Title

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Journal

Clinical Genetics, 104(2)

ISSN

0009-9163

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Publication Date

2023-08-01

DOI

10.1111/cge.14338

Received: 2 February 2023 | Revised: 10 March 2023 | Accepted: 27 March 2023

DOI: 10.1111/cge.14338

CLINICAL GENETICS WILEY

SHORT REPORT

Clinical and molecular spectrum of a large Egyptian cohort with *ALS2*-related disorders of infantile-onset of clinical continuum IAHSP/JPLS

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Funding information
NRC, Grant/Award Numbers: 11010167, 12060178

Abstract

This study presents 46 patients from 23 unrelated Egyptian families with *ALS2*-related disorders without evidence of lower motor neuron involvement. Age at onset ranged from 10 months to 2.5 years, featuring progressive upper motor neuron signs. Detailed clinical phenotypes demonstrated inter- and intrafamilial variability. We identified 16 homozygous disease-causing *ALS2* variants; sorted as splice-site, missense, frameshift, nonsense and in-frame in eight, seven, four, three, and one families, respectively. Seven of these variants were novel, expanding the mutational spectrum of the *ALS2* gene. As expected, clinical severity was positively correlated with disease onset ($p = 0.004$). This work provides clinical and molecular profiles of a large single ethnic cohort of patients with *ALS2* mutations, and suggests that infantile ascending hereditary spastic paralysis (IAHSP) and juvenile primary lateral sclerosis (JPLS) are belonged to one entity with no phenotype–genotype correlation.

KEYWORDS
ALS2, IAHSP, JPLS, UMNL

1 | BACKGROUND

Biallelic variants in *ALS2* gene are associated with a range of rare autosomal recessive motor neuron disorders (MNDs), namely IAHSP (OMIM: 607225) or JPLS (OMIM: 606353) with progressive degeneration of upper motor neurons (UMNs), and juvenile amyotrophic lateral sclerosis (JALS, OMIM: 205100) with both UMN and LMN involvement.[1] They classically manifest with spastic paraparesis, evolving to quadriparesis with bulbar and pseudobulbar affection. Nevertheless, JALS differs clinically with later age of onset, more severe presentation and shorter lifespan, due to respiratory failure.[2]

ALS2 gene comprises 34 exons (exon 1 is non-coding) spanning 83 kilobases at chromosome 2q33.1. The encoding protein, alsin, is ubiquitously expressed, with maximal expression in brain and spinal cord, particularly in motor neurons. It acts as a guanine exchange factor (GEF) activating the small GTPase Rab5, thus modulating endosome fusion and trafficking. Alsin mediates numerous cell processes including endocytosis, cytoskeletal organization and membrane dynamics.[3]

In this study, we reported the detailed neurological phenotype and molecular findings of 46 patients with *ALS2* mutations. Degeneration of motor neurons was confined to UMNs, excluding the diagnosis of JALS.

2 | SUBJECTS AND METHODS

2.1 | Clinical assessment

Patients were subjected for thorough clinical and neurological assessment and comprehensive neuroimaging analysis. We adopted a severity score ranging from 0 to 8 that was calculated for each patient at their last examination (Table S1). The correlation between patients' severity score and their age at last examination and disease-onset were assessed using the Pearson correlation coefficient.

2.2 | Molecular analysis

Whole exome sequencing for extracted DNA was carried out using Illumina platform according to the manufacturer's protocol. Identified variants met the internal QC criteria based on extensive validation processes. They were checked in different databases and their possible effects were predicted using different in silico algorithms. Familial segregation was performed using Sanger sequencing. The chi-square test was performed in an attempt to establish possible phenotype–genotype correlation.

3 | RESULTS

3.1 | Clinical findings

The study included 46 Egyptian patients (25 males and 21 females) from 23 unrelated families with consanguinity rate of 95.7%. Patients' anthropometric measurements displayed underweight (> −2SD) in eight patients (17.4%), short stature (> −2SD) in 14 (30.4%) and normocephaly (< −3SD) in all of them. The first disease symptom was recorded between the age of 10 and 30 months as tip toe walking ($n = 27$) (58.7%) or delayed and difficulty in walking ($n = 19$) (41.3%).

