 2011), as well as analyzing hypotheses for the causal underpinnings of the genetic and phenotypic associations.

Profession

Profession, occupation, and vocational interests have been associated with autism in the context of Baron-Cohen's theory that the autism spectrum is mediated by high "systemizing" (drive to understand non-social, mechanistic and rule-based systems) combined with low "empathizing" (drive to understand and connect with people, socially and emotionally; Baron-Cohen, 2009). Under this systemizing-empathizing theory, persons expressing high levels of autism spectrum psychological traits are predicted to engage in, or plan to enter, professions that involve systemizing, especially engineering and the physical, mathematical and technical sciences. This prediction of the theory has received considerable, though not fully unequivocal,

support (Baron-Cohen et al., 1997, 1998, 2007; Windham et al., 2009; Campbell and Wang, 2012; Roelfsema et al., 2012; Spek and Velderman, 2013; Wei et al., 2013). In contrast to these results, schizophrenia and mood disorders are associated with professions in the arts and humanities, across a diverse array of studies (Nettle, 2006; Kyaga et al., 2011; Campbell and Wang, 2012; Crespi et al., 2016).

Associations of autism with technical professions, in the context of autism's links with intelligence, raise the issue of whether intelligence, as measured by tests of IQ, varies in relation to profession. This controversial topic has been addressed in a suite of studies, all of which report that more-technical professions or occupational plans, especially in engineering, the physical sciences, and mathematics, are associated with relatively high IQs or strong correlates of IQ (Wolfle and Oxtoby, 1952; Hauser, 2002; Wai et al., 2009; Eysenck, 2012 p. xi). The psychological, sociological, and economic causes of these findings are ambiguous, but the results, taken at face value and in conjunction with the vocational correlates of autism, support the hypothesis that the autism spectrum is associated in some manner with relatively high intelligence.

In the context of Baron-Cohen's systemizing-empathizing hypothesis, these findings appear somewhat problematic, because systemizing, as measured by the Systemizing Quotient questionnaire, appears to be uncorrelated with IQ (Ling et al., 2009). Given the diversity of causes of autism, this condition may, however, certainly be associated with both systemizing and high, imbalanced intelligence, even if the two are not connected in simple or direct and causal ways.

Assortative Mating

Positive assortative mating, the mating between individuals who are relatively-similar for a given phenotype or genotype, results in a disproportionate concentration of the relevant alleles among offspring, an increase in additive genetic variance for the trait, and a concomitant rise in heritability (Plomin and Deary, 2015). Humans mate positively assortatively for a wide variety of phenotypes, with intelligence as one of the traits exhibiting the highest correlation between mates, on the order of 0.40–0.60 (Escorial and Martín-Buro, 2012; Plomin and Deary, 2015). To the extent that high intelligence potentiates imbalance in the components of intelligence due to either increases or reductions in its parts, strong assortative mating for intelligence is expected to intensify its effects.

Is there also assortative mating for autism or autism spectrum traits? Baron-Cohen et al. (2006) describe evidence of assortative mating between couples who are both high in the autism-associated psychological trait of systemizing. This hypothesis is supported, for example, by findings that both fathers and mothers of children with ASD exhibit elevated rates of systemizing-related occupations in their fathers (Baron-Cohen et al., 1997), as well as both showing high performance on the EFT (Baron-Cohen and Hammer, 1997). The most direct evidence for assortative mating for autistic phenotypes comes from Nordsletten et al. (2016), who reported much higher rates of assortative mating by psychiatric diagnosis for adults with ASDs (0.45–0.48), than for any other of a large set of disorders. To the extent that autism is genetically correlated with metrics of high intelligence (as described above), these findings indicate that humans mate positively assortatively not just for intelligence, but also for the autism-associated genetic underpinnings of intelligence. Genetic consequences for offspring would thus include both high intelligence and elevated risk of autism, provided that, under the intelligence-imbalance hypothesis addressed here, this process also involved dysregulation of one or more of its components. Further evaluation of this hypothesis would benefit from determining the degree to which positive assortative mating also occurs for autism-related cognitive phenotypes in non-clinical populations, data which would indicate the generality and strength of any such effects.

DISCUSSION

Risk and expression of autism is mediated by alterations to adaptive, evolved cognitive systems, and human intelligence represents one of the most important and pervasive changes along the human lineage and a principal source of cognitive variation among individuals. In this article, I have described the novel paradox that autism is positively genetically correlated with high intelligence, even though individuals with autism tend to have substantially lower IQs than controls. I then evaluated the idea that the paradox can be resolved under the hypothesis that autism involves high yet imbalanced intelligence, such that some or most components of intelligence are increased, but in such a way that overall performance is often reduced. This hypothesis extends previous studies of intelligence in relation to autism (e.g., Dawson et al., 2007; Hayashi et al., 2008; Nader et al., 2016) by providing the first comprehensive integration of the study of intelligence with the study of this condition, in the context of a novel "high and imbalanced intelligence" model that provides specific predictions and guidance for future work. The primary conclusions and implications from testing the hypothesis are four-fold.

First, the psychometric structure of human intelligence, as encompassed by the VPR model and the fluid/crystallized dichotomy, corresponds well with the differences in cognitive profiles between individual with autism and controls. Autism thus involves absolutely or relatively enhanced abilities in the Perceptual domain, but reduced or preserved Verbal and Rotation skills, and absolutely or relatively enhanced fluid intelligence, but reduced or preserved crystallized intelligence. Given that Perceptual domain tasks and tests quantify visual-spatial, sensory discrimination, mechanistic, scientific, and attentional abilities and motivations (Johnson and Deary, 2011), such enhancements are consistent with a large body of previous work on autism but can serve to unify and connect such skills with their neurological and genetic bases. The VPR model can also help to explain the male bias in autism as related to increased focus of attention, reduced verbal skills, and enhanced image rotation ability (or components thereof), given that these patterns emerge from the VPR model once the effects of *g* are controlled. Considered together, these results imply that although major aspects of intelligence

differ between individuals with autism and controls, the differences align with the evolved, neurologically-based axes of cognitive architecture that underlie human mental abilities. Finally, this model may help to frame hypotheses for the autism-related co-variation in perceptual abilities described by Meilleur et al. (2014), perhaps as a manifestation of the increased importance of this facet of intelligence in autistic cognition.

The main implications of these results are that they provide a non-arbitrary, well-validated context (the theory of intelligence) for the interpretation of differences between individuals with and without autism, and they should motivate novel and comprehensive integration of the study of intelligence with the study of autism. With regard to treatments for autism, such integration is useful because it indicates that imbalances in components of intelligence, and their neural underpinnings, may represent novel and malleable targets for individualized therapies that seek to increase the degree of balance, thereby reducing autism symptoms and enhancing everyday social and non-social functioning and well-being. In both phenotypic and genetic contexts, future studies of intelligence in autism might usefully focus on individuals with autism that is apparently mediated by polygenic (rather than monogenic, oligogenic, or syndromic) effects, given that only such causes of autism are expected to be directly relevant to its positive genetic correlation with intelligence. Do such individuals have "too many," or a biased neurological-associated set, of alleles for high intelligence? What developmental and molecular pathways are affected by such sets of genes, and can they also serve as foci for therapies?

Second, a broad swath of correlates of autism, including large brain size, fast brain growth, increased sensory and visual-spatial abilities, enhanced synaptic functions, increased attentional focus, high socioeconomic status, more deliberative decision-making, profession and occupational interests in engineering and physical sciences, and high levels of positive assortative mating, also represent strong correlates of intelligence (**Figure 4**). These findings broadly support the high, imbalanced intelligence hypothesis, although targeted tests are required for more-robust evaluation. Future studies can usefully focus on how these joint correlates of autism and intelligence are related to one another, especially across levels from genes to neurobiology and psychological traits.

Third, the theory and results described here are largely consilient with three of the major psychological theories of autism, systemizing-empathizing bias (Baron-Cohen, 2009), enhanced perceptual function (Mottron et al., 2006), and the intense world (Markram and Markram, 2010), although they ground the patterns supporting each theory in a specific domain of human adaptation, intelligence. This compatibility of theories need not imply that autism has one or few specific causes at the genetic, neurological, and psychological levels—it has many—but it focuses attention on what information will be most useful to collect, to differentially diagnose the causes of autism for each specific individual. What alleles are related to what components of intelligence under the VPR model, and what is their overlap with alleles underlying different phenotypes

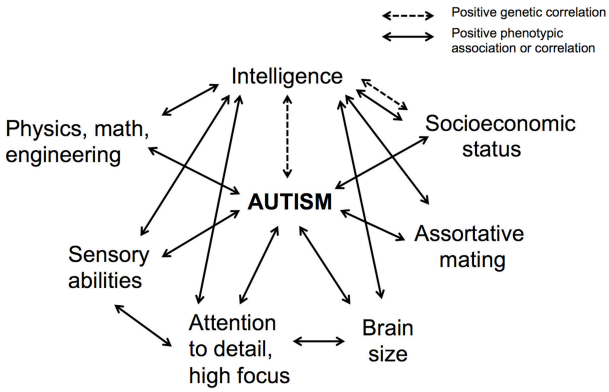

FIGURE 4 | Autism and intelligence are genetically correlated with one another, indicative of a shared genetic basis, and they share phenotypic correlations or associations with a broad suite of traits. These patterns suggest that autism risk is mediated in part by high, but imbalanced, components of intelligence.

found in autism? What neurological processes underlie negative associations between focal and diffuse attention, and verbal vs. rotational abilities (Johnson et al., 2008), and how do they relate to neurological differences among individuals with autism? More generally, what developmental and neurological components of intelligence are altered in autism, and how? And how can the genes, neurodevelopment, and psychology of each individual with autism, or subsets of individuals, be fit within these frameworks?

Fourth, comparisons of autism with schizophrenia for the genetic and phenotypic correlates of intelligence described here support the hypothesis that these two sets of conditions can be regarded as psychiatric, psychological, neurological and genetic "opposites," especially as evidenced by consistent negative genetic correlations of schizophrenia risk with measures of intelligence. Jung (2014) indeed contrasts intelligence and the autism spectrum as diametric to creativity and the schizophrenia spectrum (see also Figure 1 in Crespi et al., 2016; Krapohl and Plomin, 2016), as two major, inversely-associated domains of human cognition. Inverse associations of intelligence with personality correlates of imagination (Openness and apophenia, defined as seeing pattern where none exists) are also supported by factor-analytic studies of personality structure, and by studies that relate working memory, white matter tract integrity, and dopaminergic neurotransmission to both intelligence and imagination (DeYoung et al., 2012). To the extent that autism represents most broadly a disorder of high intelligence (and low imagination), and schizophrenia a disorder of high imagination (and low intelligence), studying these psychiatric conditions will also provide novel insights into variation among neurotypical individuals, and human cognitive architecture, at their largest and smallest scales, with important implications for such fields as artificial intelligence and cognitive enhancement (e.g., Minzenberg et al., 2008; Blaser et al., 2014).

The primary limitations of the hypotheses and predictions evaluated here are that intelligence, as measured in most

standardized tests, does not quantify aspects of social and emotional phenotypes that are also highly relevant to disorders such as autism and schizophrenia. Moreover, some key questions remain unresolved, such as how and why especially-high intelligence in one domain would tend to reduce intelligence test scores overall, how and why systemizing and empathizing are related to intelligence and its components (as well as genetic underpinnings), and how autism risk is mediated by polygenic effects, many of which apparently involve alleles for high intelligence, as well as by monogenic or oligogenic effects, which are expected to be deleterious and cause dysfunctions. Addressing these and other questions will require integration of data from evolutionary biology, genetics, the study of intelligence, and autism, and testing of hypotheses that involves spanning across these levels of analysis and theory.

AUTHOR CONTRIBUTIONS

BC conceived and wrote the paper.

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REVIEW
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Common Genetic Variants Found in HLA and KIR Immune Genes in Autism Spectrum Disorder

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The "common variant—common disease" hypothesis was proposed to explain diseases with strong inheritance. This model suggests that a genetic disease is the result of the combination of several common genetic variants. Common genetic variants are described as a 5% frequency differential between diseased vs. matched control populations. This theory was recently supported by an epidemiology paper stating that about 50% of genetic risk for autism resides in common variants. However, rare variants, rather than common variants, have been found in numerous genome wide genetic studies and many have concluded that the "common variant—common disease" hypothesis is incorrect. One interpretation is that rare variants are major contributors to genetic diseases and autism involves the interaction of many rare variants, especially in the brain. It is obvious there is much yet to be learned about autism genetics. Evidence has been mounting over the years indicating immune involvement in autism, particularly the HLA genes on chromosome 6 and KIR genes on chromosome 19. These two large multigene complexes have important immune functions and have been shown to interact to eliminate unwanted virally infected and malignant cells. HLA proteins have important functions in antigen presentation in adaptive immunity and specific epitopes on HLA class I proteins act as cognate ligands for KIR receptors in innate immunity. Data suggests that HLA alleles and KIR activating genes/haplotypes are common variants in different autism populations. For example, class I allele (HLA-A2 and HLA-G 14 bp-indel) frequencies are significantly increased by more than 5% over control populations (**Table 2**). The HLA-DR4 Class II and shared epitope frequencies are significantly above the control populations (**Table 2**). Three activating KIR genes: 3DS1, 2DS1, and 2DS2 have increased frequencies of 15, 22, and 14% in autism populations, respectively. There is a 6% increase in total activating KIR genes in autism over control subjects. And, more importantly there is a 12% increase in activating KIR genes and their cognate HLA alleles over control populations (Torres et al., 2012a). These data suggest the interaction of HLA ligand/KIR receptor pairs encoded on two different chromosomes is more significant as a ligand/receptor complex than separately in autism.

Keywords: autism, HLA, KIR, haplotype, common genetic variant

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INTRODUCTION

Understanding genetic contribution in autism spectrum disorder (ASD) is a major challenge due to genetic complexities of a multifaceted disease. ASD is a puzzling neurodevelopmental condition characterized behaviorally by deficits in social-communication and the presences of restrictive stereotyped behaviors with involvement of genetic, environmental, and neurological components. Although, multiple etiologies are purported to exist, several studies have noted a strong familial clustering suggesting that heredity is of major importance (Bolton et al., 1994; Bailey et al., 1995; Constantino et al., 2013).

The study of human genetics has advanced rapidly over the last 30 years and researchers have adopted new and better methods in an attempt to unravel the extraordinary genomic complexity of autism. An early attempt to find DNA regions that associate with ASD involved the examination of several hundred microsatellites (also known as short tandem repeats) across the genome (Lamb et al., 2000). It became apparent early on that this was not answering questions about autism genetics and this approach was largely abandoned. A significant flaw in these early studies involved the use of siblings as control populations (Philippe et al., 1999). It is now thought that siblings of subjects with autism are unsuitable controls as they have many of the same risk polymorphisms as their autistic siblings. The next attempt involved the studies of purported autism candidate genes. This research typically involved the examination of single nucleotide polymorphisms (SNPs) between subjects and controls in a candidate gene thought to be involved in the etiology of autism. Although a few candidate genes were identified with apparent association to autism, overall the studies hypothesizing the involvement of purported candidate genes were often contradictory and proved to be unreliable (State, 2010).

Genome wide association studies (GWAS) that examine over half a million SNPs have been a little more successful in identifying rare genetic variants (rare variants) in a small number of ASD cases; however, common genetic variants (common variants) have not clearly been identified in GWAS studies (Goldstein, 2009; Manolio et al., 2009; Tao et al., 2016). One interpretation is that rare variants are major contributors to genetic diseases (McClellan and King, 2010) and that ASD involves the interaction of many rare variants, especially in the brain (Irimia et al., 2014; Krystal and State, 2014). The newest research tool involves whole genome sequencing (WGS) to determine the DNA sequence of an individual's entire genome. On the surface this sounds all encompassing; however, weaknesses of this approach besides cost include distorted amplification of certain DNA targets and the difficulty in aligning SNPs to the proper chromosome (Laver et al., 2016).

Several studies have noted a strong familial clustering suggesting that heredity of risk-associated genes is about 50–60% and that about 50% of this risk resides in common variants (Gaugler et al., 2014). The "common variant-common disease" hypothesis was proposed nearly a 100 years ago to explain common diseases with a strong genetic association (Fisher, 1918). This model suggests that each common risk variant (greater or less than 5% of the control population) confers a small degree of risk and that a genetic disease is the result of the combination of several common variants. Common variants (low penetrance) are genetically old and found in multiple populations, whereas rare variants (high penetrance) are new and found in specific populations (Schork et al., 2009). The examination of whole-genome sequence data of autism quartet families (two autism affected siblings and parents) suggested that the majority (69%) of autism affected siblings carried different rare variant mutations (Yuen et al., 2015), supporting the idea that these rare variants are not established in the studied population. Over the years, much attention has been given to discovering the genetic underpinnings of ASD; however, immune abnormalities have consistently been found in this disorder, and recently the number and impact of immune studies has reached a crescendo, including in the area of immunogenetics (Westover et al., 2011; Sweeten and McDougle, 2016; Young et al., 2016).

BASIC IMMUNE SYSTEM

Immunology is the study of a system of molecules, tissue and cells that recognize and attack foreign invaders and abnormal cells that endanger the individual. The entire set of immune genes that contribute to immune function is now referred to as the "Immunome" consisting of about 900 genes across various chromosomes that encode a variety of different proteins to accomplish immune surveillance (Ortutay and Vihinen, 2009).

A major component of the immune system involves genes in the major histocompatibility complex (MHC) on chromosome 6 in humans. The MHC encodes human leukocyte antigen (HLA) proteins and the terms HLA and MHC are often used interchangeably. The human immune system has two main components, the innate immune system and the adaptive immune system. Both systems have humoral and cellular components (**Figure 1**), but innate immunity is generally considered to be non-specific whereas humoral immunity provides a specific response to pathogens and foreign antigens. Innate immunity is germ-line encoded, does not adapt after exposure to antigens, and is constitutively expressed leading to an immediate and rapid response to antigens. This is the first line of defense against pathogens, utilizing complement activation and other mechanisms to destroy most microorganisms, typically within hours of exposure.

The classical class I HLA proteins, expressed on essentially all nucleated cells of the body, serve as transducers by presenting antigens to T-cell and B-cell receptors, thereby activating the adaptive immune system for cellular cytotoxicity and antibody production, respectively. The adaptive HLA class II genes produce proteins expressed by specialized immune cells to present antigens to T-cells. HLA-class II genes can recombine by somatic mutation allowing for broad antigen adaptation. HLA-class I and II alleles are some of the most genetically variable coding loci in mammals.

INNATE IMMUNITY COMPONENTS

The innate system has soluble complement proteins that help or "complement" the clearance of pathogens in various

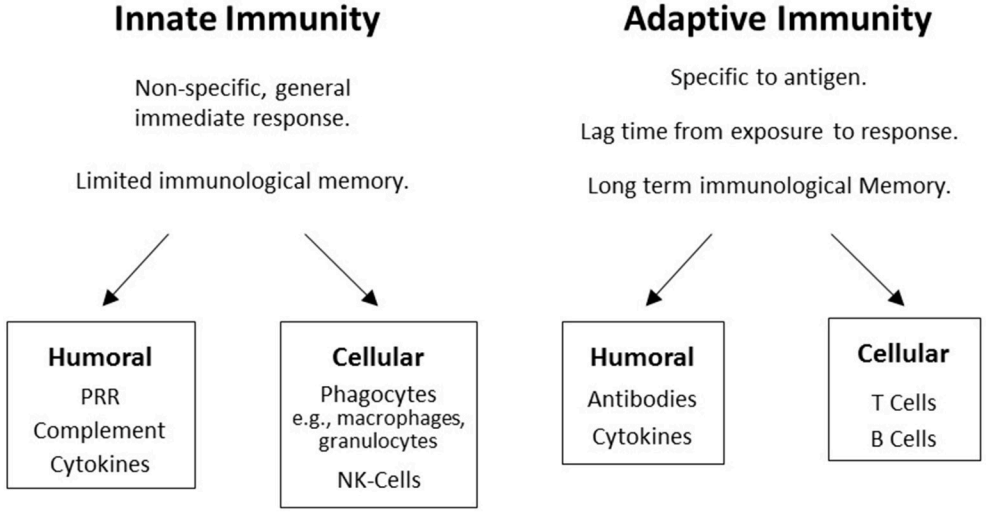

FIGURE 1 | The two branches of the immune system: Boxes display humoral and cellular components in innate and adaptive immunity.

ways, including acting as an opsonin and by forming a membrane attack complex to kill cells. The complement system contains over 25 proteins and protein fragments that are mainly synthesized in the liver, but also exist in the brain. The innate cellular components include a variety of cell surface and cytoplasmic pattern recognition receptors (PRR) expressed by granulocytes, macrophages, and dendritic cells which bind to pathogen-associated molecular patterns (PAMPs) associated with components of microorganisms. An innate cellular system important in removing aberrant cells (virally infected and transformed) involves the interaction of HLA cognate receptors with killer-cell immunoglobulin-like receptors (KIRs) on NK-cells.

ADAPTIVE IMMUNITY COMPONENTS

HLA proteins are important in presenting antigen to CD4+ (helper T-cells) and CD8+ (cytotoxic T-cells) and the extraordinary diversity in HLA alleles allows for exquisitely sensitive antigen recognition. Adaptive immune responses are slower than those elicited by the innate immune system; however, long term immunological memory is invoked. Memory involves the recognition of antigens from invading organism for extended periods of time, often decades. This is accomplished by the creation of tailor-made memory cells. Antibodies are the main soluble component of the adaptive system; however, cytokines are also important. Memory cells will continue to survive in the body for decades then re-exposure to the antigen activate them to produce the respective antibodies in unlimited numbers. This production of numerous antibodies is accomplished by gene recombination in B-cells followed by a complicated cellular selection process which results in antibodies that are very specific to the foreign antigens. The adaptive immune system is comprised of the cellular components T and B-cells. T-cells have cell-surface receptors that recognize peptides bound to HLA class I and class II proteins, a critical step in cell-mediated immunity.

The cellular components of the adaptive immune system involve T and B-cells. T-cells are important in cell-mediated immunity and are distinguished by the T-cell receptors which recognize short peptides bound to HLA class I and class II proteins. Several subsets of T-cells with distinct functions exist. For example, cytotoxic T-cells seek and destroy virally infected cells and tumor cells while helper T-cells activate macrophages and assist B-cells to mature into antibody producing plasma cells and memory B-cells.

IMMUNITY AND AUTISM

One of the earliest immune associations observed in ASD was by Stubbs et al. (1985) when they noted that pairs of ASD affected children share HLA haplotypes more often than non-affected pairs. The HLA complex is of major interest in medical research as genes/proteins in this region are involved in many immune processes such as organ transplantation, autoimmunity, resistance to specific pathogens, inflammation, ligands for immune cell receptors, and the complement cascade. There is now considerable evidence from association and linkage studies that suggests the involvement of HLA alleles/genes/haplotypes in the etiology of ASD (Torres et al., 2006, 2012a).

HLA GENE COMPLEX

The HLA complex is fascinating as there are about 200 genes that encode proteins with various functions including ligands, receptors, cytokines, signaling factors, heat shock proteins, transcription regulators, and most importantly the recognition of self from non-self. The HLA gene map in **Figure 2** (Shiina et al., 2009) illustrates this complexity. The entire 200 HLA gene region is inherited as a single 4.5 million base pair extended

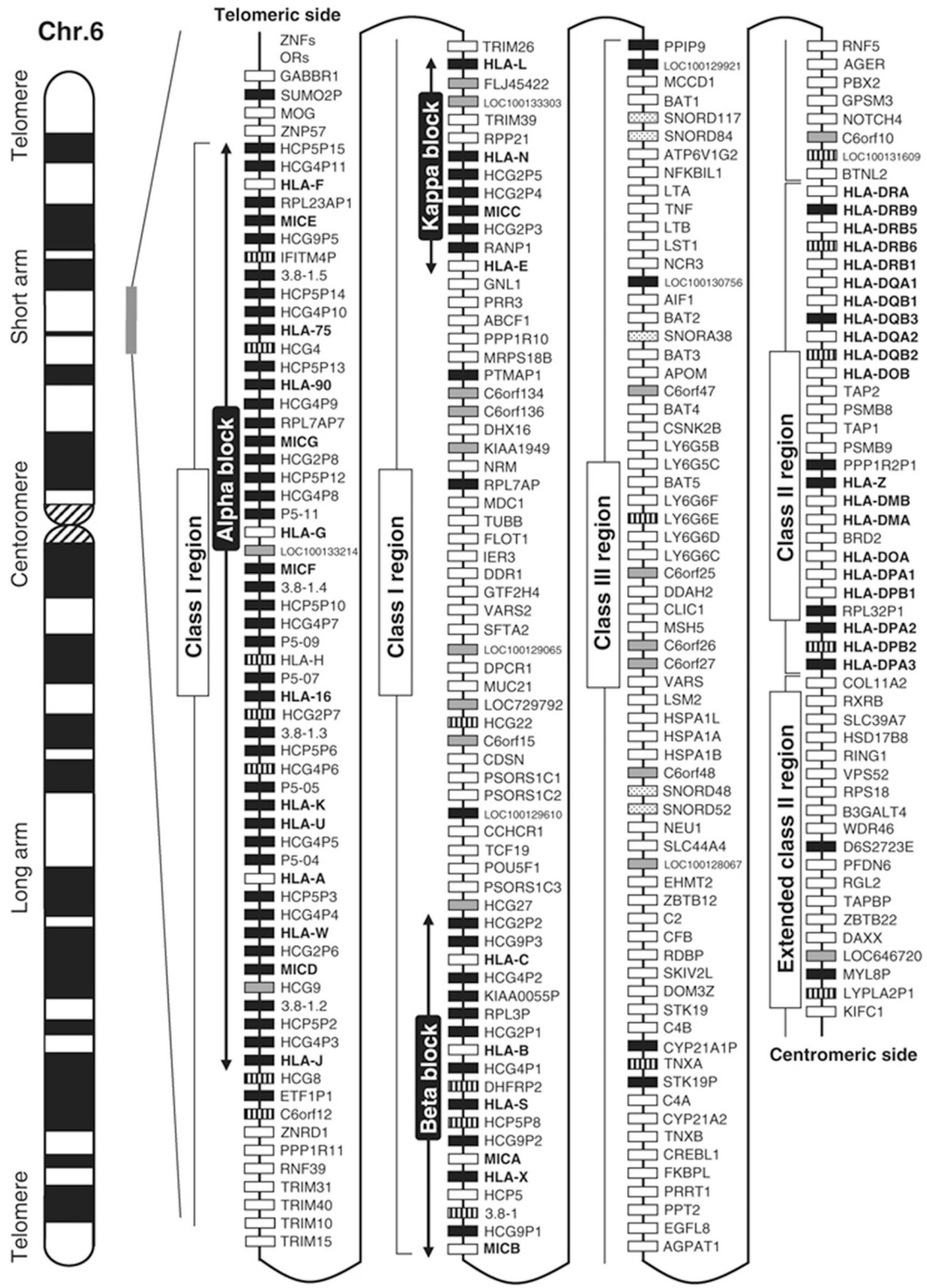

FIGURE 2 | Gene map of the human leukocyte antigen (HLA) region. The major histocompatibility complex (MHC) gene map corresponds to the genomic coordinates of 29,677,984 (GABBR1) to 33,485,635 (KIFC1) in the human genome build 36.3 of the National Center for Biotechnology Information (NCBI) map viewer. The regions separated by arrows show the HLA subregions such as extended class I, classical class I, class III, classical class II, and extended class II regions from telomere (left and top side) to centromere (right and bottom side). White, gray, striped and black boxes show expressed genes, gene candidates, non-coding genes and pseudogenes, respectively. The location of the alpha, beta, and kappa blocks containing the cluster of duplicated HLA class I genes in the class I region are indicated. Reprinted with permission from Shiina et al. (2009).

haplotype of which there are thousands; most very rare. A genetic haplotype is a contiguous combination of DNA sequences inherited on a single chromosome: eight of the more common extended haplotypes with strong disease associations have been entirely DNA sequenced (Horton et al., 2008). Various extended haplotypes containing millions of base pairs can have identical smaller haplotypes within their borders meaning that they must be carefully examined. Haplotype structure can be important in the understanding of allele-specific events such as protein structure, methylation, outcomes in transplantation, and disease prediction (McQueen et al., 2007). The HLA gene map does not fully illustrate the complexity of the class I and class II regions that have thousands of alleles important in determining self from non-self.

HLA CLASS I ASD ASSOCIATIONS

There are numerous ASD associations with alleles/genes in the HLA region. For example, three different research groups have demonstrated a significant association between ASD and the HLA-A2 (A2) allele (Ferrante et al., 2003; Torres et al., 2006; Al-Hakbany et al., 2014). Gourraud et al. (2014) noted that there are over 100,000 SNPs in the 4.5 million base pairs of HLA (1 out of every 45 bases) and concluded that whole genome screening methods like GWAS are not suited for interrogating HLA polymorphisms. They referred to this extreme HLA diversity as a "genome within a genome." **Table 1** illustrates the complexity of SNPs in a small region of the HLA-A-locus. Site specific primers (SSP) PCR assays clearly and inexpensively identify these complex HLA polymorphisms (Bunce and Passey, 2013). Other HLA class I and II loci have similar SNP complexity as do KIR genes, but none of these sets of polymorphisms are readily detected by GWAS techniques.

It has recently been reported in the Thai population that certain HLA-B alleles (B13:02, B35:02, B44:03, and B56:01) are significantly associated with ASD while two others (B18:02 and B46:12) are associated in a protective manner (Puangpetch et al., 2015). This is not surprising as certain HLA extended haplotypes which have about 200 genes including specific HLA-A, B, and C alleles have very strong associations with ASD. For example, in the Caucasian population HLA-B44 and B15 alleles as part of extended haplotypes 44.1 and 62.1, respectively, are associated with ASD (Torres et al., 2012a).

HLA-G non-classical I molecules are expressed during pregnancy on trophoblast cells that interact with leukocyte-associated immunoglobulin-like receptor (LAIR) and KIR (2DL4) molecules expressed by maternal NK-cells at the uterine fetal/maternal interface to suppress normal immune responses (Tilburgs et al., 2015). A 14 bp deletion in the HLA-G 3′-UTR is associated with higher levels of HLA-G expression whereas a 14 bp insertion associates with reduced HLA-G levels. Guerini et al. (2015) have shown that ASD subjects have an increase in the 14 bp insertion and lower levels of soluble HLA-G protein. This suggests that ASD subjects and their mothers have less HLA-G-mediated immune tolerance during pregnancy. Human non-classical HLA I molecules could be important in brain development as it has recently been shown that non-classical MHC class I molecules are expressed in the olfactory bulb, hippocampus, cerebellum, and nerve nuclei in the developing embryonic mouse brain (Liu et al., 2015).

A main function of HLA class I molecules is to present antigens to CD8+ T-cells, thus starting the complex immune process that creates cytotoxic T-cells to attack specific targets. Any gene in an extended haplotype may be the candidate ASD gene and other HLA alleles may be passengers, however, the identification of foreign peptides that bind to A2 could suggest microorganisms that may be involved in the etiology of ASD. Our data suggests that the HLA class I proteins cognate ligands for KIR are important in autism and that other NK-cell killing receptors could also be important (**Figure 4**). This interaction does not involve peptide binding as KIR receptors recognize specific amino acid sequences of certain HLA cognate protein ligands (Parham and Moffett, 2013). HLA class I A, B, C alleles all behave as antigen presenting ligands for self and nonself-peptides; however, only certain HLA-A, B, C alleles are ligands for KIR cell surface proteins. Although the HLA cognate ligand site slightly overlaps the peptide binding site, it has a separate function (**Figure 3**). Specific HLA ligands that bind to KIR activating receptors have been shown to be increased in ASD (Torres et al., 2012b, 2016; Guerini et al., 2014). It should be mentioned that the HLA-A2 does not behave as a KIR ligand.

It was only a few years ago that the brain was considered immune privileged, being protected from immune fluctuations by the blood brain barrier. Today there is increasing evidence that the systemic immune system is involved in CNS functioning. Pro-inflammatory cytokines such as TNF-α can easily cross the blood brain barrier and complement proteins such as C1q and C4 are active in brain function (Johnson et al., 1994; Torres et al., 2001). C1q appears to be especially important in eliminating unwanted synapses (Stevens et al., 2007). Perhaps the most important research from an immune-brain perspective is the involvement of HLA class I proteins in brain development. The expression of HLA class I proteins occurs throughout the brain on neurons and even synaptic membranes (Elmer et al., 2013). It is yet unknown if there are differences in class I alleles in brain development and this is an area clearly deserving of further research.

HLA CLASS II ASD ASSOCIATIONS

A maternal-fetal immune interaction has been suggested by Lee et al. (2006) in which boys with autism and their mothers were shown to have an increased DR4 frequency over the control subjects (odds ratios 4.20 and 5.54, respectively). Johnson et al. (2009) later reported significant transmission disequilibrium for HLA-DR4 (odds ratio 4.67) from maternal grandparents to mothers of children with autism, more evidence that HLA-DR4 has a maternal-fetal interaction. Finally, it has been shown in Han Chinese that the HLA-DRB1 allelic frequencies including DR4 are different in autism subjects vs. control subjects (Chien et al., 2011). It should be noted that it is common for racial groups to have different HLA alleles associated with autoimmune diseases.

Warren et al. (1996) also noted that the third hypervariable region (HVR-3) of certain class II DR4 alleles, referred to as

TABLE 1 | Demonstrates the SNP complexity in HLA alleles.

COMPLEXITY OF HLA-A ALLELE SEQUENCES

Codon position and DNA sequence

	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83
A*01	CAG	GAG	ACA	CGG	AAT	ATG	AAG	GCC	CAC	TCA	CAG	ACT	GAC	CGA	GCG	AAC	CTG	GGG	ACC	CTG	CGC	GGC
A*02	GG-	---	---	---	--A	G--	---	---	---	---	---	---	C--	---	-T-	G--	---	---	---	---	---	---
A*03	---	---	---	---	---	G--	---	---	--G	---	---	---	---	---	-T-	G--	---	---	---	---	---	---
A*11	---	---	---	---	---	G--	---	---	--G	---	---	---	---	---	-T-	G--	---	---	---	---	---	---
A*23	G--	---	---	G--	--A	G--	---	---	---	---	---	---	---	---	-A-	---	---	C--	-T-	GC-	-T-	C--
A*24	G--	---	---	G--	--A	G--	---	---	---	---	---	---	---	---	-A-	---	---	C--	-T-	GC-	-T-	C--
A*25	-G-	A-C	---	---	---	G--	---	---	---	---	---	---	---	---	-A-	-G-	---	C--	-T-	GC-	-T-	C--
A*26	-G-	A-C	---	---	---	G--	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
A*29	-T-	C--	---	---	---	G--	---	---	--G	---	---	---	---	---	---	---	---	---	---	---	---	---
A*30	---	---	---	---	---	G--	---	---	--G	---	---	---	---	---	-T-	G--	---	---	---	---	---	---
A*31	---	---	---	---	---	G--	---	---	---	---	---	-T-	---	---	-T-	G--	---	---	---	---	---	---
A*32	---	---	---	---	---	G--	---	---	---	---	---	---	---	---	-A-	-G-	---	C--	-T-	GC-	-T-	C--
A*33	-G-	A-C	---	---	---	G--	---	---	---	---	---	-T-	---	---	-T-	G--	---	---	---	---	---	---
A*34	-G-	A-C	---	---	--A	G--	---	---	--G	---	---	---	---	---	-T-	G--	---	---	---	---	---	---
A*36	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
A*43	-T-	C--	---	---	---	G--	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
A*66	-G-	A-C	---	---	---	G--	---	---	--G	---	---	---	---	---	-T-	G--	---	---	---	---	---	---
A*68	-G-	A-C	---	---	---	G--	---	---	--G	---	---	---	---	---	-T-	G--	---	---	---	---	---	---
A*69	-G-	A-C	---	---	---	G--	---	---	--G	---	---	---	---	---	-T-	G--	---	---	---	---	---	---
A*74	---	---	---	---	---	G--	---	---	---	---	---	---	---	---	-T-	G--	---	---	---	---	---	---
A*80	G--	---	---	---	---	G--	---	---	---	---	---	---	A--	---	---	---	---	---	---	---	---	---

--- = same as reference sequence (A*01)

= different from reference sequence (A*01)

Approximately one out of three DNA bases in this sequence can be a polymorphism.

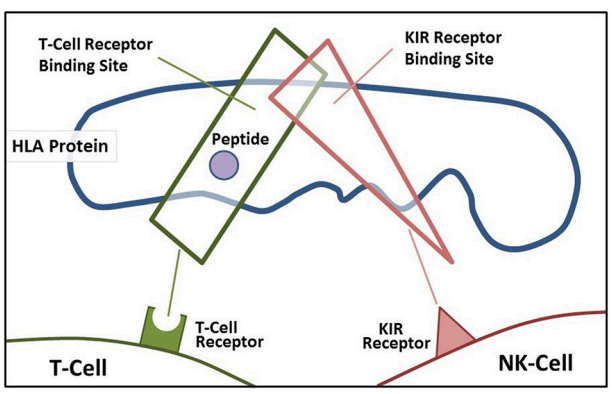

FIGURE 3 | Demonstrates the overlapping regions for KIR ligand binding and antigen-presenting portions of the HLA protein.

the shared epitope (SE), were strongly associated with ASD ($p < 0.01$; relative risk = 19.8). In this review, odds ratio and relative risk, two similar mathematical models to examine disease risk, are considered equivalent. The HLA-DRB1 SE is a 5 amino acid motif shared by 5 DRB1 alleles. The SE also strongly associates with the majority of severe rheumatoid arthritis (RA) patients. The basis for the SE association with ASD and RA is unknown; however, it has been proposed that the SE peptide acts as a signal transduction ligand that activates nitric oxide (NO) and reactive oxygen species production (de Almeida et al., 2011). It is important to mention that De Almeida et al. (2010) concluded, in an earlier publication, that the HLA DRB1 shared epitope peptide is a potent immune-stimulatory ligand that polarizes naive helper T-cell toward the potent inflammatory T_H17 lineage resulting in higher IL-17 levels detected in autism subjects (Al-Ayadhi and Mostafa, 2012; Onore et al., 2012).

It should be noted that mothers with SE alleles on either chromosome were more likely to give birth to an ASD child, referred to by Warren et al. (1996) as a "maternal attack against fetal tissue." The SE observation is important as it suggested that small peptide epitopes contributed to the high relative risk in ASD years before the T_H17 cell observation was made.

AUTOANTIBODIES IN AUTISM

One of the most interesting areas of current ASD research is the observation in at least 8 studies that up to about 10% of mothers with ASD children and only 0–2% of controls have humoral antibodies against fetal brain proteins (Croen et al.,

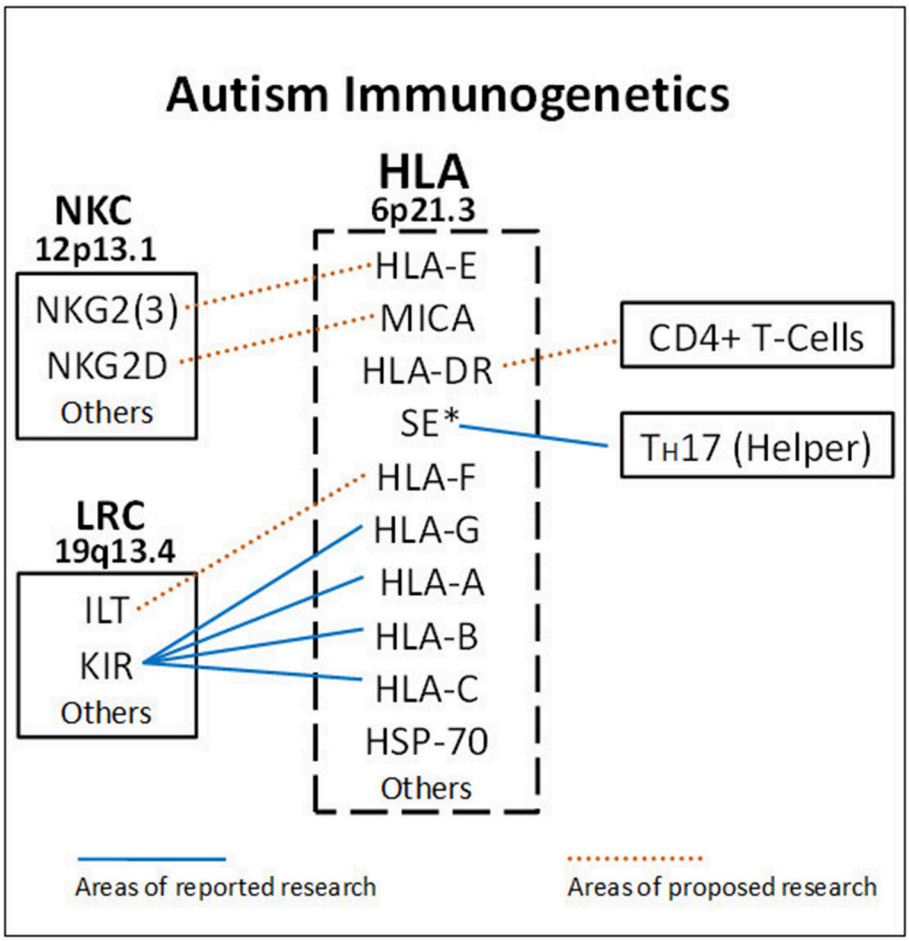

FIGURE 4 | HLA gene complex encode ligands that react with receptors on the Leukocyte Receptor Complex (LCR) and Natural Killer Cell Gene Complex (NKC) to influence killing. Blue lines indicate areas of completed research. HLA-A, B, C ligands bind to various KIR receptors encoded on chromosome 19. The DRB1*0401 shared epitope (SE*) is strongly associated with ASD (RR = 19.8) and helps increase TH17 cells. The dashed lines suggest future ASD research to get a more complete picture on NK-cell killing and antibody production. HLA-DR alleles bind to CD4+ helper T-cells to influence B-cells. HLA-E and MHC Class I like- sequence A (MICA) proteins are ligands for activating molecules NKG2(3) and NKG2D, respectively, that influence NK-cell killing (chromosome 12).

2008; Braunschweig and Van de Water, 2012). Because HLA class II molecules are important in antibody production it would be interesting to know if there is an association between mothers who make these antibodies and certain DRB1 alleles. The entire area of maternal antibodies against fetal proteins, the production of autoantibodies, as well as antibodies to microbial pathogens should be studied more in ASD (Grether et al., 2016).

CLASS III ASD ASSOCIATIONS

Four of the 25 proteins in the complement system that help or "complement" antibodies and phagocytic cells to clear pathogens from the organism are encoded within the HLA complex (C4A, C4B, C2, and Bf). The C4 complement proteins (C4A and C4B) are encoded by two separate genes in the HLA class III region (Torres et al., 2001). It was reported 25 years ago that subjects with autism had a significant increase in the deletion of the HLA class III C4B gene (C4B null allele) compared to control subjects (Warren et al., 1991; Odell et al., 2005). ASD subjects were also noted to have a significant deficiency in the plasma C4B protein as determined by an Elisa assay (Warren et al., 1994). Mostafa and Shehab (2010) have also reported a significant increase in the deletion of the C4B gene in the Egyptian ASD population. However, the gene copy number data from qPCR assays do not entirely agree with the results from the C4B protein assay, a not uncommon occurrence when comparing genomic and protein expression data (Kendrick, 2014), thus the solidity of these results are still uncertain. While the data of Warren et al. (1996) suggest that the C4B null allele is another common variant, as it is part of the 44.1 extended haplotype that also contains the SE, further clarification of the association of C4B null alleles is required, preferably using samples with known C4A/C4B DNA complotypes.

