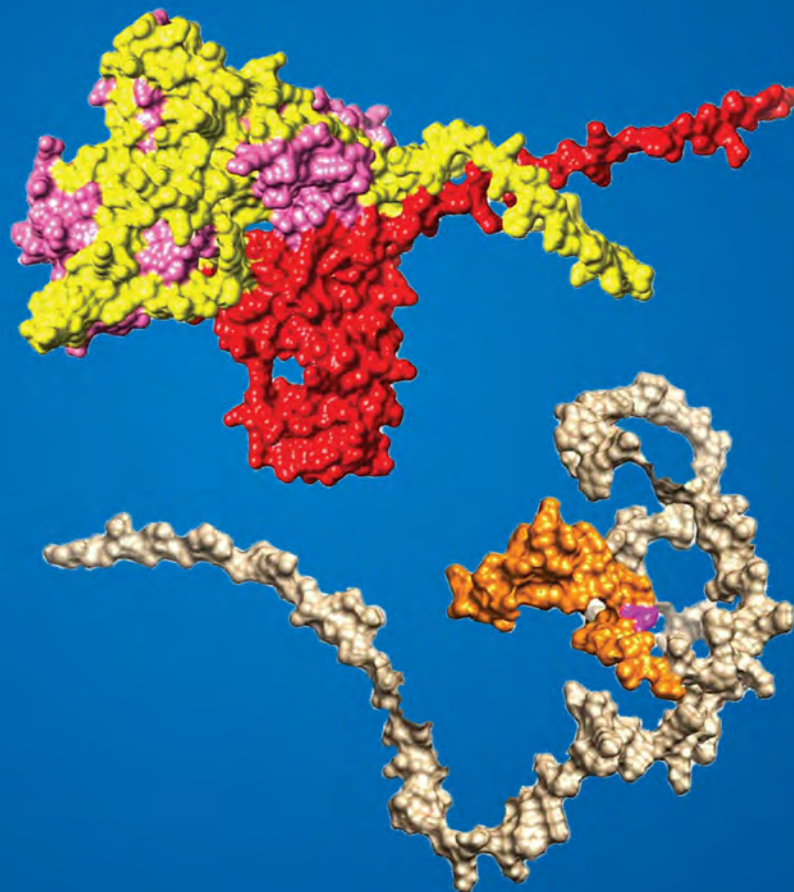
Volume 6, Issue 1, 2023 e-ISSN: 1658-8088

Journal of Biochemical and Clinical Genetics

Official Journal of the Saudi Society of Medical Genetics



SSMG
الجمعية السعودية للطب الوراثي
Saudi Society of Medical Genetics

https://jbcgenetics.com/

ORIGINAL ARTICLE

Supplementary testing after negative or inconclusive exome sequencing results

Balsam AlMaarik[1,2], Taghrid Aloraini[2,4], Roselyn Paclejan[2,4], Mohammed Balwi[2,4], Lamia Alsubaie[3,4], Abdulrahman Alswaid[3,4], Wafaa Eyiad[3,4], Fuad Al Mutairi[3,4], Faroug Ababneh[3,4], Majid Alfadhel[3,4], Ahmed A. Alfares[2,4,5]*

ABSTRACT

Background: Accurate diagnosis benefits patients and their families by directing clinical management; predicting recurrence risks; providing prognosis; and preventing the invasive, time-consuming, and costly diagnostic odyssey. The present study aimed at evaluating the usefulness and clinical utility of supplementary testing (deletion/duplication, targeted genome methylation analysis, and whole mitochondrial genome testing) after inconclusive or negative exome results and the outcome of the supplementary testing.

Methods: A total of 3,505 clinical exome sequencing results were evaluated, and cases with supplementary testing were analyzed for the accuracy and validity of the supplementary testing.

Results: The present study cohort comprised 26 cases where supplementary testing was ordered (12 inconclusive results and 14 negative results). Out of the 12 inconclusive results, only one case was positive for supplementary testing (1/12) and none of the negative cases (0/14).

Conclusion: For most cases, supplementary testing to negative exome sequencing failed to identify any possible explanation of the disorder, concluding that supplementary testing has limited clinical utility.

Keywords: Exome sequencing, deletion duplication, methylation, mitochondrial genome, Saudi Arabia.

Introduction

Accurate diagnosis benefits patients and their families by directing clinical management; predicting recurrence risks; providing prognosis; and preventing the invasive, time-consuming, and costly diagnostic odyssey. Apart from its tangible benefits, confirming a clinical diagnosis is therapeutic for the patient and the family. Establishing diagnosis in patients with complex disorders involves a stepwise approach from history taking to physical examination, with further complementary tests such as radiography and metabolite analysis, and genetic testing. However, many patients who undergo extensive genetic testing still need to be diagnosed. Exome sequencing (ES) became one of the leading diagnostic tools for genetic diseases, with a hit rate ranging from 25% to 58% (1,2). Also, ES provides further advantages, faster results, and a cost-efficient testing strategy (3,4). ES is a powerful tool to end a diagnostic odyssey. However, limitations of the current practice of ES include limited detection of copy number variation (CNV) changes based on the used bioinformatics pipeline, limited detection of variation in the mitochondrial genome and inability to detect methylation changes. Performing CNV analysis increases the diagnostic yield of ES cases by 4.2% (1). And while methylation testing is a powerful tool in cancer diagnosis with a 95% accuracy in predicting cancerous versus normal tissue (5) according to a recent study tested the utility of a new diagnostic network for methylation testing in mendelian disorders, a hit rate of 27.6% (57/207) was achieved (6). For the mitochondrial genome (mtDNA), a study published in 2018 showed that the mitochondrial genome test hit rate was 20% (23/117) (7). Furthermore, in a previous study, we showed that genome sequencing (GS) has limited clinical utility compared to the re-analysis of ES raw data (1). In this study, we aim to evaluate the usefulness and clinical utility of further testing beyond or after

Correspondence to: Ahmed A. Alfares
*Division of Translational Pathology, Department of Laboratory Medicine, King Abdulaziz Medical City, Riyadh, Saudi Arabia.
Email: fars@qu.edu.sa
Full list of author information is available at the end of the article.
Received: 03 August 2022 | **Accepted:** 08 February 2023

OPEN ACCESS
This is an open access article distributed in accordance with the Creative Commons Attribution (CC BY 4.0) license: https://creativecommons.org/licenses/by/4.0/) which permits any use, Share — copy and redistribute the material in any medium or format, Adapt — remix, transform, and build upon the material for any purpose, as long as the authors and the original source are properly cited. © The Author(s).

inconclusive or negative exome results and the outcome of supplementary testing, including deletion/duplication, targeted methylation testing and mitochondrial genome analysis post-negative or inconclusive ES results.