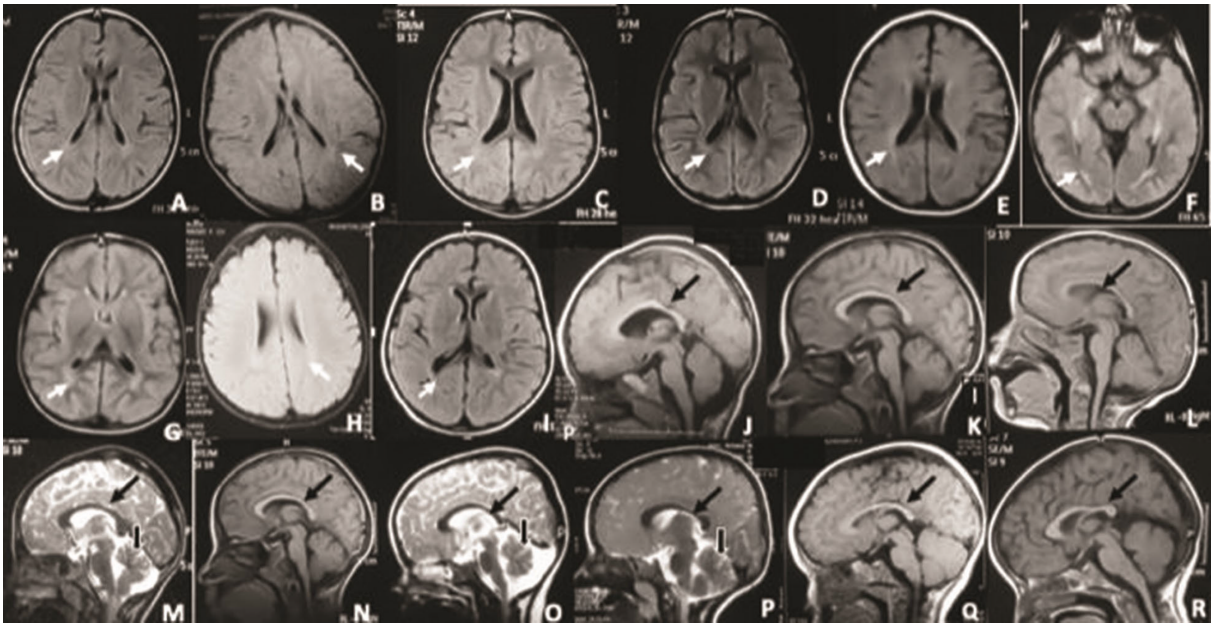

FIGURE 1 Brain MRI lesions in the studied patients. Axial T2W-FLRAIR cuts showing variable degrees of deep periventricular white matter signal mainly around the occipital horn of lateral (white arrow) (A–I). Sagittal T1W andT2W cuts showing thin corpus callosum especially the body (J–R) (long black arrow) and vermian atrophy (M,P) and hypoplasia (O) (short black arrow).

TABLE 1 Clinical findings of the enrolled subjects.