There are four proinflammatory cytokines: tumor necrosis factor-alpha (TNFα), lymphotoxin alpha and beta (LTA and

LTB), and leukocyte specific transcript-1 (LST1) in the class III region that are important in inflammation and infection. TNFα is a proinflammatory, multifunctional cytokine that is synthesized in numerous cells including NK-cells, macrophages, granulocytes and T-cells. In the CNS, TNFα is made in NK-cells, microglia (brain macrophages), astrocytes, and neurons and is necessary for neural cell differentiation and neuron maturation and may be critical for proper synaptic pruning (Cacci et al., 2005). Elevated levels of TNFα are present in ASD patients, as well as in numerous neurological disorders including multiple sclerosis, Alzheimer's Disease, Parkinson's Disease, ischemia, and traumatic brain injury (Montgomery and Bowers, 2012). The three adjacent proinflammatory cytokine genes: lymphotoxin alpha and beta (LTA and LTB) and leukocyte specific transcript-1 (LST1) have not been studied as extensively as TNFα (Ovsyannikova et al., 2010) and deserve further investigation.

There is a developing consensus that there are elevated plasma levels of proinflammatory cytokines such as IL-1β, IL-6, IL-8, as well as CCL2 and CCL5 chemokines in ASD (Grigorenko et al., 2008; Ashwood et al., 2011). Decreased levels of anti-inflammatory cytokines, most notably TGF-β and IL-10, have also been reported in ASD (Okada et al., 2007; Abdallah et al., 2013; Jyonouchi et al., 2014). In addition to these proinflammatory cytokines, it is interesting to note that an increase in interferon-gamma (INFγ) and TNFα has been observed in brain tissue and cerebral spinal fluid of in ASD subjects (Li et al., 2009). These two cytokines are of special interest, as TNFα is in the HLA class III region and activates NK-cells to make large amounts of INFγ (Schoenborn and Wilson, 2007). TNFα also may be important in ASD as it appears to be a key mediator in neuroinflammation and blood-brain barrier deterioration (McCoy and Tansey, 2008). TNFα concentrations are also elevated in several brain disorders including multiple sclerosis, Alzheimer's Disease, Parkinson's Disease, ischemia, and traumatic brain injury (Montgomery and Bowers, 2012). It is unclear if TNFα contributes to the disease state or if the higher concentrations limit brain damage. Finally, another avenue for investigation, is the class III gene encoding the heat shock protein 70 (HSP70) which is induced in many CNS disorders such as stroke, epilepsy, trauma and ASD (El-Ansary and Al-Ayadhi, 2012).

INFγ may contribute to ASD pathology by increasing the enzyme nitric oxide synthase in astrocytes and microglia, and thereby increasing brain levels of NO. It is thought that activated immune cells secrete high levels of the free radical NO to damage pathogens. In the brain however, NO is typically produced by neurons and it acts as an intercellular messenger modulating synaptogenesis, dendrite, and axonal growth and neuronal release of various neurotransmitters (Hess et al., 1993; Lizasoain et al., 1996). Problems with synaptic development and plasticity are implicated in the neuropathology of ASD (Ebert and Greenberg, 2013).

It was reported a couple of years ago that serum levels of IL-17 were elevated in ASD subjects over control subjects suggesting that T helper 17 (T_H17) cells are involved in the etiology of ASD (Al-Ayadhi and Mostafa, 2012; Onore et al., 2012). T_H17 cells are CD4+ helper T-cells characterized by a unique cytokine profile, mainly high levels of IL-17 thought to be critical for the development of autoimmunity (Bedoya et al., 2013).

THE IMPORTANCE OF HLA IMMUNITY IN AUTISM

The HLA gene complex has been considered by many to be the most important region for immune function in the human genome. However, the system is not perfect and certain HLA allotypes and haplotypes have been shown to be strongly associated with a wide spectrum of autoimmune diseases, such as Type I diabetes, celiac disease, psoriasis, rheumatoid arthritis (RA), lupus erythymatosis, myasthenia gravid, Sjogren Syndrome, and now ASD. The HVR-3 (or shared epitope) that is increased in ASD (Warren et al., 1996) is also associated with several autoimmune diseases such as RA, psoriatic arthritis, and systemic lupus erythematosus (de Almeida et al., 2011). Various HLA disease associations often have the relative risk factors 15 to 20-fold higher in subjects with autoimmune diseases than control subjects (Feitsma et al., 2008). The HVR-3 (DRB1*0401) association with ASD had a comparable relative risk of 19.8 and 31.5% increase in the ASD population relative to control subjects, fitting the criteria for a "common variant–common disease" association.

HLA/KIR COMMON GENETIC VARIANTS

There is currently an intense interest in identifying genetic variants that increase the risk of developing ASD. The goal of this paper is to summarize data which suggests that certain HLA/KIR immune alleles/genes fit the criteria as common genetic variants. For example, the HLA-A2 allele in independent studies from three different groups has a >10% increased frequency in the ASD population than controls (**Table 2A**). Interestingly, it was over three decades ago that Stubbs and Magenis (1980) first implicated HLA-A2 in ASD. A 14 base pair insertion in the class I non-classical HLA-G loci is strongly associated with ASD (Guerini et al., 2015) and is clearly a common variant at 18% over controls (**Table 2A**).

The DR4 allele has higher frequencies of 18.94 and 7.64% in two ASD populations over control populations suggesting that DR4 is a common variant (**Table 2A**). Warren et al. (1996) presented data that strongly suggested that the shared epitope is a common genetic variant with a 31.5% increase of ASD over control subjects (**Table 2A**).

Examination of KIR gene frequencies suggests that certain activating genes are common variants with ASD frequency differentials well above 5% in two different publications (Torres et al., 2012b; Guerini et al., 2014). The KIR region in the leukocyte receptor complex (LRC) is not typical as KIR haplotypes are based on different combination of activating and inhibiting genes that influence NK-cell killing (Pyo et al., 2013; Torres et al., 2016). Gene frequency analyses suggest that three activating genes (3DS1, 2DS1, and 2DS2) are common variants (**Table 2B**). Additionally, examinations of all activating KIR genes (aKIR) indicate an increase in total activating genes in ASD over

TABLE 2 | (A) Suggests that HLA class I and class II alleles are common genetic variants (>5%). (B) Suggests that KIR genes and KIR gene-content haplotypes are common genetic variants.

Variables	*p*-value	Odds ratio	Subjects (%)	Controls (%)	Common variant (>5%)
(A) HLA					
Class I alleles					
A2[1]	0.0002	2.22	101/258 (39.1%)	75/265 (28.3%)	10.8
A2[2]	0.001	3.02	30/70 (42.8%)	54/200 (27.0%)	15.8
G 14 bp(+/+)[3]	0.01	2.70	19/74 (25.7%)	3/39 (7.7%)	18.0
Class II alleles					
DR4[4]	0.003	3.24	10/32 (31.3%)	117/950 (12.3%)	19.0
DR4[5]	0.004	1.60	50/205 (24.4%)	68089/406503(16.8%)	7.6
SE[6]	<0.001	19.80	17/50 (34.0%)	2/79 (2.5%)	31.5
Class III alleles					
C4BQ0[6]	0.03	4.60	27/50 (54.0%)	16/79 (20.2%)	13.8
(B) KIR					
Kir genes					
3DS1[7]	0.004	1.89	83/158 (52.5%)	65/176 (36.9%)	15.6
2DS1[7]	0.00007	2.45	95/158 (60.1%)	67/176 (38.1%)	22.0
2DS2[8]	0.030	1.83	63/90 (70.0%)	140/250 (56.0%)	14.0
aKIR*[7]	0.0004	1.36	126/158 (79.7%)	128/176 (72.7%)	7.0
Kir haplotypes					
cA01[9]	0.00001	0.53	110/178 (61.8%)	6248/9024 (69.2%)	−7.4 (protective)
cB01[9]	0.005	1.69	41/178 (23.0%)	1184/9024 (13.1%)	9.9
cB01/tA01[9]	0.002	2.06	25/170 (14.7%)	697/9024 (7.7%)	7.0
Kir genes + HLA ligands					
aKIR + HLA[7]	0.0006	1.56	117/155 (75.5%)	104/164 (63.4%)	12.1

[1] *Torres et al., 2006.*
[2] *Al-Hakbany et al., 2014.*
[3] *Guerini et al., 2015.*
[4] *Lee et al., 2006.*
[5] *Torres et al., 2002.*
[6] *Warren et al., 1996.*
[7] *Torres et al., 2012b.*
[8] *Guerini et al., 2014.*
[9] *Torres et al., 2016.*

control subjects. KIR gene-content haplotypes have centromeric and telomeric parts that join at the junction between 3DP1 and 2DL4 (**Figure 5**). Examination of the KIR haplotypes suggests that three partial KIR haplotypes (tA01/cA01/cB01) and a complete KIR gene-content haplotype (cB01/tA01) all meet common variant criteria (**Table 2B**; Torres et al., 2016).

AUTISM IMMUNOGENETICS

Immunogenetics commonly refers to the strong association of HLA alleles/genes with autoimmune diseases (Shiina et al., 2004). We wish to propose that much of the immune dysfunction in ASD has a basis in the HLA /KIR gene complexes; thus, we have coined the term Autism Immunogenetics. Although, many of the multiple thousands of HLA alleles are rare there are hundreds of well-characterized HLA class I and class II alleles that make up a significant part of the genetic diversity of the 900 gene Immunome.

One must remember that the entire 200 genes in the HLA gene complex are inherited as large 4.5 million base pair extended haplotypes and a couple of these large extended haplotypes have been associated with autism (Warren et al., 1996). The association of the A2, DR4 alleles and C4B null allele with ASD may be due to the fact that they are part of the extended haplotype 44.1 that is ASD associated (**Figure 4**; **Table 2**). However, the important observation is that all are common genetic variants. It would be interesting to know which extended haplotypes have the HLA-G 14 bp insertion as it strongly associates with ASD. The shared epitope peptide may be the most important as it directs helper T-cells to the T_H17 lineage which appears to be important in the pathogenesis of autoimmune neuroinflammatory diseases including ASD (**Figure 4**; Onore et al., 2012; Choi et al., 2016). A recent publication suggested that maternal T_H17 cells could induce an ASD-like phenotype in newborn mice and

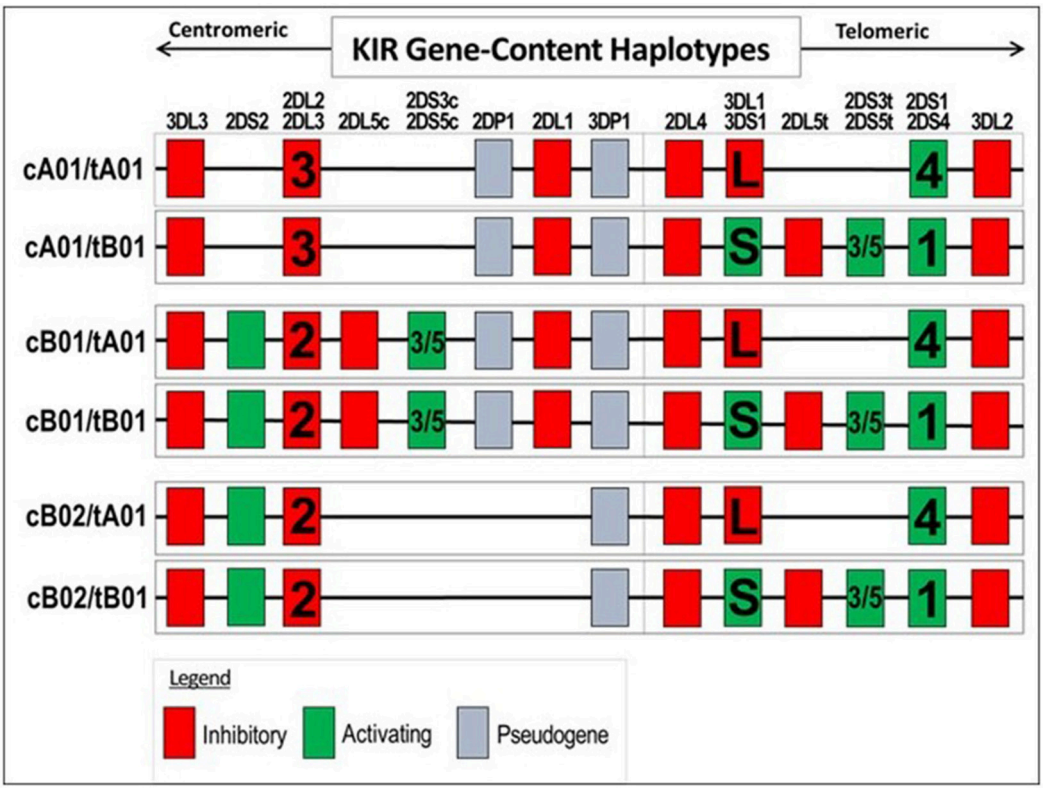

FIGURE 5 | Genes present in KIR gene-content haplotypes. Only genes present in a haplotype are listed under the gene column: for example, 2DS2 is absent in the first two haplotypes, but present in the last four haplotypes. Certain haplotype can have 2DL2 or 2DL3 and other haplotype can have 2DL1 or 2DS1 and 2DS1 or 2DS4. The complete gene-content KIR haplotype (cB01/tA01) is a proposed common variant.

that blocking antibodies to IL-17a ameliorated these symptoms (Choi et al., 2016). The high relative risk for the SE in ASD noted by Warren et al. (1996) may be due to an increase in $T_H17$cells.

CONCLUDING REMARKS

A significant body of evidence has accumulated over the past 30 plus years to demonstrate that specific variants in immune system genes, involving both innate and adaptive immunity pathways, are associated with the incidence of ASD. However, such associations have not been detected by genome wide screening methods, e.g., GWAS, and therefore have been widely ignored by the majority of investigators in the autism research field. The inability to detect immune gene ASD associations by GWAS techniques is fully understandable, considering the massive genetic complexity of the "Immunome," with more than one hundred thousand polymorphisms localized within a small segment (<5%) of the total genome. Thus, highly focused methods, usually PCR or sequence-based typing, are required to reveal disease associations with immune genes or haplotypes, and these can usually be detected using relatively small number of patient and control samples. Next-Generation Sequencing methods to rapidly sequence the HLA locus are well developed for such autism immunogenetic studies (Nelson et al., 2015; Carapito et al., in press). Indeed, as summarized in **Table 2**, several HLA and KIR gene variants that meet the "common variant–common disease" criteria have been identified using relatively small sample sizes: in the low hundreds. Perhaps the most interesting observation concerning common genetic variants is the 12% increase of the HLA ligand /KIR activating gene complex in autism over the control populations (**Table 2**). We argue strongly that additional efforts be focused on identifying and characterizing the role of immune genes in the etiology of ASD, particularly using phenotypically well-defined populations of autistic subjects.

The data summarized in this review raises numerous questions in addition to those raised earlier in this paper. For example, how do different immune gene variants influence brain development and function? While it is clear that many HLA proteins are expressed in or on neural cells and they can modulate the activity of downstream effector cells or proteins, such as NK-cells or pro-inflammatory cytokines, details on how they actually function are unknown. The fact that a single amino acid change in certain HLA class I and II proteins can alter their functionality makes such studies extremely challenging. For example, single DNA base differences in HLA-C alleles encode different amino acids which are

DNA	Ligand	Receptor
Codon AAC	HLA-C amino acid #80 ASN ——————-	KIR-2DS2 and 2DL2
Codon AAA	HLA-C amino acid #80 LYS ——————-	KIR-2DS1 and 2DL1

ligands for different KIR receptors (below) (Torres et al., 2016).

An additional question that needs to be addressed is why do up to 10% of mothers with ASD children have humoral antibodies against fetal brain proteins (compared to 0–2% of control mothers) and do these autoantibodies adversely affect fetal brain development? Furthermore, does the 14 bp insertion in the HLA G gene that occurs more frequently in ASD subjects and their mothers impair immune tolerance during pregnancy and increase the risk of *in utero* developmental problems?

HLA ALLELES AND KIR GENES AS COMMON GENETIC VARIANTS IN ASD

The observations that several HLA alleles as well as several KIR genes and haplotypes appear to be common genetic variants associated with ASD (**Table 2**) raises the question as to whether or not other immune genes (for example HLA-E, HLA-F, and MICA) have similar ASD associations. These issues should be addressed in future studies. In summary, by further understanding how immune gene variants participate in the etiology of ASD, it may be possible to: #1 develop biological markers to predict ASD at an earlier stage or even *in utero* and #2 develop targeted pharmaceutical molecules such as monoclonal antibodies, decoy peptides, and special nucleic acid molecules against SE, HLA, and KIR molecules.

AUTHOR CONTRIBUTIONS

ART conceived of the study, participated in its design and coordination, and drafted the manuscript. TS and JW participated in data collection and helped draft the manuscript. PB, DW, CD, AJT, DO, and LC helped draft the manuscript. RJ performed the statistical analyses. MB participated in data collection and statistical analyses. All authors read and approved the final manuscript.

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Paternal HLA-C and Maternal Killer-Cell Immunoglobulin-Like Receptor Genotypes in the Development of Autism

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Killer-cell immunoglobulin-like receptors (KIRs) are a family of cell surface proteins found on natural killer cells, which are components of the innate immune system. KIRs recognize MHC class I proteins, mainly HLA-C and are further divided into two groups: short-tailed 2/3DS activating receptors and long-tailed 2/3DL inhibitory receptors. Based on the Barker Hypothesis, the origins of illness can be traced back to embryonic development in the uterus, and since KIR:HLA interaction figures prominently in the maternal–fetal interface, we investigated whether specific KIR:HLA combinations may be found in autism spectrum disorders (ASD) children compared with their healthy parents. This study enrolled 49 ASD children from different Israeli families, and their healthy parents. Among the parents, a higher frequency of HLA-C2 allotypes was found in the fathers, while its corresponding ligand 2DS1 was found in higher percentage in the maternal group. However, such skewing in KIR:HLA frequencies did not appear in the ASD children. Additionally, analysis of "overall activation" indicated higher activation in maternal than in paternal cohorts.

Keywords: autism, pregnancy, natural killer, KIR, HLA

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INTRODUCTION

Autism spectrum disorders (ASD) encompass a range of neurodevelopmental syndromes defined by difficulties in social communication and stereotyped behaviors (1). Recent data indicate a prevalence of up to 1 in 66 children (2). Although studies in families and twins (3) point toward a significant genetic component with a high sibling recurrence risk (4), in only a few cases can a clear genetic component be identified. Various factors – hormonal, immunological, and biochemical – have been implicated in autism's etiology, but their roles in the symptomology of the disease remain undefined (5). Immune system dysregulation reported in ASD patients includes differential cell fractions and reactivity, autoimmune phenomena, altered cytokine and antibody profiles, and genetic correlations (6). These findings partially corroborate the theory that chronic neurological inflammation in fetal or newborn brain underlies the development of ASD. Subsequent dysregulation of the immune system may contribute to ASD development in genetically susceptible children.

Abbreviations: ASD, autism spectrum disorders; HLA, histocompatibility leukocyte antigens; KIR, killer-cell immunoglobulin-like receptor; NK, natural killer.

Altered natural killer (NK) cell activity has been reported in the periphery of ASD patients (7). However, these innate immune cells' function in the pathogenesis of autism is yet unclear. NK cells act specifically against infected or transformed cells, with their actions governed by a balance between activating and inhibitory receptors, each recognizing specific molecules on a given target (8). Killer-cell immunoglobulin-like receptors (KIRs) are a group of cell surface molecules involved in regulating activity of NK (and some T) cells. KIRs are classified as either inhibitory or activating. Their nomenclature is based on the number of extracellular Ig-like domains, and whether the cytoplasmic domain is *long* (L) or *short* (S). The KIR gene family is clustered within the LRC leukocyte receptor complex on chromosome 19q13.4, encoding a total of 14 genes and 2 pseudo genes. KIR genes exhibit allelic and haplotypic variability, while each mature NK cell expresses a specific set of KIRs.

Killer-cell immunoglobulin-like receptor counterparts, the HLA class I ligands, are also extremely polymorphic. This generates an additional level of functional diversity. Both KIRs and their HLA ligands are encoded on different chromosomes (19 and 6, respectively), and therefore segregate independently of each other. This expands the repertoire of possible profiles within any given population (9). Specific KIR–HLA combinations have been suggested as underlying susceptibility as well as resistance to various diseases and conditions (10); the functional implications are less clear. Genetic associations have been reported in infectious diseases (11), autoimmunity (12), inflammatory conditions, reproductive failure, and cancer. Biological models in such studies need to incorporate these receptor:ligand interactions, beyond mere consideration of KIR–HLA genotyping. Indeed, several methods have been proposed to analyze the overall genotyping detected in a specific subject or whole population (13, 14).

The KIR family predominantly recognizes HLA class I molecules. Specifically, HLA-C is recognized by both inhibitory receptors (KIRs 2DL1, 2DL2, and 2DL3) and activating receptors (KIRs 2DS1, 2DS2, and 2DS4) (**Figure 1**). HLA-C group 1 (C1) allotypes have an asparagine residue at position 80 (C1:N^{80}), and serve as ligands for KIRs 2DL2, 2DL3, and 2DS2. HLA-C group 2 (C2) allotypes function as ligands for KIRs 2DL1 and 2DS1; C2 allotypes have a lysine at position 80 (C2:K^{80}). The interactional affinities of these groups may differ. KIR 2DS4 can bind to both HLA-C C1 and C2 allotypes, and certain HLA-A alleles. KIRs 3DL1 and (putatively) 3DS1 interact with HLA-A BW4:I80 as well as HLA-B allotypes [with either isoleucine (BW4:I80) or threonine (BW4:T80) at position 80].

The aim of our research was to genotype the KIR receptors known to interact with HLA ligands in autistic children and their non-autistic parents (mothers and fathers, separately), and to compare KIR receptor: HLA ligand frequencies between these groups. Our hypothesis was that a non-random distribution of these genes profiles should be found if interactions between these two classes of molecules are relevant to autism.

According to the Barker Hypothesis (15), the source of pathologies – including mental disorders such as ASD – can be traced back to embryonic existence in the intrauterine environment (16, 17). KIRs are expressed by maternal NK cells (decidua, uterine lining), which are in close contact with HLA-expressing fetal tissues (placenta). Such prenatal interaction between maternal KIR and the fetal HLA in the uterus may lead to either enhanced NK activation or NK inhibition, which in turn may exert influence upon the placenta and/or developing fetus (18–20). Resulting immune activation and effects upon the central nervous system may lead to neurological and psychiatric disorders in predisposed patients. A mouse model for intrauterine maternal immune activation has already been demonstrated as a possible mechanism for autism- and schizophrenia-related behaviors in offspring (21).

MATERIALS AND METHODS

Participants

This study comprised of 49 ASD-diagnosed children (38 males, 11 females, a male:female ratio of 3.4:1) from Jewish Israeli families, and their parents. Another six sets of parents of ASD-diagnosed children were enrolled, though no DNA samples were available from their children. DNA samples were collected in Israeli special education schools and treatment centers, by the Israeli Society for Autistic Children (ALUT).[1]

Many of the probands had already been diagnosed with ASD by independent clinicians; two trained clinicians confirmed the

[1]http://www.autismaroundtheglobe.org/countries/Israel.asp

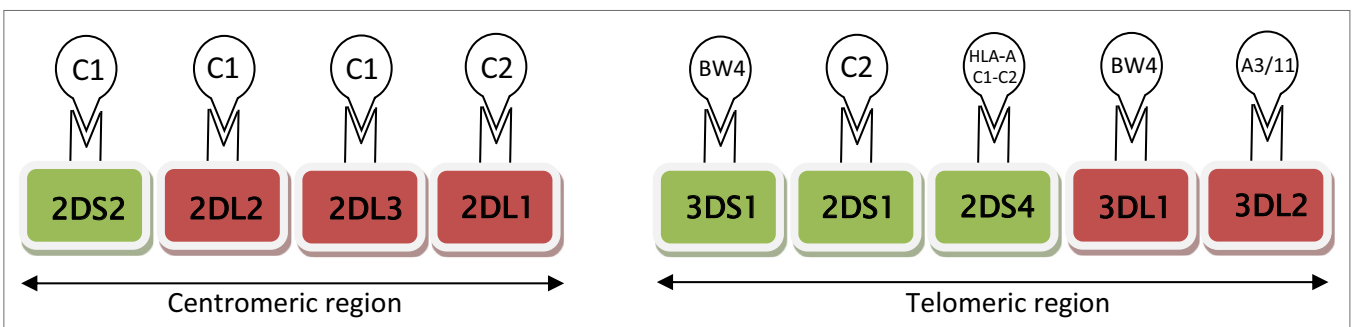

FIGURE 1 | Schematic diagram of activating (green) and inhibitory (red) receptors on natural killer cells, arranged according to centromic/telomeric location. These receptors are known to interact specifically with HLA ligands (HLA CI or C2, HLA A and B BW4). HLA-C group 1 (CI) allotypes serve as ligands for KIRs 2DS2, 2DL2, and 2DL3; HLA-C group 2 (C2) functions as ligands for KIRs 2DL1 and 2DS1.

diagnosis for autism or Pervasive Developmental Disorder-Not Otherwise Specified (PDD-NOS). All probands were diagnosed using the Autism Diagnostic Observation Scale-Generic (ADOS-G) and the Autism Diagnostic Interview-Revised (ADI-R). In addition, parents were interviewed using the Vineland Adaptive Behavior Scales-second edition (Vineland™-II) survey interview edition (VABS).

Probands ranged in age between 25 months to 33 years and 8 months. None of the subjects were known, according to parental reports, to have known genetic factors such as chromosomal aberrations, tuberous sclerosis, or other medical complications that could be related to autism. Parents were screened for psychiatric illness (including ASD); all results were found to be negative.

The Ethics Committee of the Israeli Health Ministry approved this study. All participants signed informed consent forms in advance.

Genotyping

DNA was extracted from either peripheral blood or buccal cells using a commercial DNA purification kit (Epicentre's Master Pure™, Madison, WI, USA).

The presence or absence of nine KIR genes known to interact with specific HLA ligands was determined by SSP-PCR (sequence-specific primer polymerase chain reaction), as previously described (22, 23), using nine pairs of primers specific for these KIR genes. This mapping enables verification of presence or absence of a KIR, without discriminating specific heterozygous or homozygote status. A 440/750-bp fragment of the CRP/CAMP, respectively, was included in each PCR as an internal control. The following KIR genes were amplified under identical conditions: 2DL2, 2DL1, 3DL1, 2DS2, 2DS4, and 3DS1. Their PCR thermo-profile comprised 2 min at 92°C; then 25 s at 91°C, 45 s at 65°C, and 45 s at 72°C for 4 cycles; 25 s at 91°C, 45 s at 60°C, and 45 s at 72°C for 26 cycles; 25 s at 91°C, 60 s at 55°C, and 45 s at 72°C for 5 cycles, and 10 min at 72°C (an extension step, in the case of 3DL1 and 3DS1 was set to 2 min). Other KIR amplifications (for 2DL3, 3DL2, and 2DS1) were initiated with denaturation for 2 min at 92°C; then 25 s at 91°C, 45 s at 57°C, and 45 s at 72°C for 4 cycles; 25 s at 91°C, 45 s at 54°C, and 45 s at 72°C for 26 cycles; 25 s at 91°C, 60 s at 50°C, and 45 s at 72°C for 5 cycles, and 10 min at 72°C. All primer sequences, expected PCR products and allelic recoveries are listed in **Table 1**.

The distinction of HLA-C KIR ligand groups C1 (N^{80}) and C2 (K^{80}) was also performed by SSP-PCR. The common HLA-C forward primer was either paired with the C1 (N^{80}) or C2 (K^{80}) reverse primers, leading to an amplification product of 139 bp (**Table 2**). This mapping enables discrimination between heterozygous/homozygous status, i.e., C1–C1, C1–C2, or C2–C2. In each HLA PCR reaction, a 440-bp fragment of the CRP gene was included as an internal PCR control. The thermo-profile comprised an initial denaturation of 3 min at 95°C; then 10 s at 95°C, 30 s at 65°C, and 45 s at 72°C for 10 cycles; 10 s at 95°C, 30 s at 58°C, 45 s at 72°C for 22 cycles, and 10 min at 72°C.

For HLA-C protocol validation, selected samples ($n = 30$; 10 homozygous C1/C1, 10 homozygous C2/C2 and 10 heterozygous C1/C2) that had previously been typed for HLA-C at high resolution by LIFECODES® HLA SSO Typing Kit (Gen-Probe, Inc.) (Later sequenced by Allele SEQR® HLA PCR/Sequencing Kit, Abbott Molecular) were analyzed by our SSP-PCR.

HLA-A BW4 and HLA-B BW4-I^{80}/T^{80} were identified under the same conditions as HLA-C. Validation for HLA-A and B was also performed by selected samples previously genotyped by high

TABLE 1 | KIR genotyping primers.

KIR	Forward primers (5′–3′) / Reverse primers (5′–3′)	Amplicon (bp)	Annealing temp (°C)	Control gene	Alleles not detected
2DS2	CGGGCCCCACGGTTT GGTCACTCGAGTTTGACCACTCA	240	60	CRP	
2DS4	TAGGCTCCCTGCAGTGCG GAGTTTGACCACTCGTAGGGAGC	129	63	CAMP	2DS4*003 2DS4*004 2DS4*006 2DS4*007 2DS4*009
2DS1	CTTCTCCATCAGTCGCAT AGGGTCACTGGGAGCTGACAA	102	53	CAMP	
3DS1	GCCCAGCGCTGTGGTGCCTCGC CTGCAAGGGCACGCATCATGGA	1958	71	CAMP	3DS1*047
2DL2	CTGGCCCACCCAGGTCG GGACCGATGGAGAAGTTGGCT	173	60	CRP	2DL2*004
2DL1	AACTTCTCCATCAGTCGCATGAC GGTCACTGGGAGCTGACAC	100	60	CRP	
2DL3	CTTCATCGCTGGTGCTG AGGCTCTTGGTCCATTACAA	516	54	CAMP	2DL3*007
3DL1	CTCCATCGGTCCCATGATGCT CGCTCTCTCCTGCCTGAACCT	1725	65	CAMP	
3DL2	CCCATGAACGTAGGCTCCG CACACGCAGGGCAGGG	130	54	CRP	

Expected PCR products, annealing temperature (°C), and allelic specificity are given per pair of primers. Primer reference is (22) for all KIRs except 2DS1, for which the primer reference is (23).

resolution methods. All primer sequences [based on Hong et al. (23)] and expected PCR products for HLA-A, -B, and -C genotyping are listed in **Table 2**.

All amplified SSP-PCR products were visualized by agarose gel electrophoresis using a non-mutagenic fluorescent reagent ("Novel Juice"; GeneDireX, Las Vegas City, NV, USA). Selected sample from each amplified band was sent for sequencing.

Statistical Analysis

The Wilcoxon Signed-Ranks test ($p < 0.05$) was determined using SPSS software. When combination of more than one factor to specific receptor was detected, some different aspects were detected. The first refers to all ligands equally, and compares the sum of all ligands (relevant to specific receptor) found in each person to determine the mean value. This is defined as "Ligand Composite" (**Table 7**). Secondly, an alternative designation of "Ligand One+" compares existence of at least one ligand to this specific receptor. This was the case in C1 + 2DL2/2DL3/2DS2, C2 + 2DL1/2DS1 (**Table 8**). In contrast, for overall signaling (**Table 9**) and in the case of A_BW4 + 3DL1/3DS1 and B_BW4 + 3DL1/3DS1 (**Table 7**) only the first option is shown. All HLAB genotyping analysis is described as "total," meaning having I^{80}/T^{80}.

RESULTS

HLA and KIRs Frequency Comparison

Comparison of the nine different KIR genes and HLA frequencies revealed several main differences between parents of autistic children (**Tables 3**–**5**).

The most surprising finding is higher frequency of HLA-C2 allotypes in fathers of ASD children (mean 0.73 vs. 0.52, $p = 0.038$), in contrast to C1 frequencies, which were the same in both mothers and fathers (0.88 vs. 0.91) (**Table 5**). On the other hand, 2DS1, which is known to interact specifically with HLA-C2, was found in higher percentage (0.55 vs. 0.34, $p = 0.022$) in the maternal group (**Table 4**). Additionally 3DS1, with its specificity against BW4 allotypes, was found to be higher in mothers than in their ASD children (0.51 vs. 0.36, $p = 0.044$) (**Table 4**). Other KIR frequencies (2DL2, 2DL1, 2DL3, 3DL1, 3DL2, 2DS2, and 2DS4) were similar in both mothers and fathers, and corresponded with values expected in Asian populations.[2] Most KIR and HLA frequencies in ASD children were similar to those detected in parents.

Positive correlation between parent (mother or father) and child, reflecting the likelihood of transferring the gene (**Tables 3**–**5**) were found relative to most of genes, which may reflect a high probability of homozygous genotyping for these genes.

KIR Signaling Through HLA Molecules

Relevant combinations between KIR receptors and their specific HLA ligands were detected (**Table 6**). ASD children did not exhibit

[2]http://www.allelefrequencies.net/kir6008a.asp

TABLE 2 | PCR primers for human leukocyte antigen (HLA) amplification.

HLA allotype	Direction	Forward primers (5′–3′) / Reverse primers (5′–3′)	Amplicon (bp)
HLA-C	Forward	CGCCGCGAGTCCGAGAGG	139
	Reverse (C1: N^{80})	GTTGTAGTAGCCGCGCAGG	
	Reverse (C2: K^{80})	GTTGTAGTAGCCGCGCAGT	
HLA-A	Forward	CCATTGGGTGTCGGGTTTC	569
BW4:I^{80}	Reverse	CTCTGGTTGTAGTAGCGGAGCGCG	
HLA-B	Forward	ACCCGGACTCAGAATCTCC	366
BW4:I^{80}	Reverse	CTCTGGTTGTAGTAGCGGAGCGCG	
HLA-B	Forward	ACCCGGACTCAGAATCTCC	369
BW4:T^{80}	Reverse	CTCTGGTTGTAGTAGCGGAGCAGG	

Expected PCR product sizes are given. In the case of HLA-C, forward primer was either paired with the C1 (N^{80}) or C2 (K^{80}) reverse primers, leading to an amplification product of 139 bp.
F, forward; R, reverse.

TABLE 3 | Inhibitory KIR frequencies in Israeli families with at least 1 ASD-diagnosed child.

Inhibitory KIR		$Child^{ASD}$	Father	$Child^{ASD}$	Mother	Father	Mother
2DL2	Mean	0.59	0.63	0.58	0.66	0.64	0.68
	Z	−0.577		−0.894		−0.408	
	Pearson's *r*	0.487*		0.204		NR	
2DL1	Mean	0.96	0.98	0.96	0.96	0.98	0.95
	Z	−1		0		−1	
	Pearson's *r*	0.7*		−0.039		NR	
2DL3	Mean	0.86	0.84	0.87	0.89	0.84	0.88
	Z	−0.302		−0.333		−0.535	
	Pearson's *r*	0.135		0.212		NR	
3DL1	Mean	0.94	0.94	0.94	0.89	0.93	0.89
	Z	0		−1.342		−0.707	
	Pearson's *r*	0.645*		0.428*		NR	
3DL2	Mean	1	1	1	1	1	1
	Z	0		0		0	
	Pearson's *r*	NR		NR		NR	

Z = Wilcoxon Signed-Ranks Test value.
NR = not relevant.
**p ≤ 0.01.*

TABLE 4 | Activating KIR frequencies in Israeli families with at least 1 ASD-diagnosed child.

Activating KIR		$Child^{ASD}$	Father	$Child^{ASD}$	Mother	Father	Mother
2DS2	Mean	0.61	0.63	0.6	0.62	0.64	0.66
	Z	−0.302		−0.209		−0.209	
	Pearson's *r*	0.523**		0.086		NR	
2DS4	Mean	0.96	0.96	0.94	0.89	0.94	0.91
	Z	0		−1.342		−0.707	
	Pearson's *r*	0.478**		0.428**		NR	
2DS1	Mean	0.51	0.37	0.49	0.57	0.34	0.55
	Z	−1.528		−1.069		−2.268*	
	Pearson's *r*	0.154		0.478**		NR	
3DS1	Mean	0.37	0.39	0.36	0.51	0.39	0.5
	Z	−0.728		−2*		−1.279	
	Pearson's *r*	0.281*		0.419**		NR	

Z = Wilcoxon Signed-Ranks Test value.
NR = not relevant.
**p ≤ 0.05.*
***p ≤ 0.01.*

TABLE 5 | HLA ligand frequencies in Israeli families with at least 1 ASD-diagnosed child.

HLA Ligand		Child^{ASD}	Father	Child^{ASD}	Mother	Father	Mother
HLAC1	Mean	0.86	0.9	0.83	0.85	0.88	0.91
	Z	−0.707		−0.333		−0.577	
	Pearson's *r*	0.248		0.371**		NR	
HLAC2	Mean	0.65	0.72	0.68	0.57	0.73	0.52
	Z	−0.535		−1.342		−2.058*	
	Pearson's *r*	0.353**		0.214		NR	
HLA-A-BW4	Mean	0.33	0.37	0.3	0.28	0.36	0.3
	Z	−0.577		−0.229		−0.557	
	Pearson's *r*	0.462**		0.134		NR	
HLA-B-BW4	Mean	0.88	0.86	0.88	0.81	0.87	0.8
	Z	0.33		1.272		1	
	Pearson's *r*	0.203		0.248*		NR	

Z = Wilcoxon Signed-Ranks Test value.
NR = not relevant.
$p \leq 0.05$.
**$p \leq 0.01$.*

TABLE 6 | Frequency of each KIR detected and its corresponding ligand in Israeli ASD families, consisting of both parents and an ASD-diagnosed child.

KIR		Child^{ASD}	Father	Child^{ASD}	Mother	Father	Mother
2DL2 + C1	*Z*		−0.832		−1.342		−1.177
	Pearson's *r*		0.474**		0.259		NR
2DL1 + C2	Z		−0.535		−1.091		−1.859
	Pearson's *r*		0.372**		0.176		NR
2DL3 + C1	*Z*		0		0		−0.426
	Pearson's *r*		0.227		0.321*		NR
3DL1 + A_BW4	*Z*		−0.227		−0.243		−0.392
	Pearson's *r*		0.148		0.156		NR
3DL1 + B_BW4	*Z*		−0.302		−1.941*		−1.225
	Pearson's *r*		0.283*		0.359**		NR
2DS2 + C1	*Z*		−0.577		−0.853		−1
	Pearson's *r*		0.511**		0.174		NR
2DS4 + A_BW4	*Z*		−0.577		−0.243		−0.6
	Pearson's *r*		0.393**		0.156		NR
2DS4 + B_BW4	*Z*		−0.302		−1.941*		−1.279
	Pearson's *r*		0.211		0.359**		NR
2DS1 + C2	*Z*		−0.243		−0.775		−0.784
	Pearson's *r*		0.132		0.345**		NR
3DS1 + A_BW4	*Z*		0		−0.577		−0.535
	Pearson's *r*		0.456**		0.12		NR
3DS1 + B_BW4	*Z*		−0.728		−1.414		−0.853
	Pearson's *r*		0.25		0.295*		NR

Z = Wilcoxon Signed-Ranks Test value.
NR, not relevant.
$p \leq 0.05$.
**$p \leq 0.01$.*

high frequency of the combination HLA-C2 + KIR2DS1, despite elevated paternal HLA-C2 and maternal 2DS1 frequencies.

Correlational analysis between parent (mother or father) and child reflected the likelihood of transferring a gene with its corresponding ligand. Significant values were reported in most cases (**Table 6**). It is important to mention, these gene families are located on different autosomal chromosomes, supporting homozygous status for most of the genes.

The next evaluation involves the examination of particular HLA ligands with presence/absence of all relevant activating/inhibitory KIR genes (**Table 7**). "Ligand Composite" analysis (see Materials and Methods) was compared between ASD children and their parents, as well as "Ligand One+" values (**Table 8**). Significant differences were found between parental groups in the case of C2 + 2DL1/2DS1 "Ligand One+" ($p = 0.040$).

Overall Balance of Activation and Inhibition

Final analysis (**Table 9**) presents a full view of the genotyping. It may allow insight to the overall expected interaction of KIR/HLA in Israeli autistic individuals and their non-autistic parents,

TABLE 7 | Comparison of particular HLA ligands with presence/absence of all relevant activating/inhibitory KIR genes in Israeli ASD families.

Ligand composite		Child^{ASD}	Father	Child^{ASD}	Mother	Father	Mother
C1 + 2DL2/2DL3/2DS2	*Z*	−0.363		−0.779		−1.178	
C2 + 2DL1/2DS1	*Z*	−0.213		−0.288		−0.704	
A_BW4 + 3DL1/3DS1	*Z*	−0.216		−0.378		−0.429	
B_BW4 + 3DL1/3DS1	*Z*	−0.382		−0.177		−0.28	

Z = Wilcoxon Signed-Ranks Test value.

TABLE 8 | Interactions of KIR and HLA in autistic Israeli patients and their parents, based on overall genotype mapping.

Ligand one+		Child^{ASD}	Father	Child^{ASD}	Mother	Father	Mother
C1 + 2DL2/2DL3/2DS2	*Z*	−0.707		−0.333		−0.577	
C2 + 2DL1/2DS1	*Z*	−0.535		−1.342		−2.058*	

Z = Wilcoxon Signed-Ranks Test value.
*$p \leq 0.05$.

TABLE 9 | Overall ligand and interaction summaries.

		Child^{ASD}	Father	Child^{ASD}	Mother	Father	Mother
KIRs: activating	Mean	2.4043	2.3191	2.3962	2.5849	2.2593	2.6667
	Pearson's *r*	0.331*		0.274*		0.104	
	t-Test	0.443		−1.121		−2.015*	
KIRs: inhibitory	Mean	4.3469	4.3878	4.3585	4.3962	4.4	4.3818
	Pearson's *r*	0.229		−0.023		0.153	
	t-Test	−0.362		−0.286		0.155	
Interactions: activating	Mean	1.9149	1.9787	1.9245	2.0377	1.9259	2.1111
	Pearson's *r*	0.216		0.142		−0.119	
	t-Test	−0.308		−0.636		−0.778	
Interactions: inhibitory	Mean	2.6939	2.7959	2.6981	2.6038	2.8214	2.6607
	Pearson's *r*	0.272		0.114		−0.099	
	t-Test	−0.647		0.582		0.903	

*$p \leq 0.05$.
**$p \leq 0.01$.

based on all genotype mapping. Mothers have significantly higher levels of activating signals than their male counterparts ($p = 0.049$).

DISCUSSION

Despite the increased attention garnered by autism research in recent years, the condition is currently diagnosed only after the infant reaches 2 or 3 years of age. Findings in autistic patients indicate multifactorial inheritance, while definitive genetic factors can be diagnosed in only a small percentage of cases. Immunological studies revealed a unique and distinguishing profile in ASD patients compared to controls. In the context of NK cells, altered gene expression, absolute number and function have been reported (7, 24, 25). Reports of modified NK function led us to investigate the highly polymorphic cell surface proteins on NK cells, called KIRs, to search for genetic background underlying the immune abnormalities. By interacting with MHC class I proteins expressed on all cell types, KIRs regulate the killing function of NK.

The most significant finding in this study is the unequal distribution of HLA-C2 (higher in fathers of ASD children) known to interact with KIR 2DS1 (higher in mothers of ASD children) to activate NK function. Analysis of "overall activation" (**Table 9**) signaling pointed to higher activation in maternal than paternal cohorts. Most HLA and KIR frequencies in ASD children ranged between those of their parents, indicating regular independent genetic inheritance from parents to children. This reinforced the importance of the specific HLA–KIR combinations between parents, though the mechanisms involved are still unclear. Our limitation in interpreting these findings lies in the absence of unusual prevalence of these genes or their combination in the ASD children, in comparison to their healthy parents.

Some reports correlated these initial interactions between parental cells as a possible mechanism for subsequent disease development or other complications. Hiby et al. (26) reported an increased HLA-C2 frequency in different cohorts of affected pregnancies: (a) recurrent miscarriage (RM) couples and their conception products, (b) fetal growth restriction (FGR), and (c) preeclampsia. Conversely, lack of activating KIR (in women affected by RM) or increased inhibitory KIR (in women with

preeclampsia) (26, 27) has already been reported. Specifically, KIR 2DS1 seemed to be protective because of its significant lack in RM women. We find this plausible, since activating KIR 2DS1 compete with inhibitory KIR 2DL1 in interaction with HLA-C2, such that a predominance of KIR 2DS1 can overcome the strong inhibitory effect mediated by KIR 2DL1. All these findings support the importance of the innate immune system (mediated by maternal uterine NK and fetal trophoblast cells), modulated by KIR–HLA interaction to play a beneficial role in reproductive success.