Subjects and Methods

A total of 3505 ES cases were evaluated during from 2018 to 2020. Testing was done either in-house or at other CAP-accredited laboratories. Analysis was done for patients at King Abdulaziz Medical City, Ministry of National Guard - Health Affairs, Riyadh. All clinical reports were investigated, and all cases with supplementary testing were further evaluated for accuracy and validity of the testing after the ES results (initial exome result, date of testing, clinical chart note documenting the requested test, indication of the further testing) (Figure 1). Only cases with high suspicion of specific phenotypes and negative or inconclusive exome results are considered in this analysis. Inconclusive results mean that a possible explanation of the primary indication of testing might be detected but would still need to fully explain the phenotype from a molecular point of view. For example, one pathogenic or likely pathogenic variant in a gene is related to the disorder clinically.

However, only one variant is detected, and the disorder is an autosomal recessive disorder. A negative result means ES failed to identify any variants that would explain the phenotype. The supplementary testing included in this study is one of three, either deletion/duplication analysis for a specific gene, which means a change in gene dosage that can not always be detected with next-generation sequencing testing. Deletion/duplication analysis includes looking at a missing or duplicated part of that gene in one or both strands. This is usually triggered by inconclusive exome results or a high suspicion of the specific diagnosis. The second form of supplementary testing is targeted methylation analysis which explores the epigenetic modification that causes changes in genetic regulation secondary to the addition of methyl group to the DNA, which can cause changes to DNA expression without altering the DNA sequence (8), with clinical presentation suspecting disorder related to methylation defect. The third form of supplementary testing is whole mitochondrial genome testing by analyzing mitochondrial DNA, which is of maternal origin. The mitochondria are known as the powerhouse of the cell. Changes in energy production can cause disease. This is indicated by either family history, clinical phenotype or abnormal basic laboratory testing like high lactate (9). All supplementary testing must be done at a clinical laboratory and clinical grade analysis. All research results are excluded from this study. This study was approved by the Institutional Research Board of King Abdullah International Medical Research Center, #RC19/315/R.

Results

Out of the 3,505 ES cases, there were 26 cases where supplementary testing was requested due to either inconclusive or negative exome results with high suspicion of a specific phenotype.

Deletion/duplication analysis

Out of the 26 samples we reviewed, 16 cases with either inconclusive or negative exome results in 10 cases with inconclusive ES results identified where a heterozygous variant in a gene with an autosomal recessive mode of inheritance has been detected and could explain the phenotype (Table 1: cases 1-10). However, ES failed to identify the second pathogenic variant, and supplementary testing was requested. Only 1/16 (6.25%) cases were positive for deletion/duplication analysis. Case number 5 (Table 1: case 5) presented with abnormal renal morphology with enlarged kidney and neonatal hypoglycemia with abnormal newborn screening suggestive of medium-chain acyl-CoA dehydrogenase deficiency (MCAD) (OMIM #201450), ES identifies one heterozygous, pathogenic variant in *ACADM* gene, NM_001286043.1:c.715C>T, p.(Arg239Cys), deletion/duplication analysis of *ACADM* (Figure 2) identified heterozygous deletion encompasses exon 8 of *ACADM* gene and establishes the diagnosis of MCAD. However, in 15/16 cases, the supplementary analysis results of deletion-duplication analysis were negative, for example, case number 1 (Table 1: case 1), which was tested for deletion-duplication after the inconclusive ES result presented with hypothyroidism, elevated liver enzymes and diabetes mellitus, ES showed heterozygous pathogenic variant in *ATP8B1* and associated with benign recurrent intrahepatic cholestasis (OMIM #243300), due to these results and the clinical presentation of elevated liver enzymes, the physician ordered deletion -duplication testing to exclude heterozygous CNV changes that ES did not detect. However, results are negative for deletion or duplication of the *ATP8B1* gene. A complete list of all cases is present in Table 1.

Targeted methylation analysis

Targeted methylation testing was requested in 6 cases (Table 1: cases 17-22) after initial ES results, 5 cases had negative initial ES results, and 1 was inconclusive when the initial ES testing was done. For the five negative ES cases, Silver-Russell syndrome was suspected in four cases (Table 1: Cases 18, 20-22). The four cases presented with multiple phenotypes related to Silver-Russell syndrome, including intrauterine growth retardation, small for gestational age, premature birth, short stature, abnormal facial shape, abnormal skull morphology, abnormal heart morphology, vertebral segmentation defect, failure to thrive and global developmental delay, but all four cases had negative results. The last case (Table 1: case 19) presented with delayed speech and language development, hyperactivity, intellectual disability and muscular hypotonia and was tested for Angelman syndrome but was also negative. Furthermore, an additional ES case was inconclusive for an unrelated homozygous variant in the *C1QA* gene (Table 1: case 17). However, based on the patient’s phenotype, the clinician did not pursue further testing on that gene and instead tested for Russel Silver methylation, but unfortunately, the result was negative.