Feature	Prevalence
Gender no of males (%)—no of females (%)	25 (54.3)–21 (45.7)
Consanguinity no of families (%)	22 (95.7)
weight SD (range)	−1.26 (−3.4: +2.17)
height SD (range)	−1.61 (−3.5: +1.38)
Head circumference SD (range)	−0.96 (−2.4: +0.72)
Onset of symptoms (months) mean ± SD (range)	20 ± 5 (10–30)
Motor development no of patients (%)	
Normal	18 (34.8)
Delayed	22 (47.8)
Never	8 (17.4)
Motor regression no of patients (%)	30 (65.2)
Ambulation no of patients (%)	25 (54.3)
Atrophy of lower limb muscles no of patients (%)	19 (41.3%)
Hypertonia/Spasticity of lower limbs no of patients (%)	42 (91.3)
Hyperreflexia of lower limbs no of patients (%)	44 (95.7)
Hypertonia of upper limbs no of patients (%)	24 (52.2%)
Hyperreflexia of upper limbs no of patients (%)	36 (78.3%)
lack of finger opposition no of patients (%)	19 (41.3)
Patellar reflex/ clonus no of patients (%)	46 (100)
adductor reflex no of patients (%)	42 (91.3)
Ankle clonus no of patients (%)	43 (93.5)
Babiniski sign no of patients (%)	44 (95.7)
Pseudobulbar affect no of patients (%)	21 (45.7)
Drooling no of patients (%)	27 (58.7)
Dysarthria no of patients (%)	34 (73.9)
Anarthria no of patients (%)	6 (13)
Chewing difficulties	16 (34.8)
Dysphagia no of patients (%)	6 (13)
Weakness of facial muscles no of patients (%)	17 (37)
Ocular involvement no of patients (%)	14 (30.4)
Slow eye movement	8 (17.4)
Gaze affection	11 (23.9)
Nystagmus	3 (6.5)
IQ no of patients (%)—IQ (SD)	
Average (90–109)	2 (4.3)–92.5 (±3.5)
Low average (80–89)	12 (26.1)–82.8 (±2.3)
Borderline (IQ 70–79)	10 (21.7)–75.9 (±2.5)
Mild (IQ 50–55 to approximately 70)	20 (43.5)–66.8 (±3.3)
Moderate (IQ 35–40 to 50–55)	1 (2.2)–55
Controlled tonic seizures no of patients (%)	3 (6.5)
Normal sphincteric control no of patients (%)	41 (89.1)
MRI finding no of patients (%)	24 (52.2)
Thin corpus callosum	17 (37)
White matter hyperintensity	12 (26.1)
Cerebellar vermian hypoplasia/atrophy	3 (6.5%)
Dilated lateral ventricles	1 (2.2)

(Continues)

TABLE 1 (Continued)

Feature	Prevalence
Other findings no of patients (%)	
Dystonia	3 (6.5%)
Scoliosis	1 (2.2)
Right hip displacement	1 (2.2)

Abbreviations: EMG, electromyography; IQR, interquartile range (25th quartile to 75th quartile); MRI, brain magnetic resonance; NCV, nerve conduction velocity.