Some papers have already pointed out differential segregation of KIR–HLA genes in autistic patients. Our study expands upon this by focusing within ASD families. Torres et al. (28) examined a large autistic cohort, revealing a highly significant increase in the activating KIR gene 2DS1 and its cognate HLA-C2 ligand. This increase in activating KIR may explain, at least partially, the high frequency of autoimmune diseases in autism patients. Guerini et al. (29) compared ASD children to their non-ASD mothers and showed both that activating KIR/HLA combinations were increased and inhibitory KIR/HLA were reduced in ASD children. This difference was proposed as having resulted from the genetic influence of the mothers, as these molecules' expression and interaction are relevant in the surrounding uterus.

Ethnic populations are known to differ in KIR genotype frequencies and genotype content (30). To the best of our knowledge, this is the first study to research KIR and HLA combinations in the Israeli Jewish population. As shown in the Allele Frequency Net Database,[3] our geographical location has higher frequency of HLA-C2 (around 0.4–0.7 in Africa and the Middle East). However, we showed here a significant skewing between maternal and paternal C2 frequencies. It raises an interesting question, since HLA clusters map in autosomal chromosomes, leading to independent segregation without respect to gender.

Genotype mapping of this system has certain limitations. While theoretically all gene segments identified by our SSP-PCR system may be expressed on the cell surface (meaning the functional KIR repertoire may depend on the KIR genotype), the transcription and translation processes may lead to different phenotype profiles. Another limitation of the SSP-PCR method in this context is the inability to determine the heterozygous/homozygous status of each of these KIRs. Our future plans are to determine not only genotype, but also RNA and protein levels. Another point to take into account is that NK cell function is a very complicated process determined upon the balance of an array of receptors, of which KIR is only one family. Moreover, the affinity of these interactions may differ (e.g., KIR 2DL3:HLA-C1 interaction is thought to be weak, compared to KIR 2DL2:HLA-C1 or KIR 2DL1:HLA-C2), such that one interaction may wield greater influence than another. It is also becoming increasingly clear that genetic factors, such as promoters and epigenetic mechanisms, are important in controlling NK cell receptor expression and function.

This study comprised a relatively small cohort of ASD-affected families – though statistically verified based on the minor allele frequency (MAF) of C2 (39.5%). In the absence of a control group, our findings were compared with the general population data of our geographical area. Additionally, the clinical patient data have not been thoroughly listed here, places limitations upon the reader in drawing parallels to other patient cohorts. The trends revealed in this preliminary investigation need to be further corroborated in ongoing and expanded research efforts.

Finally, we would like to emphasis another interesting aspect. On the one hand, different pregnancy complications (FGR, spontaneous abortion, placental abruption) are known to stem (at least to some degree) from immunological factors. On the other hand, perinatal factors unrelated to the immunological milieu may also be important in the pathogenesis of ASD. Certain complications during gestation were found to occur with increased frequency in mothers of ASD patients; an immunological basis has been suggested as underlying a portion of these. In some cases, intermediate factors may play a partial role in the association (e.g., reduced birth weight).

CONCLUSION

The aim of our research was to genotype the KIR receptors and their relevant HLA ligands in autistic children and their non-autistic parents, in order to screen for possible unique combinations. The most interesting finding in this study is higher frequency of HLA-C2 allotypes in the paternal group besides higher percentage of KIR 2DS1 in the maternal group. Such interaction may lead to NK activation. An absence of unusual prevalence of these genes or their combination in the ASD children, limited our ability to interpret these findings. Moreover, analysis of "overall activation" signaling pointed to higher activation in maternal than paternal cohorts.

AUTHOR CONTRIBUTIONS

Mrs. MG is a Ph.D. student. She is the major contributor to this manuscript doing the thinking, bench work, and manuscript writing. Mrs. KA contributed to the bench work as well as writing the manuscript. Prof. NY is an initiator of the original study and has performed the clinical diagnosis of all the participants of the study. Prof. RE is the initiator of original study recruited the families and the original head of the lab. Dr. DM is the head of the lab the study was conducted in, supported the study, and contributed to the manuscript writing and editing.

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[3] http://www.allelefrequencies.net/

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ASD and Genetic Associations with Receptors for Oxytocin and Vasopressin—*AVPR1A, AVPR1B*, and *OXTR*

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Background: There are limited treatments available for autism spectrum disorder (ASD). Studies have reported significant associations between the receptor genes of oxytocin (OT) and vasopressin (AVP) and ASD diagnosis, as well as ASD-related phenotypes. Researchers have also found the manipulation of these systems affects social and repetitive behaviors, core characteristics of ASD. Consequently, research involving the oxytocin/vasopressin pathways as intervention targets has increased. Therefore, further examination into the relationship between these neuropeptides and ASD was undertaken. In this study, we examined associations between variants in the receptor genes of vasopressin (*AVPR1A, AVPR1B*), oxytocin (*OXTR*), and ASD diagnosis along with related subphenotypes.

Methods: Probands were assessed using Autism Diagnostic Interview-Revised, Autism Diagnostic Observation Schedule, and clinical DSM-IV-TR criteria. Single nucleotide polymorphisms (SNPs) in *AVPR1B* and *OXTR*, and microsatellites in *AVPR1A* were genotyped in ∼200 families with a proband with ASD. Family-based association testing (FBAT) was utilized to determine associations between variants and ASD. Haplotypes composed of *OXTR* SNPs (i.e., rs53576-rs2254298-rs2268493) were also analyzed due to previously published associations.

Results: Using the additive inheritance model in FBAT we found associations between *AVPR1B* SNPs (rs28632197, $p = 0.005$, rs35369693, $p = 0.025$) and diagnosis. As in other studies, *OXTR* rs2268493 ($p = 0.050$) was associated with diagnosis. rs2268493 was also associated with ASD subphenotypes of social withdrawal ($p = 0.013$) and Insistence on Sameness ($p = 0.039$). Further analyses demonstrated that the haplotype, rs2254298–rs2268493 was found to be significantly associated with diagnosis (A-T; $p = 0.026$). FBAT was also used to analyze *AVPR1A* microsatellites (RS1 and RS3). Both length variants were found to be associated with restrictive, repetitive behaviors, but not overall diagnosis. Correction for multiple comparisons was performed for SNPs tested in each gene region, only *AVPR1B* SNPs remained significantly associated with ASD diagnosis.

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Conclusions: Autism is a heterogeneous disorder with many genes and pathways that contribute to its development. SNPs and microsatellites in the receptor genes of OT and AVP are associated with ASD diagnosis and measures of social behavior as well as restricted repetitive behaviors. We reported a novel association with ASD and *AVPR1B* SNPs. Understanding of genotype-phenotype relationships may be helpful in the development of pharmacological interventions for the OT/AVP system.

Keywords: neuropeptides, oxytocin, vasopressin, receptors, social behaviors, repetitive behaviors

INTRODUCTION

Autism spectrum disorder (ASD) is a heterogeneous disorder that is characterized by impaired social communications and interactions, and restricted, repetitive behaviors (American Psychiatric Association, 2013). The range of clinical presentations suggest multiple etiologies which makes searching for mechanisms and genetic associations challenging. While many genes and pathways have been associated with ASD, the close relationship established between the oxytocin (peptide: OT, gene: *OXT*) and vasopressin (peptide: AVP, gene: *AVP*) systems to social and restricted repetitive behaviors (RRBs) have made these systems a focal point as investigators work to elucidate potential pathophysiological mechanisms and treatments pathways for ASD.

OT and AVP, two closely related neuropeptides, are conserved in both structure and function across mammalian species. While the relationship between these neuropeptide systems and behaviors are complex (Appenrodt et al., 1998; Chang et al., 2012), in general, OT facilitates prosocial and "approaching" behaviors, social memory and recognition, and reduction in anxiety and reaction to stressors (Carter, 1998; Ferguson et al., 2000; Kosfeld et al., 2005; Seltzer et al., 2010; Neumann and Landgraf, 2012). AVP has been associated with animal territoriality and "defensive" behaviors, including sexual cues in human males, social hierarchy, and anxiogenic effects (Landgraf and Wigger, 2003; Guastella et al., 2011; Van der Kooij and Sandi, 2015). It has been noted while closely related, these systems can have opposing physiological effects. OT has primarily parasympathetic actions on the autonomic nervous system, and AVP induces reactions in the sympathetic nervous system and hypothalamic-pituitary-adrenal axis (Sawchenko and Swanson, 1985; Kenkel et al., 2012).

The systems also interact with each other: at high levels the two peptides can act as partial agonists for their homologous receptors (Chini et al., 1996). In a 2014 study of threat response in cats, AVP increased anxiety via the vasopressin receptor 1B and OT increased social affiliation during threat via the vasopressin receptor 1A (Bowen and McGregor, 2014). The receptors of OT and AVP have also been implicated in these behaviors and disorders characterized by impairments in social behaviors including ASD and related subphenotypes (Kim et al., 2002; Wassink et al., 2004; Wu et al., 2005; Lerer et al., 2008; Yang et al., 2010a,b; LoParo and Waldman, 2015). Animal studies which established the involvement of OT and AVP systems in social and repetitive behaviors as well as genetic associations between the neuropeptide systems and disorders with social impairment (i.e., ASD), strengthen the theories to target the OT and AVP systems in the treatment of disorders with social impairments.

Investigators have attempted to influence the oxytocinergic system by directly administering OT. Presently there are over a hundred clinical trials, including 11 in ASD, utilizing intranasal OT (INOT) according to clinicaltrials.gov (as of April 25, 2016). In individuals with ASD, INOT has been shown to increase social interaction (in a simulated ball game task) and improve the identification of emotions (Andari et al., 2010; Guastella et al., 2010). The other part of this equation is the target of OT, the oxytocin receptor (receptor: OTR; gene: *OXTR*). Similar to OT, protein expression of OTR has also been associated with social behaviors including maternal nurturing (Takayanagi et al., 2005). In animal studies, Takayanagi et al. (2005) found $Oxtr^{-/-}$ dams had deficits in pup retrieval, a maternal behavior. In addition, research in the last 20 years has found links between *OXTR* and ASD. In a 2005 study, evidence of transmission disequilibrium of two *OXTR* single nucleotide polymorphisms (SNPs; rs2254298 and rs53576) was observed in a Han Chinese ASD sample (Wu et al., 2005). These results were followed up by research in other ethnic populations. Jacob et al. (2007) found an association between rs2254298, but not rs53576, and ASD in a Caucasian sample. In a Japanese sample, Liu et al. (2010) observed significant differences in allelic frequency in rs2254298 between controls and individuals with ASD. In more recent studies, Skuse et al. (2014) investigated associations between 60 tagged *OXTR* SNPs and social recognition skills in ASD families from the UK and Finland. They found rs237887 associated with the Face Recognition Memory Task not only in ASD individuals, but their mothers, fathers and non-affected siblings as well. They also noted an association between rs237865 and diagnosis, but it did not remain after Bonferroni correction. Two recent meta-analyses (LoParo and Waldman, 2015; Kranz et al., 2016) reported associations between *OXTR* and ASD. LoParo and Waldman (2015) reported associations between ASD and the *OXTR* SNPs, rs7632287, rs2268491, and rs2254298 in eight studies and 11 independent samples; analyzing two independent samples and 10 additional studies, Kranz et al. (2016) found rs237889 to be associated with ASD.

Studies exploring the use of intranasally administered AVP (IN-AVP; or similar compound desmopressin, DDAVP) are

Abbreviations: RRB(s), Restrictive/Repetitive Behavior(s); SNP(s), Single Nucleotide Polymorphism(s).

fewer than INOT, yet the involvement of the AVP system in mammalian social behavior is also important. As mentioned before OT can interact with AVP receptors, making AVP receptors not only a target for AVP and pharmacology that mimic AVP, but for OT agonists as well. Often researchers have examined three nucleotide repeats: two located in the 5′ promoter region (RS1, RS3) and one in the intron (AVR) of *AVPR1A*. In both patient and non-patient populations the lengths of these repeats have been shown to modulate social behaviors including altruistic behavior in healthy adults, prepulse inhibition, and the processing of facial expressions (Knafo et al., 2008; Levin et al., 2009; Zink and Meyer-Lindenberg, 2012; Wang et al., 2016). In an ASD sample, evidence of disequilibrium of the *AVPR1A* microsatellite RS3 was observed in 115 trios (Kim et al., 2002). A few years later, Wassink et al. (2004) found evidence of linkage disequilibrium in an ASD sample as well. In 2010, Yang and colleagues noted an association between ASD and RS1 and RS3 in a Korean sample (Yang et al., 2010b). Tansey et al. (2011) found a significant association between ASD and *AVPR1A* SNPs, but only a weak association with the microsatellites in an Irish sample.

The role of *AVPR1B* has been investigated in some disorders, but not in ASD. Studies have shown that *Avpr1b* knockout mice display decreased ultrasonic vocalization in social situations throughout their lifespans, decreased aggression, and altered social behavior affiliated with dominance (Scattoni et al., 2008; Caldwell et al., 2010; Pagani et al., 2015). The relationship between *Avpr1b* and aggression was strengthened by the Pagani et al. (2015) study, which showed the rescue of aggression by targeted expression of *Avpr1b* in the hippocampus. Additionally, Caldwell et al. (2010) observed increased aggression in $Avpr1b^{-/-}$ mice under specific social conditions including competition, food deprivation, and social experience. In humans, SNPs in *AVPR1B* have been linked to child aggression, childhood-onset mood disorders (COMD), prosociality, and autistic traits (Dempster et al., 2007, 2009; Chakrabarti et al., 2009; Zai et al., 2012; Wu et al., 2015). In 2015, Wu et al. observed a significant association between prosociality (mediated through emotional empathy) and *AVPR1B* in a non-clinical Han Chinese male sample. Chakrabarti et al. (2009) noted a nominally significant association between *AVPR1B* and the empathy quotient (EQ) also in a non-clinical sample.

OXTR, *AVPR1A,* and *AVPR1B* are genes that emerged evolutionarily through duplication events and have both paralogous and orthologous relationships in placental mammals (Paré et al., 2016). Given their evolutionary connections, roles in social behavior, and potential as treatment target pathways in ASD, we investigated their genetic variation within an ASD population. *OXTR*, *AVPR1A,* and *AVPR1B* SNPs or microsatellites were genotyped in ∼200 families and analyzed using family based association testing (FBAT). *OXTR* and *AVPR1A* genetic associations have been investigated in ASD previously, but to our knowledge *AVPR1B* variants have not been studied.

MATERIALS AND METHODS

Subjects were recruited through the Developmental Disorders Clinic of the University of Illinois at Chicago (UIC) Institute for Juvenile Research, referral from providers of autism services, a website providing information about the study, and parent advocacy organizations with the approval of the UIC Institutional Review Board [IRB#: 2007-0239; Title: Interdisciplinary Studies of Insistence on Sameness (IS) in Autism Spectrum Disorders (ASD)]. All participants were provided with a description of the study to obtain informed written consent from adults able to consent for themselves, and parents or guardians of minors and individuals unable to consent prior to their first session.

Participants

For each participant, a medical history and physical examination were performed by a pediatric neurologist or child psychiatrist and psychiatric evaluation by a child psychiatrist experienced in ASD. Participants were diagnosed using the Autism Diagnostic Observation Schedule (ADOS; Lord et al., 1999; Gotham et al., 2007), Autism Diagnostic Interview-Revised (ADI-R; Rutter et al., 2003; Risi et al., 2006), and confirmed by a physician according to the DSM-IV-TR (American Psychiatric Association, 2000) ASD classification (including autism, Asperger disorder, or pervasive developmental disorder-not otherwise specified (PDD-NOS)). While subjects were not diagnosed with comorbidities, individuals were selected to enrich the IS traits of the sample, which may have increased the probability of comorbidity with certain traits and disorders (i.e., OCD, high anxiety). Additionally, individuals on psychotropic medications were excluded. We performed SNP and microsatellite analysis on two nearly identical samples (**Table 1**). Two hundred and seven probands including 156 trios and 51 single parent-child duo families were genotyped for SNP analysis. The microsatellite sample consisted of 210 probands of which 206 probands overlapped with the SNP sample (**Table 1**).

Instruments and Assessments

The instruments utilized to assess clinical presentation, including social abilities and RRBs, were appropriate for the subjects' ages and abilities.

Autism Diagnostic Observation Schedule (ADOS): The ADOS is a standardized, interactive, semi-structured assessment administered by a trained professional resulting in a standardized score that encompasses social interaction, communication, and restricted interests and repetitive behaviors. The Social Affect domain evaluates communication and reciprocal social interaction including behaviors such as: unusual eye contact, quality of social overtures, initiation of joint attention, amount of facial expression directed at others, and frequency of spontaneous vocalization directed to others gestures (Hus et al., 2014; Hus Bal and Lord, 2015).

Autism Diagnostic Interview-Revised (ADI-R): The ADI-R is a structured interview between a trained professional and the parent or guardian of the individual. The assessment

TABLE 1 | Demographic description of the probands analyzed.

	SNP analysis	MS Analysis
Number of participants	207	210
Average age (years)	9.89 (±5.48 *SD*)	10.23 (±6.25 *SD*)
Gender	M: 170 (82.1%)	M: 173 (82.4%)
	F: 37 (17.9%)	F: 37 (17.6%)
Race	*N* = 207	*N* = 208
Caucasian/Western European	154 (74.4%)	155 (73.8%)
African American/African	35 (16.9%)	35 (16.7%)
Asian	8 (3.9%)	8 (3.8%)
More than one race	10 (4.8%)	10 (4.8%)
IQ		
FSIQ	79.93 (±24.03 *SD*; *N* = 193)	80.70 (±24.49 *SD*; *N* = 194)
VIQ	77.50 (±26.01 *SD*; *N*=196)	78.41 (±26.64 *SD*; *N* = 197)
NVIQ	82.12 (±23.86 *SD*; *N* = 204)	82.63 (±24.12 *SD*; *N* = 205)

Demographic data describing the probands utilized for SNP and microsatellite (MS) analyses. The two samples overlap, 206 probands are in both the SNP and microsatellite analyses.

measures behaviors that include reciprocal social interaction, communication and language, and patterns of behaviors. The Insistence upon Sameness domain is comprised of three ADI-R items: (1) compulsions and rituals, (2) resistance to changes in personal routine, and (3) resistance to change in environment (Hus et al., 2007).

Repetitive Behavior Scale-Revised (RBSR): The RBSR assesses the presence, characterization, and severity of RRBs in individuals with developmental disorders (Bodfish et al., 2000). It is a 43-item form that categorizes RRBs into five factors—compulsive behaviors, ritualistic and sameness behaviors, restricted interests, stereotyped behaviors, and self-injurious behaviors (Lam and Aman, 2007).

Aberrant Behavior Checklist—Community Version (ABC-CV): The ABC-CV (Aman et al., 1985) is a five-factor, 53-item questionnaire completed by the parent or guardian. The ABC assesses symptoms of irritability and agitation, social withdrawal (lethargy), stereotypic behavior, hyperactivity and non-compliance, and inappropriate speech in individuals aged 6–54 years.

Genotyping

SNPs

We selected two *AVPR1B* and 13 *OXTR* SNPs to genotype based on the literature. DNA was extracted from 10 mL of blood using PureGene® DNA Purification Kit. Next, DNA was quantified with Quant-iT™ PicoGreen® dsDNA Assay (Invitrogen, Carlbad, CA) and the samples normalized to 10 ng/mL. TaqMan® SNP genotyping assays (Applied Biosystems™, Foster City, CA) were then utilized to perform blinded genotyping of the samples. Standard TaqMan® SNP genotyping protocols were used. TaqMan® PCR reactions were performed in 5–2.50 μL Universal Master Mix Amperase® UNG, 0.125 μL TaqMan® probe mix, and 2.375 μL water. PCR conditions (Applied Biosystems™ GeneAmp® PCR System 9700; Foster City, CA) were: OneAmpErase® step at 50.0°C for 2 min, one enzyme activation step at 95.0°C for 10 min, 40–99 alternating cycles of denaturation at 92.0°C for 15 s, and annealing and extension at 58.0°C for 1 min. We used a Roche Light Cycler Model 480-II and Roche Light Cycler 480 Gene Scanning Software v.1.5 (Hoffmann–La Roche AG, Basel, Switzerland) to measure fluorescence intensity of each allele of the final PCR product.

Microsatellites

Utilizing primers and protocols from Kim et al. (2002), we genotyped probands, mothers, and fathers blinded to the affect status, family relationship, and demographic data. To summarize, the protocol consisted of multiplex PCR reactions containing 50 ng of DNA, 200 _M dNTPs, 2.5 mM MgCl2, and 0.3 units of AmpliTaq Gold DNA Polymerase (Applied Biosystems™, Foster City, CA) in a 10 μl volume. Microsatellite peaks were measured on the Applied Biosystems 3730xl and sized with Genemapper 3.7 (Applied Biosystems™, Foster City, CA, USA).

Analysis

Prior to analyzing the SNP data, we checked for genetic Mendelian errors and Hardy-Weinberg equilibrium. Utilizing PLINK v1.07 (Purcell et al., 2007), all but two SNPs were in Hardy-Weinberg equilibrium (p-values > 0.05) in the overall sample (note rs237851 and rs2268493: $p < 0.05$ in all parents). Mendelian errors were rare and excluded from analysis on a per SNP basis (the most Mendelian errors were observed in *OXTR* rs11720238 with a total of three). We tested for associations between *OXTR* and *AVPR1B* SNPs, ASD diagnosis, and scores from assessments of social behavior and RRBs. Tests were carried out using *FBAT v2.0.3* (Laird et al., 2000; Rabinowitz and Laird, 2000). FBAT is a non-parametic test of linkage or association between the genotype and the phenotype in which the covariance between the phenotype and offspring genotype is estimated. Tests were conducted assuming an additive inheritance model. Three *OXTR* SNPs (rs53576, rs2254298, and rs2268493) were selected based upon their previous associations with ASD in the literature for haplotype analysis (bi-allelic mode; additive inheritance model). We tested the three marker haplotype and the two marker permutations. The nucleotide repeats of *AVPR1A* were analyzed using a similar methodology. Using FBAT, microsatellite data were checked for Mendelian errors. Mendelian errors were excluded from analysis on a per marker (RS1 and RS3) basis. Next using an additive inheritance model, we used FBAT to examine associations between the genetic variants of *AVPR1A* and ASD diagnosis and other related phenotypes.

RESULTS

The relationships between genetic variations in the receptor genes of OT and AVP, and ASD diagnosis and ASD-related phenotypes were examined in our analysis. *AVPR1B* SNPs,

rs35369693 ($p = 0.025$) and rs28632197 ($p = 0.006$), were associated with ASD diagnosis and remained significant after correction for multiple comparisons for SNPs tested in *AVPR1B* (**Table 2A**). Next, we explored possible relationships with ASD-related phenotypes. No further associations were noted with *AVPR1B* SNPs (*p*-values were $p > 0.100$ for social communication and restrictive/repetitive subphenotype scores).

OXTR rs2268493 ($p = 0.050$) was associated with ASD diagnosis, but did not remain significant after multiple comparison correction for the number of *OXTR* SNPs that were tested. We also examined ASD-related subphenotypes and found associations with Several *OXTR* SNPs (**Table 2B**). rs2268493 was not only associated with ASD diagnosis, but with measures of social behaviors as well as RRBs (ABC-Social Withdrawal: $p = 0.013$; ADI-R IS as a restricted behavior: $p = 0.039$). Similarly, rs4686302 was another SNP found to be significantly associated with both subphenotype categories within ASD (ABC-Social Withdrawal: $p = 0.036$; ABC-Stereotypy: $p = 0.018$). We also found rs2254298 to be significantly associated with ADOS-Social Affect ($p = 0.023$). No further associations were noted for the remaining *OXTR* SNPs and the characterized subphenotypes (*p*-values were $p > 0.054$). None of the subphenotype associations were significant after correcting for multiple comparisons. Additionally, we performed a haplotype analysis utilizing FBAT. Both rs53576 and rs2254298 have been actively studied variants in the ASD literature (Wu et al., 2005; Jacob et al., 2007; Lerer et al., 2008; Liu et al., 2010; Wermter et al., 2010; Campbell et al., 2011; Di Napoli et al., 2014), therefore, they were used along with rs2268493 to create our haplotype. Haplotype analysis yielded significant associations with ASD diagnosis and both ASD-related phenotypes (**Table 3**). The two marker haplotype rs2254298–rs2268493 (A-T: $p = 0.026$) was significantly associated with ASD diagnosis. This same haplotype was significantly associated with social behaviors as measured by ADOS (Social Affect: $p = 0.016$) and ABC (Social Withdrawal: $p = 0.036$). Overall, haplotypes were associated with ASD diagnosis, RRBs as assessed by ADOS, and social behaviors measured by ADOS and ABC.

FBAT was also utilized to examine relationships between microsatellites in *AVPR1A*, *ASD,* and subphenotypes. No main effects were noted; however, significant associations were observed with assessments measuring RRBs. Length variants in both RS1 and RS3 were found to be significantly associated with different measures of RRBs (**Table 4A**). Some studies have categorized the variant lengths as short (S) and long (L) due to the variation of defining lengths across studies (i.e., RS1 306 was analogous to RS1 308 and RS1 312 in different studies—see Table 2 in Kantojärvi et al., 2015). While we analyzed the microsatellite data using individual lengths, afterwards for ease of comparison with other published studies we categorized the length variant values as S or L. The cutoff was determined by creating two approximately equal groups (Knafo et al., 2008). The split occurred between the highest and second highest frequencies reported in our sample (**Table 4B**). Utilizing S/L categories, we noted long variants of RS1 and RS3 were associated with different measures of RRBs in our sample. We found a significant association with a short length variant (RS1 S) and RRBs as measured by the RBSR.

DISCUSSION

The core symptoms of ASD include impairments in social communication and the presence of RRBs. Given the well-established links between the OT/AVP system and these behaviors, genetic variants and associations were investigated in this ASD sample. We used FBAT to examine associations between receptor genes of these neuropeptides and ASD. The variants in *AVPR1B* (SNPs—rs35369693 and rs28632197), *AVPR1A* (two microsatellites—RS1 and RS3), and *OXTR* (13 SNPs) were

TABLE 2 | Significant SNP associations.

A

SNP	*p*-value	Z	Risk Allele
AVPR1B rs35369693*	0.025	2.24	G
AVPR1B rs28632197*	0.006	2.77	A
OXTR rs2268493	0.050	1.96	T

B

Phenotype		SNP	*p*-value	Z	Allele	Risk Allele
Social communications	ABC—Social withdrawal	OXTR rs4686302	0.036	–2.09	T	C
		OXTR rs2268493	0.013	–2.49	C	T
	ADOS—Social affect	OXTR rs2254298	0.023	2.27	A	A
Restricted/Repetitive behaviors	ABC—Stereotypy	OXTR rs4686302	0.018	2.37	C	C
	ADOS—RRB	OXTR rs1042778	0.011	–2.54	T	G
	ADI—IS	OXTR rs2268493	0.039	–2.07	C	T

*(A) list the SNPs in both OXTR and AVPR1B that were found to be significantly associated with ASD diagnosis. (B) notes the SNP significantly associated with ASD-related phenotypes, mainly social communications and RRBs, in our sample using the additive inheritance model. None of the AVPR1B SNPs were significantly associated with the different subphenotypes ($p > 0.100$). The remaining OXTR SNPs were also not significantly associated with the subphenotypes ($p > 0.054$). * denotes significant after correcting for multiple comparisons.*

TABLE 3 | Haplotype analysis using three markers from *OXTR*.

Phenotype	Markers			Haplotype			*p*-value	Z
ASD		rs2254298	rs2268493		A	T	0.026	2.23
ADOS−-Social affect	rs53576	rs2254298	rs2268493	G	A	T	0.008	2.63
	rs53576	rs2254298		G	A		0.017	2.40
		rs2254298	rs2268493		A	T	0.016	2.41
ABC−-Social withdrawal	rs53576	rs2254298	rs2268493	G	G	C	0.009	−2.59
	rs53576		rs2268493	G		C	0.009	−2.62
		rs2254298	rs2268493		A	T	0.036	2.10
ADOS−-RRB	rs53576	rs2254298	rs2268493	A	G	C	0.032	−2.15
	rs53576		rs2268493	A		C	0.024	−2.25

Haplotypes were created from three SNPs (rs53576, rs2254298, and rs2268493) previously associated with ASD. Data were analyzed for associations with ASD diagnosis and subphenotypes.

studied in association with ASD diagnosis, as well as scores from assessments measuring deficits in social behaviors and RRBs.

To our knowledge, there are no published studies reporting *AVPR1B* associations with ASD. Our results showed that both *AVPR1B* SNPs, rs35369693, and rs28632197, were significantly associated with ASD diagnosis (**Table 2A**). Variants within *AVPR1B* have been linked to social behavior in humans and other mammals. As mentioned earlier, *Avpr1b*$^{-/-}$ mice show decreased aggression and altered dominance behavior (Caldwell et al., 2010; Pagani et al., 2015). Human studies including our results have linked *AVPR1B* to disorders with social components including bipolar type I, depression, autistic traits as measured by EQ, childhood aggression, COMD, suicidal attempts, prosociality, and emotional empathy (Dempster et al., 2007; Chakrabarti et al., 2009; Leszczynska-Rodziewicz et al., 2012; Zai et al., 2012; Szczepankiewicz et al., 2013; Luppino et al., 2014; Wu et al., 2015). Specifically, Zai et al. (2012) found rs3536969C to be underrepresented in 177 aggressive child cases as compared to adult matched controls. This finding remained significant when a homogenous European Ancestry subsample was analyzed. In a Hungarian sample (382 nuclear families), Dempster et al. (2007) found rs35369693 to be significantly associated with COMD. They also noted a sex difference: when divided by sex, the association remained significant for affected females. Additionally, associations between anxiety and panic disorders and *AVPR1B* rs28632197 have been observed (Keck et al., 2008). Keck and colleagues compared individuals with panic disorder with matched controls and found nominal associations with panic disorder and *AVPR1B*, including rs28632197 ($p = 0.046$), in their main sample (not significant in their replication sample or the combined sample). They also examined interactions between *AVPR1B* and *CRHR1* in their samples. Two SNP pairs, *AVPR1B* rs28632197—*CRHR1* rs878886 and *AVPR1B* rs28632197—*CRHR1* rs187631, were also found to be significantly associated with panic disorder. While previous findings were not conducted in an ASD sample, anxiety, aggression, and depression are often co-morbid issues along with restrictive behaviors and social challenges in ASD (Giles and Martini, 2016; Russell et al., 2016).

The *OXTR* SNP, rs2268493, was not only related to diagnosis, but also with scales measuring impairments in social behaviors and RRBs (**Tables 2A,B**). Both subphenotypes, social behaviors and RRBs, are characteristic of ASD. While these findings did not withstand correction for multiple comparisons, they replicated similar findings in the literature. Yrigollen et al. (2008) found rs2268493 to be significantly associated with diagnosis, communication skills, and stereotyped behaviors. Subsequently, Campbell et al. (2011) used a narrow diagnosis of autism in an Autism Genetic Resource Exchange (AGRE) sample and found rs2268493T to be significantly associated with their diagnosis criteria. In schizophrenia, rs2268493T has also been associated with social cognition (Davis et al., 2014). Decreased activity in the mesolimbic reward circuitry during reward anticipation in typically-developed adults was associated with the T homozygotes of rs2268493 (Damiano et al., 2014). Additionally, as part of a haplotype rs2268493 was associated with Asperger Syndrome (Di Napoli et al., 2014).

Other significant associations were noted between variants in *OXTR* and assessments of core characteristics of ASD in the entire sample (**Table 2B**). Besides rs2268493, which was associated with social behaviors and RRBs, rs4686302 had significant associations across both categories of phenotypes. rs4686302 has been associated with emotional empathy in a non-clinical Chinese sample (Wu et al., 2012). Wu et al. (2012) found the CC homozygotes to have less cognitive and trait empathy than the CT heterozygotes in their sample. We found rs1042778 was significantly associated with RRBs as measured by ADOS. This SNP (G-allele) has also been associated with ASD diagnosis in an AGRE sample (Campbell et al., 2011) and creative cognition in a non-patient Han Chinese sample (De Dreu et al., 2014). In the study conducted by De Dreu et al. (2014), creativity gives the individual the ability to adapt and be flexible to changing circumstances and social situations.

A significant association was also observed between rs2254298 and ADOS-Social Affect. This SNP has been affiliated with social impairment in two recent studies, Parker et al. (2014) found rs2254298 to be associated with social impairment in an ASD and typically developing sample. Also, in a meta-analysis conducted

TABLE 4 | FBAT analysis for *AVPR1A* microsatellites and allelic frequencies.

A

		Marker	Allele	*p*-value	Z
Restricted/Repetitive behaviors	ABC–Stereotypy	RS1	318	0.015	2.44
		RS1	322	0.027	−2.21
	ADOS–RRB	RS3	344	0.003	2.93
	RBSR–Compulsive and Ritualistic/Sameness subscale total	RS1	310	0.020	−2.32
		RS3	336	0.014	−2.45
	RBSR–Total	RS1	310	0.047	−1.98
		RS3	336	0.008	−2.65
		RS3	338	0.042	−2.03

B

RS1		RS3	
Allele	**Percent**	**Allele**	**Percent**
306	0.005	317	0.012
310	0.100	326	0.003
314	**0.387(S)**	328	0.063
318	0.263(L)	330	0.104
322	0.106	**332**	**0.236(S)**
326	0.079	334	0.195(L)
330	0.023	336	0.130
334	0.032	338	0.110
338	0.006	340	0.041
342	0.001	342	0.015
		344	0.046
		346	0.029
		348	0.015
		350	0.002
		353	0.001

(A) lists the significant associations with ASD-related phenotypes. (B) displays allelic frequencies of AVPR1A in our sample. The bold text denotes the allele with the highest frequency. The underlined text identifies the second highest allelic frequency for the markers. This "border" is often used to delineate short (S) and long (L) allelic categories. The categories are determined by creating approximately equal percentages in each category (Knafo et al., 2008).

by LoParo and Waldman (2015) they found rs2254298 and two other *OXTR* SNPs to be associated with ASD in analysis of eight studies and 11 independent samples. In 2008, Lerer et al. found rs2254298 to be associated with Vineland Adaptive Behavior Scales-2nd Edition (VABS-II) and an active marker in their haplotype analysis in a male ASD Israeli sample (Lerer et al., 2008). Given these previous ASD findings and the relationship between diagnosis and both categories of subphenotypes in our sample, rs2254298 and rs2268493 in addition to rs53576 were used in our haplotype analysis. rs53576 has had previous associations in ASD samples. rs53576 was found to be associated with ASD, social behavior, and emotional withdrawal in a patient population (Wu et al., 2005; Chang et al., 2014; Haram et al., 2015). However, in this sample we did not find associations with rs53576 as an individual SNP. The two marker haplotype, rs2254298A–rs2268493T was found to be associated with ASD diagnosis (**Table 3**).

In addition to SNPs, we studied the microsatellites located in the 5′ flanking region of *AVPR1A*, RS1, and RS3, in association with ASD-related phenotypes (**Table 4A**). Our significant associations were observed with several measures of RRBs. *AVPR1A* RS3 length variants that can be categorized as L were associated with these assessments. While we did not find associations with diagnosis or social behavior in the whole sample, others have found affiliations in ASD samples (Kim et al., 2002; Wassink et al., 2004; Yang et al., 2010b; Kantojärvi et al., 2015). Associations between *AVPR1A* microsatellites and social behaviors were also noted in both patient and non-patient samples in several studies (Knafo et al., 2008; Ebstein et al., 2009; Levin et al., 2009; Meyer-Lindenberg et al., 2009).

Future research should address the limitations of our study along with the inconsistencies in the literature. Firstly, our findings (SNPs, haplotypes, and microsatellites) need to be replicated in a larger sample, particularly given the small effect of specific inherited common variants in ASD based on genome-wide association studies of ASD (Anney et al., 2012; Chaste et al., 2015). A larger sample would also allow additional analysis of other subgroups, such as sex differences. This is of interest, because there are sex differences between the OT and AVP systems and ASD is diagnosed in males more often than females (Chakrabarti and Fombonne, 2005; Carter et al., 2008). Another factor that can contribute to inconsistent results is the use of differing diagnostic tools and criteria, especially because of the heterogeneity of the disorder. Alternative tools in both international and national studies could be measuring slightly different aspects of behaviors. Lastly, varying methodologies can be a contributing factor as well. In the analysis of microsatellites, differing groups define the variant lengths differently (see Table 2 in Kantojärvi et al., 2015).

OT and AVP systems influence mammalian brain pathways related to social and adaptive (vs. rigid, restricted) behaviors. There is a growing literature about OT and AVP in a range of disorders including schizophrenia, COMD, bipolar disorder as well as neurodevelopmental disorders (Dempster et al., 2007; Leszczynska-Rodziewicz et al., 2012; Davis et al., 2014; Francis et al., 2014). There are currently many clinical trials exploring the therapeutic value of OT and AVP (e.g., in ASD, schizophrenia, major depressive disorder, and substance dependence). With shifts from disorder-based to circuit-based therapeutic targets, studies of OT and AVP genetics need to explore associations with social and rigid behavior subphenotypes in larger samples. Overall, our results suggest further research of the OT/AVP system, including *OXTR, AVPR1A*, and especially *AVPR1B* in ASD and other disorders with social impairment and/or RRBs.

AUTHOR CONTRIBUTIONS

Genotyping, analysis, and manuscript preparation were performed by SF and SJ. EK contributed to genetics data analysis throughout the study. SK assisted with microsatellite methods and analyses. SG assisted with phenotype data collection and

data management. EC contributed to sample collection and to manuscript preparation. SJ was the principal investigator for the study and coordinated the project. All authors read and approved of the final manuscript.

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Conflict of Interest Statement: SJ has been a site-investigator for a Roche multisite ASD clinical trial, a site-investigator for a federally funded oxytocin ASD clinical trial, and a consultant for Genentech. EC has been a consultant for a Seaside Therapeutics multisite clinical trial. SK serves as part of Pfizer, Shire, Roche, and Ironshore Pharmaceuticals clinical trials. For the remaining authors there are no conflicts of interest.

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REVIEW
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Variation in Gene Expression in Autism Spectrum Disorders: An Extensive Review of Transcriptomic Studies

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Autism spectrum disorders (ASDs) are a group of complex neurodevelopmental conditions that present in early childhood and have a current estimated prevalence of about 1 in 68 US children, 1 in 42 boys. ASDs are heterogeneous, and arise from epigenetic, genetic and environmental origins, yet, the exact etiology of ASDs still remains unknown. Individuals with ASDs are characterized by having deficits in social interaction, impaired communication and a range of stereotyped and repetitive behaviors. Currently, a diagnosis of ASD is based solely on behavioral assessments and phenotype. Hundreds of diverse ASD susceptibility genes have been identified, yet none of the mutations found account for more than a small subset of autism cases. Therefore, a genetic diagnosis is not yet possible for the majority of the ASD population. The susceptibility genes that have been identified are involved in a wide and varied range of biological functions. Since the genetics of ASDs is so diverse, information on genome function as provided by transcriptomic data is essential to further our understanding. Gene expression studies have been extremely useful in comparing groups of individuals with ASD and control samples in order to measure which genes (or group of genes) are dysregulated in the ASD group. Transcriptomic studies are essential as a key link between measuring protein levels and analyzing genetic information. This review of recent autism gene expression studies highlights genes that are expressed in the brain, immune system, and processes such as cell metabolism and embryology. Various biological processes have been shown to be implicated with ASD individuals as well as differences in gene expression levels between different types of biological tissues. Some studies use gene expression to attempt to separate autism into different subtypes. An updated list of genes shown to be significantly dysregulated in individuals with autism from all recent ASD expression studies will help further research isolate any patterns useful for diagnosis or understanding the mechanisms involved. The functional relevance of transcriptomic studies as a method of classifying and diagnosing ASD cannot be underestimated despite the possible limitations of transcriptomic studies.

Keywords: autism spectrum disorders (ASD), gene expression, immune system, lymphoblastoid cell lines, monozygotic twins, Fragile X Syndrome, neurogenesis and inflammation

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BACKGROUND

Autism spectrum disorders (ASDs) are a group of complex neurodevelopmental conditions that present in early childhood. Individuals with ASDs are characterized by having deficits in social interaction, impaired communication and a range of stereotyped and repetitive behaviors (Lord et al., 1994). In 2012, ASD has a current estimated prevalence of about 1 in 68 US children aged 8 years; estimated prevalence was significantly higher among boys (23.6 per 1000) than among girls (5.3 per 1000) (Christensen et al., 2016). ASDs are heterogeneous, and arise from epigenetic, genetic and environmental origins. The exact etiology of ASDs still remains unknown and ASD cases with a genetic etiology only collectively account for 10–20% at most (Abrahams and Geschwind, 2008). The precise role of genetics in the pathogenesis of ASD remains unclear. On the one hand, there are several hints that genetics play a role (Wang et al., 2009). For example, there are much higher concordance rates of ASDs in monozygotic twins (70–90%) than dizygotic twins (0–10%) (Abrahams and Geschwind, 2008). Similarly, the recurrence risk in families ranges from 12.9% (Sandin and Reichenberg, 2014) to 18.7% (Ozonoff et al., 2011; Yuen et al., 2015). Furthermore, more than 100 ASD-susceptibility genes have already been identified (Yuen et al., 2015). On the other hand, these specific genetic mutations account for less than 8% of cases (Alter et al., 2011). There are a number of explanations for this ambiguity ranging from gene-gene interactions, to the heterogeneity of the disease, to epigenetic factors (Alter et al., 2011). Broad gene expression screening of children diagnosed with ASD is one approach toward mitigating the challenge of the heterogeneity of ASD by separating those diagnosed with ASD into subclasses according to gene expression profiles.

In the most recent comprehensive review of gene expression studies in ASD in 2012, Voineagu et al. surveyed 10 major mRNA studies across two types of samples [lymphoblastic (LBL) and post-mortem brain tissue]. They found that transcriptome analysis was more efficient than DNA studies in identifying differences between ASD and controls. Focusing on human mRNA studies since 2011 in English that monitor up or down regulation of multiple genes in ASD, without comparing to other disorders, we find that 27 major new studies have been published. By considering both single gene studies and more complex studies looking at pathways, researchers will be able to compare their findings to individual lists of genes but also to attempt to place their findings in a broader context by looking at pathway networks implicated in ASD. For example, if a researcher identifies a single specific gene to be upregulated in a specific type of tissue in ASD, that can then be compared to other single gene studies across different tissue types to see if there is consistency in the directionality of regulation and across tissue types. Additionally, by looking at a variety of networks and pathways, researchers can see the interaction between various neurodevelopmental and immune processes. These studies follow similar methodology to the studies previously discussed; though some expand to a third tissue type namely, intestinal biopsy samples.

Abbreviations: ADI-R, autism diagnostic interview revised; ANOVA, analysis of variance; ASD, autism spectrum disorders; asdMO, mothers having children with ASD; CNV's-copy number variants; DAVID, Database for Annotation, Visualization and Integrated Discovery; DSM-5, Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition; ELISA, Enzyme-Linked Immunosorbent Assay; IFNγ, interferon gamma; IL, interleukin; LCL, lymphoblastoid cell lines; mRNA, messenger ribonucleic acid; MGAM, maltase glucoamylase; NOS, nitric oxide synthetase; PCs, Purkinje cells; RT-qPCR, Real-time quantitative polymerase chain reaction; SAM, significance analysis of microarrays; SNP's, single nucleotide polymorphism; TD, typically developing/developed.

GENE EXPRESSION STUDIES IN ASD BY TISSUE TYPE

In reviewing the new gene expression studies since Voineagu's paper (**Table 1**), the classification according to sample source will prove helpful. Five sample sources were studied: post-mortem brain, peripheral blood, gastrointestinal tissue, adult olfactory stem cells, and scalp hair follicles. Before beginning with the post-mortem brain tissue studies, it is relevant to mention gross pathological and radiological brain findings that might provide context for the brain-related expression findings.