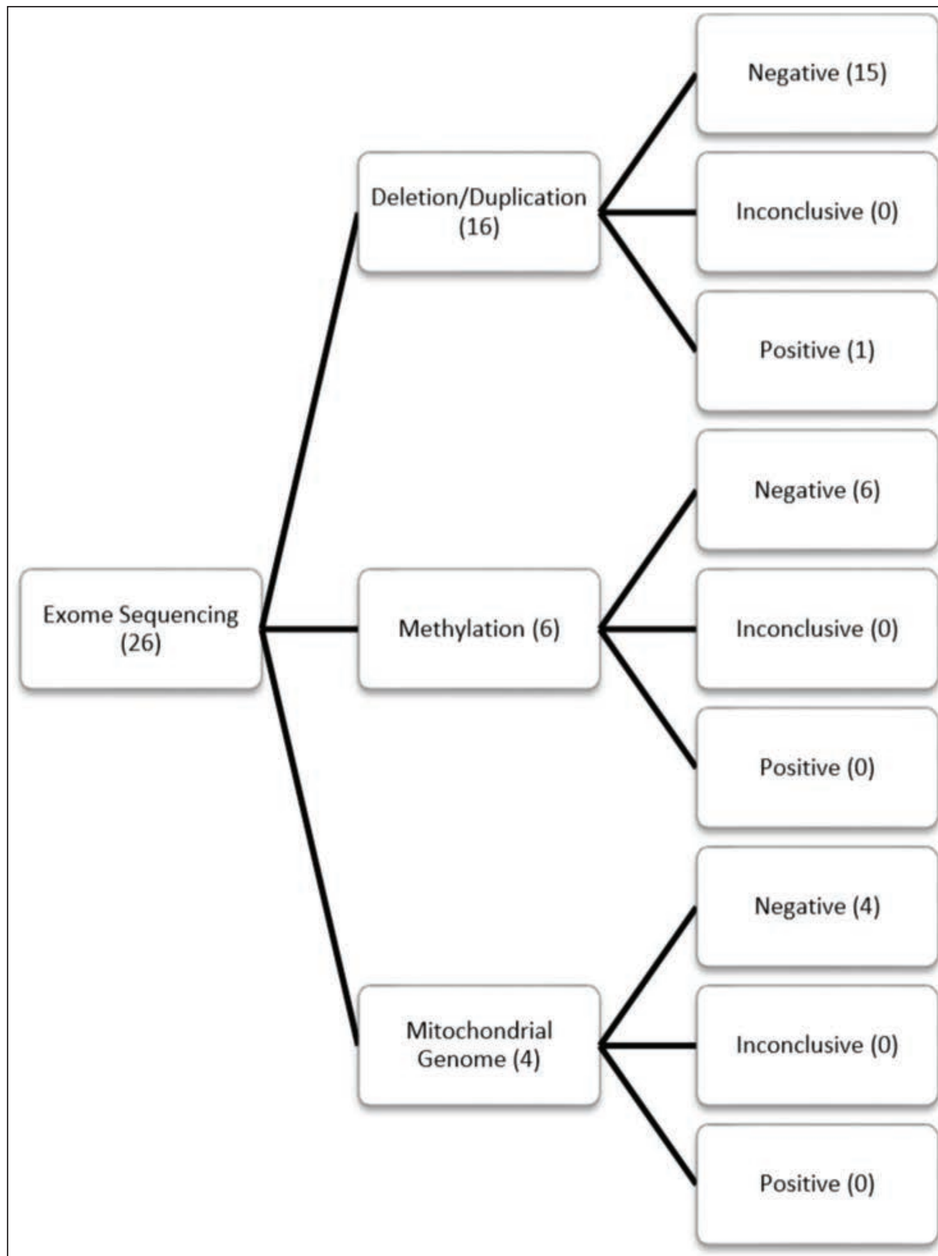


Figure 1. *Number of patients that underwent supplementary testing after ES flow diagram.*

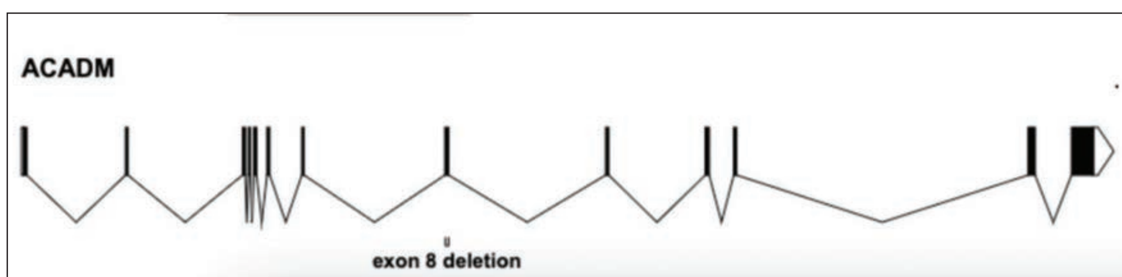


Figure 2. *ACADM: exon 8 deletion.*

Mitochondrial genome

There were 4 cases in total tested for mitochondrial genome after the ES results (Table 1: case 23-26). Three of these cases (Table 1: case 24-26) were ES-negative and were followed by mitochondrial testing. However, all 3 had negative results. Phenotypically all three cases were heterogeneous and had no specific phenotype. For instance, one case (Table 1: case 24) presented with lactic acidosis, and another presented with hypoglycemia (Table 1: case 25). Moreover, a case (Table 1: case 23) was ES inconclusive for the *GRIN2A* gene. However,

Table 1. 26 cases with initial test and supplementary testing.