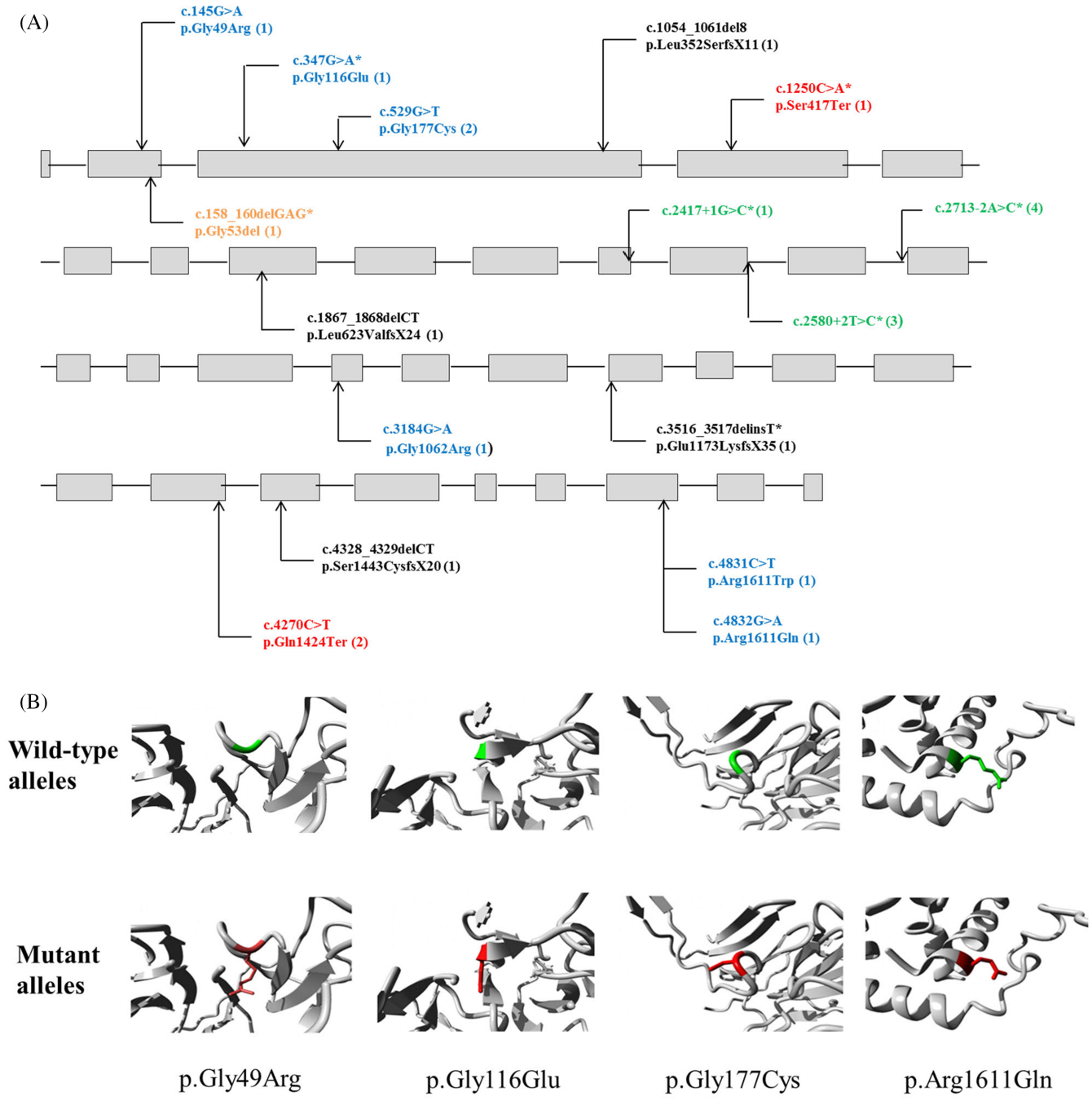

FIGURE 2 (A) Spectrum of the disease-causing variants in our patients. Coding exons (boxes) are proportionate with their size. Introns (lines) are not scaled. Base and amino acid changes are indicated (missense: blue; splice-site: green; frameshift: black; nonsense: red; In-frame: orange).* denotes for novel mutations. (B) Protein 3D model with mutation close-up. A protein model was built based on a homologous structure. No solved models were generated for p.Gly1062Arg, p.Arg1611Trp and p.Asn1320Asp.

Eight patients (17.4%) were never achieved independent walking and motor skills were retracted in 30 patients (65.2%). Twenty-five patients (54.3%) were still ambulatory at their last evaluation. With the advancement of age, more motor deficits, upper limbs involvement, speech regression, pseudobulbar effects and bulbar symptoms became more evident. Abnormal eye movement was recorded in 30.4% and sphincter control was well preserved in ~90%. Cognitive functions were normal to mildly impaired with average IQ of 73.8 (±8.6). Sixteen patients (34.8%) had one or two tendon release surgical interventions with only short-term improvement. Abnormal neuroimaging findings were in 52.2% dominated by thin corpus callosum (37%) and hyperintensities in white matter (26.1%) (Figure 1). Normal EMG and NCV excluded signs of denervation in all patients (Tables 1, S2).

Statistical analysis showed that disease severity was significantly associated with increased age ($p = <0.001$) and a positive correlation was documented between the severity score and age of disease onset ($p = 0.004$) (Figure S1). Due to disparity of age among our patients, we could not estimate an accurate correlation within similar ages.