Neurological Background

Evidence for neurological involvement in ASD can be divided into neuroimaging and post-mortem pathology.

Neuroimaging

Neuroimaging studies of children with autism have revealed abnormal brain overgrowth in prefrontal, temporal and amygdala regions and abnormal functional asymmetry and activation in the cortex and cerebellum (Chow et al., 2012). Neuroimaging techniques such as functional MRI has shown altered patterns of functional specialization in autism in several domains of thinking, such as cognitive, linguistic, social, and visuospatial processing in children and adults with ASD (Maximo et al., 2014). Impaired functional connectivity may demonstrate inefficiency in maximizing network connections to execute tasks, yet the findings of over-connectivity have been interpreted to reflect hyper-specialized, rather than more efficient connectivity. Based on the abundance of information, inefficient connectivity may be the hallmark of ASD.

Pathology

Pathology on postmortem brains from subjects diagnosed with ASD have demonstrated abnormalities in neuronal organization of the cerebral cortex and decreased number of Purkinje cells in the cerebellum (Garbett et al., 2008). Children between 2 and 4 years old diagnosed with ASD have been found to have increased total cerebral gray and white matter, excess neurons in the pre-fontal cortex, and an increase in brain weight at autopsy (Courchesne et al., 2011; Hazlett et al., 2011). In some children diagnosed with ASD, the rate of head growth and brain growth is exceptionally rapid during the first few years of life. Additionally, although most children with autism are born normocephalic, during the first years of life, 15–20% will develop macrocephaly (Lainhart et al., 2006). However, by late adolescence and early

TABLE 1 | Gene Expression Studies of Autism: 2011 onwards (Year chosen since Voineagu et al. review paper is from 2012).

Study	Number of samples	Tissue source	Number of dysregulated genes listed
Alter et al., 2011	82 children with autism (mean: 5.5 years SD 2.1 $p < 0.0001$) and 64 controls (mean: 7.9 years SD 2.2 $p < 0.0001$)	PBL	Children w/autism/Children w/younger fathers: 2093 significantly downregulated & 641 significantly upregulated genes; Children w/older fathers: Children w/younger fathers: 1476 significantly downregulated & 764 significantly upregulated genes; 593 genes were downregulated and 145 genes were upregulated in both children with autism and children of with older fathers
Anitha et al., 2012	9 autism patients ant 8 controls	ACG, MC, THL	28 genes showed brain region-specific reduced expression in autism
Chien et al., 2013	PART I: Conducted comparative total gene expression profiling analysis between 16 male patients with ASD (age range 4–18 years) and 16 male control subjects (age range 18–67 years); PART II: compared transcript level of one particular gene (FOXP1) between 83 male patients with ASD and 83 male healthy controls	LCL derived from the EBV transformation of lymphocytes of peripheral blood	202 genes were differentially expressed in the ASD group, including 89 upregulated and 113 downregulated
Chow et al., 2012	16 from young postmortem males (2–14 years; autism = 9, control = 7) and 17 adult males (15–56 years, autism = 6, control = 11)	DLPFC	2017 genes across all autistic and control cases independent of age
Féron et al., 2016	9 adults with severe autism and low to very low developmental disabilities, plus two adults with mild or moderate autism and no or mild cognitive abilities (Asperger syndrome or high functioning autism) paired with 11 age and gender matched controls	Adult nasal olfactory stem cells	156 genes that were differentially expressed in at least one ASD patient, of which 31 were dysregulated in more than a third of the cohort
Ginsberg et al., 2012	9 autism and 9 control subjects	BA19 (occipital) brain tissues	876 unique, annotated genes differently expressed between autistic and control brains
Glatt et al., 2012	60 infants and toddlers at risk for ASDs (autistic disorder and pervasive developmental disorder), 34 at risk for LD, 17 at risk for DD, and 68 TD children	PBMCs	154 probes showed significant dysregulation in ASD
Chana et al., 2015	Utilized published microarray data from 30 control and 27 ASD individuals	DLPFC	3 downregulated genes and 1 upregulated gene in ASD samples
Ivanov et al., 2015	30 subjects with idiopathic autism (24 male, 6 female) aged 3 to 11 years (mean age of sample 6.86 years) and 30 healthy children age and sex matched	Peripheral blood	23 differentially expressed genes (10 upregulated, 13 downregulated)
James et al., 2014	13 autism and 13 unaffected control individuals	Cerebellar cortex	7 genes
Khan et al., 2014	11 control (4F,7M) and 10 ASD (3F,7M) cases were examined (Controls ranged between 5 and 16 years of age, while ASD ranged from 4 to 15 years of age)	CB, BST, CG, ORC, PT, Wer	15 genes showed brain-region specific dysregulated expression in ASD samples
Kong et al., 2013	20 proband-unaffected sibling pairs (5 probands-sib pairs were of the same gender, i.e., males, while 15 pairs were of the opposite gender including 12 male and 3 female probands) and 18 unrelated control (11 males, 7 females) individuals	Peripheral blood	163 unique genes were significantly changed between probands and siblings
Kuwano et al., 2011	GROUP 1: 21 Young adults with ASD (17 males and 4 females) aged 26.7 ± 5.5 years, age range: 18-38 years; GROUP 2: 21 age and gender matched healthy controls aged 27.0 ± 5.5 years, age range: 19–39; GROUP 3: 21 Healthy mothers having children with ASD (asdMO), aged 44.7 ± 6.7 years, age range: 33–58 years; GROUP 4: asdMO control, aged 44.7 ± 6.7 years, age range: 31–59 years	Peripheral blood	ASD/control: 19 genes were found to be significantly dysregulated (18 upregulated and 1 downregulated); asdMO/asdMO control: 57 genes were found to be significantly dysregulated (17 upregulated and 40 downregulated); 3 genes overlapped and were dysregulated both in individuals with ASD and in asdMO
Maekawa et al., 2015	24 male control subjects (Aged 32.60 ± 3.91) and 18 Autism subjects (16 male, 2 female; aged 25.61 ± 4.95)	Scalp hair follicles	1 gene

(Continued)

TABLE 1 | Continued

Study	Number of samples	Tissue source	Number of dysregulated genes listed
Prandini et al., 2014	Two separate series of sib-pairs totaling 36 children and adolescents between 4 and 18 years of age	LCLs	none found
Segura et al., 2015	21 adolescents and adults diagnosed as ASD (20 males, 1 female) as well as from 10 healthy controls (10 males)	Whole blood	3 genes
Stamova et al., 2011	33 boys with AU (mean age 45.3 months; age range of 31–60 months) and 51 age-matched TD control boys (mean age 43.3 months; age range 28–57 months)	Whole blood	11 genes
Talebizadeh et al., 2014	Autistic group included three females (6, 11, and 13 years old) and two males (5 and 12 years old) diagnosed with classical autism and 5 age and sex matched unrelated controls	LCL derived RNAs	57 genes
Taurines et al., 2011	51 children with ADHD, 26 children with ASD (19/26 comorbid with ADHD) and 39 TD	Whole blood cells	2 genes
Tian et al., 2011	37 children with autism (32 males, 5 females; average age 44.2 ± 10 months) compared to 15 typically developing controls (11 males, 4 females; average age 41.2 ± 6 months)	Whole blood	31 genes
Voineagu and Eapen, 2013	19 autism cases and 17 controls	STG, prefrontal cortex (BA9) and cerebellar vermis	444 genes showing significant expression changes in autism cortex samples, 2 genes differentially expressed in cerebellum
Walker et al., 2013	25 children with a diagnosis of ASD (mean age 5.0862.06 years; 23 male and 2 female, 16 had a diagnosis of autism; 9 had a diagnosis of autism spectrum disorder); 3 TD groups: (1) 15 children with no chronic GI symptoms (mean age 12.263.07 years; 6 male and 9 female); (2) children with a diagnosis of Crohn's disease ($n = 8$, mean age 12.9763.07 years; 3 male and 5 female); (3) children with a diagnosis of ulcerative colitis ($n = 5$, mean age 12.064.0 years; all female)	Tissue specimen from 7 anatomic locations (from terminal ileum to rectum)	Ileal mucosa: ASD-GI/TD -1409 differentially expressed transcripts • Colonic mucosa: ASD-GI/TD -1189 differentially expressed transcripts: Overlap between both sets ASD-GI (Ileum and Colon)/ TD -178 transcripts exclusively differentially-expressed
Williams et al., 2011	15 AUT-GI children (mean onset age 13.4+/25.4 months, median age at biopsy 4.5), 7 Control-GI patients (median age at biopsy 4.0)	Ileum and Cecum	6 genes
Yasuda et al., 2011	35 patients with ASD (mean age 12.9 years ± 12.4 SD) 35 healthy controls (mean age 34.8 years ± 9.7 SD)	LCLs	2 genes
Zhubi et al., 2014	10 ASD and 10 control samples	Cerebellar cortex	4 genes
Ziats and Rennert, 2013	Re-analyzed sex-specific gene-expression from a recent large transcriptomic study (Kang et al., 2011); $N = 57$, including 39 with both hemispheres; age, 5.7 post-conceptual weeks to 82 years; sex, 31 males and 26 females;	Transient prenatal structures and immature and mature forms of 16 brain regions (Kang et al., 2011; **Table 2**)	37 Female and 123 Male genes found to be differentially expressed by sex, and their brain region and developmental time point

PBL, Peripheral blood lymphocytes; LCL, lymphoblast cell line; PBMCs, peripheral blood mononuclear cells.
ACG, anterior cingulate gyrus; MC, motor cortex; THL, thalamus; DLPFC, Dorsolateral Prefrontal Cortex; CB, Cerebellar; BST, Brain stem.
CG, Cingulated gyrus; ORC, Orbitofrontal cortex; PT, Putamen; Wer, Wernicke's; STG, superior temporal gyrus.
LD, language delay; DD, global developmental delay; TD, typically developing.

adulthood, the autistic brain commonly displays neuron loss and cortical thinning and is no longer enlarged (Kates et al., 2004).

POST-MORTEM BRAIN TISSUE GENE EXPRESSION STUDIES

With the background provided by the neuroimaging and pathology findings in mind, gene expression studies in brain tissue can now be reviewed. There are nine gene expression studies that look at a variety of brain regions. The studies are all very small but produced large numbers of genes with variable expression between ASD and control. Chow et al. analyzed frozen samples of dorsolateral prefrontal cortex from 16 young postmortem males (9 autism, 7 control) and 17 adult males (6 autism, 11 control) in order to study age-dependent brain gene expression (Chow et al., 2012). They found 102 genes, which

TABLE 2 | Gene expression changes in ASD detected in multiple independent studies in various tissues.

Gene symbol	Blood/LCL study	Brain study	Intestinal biopsy study	Expression change in ASD
ABHD3	Kong et al., 2013	Garbett et al., 2008		Upregulated
ACTG2		Ziats and Rennert, 2013	Walker et al., 2013	Downregulated
ADM		Garbett et al., 2008; Voineagu and Eapen, 2013		Upregulated
AHI1		Garbett et al., 2008; Voineagu and Eapen, 2013		Downregulated
ALAD		Chow et al., 2012	Walker et al., 2013	Downregulated
ALPK1	Nishimura et al., 2007; Talebizadeh et al., 2014			Upregulated
ANKRD22	Glatt et al., 2012; Ivanov et al., 2015			Upregulated in Ivanov et al. Downregulated in Glatt et al.
ANXA1	Chien et al., 2013	Garbett et al., 2008; Voineagu and Eapen, 2013; Ziats and Rennert, 2013		Upregulated in Garbett et al. and Ziats et al. Downregulated in Chien et al.
AQP4		Garbett et al., 2008; Voineagu and Eapen, 2013		Upregulated
ATF3	Hu et al., 2006		Walker et al., 2013	Upregulated in Hu et al. Downregulated in Walker et al.
BAG3		Garbett et al., 2008; Voineagu and Eapen, 2013		Upregulated
C20orf7		Chow et al., 2012; Ginsberg et al., 2012		Downregulated
C5orf16		Garbett et al., 2008; Voineagu and Eapen, 2013		Downregulated
CCL17	Nishimura et al., 2007		Walker et al., 2013	Upregulated
CD160	Gregg et al., 2008; Enstrom et al., 2009			Upregulated
CHI3L1	Chien et al., 2013	Garbett et al., 2008		Upregulated in Garbett et al. Downregulated in Chien et al.
CLIC1		Garbett et al., 2008; Voineagu and Eapen, 2013		Upregulated
CMKOR1	Nishimura et al., 2007	Garbett et al., 2008; Voineagu and Eapen, 2013		Upregulated
CNN3		Garbett et al., 2008; Voineagu and Eapen, 2013		Upregulated
COL4A1		Garbett et al., 2008; Voineagu and Eapen, 2013		Upregulated
COX7B		Ginsberg et al., 2012; Anitha et al., 2012		Downregulated
CSDA		Garbett et al., 2008; Voineagu and Eapen, 2013		Upregulated
CTNNB1	Kong et al., 2013	Chow et al., 2012		Upregulated in Kong et al. Downregulated in Chow et al.
CX3CR1	Gregg et al., 2008; Enstrom et al., 2009	Ziats and Rennert, 2013		Upregulated
CXCL10	Chien et al., 2013	Chow et al., 2012		Upregulated
CXCR4	Chien et al., 2013	Chow et al., 2012		Upregulated
CYC1		Ginsberg et al., 2012; Anitha et al., 2012		Downregulated
CYFIP1	Nishimura et al., 2007; Talebizadeh et al., 2014			Upregulated
DLX1		Garbett et al., 2008; Voineagu and Eapen, 2013		Downregulated
DNASE1L3	Chien et al., 2013	Chow et al., 2012		Downregulated
DRD4	Emanuele et al., 2010; Taurines et al., 2011			Upregulated in Emanuele et al. Downregulated in Taurines et al.

(Continued)

TABLE 2 | Continued

Gene symbol	Blood/LCL study	Brain study	Intestinal biopsy study	Expression change in ASD
FAM46C	Nishimura et al., 2007; Chien et al., 2013			Upregulated in Chien et al. Downregulated in Nishimura et al.
FOSL1	Ivanov et al., 2015	Chow et al., 2012		Upregulated in Chow et al. Downregulated in Ivanov et al.
GAD1	Chien et al., 2013	Zhubi et al., 2014		Downregulated
GADD45B		Garbett et al., 2008; Voineagu and Eapen, 2013		Upregulated
GPR56	Gregg et al., 2008	Ginsberg et al., 2012		Upregulated
GRIA3	Chien et al., 2013	Chow et al., 2012		Downregulated
GZMB	Gregg et al., 2008; Enstrom et al., 2009; Chien et al., 2013			Upregulated
HCK	Hu et al., 2006; Chien et al., 2013; Talebizadeh et al., 2014			Upregulated in Talebizadeh et al., Downregulated in Hu et al. and Chien et al.
HIST1H1C		Garbett et al., 2008; Voineagu and Eapen, 2013		Upregulated
HIST1H2BD		Garbett et al., 2008; Voineagu and Eapen, 2013		Upregulated
HIST1H3H	Nishimura et al., 2007	Chow et al., 2012		Upregulated in Chow et al. Downregulated in Nishimura et al.
HLA-DQA1	Gregg et al., 2008; Stamova et al., 2011			Downregulated
HSPB1		Garbett et al., 2008; Ginsberg et al., 2012		Upregulated
IFITM2		Garbett et al., 2008; Voineagu and Eapen, 2013		Upregulated
IFITM3		Garbett et al., 2008; Voineagu and Eapen, 2013		Upregulated
IGF2BP1	Ivanov et al., 2015		Walker et al., 2013	Upregulated in Walker et al. Downregulated in Ivanov et al.
IGHA1	Gregg et al., 2008; Chien et al., 2013			Upregulated
IGHG1	Hu et al., 2006; Gregg et al., 2008; Chien et al., 2013			Upregulated in Chien et al. and Gregg et al. Downregulated in Hu et al.
IL2RA	Chien et al., 2013		Walker et al., 2013	Upregulated
IL2RB	Gregg et al., 2008; Enstrom et al., 2009			Upregulated
ITGB2	Gregg et al., 2008; Enstrom et al., 2009			Upregulated
KIF1B	Hu et al., 2006; Talebizadeh et al., 2014	Garbett et al., 2008		Upregulated in Garbett et al. and Talebizadeh et al. Downregulated in Hu et al.
KIR3DL2	Gregg et al., 2008; Enstrom et al., 2009			Upregulated
KSP37	Gregg et al., 2008; Enstrom et al., 2009			Upregulated
LAMP2		Chow et al., 2012	Walker et al., 2013	Downregulated
LRP6	Chien et al., 2013; Talebizadeh et al., 2014			Downregulated
MeCP2	Kuwano et al., 2011	James et al., 2014; Zhubi et al., 2014**		Upregulated in Kuwano et al. and Zhubi et al. Downregulated in James et al.
MIA	Nishimura et al., 2007		Walker et al., 2013	Upregulated in Nishimura et al. Downregulated in Walker et al. (Colon)
MKNK2		Garbett et al., 2008; Voineagu and Eapen, 2013		Upregulated
MSI2		Garbett et al., 2008; Voineagu and Eapen, 2013		Upregulated

(Continued)

TABLE 2 | Continued

Gene symbol	Blood/LCL study	Brain study	Intestinal biopsy study	Expression change in ASD
MSN		Garbett et al., 2008; Voineagu and Eapen, 2013		Upregulated
NDUFA2		Ginsberg et al., 2012; Anitha et al., 2012		Downregulated
NDUFB3		Ginsberg et al., 2012; Anitha et al., 2012		Downregulated
NDUFB5	Talebizadeh et al., 2014	Anitha et al., 2012		Upregulated in Talebizadeh et al., Downregulated in Anitha et al.
NEURL3	Kong et al., 2013; Chien et al., 2013			Upregulated in Chien et al. Downregulated in Kong et al.
NKG7	Gregg et al., 2008; Enstrom et al., 2009			Upregulated
NP		Garbett et al., 2008; Voineagu and Eapen, 2013		Upregulated
P2RX5	Hu et al., 2006; Chien et al., 2013			Upregulated
P4HA1		Garbett et al., 2008; Voineagu and Eapen, 2013		Upregulated
PALLD		Garbett et al., 2008; Voineagu and Eapen, 2013		Upregulated
PAM	Gregg et al., 2008; Enstrom et al., 2009			Upregulated
PARP9	Glatt et al., 2012	Garbett et al., 2008		Upregulated in Garbett et al. Downregulated in Glatt et al.
PIR		Garbett et al., 2008; Voineagu and Eapen, 2013		Upregulated
PITPNC1	Hu et al., 2006; Nishimura et al., 2007	Garbett et al., 2008; Voineagu and Eapen, 2013		Upregulated in Nishimura et al. Garbett et al. and Voineagu et al. Downregulated in Hu et al.
PLEKHC1		Garbett et al., 2008; Voineagu and Eapen, 2013		Upregulated
PRF1	Gregg et al., 2008; Enstrom et al., 2009			Upregulated
PTGDR	Gregg et al., 2008; Enstrom et al., 2009			Upregulated
PTTG1IP		Garbett et al., 2008; Voineagu and Eapen, 2013		Upregulated
PXDN	Stamova et al., 2011; Chien et al., 2013			Upregulated in Chien et al. Downregulated in Stamova et al.
RELN		Chow et al., 2012; Khan et al., 2014; Zhubi et al., 2014		Upregulated in Khan et al. Downregulated in Chow et al. and Zhubi et al.
RPS21		Garbett et al., 2008; Ginsberg et al., 2012		Upregulated in Garbett et al. Downregulated in Ginsberg et al.
S100A10		Garbett et al., 2008; Voineagu and Eapen, 2013		Upregulated
SCARA3		Garbett et al., 2008; Voineagu and Eapen, 2013		Upregulated
SDC2		Garbett et al., 2008; Ziats and Rennert, 2013		Upregulated
SERPINA1	Chien et al., 2013	Chow et al., 2012		Upregulated in Chow et al. Downregulated in Chien et al.
SERPINH1		Garbett et al., 2008; Chow et al., 2012; Ziats and Rennert, 2013		Upregulated
SERTAD1		Garbett et al., 2008; Voineagu and Eapen, 2013		Upregulated
SFTPA2		Chow et al., 2012	Walker et al., 2013	Upregulated in Chow et al. Downregulated in Walker et al.

(Continued)

TABLE 2 | Continued

Gene symbol	Blood/LCL study	Brain study	Intestinal biopsy study	Expression change in ASD
SH2DIB/EAT2	Gregg et al., 2008; Enstrom et al., 2009			Upregulated
SHANK3		Yasuda et al., 2011; Chana et al., 2015		Downregulated
SLC38A2	Hu et al., 2006; Kong et al., 2013			Upregulated
SLC9A9	Talebizadeh et al., 2014	Garbett et al., 2008; Voineagu and Eapen, 2013		Upregulated
SPON2	Gregg et al., 2008; Enstrom et al., 2009			Upregulated
STOM	Glatt et al., 2012	Garbett et al., 2008; Ziats and Rennert, 2013		Upregulated in Garbett et al. Downregulated in Glatt et al. and Ziats et al.
SYCE1	Kong et al., 2013	Chow et al., 2012		Downregulated
TAGLN2		Garbett et al., 2008; Voineagu and Eapen, 2013		Upregulated
TAP1	Glatt et al., 2012	Garbett et al., 2008; Voineagu and Eapen, 2013		Upregulated in Garbett et al. Downregulated in Glatt et al.
TBX21	Gregg et al., 2008; Enstrom et al., 2009			Upregulated
TET1		James et al., 2014; Zhubi et al., 2014		Upregulated
TIMP1		Garbett et al., 2008; Voineagu and Eapen, 2013		Upregulated
TMBIM1		Garbett et al., 2008; Voineagu and Eapen, 2013		Upregulated
TMEM40	Kong et al., 2013; Ivanov et al., 2015			Downregulated
TNFRSF19	Chien et al., 2013	Chow et al., 2012		Downregulated
TNPO1		Garbett et al., 2008; Voineagu and Eapen, 2013		Upregulated
UBD	Chien et al., 2013		Walker et al., 2013	Upregulated
WWTR1	Chien et al., 2013	Garbett et al., 2008		Upregulated in Garbett et al., Downregulated in Chien et al.
YAP1		Garbett et al., 2008; Voineagu and Eapen, 2013		Upregulated
ZFP36L1		Garbett et al., 2008; Voineagu and Eapen, 2013		Upregulated

***James et al. (2014). looked at MeCP2 binding to the EN-2 promoter, while Zhubi et al. (2014) looked at MeCP2 binding to the GAD1 & RELN promoters.*

contrasted between young children diagnosed with ASD and control groups, and 736 genes, which contrasted between adults diagnosed with ASD and control groups. Ginsberg et al. analyzed cerebellar and BA19 (Brodmann Area 19, occipital) brain tissues from 9 control and 9 autism patients (Ginsberg et al., 2012). After correcting for the region of the brain, they discovered 876 unique, annotated genes expressed differently between autistic and control brains. This was a false discovery rate of five percent. Anitha et al. compared the expression of 84 electron transport chain genes belonging to the 5 complexes in the post-mortem brains of 8 autism patients and 10 controls. They found that 11 genes of Complex I, 5 genes each of Complex III and Complex IV, and 7 genes of Complex V (28 genes in total) showed brain region-specific reduced expression in autism (Anitha et al., 2012). Voineagu et al. profiled post-mortem samples of the superior temporal gyrus, prefrontal cortex, and cerebellar vermis regions from 19 autism and 17 control cases (Voineagu and Eapen, 2013). They identified 444 genes, which showed significant expression changes in autism cortex samples as compared to the controls, while only 2 genes were differentially expressed in the cerebellum. Ziats et al. re-analyzed a large transcriptomic study in the developing brain that was done by (Kang et al., 2011; Ziats and Rennert, 2013). The study performed genome-wide microarray analysis on postmortem human brain tissues from 16 brain regions spanning preconception to adulthood, specifically looking to identify sex-biased gene expression in the developing brain. Thirty-seven female, and 123 male genes were found to be differentially expressed by sex, brain region, and developmental time point. Khan et al. analyzed altered thyroid hormone-dependent brain gene expression by studying various brain regions from 10 ASD and 11 control cases (Khan et al., 2014). They found 14 genes that were differentially expressed

based on sex-specific brain area. For example, expression of the DIO2 gene, a gene involved in thyroid hormone activation, was increased in the putamen ($p < 0.05$) and there was a trend toward increase in cingulated gyrus ($p = 0.08$) of the female ASD cases, while expression of the Cirbp gene, a gene responsible for stabilizing transcripts of genes involved in cell survival, was decreased ($p < 0.05$) in the putamen of ASD male cases only. James et al. compared 1 sq cm blocks of cerebellar cortex from 13 individuals with ASD and 13 controls (James et al., 2014). They observed a significant increase in both 5-mC and 5-hmC in the cerebellum of individuals with ASD relative to control samples. DNMT3A, DNMT3B, TET1, TET3, which are all genes related to methylation and 8-oxo-deoxyguanosine (8-oxo-dG) (a major product of DNA oxidation) expression levels were significantly increased in the cerebellum of individuals with ASD relative to control samples. They also found that within the EN-2 promoter sequence there was a statistically significant positive association between 5-hmC (a gene important in epigenetics) and EN-2 gene expression. Additionally an association between 5-hmC and EN-2 gene expression in the 5′ promoter CpG Island was found in people diagnosed with ASD but not in controls. Studies of MeCP2 (a chromosomal protein that binds to methylated DNA) binding in the EN-2 promoter demonstrated a significant decrease in repressive MeCP2 binding to the identical 5′ promoter region that contained increased levels of 5-hmC in individuals with ASD relative to control samples. Zhubi et al. also compared blocks of cerebellar cortex from 10 individuals with ASD and 10 control samples. They found a one and a half to two time increase in binding of MeCP2 to GAD1 and RELN promoters in the cerebellum of individuals diagnosed with autism when contrasted to controls. RELN plays a role in layering of neurons in the cerebral cortex and cerebellum. They detected that levels of 5-hmC were significantly enriched at GAD1 and RELN promoters in individuals with ASD. The methylation changes they found lead to a remarkable increase in the amounts of 5-hmC relative to 5-mC. The 5-hmC/5-mC ratio at the GAD1 promoter is 5.5 in people diagnosed with ASD and only 1.2 in controls. Similar to James et al. (2014), they also found TET1 mRNA levels to be increased in the cerebella of individuals with ASD (Zhubi et al., 2014). Chana et al. (2015) compared data from brain tissue from 30 controls and 27 individuals diagnosed with ASD. They found that expression of mGluR5 was decreased in ASD when compared to controls. This gene has been found to be associated with the forming of synapses, activation of microglia, and other processes.

Immune System

One study demonstrated findings in post-mortem brain tissue that were specific to the immune system. Considering the extensive literature analyzing the relationship between the immune system and ASD (Gesundheit et al., 2013), this demands further attention. Garbett et al. analyzed frozen samples of superior temporal gyrus from 6 subjects with ASD and 6 controls (Garbett et al., 2008). Based on four parameters, they identified 152 differentially expressed gene products, and of these, 130 demonstrated increased expression while 22 showed decreased levels in the brains of individuals with ASD. Seventy-two annotated differentially expressed transcripts were either cytokine responsive transcripts or transcripts related to the immune system. Additionally, they noticed decreased transcript levels for a number of genes involved in outgrowth and neuronal differentiation. They used Gene Set Enrichment Analysis, which marks functional pathways in which gene expression changes are grouped together. This enabled the identification of 31 gene sets that were differentially expressed between individuals with autism and control samples. Of the 31, 19 genes were involved in immune system function.

PERIPHERAL BLOOD GENE EXPRESSION STUDIES

The second and most common source used in gene expression studies for autism was lymphoblastoid cell lines (LCLs). There is an apparent discrepancy between neuroanatomical and cellular abnormalities observed for autism at younger ages and molecular pathologies at more advanced ages. Hu et al. analyzed LCLs derived from lymphocytes of 3 pairs of monozygotic twins that were discordant with respect to clinical diagnosis of ASD (Hu et al., 2006). Twelve hundred genes were identified as significant with a false discovery rate of 26%, 25 genes were found to be up-regulated at least 1.5-fold in the more severely affected twin relative to the other twin (log2 (ratio) = 0.58) and 19 genes were down-regulated by at least 1.5-fold. Of these, eight of the 26 genes match genes connected to neurological function, development, or disease. In 2009, Hu et al. conducted microarray analysis on 116 LCLs from individuals with idiopathic autism who were separated into three phenotypic subcategories according to severity scores from the ADI-R questionnaire and age-matched, typically developing controls. They identified 530 significantly differentially expressed genes that distinguished controls from all samples with ASD. Hu et al. analyzed gene expression profiling and how it differentiates ASD from controls and phenotypic variants of ASD (Hu et al., 2009). Microarray analyses were conducted on 116 LCLs from individuals with idiopathic ASD who were separated into three phenotypic subgroups according to severity scores from the ADI-R questionnaire and age-matched, non-autistic controls. Five hundred and thirty significantly differentially expressed genes were found that distinguished the samples of ASD from controls. They also identified 123 significant genes from 4-class significance analysis of microarrays (SAM) analysis of data from gene expression from severe, mild, and savant subgroups and the non-autistic control groups. When the pathways of the overlapping genes were analyzed between the severe language and mild autism subgroups, a network of genes that affect common functional targets, such as synaptic transmission, neurogenesis, neurulation, long-term potentiation (learning), protein ubiquitination, and brain function was revealed. Additionally, 15 significant differentially expressed genes that regulate circadian rhythm were found, unique to the most severely affected ASD subgroup. Differential expression of these genes was observed only in the samples

from severely language-impaired individuals (L subgroup), with each individual showing altered expression of multiple (but not all 15) genes. Emanuele et al. investigated increased dopamine DRD4 receptor mRNA expression in lymphocytes of individuals diagnosed with autism and musicians in order to explore the music-autism connection. The DRD4 receptor is known to be responsible for neuronal signaling in the mesolimbic system of the brain. They studied 20 ASD patients, 19 professional adult musicians, and 19 gender and age matched control individuals who were not interested in playing or listening to music. Analysis of variance (ANOVA) demonstrated significant differences in DRD4 mRNA expression between the groups ($P = 0.008$). *Post-hoc* analysis highlighted significant differences between the control group and both musicians ($P < 0.05$) and individuals diagnosed with ASD ($P < 0.05$) (Emanuele et al., 2010). Yasuda et al. measured mRNA expression levels in lymphoblastoid cells from 35 subjects with ASD and 35 controls. They found that in individuals diagnosed with autism, B-actin normalized NLGN3 expression levels or TATA-binding protein were decreased by 35 or 26% respectively. They also found that gene expression levels of the SHANK3 gene, a gene that codes for a major scaffold postsynaptic density protein, regulated by B-actin or TATA binding protein were also decreased in individuals with autism by 39 or 40% respectively (Yasuda et al., 2011).

Stamova et al. looked for correlations between gene expression and mercury level in blood of boys with and without ASD. They collected whole blood from 33 boys with ASD and 51 age-matched typically developed (TD) control boys. There was no significant difference in Hg levels between the ASD and TD groups. They found 11 genes whose expression correlated inversely with mercury levels in boys diagnosed with autism compared to typically developing children. One limitation of this study was that samples collected from children ages 2–5 do not consider the possible direct role of mercury as a causal factor for autism, which likely starts *in utero* or shortly after birth (Stamova et al., 2011).

Tian et al. looked for correlations of gene expression with blood lead levels in children diagnosed with autism contrasted to typically developed controls. They looked at 37 children with ASD, and 15 TD controls. There was no significant difference in blood lead levels. Forty-eight probe sets represent 31 genes, whose expression correlated with lead levels in each group, and the partial correlation coefficients were statistically different between the groups; most of the genes are negatively correlated with blood lead levels in typically developed children and positively correlated with blood lead levels in children diagnosed with autism. The conclusion of their study, however was that lead most likely does not explain the increasing incidence in ASD (Tian et al., 2011).

Alter et al. looked at ASD and changes related to paternal age in overall levels of gene expression regulation. They performed gene expression microarrays on RNA from peripheral blood lymphocytes of 82 children with ASD and 64 controls where parental age was similar between the 2 groups. They then performed a secondary analysis by analyzing paternal age difference as a risk factor for ASD and whether it was associated with variance. They found that the distribution of gene expression levels on microarrays from individuals with autism had a decreased variance when compared to microarrays from controls ($p = 0.006$). They also found that in controls, but not in children with ASD, overall variance in gene expression was found to be significantly and negatively associated with the age of the father ($p = 0.03$), so as predicted, the overall variance was the same in children of fathers who were older and children with ASD with fathers of any age. In the comparison of children with ASD to children with fathers of a younger age, there were 2093 genes that were significantly downregulated by at least 1.1-fold, and only 641 that were upregulated. In the blood of children with fathers who were older compared to the children with younger paternal age, there were 1476 downregulated and 764 upregulated genes. There were 593 genes that were downregulated in both children with ASD and children of with older fathers ($p< 0.000001$) and 145 genes that were upregulated in both comparisons (Alter et al., 2011).

Chien et al. looked at increased gene expression of FOXP1 by comparing LCL between 16 males diagnosed with ASD and 16 male controls. FOXP1 is a transcriptional repressor. A total of 252 differentially expressed probe sets corresponding to 202 genes were detected between the 2 groups, including 89 up- and 113 down-regulated genes in the group diagnosed with autism. Real-time quantitative polymerase chain reaction (RT-qPCR) verified significant elevation (1.89 ± 2.64, $P = 0.005$) of the FOXP1 gene transcript of LCL in a sample of 83 male patients, compared with 83 male controls. Using three platforms, they found several immune-related pathways showing significant differences between the ASD patients and controls (Chien et al., 2013).

Prandini et al. analyzed RBFOX1 gene expression in LCL of Italian discordant ASDs sib-pairs totaling 36 children and adolescents. Their data showed however, that RBFOX1 normalized mean values were not significantly different between controls and those diagnosed with ASD, they suggested a possible cause for this might have been due more subtle transcription level differences in RBFOX1 gene expression in LCL than in brain samples (Prandini et al., 2014).

Talebizadeh et al. performed a pilot study and looked at exon-level expression profiling and alternative splicing in ASD using LCL. They found 57 genes that were differentially expressed at the exon level between ASD and control samples. They also found differential splice variants of the gene CYFIP1 (which has 2 protein-coding transcripts in the literature), exon array analysis demonstrated a higher expression for the probe sets which binds to exon 16 in variant 1 (encoding a long form) in subjects with ASD vs. controls. DNA sequencing following RT-PCR for variant 1 also detected a product missing exon 16 inducing a premature stop codon, and qRT-PCR showed a higher expression of variant 1 in ASD compared with control samples. RT-PCR reactions were run for TRAP150 and ZMYM6 and DNA sequencing of the amplified products supported the fact that these exons undergo alternative splicing and enabled the indication of previously unreported alternative splicing isoforms for these two genes. These new variants included one isoform of TRAP150 (missing exon 4 resulting in an in-frame loss of 301 amino acid residues) and five alternatively spliced ZMYM6 variants that are labeled on

the basis of the missing exons (isoforms missing exon 2, exon 4, exons 2&4, exons 2&5, and exons 2, 4, &5). ZMYM6 variants missing exon 2, the location of the start codon, most likely do not code for proteins and the exclusion of only exon 4 introduces a premature stop codon (Talebizadeh et al., 2014).

Immune System-Background

The role of the immune system in ASD is an active area of research. Evidence of an immune role in at least a subset of children diagnosed with ASD can be divided into brain antibodies, serum cytokines, family history and immunogenetics (Gesundheit et al., 2013).

Immune System-Gene Expression Studies

Gregg et al. subdivided the children into three groups based on various clinical criteria. Their sample size included 49 children on the autism spectrum and 12 controls. Unpaired *t*-tests detected a number of genes that were regulated more than 1.5-fold for autism vs. general population ($n = 55$ genes), for history of early onset vs. general population ($n = 140$ genes), and for developmental regression vs. general population ($n = 20$ genes). The three gene lists from the analysis were used to identify a small group of 11 genes that are shared between the three groups. These genes were all expressed in natural killer cells and many belonged to the KEGG natural killer cytotoxicity pathway. Database for Annotation, Visualization and Integrated Discovery (DAVID) and Ingenuity Pathway Analysis were used to analyze pathways, and notable pathway overlaps included natural killer cell signaling in all three comparisons, IL-2 signaling and serotonin receptor and dopamine receptor signaling in autism vs. general population and early onset vs. general population, and retinol and methionine metabolism in the early onset vs. general population analysis (Gregg et al., 2008).

Enstrom et al. analyzed peripheral blood from 35 children with ASD and 11 age and gender matched controls. They discovered that a total of 626 probes showed differential gene expression between the two groups (82 significantly higher and 544 significantly lower in the ASD group). The 82 upregulated probes in ASD correlated to 59 known genes, most of which have been connected to leucocyte function, more specifically the function of natural killer cells. Using microarray analysis, their studies demonstrated that 12 gene probes, corresponding to 11 different genes were differentially expressed in early onset and regressive types of ASD when compared with the control group. Flow cytometric analysis of natural killer cells demonstrated increased production of granzyme B, perforin, and interferon gamma (IFNγ) under resting conditions in children diagnosed with ASD (Enstrom et al., 2009).

Kuwano et al. looked at ASD-associated gene expression in peripheral leucocytes that were often noticed between subjects with ASD, and healthy mothers of children with ASD. They used DNA microarray to perform gene expression profiling in peripheral blood on 21 individuals from 4 groups: young adults with ASD, age and gender matched controls, mothers having children with ASD (asdMO), and age matched controls having healthy children. They found 19 genes that were significantly differentially expressed (18 up and 1 down-regulated) when comparing the ASD to the control group, and 57 genes that were differentially expressed between the asdMO group and the asdMO control group (17 up-regulated and 40 down-regulated genes fold change >2.0). Three genes overlapped and were dysregulated in both individuals diagnosed with ASD and in asdMO. An ASD-associated gene expression pattern was often observed in both asdMO and individuals with ASD even though they had no symptoms above clinical threshold of ASD (expression of the 19 and 57 genes was changed in a parallel direction; Kuwano et al., 2011).

Glatt et al. identified 60 infants and toddlers at risk for ASDs, 34 at risk for language delay, 17 at risk for global developmental delay, and 68 typically developing children. One hundred and fifty four probes showed significant dysregulation in ASD, and a log 2-fold change. The most accurate support vector machine utilized the magnitude of the expression of 48 probes to classify 71% of ASD and control subjects across 10 subsets of discovery sample into their appropriate diagnostic categories. Of 30 individuals diagnosed with ASD, 27 were correctly classified by this support vector machine as having ASD, 23/34 control subjects were correctly classified as controls. The list of 48 probes making up the best support vector machine classifier of ASDs was most significantly enhanced with genes related to immune responses, genes of the hemoglobin complex, and genes with guanine- or guanylate-binding affinity (Glatt et al., 2012).

Kong et al. performed a genome-wide expression profile of the blood from 20 proband-unaffected sibling pairs, and 18 unrelated controls. One hundred and eighty nine probe sets that represented 163 unique genes (including 2 previously reported ASD candidate genes) were significantly changed between probands and siblings—84 probands were up-regulated compared to unaffected siblings (Kong et al., 2013).

Segura et al. looked at neurotrophin blood-based gene expression and social cognition analysis by obtaining whole blood from 21 adults and adolescents diagnosed as ASD, as well as from 10 controls. Social cognition abilities of subjects with ASD and controls were determined according to three Theory of Mind tests (RME, Faux pas test, The Happé stories). They found that NT3 and NT4 mRNA expression in the whole blood was significantly lower in ASD patients compared to healthy controls ($P < 0.05$). They also found that P75NTR mRNA expression was significantly higher in ASD patients than in controls. The ASD group received lower scores in three Theory of Mind tasks compared to the control group, which indicates that social cognition impairments in association with the ASD phenotype, yet no correlations were observed between neurotrophins and their receptors expressions and measures of Theory of Mind (Segura et al., 2015).

GI AND ASD BACKGROUND

Gastrointestinal (GI) symptoms are common in children with ASD compared with typically developing children and those with other developmental delays. Some controversial studies suggest

that as many as 70% of children with ASD exhibit chronic GI-related symptoms (Walker et al., 2013).

GASTROINTESTINAL TISSUE GENE EXPRESSION STUDIES

Williams et al. looked at impaired carbohydrate digestion and transport and mucosal dysbiosis in the intestines of children diagnosed with ASD and gastrointestinal disorders. They found that the levels of three brush border disaccharidases (sucrase isomaltase [SI], maltase glucoamylase [MGAM], and lactase [LCT]) were all significantly decreased in children with ASD and GI complaints (ASD-GI). Within the ASD-GI group, 86.7, 80, and 80% of children had lower transcript levels in SI, MGAM, and LCT respectively. Almost all (14/15, or 93.3%) ASD-GI children had deficiencies in at least one disaccharidase enzyme; 80% had deficiencies in 2 or more enzymes; and 73.3% had deficiencies in all three enzymes. Real-time polymerase chain reaction (RT-PCR) revealed a significant decrease in 2 hexose transporters: ileal SGLT1 mRNA and GLUT2 mRNA in ASD-GI children. For SGLT1, 73.3% of ASD-GI children had deficient transcript levels, and 73.3% of ASD-GI children had deficient GLUT2 transcript levels, relative to control-GI children. In total, 93.3% (14/15) of ASD-GI children had mRNA deficiencies in at least one of the five genes involved in carbohydrate digestion or transport; 66.7% (10/15) had mRNA deficiencies in all five genes (Williams et al., 2011).

Similarly, Walker et al. looked at subjects including children with ASD and three typically developing groups including (1) children who underwent diagnostic ileocolonoscopy for chronic GI symptoms in which no histopathology; (2) children with Crohn's disease (3) and children with ulcerative colitis. Pairwise analysis between the ileal mucosa from ASD-GI and non-inflamed control samples resulted in 1409 differentially expressed transcripts unique to the ASD-GI samples. Pairwise analysis between inflamed colonic mucosa from ASD-GI children and non-inflamed control samples resulted in 1189 differentially expressed transcripts unique to ASD-GI samples. The overlap between the 2 sets (ileum and colon) resulted in 178 transcripts that were exclusively differentially expressed in both ileal and colonic tissues form the ASDGI population. When these 178 transcripts were analyzed using Ingenuity Pathway Analysis software, three of the top associated biological functions were inflammatory disease, endocrine system development and function, and digestive system development and function (Walker et al., 2013).

ADULT OLFACTORY STEM CELL GENE EXPRESSION STUDIES

Féron et al. used adult nasal olfactory stem cells from nine adults with severe ASD and low developmental disabilities (DSM-5), plus two adults with mild ASD and no or mild cognitive abilities (Asperger syndrome or high functioning ASD) paired with 11 age and gender matched controls. Gene microarray analysis highlighted 156 genes that were differentially expressed in at least one ASD patient, of which 31 were dysregulated in more than 33% of the cohort (9 out of the 156 genes have been previously associated with ASD). They found that MOCOS, an enzyme involved in purine metabolism, is downregulated in most ASD individuals (8/11), compared to controls (Féron et al., 2016).

HAIR FOLLICLE GENE EXPRESSION STUDIES

Maekawa et al. utilized scalp hair follicles as a source of biomarker genes and found that the gene CNTNAP2 showed significantly decreased expression in samples from subjects with ASD compared with control follicles (Maekawa et al., 2015).

GENE EXPRESSION ANALYSIS: COMORBIDITIES

A few of the gene expression studies looked at people diagnosed with ASD and comorbidities.

ASD and Fragile X/Dup 15q

Nishimura et al. performed genome-wide expression profiling of LCL in order to distinguish different forms of ASD and to reveal shared pathways. Individuals with ASD both with and without FMR1-FM or dup (15q) were compared to TD male controls. The combination of ANOVA, SAM and RankProd, isolated 120 genes in ASD with FMR1-FM, and 80 genes in ASD dup (15q), 68 genes were found to be dysregulated in both ASD with FMR1-FM and dup (15q) (so 52 genes were selectively dysregulated only in ASD with FMR1-FM, and 12 genes were selectively dysregulated only in ASD with dup (15q). They also found a potential molecular connection between FMR1-FM and dup (15q), the cytoplasmic FMR1 interaction protein 1 (CYFIP1), which was up-regulated in dup (15q) patients. Expression of JAKMIP1 and GPR155 was significantly dysregulated in the 27 males with ASD when compared with their siblings without ASD. JAKMIP1 is a gene known to be related to microtubule transport. Genes related to chaperone and protein folding were enriched in the 52 genes selectively dysregulated in ASD with FMR1-FM; genes related to RNA binding and mRNA metabolism were also enriched in this set (this is consistent with FMRP protein's function as an RNA binding protein important in regulatory translation) (Nishimura et al., 2007).