Case number	HPO	Initial test	Initial result	Initial result	Supplementary test	Supplementary test result
1	Hypothyroidism (HP:0000821), Elevated liver enzymes (HP:0002910), Diabetes mellitus (HP:0000819)	ES	Inconclusive	Heterozygous, pathogenic (PS4, PM2, PP2, PP3, PP4, PP5) variant in ATP8B1 gene NM_005603:c.1594G>A, p.(Ala532Thr), (AR)	ATP8B1 Deletion-Duplication	Negative
2	Direct hyperbilirubnemia (HP:0002904), Rickets (HP:0002748)	ES	Inconclusive	Heterozygous, VUS (PM2, PP2, PP3) variant in ABCB11 gene NM_003742.2:c.2092C>T, p.(Arg698Cys), (AR)	ABCB11 Deletion-Duplication	Negative
3	Apnea (HP:0002104), Hypotonia (HP:0001290), gastro-esophageal reflux (HP:0002020), vesicoureteral reflux (HP:0000076)	ES	Inconclusive	Heterozygous, pathogenic (PS4, PM2, PP1, PP3, PP4, PP5) variant in POMT1 gene NM_007171.3:c.1241+4_1241+7del, (AR)	POMT1 Deletion-Duplication	Negative
4	Bone marrow failure (HP:0005528)	ES	Inconclusive	Heterozygous, VUS (PM2,PP2) variant in FANCA gene NM_000135.2:c.3697G>A, p.(Ala1233Thr), (AR) Heterozygous, VUS variant in FANCG gene NM_004629.1:c.1156C>G, p.(Pro386Ala), (AR) Heterozygous, VUS (PM2,BP4) variant in PALB2 gene NM_024675.3:c.1102A>T, p.(Asn368Tyr), (AR,AD)	FANCA, FANCG, PALB2 Deletion-Duplication	Negative
5	Abnormal renal morphology (HP:0012210), Abnormal renal physiology (HP:0012211), Enlarged kidney (HP:0000105), Hyperinsulinemic hypoglycemia (HP:0000825), Hypoglycemia (HP:0001943), Lethargy (HP:0001254), Multicystic kidney dysplasia (HP:0000003), Neonatal hypoglycemia (HP:0001998)	ES	Inconclusive	Heterozygous, pathogenic (PP5, PM2, PM5, PP2, PP3) variant in ACADM gene NM_001286043.1:c.715C>T,p.(Arg239Cys), (AR)	ACADM gene Deletion-Duplication Heterozygous deletion encompassing exon 8 of the ACADM gene	Positive
6	Facial dysmorphism (HP:0001999), global developmental delay (HP:0001263), agenesis of corpus callosum (HP:0001274) , seizure disorder (HP:0001250), tetralogy of Fallot (HP:0001525), congested lung with frequent lung collapse (HP:0002107)	ES	Inconclusive	Heterozygous, likely pathogenic variant in SMG9 gene NM_019108.3:c.701+4A> G, (AR)	SMG9 gene Deletion-Duplication	Negative
7	Abnormality of blood and blood-forming tissues (HP:0001871), Behavioral abnormality (HP:0000708), Bone marrow hypocellularity (HP:0005528), Depressivity (HP:0000716), Fever (HP:0001945), Leukopenia (HP:0001882), Specific learning disability (HP:0001328)	ES	Inconclusive	Heterozygous, VUS (PM2, PP3) variant in NBN gene NM_002485.4:c.737G>A, p.(Gly246Asp), (AR)	NBN gene Deletion-Duplication	Negative

Continued

Case number	HPO	Initial test	Initial result	Initial result	Supplementary test	Supplementary test result
8	Dysmorphic features (HP:0001999), hydrocephalus (HP:0000238), large VSD (HP:0001629), multicystic dysplastic left kidney (HP:0000003), ectopic echogenic right kidney (HP:0000086), repaired inguinal hernia (HP:0000023), right periventricular white matter punctate lesions.	ES	Inconclusive	Heterozygous, pathogenic (PS4, PM2, PM5, PP2, PP3) variant in FKRP gene NM_001039885.2:c.898G>A, p.(Val300Met), (AR)	FKRP gene Deletion-Duplication	Negative
9	Abnormality of the cerebral white matter, cafe-au-lait spot (HP:0000957), delayed fine motor development (HP:0010862), delayed gross motor development (HP:0002194), delayed speech and language development (HP:0000750), global developmental delay (HP:0001263), hyporeflexia (HP:0001265), infantile-onset (HP:0003593), motor delay (HP:0001270), muscular hypotonia (HP:0001252), pointed chin (HP:0000307), protruding ear (HP:0000411), triangular face (HP:0000325).	ES	Inconclusive	Heterozygous, pathogenic (PP5, PM1, PM2, PM5, PP2, PP3) variant in SMPD1 gene NM_000543.4:c.1267C>T, p.(His423Tyr), (AR)	SMPD1 gene Deletion-Duplication	Negative
10	Abnormal facial shape (HP:0001999), Brachydactyly (HP:0001156), Central hypotonia (HP:0001252), Depressed nasal bridge (HP:0005280), Generalized hypotonia (HP:0001290), Global developmental delay (HP:0001263), Hypertelorism (HP:0000316), Left ventricular outflow tract obstruction (HP:0032092), Left-to-right shunt (HP:0012382), Low-set ears (HP:0000369), Mitral regurgitation (HP:0001653), Patent ductus arteriosus (HP:0001643), Patent foramen ovale (HP:0001655), Sandal gap (HP:0001852), Single transverse palmar crease (HP:0000954), Systolic heart murmur (HP:0031664), Tricuspid regurgitation (HP:0005180), Umbilical hernia (HP:0001537), Upslanted palpebral fissure (HP:0000582)	ES	Inconclusive	Heterozygous, pathogenic (PVS1, PP5, PM2, BP4) variant in ACADVL gene NM_001270447.1:c.134C>A, p.(Ser45*), (AR)	ACADVL gene Deletion-Duplication	Negative

Continued

Case number	HPO	Initial test	Initial result	Initial result	Supplementary test	Supplementary test result
11	Adrenal Insufficiency (HP:0000846), Recurrent hypoglycemia (HP:0001988)	ES	Negative	Negative	TBX19 gene Deletion-Duplication	Negative
12	Atrial septal defect (HP:0001631), Hydronephrosis (HP:0000126), congenital bilateral hip dislocation (HP:0008780), supraventricular tachycardia (HP:0004755)	ES	Negative	Negative	KCNQ1 gene Deletion-Duplication	Negative
13	11 pairs of ribs (HP:0000878), Abnormal facial shape (HP:0001999), Abnormality of the dentition (HP:0000164), Abnormality of the nasal bridge (HP:0000422), High, narrow palate (HP:0002705), Kyphosis (HP:0002808), Large fontanelles (HP:0000239), Low-set ears (HP:0000369), Metatarsus adductus (HP:0001840), Microretrognathia (HP:0000308), Muscular hypotonia (HP:0001252), Poor head control (HP:0002421), Retrognathia (HP:0000278), Talipes equinovarus (HP:0001762), Webbed neck (HP:0000465), Wide intermamillary distance (HP:0006610)	ES	Negative	Negative	SMN1 gene Deletion-Duplication	Negative
14	Abnormal enzyme/coenzyme activity (HP:0012379), Bacteremia (HP:0031864), Cesarean section, Failure to thrive (HP:0001508), Fever (HP:0001945), Food intolerance (HP:0012537), Gastroparesis (HP:0002578), Laryngomalacia (HP:0001601), Left ventricular hypertrophy (HP:0001712), Muscular hypotonia (HP:0001252), Muscular hypotonia of the trunk (HP:0008936), Patent ductus arteriosus (HP:0001643), Patent foramen ovale (HP:0001655), Polyhydramnios (HP:0001561), Small for gestational age (HP:0001518), Stridor (HP:0010307), Vocal cord paralysis (HP:0001605), Vomiting (HP:0002013).	ES	Negative	Negative	SMN1 gene Deletion-Duplication	Negative