3.2 | Molecular results

Sixteen homozygous pathogenic variants were detected in the enrolled subjects; six missense, four frameshift, three splice-site, two nonsense and one in-frame (Figure 2A). A homozygous missense variant (p.Asn1320Asp) was coexisted and segregated with p.Gly49Arg in one family and was classified as a likely benign variant with reduced CADD and REVEL scores (24.2 and 0.241, respectively) and low degree GVGD class (C15). It is noteworthy that residues sharing some common properties with Asp1320 were observed at this position in other homologous proteins. While, Gly49 has high pathogenicity scores and it is evolutionarily conserved; neither the mutant residue nor any other variation with similar properties was observed at this site in other species. The wild-type residues of the other five missense variants were 100% conserved among other homologous sequences and they were predicted to have a deleterious effect on protein structure and/or function (Figure 2B). The reported frameshift and in-frame variants had deletions of up to eight nucleotides, and the latter was strongly classified as a pathogenic variant by PROVEAN (score = −7.27) and MutationTaster. Two missense variants encompassed the same amino acid (Arg1611). Glycine residues are mutated in 83.3% of *ALS2* missense variations ($n = 5$). The detected splice-site variants were all novel with substantial potential to be disease causing (Table S3). Four other novel variants were identified (p. Gly53del, p.Gly116Glu, p.Ser417Ter, and p.Glu1173LysfsX35). The splice-site variants c.2713-2A > C and c.2580 + 2 T > C were repeated in four and three families, respectively, accounting for 15 cases (32.6%) with possible founder effect in the Egyptian population. *ALS2* variants identified in this study are scattered throughout the whole gene, without hotspot variations or regions. We did not observe association between the degree of severity and genotype in terms of mutation type ($p = 0.838$) and location ($p = 0.098$), suggesting that all mutations impact function to a similar degree.

4 | DISCUSSION

This study presents the largest series of *ALS2*-related disorders from same ethnicity, encompassing 16 different disease-causing mutations in 23 different families. Some conflicting issues have been raised about the various clinical and neurophysiological manifestations among the *ALS2*-related phenotypes. Few articles stated that UMNs and LMNs are compromised in both IAHSP and JALS with delayed onset in JALS.[2,4] On the other hand, most of the literature delineated that LMN degeneration is only restricted to JALS.[5–9] In this context, patients without involvement of LMNs were either described as JPLS (few cases) or as IAHSP (the majority of cases)[2] with no clear difference in the disease onset between the two entities[6,7,9,10] where the same entity may be referred to either JPLS or IAHSP.[1] Nogueira et al. in two consequent reports, assumed that JPLS is much more severe with relatively slow progressive phenotype of IAHSP.[8,11] Of utmost importance, Orrell et al., (2022) precisely described *ALS2*-related disorders as a clinical continuum ranging from IAHSP to juvenile forms without LMN involvement (JPLS) or with LMN involvement (JALS).[1] Here, we identified 46 patients with homozygous *ALS2* mutations without LMN involvement justified by absence of denervation. Therefore, all patients were diagnosed as IAHSP or its allelic phenotype JPLS. The prevalence of *ALS2*-related disorders is currently unknown. In the literature, about 35 families with IAHSP and/or JPLS were documented from 23 studies, with few families in each. On the other hand, only 8 families were diagnosed with JALS with delayed and more severe disease progression.[2,4,10]

The enrolled subjects were typically normal at birth, and then symptoms appeared during the first few years of life (10–30 months) with an average of 20 months, highly comparable to the literature on IAHSP/JPLS. Conversely, the average age of JALS onset was about 5 years.[5] Short stature and underweight were present in 30.4% and 17.4% of our patients, respectively. These could be referred to the spastic nature of the disease that hinders the achievement of regular growth, specifically the stature. Disease initially led to stiffness of lower limbs, that about half of the enrolled subjects were bounded to wheelchair by late childhood or early adolescence. Further manifestations became evident with disease progression, as weakness ascended to include arms, neck and face. We have adopted a scoring system retrieving individual clinical severity which found to be significantly correlated with patient's age ($p = <0.001$). Patients who underwent tendon release surgical interventions had temporary improvement that consistently attributed for the progressive nature of the disease.[7] NCV and EMG were normal for all patients, as a distinguishable sign between IAHSP/JPLS and JALS.[1] It is noteworthy that the frequency of disease clinical and neuroimaging features are currently underestimated in the literature due to paucity of published cases, without full clinical details in most.[2,10] It has been reported that cognitive function is preserved in *ALS2*-related disorders.[6] However, in our study, cognitive functions were either low average, borderline, or impaired (26.1%, 21.7% and 45.7%, respectively) according to Wechsler IQ classification. Only two patients with IQ 95 showed acceptable

scholastic performance. This highlights on precise evaluation of cognitive functions in *ALS2*-related disorders. However, reduced cognition abilities could be endorsed to the disease burden that hinders learning achievement.