ASD and ADHD

Taurines et al. looked at altered mRNA expression of monoaminergic specific genes in the blood of children with ADHD and ASD. They found a significant group difference with decreased DRD5- level in ASD patients when compared with controls and to patients diagnosed with ADHD. *Post-hoc* analyses demonstrated reduced DRD4-levels in the group of both ADHD patients and ASD patients when compared with healthy controls (Taurines et al., 2011).

GENE EXPRESSION IN ASD-SUMMARY (TABLE 2)

Sample Source Types

When the gene expression studies are viewed together independent of source type, over 100 genes are found in more than one study. Interestingly, whether the gene is up-regulated or down-regulated is independent of the source of the gene sampled and can even vary within the same source type in different studies. For example, the gene NDUFB5 was found to be upregulated in LCL derived cell line RNA's (Talebizadeh et al., 2014), but downregulated in three different regions of post-mortem brain tissue (Anitha et al., 2012). The gene NEURL3 was up-regulated in peripheral blood in a study conducted by Chien et al. (2013), but down-regulated in peripheral blood in a study conducted by Kong et al. (2013). However, most genes that were up or down-regulated followed the same pattern in different studies across sample source types.

Pathways (Table 3)

One of the aims of gene expression studies is to look for multiple genes in the same pathway. This should provide some clues to the underlying mechanism of the disease. From the studies surveyed, five pathways were found three times across different studies, and eight pathways were found twice. The pathways that were found three times included: cell cycle, cell death, GI disease, immune function, and neurogenesis. All of these areas are already areas of extensive research in ASD and therefore, they are not-surprising. The pathways that were found twice were: alternative splicing, arrhythmogenic right ventricular cardiomyopathy, cellular assembly and organization, cell-to-cell signaling and interaction, gap junction, inflammation, small molecule biochemistry, and ubiquitin mediated proteolysis. While inflammation and cell signaling are already areas of research for ASD, the potential relationship between the other pathways and ASD deserves further consideration. A more recent paper by Ivanov et al. (2015) highlights a number of other pathways and their importance in understanding the function of genes including the Wnt pathway and the calcium pathway, which is involved in the development of the nervous system and deserves further investigation regarding its potential role in ASD. Similarly, Wen et al. (2016) found the calcium signaling pathway to be a very active pathway in ASD. The precise role of calcium signaling in ASD and its potential relationship to other common metabolic disturbances in ASD demands further research.

Specific Genes/Pathways Already Implicated in ASD or in Processes Relevant to ASD

There are numerous examples of specific genes that have been shown to be altered between ASD and control samples. All have been shown to be directly related to important processes that when reduced or altered, can be connected to ASD. These genes can be roughly subdivided into genes related to the brain and genes related to the immune system.

Brain Related Genes

Hu et al. found the protein ASS to be upregulated in the autism samples. ASS controls the rate-limiting step involved in nitric oxide (NO) production via regeneration of arginine from citrulline, a by-product of the nitric oxide synthetase (NOS) reaction. Since NO is a important signaling molecule in the brain and has been implicated in several disorders, including ASD, thus the increased expression of ASS may be potentially relevant to the ASD phenotype (Hu et al., 2006).

Based on the findings of Nishimura et al., since CYFIP1, which was shown to be upregulated in dup (15q) patients is known to counteract FMRP, they reason that the induction of CYFIP1 in dup (15q) might elucidate some of the significant overlap between ASD with FMR1-FM and with dup (15q). They also found that JAKMIP1 was significantly induced in ASD with FMR1-FM and had a positive trend in ASD with dup (15q), suggesting that JAKMIP1 could represent a commonly dysregulated pathway. This gene is a particularly biologically important candidate, given its putative role in GABAB receptor expression and microtubule networks (Nishimura et al., 2007).

Since Taurines et al. found reduced expression in ASD probands of DRD5 which is expressed in the hippocampus associated areas and is thought to be important in the induction of long term potentiation related to novel events, it can be suggested that the reduced expression could give insight into the fact that probands have less expression of an important hippocampal gene (Taurines et al., 2011).

Kuwano et al. found that mRNA levels of ITGA2B encoding glycoprotein (GP) αIIβ were upregulated both in individuals with ASD and in asdMO; GPαIIβ forms αIIbβ3 integrin with ITGB3, an ASD-susceptible gene. Since αIIbβ3 integrin has an critical role in cell morphology, including synapse maturation, the increased expression of ITGA2B mRNA might change cellular morphology of peripheral cells in mothers having children with ASD as well as subjects with ASD (Kuwano et al., 2011).

Chow et al. showed that the A2A receptor-signaling pathway was the top dysregulated pathway in the young autistic brain. Adenosine receptors are crucial for both brain development and function including the regulation of neuronal stem cell proliferation, synaptic plasticity, motor function, cognition and emotion-related behaviors (Chow et al., 2012).

Voineagu et al. identified that A2BP1/FOX1, a neural and muscle specific alternative splicing regulator (and the only splicing factor previously implicated in ASD) was down-regulated in several individuals with ASD (Voineagu and Eapen, 2013).

The reports of James et al. showed that elevated 5-hmC in the EN-2 promoter is correlated with a significant decrease in repressive MeCP2 and histone H3K27me3 which appear to override 5-mC hypermethylation. These epigenetic changes are thought to loosen enhancer region chromatin which would facilitate enhancer binding and promote sustained upregulation of EN-2 expression. Since perinatal EN-2 downregulation is crucial for normal Purkinje cell differentiation and cerebellar patterning, the consistent postnatal overexpression of EN-2 suggests that the shutting of this programmed developmental

TABLE 3 | List of main pathways affected.

Paper	Software	List of main pathways affected
Alter et al., 2011	DAVID analysis	Alternative splicing, splice variant, zinc-finger, phosphoprotein, zinc, metal-binding, zinc-ion binding, dna-binding, ubiquitin mediated proteolysis, nucleus, transcription, transition metal ion binding, chromosomal rearrangement, ubl conjugation pathway, transcription regulation, coiled coil, regulation transcription, compositionally biased region: Ser-rich
Chien et al., 2013	DAVID analysis	Long-term depression, Cytokine-cytokine receptor interaction, Vascular smooth muscle contraction, Arrhythmogenic right ventricular cardiomyopathy (ARVC), glycerophospholipid metabolism, allograft rejection, Jak-STAT signaling pathway, Hematopoietic cell lineage, Gap junction, T cell receptor signaling pathway, RIG-I-like receptor signaling pathways, Ubiquitin mediated proteolysis, Intestinal immune network for IgA production, Type II diabetes mellitus, Leukocyte transendothelial migration, GnRH signaling pathway
	EMA (Easy Microarray data Analysis)	Long-term depression, Amoebiasis, Vascular smooth muscle contraction, Cytokine-cytokine receptor interaction, Arrhythmogenic right ventricular cardiomyopathy (ARVC), Intestinal immune network for IgA production, Endocytosis, Aldosterone-regulated sodium reabsorption, African trypanosomiasis, Graft-vs.-host disease
	GSEA Gene Set Enrichment Analysis	Arrhythmogenic right ventricular cardiomyopathy ARVC, terpenoid backbone biosynthesis, glycerophospholipid metabolism, vascular smooth muscle contraction, alpha linolenic acid metabolism, tight junction, inositol phosphate metabolism, cytosolic dna sensing pathway, renal cell carcinoma, cell receptor signaling pathway, chronic myeloid leukemia
Chow et al., 2012		
Young autistic/control	MetaCore software suite	DNA damage-response, cell cycle and apoptosis-related pathways
	DAVID analysis	DNA damage/cell cycle, apoptosis, and immune signaling and neurogenesis and neural development
Adult autistic/control	MetaCore software suite	Cell differentiation, mitogenic signaling and apoptosis genes
All autistic and control cases independent of age		DNA-damage response, apoptosis and immune system response functions
Enstrom et al., 2009	DAVID analysis	NK function, cellular proliferation, and leukocyte function
Féron et al., 2016	IPA analysis	Developmental disorders, gastrointestinal diseases, purine metabolism, inflammation
Garbett et al., 2008	GSEA Gene Set Enrichment Analysis	Antigen-specific immune response, inflammation, cell death, autoimmune diseases, migration, and targeting of the immune response to specific cells
Ginsberg et al., 2012	DAVID analysis	Mitochondrial oxidative phosphorylation, protein translation, synapse/neurotransmitters, vesicle transport, brain patterning,
	IPA analysis	Oxidative phosphorylation
Glatt et al., 2012	DAVID analysis	Genes related to immune response, genes of the hemoglobin complex, and genes with guanine- or guanylate-binding affinity
Gregg et al., 2008	DAVID analysis	Natural killer cell-mediated cytotoxicity
	IPA analysis	Natural killer cell signaling, IL-2 signaling, serotonin receptor and dopamine receptor signaling, retinol and methionine metabolism
Hu et al., 2006	IPA analysis	Neurological development and function, neuronal signaling, extension of neurites, myelination, VEGF-induced release of nitric oxide, neurogenesis, survival of Purkinje cells, apoptosis of neurons, development of septum, TNF and other cytokines
Hu et al., 2009	IPA analysis	Synaptic transmission, neurogenesis, neurulation, long-term potentiation (learning), protein ubiquitination, brain function, molecular and cellular functions, cell death, small molecule biochemistry, free radical scavenging, cellular function and maintenance, liver toxicity, circadian rhythm, and androgen sensitivity
Ivanov et al., 2015	KEGG pathway analysis	Calcium signaling pathway, Amphetamine addiction, Leishmaniasis, GABAergic synapse, Retrograde endocannabinoid signaling, MAPK signaling pathway, Arrhythmogenic right ventricular cardiomyopathy (ARVC), Cholinergic synapse, GnRH signaling pathway, Glutamatergic synapse, Serotonergic synapse, Dopaminergic synapse, Steroid biosynthesis, Steroid biosynthesis, Wnt signaling pathway, Leukocyte transendothelial migration, Hypertrophic cardiomyopathy (HCM), Dilated cardiomyopathy, Asthma, Influenza A, Carbohydrate digestion and absorption and Endocrine and other factor-regulated calcium reabsorption
Kong et al., 2013	GSEA Gene Set Enrichment Analysis	Ribosome and spliceosome pathways, neuroactive ligand receptor interaction pathway, calcium signaling pathway, and Gap junction
Kuwano et al., 2011		
ASD/control group	IPA analysis	Cell morphology, cellular assembly and organization, nerve system development and function
asdMO/asdMO	IPA analysis	Cancer, RNA post-transcriptional modification, reproductive system disease, protein synthesis, immune functions

(Continued)

TABLE 3 | Continued

Paper	Software	List of main pathways affected
Mahfouz et al., 2015	DAVID analysis	Synaptogenesis, regulation of apoptosis, regulation of cell death, GABAergic neurons, neuron projection, neuron differentiation, cell morphogenesis, learning/memory, behavior, mental retardation, epilepsy, mitochondrial function, protein translation, ubiquitination, synapse formation and elimination, protein turnover, ion channel, neurotransmitter receptor activity, and mitochondrial function
Nishimura et al., 2007	DAVID analysis	Cell communication and signal transduction, immune response, defense response
	IPA analysis	Cell cycle, cellular movement, and cell-to-cell signaling and interaction
Pramparo et al., 2015	Metacore GeneGo analysis	Apoptosis/Apoptotic nucleus, Immune response/Antigen presentation, Immune response/Phagocytosis, Immune Response/TCR signaling, Translation/Translation initiation, Inflammation/Interferon signaling, Apoptosis/Anti-Apoptosis via NF-kb, Cell Adhesion/Leukocyte chemotaxis, Inflammation/IFN-gamma signaling
Stamova et al., 2011	IPA analysis	Cellular Assembly and organization, cellular compromise, small molecule biochemistry, vitamin and mineral metabolism, cell death, neuronal development, neuronal survival
Talebizadeh et al., 2014	DAVID analysis	Protein-lipid modification
Tian et al., 2011	IPA analysis	Immunological and inflammatory disease processes, cell-cell signaling, antigen presentation, cell cycle, development and growth, proliferation, mitochondrial dysfunction pathways
Voineagu and Eapen, 2013	GO gene ontology enrichment analysis	Synaptic function, immune and inflammatory function
Walker et al., 2013		
Ileal Mucosa	PCA (Principal component analysis)	Gastrointestinal disease, inflammatory response, humoral immune response, tissue morphology, digestive system development and function, O-Glycan Biosynthesis, Propanoate Metabolism, Arginine and Proline Metabolism, and Alanine and Aspartate Metabolism
Colonic Mucosa	PCA (Principal component analysis)	Gastrointestinal disease, neurological disease, behavior, organ development, Atherosclerosis Signaling, Factors Promoting Cardiogenesis in Vertebrates and Mitotic Roles of Polo-Like Kinase
	IPA analysis	Inflammatory disease, endocrine system development and function and digestive system development and function, Granzyme A Signaling, Atherosclerosis Signaling, Valine, Leucine and Isoleucine Degradation and Clathrin-mediated Endocytosis Signaling
Wen et al., 2016	KEGG Pathway Analysis	MAPK signaling pathway, Calcium signaling pathway, Cell signaling, cell structure/transport, metabolism, neural, immune, cancer, cardiac disease, metabolic disease, Cell Adhesion Molecules, Wnt signaling pathway, mTOR signaling pathway, Focal adhesion, Regulation of actin cytoskeleton, Ubiquitin mediated proteolysis, Long-term potentiation, Axon guidance, and neurodegenerative diseases
Ziats and Rennert, 2013	DAVID analysis	Extracellular matrix formation/glycoproteins, immune response, chromatin, and cell cytoskeleton

window may have been missed in some individuals with ASD because of epigenetic abnormalities (James et al., 2014).

NT3&NT4, which were down to have lower expression in ASD patients compared to controls by Segura et al. play a crucial role in the development of the climbing fiber system of the cerebellar Purkinje cells (PCs), and NT3 selectively increased their survival. PC's are the primary efferent neurons of the cerebellar cortex, and its potential involvement in ASD has long been proposed. Neuropathological studies have shown significant reductions in the number and size of PCs in ASD post-mortem brain. Therefore, they hypothesize that reduced NT3 levels in the periphery in ASD patients might reflect altered expression in the CNS which may be associated with a loss of PC that result in altered cerebellar cortical efferent signals (Segura et al., 2015).

CNTNAP2, which encodes the contactin associated protein-like 2, which was found have significantly decreased gene expression in Maekawa et al. is one of the most intense ASD susceptibility genes with supporting evidence from several independent studies (Peñagarikano and Geschwind, 2012). CNTNAP2, a neurexin family protein that acts as a neuronal adhesion molecule and receptor. It was found to be a direct neural target of the human FOXP2 protein, and mutations of FOXP2 and CNTNAP2 were linked to language and speech disorders in ASD (Maekawa et al., 2015). The FOXP1 gene, which was found to be elevated in ASD subjects according to Chien et al. functions as a transcription repressor, forms a heterodimer with FOXP2, and is co-expressed with FOXP2 in numerous brain regions, suggesting close functional cooperation between the two proteins. FOXP1 is extensively expressed in the developing and mature brain and has been suggested to be important for brain development and function. Based on their data, it indicates that associations among FOXP1, FOXP2, and CNTNAP2 genes may play an important role underlying the pathogenesis of syndromic and non-syndromic ASD. They inferred that increased FOXP1 gene expression may lead to increased FOXP2 gene expression through a feedback mechanism, which may in turn reduce the gene expression of CNTNAP2 in patients with ASD (Chien et al., 2013).

Feron et al. found MOCOS to be downregulated in most ASD individuals as compared to controls. *In vivo* and *in vitro*

engineered models indicate that altered expression of MOCOS results in neurotransmission and synaptic defects. MOCOS expression also induces increased oxidative stress sensitivity. Metabolic disorders of purine metabolism have been shown to affect the nervous system and are able to induce autistic features (Féron et al., 2016).

Immune Related Genes

Gregg et al. found that SH2D1B/EAT2, one of the 11 differentially expressed genes they found to overlap in all 3 groups (AU vs. GP, A-E vs. GP, and A-R vs. GP), is mostly expressed in natural killer cells as well as macrophages, B cells, and dendritic cells, and has been theorized to suppress natural killer cell activity through the binding of protein tyrosine phosphatases, inhibitory kinases, or ubiquitin ligases. Abnormalities in RUNX3 (one of the 55 differentially expressed genes in AU vs. GP) function in leukocytes and is associated with sudden development of colitis and gastric mucosal hyperplasia and might be relevant to ASD since a small group of children with ASD appear to have gastrointestinal abnormalities (Gregg et al., 2008).

The findings of Enstrom et al. suggest possible dysfunction of natural killer cells in children with ASD, and the data suggests that circulating natural killer cells in ASD are persistently activated rather than quiescent (Enstrom et al., 2009).

Reduced expression of NLGN3 and SHANK3 genes in lymphoblasts of individuals with ASD is consistent with previous reports indicating that mutations of these genes cause reduced expression or loss of function of the protein. Yasuda et al. found that both these genes were found to be decreased in individuals in ASD (Yasuda et al., 2011).

One of the 48 biomarkers in the optimized support vector machine classifier by Glatt et al. IF116, was previously found to be dysregulated in the postmortem temporal cortex of subjects with ASD (Garbett et al., 2008; Glatt et al., 2012).

GENERAL LIMITATIONS IN RNA-GENE EXPRESSION STUDIES: A CRITICAL APPRAISAL OF THE DATA

Variance

One limitation of mRNA gene expression studies is variance. While gene specific approaches are helpful, they may ignore changes known as variance occurring at the global level of gene expression regulation. Global levels of gene expression regulation are crucial for understanding the underlying basis of diseases such as ASD where multiple systems are affected. For example, the associated increased risk of ASD in children of older fathers could be mediated by changes in global levels of gene expression regulation, or by paternally transmitted age related factors that are linked to changes in the global regulation of gene expression (Alter et al., 2011). Thus, it is possible that a common mediator, a change at the global level of gene expression regulation, could offer an all-encompassing explanation for multi-systemic effects of the disease.

Tissue Source

According to Chien et al. there are several disadvantages with the use of post-mortem brain tissue in gene expression studies (Chien et al., 2013). They point out that using fresh brain tissue from living patients is not always practical, and as a surrogate for brain tissue, several studies have instead utilized peripheral blood cells and LCL. They also state that there is a moderate correlation of gene expression has been reported between peripheral blood cells and brain tissue in humans which supports the usefulness of peripheral blood cells instead of brain-tissue for gene expression studies. Yet Mahfouz et al. (2015), argue that due to the nature of the pathology of ASD, which affects brain regions and the connection between various brain regions, there are advantages to post-mortem gene studies from brain tissue over peripheral blood studies. Kuwano et al. concluded that gene expression profiling of LCL are well documented because of their homogeneity (Kuwano et al., 2011).

Segura et al. explained that their rationale to use blood as opposed to post-mortem brain tissue was due to the limited access of tissue from the central nervous system (CNS) in humans (Segura et al., 2015). They also brought reports of a potential correlation between neurotrophin expression in CNS and the periphery, which would suggest that taking blood in order to study neurotrophins would be similarly effective to using brain tissue. Similarly, Pramparo et al. (2015) emphasized the advantages of peripheral blood, and were successful in identifying 2765 genes from a peripheral blood source from a variety of pathways including apoptosis, the immune response, and genes involved in translation.

According to Hu et al. while studies that used brain tissue to better understand the mechanistic basis for ASD could be informative; this method of study is not an appropriate target for diagnostic assays. They counter that diagnostic assays should ideally be taken from easily obtainable samples such as the patient's blood (Hu et al., 2006). However, Talebizadeh et al. acknowledge that LCL samples may not be ideal to use in order to investigate brain-related genes, but they may still be helpful for understanding at least a subset of brain-related changes (Talebizadeh et al., 2014).

LIMITATIONS SPECIFIC TO ASD GENE EXPRESSION STUDIES: A CRITICAL APPRAISAL OF THE DATA

The clinical heterogeneity of ASD presents a challenge any time studies attempt to find patterns across the spectrum. Complicating matters even more, the ASD gene expression studies relied on a variety of diagnostic methods to define ASD (**Tables 4A,B**). The lack of consistency between the diagnostic criteria and the subjectivity of the behavioral methods of diagnosis limit the ability to extrapolate the data to the broader ASD population.

Additionally, demographic variability between subjects and between subjects and controls complicates analysis. Considering that ASD is 4.5 times more common in males than females, the ratio of subjects in each study is of great importance if it is meant

TABLE 4A | Diagnostic tests part 1.

Paper/diagnostic test	DSM-4	DSM-5	ADOS	ADI-R	CARS	IQ scores	SCQ	CSBS DP	Mullen scales of early learning	Bayley scale of infant and toddler development	Vineland adaptive behavior scales	ToM social cognition test	Peabody Picture Vocabulary Test (PPVT)
Alter et al., 2011	✓		✓	✓									
Chien et al., 2013	✓			✓									
Chow et al., 2012			✓	✓									
Emanuele et al., 2010	✓				✓								
Enstrom et al., 2009	✓		✓	✓			✓						
Féron et al., 2016		✓									✓		
Ginsberg et al., 2012	✓		✓	✓		✓							
Glatt et al., 2012			✓					✓	✓				
Gregg et al., 2008	✓		✓	✓									
Hu et al., 2006			✓	✓									✓
Hu et al., 2009				✓									
Ivanov et al., 2015				✓	✓								
James et al., 2014	✓			✓									
Kong et al., 2013			✓	✓									
Kuwano et al., 2011	✓					✓							
Maekawa et al., 2015	✓			✓									
Nishimura et al., 2007			✓	✓		✓							
Pramparo et al., 2015	✓		✓						✓		✓		
Prandini et al., 2014	✓		✓	✓									
Segura et al., 2015	✓		✓	✓		✓						✓	
Stamova et al., 2011			✓	✓									
Talebizadeh et al., 2014 (taken from AGRE)			✓	✓							✓		
Taurines et al., 2011			✓	(ADI)									
Tian et al., 2011			✓	✓					✓		✓		
Walker et al., 2013	✓		✓	✓					✓	✓	✓		
Williams et al., 2011	✓			✓									
Yasuda et al., 2011	✓												

TABLE 4B | Diagnostic tests part 2.

Paper/ diagnostic test	Social communication questionnaire	Shortened CPEA regression interview	ICD-10	PARS	Japanese version of the Asperger's questionnaire	Japanese version of the autism spectrum Quotient (AQ-J)	Japanese versions of WAIS-III	PPVT	Inclusion criteria	Exclusion criteria
Alter et al., 2011									For Probands: A negative Fragile X DNA test, impairment in language	For Probands: significant prenatal history (prematurity, 35 weeks, intraventricular hemorrhage, severe asphyxia, or cerebral palsy), serious CNS abnormality, known genetic or metabolic disorder, non-classic forms of autism were excluded, including autism with regression and Asperger's syndrome, a higher functioning form of autism where individuals have language skills within the normal range
Emanuele et al., 2010									For controls: no past or present history of any psychiatric disorder and none of them had ever taken medications for psychiatric conditions	For controls: subjects with axis-I diagnosis of first-degree relatives
Enstrom et al., 2009									ASD: children who scored above the cut-off for the ADOS modules 1 and 2 for ASD and met the criteria for autism	Children who were ill at the time of the study, or had a temperature above 98.9°F, or were prescribed anti-psychotics, or had a known medical disorder or primary diagnosis (e.g., Fragile X or Rett syndrome)
Féron et al., 2016			✓						For controls: neither presenting a neuropsychiatric disorder nor taking medication	
Ginsberg et al., 2012									Male gender; autism diagnosis by a validated psychiatric/psychologic instrument; and the availability of sufficient fresh frozen tissue available for genome-wide methylation analysis, bisulfite sequencing, and gene expression studies	Formalin-fixation of brains, brains from individuals with a medication history of medications known or suspected to have effects on methylation; gross structural abnormalities of the brain; brains from individuals with a complicated birth history and/or evidence of pre- or perinatal hypoxia; history of major head trauma; diagnosis of Rett syndrome, Fragile X syndrome, tuberous sclerosis, or other syndromic process; or any known or likely pathologic cytogenetic abnormality identified by either routine karyotyping or chromosomal microarray analysis
Hu et al., 2006								✓		

(Continued)

TABLE 4B | Continued

Paper/ diagnostic test	Social communication questionnaire	Shortened CPEA regression interview	ICD-10	PARS	Japanese version of the Asperger's questionnaire	Japanese version of the autism spectrum Quotient (AQ-J)	Japanese versions of WAIS-III	PPVT	Inclusion criteria	Exclusion criteria
Hu et al., 2009										All females, individuals with cognitive impairment, those with known genetic or chromosomal abnormalities (e.g., Fragile X, Retts, tuberous sclerosis, chromosome 15q11-q13 duplication), those born prematurely (<35 weeks gestation), those with diagnosed comorbid psychiatric disorders (e.g., bipolar disorder, obsessive compulsive disorder, severe anxiety)
James et al., 2014										For Case Donors: PDD-NOS, Asperger's, Rett or Fragile X For Control Donors: Previous medical history of neurologic disorders, seizures or mental retardation
Kong et al., 2013									ASD: No known genetic or syndromic disorders	For controls: chronic disease such as infectious disease, diabetes, cardiovascular disease, and developmental disorder or neurological disorder
Kuwano et al., 2011						✓	✓			For controls: serious physical or mental disorders including ASD in the past and at present
Prandini et al., 2014									ASD: meets Diagnostic and Statistical Manual of Mental Disorders, fourth edition criteria for Autistic Disorder, Asperger's Disorder, or Pervasive Developmental Disorder Not Otherwise Specified (PDD NOS); reaches the score cutoff in Autism Diagnosis Interview-Revised (ADI-R) and Autism Diagnostic Observation Schedule (ADOS); is at least 4 years old at the time of entering the research project; has at least one parent or legal guardian giving voluntary written consent for him/her to participate in the research project, and gives his/her assent when possible	For ASD: diagnosis of Rett syndrome or childhood disintegrative disorder, history of serious head injury, encephalitis or tumors, profound mental retardation (intelligence quotient <20), and age more than 18 years

(Continued)

TABLE 4B | Continued

Paper/ diagnostic test	Social communication questionnaire	Shortened CPEA regression interview	ICD-10	PARS	Japanese version of the Asperger's questionnaire	Japanese version of the autism spectrum Quotient (AQ-J)	Japanese versions of WAIS-III	PPVT	Inclusion criteria	Exclusion criteria
Segura et al., 2015									For ASD: Had a total IQ score above 70 For controls: no somatic or neurological disease and without medication	
Talebizadeh et al., 2014 (taken from AGRE)	✓									
Taurines et al., 2011										For controls: if had somatic or neurological disease or were taking medication
Tian et al., 2011	✓								For Autism: meeting criteria on the communication, social, and repetitive behavior domains of the ADI-R, and scoring at or above the total cutoff for autistic disorder on the ADOS module 1 or 2	
Walker et al., 2013										For controls: identifiable gastrointestinal pathology
Williams et al., 2011		✓								For controls: Developmental disturbances, including ASD
Yasuda et al., 2011				✓	✓					For healthy controls: neurological or medical conditions that could potentially affect the central nervous system, had any psychiatric diseases and/or received psychiatric medication, had first- or second-degree relatives with psychiatric disease or presented with an IQ < 70
Zhubi et al., 2014- brain samples taken from the Harvard Brain Tissue Resource Center									For Control donors: free of neurological disorders, seizures, mentalretardation, dementia	For Case Donors: Asperger's syndrome Fragile–X syndrome, RTT, pervasive developmental disorder not otherwise specified, and 15q11-q13 duplication

to represent the broader ASD population. While some studies took the disproportionate male incidence into consideration in choosing their subjects, others did not. The clinical presentation of ASD also varies by age. Therefore, significant age differences between ASD subjects and controls such as those in the study by Chien (Chien et al., 2013) deserve further attention.

CONCLUSION

One of the advantages of gene expression studies over whole genome sequencing studies or Enzyme-Linked Immunosorbent Assay (ELISA) based protein studies for ASD is that it allows for broad screening for unique aspects of the disorder while maintaining a level of specificity that the other modalities cannot provide. In whole genome sequencing studies, millions of base pairs are analyzed and often nothing significant is found, or the few sporadic single nucleotide polymorphisms (SNP's) or copy number variants (CNV's) lack any useful context. While admittedly, more than 100 ASD-susceptibility genes have been found, the utility of this information remains elusive. In gene expression studies, on the other hand, despite analyzing large amounts of genes, the thresholds for differences in expression enable a level of specificity and the ability to group specific genes together in order to identify specific pathways. Approximately 12,000 genes were differentially expressed between ASD compared to controls in gene expression studies since 2011. Most of studies can be subdivided by the source into three categories: brain (∼3500 dysregulated genes), LCL (∼5600 dysregulated genes), and GI (∼2600 dysregulated genes). More specifically, in the gene expression studies of ASD surveyed, cell cycle, GI disease, immune function, and neurogenesis, were found to be the most common implicated pathways.

Most of the genes surveyed were shown to be consistently down or up-regulated across different source types in different studies. This strongly suggests that, in fact, these genes are not coincidentally higher or lower in ASD but might actually be active players in the underlying pathogenesis of the disorder.

Future Directions

Researchers might want to consider testing more than one sample source (peripheral blood, intestine, olfactory stem cell, and hair follicles) for each subject to help determine if up or downregulation is consistent amongst tissue types. In the absence of comparing across sample source types it remains unclear if differences between subjects in gene regulation are due to the different subjects or the different sample source.

Furthermore, researchers might consider including more healthy mothers of children with ASD in the gene expression studies and TD siblings in order to help isolate potential immunogenetic processes. This might help clarify why certain immune mechanisms affect only the child with ASD and not the mother or the siblings. Another idea could be to analyze the blood of daughters of women who have given birth to children with ASD in order to test whether they too have abnormal levels of certain proteins or immune markers that their healthy mothers have. If found, the abnormal levels could suggest that the healthy mother is passing something on to her daughter that would then make her more susceptible to having a child with ASD herself.

Additionally, now that certain pathways have been identified as being associated with ASD, researchers can work backwards and look for other genes involved in those pathways and test whether these specific genes play a role in ASD.

Finally, in order to help identify potential sub-groups of ASD, it might be fruitful to study correlations between subsection scores on ADOS and gene expression studies. This might unravel the heterogeneity of ASD into individual strands whose underlying pathology can be better understood at the genetic and epigenetic levels in order to develop targeted therapeutic approaches.

AUTHOR CONTRIBUTIONS

AA drafted the article, reviewed the relevant literature, made substantial contributions to conception and design, interpreted the data and approved final version. JR drafted drafted the article, reviewed the relevant literature, made substantial contributions to conception and design, interpreted the data, revised the article critically and approved final version. PZ and MM made substantial contributions to conception and design, participated in revising it critically, interpreted the data, and approved final version. BG participated in revising it critically, interpreted the data, and approved final version.

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REVIEW
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Genetic Syndromes, Maternal Diseases and Antenatal Factors Associated with Autism Spectrum Disorders (ASD)

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Autism spectrum disorder (ASD) affecting about 1% of all children is associated, in addition to complex genetic factors, with a variety of prenatal, perinatal, and postnatal etiologies. In addition, ASD is often an important clinical presentation of some well-known genetic syndromes in human. We discuss these syndromes as well as the role of the more important prenatal factors affecting the fetus throughout pregnancy which may also be associated with ASD. Among the genetic disorders we find Fragile X, Rett syndrome, tuberous sclerosis, Timothy syndrome, Phelan–McDermid syndrome, Hamartoma tumor syndrome, Prader-Willi and Angelman syndromes, and a few others. Among the maternal diseases in pregnancy associated with ASD are diabetes mellitus (PGDM and/or GDM), some maternal autoimmune diseases like antiphospholipid syndrome (APLS) with anti-β2GP1 IgG antibodies and thyroid disease with anti-thyroid peroxidase (TPO) antibodies, preeclampsia and some other autoimmune diseases with IgG antibodies that might affect fetal brain development. Other related factors are maternal infections (rubella and CMV with fetal brain injuries, and possibly Influenza with fever), prolonged fever and maternal inflammation, especially with changes in a variety of inflammatory cytokines and antibodies that cross the placenta and affect the fetal brain. Among the drugs are valproic acid, thalidomide, misoprostol, and possibly SSRIs. β2-adrenergic receptor agonists and paracetamol have also lately been associated with increased rate of ASD but the data is too preliminary and inconclusive. Associations were also described with ethanol, cocaine, and possibly heavy metals, heavy smoking, and folic acid deficiency. Recent studies show that heavy exposure to pesticides and air pollution, especially particulate matter <2.5 and $10\,\mu m$ in diameter (PM2.5 and PM10) during pregnancy is also associated with ASD. Finally, we have to remember that many of the associations mentioned in this review are only partially proven, and not all are "clean" of different confounding factors. The associations described in this review emphasize again how little we know about the etiology and pathogenesis of ASD. It is obvious that we need more epidemiologic data to establish many of these associations, but if proven, they might be promising avenues for prevention.

Keywords: ASD, genetic syndromes, autoimmune diseases, prenatal factors, inflammation, drugs, chemicals, pollution

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INTRODUCTION

ASD is defined by the DSM 5 as a neurobehavioral disorder manifested by persistent deficits in social and communication interaction, deficits in developing, understanding and maintaining relationships, as well as abnormal and fixed interests and repetitive behavior (Kogan et al., 2009; American Psychiatric Association, 2013). Symptoms must be present at early childhood and interfere with daily function. ASD is 4 times more prevalent in males than in females, but in experimental animal models where ASD is induced by neuro-teratogens, there are little gender differences. Mental impairment is common among children with ASD, and a variety of learning and behavioral changes are also prominent in autistic animals. The etiology is diverse, largely unknown, and seems to be the result of genetic and environmental interaction (Kogan et al., 2009; Tchaconas and Adesman, 2013).

Environmental exposures are increasingly being recognized as potential risk factors for ASD, and the possibility that the prenatal and perinatal environment affect fetal programming is an expanding direction for research. Prenatal environment include maternal use of medication, maternal infection and inflammations, and prenatal or perinatal exposure to various substances such as alcohol and heavy air pollution. Of special relevance are prematurity and maternal diabetes.

Epidemiology

The reported prevalence of autism has increased dramatically over time, from 4 to 5 cases per 10,000 in 1966 to ∼1% today (Kogan et al., 2009; Tchaconas and Adesman, 2013). The increase is thought to result increased public awareness, changing diagnostic standards, earlier age of diagnosis, and development of treatment modes. It was also shown that many of the children and adults who were previously diagnosed as having mental retardation meet the DSM 4 and DSM 5 diagnostic criteria of ASD (Shattuck, 2006; Elsabbagh et al., 2012). Some increase, however, may result from "new" environmental causes such as pollution and changing life style.

In the US, for example, the autism and developmental disabilities monitoring network (ADDM network) found in children aged 8 years in 2008 an increase from 1/150 in year 2000 to 1/88 in 2008 and in the 2010 monitoring the rate increased to 1/68 children (Autism Developmental Disabilities Monitoring Network Surveillance Year Principal Centers for Disease Prevention, 2012).

In Israel, judging from the number of children who received childhood disability benefits by the Israeli Insurance Institute because of ASD, the cumulative incidence at 8 years of age at 2011 has increased 10-folds from 1991 and reached 0.49% with a ratio of males/females of about 5 (Raz et al., 2015b).

The more recent global prevalence of autism was estimated to be 0.62% (Autism Developmental Disabilities Monitoring Network Surveillance Year Principal Centers for Disease Prevention, 2012; Elsabbagh et al., 2012). In spite of a wide variation in prevalence between the studies, the authors conclude that there is no evidence of a significant impact of ethnic or socioeconomic factors on the rate of ASD.

It can be presumed that in developed countries the great progress in the diagnosis and treatment of ASD reached a relatively steady state at least in the last 10 years. However, both the incidence and prevalence of ASD continued to rise, implying that some of the increase results from a true increase in ASD rate. For example, in the UK in 1988–92 the incidence was 0.40/10,000 person years and it raised to 2.98/10,000 person years in 2000–2001 (Smeeth et al., 2004) and an in the US an increase was reported in the same states in the US between 2000 and 2008 and a further increase between 2008 and 2010 (Fombonne, 2009; Autism Developmental Disabilities Monitoring Network Surveillance Year Principal Centers for Disease Prevention, 2012). An increased incidence was also reported in Israel (Davidovitch et al., 2013). If this is correct, it may largely result from prenatal environmental causes because genetic, ethnic, socioeconomic, and geographic factors did not change significantly during that time. Recent meta-analysis of seven studies including 1,140,210 children evaluated the association between inter-pregnancy interval and increased risk of ASD, and found OR of 1.9 for interval <12 months and 1.37 interval >5 years. Proposed mechanisms included folate deficiency, maternal stress, and sustained post-partum inflammation from previous pregnancy for short interval and infertility, unintended pregnancy, and inflammation for long one (Conde-Agudelo et al., 2016). Genetic predisposition was offered by Darbro et al. who found decreased risk of cancer among ASD patients. Despite increased prevalence of rare coding single-nucleotide variations in oncogenes they tend to develop fewer neoplasms. They hypothesized that the protective effect may be due to defects in cellular proliferation and aging (Darbro et al., 2016).

The prenatal causes of ASD can be divided into environmental chemicals (i.e., drugs such as valproic acid, thalidomide, misoprostol; alcohol, cocaine, and toxic metals taken by the mother during pregnancy), exposure to particulate matter air pollution of up to 2.5 micron in diameter (PM2.5), maternal infections during pregnancy (i.e., rubella, CMV), maternal and fetal inflammation (Fox et al., 2012) and maternal diseases (i.e., diabetes mellitus), including autoimmune diseases (Brown et al., 2015) or allergic diseases such as asthma (Croen et al., 2005). In addition, perinatal factors such as perinatal asphyxia, IUGR, RDS, and others were also associated with an increase in the prevalence of ASD (Gardener et al., 2011).

Numerous mechanisms for ASD have been offered, based also on experimental animal models. In addition to complex genetic susceptibility, epigenetic changes have also been proposed (Kogan et al., 2009). Other mechanisms are: immune dysregulation that include abnormal levels of cytokines and growth factors and various fetal and maternal antibodies to brain tissue (Rossignol and Frye, 2012). Additional proposed mechanisms are increased oxidative stress, mitochondrial dysfunction, abnormalities in brain serotonin, abnormal white matter connectivity, decrease number of Purkinje cells in the cerebellum, and neuronal migration defects (Billeci et al., 2012; Grabrucker, 2012; Rossignol and Frye, 2012).

The purpose of the present review is to summarize the data describing genetic syndromes with ASD like behavior and the maternal diseases associated with increased prevalence of ASD.

In addition, we summarize the antenatal injurious factors that are known to be related to the etiology and pathogenesis of ASD.

GENETIC SYNDROMES ASSOCIATED WITH OR PREDISPOSING TO ASD

ASD phenomenology varies between syndromes, but in some it is more common than in the general population (Richards et al., 2015). Several human syndromes derived from a single gene mutation increase the risk for ASD. The more common aberrations are Fragile X syndrome, a mutation in FMR1 (Kaiser-McCaw et al., 1980), Rett syndrome, a mutation in MECP2 (Meloni et al., 2000), tuberous sclerosis, mutations in TSC1 or TSC2 (Green et al., 1994), Timothy syndrome, a mutation in CACNA1C (Gillis et al., 2012), and Hamartoma tumor syndrome, a mutation of the PTEN gene. Copy-number variants that lead to inherited maternal 15q11–13 duplication resulting in Prader-Willi and Angelman syndromes (Ogata et al., 2014) and other duplications like the NPHP1 gene (Yasuda et al., 2014) are also associated with autistic traits. Patients with other syndromes like 22q.11.2 deletion—DiGeorge syndrome (Swillen and McDonald-McGinn, 2015) and neurofibromatosis type 1 (Huijbregts et al., 2015) show behavioral changes that are also common among ASD patients. Multiple other syndromes were associated with ASD, however cases are sporadic (Moss and Howlin, 2009). The development in strategies for the identification of genetic variants led to the description of new syndromic forms of ASD and enabled the association between phenotype and genetic traits.

Fragile X (FMR1)

Children with Fragile X syndrome which is the most frequent inherited cause of mental retardation have increased rates of ASD. Current estimates suggest that ASD occurs in 21–50% of males with fragile X syndrome, with increased prevalence among individuals with a greater degree of intellectual disability (Moss and Howlin, 2009; Wadell et al., 2013). The Fragile Mental Retardation 1 locus (FMR1) resides in the X chromosome and expansion of triplet repeats in the untranslated region of the FMR1 gene prevents synthesis of the FMR1 gene product FMRP. FMRP is a RNA-binding protein that modulates mRNA trafficking, dendritic maturation and synaptic plasticity. FMRP deficiency leads to dysregulation of other pathways including metabotropic glutamate receptors (mGluR1 and 5), aminobutyric acid A and B pathways, phosphatidylinositol 3-kinase, and mammalian target of rapamycin pathways. FMRP regulates proteins known to be mutated in autism including neuroligins, neurorexins, SHANK, phosphatase, and tensin homolog (Darnell et al., 2011). Deficits in global network organization and decreased clustering with connectome that shift toward randomness were found by Bruno et al among fragile X patients by structural brain network topology evaluation with magnetic resonance imaging (MRI), a finding that was qualitatively similar to what has been described in ASD (Bruno et al., 2016). When 47 children and adolescents with fragile X were assessed for ASD symptoms, most were engaged in self injury behavior, aggression, and stereotypy (Newman et al., 2015). Compared to children with fragile X, those who also had ASD developed slower, had lower developmental level for age that improved over time and performed worse at language tasks (Ballantyne and Nunez, 2016). Boys with fragile X were found to have social smiling less impaired and complex mannerisms more impaired compared to boys with non-syndromic ASD (McDuffie et al., 2015). Besides behavioral Interventions that were proven beneficial, medical therapy based on the pathophysiology of fragile X is currently investigated (Newman et al., 2015). Waddell et al revised the medical treatment modalities based on the up-regulation of the metabotropic glutamate receptor 5 (mGluR5) pathway and down-regulation of the GABA A pathway. The mGluR5 antagonists evaluated in human studies, Fenobam and AFQ056, improved behavior. The GABA B agonist Arbaclofen which is the R-isomer of baclofen and a GABA B agonist that works at a presynaptic receptor improved social behavior of patients with fragile X and ASD. Minocycline, which lowers the levels of matrix metalloproteinase 9 (MMP9), one of a family of proteins important for synaptic plasticity, improved language and behavior among adolescents and young adults with fragile X, however minocycline may cause in young children graying of the permanent teeth when they emerge (Wadell et al., 2013). Human studies with antioxidants like melatonin (Karaaslan and Suzen, 2015; Kwon et al., 2015) and omega 3 fatty acid, vitamin C, vitamin E, and N acetyl cysteine have been tried in single cases. Acetylcarnitine (LAC) was proven beneficial among fragile x patients with ADHD (Wadell et al., 2013).