Continued

Case number	HPO	Initial test	Initial result	Initial result	Supplementary test	Supplementary test result
15	Abnormality of lateral ventricle (HP:0030047), Abnormality of the choroid plexus (HP:0007376), Abnormality of the musculature (HP:0003011), Absent speech (HP:0001344), Delayed ability to walk (HP:0031936), Delayed gross motor development (HP:0002194), Feeding difficulties (HP:0011968), Full cheeks (HP:0000293), Generalized hypotonia (HP:0001290), Global developmental delay (HP:0001263), Growth delay (HP:0001510), Intracranial hemorrhage (HP:0002170), Moderate global developmental delay (HP:0011343), Muscle weakness (HP:0001324), Neonatal hypotonia (HP:0001319), Polyhydramnios (HP:0001561), Premature birth (HP:0001622), Proptosis (HP:0000520), Skeletal muscle atrophy (HP:0003202), Small for gestational age (HP:0001518), Triangular mouth (HP:0000207), Turricephaly (HP:0000262), Wide nasal bridge (HP:0000431)	ES	Negative	Negative	SMN1 gene Deletion-Duplication	Negative
16	Ataxia (HP:0001251), Brachycephaly (HP:0000248), Broad-based gait (HP:0002136), Delayed fine motor development (HP:0010862), Delayed gross motor development (HP:0002194), Delayed speech and language development (HP:0000750), Dermoid cyst (HP:0025247), Frequent falls (HP:0002359), Gait ataxia (HP:0002066), Generalized-onset seizure (HP:0002197), Global developmental delay (HP:0001263), Hirsutism (HP:0001007), Hyperalaninemia (HP:0003348), Lactic acidosis (HP:0003128), Long eyelashes (HP:0000527), Mongolian blue spot (HP:0011369), Motor delay (HP:0001270), Neonatal onset (HP:0003623),	ES	Negative	Negative	SYNE1 gene Deletion-Duplication	Negative

Continued

Case number	HPO	Initial test	Initial result	Initial result	Supplementary test	Supplementary test result
	Neonatal respiratory distress (HP:0002643), Periorbital dermoid cyst (HP:0030668), Premature birth (HP:0001622), Seizures (HP:0001250), Small for gestational age (HP:0001518), Synophrys (HP:0000664), Tremor (HP:0001337), Triangular face (HP:0000325)					
17	Deeply set eye (HP:0000490), Frontal bossing (HP:0002007), Hypodontia (HP:0000668), Micrognathia (HP:0000347), Pointed chin (HP:0000307), Prominent supraorbital ridges (HP:0000336), Short nose (HP:0003196), Systemic lupus erythematosus (HP:0002725), Thin upper lip vermilion (HP:0000219), Triangular face (HP:0000325)	ES	Inconclusive	Homozygous, VUS (PM1,PM2,PP5) variant in C1QA gene NM_001347465.1:c.470G>A p.(Gly 157Asp), (AR)	Russell Silver Methylation	Negative
18	Abnormal heart morphology (HP:0001627), abnormality of the genitourinary system (HP:0000119), atrial septal defect (HP:0001631), hydronephrosis (HP:0000126), medullary nephrocalcinosis (HP:0012408), nephrocalcinosis (HP:0000121), premature birth (HP:0001622), scoliosis (HP:0002650), small for gestational age (HP:0001518), ventricular septal defect (HP:0001629), vertebral fusion (HP:0002948), vertebral segmentation defect (HP:0003422)	ES	Negative	Negative	Russell Silver Methylation	Negative
19	Attention deficit hyperactivity disorder (HP:0007018), Autism (HP:0000717), Autistic behavior (HP:0000729), Delayed speech and language development (HP:0000750), Hyperactivity (HP:0000752), Intellectual disability (HP:0001249), mild Joint hypermobility (HP:0001382), Muscular hypotonia (HP:0001252), Pulmonic stenosis (HP:0001642), Systolic heart murmur (HP:0031664), Violent behavior (HP:0008760)	ES	Negative	Negative	Angelman Methylation	Negative

Continued

Case number	HPO	Initial test	Initial result	Initial result	Supplementary test	Supplementary test result
20	Abnormal facial shape (HP:0001999), Abnormal skull morphology (HP:0000929), Clubbing (HP:0001217), Delayed ability to sit (HP:0025336), Delayed ability to walk (HP:0031936), Delayed fine motor development (HP:0010862), Delayed speech and language development (HP:0000750), Dry skin (HP:0000958), Ectopic kidney (HP:0000086), Growth hormone deficiency (HP:0000824), Motor delay (HP:0001270), Reduced bone mineral density (HP:0004349), Reduced subcutaneous adipose tissue (HP:0003758), Severe failure to thrive (HP:0001525), Short nail (HP:0001799), Short stature (HP:0004322)	ES	Negative	Negative	Russell Silver Methylation	Negative
21	Abnormal liver morphology (HP:0410042), Abnormal pulmonary valve morphology (HP:0001641), Abnormal respiratory system morphology (HP:0012252), Abnormal skull morphology (HP:0000929), Abnormality of the gallbladder (HP:0005264), Abnormality of the ribs (HP:0000772), Cesarean section, Global developmental delay (HP:0001263), Hyperechogenic kidneys (HP:0004719), Inguinal hernia (HP:0000023), Intrauterine growth retardation (HP:0001511), Muscular hypotonia (HP:0001252), Nasogastric tube feeding (HP:0040288), Nephrolithiasis (HP:0000787), Oral-pharyngeal dysphagia (HP:0200136), Patent ductus arteriosus (HP:0001643), Poor suck (HP:0002033), Premature birth (HP:0001622), Recurrent lower respiratory tract infections (HP:0002783), Renal hypoplasia/aplasia (HP:0008678), Respiratory distress (HP:0002098), Severe failure to thrive (HP:0001525), Small for gestational age (HP:0001518)	ES	Negative	Negative	Russell Silver Methylation	Negative