No genotype–phenotype correlation was observed in our cohort with clear clinical heterogeneity among unrelated patients with the same mutation and even within the same family. Consistently, intra- and interfamilial phenotypic variability in patients with *ALS2* mutations was previously demonstrated in terms of disease severity, clinical features, and imaging findings.[5] Intra- and interfamilial clinical heterogeneity in our series would be best exemplified by the variant c.2713-2A>C identified in nine patients from four unrelated families. They had significant difference in terms of disease onset (18–30 months) and degree of severity (0–8). Clinical heterogeneity could be attributed to the possible influence of certain modifier genes, epigenetic changes or environmental factors.[5,6] This could also illustrate why the same *ALS2* mutation can mediate different pathologies.

To date, about 120 pathogenic *ALS2* variants have been reported in patients with MNDs. The majority were small deletions and nonsense variants, followed by missense mutations.[12] In the current study, 16 homozygous pathogenic *ALS2* variants were identified, seven of them were novel. The variants were mainly missense and splice-site in seven and eight families, respectively. However, splice-site mutations seem to be relatively rare in the literature.[12] Significant proportions of our reported missense variations (83.3%) involved glycine residues. It should be noted that glycine residues are the most flexible protein residues, where loss of this flexibility most probably affect the proper protein's structure and/or function.[13] Four frameshift, two nonsense and an in-frame variants were also detected in four, three, and one families, respectively. It was demonstrated that mutant alsin molecules with in-frame deletions existed as abnormally higher molecular weight complexes.[14] All our variants were detected in a homozygous state similar to the majority of the reports[2] that highlighted the significant effect of consanguinity on disease origination.

5 | CONCLUSION

The current study presented the detailed phenotype and genotype of a large cohort of Egyptian patients with *ALS2* mutations with findings compatible with infantile cases of the *ALS2*-clinical continuum IAHSP/JPLS. We strongly believe that the clinical criteria of this spectrum of alsin mutations are well characterized and easily differentiated from other related disorders.

"The current study presented the detailed phenotype and genotype of a large cohort of Egyptian patients with ALS2 mutations with findings compatible with infantile cases of the ALS2-clinical continuum IAHSP/JPLS.

AUTHOR CONTRIBUTIONS

Maha S. Zaki, Karima Rafat, Hasnaa M. Elbendary, Mona Kamel, Nour Elkhateeb, Mahmoud M. Noureldeen, Mohamed A. Abdeltawab, Abdelrahim A. Sadek, and Mahmoud Y. Issa: Patient evaluation, data acquisition. Wessam E. Sharaf-Eldin, Mona L. Essawi, Tracy Lau, David Murphy, Henry Holuden, Mohamed S. Abdel-Hamid, and Joseph G. Gleeson: Molecular analysis, data interpretation. Maha S. Zaki: Conceptualization. Maha S. Zaki and Wessam E. Sharaf-Eldin: Writing the original draft. Tracy Lau, Joseph G. Gleeson, and Maha S. Zaki: Reviewing the manuscript.

ACKNOWLEDGMENTS

The work was partially supported by the NRC grants 12060178 and 11010167. We are thankful to Dr. Reza Maroofian, UCL Queen Square Institute of Neurology, UK, for his unlimited help to complete this work.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

PEER REVIEW

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/cge.14338.

DATA AVAILABILITY STATEMENT

Datasets are included within the article and its supplementary files and upon request to corresponding author.

ETHICS STATEMENT

Patients' guardians provided informed consent in accordance with the Declaration of Helsinki and the study was approved by NRC Medical Research Ethics Committee (ID 12060178).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Zaki MS, Sharaf-Eldin WE, Rafat K, et al. Clinical and molecular spectrum of a large Egyptian cohort with *ALS2*-related disorders of infantile-onset of clinical continuum IAHSP/JPLS. *Clinical Genetics*. 2023;1-7. doi:10.1111/cge.14338