Rett Syndrome and MECP2 Mutations

Rett syndrome, an X linked disease that affects girls, is characterized by neurodevelopmental delay, ASD and seizures. Estimates of rates of ASD in Rett syndrome range from 25 to 40% and up to 97% in individuals with the preserved speech variant of Rett syndrome (Moss and Howlin, 2009). It is caused by mutations in the gene encoding for the methyl-CpG binding protein 2 (MeCP2) that binds to methylated-CpG dinucleotides and influences gene expression. MeCP2 is expressed widely, but is most abundant in neurons of the mature nervous system. MeCP2 duplication syndrome is characterized by autism, intellectual disability, motor dysfunction, anxiety, epilepsy, recurrent respiratory tract infections and early death. The MET receptor tyrosine kinase gene which is a part of the biological network that includes several ASD-associated transcriptional regulators, including FOXP2 and MeCP2 was found reduced in the temporal lobe of subjects with ASD. Regulation of MET transcription by MeCP2 was found *in vitro* in primary human neural progenitors from the olfactory epithelium. Analyses of postmortem temporal cortex samples from Rett syndrome and ASD cases demonstrated sex-based differences in reduced MET expression, with males' perturbation in ASD and females in Rett syndrome (Plummer et al., 2013). In a UK national sample of 91 women with Rett syndrome aged from 4 to 47 years behavioral disturbances included hand stereotypies (99%), low mood/changeable mood (77%), anxiety

or inappropriate fear (73%), sleeping difficulties and night-time laughing (64%), teeth grinding (58%), and breath holding (63%) (Cianfaglione et al., 2015). Despite autistic traits Rett syndrome patients have some phenotypic features that differ from ASD patients like increased visual fixation at social stimuli (Schwartzman et al., 2015). Rett patients exhibited a decrease in visual evoked potential (VEP) amplitude that was most striking in the later stages of the disorder and a slower recovery from the peak of the VEP response that was impacted by MeCP2 mutation type (Leblanc et al., 2015). Based on animal studies in which IGF-1 and BDNF supplementation therapies improved neuronal developmental, synaptic maturation and plasticity exerted through the key cell-signaling pathways, PI3K/Akt and MAPK in MeCP2 mutant mice (Castro et al., 2013), a clinical trial in 10 Rett syndrome patients showed improvement in social and cognitive ability with recombinant human Insulin-Like Growth Factor 1 supplementation (Pini et al., 2016).

Tuberous Sclerosis TSC1 or TSC2

Tuberous sclerosis complex is an autosomal dominant disorder caused by mutations in either the TSC1 or TSC2 gene. The syndrome is associated with cerebral cortical tubers and may be complicated by astrocytomas. About 90% suffer from behavioral, intellectual, psychiatric and psychosocial difficulties and up to 60% have the diagnosis of ASD. There is disagreement regarding the association of ASD with the presence of temporal-lobe tubers (Moss and Howlin, 2009). The protein products of TSC1, hamartin and TSC2, tuberin, inhibit Ras homolog enriched in the brain (Rheb) that activates mammalian target of rapamycin (mTOR) complex 1 (mTORC1). Heterozygous defects in either TSC gene prevents the Rheb inhibition and allows for excessive mTOR activation, which induce cell growth and proliferation. Upregulation of the mTOR pathway leads to abnormal dendritic protein synthesis with reduced or dysmorphic dendritic spines and alterations in postsynaptic glutamate receptor-mediated long-term depression, similar to synaptic abnormalities described in ASD. Owing to the presence of cardiac rhabdomyomas being detected by pre/perinatal ultrasonography patients are diagnosed early, allowing prompt detection of ASD symptoms (Davis et al., 2015). In a recent study evaluating 42 patients aged 4–44 years that were diagnosed with Tuberous sclerosis 17 (40%) had ASD that worsened with intellectual disabilities. None of the patients with normal or borderline mental abilities had ASD. The Social Communication Questionnaire (SQL) score was positively associated with epilepsy, seizure onset before age 1 year, infantile spasms and mutations in TSC2, but not tubers localization and gender (Vignoli et al., 2015). The mTOR inhibitors used in treatment of TSC patients for various manifestations were also evaluated for ASD symptoms. Clinical trials with Rapamycin supplementation were inconclusive, some due to the severe intellectual deficits of the evaluated patients. TSC patients with infantile spasms had higher response rates compare to other patients when treated with Vigabatrin which increase GABA availability in the synaptic cleft. It is hypothesized that it may be associated with mTOR suppression found in a murine model (Davis et al., 2015)

Timothy Syndrome—CACNA1C Mutation

Timothy syndrome (TS) is a rare dysmorphic disease presenting with cardiac arrhythmias, syndactyly; immune deficiency, intermittent hypoglycemia and neurologic morbidities including seizures, mental retardation, and autism. It is a single nucleotide mutation in the gene encoding the pore-forming subunits of an L-type calcium channel (CaV1.2). The sporadic glycine-to-arginine mutation is located at position 406 in exon 8A (Splawski's terminology). Both variants of TS (the milder TS1 and the more severe TS2) arise from missense mutations in alternatively spliced exons that cause the same G406R replacement in the CaV1.2 L-type calcium channel. Association between non-syndromatic ASD and two SNPs in CACNA1C was found in families of Chinese Han ancestry (Li et al., 2015). Currently there is no specific treatment for ASD in patients with Timothy syndrome. Mexiletine, a voltage-gated sodium channel blocker improved cardiac symptoms in a 2 years old girl; however no data concerning her psychological situation is given (Gao et al., 2013).

Phelan–McDermid Syndrome—*SHANK3* Deletion

Deletion of the human *SHANK3* gene near the terminus of chromosome 22q13 is associated with Phelan–McDermid syndrome and autism. Clinical manifestations include dysmorphic face with wide nasal bridge, pointed chin, deep-set eyes, flat mid-face, large ears, long eyelashes, bulbous nose and high-arched palate, hypotonia, developmental delay, and delayed or absent speech. SHANK genes code for large synaptic scaffold proteins of the post-synaptic density. SHANK deletions or mutations were found in about 1% of cases of ASD. Among 760 ASD patients evaluated SHANK1 mutations were found in 0.04% and were present in males with normal IQ and autism, SHANK2 mutations were present in 0.17% of patients with ASD and mild intellectual disability and mutations in SHANK3 were present in 0.69% of the patients with ASD. Those with SHANK3 deletions presented manifestations of the Phelan–McDermid syndrome (Leblond et al., 2014). Using functional MRI to evaluate neural response to communicative vocal sounds, infants with Phelan–McDermid syndrome differed from infants with ASD by their better neural response to communicative vocal sounds in the right superior temporal gyrus (Wang et al., 2016). Children with Phelan–McDermid syndrome were found to have less sensory sensitivity as compared to children with ASD (Mieses et al., 2016).

Hamartoma Tumor Syndrome—PTEN Mutation

Phosphatase and tensin homolog on chromosome 10 (PTEN) is a tumor suppressor gene that has been reported in autistic individuals with macrocephaly, and also in some syndromes including the Hippocampus Cowdon, the Lhermitte-Duclos disease, and the Bannayan Riley Ruvalcaba Syndrome. More recently, the term PTEN Hamartoma Tumor Syndrome (PHTS) has been used to encompass the range of symptoms identified in PTEN mutation carriers (Leslie and Longy, 2016). McBride

et al found in their ASD cohort that 7/99 (7.1%) of the ASD patients and 8/100 (8.0%) of those with mental retardation or developmental delay had PTEN mutations, all of them also had macrocephaly (McBride et al., 2010).

CNTNAP2 Mutations

The CNTNAP2 gene is located in chromosomal region 7q35. A recessive nonsense mutation in the contactin associated protein-like 2 (CNTNAP2) gene was shown to cause a syndromic form of ASD, cortical dysplasia and focal epilepsy syndrome (CDFE). This is a rare disorder resulting in epileptic seizures, language regression, intellectual disability, hyperactivity, and ASD. Neuronal migration defects were found in about half of the patients (Strauss et al., 2006). The CNTNAP2 variant that increases risk for the language impairments in autism was shown to lead to abnormal functional brain connectivity in human subjects (Scott-Van Zeeland et al., 2010). Besides ASD, mutations in the CNTNAP2 gene were also found among patients with Gilles de la Tourette syndrome, ADHD, language disturbances, epilepsy, and schizophrenia (Poot, 2015).

15q11-13 Deletion or Duplication Maternal/Paternal

Mutations in 15q11-13 are associated with either duplication or gene deletion. Prader-Willi syndrome results from the loss of imprinted genomic material within the paternal 15q11.2-13 locus and deletions, unbalanced translocations, or uniparental maternal disomy. The loss of maternal genomic material at the 15q11.2-13 locus results in Angelman syndrome since normally, only the maternal copy of the Ube3A gene is active in the brain (Lasalle et al., 2015). Region duplications are the most frequent genetic abnormality in autism and the penetrance rate in individuals with a maternally derived duplication is >85% (Cook and Scherer, 2008). Maternally inherited 15q11-13 duplications and triplications are among the most common genomic copy number variants identified in patients with autism. The dosage of an imprinted gene or genes within the duplicated region underlies the autism risk in these patients. E3 ubiquitin-protein ligase, Ube3a is the only gene within the 15q11-13 duplicated segment consistently expressed solely from the maternal allele in mature neurons, and inactivating mutations or deletions of Ube3a cause Angelman syndrome. Treatment attempts to hypermethylate the maternal locus by using pro-methylation oral supplements with folic acid and betaine (Peters et al., 2010) or L-5-methyltetrahydrofolate, vitamin B12, betaine, and creatine (Bird et al., 2011) failed to improve biochemical or behavioral parameters.

Oxytocin (OXT Gene)

Oxytocin is a hormone known for its role in childbirth and lactation. It is synthesized in neurons in the paraventricular and supraoptic nuclei of the hypothalamus and transported via axonal processing to the posterior pituitary, where it is stored in secretory vesicles for release into the portal vascular system to affect target organs, mainly the uterine and mammary glands. Additionally, oxytocin neurons directly project from the hypothalamus to the forebrain, the amygdala, the hippocampus, and the nucleus accumbens. Recent publications showed its role in interpersonal bonding behaviors and psychiatric disorders (Kirsch, 2015). The oxytocin receptor gene is a neuropeptide that contains many single-nucleotide polymorphisms (SNPs) with inconsistency in the effect of these variants on ASD. LoParo et al. revised data from eight studies evaluating 16 SNPs in oxytocin receptor using data from 3941 individuals with ASD from 11 independent samples and found significant associations between ASD and the SNPs rs7632287, rs237887, rs2268491, and rs2254298 (Loparo and Waldman, 2015). Another meta-analysis which also included data bases from Germany found an association with rs237889-A (Kranz et al., 2016). Studies on plasma oxytocin concentrations in children with ASD reported conflicting results, either lower levels (Modahl et al., 1998) or without significant difference to healthy controls (Taurines et al., 2014). Recently Francis et al showed association between OXT rs6084258 and ASD (Francis et al., 2016). Despite no difference between ASD children and controls, higher oxytocin blood levels were found in girls, and were associated with anxiety among the girls and less language impairment in the boys and girls of both groups (Miller et al., 2013). The validity of plasma oxytocin as a potential marker for brain levels was not evaluated among ASD patients, however, blood and CSF levels among non-psychiatric patients undergoing spinal anesthesia for minor procedures failed to show correlation between blood and CSF levels (Kagerbauer et al., 2013).

Oxytocin supplementation can be given either intravenously or intranasal with intranasal being superior due to its being well tolerated and easy to use. Reduction in repetitive behaviors (Hollander et al., 2003) and improved affective speech comprehension (Hollander et al., 2007) were found in ASD adults following oxytocin infusion. Studies evaluating ASD patients showed tasks improvement following a single dose (Aoki et al., 2015). However, some failed to show treatment effects (Althaus et al., 2015). Recent studies used continuous administration in order to evaluate treatment effects on daily life. Continuous intranasal administration over 6-weeks reduced autism core symptoms specific to social reciprocity among adults (Watanabe et al., 2015). Another study that failed to show improvement in social cognition and repetitive behaviors after 6 weeks of treatment found improvements in measures of social cognition and quality of life (Anagnostou et al., 2012). In a double-blind, randomized, placebo-controlled, crossover clinical trial, children with ASD at the age 3–8 years showed global improvement and significant improvement in caregiver-rated social responsiveness following a 5 week course of oxytocin (Yatawara et al., 2015).

The possible role of oxytocin in the etiology of ASD, and its potential use for the improvement of symptoms, is not yet understood and needs more studies.

MATERNAL DISEASES AND ASD IN THE OFFSPRING

Diabetes, several autoimmune diseases, infections and inflammatory diseases during pregnancy have been

associated with an increased rate of ASD in the offspring. These diseases may apparently occur at any time during pregnancy.

ASD in Offspring of Diabetic Mothers

Diabetic pregnancies, both pregestational diabetes (PGD) and gestational diabetes (GD) are associated with a large number of pregnancy complications. While PGD may increase the rate of congenital anomalies in the offspring and affect fetal well-being and growth, GD is mainly associated with disturbed fetal growth and increased rate of a variety of pregnancy complications. Both PGD and GD are associated with slight disturbances in postnatal growth and development, also affecting fine and gross motor development and increasing the rate of learning difficulties and Attention Deficit Hyperactivity Disorder (Ornoy et al., 2001). Both also seem to be associated with a slight but significant increase in the rate of ASD in the offspring (Ornoy et al., 2015a).

Most, but not all studies on the possible association of maternal diabetes and ASD show an increased rate, and only recently is the type of diabetes in the mother (T1DM or T2DM or GDM) in relation to ASD in the offspring being considered. Lyall et al. (2012) assessed the possible association of maternal diabetes and ASD in 793 children with ASD from a cohort of 66,445 pregnancies. The highest association was found with maternal GD, as the OR of ASD among children born to mothers with GD was 1.76. This is in slight contrast to other studies which found that the risk for ASD is higher among offspring of mothers with PGD compared to mothers with GDM. Gardener et al. (2009) assessed the possible association of a variety of antenatal maternal factors with ASD in the offspring. Maternal diabetes was among the leading factors associated with ASD, with an odds ratio of 2.07 (95% CI 1.24–3.47). In a very recent study by Li et al. (2016) using the Boston Birth Cohort, both maternal diabetes and pregestational obesity were highly associated with ASD in the offspring. In mothers with PGD and obesity, the hazard ratio (HR) for ASD was 3.91 (95% CI 1.76–8.68) and in obesity and GD the HR was 3.04 (95% CI 1.21–7.63). As cohort and case-control studies are being published, there appears to be a more significant trend toward an association with ASD (Krakowiak et al., 2012). However, there are also studies that could not demonstrate such associations. For example, Hultman et al. (2002) in their case control study on 408 Swedish children with ASD compared to 2040 matched controls, found an association of ASD with a variety of pregnancy associated factors but not with maternal diabetes. Xiang et al. (2015) found in a recent study on pregnant women with preexisting type 2 diabetes. They found an adjusted odds ratio for ASD incidence in the offspring of only 1.21, that was insignificant, but in the offspring of women with GD diagnosed before the 26th week of pregnancy the HR was significant—1.42 (95% CI 1.15–1.74). Guinchat et al. (2012) in their review of 85 studies on the possible association between prenatal, perinatal and neonatal factors and ASD did not find a strong association of maternal diabetes and ASD. In contrast to this review, Xu et al. (2014) culled from 167 publications twelve; three cohort and nine case control studies. For the cohort studies, the pooled risk of maternal diabetes was 1.48 (1.25–1.75, $p < 0.001$), and for the case-control studies, the pooled odds ratio was 1.72 (1.24–2.41, $p = 0.001$). The OR for offspring of mothers with GD was generally lower than for that of mothers with PGD.

In a very recent study looking at the neuropsychiatric morbidity in offspring of 12,642 women with gestational diabetes compared to 218,629 non-diabetic women the investigators found an adjusted odds ratio of 4.4 (95% confidence interval: 1.55–12.69) for ASD. They examined children born during 1991–2014, and excluded prematurity, congenital anomalies, offspring of mothers with pregestational diabetes and other possible etiologic factors (Nahum Sacks et al., 2016).

The mechanism of the association between diabetes and ASD is largely unknown. We should remember that the increased risk found may be related to a variety of pregnancy complications that are common in diabetes, or to effects on fetal growth rather than to complications of hyperglycemia. Judging from the proposed mechanisms of the effects of maternal diabetes on the embryo and fetus, the increase in the rate of ASD in diabetic pregnancies may result from increased fetal oxidative stress, from epigenetic changes in the expression of several genes and it may also be related to the other neurodevelopmental changes induced by maternal diabetes (Ornoy et al., 2015b). It was shown repeatedly that good control of diabetes in pregnancy may reduce the different diabetic complications, but apparently will not completely prevent them. Hence, this is probably also the best way to reduce the diabetic—related ASD. There seem to be no studies relating the prevalence of ASD to the degree of diabetic control.

Maternal Autoimmune Diseases

In utero exposure to maternal antibodies and cytokines are known potential risk factors for ASD. Recent studies associated familial autoimmune diseases with the development of ASD. Most results come from cohort studies. However, due to the limited number of infants with ASD born to mothers with autoimmune diseases, many studies are small. Three Swedish registries including 1227 children with ASD [childhood autism, Asperger syndrome, or pervasive developmental disorder (PDD)] and 30,693 matched controls (25 controls for each case) evaluated linkage for 19 parental autoimmune disorders and found them weakly associated (maternal OR = 1.6, paternal OR = 1.4). A positive history of rheumatic fever was increased in both parents of ASD children, while type 1 diabetes, idiopathic thrombocytopenic purpura and myasthenia gravis were increased only among mothers (Keil et al., 2010). Out of 689,196 children born in Denmark from 1993 through 2004, a total of 3325 children were diagnosed with ASD. ASD diagnoses included infantile autism (1089 children), atypical autism, Asperger syndrome, and PDD. Children were evaluated for parental autoimmune diseases diagnosed before pregnancy. Increased ASD risk was found in offspring of families with type 1 diabetes, offspring of fathers with endocrine autoimmune diseases and mothers with rheumatoid arthritis and celiac disease. Thyrotoxicosis in the family and the mother, specifically, were related to lower risk of ASD (Atladottir et al., 2009).

Studies on the inter-relation of maternal autoimmune disorders with ASD also showed increased susceptibility to ASD among the offspring. A population-based cohort of 719 children

born to 509 mothers with SLE, and a matched control group of 8493 children born to 5824 mothers without SLE found that children born to women with SLE had 1.4% recorded ASD diagnoses compared to 0.6% among the controls (OR 2.25). However, the numbers were small with 10 children of mothers with SLE and 53 children of controls. In addition to maternal SLE, other potential predictors of ASD in the adjusted multivariate analysis were gestational diabetes and male sex (Vinet et al., 2015). The association between maternal autoimmune thyroid disease and childhood autism was evaluated in 1.2 million singleton births with 1132 cases of childhood autism (not including Asperger disorder or PDD) in the Finnish cohort. The cases were matched 1:1 to comparison subjects drawn from the birth cohort who were without ASD. The odds of childhood autism were increased by nearly 80% among offspring of mothers who were positive for anti thyroperoxidase antibodies (TPO-Ab+) during pregnancy, compared to mothers negative for this autoantibody. The association was similar in males and females. There were no associations between maternal clinical or subclinical hypothyroidism or hyperthyroidism, maternal TSH or free T4 and autism (Brown et al., 2015). A follow-up study of 23 mothers with antiphospholipid syndrome (APLS) and their 36 children, and nine SLE mothers with 12 children revealed ASD in 3 children of the APLS mothers. Four children of the APLS mothers, of them the 3 ASD children had persistent anti-β2GP1 IgG antibodies. None of the offspring of the SLE mothers had either neurodevelopmental disorders or anti-β2GP1 IgG antibodies. The ASD cases born to mothers with APLS had normal birth weight, and normal genetic and metabolic work-up. Cerebral MRI performed at the ages of 2.5, 2.6, and 3.4 years were normal in two cases and revealed punctuate hyperintensities in the white matter of one case. None of the children had thrombosis or other antiphospholipid symptomatology (Abisror et al., 2013).

A prospective follow-up of a European multicenter cohort evaluated the long-term outcome and immunological status of children born to mothers with APLS at 3, 9, 24 months and 5 years. Of 134 children, four displayed behavioral abnormalities, which consisted with: autism (1), hyperactive behavior (1), feeding disorder with language delay (1), and axial hypotonicity with psychomotor delay (1). It is noteworthy that the mother of the ASD offspring also suffered from gestational diabetes (Mekinian et al., 2013).

Two large meta-analyses published lately found a significant increase in ASD prevalence among children born to parents with autoimmune diseases. Chen et al. evaluated nine case-control studies and one cohort study published from 1999 to 2015 and comprising of 9775 ASD cases born to mothers with autoimmune diseases and 952,211 controls. Of them, six studies reported a positive association while four did not reveal a substantially significant association. While evaluating the entire cohort they found that children of mothers with autoimmune diseases during pregnancy had a 30% greater risk of ASD. A positive association was identified between maternal autoimmune thyroid disease and ASD in the offspring. No significant difference was found for maternal SLE, inflammatory bowel disease, idiopathic thrombocytopenic purpura (ITP), psoriasis. and rheumatoid arthritis (Chen et al., 2016).

Wu et al evaluated the relationship between family history of autoimmune diseases and risk of ASD in children by a meta-analysis of 11 articles, including 3 cohort studies, 6 case-control studies. and 2 cross-sectional studies published up to Dec 2014. They found in the pooled analysis that family history of all autoimmune diseases combined was associated with a 28% higher risk of autism. Maternal autoimmune diseases were not associated with childhood autism and significant relative risks were only found in case-control studies. Type 1 diabetes in either of the parents, family history of rheumatoid arthritis, hypothyroidism, and psoriasis were significantly associated with higher risk of ASD in children (Wu et al., 2015).

In summary: there is insufficient data to associate maternal autoimmune diseases with increased rate of ASD. However, autoimmune diseases with specific IgG antibodies like TPO-Ab, or anti-β2GP1 that cross the placenta seem to increase significantly the rate of ASD in the offspring

Pre-Eclampsia

Preeclampsia may trigger aberrant neurodevelopment through placental, maternal, and fetal pathophysiologic mechanisms. Preeclampsia results from shallow placentation that may lead to fetal hypoperfusion. Association between pre-eclampsia and ASD was offered when brain MRI of 7–10 years old children (5 boys and 5 girls), offspring of pre-eclamptic pregnancies, revealed enlarged brain regional volumes in the cerebellum, temporal lobe, brain stem, and right and left amygdalae. These offspring also displayed reduced cerebral vessel radii in the occipital and parietal lobes. Enlarged left and right amygdalae were described in ASD and temporal lobe epilepsy (Ratsep et al., 2016).

Recent cohort studies suggested an association between pre-eclampsia and ASD. In the Swedish, population-based, case-control study that included 1216 subjects with autism who were born between 1987 and 2002 and 6080 controls, preeclampsia was associated with 50% increased risk of an autism spectrum disorder (Buchmayer et al., 2009). A recent study from the California Department of Developmental Services that compared 517 ASD cases to 350 controls found that pre-eclampsia complicated the pregnancy of children with ASD more than twice as often as those of controls and the association was more robust in those pregnancies complicated by severe disease (Walker et al., 2015). Two cohort data showed increased prevalence of ASD among pre-eclamptic pregnancies. Analysis of 87,677 births between 1996 and 2002, of the South Carolina Medicaid program, found greater odds of ASD with (OR = 1.69) or without (OR = 1.85) controlling for birth weight (Mann et al., 2010). Analysis of 218,890 singleton live births in Alberta, Canada, between 1998 and 2004 also found greater odds of ASD (OR = 1.49; Burstyn et al., 2010). Compared to these studies there was no association between pre-eclampsia and ASD among 28,967 children born between 1995 and 2008 in Aberdeen city and district (Love et al., 2012). A meta-analysis of 85 studies of pre-, peri,- and neonatal hazards related to PDD, including autism, concluded that there was not enough data in order to determine risk for PDD (Guinchat et al., 2012).

In conclusion: Preeclampsia seems to be an additional risk factor for ASD, but more studies are needed for the conclusive confirmation of such an association.

THE INFLAMMATORY AND INFECTIOUS ORIGIN OF ASD

Adverse intrauterine environment resulting from maternal bacterial and viral infections during pregnancy represent a significant risk factor for several neuropsychiatric disorders including ASD (Adams Waldorf and McAdams, 2013). The association between intrauterine inflammation, infection and ASD is based on both epidemiological studies and case reports.

Many studies associate maternal infection with ASD, and some studies even found that the season or month of birth was significantly related to the risk of ASD. For example, Gardener et al. (2011) found in a meta-analysis that March and August were both suggested as birth months associated with an elevated risk of ASD and hypothesized that a relationship may be caused by seasonal variation in viral or other infections.

Epidemiological Population Studies

An increased rate of ASD was found among children born to mothers that were hospitalized due to infection during pregnancy. Atladottir et al. (2010) matched the Danish Medical Birth Register of children born between 1980 and 2005, the Danish National Hospital Register and the cohort data in the Danish Psychiatric Central Register (1,612,342 children with 10,133 cases of ASD). They found that admission to hospital due to maternal viral infection in the first trimester of pregnancy increased the adjusted hazard ratio for ASD in the offspring by 2.98, and due to maternal bacterial infection in the second trimester by 1.42. No specific infectious category was associated with ASD in the offspring. Later, these authors (Atladottir et al., 2012) evaluated in the Danish cohort the association between self-reported maternal common infections and ASD among children born between years 1997–2003, and found that infection with influenza resulted in a hazard ratio of 2.3.

Lee et al. (2015) investigated the Swedish nationwide register-based birth cohort of children born in Sweden in 1984–2007 and followed until December 31, 2011. They found that 3.7% of ASD cases and 2.6% of non-ASD cases had mothers that were hospitalized with a diagnosis of infection during pregnancy. Maternal inpatient diagnosis of infection during pregnancy was associated with increased OR of ASD regardless of whether the infection was bacterial, viral, or other/unknown. The higher risk of ASD was observed during all trimesters: the 1st trimester (OR = 1.24), 2nd trimester (OR = 1.38), and 3rd trimester (OR = 1.36). In a subsample of the total Swedish population, the Stockholm Youth Cohort, the odds ratio for ASD with intellectual disability was 1.50, not related to the type of infection. Contradictory to these findings, Dodds et al. (2011) found that maternal infection during pregnancy did not increase the rate of ASD.

In a meta-analysis of 40 papers published until 2007 Gardener et al. (2009) evaluated the relationships between autism and pregnancy-related factors. Combined together there was no statistical increase in ASD except for maternal rubella with an OR of 1.66. However, when the analysis was limited to the four studies that controlled for multiple covariates or used sibling controls exposure to intra-uterine infections, a significant increase in risk for autism in OR was observed: 1.82 (1.01–3.30).

Case control studies comparing complications of pregnancy including maternal infection have also shown an association between viral maternal infections and ASD (Wilkerson et al., 2002). A similar association was found in women hospitalized during pregnancy due to bacterial infection (Zerbo et al., 2013). There were, however, case control studies that did not find any association of ASD with either maternal viral or bacterial infections (Langridge et al., 2013).

In summary: many but not all population or case control studies have shown a slight to moderate association of maternal infections with ASD. The controversy might be related to the fact that only several specific maternal infections (subgroups of women with infection during pregnancy) are associated with ASD. These are mainly Rubella, CMV and possibly influenza.

Congenital Rubella

The association between Rubella and ASD is based on few population studies and case reports. Berger et al. (2011) calculated that the rubella vaccination prevented an estimated number of congenital rubella cases that ranged from 8300 to 62,250 and that the corresponding ASD prevention estimates ranged from 614 to 4607 cases, depending on the infectious rate and prevalence of ASD among offspring of infected mothers. We should remember that at the time of the rubella epidemics, ASD definition was not the same as nowadays.

The largest study associating congenital rubella with ASD was reported in 1978 by Chess et al. (1978) who evaluated 243 children with congenital rubella, most of them followed until 9 years. Eighteen (7.4%) had autism compared to expected 0.07 (0.35/1000) in the general population at that time. All the children with autism also had other rubella associated anomalies including cardiac, neurologic, hearing and visual problems. Desmond et al. (1978) reported behavioral disturbances among 45% of *29* non-retarded children with congenital rubella at the age of 9–12 years. Carvill et al. (Carvill and Marston, 2002) described 12 young males with congenital Rubella who suffered from sensory imbalance and ASD. Associated morbidities included: visual and hearing impairment and seizures in three. Feldman et al. (1973) evaluated 12 children with autism or autistic traits and compared them with 25 children with a variety of psychiatric diagnoses, 21 children with language delay and 26 normal controls. None had a history of rubella. Sero-positivity for rubella was found to be significantly higher among the children with language delay, 8/21 and among children with autism, 3/12 compared to the other groups and the general population. All the mothers of the sero-positive children were sero-positive for rubella as well. Other reported cases of congenital rubella also described ASD children who suffered from hearing and visual impairment, iris hypoplasia, heart malformations, retinopathy, and seizures (Assumpcao and Kuczynski, 2002; Hwang and Chen, 2010).

CMV

The diagnosis of maternal CMV is based on medical history, serological studies for CMV or late diagnosis by preserved blood, mostly from Guthrie cards or dried umbilical cord. Koyano et al. (2004) and Sakamoto et al. (2015) used dried umbilical cords for retrospective diagnosis of congenital CMV infection in Japan where obstetric hospitals customarily provide dried umbilical cord to every parent as a symbol of the bond between mother and child.

Yamashita et al. studied seven children with congenital CMV, two of them had ASD. Neuroimaging studies did not vary between those who developed or did not develop autism when seven infants with congenital CMV were evaluated. Three had sub-ependymal cysts and two had calcifications. All had mental retardation (Yamashita et al., 2003).

When children with ASD were assessed, those seropositive for CMV tended to test worse in the major severity scales than the seronegative ones (Gentile et al., 2014). Additionally, Yamazaki et al. found that ASD cases with cochlear implants had lower language and social function than other children with corresponding developmental quotients (Yamazaki et al., 2012). Some studies showed that CMV DNA was detected among ASD cases in a higher prevalence than in the general population; however the numbers were small: 2/27 by Sakamoto et al. compared to incidence of ASD in Nagasaki which was 0.31%, $p = 0.004$ (Sakamoto et al., 2015); 3/76 by Stubbs et al. even though two had multifactorial prenatal causes for autism (Stubbs et al., 1984); 4/26 by Engman et al. with two of them also having cerebral cortical malformations (Engman et al., 2010). Townsend et al. (2013) evaluated 176 infants with congenital CMV born in Sweden and the United Kingdom between 1977 and 1986, and followed them until at least 5 years; none was diagnosed with ASD.

The role of individual placentas in the protection of the fetus against congenital CMV infection is exemplified by the different outcome in association with CMV stigmata, as described by Kitajima (Kitajima et al., 2012) in a triamniotic- trichorionic triplet born at 31 weeks after maternal febrile illness at 24 weeks. Two girls were asymptomatic; the third, a boy, had lower birth weight, increased IGM titer, thrombocytopenia, hepatosplenomegaly, retinitis, brain calcifications, and suffered from ASD. The triamniotic- trichorionic placentas had no fusion or structural abnormalities. Examination of the placenta of the third-born triplet showed that it was paler than the other two and had more severe villitis.

Other reported cases of ASD and congenital CMV also had associated anomalies including brain calcifications and chorioretinitis (Stubbs, 1978; Stubbs et al., 1984; Ivarsson et al., 1990; Sweeten et al., 2004; Lopez-Pison et al., 2005; Ikeda et al., 2006; Kawatani et al., 2010; Dogan et al., 2011).

In summary: There seem to be sufficient studies to associate maternal CMV infection in pregnancy to an increased rate of ASD in the offspring, especially in the children who suffer from other brain manifestations of congenital CMV.

Influenza

Studies associating maternal influenza and ASD are based on maternal reports. As stated above, Atladottir et al. (2012) found self-reported maternal infection with influenza to be associated with increased risk for infantile autism (adjusted HR: 2.3 [95% CI: 1.0–5.3]). The highest association was found with prolonged episodes of fever.

Zerbo et al. (2013) in a telephone interview, evaluated cases and controls in the Childhood Autism Risk from Genetics and Environment (CHARGE) Study. While comparing data from 538 children with ASD and 421 normal controls in association with maternal influenza during pregnancy, they found that the weighted odds ratio (wOR) between mothers of ASD children and those of children with normal development was 1.26. When they analyzed the association between fever and ASD, the odds ratio for mothers of children with ASD and fever during pregnancy doubled that of mothers of normal controls (wOR = 2.12). They also found that the wOR for ASD was 2.55, for children whose mothers reported fever but did not take anti-pyretic medication. For women who reported fever and took anti-pyretic medications, the wOR for ASD was only 1.30. Hence, if there is an association, it is weak, and apparently due to fever.

Inflammation

Maternal immune activation and cytokine dysregulation was also shown to be a mediator in the neuro-pathological behavior observed in autism. Maternal immune system and animal models were used to evaluate the role of cytokines in brain dysregulation together with *in-vitro* studies that evaluated the association between inflammatory mediators and neurological components.

Studies Evaluating Maternal Immune System

Increased levels of inflammatory mediators obtained from stored samples were found in large population studies. Abdallah et al. (2012, 2013) used a pool of samples from more than 100,000 pregnant women in Denmark and had their biologic samples stored from 1980 to 2004, and matched 421 singleton ASD cases born between 1982 and 2000 for gender and birth year with 820 controls. Levels of Monocyte Chemotactic Protein-1 (MCP-1) which play a role in the maturation of cerebellar Purkinje cells and may serve as a useful marker of abnormal neuronal development, were significantly elevated among ASD cases compared to controls. Using the same cohort amniotic fluid they found that samples from 331 ASD cases compared to 698 controls, had significantly elevated levels of IL-4, IL-10, TNF-α, and TNF- β. Goines et al. (2011) also found Increased levels of IL-4, IL-5, and IFN-gamma in banked serum collected from women at 15–19 weeks of gestation who gave birth to a child ultimately diagnosed with ASD ($n = 84$). Grether et al. (2010) found by examining screening filter paper blood specimens from the California Genetic Disease Screening Program of 213 ASD cases and 265 controls that children with ASD had lower total IgG and that the median value, for Herpes Simplex type 1 Virus IgG was significantly lower compared to controls.

Braunschweig et al. (2008) found reactivity to two protein bands against fetal brain at approximately 73kDa and 37kDa in plasma from 7 of 61 (11.5%) mothers of children with autism. These bands were not found in controls. These children also showed behavioral regression. It is assumed that the presence of specific anti-fetal brain antibodies in the circulation of mothers during pregnancy is a potential trigger to induce a downstream

effect on neurodevelopment that may lead to autism. Mazina et al. (2015) investigated the gene-environment interaction by evaluating the interactive effects of maternal infection in pregnancy and the presence of copy number variants which are considered likely to play a contributing role in symptoms of ASD. Participants included 1971 children with ASD between the ages of 4 and 18 years with complete genetic, maternal pregnancy history, and phenotypic information. A statistically significant interactive effect of the presence of copy number variants and maternal infection on autistic symptomatology was observed.

C reactive protein (CRP) is an inflammatory marker that rises in response to infection or inflammation. Analysis of early gestational CRP levels in maternal sera and childhood autism in the Finish birth cohort (consisting of 1.2 million births from 1987–2007) revealed that increased maternal CRP levels were significantly associated with autism in the offspring (OR = 1.12). The increased risk was found in both sexes (Brown et al., 2014). Contradictory to these results, no association between maternal CRP blood levels during the first trimester of pregnancy and ASD in their offspring (children evaluated at 6 years) was found among 4165 pregnant women in Rotterdam that gave birth between 2002 and 2006 (Koks et al., 2016).

In Conclusion

In children affected by intrauterine infections with rubella or CMV, the association with ASD seems to result from the insult to the nervous system, as all also had other neurological problems as well as visual and hearing impairment, suggesting that ASD is secondary to the primary morbidity. Large population studies did not find a specific maternal infection to be associated with ASD, implying that the association between maternal infection during pregnancy and ASD is apparently related to maternal inflammatory process; hence, maternal immune activation may play a role in neuro-developmental perturbation.

EXPOSURE TO DRUGS AND CHEMICALS DURING PREGNANCY AND ASD IN THE OFFSPRING

Several drugs and chemicals have been associated with an increased rate of ASD following intrauterine exposure. However, the association was clearly demonstrated only in few drugs. In others, there are either few reports, or the existing literature is contradictory.

Exposure to SSRI's

Selective serotonin reuptake inhibitors (SSRIs) are among the most used drugs for the treatment of depression especially during pregnancy (Evans et al., 2001; Pereira et al., 2009). They increase extracellular serotonin and are recommended for first-line pharmacological management of depression because they are considered safer and better tolerated than other types of antidepressants. SSRIs and other antidepressant medications cross the placenta and are secreted in breast milk, thus raising concerns about possible adverse effects from fetal and infant exposure. Several studies show a possible association between SSRIs exposure during pregnancy and a higher risk of ASD in children (Croen et al., 2011b; Rai et al., 2013; Gidaya et al., 2014; Harrington et al., 2014). Three out of six recently published case–control studies reported a significantly positive association between SSRI exposure during pregnancy and ASD in children. Odds ratio in the different studies ranged from 2.2 (Croen et al., 2011b) with the strongest effect associated with treatment during the first trimester of pregnancy (OR, 3.8)—to 2.34 (Rai et al., 2013) and the highest was 2.91(Harrington et al., 2014). They found that the strongest association was following exposure in the first trimester of pregnancy. In addition, Gidaya et al. (2014) studied in Denmark a group of 5215 children diagnosed with ASD and 52,150 controls, all born between 1997 and 2006. The OR for ASD doubled among the children born to mothers who used SSRIs at any time during pregnancy.

Recently published large population based retrospective study by Boukhris et al. (2016) reported a significant association between maternal use of SSRI's during pregnancy and the risk of ASD in children. The study included 145,456 children born in Quebec, Canada from 1998 to 2009, of which1054 (0.72%) had at least one diagnosis of ASD. The authors reported that use of antidepressants during the second and/or third trimester was associated with the risk of ASD (31 exposed infants; adjusted hazard ratio, 1.87; 95% CI, 1.15–3.04). Stronger association was found if the mother used SSRI's antidepressants (22 exposed infants; adjusted hazard ratio, 2.17; 95% CI, 1.20–3.93) or used more than one class of antidepressants (5 exposed infants; adjusted HR, 4.39; 95% CI, 1.44–13.32). The risk was persistent even after taking into account maternal history of depression (29 exposed infants; adjusted hazard ratio, 1.75; 95% CI, 1.03–2.97). The use of other antidepressants during the first trimester or the year before pregnancy was not associated with the risk of ASD.

In contrast to these studies, Hviid et al. (2013) found no connection between the use of SSRIs during pregnancy and the increased risk for ASD (OR, 1.20; 95%CI, 0.90–1.61). Similarly, Sorensen et al. (2013) found that children who were exposed to SSRIs during pregnancy have indeed a 50% higher risk for ASD compared with unexposed children, but after controlling for important confounding factors such as maternal history of affective disorder and familial risk factors, they did not detect any significant association between maternal use of SSRI's during pregnancy and ASD in the offspring. Both researchers used data from the Danish civil registration system and Danish health registry systems about children who were born in Denmark between 1.1.96 and 31.12.2005 (Hviid) and until 31.12.2006 (Sorensen). The latest cohort study by Malm et al. from Finland (Malm et al., 2016) examined the rate of ASD among the offspring of 15,729 pregnant women exposed to SSRIs during pregnancy compared to 9651 women with psychiatric disease but no antidepressant treatment and 31,394 non-exposed women and without psychiatric disease. They did not find any increase in the rate of ASD among the SSRIs exposed children, as the rates of ASD were similar to the rate observed in the offspring of mothers with a psychiatric disorder but without treatment. The main finding in this study was an increase in the rate of depression in the SSRI exposed children at adolescence.

In summary: It is still unclear whether there is a direct association between the use of SSRIs during pregnancy and increased rate of ASD in the offspring. Alongside studies which show an association with ASD there are also negative studies. Of special importance is the recent negative study by Malm et al. (2016) as it examined the largest population of SSRI exposed children and adequately adjusted for confounders. It seems more reasonable that maternal depression itself, which is expressed by the use of antidepressants, or other causes, might be responsible for the association. Medical treatment during pregnancy of women with depression should apparently continue in spite of these, rather conflicting studies.

Exposure to Paracetamol

Several studies suggested a possible association between maternal use of paracetamol during pregnancy and a higher risk of ASD among the children. Bauer et al. (Bauer and Kriebel, 2013) examined the association between maternal paracetamol usage or early life paracetamol exposure in males and the prevalence of ASD. They reported positive correlations between ASD prevalence and indicators of both prenatal ($r = 0.80$) and very early life ($r = 0.98$) paracetamol exposures. Liew et al. (2015) followed 64,322 children and mothers enrolled in the Danish National Birth Cohort. They reported that maternal use of paracetamol in pregnancy was associated with ASD with hyperkinetic symptoms only (HR = 1.51 95% CI 1.19–1.92), but not with other types of ASD (HR = 1.06 95% CI 0.92–1.24). More than half of pregnant women in the USA and Europe report using paracetamol and it is the most common drug administered to children; as a result it is difficult to connect between these exposure and ASD). It is obvious that the data is insufficient to draw any conclusion from these studies.

Exposure to β2-Adrenergic Receptor Agonists

Connors et al. (2005) was the first to examine the effects of prenatal overstimulation of the beta2-adrenergic receptor (B2AR), in 37 dizygotic pair of ASD twins who were exposed to terbutaline, a B2AR agonists, commonly prescribed to treat asthma and other pulmonary disorders and to delay preterm labors. They found increased concordance for autism spectrum disorders in dizygotic twins (relative risk = 2.0), with a further increase in the risk for male twins with no other affected siblings (relative risk = 4.4) related to terbutaline exposure overall.

Croen et al. (2011a) examined maternal use of B2AR agonists during pregnancy among 291 children with and 284 without ASD. No evidence linking B2AR exposure during pregnancy with ASD risk was found. However, they reported that exposure to terbutaline only during the third trimester for more than 2 days may be associated with an increased risk of ASD.

A new case control study by Gidaya et al. (2016) investigated the associations between prenatal use of B2AR agonist drugs and the risk for ASD. They studied 5200 ASD diagnosed cases and 52,000 controls from the Denmark's health and population register. The frequency of B2AR agonist drug exposure in pregnancy was 3.7% (190) for cases and 2.9% (1489) in controls. B2AR agonist use during pregnancy was associated with increased risk of ASD, even after adjustment for maternal asthma and other covariates (OR: 1.3, 95% CI: 1.1–1.5). There was no difference regarding the trimesters of pregnancy during exposure.

Exposure to Valproic Acid

Antiepileptic drugs are among the most common teratogens prescribed to women of childbearing age, especially as some of them are also used for psychiatric indications and pain management. Prenatal exposure to antiepileptic drugs (AEDs) is associated with an increased risk of major congenital malformations and delayed cognitive development among the offspring, in a dose dependent manner (Meador et al., 2007; Tomson et al., 2011). Valporic acid (VPA), apparently the most teratogenic AED (Werler et al., 2011), was also found to be associated with impaired cognitive outcomes (Meador et al., 2009). Many reports demonstrate that prenatal exposure to VPA is also associated with a high risk for ASD in the prenatally exposed child in addition to other neurodevelopmental disorders, especially in language development (Moore et al., 2000; Williams et al., 2001; Rasalam et al., 2005; Bromley et al., 2008).

Christianson et al. (1994) were apparently the first to point to of a possible association between intrauterine exposure to VPA and ASD, as observed in four children exposed to VPA during pregnancy. All children had developmental delay and one of them had ASD.

Several years later, Williams et al. (2001) examined five children with ASD and found that VPA was used during pregnancy in all five, but in three in combination with another anticonvulsant. Moore et al. (2000) found among 57 children affected by antiepileptic drugs that four had autistic syndrome and two had Asperger syndrome. Five of them (10.8% of 46 exposed to VPA) were exposed to VPA alone or combined with another AED. Rasalam et al. (2005) evaluated the clinical features and frequency of autistic disorder or Asperger syndrome in 260 children exposed to anticonvulsant medication during pregnancy, by using the DSM IV criteria. They found that VPA was the drug most commonly associated with autistic disorder. The prevalence of ASD in children exposed to VPA alone was 8.9 and 11.7%, among children exposed to sodium valproate in combination with other AEDs. Bromley et al. (2008) examined 296 children of epileptic mothers and 336 children born to mothers without epilepsy. Out of the 632 children from both groups 10 have been diagnosed with ASD, 7 of them were exposed to AEDs. Of those seven children, four were exposed to VPA monotherapy (4/64 6.3%). In addition to these prospective studies, several large retrospective studies have also supported the findings that *in utero* VPA exposure may be linked to an increased risk of ASD (Ornoy, 2009).