Continued

Case number	HPO	Initial test	Initial result	Initial result	Supplementary test	Supplementary test result
22	Blue nevus (HP:0100814), Coxa valga (HP:0002673), Cyanosis (HP:0000961), Delayed speech and language development (HP:0000750), Diarrhea (HP:0002014), Dry skin (HP:0000958), Dyspnea (HP:0002094),(HP:0100559), Premature birth (HP:0001622), Respiratory failure requiring assisted ventilation (HP:0004887), Snoring (HP:0025267), Lower limb asymmetry (HP:0100559), Eczema (HP:0000964) Elevated alkaline phosphatase (HP:0003155), Elevated serum aspartate aminotransferase (HP:0031956), Fetal distress (HP:0025116), Flushing (HP:0031284), Global developmental delay (HP:0001263), Lactic acidosis (HP:0003128), Low anterior hairline (HP:0000294)	ES	Negative	Negative	Russell Silver Methylation	Negative
23	Abnormality of higher mental function (HP:0011446), elevated urinary 3-hydroxybutyric acid (HP:0040155), hepatic steatosis (HP:0001397), hypoglycemia (HP:0001943), increased circulating free fatty acid level (HP:0030781), recurrent hypoglycemia (HP:0001988), seizure (HP:0001250), tonic seizure (HP:0032792)	ES	Inconclusive	Heterozygous, VUS (PM2, PP2, PP3) variant in GRIN2A gene NM_000833.3:c.905C>T p.(Ala302Val), (AD)	Mitochondrial Genome	Negative
24	Ataxia (HP:0001251), Brachycephaly (HP:0000248), Broad-based gait (HP:0002136), Delayed fine motor development (HP:0010862), Delayed gross motor development (HP:0002194), Delayed speech and language development (HP:0000750), Dermoid cyst (HP:0025247), Frequent falls (HP:0002359), Gait ataxia (HP:0002066), Generalized-onset seizure (HP:0002197), Global developmental delay (HP:0001263), Hirsutism (HP:0001007), Hyperalaninemia (HP:0003348), Lactic acidosis (HP:0003128), Long eyelashes (HP:0000527), Mongolian blue spot (HP:0011369), Motor delay (HP:0001270), Neonatal onset (HP:0003623),	ES	Negative	Negative	Mitochondrial Genome	Negative

Continued

Case number	HPO	Initial test	Initial result	Initial result	Supplementary test	Supplementary test result
	Neonatal respiratory distress (HP:0002643), Periorbital dermoid cyst (HP:0030668), Premature birth (HP:0001622), Seizures (HP:0001250), Small for gestational age (HP:0001518), Synophrys (HP:0000664), Tremor (HP:0001337), Triangular face (HP:0000325)					
25	Hypoglycemia (HP:0001943), Seizures (HP:0001250), Gastroesophageal reflux (HP:0002020), Vomiting (HP:0002013), Choking episodes (HP:0030842), Premature birth (24 weeks) (HP:0001622), Pulmonary hemorrhage (HP:0040223), Sepsis (HP:0100806), Grade II preterm intraventricular hemorrhage (HP:0030750), Patent ductus arteriosus (HP:0001643), Small for gestational age (HP:0001518), Arterial thrombosis (HP:0004420), Gastroparesis (HP:0002578), Enterocolitis (HP:0004387), Hypopigmentation of the skin (HP:0001010), Hyperpigmentation of the skin (HP:0000953), Lichenoid skin lesion (HP:0031452), Nevus (HP:0003764), Hydronephrosis (HP:0000126), Abnormality of the lower limb (HP:0002814), Coarse facial features (HP:0000280), Depressed nasal bridge (HP:0005280), Frontal bossing (HP:0002007), Hypertelorism (HP:0000316).	ES	Negative	Negative	Mitochondrial genome	Negative
26	Gait disturbance (HP:0001288), Hip dysplasia (HP:0001385), Seizure (HP:0001250), Spastic diplegia (HP:0001264), Spasticity (HP:0001257)	ES	Negative	Negative	Mitochondrial Genome	Negative

AD: Autosomal dominant; AR: Autosomal recessive; VUS: Variant of uncertain significance; ES: Exome sequencing.

this gene could not explain the phenotype, leading the clinician to exclude the gene and pursue mitochondrial genome testing instead, but the result was negative.

Inconclusive versus negative ES results

The hit rate of positive results after inconclusive ES results is 1/12 (8.3%) for supplementary testing to ES. However, if the ES results are negative, supplementary testing fails to identify any further possible explanation of the disorder.

Discussion

Establishing a clinical diagnosis is one of the main goals in health care - advances in molecular testing aid for better diagnosis. For example, the diagnostic yield for ES is clearly in the lead with a 25% to 58% hit rate (2), supplementary testing after inconclusive or negative exome like deletion/duplication testing, targeted methylation analysis or mitochondrial genome testing has low or no hit rate (10). Previous studies estimated the hit rate of methylation analysis in mendelian disorder to be around 27% (6). However, we could not establish the diagnosis of any disorder related to methylation defect despite the suspected phenotype of disorders related to methylation defect and the possible clinical phenotype presentation in the included cohort.

One major consideration of this study is the targeted population. The majority of marriages in the included cohort are consanguineous marriages (~75%), previously we showed that around 84% of detected disorders in our population results from homozygous variants in autosomal recessive disorders (11), which might explain the lower hit rate of any supplementary testing for disorders that are unlikely related to consanguinity like heterozygous deletion, or disorders in the mitochondrial genome which is maternally inherited or defects in methylation or imprinting that are unlikely linked to the autosomal recessive mode of inheritance.

Even though supplementary testing is essential, how crucial and beneficial it is when both results and testing cost are considered. Previously we showed that ES is the most cost-effective diagnostic testing even compared to GS (1). Furthermore, we showed that solo ES compared to extended family testing, also has limited clinical advantages (12). In this study, we showed supplementary testing triggered either by initial exome results or suspected phenotype with negative or inconclusive results also has no major advantages on top of ES and hence until the price of GS is equal to or lower than ES, solo ES would consider as the most cost-effective testing approach.

Conclusion

ES is considered the best approach in terms of diagnostic yield and cost-effectiveness. Supplementary testing (deletion/duplication testing, targeted methylation analysis or whole mitochondrial genome testing) beyond negative or inconclusive ES has lower diagnostic yield with limited clinical advantages. It is recommended only when another biomarker establishes the diagnosis and as a confirmatory tool for the molecular defects of disorders diagnosed by other methods.

Acknowledgements

We thank the patients, their families, and referring physicians for their important contributions. This work was supported by King Abdullah International Medical Research Center.

Funding

None.

Declaration of conflicting interests

The authors of this article have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

Ethical approval

Ethical approval was granted by the Institutional Research Board of King Abdullah International Medical Research Center with approval number RC19/315/R.

Author details

Balsam AlMaarik[1,2], Taghrid Aloraini[2,4], Roselyn Paclejan[2,4], Mohammed Balwi[2,4], Lamia Alsubaie[3,4], Abdulrahman Alswaid[3,4], Wafaa Eyiad[3,4], Fuad Al Mutairi[3,4], Faroug Ababneh[3,4], Majid Alfadhel[3,4], Ahmed A. Alfares[2,4,5]

1. Molecular Genetic Pathology Unit, Pathology Department, King Saud University, Riyadh, Saudi Arabia
2. Division of Translational Pathology, Department of Laboratory Medicine, King Abdulaziz Medical City, Riyadh, Saudi Arabia
3. Division of Genetics, Department of Pediatrics, King Abdullah Specialized Children Hospital, King Abdulaziz Medical City, MNGHA, Riyadh, Saudi Arabia
4. King Abdullah International Medical Research Center, King Saud bin Abdulaziz University for Health Sciences, Riyadh, Saudi Arabia
5. Department of Pediatrics, College of Medicine, Qassim University, Qassim, Saudi Arabia

References

1. Alfares A, Aloraini T, subaie L Alissa A, Qudsi AA, Alahmad A, et al. Whole-genome sequencing offers additional but limited clinical utility compared with reanalysis of whole-exome sequencing. Genet Med. 2018;20(11):1328–33. https://doi.org/10.1038/gim.2018.41
2. Fung J, Yu M, Huang S, Chung CCY, Chan MCY, Pajusalu S, et al. A three-year follow-up study evaluating the clinical utility of exome sequencing and diagnostic potential of reanalysis. NPJ Genom Med. 2020;5(1):37. https://doi.org/10.1038/s41525-020-00144-x
3. Vissers LELM, van Nimwegen KJM, Schieving JH, Kamsteeg E-J, Kleefstra T, Yntema HG, et al. A clinical utility study of exome sequencing versus conventional genetic testing in pediatric neurology. Genet Med. 2017;19(9):1055–63.
4. Stark Z, Tan TY, Chong B, Brett GR, Yap P, Walsh M, et al. A prospective evaluation of whole-exome sequencing as a first-tier molecular test in infants with suspected monogenic disorders. Genet Med. 2016;18(11):1090. https://doi.org/10.1038/gim.2016.1
5. Hao X, Luo H, Krawczyk M, Wei W, Wang W, Wang J, et al. DNA methylation markers in common cancers. Proc Natl

Acad Sci. 2017;114(28):7414–9. https://doi.org/10.1073/pnas.1703577114

6. Haghshenas S, Bhai P, Aref-Eshghi E, Sadikovic B. Diagnostic utility of genome-Wide DNA methylation analysis in mendelian neurodevelopmental disorders. Int J Mol Sci. 2020;21(23):9303. https://doi.org/10.3390/ijms21239303

7. Theunissen T, Nguyen M, Kamps R, Hendrickx AT, Sallevelt SCEH, Gottschalk RWH, et al. Whole exome sequencing is the preferred strategy to identify the genetic defect in patients with a probable or possible mitochondrial cause. Front Genet. 2018;9. https://doi.org/10.3389/fgene.2018.00400

8. Sant KE, Nahar MS, Dolinoy DC. DNA methylation screening and analysis. Methods Mol Biol. 2012;889:385–406. https://doi.org/10.1007/978-1-61779-867-2_24

9. Dimmock DP, Lawlor MW. Presentation and diagnostic evaluation of mitochondrial disease. Pediatr Clin North Am. 2017;64(1):161–71. https://doi.org/10.1016/j.pcl.2016.08.011

10. Alfadhel M, Umair M, Al Tuwaijri A, Al Mutairi F. A patient with coarse facial features and molecular odyssey: lessons learned and best practice. Clin Chem. 2023;69(1):17–20. https://doi.org/10.1093/clinchem/hvac187

11. Alfares A, Alfadhel M, Wani T, Alsahli S, Alluhaydan I, Al Mutairi F, et al. A multicenter clinical exome study in unselected cohorts from a consanguineous population of Saudi Arabia demonstrated a high diagnostic yield. Mol Genet Metab. 2017;121(2):91–5. https://doi.org/10.1016/j.ymgme.2017.04.002

12. Alfares A, Alsubaie L, Aloraini T, Alaskar A, Althagafi A, Alahmad A, et al. What is the right sequencing approach? Solo VS extended family analysis in consanguineous populations. BMC Med Genomics. 2020;13(1):130. https://doi.org/10.1186/s12920-020-00743-8

Journal of Biochemical and Clinical Genetics

ORIGINAL ARTICLE

Erythropoietin resistance in patients with regular hemodialysis in Sohag university hospital

Nagwa S. Ahmed[1], Aida A Mahmoud[1], Nayel Abd Elhamed Zaki[2], Amera A.Genedy[1*]

ABSTRACT

Background: A definite anemia [hemoglobin (HB) 10-12 g/dl] or the need for significantly higher Erythropoietin (EPO) dosages of epoetin alfa are symptoms of EPO resistance, respectively. The recommended restorative goal is to maintain HB levels between 11 and 12 g/dl. Fe deficiency, concurrent inflammation, inadequate dialysis, hyperparathyroidism, hemolysis, vitamin B12, and folate deficiency are the main causes of EPO resistance. The objective of this study was to evaluate patients who receive frequent hemodialysis for EPO resistance.

Methods: This study was performed at Sohag University Hospital on 50 hemodialysis patients compared to 40 healthy adult subjects from June 2021 to January 2022. Serum EPO was analyzed by enzyme-linked immunosorbent assay and Angiotensin Converting Enzyme (*ACE)* rs1799752 polymorphism was assessed using the Genomic TaqMan genotyping test.