Christensen et al. (2013) in a much larger Danish population-based study reviewed the National Population Register with prescription data, psychiatric register and birth records. Records were collated for 655,615 eligible children, with 508 exposed to VPA and 2136 to other anticonvulsants. An increased risk of ASD (Hazard Ratio-HR = 2.9, 95% CI, 1.7–4.9) was found in children exposed to VPA during pregnancy. The risk of ASD was

elevated compared to non-exposure when VPA was taken in the first trimester of pregnancy or after the first trimester.

Animal models in rodents using different neurobehavioral measures fully support the human data. Exposure of mice and rats to VPA produced autism-like behaviors in the offspring (Schneider and Przewlocki, 2005; Wagner et al., 2006). Maternal exposure to VPA induced developmental delay, lifelong deficits in social behavior as well as motor deficits, anxiety-like behavior and alterations in postnatal growth and development in the offspring (Schneider and Przewlocki, 2005; Kolozsi et al., 2009; Bambini-Junior et al., 2011). As in humans, anatomical alterations such as reduced number of cerebellar Purkinje cells, damage to cranial nerve nuclei have been described (Rodier et al., 1997; Ingram et al., 2000) as well as enhanced synaptic plasticity of the prefrontal cortex (Sui and Chen, 2012).

In summary: VPA seems to be the drug with the highest association to ASD as proven in human and animal models and should therefore not be used as a first-line antiepileptic drug in pregnant women or in those who plan pregnancy. Similarly, it should not be used as a mood stabilizer for the treatment of psychiatric patients.

Exposure to Thalidomide

Thalidomide is a well-known human teratogen that caused multiple birth defects including limb reduction defects, ocular and cardiovascular anomalies. Thalidomide is still used in the treatment of leprosy and multiple myeloma (Ito et al., 2010). Stromland et al. (1994) have reported increased number of incidences of autism in children exposed prenatally to thalidomide. At least four of the 100 thalidomide embryopathy patients met full criteria for DSM III R autistic disorder- much higher prevalence then in the general population. The critical window of exposure to thalidomide was in the first trimester, mainly days 20–35 post fertilization. Because thalidomide is generally used in elder patients, no newer data was published regarding this possible effect, making it difficult to accept or deny this possible association, or to calculate the relative risk.

Exposure to Cocaine

Cocaine use during pregnancy has many deleterious effects on both the mother and the fetus, (Singer et al., 1993; Fox, 1994). Cocaine, which is used nowadays mainly as a recreational drug crosses the placenta and the fetal blood-brain barrier readily, and may affect the fetal central nervous system (Roe et al., 1990). Its use during pregnancy may have a variety of deleterious effects on the fetus including preterm delivery, intrauterine growth retardation, placental abruption, stillbirth, and neonatal death (Singer et al., 1993; Fox, 1994). Several studies reported a possible association between the use of cocaine during pregnancy and the development of ASD in the offspring.

Davis et al. (1992) examined 70 children with a history of prenatal cocaine exposure, in Harlem, New-York and found that nine of the children met the DMS III R criteria for autistic disorder—11.4%, much higher than the national average at that time. Harris et al. (1995) examined three, 25–36 month old children with prenatal exposure to cocaine, alcohol and other illicit drugs. They reported that all three children showed a notable pattern of autistic-like behaviors.

We could not find other studies associating prenatal cocaine exposure with ASD. Hence, judging from the paucity of studies, in spite of relatively high use, there seems to be insufficient data to support such association. Hence, the relative risk could not be calculated.

Exposure to Ethanol

Fetal alcohol syndrome (FAS) is a pattern of physical and neurodevelopmental abnormalities which develop in some of the children who were exposed to high levels of alcohol during pregnancy. It is characterized by prenatal and postnatal growth deficiency, CNS dysfunction including mental retardation and behavioral abnormalities, a distinctive pattern of facial features and major malformations of several organs. Some studies have shown a connection between FAS and an increased risk of ASD.

Nanson (1992) described six children aged 6–15 years, out of a data base of 326 FAS individuals who showed the physical phenotype of FAS and also presented a behavioral phenotype typical of autism spectrum disorder. The children also suffered from significant retardation of their cognitive and social skills. These data is representing an incidence of ASD in 1 of 54 cases of FAS.

Aronson et al. (1997) found that among 24 children born to mothers who used high doses of alcohol during pregnancy, two had Asperger syndrome and one had autistic-like behavior. There was a clear correlation between the occurrence and severity of ASD and the degree of alcohol exposure *in utero*. Landgren et al. (2010) assessed 71 children adopted from Eastern Europe between 4.8 and 10.5 years of age and found fetal alcohol syndrome in 37 of the children (52%) and autism in 6 of the children (9%), suggesting a positive association between prenatal alcohol consumption and ASD.

The association between maternal alcohol consumption in pregnancy and ASD does not seem to exist with low alcohol intake. In a large population-based cohort study Eliasen et al. (2010) evaluated a total of 80,522 mother-child pairs. Almost half of the women reported an average weekly intake of at least half a standard alcoholic drink (45%), and approximately one-quarter of the women reported at least one episode of binge drinking during pregnancy. They found no positive associations between ASD and average alcohol consumption or number of binge drinking episodes during pregnancy Adjusted hazard ratio for alcohol consumption of 0.5–1.5 glasses of wine per week (119 cases) = 0.84, 95% CI: 0.68–1.05; adjusted hazard ratio for single binge drinking episode (51 cases) = 0.72, 95% CI: 0.53–0.97.

In summary: Although the existing data of the association between high amounts of alcohol consumption in pregnancy and the risk for ASD in children (especially those with FAS) is well established, the relation to lower amounts ingested is not yet established.

Exposure to Misoprostol and Moebius Sequence

Misoprostol is a prostaglandin analog drug widely used to induce medical abortions (Gonzalez et al., 1998). It is also often

prescribed for the prevention and treatment of gastric ulcers. Möbius sequence is a rare congenital disorder, characterized by uni-or bilateral eye-face palsy due to damage to cranial nerve nuclei, associated with muscle or skeletal malformations in the upper or lower limbs, with an estimated prevalence of 1:50,000 cases in the general population (Stromland et al., 2002; Verzijl et al., 2003). Among the etiological factors responsible for Möbius sequence, apart from genetic causes, is also intrauterine exposure to teratogens mainly misoprostol (Bandim et al., 2003; Vauzelle et al., 2013). Judging from the literature, it is expected that about 1% of children exposed *in utero* to misoprostol will have Möbius sequence (Gonzalez et al., 1993; Marques-Dias et al., 2003).

Johansson et al. (2001)analyzed 25 Swedish individuals with Möbius sequence. Six patients met all diagnostic DSM III R criteria for autism which is a much higher frequency than that of the general population. These findings suggest a strong association between Möbius sequence and ASD. All these patients also had mental retardation.

Bandim et al. (2003) examined 23 Brazilian patients with the age range of 1–11 years, which had been diagnosed as having Möbius sequence based on clinical findings. They identified seven children with ASD of whom four (57.1%) had a positive history of prenatal exposure to misoprostol during the first trimester of pregnancy. They concluded that the prevalence of ASD found in Möbius patients is around 26.1%, much higher than the general population, thus suggesting a strong association linking the two pathologies.

The correlation between misoprostol exposure during pregnancy and ASD is suggested on the basis of reports published in the literature. However, since most, if not all affected children also had Mobius syndrome, it is difficult to directly associate misoprostol exposure to ASD. We found no reports on an association of ASD to misoprostol exposure in exposed children that do not present the symptoms of Moebius syndrome. It should be remembered that the experimental models of VPA—induced ASD in rats and mice have also caused damage to the cranial nerve nuclei, similar to the damage observed in Mobius syndrome (Rodier et al., 1997; Ingram et al., 2000; Sui and Chen, 2012). Hence, it is possible that the relation of misoprostol—induced Mobius syndrome to ASD is not a result of the direct effects of misoprostol but rather from the damage to the cranial nerve nuclei.

Folic Acid Deficiency

Folic acid deficiency is associated with a variety of adverse outcomes in the offspring, especially Neural Tube Defects (NTD). Folic acid deficiency was therefore also proposed as a possible risk factor for ASD.

Schmidt et al. were the first to report that mothers of children with autism were less likely, than those of typically developing children, to report having taken prenatal vitamins, including folic acid, during the 3 months before pregnancy or the first month of pregnancy (OR, 0.62; 95% CI, 0.42–0.93). Later, Schmidt et al. (2012) examined the effect of maternal folic acid intake on the ASD risk in a group of 429 children with ASD and 278 normal children and found that mothers of normal children had a significantly greater mean folic acid intake during the first month of pregnancy compared to mothers of children with ASD. A mean daily folic acid intake of $\geq$600 μg during the first month of pregnancy was associated with reduced ASD risk (OR, 0.62), and risk estimates decreased with increased folic acid (P-trend = 0.001). The association between folic acid and reduced ASD risk was strongest for mothers and children with MTHFR 677 C > T variant genotypes.

In a prospective Norwegian cohort study, Surén et al. (2013) followed up 85,176 children of whom 270 had been diagnosed with ASD and found a significantly higher rate of Autism in children who were not exposed *in utero* to folic acid (0.21%) compared to children of mothers who took 400 μg or more folic acid (0.10%) during a month before and 2 month after the start of pregnancy (OR, 0.6).

Recently, DeVilbiss et al. (2015) summarized the existing literature regarding folic acid deficiency and ASD and discussed the limitations of the different studies. He concluded that the data associating Folate deficiency with a higher risk of autism is yet inconclusive. Hence, it is obvious that more studies are needed to elucidate the possible association of maternal folic acid deficiency with ASD.

EXPOSURE TO HIGH LEVELS OF ENVIRONMENTAL AGENTS

Exposure to Air Pollution

Increased air pollution is one of the more common signs of our modern life, and its possible effects on health are extensively investigated. In spite of great difficulties in measuring the extent of air pollution and the elucidation the large number of possible confounding factors, more and more studies are being published associating heavy air pollution to ASD.

The first study was apparently conducted in San Francisco Bay area in 2006 by Windham et al. (2006). They examined the effects of prenatal exposure to 19 hazardous air pollutants in 284 children with ASD compared to 657 controls. Elevated risk for ASD was found in adjusted analyses of the top quartile of exposure to chlorinated solvents and heavy metals [95% CI, 1.1–2.1]. In this study, however, the exposure was estimated 2 years after birth when ASD diagnosis was established. Hence the effects might be attributed to postnatal effects of these pollutants.

Kalkbrenner et al. (2010) examined prenatal exposure to 35 hazardous air pollutants in 383 children with ASD and 2829 control children with speech and language impairment. They found that prenatal exposure to hazardous air pollutants including methylene chloride (OR, 1.4; 95% CI, 0.7–2.5), quinoline (OR, 1.4; 95% CI, 1.0–2.2), and styrene (OR, 1.8; 95% CI, 1.0–3.1) were associated with a higher risk for ASD.

Volk et al. (2011) investigated the association between ASD and proximity of residence to freeways and major roadways during pregnancy and near the time of delivery, in a group of 304 children with ASD and 259 controls. They found that maternal residence during the third trimester (OR, 2.22; 95% CI, 1.16–4.42) and at the time of delivery (OR, 1.86; 95%CI, 1.04–3.45) were more likely to be near a freeway ($\leq$309 m) for the ASD children than for the controls. They also found (Volk et al., 2013) in an additional study that, during gestation, children with

ASD were more likely to live at residencies that had the highest quartile of exposure to traffic-related air pollution (OR, 1.98; 95% CI, 1.20–3.31) compared with control children. Regional exposure during pregnancy with nitrogen dioxide and particulate matter <2.5 and 10 μm in diameter (PM2.5 and PM10) were also associated with autism.

Similarly, Becerra et al. (2013) studied the influence of exposures to traffic-related air pollution during pregnancy on the occurrence of ASD with controls matched by sex, birth year, and gestational age. They found a small but significant 12–15% relative increase, per interquartile range, in odds of autism for ozone (OR, 1.12, 95% CI: 1.06–1.19; per 11.54-ppb increase) and particulate matter ≤2.5 μm when mutually adjusting for both pollutants.

Roberts et al. (2013) estimated the association between levels of hazardous air pollutants at the time and place of birth and ASD in 325 children with ASD and 22,101 controls. Prenatal exposures to the highest quintile of air pollutants, such as diesel, lead, manganese, mercury, and methylene chloride, were significantly associated with ASD, with odds ratios ranging from 1.5 (for overall metals measure) to 2.0 (for diesel and mercury). For most pollutants, associations were stronger for boys (279 cases) than for girls (46 cases) suggesting a significant gender difference.

Raz et al. (2015a) examined the association between maternal exposure to particulate matter (PM) air pollution during pregnancy and the risk of ASD compared to controls. They found that PM2.5 exposure during pregnancy was associated with increased risk of ASD, with an adjusted odds ratio for ASD per interquartile range of 1.57 (95% CI: 1.22, 2.03). The association between ASD and PM2.5 was stronger for exposure during the third trimester (OR, 1.42 per inter-quartile range increase in PM2.5, 95% CI, 1.09–1.86).

In a recent, population based case–control study in Pennsylvania, Talbott et al. (2015) found that living in areas with higher ambient levels of styrene and chromium during pregnancy and the first and second years of life is associated with increased risk of ASD with borderline effects for polycyclic aromatic hydrocarbons (PAHs) and methylene chloride.

In contrast to these studies mostly carried out in the United States, showing a consistently positive association between exposure to several air pollutants during pregnancy and diagnosis of ASD, a much larger, recently published analysis of four European population-based cohorts appears to contradict those findings, reporting no associations between ASD and prenatal air pollution exposure (Guxens et al., 2016).

This research compared between nitrogen oxides and various sizes of particulate matter concentrations at the participants' birth home addresses during pregnancy and the presence of autistic traits, but not formal diagnoses of ASD, in more than 8000 children. No associations were found between the presence of autistic traits and prenatal exposure to any of the air pollutants (odds ratio = 0.94; 95% CI: 0.81, 1.10 per each 10-μg/m3 increase in NO2 pregnancy levels). The researchers explained the contradictory findings by the fact that previous case–control studies selected children with a diagnosis of ASD, whereas this study examined children with autistic traits from population-based birth/child cohorts. An alternative explanation could be the possible differences in air pollution levels and sources.

It can therefore be concluded that air pollution might be a contributing factor in the risk for ASD, but the data is as yet inconclusive.

In summary: these case control studies suggest that there is a positive association between maternal exposure to air pollution during pregnancy and ASD in children, especially following exposure in the third trimester. Although small PM may cross the placenta, there might also be other factors in the air pollutants that are associated and were not measured.

Exposure to Pesticides

Few studies have examined the effects of *in utero* exposure to pesticides such as organophosphates and carbamates on the neurobehavioral development and the risk of ASD in children. Each study reported an association with ASD.

Roberts et al. (2007) examined the association between maternal residence near agricultural pesticide applications during key periods of gestation and ASD in children. They reported that ASD risk was higher in children whose mothers lived near agricultural applications of organochlorines during the first trimester of pregnancy (OR 6.1) and decreased with increasing distance from such fields.

Shelton et al. (2014) evaluated whether residential proximity to agricultural areas and therefore increased exposures to pesticides during pregnancy is associated with ASD. They found that residence near organophosphates at some point during gestation was associated with a 60% increased risk for ASD, higher for third-trimester exposures (OR, 2.0), and second-trimester chlorpyrifos applications (OR, 3.3). Also, children of mothers residing near pyrethroid insecticide applications just before conception or during the third trimester were at greater risk for ASD with ORs ranging from 1.7 to 2.0. We can summarize that due to the paucity of studies in spite of extensive use, an association between ASD and pesticides is plausible, but it was not yet established.

Exposure to Heavy Metals

Maternal exposure to heavy metals, such as mercury, lead, and cadmium, has been suggested to be associated with neurodevelopmental disorders including mental retardation, inattention, learning difficulties, and possibly ASD (Mendola et al., 2002). Although there is more data on mercury, most available data suggests that maternal exposure to mercury does not play an important role in the development of ASD. Moreover, several of the studies were carried out on the affected children and not on their mothers, hence making the association with pregnancy an unproven possibility (Kern et al., 2007). In that respect we should add that Kern et al. (2007) found that sulfhydryl reactive metals: arsenic, cadmium, and lead were reduced in the hair of 45 children with ASD compared to controls. They suggested that the children with ASD have a problem in secreting these sulfhydryl reactive metals and their accumulation may contribute to the symptoms of ASD.

Geier et al. (2009) examined the association between maternal dental amalgams (containing 50% mercury) and the severity of ASD diagnosis in a group of 100 autistic children. They found that a rise in autism severity correlated with an elevation in the number of dental amalgams in the mother during pregnancy.

However, Yau et al. (2014) investigated the association between ASD and levels of total mercury measured in maternal serum from mid-pregnancy and infant blood shortly after birth in 84 children with ASD and 159 control children. They found no significant associations between ASD and mercury levels in maternal serum samples (OR, 0.96; 95% CI, 0.49–1.90) or in newborn blood samples (OR, 1.18; 95% CI, 0.71–1.95).

Van Wijngaarden et al. (2013) examined prenatal exposure to methyl mercury in 1784 children and young adults, by measuring maternal hair samples collected at or near the time of birth. They found no significant association between ASD and prenatal methyl mercury exposure.

It can therefore be concluded that there in insufficient data to associate prenatal exposure to heavy metals, especially mercury, to a higher occurrence of ASD in the offspring.

Exposure to Cigarette Smoking

Heavy maternal cigarette smoking during pregnancy is associated with an increased rate of spontaneous abortions, preterm delivery, reduced birth weight, immune system difficulties such as asthma and allergies and a relatively high rate of learning disabilities and attention deficit disorders later in life (Castles et al., 1999; Stene-Larsen et al., 2009; Kiechl-Kohlendorfer et al., 2010). Several studies have examined whether prenatal exposure to heavy tobacco smoke is also associated with ASD, and the findings are inconsistent. Hultman et al. (2002) and Larsson et al. (2009) found a mild association between smoking during pregnancy and the risk of childhood autism (OR, 1.4 and 2.09, respectively).

Kalkbrenner et al. (2012) assessed the association between maternal smoking during pregnancy and ASD among 3315 children with ASD and 630,674 control children at 8 years of age. A slightly positive association (OR 1.26) was found only for "ASD not otherwise specified" (ASD-NOS), which disappeared after correcting for possible confounding factors. Similarly Tran et al. (2013) examined 4019 ASD cases and 16, 123 controls, in a nested case-control study based on the Finnish Prenatal Study of Autism (FIPS-A) and found no connection between maternal smoking and childhood autism or Asperger syndrome, but found a slight association with PDD (OR, 1.2, 95% CI, 1.0- 1.5).

Other large studies also did not find any association between tobacco exposure and ASD. In a large registry based Swedish case-control study, that included 3958 ASD cases and 38,983 controls, Lee et al. (2012) found that maternal smoking during pregnancy is not associated with increased risk of ASD after adjustments for parental education, occupation, and income.

Several additional studies (Maimburg and Vaeth, 2006; Burstyn et al., 2010) and two recently published meta-analyses by Rosen et al. (2015) and Tang et al. (2015), also reported no significant association between maternal smoking during pregnancy and ASD.

It can be summarized maternal smoking does not increase the rate of ASD in the offspring.

CONCLUSIONS

The observed increase in the incidence of ASD in the last decades is considered to result directly from changes in definitions and better ascertainment. However, it is also possible that it reflects a real increase in the occurrence of ASD. In addition to well-defined genetic causes for ASD, examples of which we discussed in this review, the current search for prenatal environmental etiologic factors demonstrated real associations with a number of causes that may affect the developing fetal brain raising the vulnerability for ASD. Based on the findings reviewed here, we may conclude—with caution—that: (1) A possible association exists with maternal influenza in pregnancy, exposure to pesticides and insecticides, exposure to misoprostol, thalidomide, cocaine, SSRIs, or folic acid deficiency. (2) A probable association was found for maternal fever, autoimmune diseases, diabetes, preeclampsia, and exposure to heavy air pollution. (3) A definite association of ASD with maternal Rubella and CMV infections in pregnancy, maternal inflammation and immune activation, or exposure in pregnancy to VPA, and high levels of ethanol. (4) Although sometimes suggested, there seems to be sufficient evidence that there is no association with ASD for many maternal infections in pregnancy (i.e., herpes viruses, Epstein Barr virus, varicella—zoster virus, parvovirus), smoking, exposure to heavy metals or vitamin D deficiency.

Despite the increasing efforts in recent years, and support from animal studies, we still seem to be in a stage where the etiology of ASD is largely unknown and the associations described in the literature are, for many agents, inadequately proven. Hence, more research is needed to unravel environmental contribution to the genetic etiology of ASD. This type of research may ultimately lead to the establishment of possibilities for prevention.

AUTHOR CONTRIBUTIONS

Following the request by Dr. Joshua Rosenzweig and Dr. Benjamin Gesundheit, we attach our manuscript entitled Genetic syndromes, maternal diseases and antenatal factors associated with Autism Spectrum Disorders (ASD) by EZ, WL, and OA for consideration for publication in the special topic of the Journal-Autism Spectrum Disorders (ASD)—searching for the biological basis for behavioral symptoms and new therapeutic targets.

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REVIEW
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The Interaction between the Immune System and Epigenetics in the Etiology of Autism Spectrum Disorders

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Recent studies have firmly established that the etiology of autism includes both genetic and environmental components. However, we are only just beginning to elucidate the environmental factors that might be involved in the development of autism, as well as the molecular mechanisms through which they function. Mounting epidemiological and biological evidence suggest that prenatal factors that induce a more activated immune state in the mother are involved in the development of autism. In parallel, molecular studies have highlighted the role of epigenetics in brain development as a process susceptible to environmental influences and potentially causative of autism spectrum disorders (ASD). In this review, we will discuss converging evidence for a multidirectional interaction between immune system activation in the mother during pregnancy and epigenetic regulation in the brain of the fetus that may cooperate to produce an autistic phenotype. This interaction includes immune factor-induced changes in epigenetic signatures in the brain, dysregulation of epigenetic modifications specifically in genomic regions that encode immune functions, and aberrant epigenetic regulation of microglia. Overall, the interaction between immune system activation in the mother and the subsequent epigenetic dysregulation in the developing fetal brain may be a main consideration for the environmental factors that cause autism.

Keywords: autism, epigenetics, immune system, maternal immune activation, DNA methylation, microbiome

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INVOLVEMENT OF MATERNAL IMMUNE ACTIVATION IN AUTISM

The etiology of autism spectrum disorders (ASD) is widely defined by the interaction between genetics and environmental factors. Within the past two decades, a large number of resources have been invested in understanding the genetic underpinnings of ASD. Several large scale genetic studies have uncovered single nucleotide variations (SNVs; Gaugler et al., 2014; De Rubeis and Buxbaum, 2015; LoParo and Waldman, 2015), copy-number variations (CNVs) (Shishido et al., 2014) and *de novo* mutations (De Rubeis et al., 2014a; Iossifov et al., 2014) that are associated with ASD risk. Recent studies have found that common genetic variations account for a high percentage of the genetic risk for ASD (Gaugler et al., 2014). Of particular importance in this current discussion, the genetic variations associated with autism are also often present in individuals without an ASD diagnosis. One investigation reported that the presence of autism-risk alleles in individuals that are not diagnosed with ASD was associated with a higher IQ (Clarke et al., 2016), while another study found that genetic risk for ASD is associated with changes in social

behavior even in individuals without an ASD diagnosis (Robinson et al., 2016). Overall, these studies highlight the fact that genetic risk for autism can influence multiple aspects of behavior, but it is often not sufficient to induce the full spectrum of behavior needed for a diagnosis of ASD. Therefore, a genetic contribution is likely to introduce a predisposition to develop ASD, while its actual onset requires a further environment agitation.

Current studies have been exploring the possible environmental factors that may be involved in the etiology of ASD. An expanding list includes viral infection and exposure to environmental toxins during pregnancy, gestational diabetes, among others (Grabrucker, 2012). In particular, compounding evidence supports a role for immune system activation at specific time frames in the pregnant mother as a risk factor for autism (**Table 1**). The first evidence for this connection was provided from the Rubella outbreak of the 1960s. Autism was diagnosed in ∼5–10% of children born to mothers that were infected by Rubella virus (Chess, 1971, 1977; Hutton, 2016). Subsequently, a study, surveying Danish births from 1980 to 2005 determined a three-fold increase in the incidence of ASD in children whose mothers were hospitalized for viral infection, specifically during the first trimester of pregnancy (Atladóttir et al., 2010). A report of Swedish births found a 30% increase in ASD when the mothers were hospitalized for viral infections during pregnancy (Lee et al., 2014). However, unlike the Danish study, this investigation reported a significant effect for an association between ASD diagnosis and viral infection in all three trimesters.

Additional support for the role of maternal immune activation (MIA) in the development of autism has come from animal experimentation. MIA can be simulated by injecting pregnant rodents with Poly I:C, a synthetic double-stranded mimetic of the RNA molecule, which triggers an immune response through the activation of Toll like Receptor 3, and subsequent expression of interferon-1 (Alexopoulou et al., 2001). This process induces activation of both innate and adaptive immune regulatory mechanisms in the pregnant rodent female. Offspring can then be tested for behavioral abnormalities and changes in neurodevelopment. Malvoka et al. determined that the offspring of pregnant mice treated with Poly I:C displayed all the core deficits associated with ASD including problems in social, communication, and repetitive behaviors (Malkova et al., 2012). Several follow up studies verified these findings and went on to suggest other related deficits in neurodevelopment that are responsible for this behavior (Smith et al., 2007; Canetta et al., 2016; Choi et al., 2016). In addition to rodent models, MIA has recently been induced in monkeys. Offspring of Rhesus monkeys subjected to MIA displayed deficits in social behavior and an increase in repetitive behaviors (Bauman et al., 2014; Machado et al., 2015). Therefore, both epidemiological and animal modeling studies support a causative role for MIA in the etiology of ASD.

Other studies have shown another possible trigger of an immunity-induced autism phenotype could be due to the presence of an autoimmune disorder in the mother. A study of the Swedish health registry uncovered an increase of 60% in the odds for developing autism among those children of mothers diagnosed with an autoimmune disorder (Keil et al., 2010). Interestingly, an increase of 40% was detected among children whose fathers had an autoimmune disorder. A separate study in Northern California found an association between maternal psoriasis and the insurgence of autism, although the association between autoimmune diseases in general and autism was not significant (Croen et al., 2005). In a large study examining nearly 700,000 Swedish births, Atladottir et al. found that autism was associated with maternal Rheumatoid Arthritis and Celiac Disease, as well as those with a family history of Type 1 Diabetes (Atladóttir et al., 2009). An increase in autism was also identified in a cohort of Canadian children from mothers with Systemic Lupus Erythematosus (Vinet et al., 2015). A recent meta-analysis of studies performed in multiple locations confirmed a significant association between maternal autoimmune diseases and ASD (Chen et al., 2016). Generally, it has become apparent that both MIA and maternal autoimmune disease are associated with ASD.

EPIGENETICS IN AUTISM

Epigenetics is responsible for the proper development of the nervous system and is highly regulated by environmental factors, such as an inflammatory response (Jirtle and Skinner, 2007). Epigenetics refers to heritable changes in gene regulatory mechanisms that are independent from alteration of the underlying DNA sequence. Two of the most commonly studied epigenetic markers include DNA methylation and histone modifications (Jones, 2001; Goldberg et al., 2007). Both markers influence the establishment of gene transcription patterns through multiple mechanisms, including the regulation of genomic, structure, and the accessibility of genomic loci to diverse regulatory factors (e.g., transcription factors, enhancers, silencers). Temporal changes in epigenetic signature during the developmental stage finely tunes the differentiation of precursor cells into their specific mature state (Kiefer, 2007). Therefore, epigenetic markers display a relatively high level of plasticity during periods of cellular specification, including in brain development (Spiers et al., 2015). As such, it is likely that environmental disturbances during pregnancy may induce stable and long-term modifications in epigenetic patterns that probably survive until adulthood.

Genetic studies demonstrated preliminary evidence of a role for epigenetic mechanisms in the etiology of ASD. Analysis of hundreds of genes associated with ASD reveal enrichment for two biological functions: synaptic plasticity or chromatin binding factors (Lasalle, 2013; De Rubeis et al., 2014b). The enrichment of chromatin binding genes in autism studies suggests their potential role in the etiology of ASD. Rett Syndrome is one very well-known example of a genetic neurodevelopmental condition that includes autistic behavior, and whose etiology is directly related to epigenetic regulation. Rett Syndrome is caused by mutations in the gene MECP2, which encodes for the methylated DNA binding protein MeCP2 (Amir et al., 1999). Upon binding methylated DNA, MeCP2 either activates or inhibits gene transcription depending on the genomic context (Chahrour et al., 2008). The fact that mutations in the MECP2

TABLE 1 | Main manuscripts that investigated the association between MIA and autism, epigenetic changes in the autism postmortem brain, or MIA-induced changes in brain epigenetic patterns in mouse models.

Geographic region	Association with autism	References
MATERNAL IMMUNE ACTIVATION IN ASD-EPIDEMIOLOGICAL STUDIES		
United States	Positive association with Congenital rubella syndrome	Chess, 1977
Denmark	Positive association with hospitalization for viral infections in first trimester and bacterial infections in second trimester	Atladóttir et al., 2010
Sweden	Positive association with hospitalization for viral infections in all three trimesters	Lee et al., 2014
Sweden	Positive association with maternal autoimmune disease in general. In addition, association with maternal Myasthenia Gravis and Rheumatic Fever	Keil et al., 2010
United States, CA	No association with maternal autoimmune diseases in general. Positive association with maternal psoriasis	Croen et al., 2005
Denmark	Positive association with maternal Rheumatoid Arthritis, Celiac Disease, and Type1 Diabetes	Atladóttir et al., 2009
Canada	Positive association with Systemic Lupus Erythematosus	Vinet et al., 2015

Brain Area	Method	Main differences between control and autism cohorts	References
EPIGENETIC CHANGES IN ASD BRAIN-POSTMORTEM STUDIES			
Frontal cortex, temporal cortex and cerebellum	Genome-wide 450K Illumina BeadArray	Hypomethylation in PRRT1 and C11orf21; Hypermethylation in ZFP57 and SDHAP3	Ladd-Acosta et al., 2014
Frontal and cingulate cortex	Genome-wide 450K Illumina BeadArray	Hypomethylation in genes implicated in immune response. Hypermethylation in genes implicated in synaptic processes	Nardone et al., 2014
Cerebellum and occipital cortex	Genome-wide 27K Illumina BeadArray	No significant differences	Ginsberg et al., 2012
Cerebellum and cortex	Gene-specific sodium bisulfite sequencing	Hypermethylation in Shank3	Zhu et al., 2014
Temporal cortex	Gene-specific sodium bisulfite sequencing	Hypermethylation in RELN	Lintas et al., 2016
Cerebellum	Methylation-sensitive PCR; Chip-real time PCR	Hypermethylation and increased H3K27me3 in EN-2 gene; global DNA hypermethytation	James et al., 2013
Cerebellum	hMeDIP-real time PCR; Chip-real time PCR	Hyperhydroxymethylation and decreased MeCP2 binding In EN-2 gene promoter	James et al., 2014
Frontal cortex	H3K4me3 Chip-seq on FACS-sorted neuronal nuclei	No significant differences; subset of autism cases displays spreading of H3K4me3 from transcription start sites to adjacent regions	Shulha et al., 2012

Brain area	Method	Main findings	References
MIA-INDUCED EPIGENETIC CHANGES IN BRAIN-RODENT STUDIES			
Hypothalamus	Gene-specific sodium bisulfite sequencing	Decrease in MECP2 and LINE1 methylation	Basil et al., 2014
Frontal cortex	Gene-specific sodium bisulfite sequencing	Increase in GAD1 and GAD2 methylation	Labouesse et al., 2015
Frontal cortex and hippocampus	Western blot; Chip-real time PCR	Global histone hypoacetylation in the cortex of juvenile offspring; promoter-specific hypoacetylation in adult cortex and hyperacetylation in adult hippocampus	Tang et al., 2013
Frontal cortex	Chip-seq	No significant changes in H3K4me3 marks	Connor et al., 2012

gene induce a syndrome with autistic-like behavior, indicates a direct link between DNA methylation-regulated gene expression and ASD symptomatology. Other chromosome binding genes that have been found to be strongly associated with ASD include CHD8, MBD5, and AUTS2 (Sultana et al., 2002; Cukier et al., 2012; Bernier et al., 2014). Therefore, genetic studies have long proposed an involvement of epigenetics in the etiology of ASD.

While genetic studies have provided preliminary evidence for the involvement of epigenetics in the etiology of ASD, epigenetic-based studies were necessary to finally prove a direct association between dysregulation of epigenetic signatures, both in peripheral tissues and the brain, and ASD (Ciernia and LaSalle, 2016). Post-mortem brain epigenetic studies have been critical in understanding how epigenetic patterns may be affected in the ASD brain. This is due to the fact that each tissue and cell type are characterized by highly specific epigenetic signatures. Accordingly, the interrogation of peripheral tissues may not truly represent changes seen in brain tissues. Previous reviews have thoroughly discussed the literature on epigenetic dysregulation in ASD, including the periphery (Tordjman et al., 2014; Loke et al., 2015; Ciernia and LaSalle, 2016). Here, we will focus specifically on epigenetic dysregulation in the brain, in order to later discuss

the possible role of MIA in brain epigenetics and development of ASD (**Table 1**).

Several gene-specific studies have been performed to provide preliminary evidence of DNA methylation dysregulation in the ASD brain. An initial study analyzed the DNA methylation profile of the autism-related gene Shank3. This study determined a hypermethylation of multiple intragenic regions in the Shank3 gene (Zhu et al., 2014). While mutations in Shank3 may be found in ∼0.5% of individuals with autism, this study suggests that epigenetic changes in Shank3 may actually be more widespread. Therefore, epigenetic alterations may significantly affect the function of autism-related genes in the absence of those rare genetic changes. Other studies have also determined dysregulated DNA methylation signatures in the autism-related genes RELN and EN-2 (James et al., 2013, 2014; Lintas et al., 2016).

To date, only a few epigenetic studies have investigated in a genome-wide fashion the DNA methylation profile of post-mortem brain tissue from individuals with ASD diagnosis and matched controls. These studies have been performed using the Illumina 450K BeadArray, which profiles the methylation level of ∼480,000 CpGs throughout the entire human genome. Using this method, Ladd Acosta et al. discovered four differentially methylated regions in the DNA when comparing the control and autism groups (Ladd-Acosta et al., 2014). These methylated regions containing multiple CpGs included the genes PRRT1, ZFP57, and TSPAN32 in the cortex, and SDHAP3 in the cerebellum. In a separate study, we found that over 5000 individual CpGs were differentially methylated in the frontal cortex when comparing the control and autism cohorts (Nardone et al., 2014). Hypermethylated and hypomethylated CpGs were mainly found in genomic regions that were associated to genes enriched with synaptic functions and immune response, respectively. Of interest, the gene TSPAN32 had about an 8% decrease in DNA methylation levels in the ASD vs. control group in both studies. An additional genome-wide study investigating the DNA methylation in both the cerebellum and occipital cortex found no significant changes between the control and autism groups, suggesting that the effects in the brain are region-specific (Ginsberg et al., 2012). Overall, these findings corroborate the aforementioned gene-specific studies, further reinforcing the evidence for an involvement of epigenetics in the etiology of ASD. In addition, they highlight the brain-area specificity of epigenetic signature, pinpointing the forebrain as one of the brain regions more liable to environmental insults.

AUTISM AND EPIGENETIC REGULATION OF IMMUNE GENES AND IMMUNE CELLS

There is converging evidence that the epigenetic regulation of the immune system may be involved in the etiology of ASD. First, as mentioned above, hypomethylated CpGs detected in frontal cortex of autistic individuals were enriched in genes involved in immune response (Nardone et al., 2014). The genes associated to those CpGs displayed a significant increase in their transcription: of particular interest were C1qA, C3, TNF-alpha, and other transcription factors that are known to be essential in the development of the microglia. These findings correlate very well with genome-wide transcriptomic studies that demonstrated an increased expression of microglia and innate immune response-related genes in the brain of individuals with autism (Voineagu et al., 2011; Gupta et al., 2014). Therefore, there is evidence for dysregulation of the immune response in the autism brain at both the level of gene transcription and DNA methylation.

Mouse studies have demonstrated that the malfunctioning of the epigenetic machinery, specifically in microglia cells, can cause autistic-like behavior. Much of this evidence stems from studies on Rett Syndrome and the MECP2 gene. As aforementioned, MeCP2 binds methylated CG sites and contributes to gene transcription regulation. A major study demonstrated that a Rett Syndrome-like phenotype, present in microglia-specific MECP2 KO mice, could be reversed by replenishing the MECP2 KO with wild type microglia (Derecki et al., 2012). Furthermore, Maezawa et al. proved that deletion of MECP2 in microglia induced dysregulation of extracellular glutamate levels and neuronal dendrites (Maezawa and Jin, 2010). In a follow-up study, the same research group demonstrated that inhibition of the interaction between microglia and neurons in the MECP2 KO mice can attenuate many of the Rett Syndrome-like behavioral manifestations (Horiuchi et al., 2016). These studies suggest that MeCP2 influences mouse behavior by regulating epigenetic machinery in microglia. It is important to note, however, that a recent study was not able to entirely replicate those main findings (Wang et al., 2015). So it remains unclear whether microglia is the main or only one of many factors that play a role in the etiology of Rett Syndrome. while the exact role of microglia is not completely defined, there is much evidence to suggest the epigenetic regulation of microglia plays an important role in the etiology of ASD.

Although there is sufficient evidence for increased immune activation in the brains of autistic subjects, immune system genes are not among those that are often mutated in autism. Therefore, immune activation is unlikely to be explained by genetic etiology, but rather via epigenetic machinery. In conclusion, the immune activation detected in brain and blood samples of autistic subjects may be due to environmental factors and mediated by epigenetic mechanisms.

CAN THE PRENATAL ENVIRONMENT INDUCE DNA METHYLATION CHANGES IN THE OFFSPRING?

The primary question that we aim to address is whether the epigenetic differences detected in the brain and peripheral tissues of autism individuals are due to environmental insults during development, such as MIA. In order to consider this possibility, we must first establish the extent to which the prenatal environment may generally affect the epigenetic machinery. Multiple human studies have been performed to determine a link between environmental insults during pregnancy and altered methylation patterns in the offspring. A well-known example is the study based on the Dutch Famine Winter, which investigated the offspring of mothers exposed to famine conditions during

pregnancy. The offspring displayed dysregulation of DNA methylation in genomic regions associated to the gene IGF2, an imprinted gene that has primary roles in metabolism (Heijmans et al., 2008). This epigenetic alteration was maintained for several decades after the famine, showing that environmental insults during pregnancy may have a very longlasting effects on epigenetic patterning. Similarly, multiple studies have found dysregulation in DNA methylation in the cord blood of offspring from mothers diagnosed with gestational diabetes (Quilter et al., 2014; Finer et al., 2015). In addition, other environmental cues that can influence the stress response during pregnancy and the insurgency of maternal depression episodes (Nemoda et al., 2015) have been associated with DNA methylation changes in offspring. Yet some of the most meaningful data came from studies investigating the effects of maternal smoking. Maternal smoking during pregnancy has been associated with dysregulation of DNA methylation in multiple genes in newborns, including NeuroG1 and CNTNAP2, which are known to play a pivotal role in language development and have been associated with ASD by several studies (Küpers et al., 2015; Lee et al., 2015; Richmond et al., 2015). An additional factor that has the potential to influence the epigenetic machinery is the level of maternal serum folate during pregnancy. Folic acid deficiency during pregnancy may be accountable for the occurrence of neurodevelopmental abnormalities, such as spina bifida. Nowadays folic acid is a widely-prescribed supplement during pregnancy, and has been associated with a decreased risk of developing ASD, despite its mechanism of action not being completely understood (Schmidt et al., 2012; Surén et al., 2013). Recently, it has been shown that a mother's folate level is correlated to differential methylation in several developmental-related genes in offspring (Joubert et al., 2016). As such folic acid supplementation may be an additional environmental factor that regulates epigenetic programming during development. Together, these human studies provide a firm basis for the linkage between the *in utero* environment and dysregulation of DNA methylation patterning.

COULD MIA BE RESPONSIBLE FOR EPIGENETIC DYSREGULATION IN ASD?

Considering the association of both MIA and epigenetic dysregulation in autism, immune activation in pregnant mothers may be instrumental in epigenetic dysregulation and the downstream behavioral phenotypes observed in autistic children. In support of this idea, recent animal studies have proven that MIA can alter epigenetic patterns associated to autism-candidate genes in the offspring brain (**Table 1**). An initial study found that MIA induced a decrease in global histone acetylation levels in the cortex of juvenile offspring. Also detected were gene-specific changes of histone acetylation at several neuron-related gene promoters in both the cortex and hippocampus of juvenile offspring (Tang et al., 2013). Although these epigenetic patterns did not survive till adulthood, it is conceivable that their transient effect on gene transcription may cause permanent effects that persist into adulthood. The authors did in fact show that adult offspring displayed abnormalities in exploratory behavior. A separate study found only few changes in histone acetylation levels in adult mice after MIA (Connor et al., 2012). So it appears most likely that changes in histone modifications occur mainly during developmental stages and are no longer present in adulthood. Preliminary studies have recently been performed in order to investigate the relationship between MIA and DNA methylation in the brain of offspring. The injection of pregnant mice with Poly I:C induced a decrease of DNA methylation of CpGs associated to MECP2 and LINE-1 in the hypothalamus of the newborns (Basil et al., 2014). While the biological meaning of dysregulation in MECP2 is well understood, the implications of LINE-1 dysregulation remainunclear, especially in light of the fact that LINE-1 methylation levels are often employed to detect any variation in global DNA methylation. Further studies are necessary to fully understand if a decrease in DNA methylation detected in LINE-1 can be indicative of genome-wide hypomethylation after MIA. In a more recent study, offspring of Poly I:C treated mothers displayed hypermethylation at the promoter of Glutamic Acid Decarboxylase 1 (GAD1) and Glutamic Acid Decarboxylase 2 (GAD2) genes in the prefrontal cortex (Labouesse et al., 2015). An increase in DNA methylation levels was associated with augmented MeCP2 binding and lower gene expression. GAD1 and GAD2 are responsible for the production of the inhibitory neurotransmitter Gamma-Amino Butyric acid (GABA) from its precursor Glutamate. Downregulation of the GABAergic system has been characterized extensively in both autistic children and animal models. Levels of GABA and its receptors have been found to be downregulated in the cortex of individuals with autism (Harada et al., 2011; Crider et al., 2014), and pharmacological activation of GABA receptors improved social behavior in the BTBR autism mouse model (Han et al., 2014). Therefore, the link between MIA and regulation of GABAergic gene-expression operated by DNA methylation, can represent a possible mechanism leading toward GABAergic dysfunction in ASD. In summary, animal studies have provided evidence that MIA induces long term changes in DNA methylation patterns in the brain of offspring.

Although there is an extreme complexity in conducting such studies, there is a need for a human investigation of the relationship between MIA and epigenetics in young children. However, other environmental factors that have been associated with autism—and are known to affect the epigenetic machinery—can also regulate the maternal immune response during pregnancy. For example, as mentioned earlier, gestational diabetes has been associated with ASD, and has been correlated with increased immune activation in the mother and dysregulated placental DNA methylation. Therefore, it is plausible that environmental cues such as gestational diabetes can affect epigenetics modulating the activation of immune system.

MECHANISMS THROUGH WHICH THE IMMUNE RESPONSE MAY AFFECT EPIGENETIC PATTERNING

If immune system activation leads to epigenetic dysregulation seen in autism, there should be a direct molecular mechanism

that links the two phenomena. Animal studies have focused on determining the specific immune factors that might be responsible for the MIA-induced autism phenotype. Smith et al. showed the inhibition of the maternal IL-6 mediated pathway attenuates the MIA-induced autism behavior in offspring (Smith et al., 2007). Maternal IL-6 was directly responsible for MIA-induced gene transcription changes in the offspring frontal cortex. In addition, it has been previously demonstrated that IL-6 is transferred across the human placenta (Zaretsky et al., 2004), which suggests that maternal IL-6 could directly affect the development of the fetal brain. Multiple studies have independently determined that IL-6 can activate DNA methyltransferase 1 (DNMT1), thus providing a direct interaction between MIA and epigenetic regulation. IL-6 induces the nuclear translocation of DNMT1 through AKT-dependent phosphorylation of a nuclear location sequence on the DNMT1 protein (Hodge et al., 2007). IL-6 triggers the DNMT1-mediated hypermethylation at specific promoters in cancer cell lines, that in turn increases the cellular growth rate along with other oncogenic properties. While these effects were witnessed in cancer cells, it is likely that IL-6 can regulate DNMT1 in other cell types such as brain cells, particularly during development. DNMT1 is considered a maintenance methyltransferase, which functions mostly to maintain methylation patterns during cellular proliferation. As such, it is most likely that IL-6-induced DNMT1 activation would have effects during neurogenesis, which takes place mostly during developmental time periods, such as *in utero*. Therefore, there may be a direct interaction between IL-6 and epigenetic machinery, which in turn can regulate gene expression after MIA. A recent study has found that IL-17 activation is also mandatory for an MIA-induced autism-like phenotype in mice (Choi et al., 2016). In fact, it was demonstrated that IL-17-producing T-cells were recruited to the placenta and IL-17 was activated in the developing neocortex. Possible effects of IL-17 on epigenetic machinery have not been extensively studied, however, one study did find that IL-17 inhibits HDAC activity, most likely through PI-3Kinase signaling pathway (Zijlstra et al., 2012). So while IL-6 may directly mediate changes in DNA methylation, IL-17 may affect histone acetylation. Further investigations are still required to really understand if the effect of prenatal IL-6 and IL-17 on offspring behavior is mediated through epigenetic mechanisms.

The mechanism by which most cytokines are likely to influence epigenetic patterns is through regulation of signal transduction pathways that activate epigenetic enzymes or recruit chromatin regulators to the DNA. Two of the main signal transduction pathways that are activated by cytokines are JAK/STAT and MAPK/ERK signaling pathways (Heinrich et al., 2003). STAT proteins act as transcription factors, and also mediate the remodeling of histone acetylation at STAT-binding sites (Wei et al., 2010). The MAP/ERK signaling pathway affects multiple epigenetic modulators, such as CREB. The activation of CREB by phosphorylation involves the recruitment of a chromatin modifying complex, the histone acetyltransferase CBP to the chromatin (Ogryzko et al., 1996). CREB has been shown to be activated by various interleukins, including IL-18 (Zhou et al., 2014) and IL-6 (Melemedjian et al., 2014). While we have just shown that two specific pathways through which IL-6 and IL-17 may affect epigenetic enzymes, it is probable that cytokines or other soluble mediators of the immune response can actually affect epigenetic marks through multiple signaling pathways.

THE MICROBIOME AS A POSSIBLE MODULATOR BETWEEN MIA AND THE AUTISM PHENOTYPE

Of notable interest to neuroscience in general, and to neurodevelopment in particular, is the contribution of the microbiome to human health. The population of bacteria that lives in symbiosis with the human body, collectively known as the microbiome, has recently been shown to influence behavior (Cryan and Dinan, 2012). Of particular interest to this review, the microbiome conducts a very tight crosstalk with the host immune system (Kau et al., 2011). In fact, the microbiome population affects the activity of the immune system, and vice-versa. In addition, recent studies have found that autistic individuals own a distinct microbiome signature compared to non-autistic individuals and even to individuals who were previously diagnosed with Pervasive Developmental Disorder (De Angelis et al., 2013). A recent rodent study suggested that MIA-induced autism phenotypes are caused by an alteration in the number and composition of the wild type mouse microbiota; in particular *B. fragilis* played a fundamental role (Hsiao et al., 2013). Hsiao et al. found that *B. fragilis* specifically affects repetitive behaviors while not having any influence on social behaviors. Therefore, MIA may affect the development of ASD by partially regulating the microbiome composition, even though the molecular mechanisms by which the microbiome may trigger ASD-related behavior are still unknown.

One of the main metabolic products of the microbiome is short-chained fatty acids, including sodium butyrate. Sodium butyrate is well characterized as a histone deacetylase (HDAC) inhibitor (Davie, 2003), and can easily cross the blood-brain barrier. Therefore, sodium butyrate could represent a direct link between the microbiome and epigenetic machinery in the brain. In a previous study it was found that sodium butyrate can also attenuate autism-like behavior in the BTBR mice, which is another autism mouse model often used in pharmacological studies (Kratsman et al., 2015). It was also shown that sodium butyrate specifically affected the transcription of genes involved in the excitation/inhibition balance and GABAergic signaling in mouse prefrontal cortex. So aside from being a common bacterial metabolite, sodium butyrate, is also a very efficient epigenetic modulator that can possibly influence processes that rely on a subtle balance of neurotransmitters. Further studies are needed to understand if immune regulation of the microbiome can induce epigenetic changes in the brain through modulation of fatty acids.

CONCLUSIONS

Separate studies have implicated both immune and epigenetic dysregulation in the etiology of ASD. We set out to review

the evidence indicating that the two phenomena are not independent, and that there could actually be a causal relationship between them. This relationship may have different aspects including immune activation-induced alterations in epigenetic patterns in the fetus brain, and epigenetic regulation of immune cells and immune-related genes in the brain. As was pointed out early in this discussion, the development of ASD is a multifactorial process and it is therefore possible that a genetic predisposition increases the likelihood of developing ASD after MIA. In support of this statement, one study found that MIA induced a phenotype characterized by strong social impairment specifically in mice overexpressing a double negative form of DISC1 (Ibi et al., 2010), a gene strongly associated to Schizophrenia and ASD (Ishizuka et al., 2006; Crepel et al., 2010; Zheng et al., 2011; Turner et al., 2016). In addition, a recent study found that autistic individuals who bear both CNVs and a history of MIA displayed more severe social deficits and repetitive behaviors (Mazina et al., 2015). This means MIA-induced epigenetic changes and genetic susceptibility are likely required to interact to promote autistic behavior. The study, by means of a simplistic example of GxE interaction, puts forth a two-hit model that combines the genetic makeup, as predisposing factor, and environmental cues, as trigger, for explaining the etiology of complex diseases, such in psychiatry.

There are many follow-up studies that are needed to further establish and understand the model of MIA-induced epigenetic changes in the etiology of ASD. In particular, studies are needed that look at the epigenetic studies of children whose mother's experienced MIA during pregnancy. In addition, it is necessary to understand why GABAergic genes seem to be particularly susceptible to MIA and to epigenetic modulation in the brain. Looking to the future, these studies may lead to a more integrated model of ASD biology and etiology, which will help determine the exact relationships between immune response activation and epigenetic regulation, and how they lead to specific autism phenotypes.

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Prenatal and Newborn Immunoglobulin Levels from Mother-Child Pairs and Risk of Autism Spectrum Disorders

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Background: An etiological role for immune factors operating during early brain development in children with autism spectrum disorders (ASD) has not yet been established. A major obstacle has been the lack of early biologic specimens that can be linked to later diagnosis. In a prior study, we found lower risk of ASD associated with higher levels of maternally-derived total IgG and *Toxoplasmosis gondii* (Toxo) IgG in newborn blood spot specimens from children later diagnosed with ASD compared to population controls.

Methods: We obtained maternal mid-gestational serum specimens and newborn screening blood spots from the California Genetics Disease Screening Program (GDSP) for linked mother-baby pairs for 84 children with ASD and 49 children with developmental delay but not ASD (DD) identified from California Department of Developmental Services records and for 159 population controls sampled from birth certificates.Immunoglobulin levels in maternal and newborn specimens were measured by solid phase immunoassays and analyzed in logistic regression models for total IgG, total IgM, and Toxo IgG, and, for maternal specimens only, Toxo IgM. Correlations between maternal and newborn ranked values were evaluated.

Results: In both maternal and newborn specimens, we found significantly lower risk of ASD associated with higher levels of Toxo IgG. In addition, point estimates for all comparisons were <1.0 suggesting an overall pattern of lower immunoglobulin levels associated with higher ASD risk but most did not reach statistical significance. We did not find differences in maternal or newborn specimens comparing children with DD to controls.

Discussion: These results are consistent with evidence from our prior study and other published reports indicating that immune factors during early neurodevelopment may be etiologically relevant to ASD. Lowered immunoglobulin levels may represent suboptimal

function of the maternal immune system or reduced maternal exposure to common infectious agents.

Conclusion: Patterns seen in these selected immunoglobulins may provide clues to mechanisms of early abnormalities in neurodevelopment contributing to ASD. We recommend further study of immunoglobulin profiles in larger samples of linked mother-baby pairs to evaluate possible etiologic relevance.

Keywords: autism, maternal infection, biomarkers, immune function

INTRODUCTION

Whether and how immune factors operating during fetal brain development are etiologically relevant to autism spectrum disorders (ASD) has not yet been determined. Numerous studies have documented atypical immune findings in some individuals already diagnosed with ASD. These include post-mortem brain tissue studies demonstrating evidence of chronic CNS inflammation in individuals aged 4–40 years old with ASD (Goines and Van de Water, 2010; Onore et al., 2012). More directly implicating a prenatal etiologic pathway, animal model studies have shown that autism-relevant behaviors can be induced in offspring following in utero exposure to antibodies derived from mothers with a child already diagnosed with ASD (Martin et al., 2008; Singer et al., 2009; Braunschweig et al., 2012), but not prenatal exposure to antibodies derived from serum of children with ASD (Morris et al., 2009). Other animal studies provide evidence that activation of the maternal immune system during pregnancy, either through infectious or non-infectious agents, can lead to atypical behaviors similar to those in ASD-affected children (Patterson, 2011). These findings are consistent with observational human studies that report maternal viral exposure (Chess, 1971; Deykin and MacMahon, 1979; Markowitz, 1983; Stubbs et al., 1984; Yamashita et al., 2003; Atladottir et al., 2012a; Zerbo et al., 2013) or inflammation (Brown et al., 2014) during pregnancy to be associated with increased risk of ASD in some children. Evidence for an association between prenatal infection and risk of other psychiatric disorders, such as schizophrenia and bipolar disorder, has also been reported (Buka et al., 2001a, 2008; Brown, 2011; Mortensen et al., 2011; Kneeland and Fatemi, 2013).

Despite this growing and complex body of research, questions regarding an etiologic role for immune factors in ASD remain to be resolved, impeded, in part, by a paucity of biologic specimens obtained during the presumed vulnerable period of fetal brain development and linked to later behaviorally-based diagnostic information. Routinely collected and archived specimens in California provide a resource for addressing some of these questions.

Using archived maternal specimens from California originally obtained during routine prenatal screening (Early Markers for Autism Study/EMA), Croen et al. (2008a) found differences in maternal mid-gestation antibody reactivity to human fetal brain protein in specimens from mothers of children with ASD compared to population-based controls. Although, the differences did not reach statistical significance, they are generally consistent with those based on maternal specimens obtained after an ASD diagnosis of an affected child (Dalton et al., 2003; Zimmerman et al., 2007; Braunschweig et al., 2008). Using the same prenatal specimens from the EMA project, Goines et al. (2011) reported a pro-inflammatory cytokine profile in mothers of children with ASD that was different from that of mothers of children without ASD.

In another California study, we measured neonatal levels of selected IgG, IgM, and IgA antibodies in archived newborn screening blood specimens to evaluate the hypothesis that higher antibody levels would be associated with a risk of ASD (Grether et al., 2010). In newborns, IgG antibodies are largely derived from the mother and transferred across the placenta to the fetus (Simister, 2003). Neonatal IgM and IgA antibodies are generally made by the fetus or neonate and serve as one set of putative markers of perinatal infection. In this earlier California study (Grether et al., 2010) in which we evaluated total IgG, IgM, and IgA as well as common viral antigen-specific immunoglobulins, we found no evidence of elevated immunoglobulin levels that would indicate increased prenatal infectious exposure in children with ASD, but we could not confidently rule out a role for all possible antigens or early transient exposures. Contrary to our hypothesis and of uncertain clinical significance, we found significantly lower ASD risk associated with higher levels of total IgG and *Toxoplasmosis gondii* (Toxo) IgG in the newborn specimens from the children with ASD. If supported by further research, these results may plausibly represent suboptimal humoral function in the maternal immune system and/or impaired transplacental transfer; the findings may also represent a protective effect in the controls in the postnatal period from earlier maternal exposure. As maternal specimens were not available for the children in that study, we were unable to further explore possible pathways.

We here report a case-control study using maternal mid-gestational as well as neonatal specimens from linked

Abbreviations: ASD, autism spectrum disorders; CNS, central nervous system; DDS, California Department of Developmental Services; CAT, category; CPD, The Center for Persons with Disabilities (CPD); DD, developmental delay; DSM IV, Diagnostic and Statistical Manual of Mental Disorders, 4th Edition; ELISA, enzyme-linked immunosorbent assay; EMA, Early Markers for Autism Study; GA, gestational age; GDSP, California Genetics Disease Screening Program; HIGH, the three higher quartiles (or categories); IgA, Immunoglobulin A; IgG, Immunoglobulin G; IgM, Immunoglobulin M; IRB, institutional review board; LOW, lowest quartile (or category); MDL, minimum detection level; OR, odds ratio; PBB, Project Baby's Breath; Q, quartile; RCOC, Regional Center of Orange County; SI, standardized international units; Toxo, Toxoplasmosis gondii; XAFP, expanded alpha fetoprotein.

mother-child pairs to re-evaluate total IgG, total IgM, and *Toxoplasma gondii* immunoglobulins in children with ASD compared to population-based controls. To assist with interpretation of results, we also included a group of children with other developmental disabilities, comparing their assay results to control values. The study sample comes from the Early Markers for Autism (EMA) Study, a population-based, nested case-control study designed to evaluate biologic markers of susceptibility and exposure in archived maternal mid-gestational and neonatal blood specimens from linked mother-baby pairs.

METHODS

Subjects

Study subjects were selected from the population of offspring born to women living in Orange County, California who were pregnant in 2000–2001 and enrolled in the State's Prenatal Expanded Alphafetoprotein Screening Program (Croen et al., 2008a) and for which specimens were available in the Project Baby's Breath (PBB) special research archive (see below). Three groups of children were identified: children with ASD, children with other developmental delay but not ASD (DD), and general population controls. Children with ASD or DD represent all children identified with these conditions who met the above criteria and who were enrolled with the Regional Center of Orange County (RCOC), one of 21 Regional Centers operated by the California Department of Developmental Services (DDS) to coordinate services for persons with ASD, developmental delay, and other developmental disabilities. Possible ASD cases were initially ascertained as clients receiving DDS services for autistic disorder or clients receiving services for other DDS-eligible conditions but who also had a code indicating ASD in the electronic record. Possible DD cases were initially ascertained as DDS clients without any evidence of ASD in the electronic records and with evidence of intellectual disability.

To confirm ASD or DD case status, we followed a protocol initially developed by the Metropolitan Atlanta Developmental Disabilities Surveillance Program (Autism and Developmental Disabilities Monitoring Network Surveillance Year 2006 Principal Investigators; Centers for Disease Control and Prevention (CDC), 2009), employing trained medical record abstractors to compile detailed diagnostic and clinical data from RCOC records for all children ascertained as possible ASD or possible DD. A pediatrician with certification in developmental and behavioral pediatrics (RH) then conducted expert clinical review of abstracted data to confirm ASD or DD case status using DSM IV criteria. Because of etiologic questions regarding co-morbid intellectual disability in children with ASD, children with ASD were further categorized by presence or absence of intellectual disability using DSM-IV criteria and based on standardized cognitive and adaptive assessments. Children with all scores <70 were coded as having ID; children with all scores ≥ 70 or some scores <70 and others ≥ 70 were coded as not having ID; children with no standardized scores in their chart were coded as "unknown."

Controls were randomly sampled from the birth certificate files, frequency matched to ASD cases by sex, birth month, and birth year in a 2:1 ratio; all past or current DDS clients were identified through statewide electronic files and excluded from the control population. Demographic variables for all subjects were obtained from live birth certificates.

Specimen Collection

Maternal mid-gestational specimens were retrieved from the Project Baby's Breath (PBB) prenatal screening specimen archive maintained by the California Genetic Disease Screening Program (GDSP), California Department of Public Health. Following the completion of routine prenatal screening conducted by GDSP, PBB had retained any remaining portions of specimens in three selected southern California counties and selected birth years for analysis in approved research studies. During the study period, prenatal screening was conducted for approximately 70% of pregnancies in the state (state law mandates that all women in the first half of pregnancy be offered the voluntary screening). Venous blood was collected at 15–20 weeks gestation in serum separator tubes by obstetrical care service providers and laboratories, and underwent expanded alpha fetoprotein (XAFP) screening at a single regional laboratory, typically within 7 days of collection (median time = 3 days). During transit via US Postal Service to the regional screening laboratory, no effort was made to control the temperature of the specimens. After testing, remaining portions of specimens were kept under refrigeration for 1–2 days and then stored at -20°C by the regional laboratory and PBB. Aliquots of the samples used for this study were shipped to the laboratory of Dr. Judy van de Water, UC Davis, and stored at -80°C until use with a single thaw prior to immunoglobulin testing. All samples were exposed to the same collection and storage protocols.

Newborn screening filter paper blood specimens were obtained from the newborn archive maintained by GDSP that conducts routine metabolic screening for all newborns in the state. Specimens from cases and controls were located, one spot bar-coded with a study identifier, and shipped on dry ice, without regard to case-control status, to The Center for Persons with Disabilities (CPD) at Utah State University. Working under aseptic conditions, researchers in the CPD lab prepared 96-well plates, each well containing two 3 mm diameter punches from one archived specimen. Since original blood draw and newborn screening, specimens have been stored at -20°C.

All prenatal and newborn specimens were then shipped on dry ice to the Neurovirology Laboratory, Johns Hopkins School of Medicine, for analysis, blinded to case-control status, using enzyme immunoassays as described below.

Laboratory Assays

We measured antibodies in both maternal and newborn specimens by solid phase immunoassays (ELISA). Maternal and newborn total IgG and total IgM levels were expressed as μg/ml. In maternal specimens, we also measured *Toxoplasma gondii* (Toxo) IgG with resulting levels expressed as standardized international units (SI) and Toxo IgM with resulting levels expressed as blank-adjusted optical density units. In newborn specimens, we measured Toxo IgG with resulting levels expressed as blank-adjusted optical density units. Assay results were not

corrected for hematocrit or total protein and specimens lacking detectable signals for a specific assay were considered at the minimum detection level (MDL) for that assay.

Exclusions

Subjects (1 child with ASD, 3 children with DD, 1 control child) were excluded due to estimated gestational age <26.0 completed weeks (182 days; recorded on the birth certificate and based on LMP) or because age at blood draw was >7.0 days after birth according to the newborn screening record.

Statistical Methods

To take into consideration assay detection limits, unknown distributions of the immunoglobulins, and inadequate knowledge of the biologic significance of immunoglobulin levels measured in archived specimens, we conducted primary analyses treating the measured immunoglobulin values as categorical variables. Categories were constructed separately for each of the analytes based on percent of signals at the MDL for these assays: if ≤25% of controls were at the MDL, observations were divided into quartiles based on control values, with MDL values included in lowest quartile (Q1); if >25% of controls had values at the MDL, then MDL values were designated as the lowest category (CAT1) and remaining values were divided into three equal-sized categories based on control values. The exception to this was newborn total IgM, which had only a low and a high category due to limited distribution.

For comparisons between ASD and controls, and separately between DD and controls, we then conducted crude and adjusted analyses using logistic regression to estimate the risk of ASD (or DD) associated with each analyte separately, comparing the lowest category to each higher category using odds ratios (ORs) considered statistically significant if 95% confidence intervals did not include 1.0. Adjusted models included assay plate (5 maternal and 5 newborn) as additional independent variables and covariates associated with ASD (or DD) in these data ($p < 0.5$; **Table 1**). Due to small numbers of children with DD, we collapsed the three higher quartiles (or categories) into one group (HIGH) and used the lowest quartile (or category) as the reference group (LOW). We explored differences in immunoglobulin levels between children with ASD with ID and those with ASD without ID, comparing each of these subgroups to the control group. Small numbers of subjects in subgroups precluded the use of adjusted models.

To evaluate the association between maternal mid-gestational analyte signals and those detected in their children's newborn specimens, we computed Spearman rank-order correlation coefficients separately for cases and controls for the three analytes measured in both mothers and newborns (total IgG, total IgM, and Toxo IgG). Values at the MDL were treated as the lowest category, with measured signals above the MDL for each individual treated as categorical variables in the computations.

Institutional Review Board

This study was approved by the California Health and Human Services Agency Committee for the Protection of Human Subjects (IRB) and the Kaiser Permanente of Northern California Institutional Review Board, who granted a waiver of informed consent. The analytic laboratories operated under an IRB exemption as the samples were preexisting and did not have personal identifiers.

RESULTS

The final study group included 84 children with ASD, 49 children with DD, and 159 controls. No significant differences were found between ASD and controls on the matching variables (gender, birth month, birth year), nor for single or multiple birth, gestational age (GA), birth weight, days between birth and blood draw, and protein concentration in the newborn specimen (**Table 1**). Mothers of children with ASD were similar to controls with regard to parity, inter-pregnancy interval (number of months between birth of index child and prior live birth), GA at mid-gestational blood draw, and maternal weight at blood draw (**Table 1**). ASD cases were more likely than controls to have mothers who self-identified as white non-Hispanic, were older, and more educated.

Compared to the control children, children with DD were less likely to be male and born in the first study year (2000) or during the same birth months due to matching of the controls with the ASD group on these variables. Children with DD were also of lower birth weight and had a longer time between birth and blood draw than controls (**Table 1**). No differences in maternal characteristics were observed.

For the ASD study group, both newborn and maternal specimens were available for 80 mother-baby pairs and only maternal specimens were available for the remaining 4 mother-baby pairs; for the DD study group, both specimens were available for 45 pairs, only maternal specimen for 1 pair, and only newborn specimens for 3 pairs; for the controls, both specimens were available for 141 pairs, only maternal specimens for 6 pairs, and only newborn specimens for 12 pairs. Signals above the MDL were detected for total IgG and total IgM in all maternal and newborn specimens and for Toxo IgM in all maternal specimens. For the Toxo IgG assay, 93 maternal specimens were at the MDL (45% of ASD, 33% of DD, 27% of controls), as was one newborn specimen for a child with DD.

ASD vs. Controls

The distributions of each analyte for ASD cases and controls are shown in **Table 2**. In unadjusted analyses, measured levels of total IgG, total IgM, and Toxo IgM in mid-gestational specimens of mothers of children diagnosed with ASD were not significantly different from population control values but virtually all point estimates were substantially below 1.0 (**Table 2**). For Toxo IgG, significantly fewer mothers of children with ASD were represented in CAT3 and CAT4 than were mothers of controls (**Table 2**), indicating lower risk of ASD with higher measured antibody.

In unadjusted analyses, newborn specimens from children with ASD did not show significantly different levels from controls for total IgG or total IgM. For Toxo IgG, risk was reduced for signals above Q1, but only significantly so for Q2 and Q4 (**Table 2**). All ORs were below 1.0 in the newborn comparisons.

TABLE 1 | Demographic characteristics of children with Autism Spectrum Disorders (ASD), developmental delay without ASD (DD), and Control Subjects. Early Markers for Autism (EMA) Study, births 2000, 2001.

	Controls (*N* = 159)		Children with ASD (*N* = 84)			Children with DD (*N* = 49)		
Baby characteristics	***N***	**%**	***N***	**%**	***P*-value[a]**	***N***	**%**	***P*-value[a]**
GENDER								
Male	139	87.42	73	86.90	0.91	29	59.18	<0.0001
Female	20	12.58	11	13.10		20	40.82	
BIRTH YEAR								
2000	31	19.5	23	27.38	0.16	26	53.06	<0.0001
2001	128	80.5	61	72.62		23	46.94	
Birth Month					0.96			0.01
PLURALITY								
Singleton	156	98.11	81	96.43	0.42	47	95.92	0.34
Multiple	3.00	1.81	3	3.57		2	4.08	
	Mean	**(SD)**	**Mean**	**(SD)**	***P*-value[b]**	**Mean**	**(SD)**	***P*-value[b]**
Gestational age at birth (days)	271.01	(14.27)	272.12	(18.47)	0.63	266.33	(28.12)	0.27
Birth weight	3348.1	(616.09)	3416.1	(699.1)	0.44	2897.7	(827.22)	0.0008
Days between birth and blood draw	1.46	(0.94)	1.48	(0.71)	0.80	1.92	(1.43)	0.036
Protein concentration in newborn specimen (ug/ml)	6520.3	(1377.2)	6495.5	(1222.1)	0.89	6404.4	(1551.5)	0.62
Maternal characteristics	***N***	**%**	***N***	**%**	***P*-value[a]**	***N***	**%**	***P*-value[a]**
MATERNAL RACE/ETHNICITY								
White non-Hispanic	53	33.33	37	44.05	0.0005	9	18.37	*0.43*
White Hispanic: US Born	13	8.18	10	11.9		5	10.20	
White Hispanic: Not US Born	60	37.74	10	11.9		23	46.94	
Black	1	0.63	0	0.00		1	2.04	
Asian	28	17.61	19	22.62		9	18.37	
Other	4	2.52	8	9.52		2	4.08	
MATERNAL AGE								
<20	9	5.66	2	2.38	0.004	3	6.12	0.90
20–24	33	20.75	7	8.33		8	16.33	
25–29	48	30.19	18	21.43		18	36.73	
30–34	51	32.08	38	45.24		14	28.57	
35+	18	11.32	19	22.62		6	12.24	
MATERNAL EDUCATION								
< High school	49	30.82	7	8.43	<0.0001	22	44.90	0.23
High school graduate	39	24.53	15	18.07		9	18.37	
College	46	28.93	40	48.19		14	28.57	
Post graduate	25	15.72	21	25.3		4	8.16	
PARITY								
Primiparous	67	42.14	42	50.00	0.24	16	37.65	0.24
Multiparous	92	59.86	42	50.00		33	67.35	
INTER-LIVEBIRTH INTERVAL (MONTHS)								
0–12	16	17.58	8	20.00	0.68	4	12.50	0.52
13–24	19	20.88	10	25.00		10	31.25	
25–60	31	34.07	15	37.50		12	37.50	
>60	25	27.47	7	17.50		6	18.75	
	Mean	**(SD)**	**Mean**	**(SD)**	***P*-value[b]**	**Mean**	**(SD)**	***P*-value[b]**
Gestational age at blood draw (days)	118.78		119.76		0.35	118.37		0.75
Mother's weight at XAFP blood draw (lbs)	146.89	(33.78)	145.07	(26.72)	0.65	150.3	(39.3)	0.56

[a] *Chi-square test of association, comparing to controls.*
[b] *t-test for equality of means, comparing to controls.*

TABLE 2 | Association of antibodies and risk of ASD, distribution and crude odds ratios, EMA study, births 2000–2001.

	N at MDL	Category	Analyte	ASD	Controls	OR_{unadj}	95% CI
MATERNAL							
Total IgG (μg/ml)		Q4	>53.63	18	36	0.77	(0.36, 1.66)
		Q3	>46.59–53.63	28	37	1.17	(0.57, 2.37)
		Q2	>43.11–46.59	14	37	0.58	(0.26, 1.30)
	0	(Ref*) Q1	0–43.11	24	37		
Total IgM (μg/ml)		Q4	>65.96	15	35	0.55	(0.25, 1.19)
		Q3	>56.80–65.96	18	38	0.60	(0.29, 1.27)
		Q2	>29.56–56.80	22	37	0.76	(0.37, 1.56)
	0	(Ref*) Q1	0–29.56	29	37		
Toxo IgG (SI)		Cat 4	>18.6	8	37	**0.23**	**(0.09, 0.55)**
		Cat 3	>6.3–18.6	15	35	**0.45**	**(0.21, 0.96)**
		Cat 2	>0–6.3	23	35	0.69	(0.35, 1.38)
	78	(Ref**) Cat 1	0	38	40		
Toxo IgM (odu***)		Q4	>0.202	15	35	0.57	(0.26, 1.23)
		Q3	>0.172–0.202	22	38	0.77	(0.38, 1.56)
		Q2	>0.158–0.172	17	34	0.67	(0.32, 1.41)
	0	(Ref*) Q1	0–0.158	30	40		
NEWBORN							
Total IgG (μg/ml)		Q4	>61.18	18	38	0.66	(0.31, 1.38)
		Q3	>55.03–61.18	16	38	0.59	(0.27, 1.25)
		Q2	>51.63–55.03	18	38	0.66	(0.31, 1.38)
	0	(Ref*) Q1	0–51.63	28	39		
Total IgM (μg/ml)		Cat2	>1.20	29	66	0.75	(0.43, 1.31)
	0	(Ref**) Cat 1	0–1.20	51	87		
Toxo IgG (odu***)		Q4	>0.097	5	38	**0.14**	**(0.05, 0.39)**
		Q3	>0.066–0.097	23	38	0.64	(0.32, 1.27)
		Q2	>0.057–0.066	15	38	**0.42**	**(0.20, 0.88)**
	0	(Ref*) Q1	0–0.057	37	39		

Reference quartile (Q1) includes signals at and above the MDL based on control values.

**Reference category (Cat 1) only includes signals at the MDL. Values above the MDL divided into equal-sized categories based on control values.*

***Optical density units (blank adjusted).*

Bold means statistically significant.

After adjustment for laboratory plate, maternal age, and maternal education (**Table 3**$_{\text{Adj Column 1}}$), maternal specimens did not show significant difference between cases and controls for total IgG, total IgM, or Toxo IgM, although, as in the unadjusted analyses, virtually all point estimates were below 1.0. Higher levels of Toxo IgG were significantly associated with lower risk of ASD (Category 4 vs. Category 1) and the dose-response trend across the Toxo IgG categories reached statistical significance ($p = 0.038$). With further adjustment for maternal race-ethnicity (**Table 3**$_{\text{Adj Column 2}}$), the Toxo IgG results continued to indicate lower risk with higher levels with a dose-response pattern ($p = 0.039$). Further adjustment that included place of birth for white Hispanic mothers (US-born or not US-born) showed similar patterns that did not reach statistical significance (**Table 3**$_{\text{Adj Column 3}}$).

In adjusted models with newborn specimens, all ORs were below 1.0, with the comparison of Toxo IgG Q4 vs. Q1 reaching statistical significance (**Table 3**$_{\text{Adj Columns 1 and 2}}$). No dose-response pattern for newborn Toxo IgG was observed.

ASD with and without Intellectual Disability

Data on presence/absence of intellectual disability was available on 76% of the children with ASD, permitting identification of two subgroups: ASD with ID ($n = 34$) and ASD without ID ($n = 30$). Each of these subgroups was then compared separately to the control group and the resulting ORs for each analyte compared across the two subgroups. No statistically significant differences between the two subgroups were observed (data not shown). Small numbers of children precluded adjustment for covariates.

DD vs. Controls

In models adjusted for laboratory plate, gender, birth year, and days to blood draw, we found no association between risk of DD and any of the analytes when comparing the LOW and HIGH exposure categories. Odds ratios for the different analytes ranged from 0.71 to 1.28 with no apparent pattern and all confidence intervals included the null value (data not shown).

Correlations within Mother-Baby Pairs

Within mother-baby pairs, levels of total IgG measured in mothers during mid-gestation and in their newborns were not

TABLE 3 | Association of antibodies and risk of ASD, EMA study, births 2000–2001.

	OR^{a}_{adj}	95% CI	OR^{b}_{adj}	95% CI	OR^{c}_{adj}	95% CI
MATERNAL						
Total IgG						
Q4 vs. Q1	0.92	(0.39, 2.17)	0.87	(0.37, 2.08)	0.89	(0.37, 2.13)
Q3 vs. Q1	1.41	(0.63, 3.14)	1.39	(0.62, 3.12)	1.34	(0.59, 3.01)
Q2 vs. Q1	0.53	(0.22, 1.30)	0.51	(0.21, 1.25)	0.51	(0.21, 1.27)
Total IgM						
Q4 vs. Q1	0.46	(0.17, 1.27)	0.44	(0.16, 1.23)	0.42	(0.15, 1.21)
Q3 vs. Q1	0.63	(0.25, 1.61)	0.63	(0.25, 1.60)	0.71	(0.27, 1.85)
Q2 vs. Q1	0.68	(0.29, 1.62)	0.69	(0.29, 1.66)	0.75	(0.31, 1.82)
Toxo IgG						
CAT4 vs. CAT1*	**0.35**	**(0.13, 0.91)**	**0.33**	**(0.12, 0.86)**	0.40	(0.15, 1.11)
CAT3 vs. CAT1*	0.58	(0.25, 1.32)	0.53	(0.22, 1.24)	0.55	(0.23, 1.30)
CAT2 vs. CAT1*	0.86	(0.40, 1.86)	0.83	(0.38, 1.81)	0.86	(0.39, 1.87)
Toxo IgM						
Q4 vs. Q1	0.80	(0.32, 1.99)	0.78	(0.31, 1.94)	0.71	(0.28, 1.77)
Q3 vs. Q1	0.82	(0.36, 1.84)	0.78	(0.34, 1.78)	0.70	(0.30, 1.64)
Q2 vs. Q1	0.78	(0.33, 1.84)	0.74	(0.31, 1.78)	0.69	(0.28, 1.69)
NEWBORN						
Total IgG						
Q4 vs. Q1	0.77	(0.31, 1.89)	0.76	(0.31, 1.86)	0.70	(0.28, 1.76)
Q3 vs. Q1	0.65	(0.28, 1.48)	0.61	(0.27, 1.42)	0.64	(0.27, 1.48)
Q2 vs. Q1	0.75	(0.33, 1.69)	0.72	(0.32, 1.65)	0.72	(0.31, 1.66)
Total IgM						
HIGH vs. LOW**	0.67	(0.30, 1.49)	0.67	(0.30, 1.48)	0.77	(0.34, 1.75)
Toxo IgG						
Q4 vs. Q1	**0.25**	**(0.08, 0.79)**	**0.25**	**(0.08, 0.78)**	0.31	(0.10, 1.01)
Q3 vs. Q1	0.89	(0.41, 1.94)	0.90	(0.41, 1.96)	0.87	(0.39, 1.92)
Q2 vs. Q1	0.52	(0.23, 1.17)	0.52	(0.23, 1.18)	0.50	(0.22, 1.14)

[a] *Adjusted for laboratory plate, maternal age, maternal education.*

[b] *Adjusted for laboratory plate, maternal age, maternal education, maternal race/ethnicity (White non-Hisp., White Hisp., Other).*

[c] *Adjusted for laboratory plate, maternal age, maternal education, maternal race/ethnicity (White non-Hisp., White Hisp.-US Born, White Hisp.-Not US Born, Other).*

[*] *Reference category (CAT1) only includes signals at the MDL. Values above the MDL divided into 3 equal-sized categories based on control values.*

[**] *Reference category (LOW) includes all signals above but close to the minimum observed value for the assay, with remaining values lumped into one higher category.*

Bold means statistically significant.

correlated in the group of ASD cases ($r_{\varphi} = -0.03$), in controls ($r_{\varphi} = 0.04$), or in the DD group ($r_{\varphi} = 0.08$). Similarly, IgM showed no correlation between levels in maternal and newborn specimens in the ASD group ($r_{\varphi} = 0.04$), the control group ($r_{\varphi} = -0.03$) or the DD group ($r_{\varphi} = -0.0.26$, $p = 0.08$). In contrast, Toxo IgG levels in maternal and newborn specimens were correlated within ASD cases ($r_{\varphi} = 0.34$, $p < 0.002$) and within population controls ($r_{\varphi} = 0.53$, $p < 0001$) but not in the DD group ($r_{\varphi} = 0.11$, $p = 0.47$).

To further explore an association between low Toxo IgG and ASD, we evaluated mother-baby ASD and control pairs in which both mother and newborn Toxo IgG levels were in the reference range (see **Table 2**) compared to pairs in which either or both the maternal or the newborn values were above the reference range. The logistic model (adjusted for laboratory plate only) yielded an OR of 0.24 (95% CI 0.10, 0.56), consistent with a lower risk of ASD associated with higher levels of Toxo IgG.

DISCUSSION

This ASD case-control study is the first to report immunoglobulin levels measured in both maternal mid-gestational and newborn specimens from linked mother-baby pairs. The immunoglobulins were selected based on our prior California study using newborn specimens in which lower risk of ASD was associated with higher assay levels for total IgG and Toxo IgG (Grether et al., 2010).

The results reported here are consistent in many, but not all, respects to those we reported earlier. In the current study, the results for both total IgG and Toxo IgG suggest, but do not statistically confirm, a lower risk of ASD associated with higher levels of the analytes in newborn specimens, similar to the earlier findings but not reaching statistical significance, perhaps because of the smaller number of subjects available for this current study. In the maternal specimens, lower risk of ASD appeared to be

associated with higher measured levels of Toxo IgG, reaching statistical significance for several comparisons with a dose-response pattern indicating a protective association. However, this association no longer reached statistical significance in models controlling for maternal place of birth, suggesting that the ASD case and controls differences we see with Toxo IgG may be a function of place of birth, as country of origin is a significant factor influencing exposure to Toxo (Jones et al., 2001).

The ORs for most comparisons, both maternal and newborn, were substantially below the null value, suggesting an overall pattern of lower measured levels of these immunoglobulins in ASD mother-baby pairs, even though some risk estimates did not reach statistical significance. Whether these patterns would become more statistically robust with a larger number of subjects cannot be determined from this one study. No differences were detected in immunoglobulin levels between subgroups of ASD defined by presence/ absence of intellectual disability, but sample sizes were small. Analyses of the analytes in specimens from a heterogeneous group of children with DD and their mothers failed to show any associations or possible patterns when compared to the controls, and small numbers of children with DD prevented detailed analysis.

Within mother-baby pairs, the levels of total IgG for mothers and their offspring were not statistically correlated for any of the three groups of subjects; nor were mother-baby values correlated for total IgM. Absence of correlation is, perhaps, not surprising given the elapsed time between maternal mid-gestational and newborn blood draws, the limitation of one time-point during pregnancy, and the multitude of antigens and immune response represented in these total measurements (Buka et al., 2001b). In contrast, signals for antigen-specific Toxo IgG show moderate and statistically significant correlation between maternal mid-gestational and newborn values for both ASD and population control pairs, despite the limitations related to time of specimen collection. Within the context of this study, we are unable to determine if the presence of Toxo IgG represents recent or distant exposure (Toxo IgG can persist for several years), but to our knowledge, none of the children in either the ASD or population control groups showed clinical manifestations of congenital Toxoplasmosis. For the DD group, no correlations were observed within mother-baby pairs for any analytes, perhaps because of the greater number of children of low gestational age in this subgroup (transfer of IgG antibodies from mother to baby occurs late in gestation).

Because this study employs different specimens and assays than those used in clinical settings, the clinical significance of the differences observed here is unclear. However, the overall consistency of our results with those found in our prior study (Grether et al., 2010), of higher ASD risk associated with lower immunoglobulin signals, is potentially informative. Taken together, these results are consistent with the evidence from other studies indicating that atypical maternal immune function during fetal life may contribute in some way to the development of ASD in a subset of children (Croen et al., 2008a; Goines et al., 2011; Brown et al., 2014).

In the prior study, we raised the question of whether the higher risk of ASD associated with lower newborn immunoglobulin signals might represent impaired placental transport. Based on the current study, this does not seem likely since the ASD maternal specimens showed the same overall pattern of lower immunoglobulin levels compared to controls as seen in the newborn specimens.

A plausible explanation for lower immunoglobulin levels associated with ASD is suboptimal function of the maternal immune system; exposure to common antigens may not elicit a full or typical maternal immune response, contributing to development of ASD in offspring. Whether and how suboptimal maternal immune function may impact fetal neurodevelopment remains to be explicated. Another possible explanation is that the lower immunoglobulin levels associated with ASD may simply represent reduced maternal exposure to common infectious agents. That some version of the "hygiene hypothesis" during early neurodevelopment may be relevant to ASD risk was previously offered by Becker (2007) based on parallel epidemiological, morphometric, molecular, and genetic patterns between ASD and other inflammatory disorders such as asthma.

Infants and young children who lack the robust immune protection provided by maternally-derived immunoglobulins, whether from suboptimal maternal immune function or reduced maternal exposure, might be expected to be more vulnerable to infectious exposures after birth, perhaps contributing to higher risk for ASD. However, studies have failed to consistently show an association between increased frequency of postnatal infectious illnesses and risk for ASD (Rosen et al., 2007; Atladottir et al., 2010, 2012b). Whether postnatal exposure to *Toxoplasmosis* as a specific antigen may be related to higher risk for ASD has not, to our knowledge, been investigated.

Animal studies have documented a link between maternal infectious exposure (and non-infectious activation of the maternal immune system) during pregnancy and autistic-like behaviors in offspring (Patterson, 2011). A number of early human studies also indicated that maternal infectious illnesses during pregnancy, although uncommon, may be more frequent in the history of children with ASD than in controls (Chess, 1971; Deykin and MacMahon, 1979; Markowitz, 1983; Yamashita et al., 2003). More recent studies are inconsistent (Atladottir et al., 2012a; Zerbo et al., 2013; Brown et al., 2014). Further studies based on medical record documentation may be informative, at least for more severe infections/fevers, as would prospective studies that document maternal illness during pregnancy.

In interpreting the results of our study, several strengths and limitations need to be considered. Strengths include population-based identification of subjects and the prospective specimen collection, permitting analysis of biological markers of infections during early fetal and neonatal neurodevelopment. However, our modest sample size limited our statistical power, especially for DD group for which our null results may not be generalizable. The diagnostic information we used to select the ASD and DD groups relied on DDS service agency reports with expert review by experienced clinicians. Other studies, in which the ASD diagnosis has been validated through comprehensive clinical assessment, have indicated a very high level of diagnostic validity for children enrolled in DDS with an ASD (Hertz-Picciotto et al., 2006; Croen et al., 2008b; Hallmayer et al., 2011). Our laboratory

assays were fully blinded to case-control status and conducted in research laboratories with extensive experience in these research assays. However, the assays are not comparable to clinical ones, limiting our ability to interpret the biologic relevance of the results in a clinical framework. Since we did not measure all antigen-specific immunoglobulins, our results should not be interpreted as ruling out the possibility that exposure to some specific antigens not evaluated here might increase risk of ASD. Bias related to laboratory procedures can reasonably be ruled out as an explanation for our overall patterns of lower antibody levels in the ASD group, but undetected bias in initial selection of subjects must be considered as a possible explanation.

CONCLUSION

Taken as a whole, and if supported by further research, our results suggest that early immunoglobulin patterns in ASD mother-baby pairs may be etiologically informative; we recommend further studies that include prenatal and newborn specimens from linked mother-baby pairs to evaluate these immunoglobulins and other immune markers and possible effect modification associated with gene-pool factors or geographic differences in maternal exposure.

AUTHOR CONTRIBUTIONS

JG provided direction to the conception and design of the study, acquisition of data, conducted analysis and interpretation of data, and prepared the manuscript. PA contributed to conception and design of the study, analysis and interpretation of data, and revision of the manuscript for important intellectual content. JV contributed to conception and design of the study, preparation of specimens, analysis and interpretation of data, and revision of the manuscript for important intellectual content. RY contributed PA contributed actively to conception and design of the study, analysis and interpretation of data, and revision of the manuscript for important intellectual content. MA contributed to conception and design of the study, analysis and interpretation of data, and revision of the manuscript for important intellectual content. AT contributed to conception and design of the study, preparation of specimens, analysis and interpretation of data, and revision of the manuscript for important intellectual content. JW contributed to preparation of specimens, analysis and interpretation of data, and revision of the manuscript for important intellectual content. TS contributed to preparation of specimens, analysis and interpretation of data, and revision of the manuscript for important intellectual content. RH contributed to conception and design of the study, acquisition of data, and revision of the manuscript for important intellectual content. MK contributed to conception and design of the study, acquisition of data, analysis and interpretation of data, and revision of the manuscript for important intellectual content. LC contributed to the conception and design of the study, acquisition of data, analysis and interpretation of data, and review and revision of the manuscript. All authors read and approved the final manuscript.

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