Results: This study found insignificant relation between EPO Resistance Index (ERI) and diverse *ACE* genotype groups and para thyroid hormone. A further significant direct proportional relationship was found between ERI and Ferritin. EPO and parathyroid hormone did not show any significant relationship.

Conclusion: Considering the non-critical connection between ERI and our components, it is vital to enhance the treatment of anemic patients with chronic kidney diseases to recognize the potential causes of resistance and ponder other variables for resistance before proposing an expanded EPO-stimulating agent administration.

Keywords: Erythropoietin, hemodialysis, erythropoietin resistance index, erythropoietin stimulating agents.

Introduction

Erythropoietin (EPO) is a glycoprotein hormone. During mammal development, EPO formation in mice begins in the neural crest cells during mid-gestation. Then it stimulates the yolk sac for primitive erythropoiesis for oxygen transport in mid-stage embryos (1-3). As development advances, EPO formation and subsequent erythropoiesis shift to the liver (4,5). By the final trimester of development in mammals, EPO formation shifts to the kidneys, which become the primary site of EPO generation in the developing blood cells. Then, erythropoiesis shifts from the fetal liver to bone marrow, the site of future hematopoiesis (6). EPO synthesis is stimulated by decreased oxygen tension at the renal sensor and renal tubular and interstitial cells. This includes many causes of hypoxia, such as pneumonia (resulting in less oxygen uptake), arteriovenous shunts mixing arterial and venous blood through a right to left shunt, high attitudes, low renal blood flow, etc. Others causes include breathing a gas mixture under high oxygen pressure, hypothyroidism, and hypopituitarism. The oxygen sensor is near the EPO generation site and may comprise a heme-containing protein (7). In spite of the fact that the decrease in oxygen is the essential physiologic controller of EPO synthesis, other factors exist, such as androgenic steroids, anabolic steroids, and cobalt chloride, stimulating EPO synthesis by an obscure mechanism. Protein deprivation and inflammatory cytokines, e.g., interleukin-6 and tumor necrosis factor-α, cause decreased EPO synthesis (8,9)

Correspondence to: Amera Ahmed Genedy
*Medical Biochemistry Department, Faculty of Medicine, Sohag University, Sohag, Egypt.
Email: ameragenedy_2015@yahoo.com
Full list of author information is available at the end of the article.
Received: 09 December 2022 | **Accepted:** 23 January 2023

This is an open access article distributed in accordance with the Creative Commons Attribution (CC BY 4.0) license: https://creativecommons.org/licenses/by/4.0/) which permits any use, Share — copy and redistribute the material in any medium or format, Adapt — remix, transform, and build upon the material for any purpose, as long as the authors and the original source are properly cited. © The Author(s).

and may play a role in EPO resistance. Recombinant human EPO (rHuEPO) resistance is characterized by persistent anemia (hemoglobin <10-12 g/dl) that needs an exceptionally high EPO dose (300 IU/kg/week subcutaneously or 450 IU/kg/week intravenously). Alpha EPO should be taken at a dosage of 50-100 IU/kg subcutaneously, one to three times a week. The treatment objective is to attain a weekly increment in hemoglobin levels by 0.3 g/dl. After 4 weeks of treatment, if the hemoglobin (HB) level remains below 11 g/dl, the dosage should be expanded by 25%, and if the hemoglobin level exceeds 13 g/dl, EPO should be discontinued. The target is to maintain hemoglobin levels from 11 to 12 g/dl or hematocrit values from 33% to 36% (10). EPO resistance index (ERI) is characterized by a weekly weight-adjusted αEPO dosage (U/kg/week) divided by the HB level (g/dl), and it is calculated month to month to examine resistance to αEPO treatment (11). The causes of EPO resistance in dialysis patients are mainly iron insufficiency. However, after satisfactory iron supplementation, few patients may remain anemic, possibly due to concomitant inflammation, intense or chronic disease, lack of nutritional sustenance, inadequate dialysis, severe hyperparathyroidism, aluminum intoxication, malignancy, hemolysis, vitamin B12 and folate insufficiencies, pure red cell aplasia, and myelosuppressive operators. Dialysis methodology and biocompatibility of dialysis machines are essential contributing factors (12). Antihypertensive intake, e.g., angiotensin-converting enzyme inhibitors (ACEIs) and angiotensin receptor blockers (ARBs), can diminish the hematopoietic reaction to EPO stimulating agents (ESA), where the former can lead to a high level of negative erythropoiesis control (13). Angiotensin Converting Enzyme (*ACE*) gene polymorphisms may affect ACE serum levels and pharmacological actions (14). This way, a few patients may be more vulnerable to ESA resistance upon utilizing ACE and ARB inhibitors (15).

Subjects and Methods

This prospective study was performed at Sohag University Hospital, Sohag, Egypt. Fifty Egyptian adult patients on regular hemodialysis were enrolled, and 40 healthy adult subjects served as the negative control. This study lasted from June 2021 to January 2022. We used a sample size of 90 respondents (50 cases and 40 controls) to attain an 80% power and a 5% confidence interval of significance upon using a two-tailed test (type 1 error). Before performing the study, ethical committee approval was gained from the Sohag faculty of medicine. All participants signed written agreements and consented to participate in the study. All individuals were informed about the biochemical blood tests, and their clinical evaluations were performed before the study. Serum levels of parathyroid hormones (parathormone, PTH) and ferritin were assayed using commercially available kits. Other data were obtained by history. Inclusion criteria included patients having chronic renal failure and receiving hemodialysis for 3 months or more; age >18 years or more, and receiving EPO for anemia due to kidney diseases; and individuals with persistent blood loss that requires a blood transfusion, intense kidney disease, blood diseases, people having malignancy, and intercurrent infections.

About 3 ml of venous blood was withdrawn from the patients in the early morning after an overnight fast before the dialysis sessions. Another 2 ml of blood was collected in plain tubes for serum analysis, and 1 ml was collected in ethylene diamine tetraacetic acid (EDTA) tubes for genomic DNA extraction. Blood samples were centrifuged, and serum was collected and stored at (-80) until the biochemical assays. DNA extraction was done using QIAamp blood pack (cat.nos.51104 and 51106) according to the manufacturer's instructions. Enzyme-linked immunosorbent assay (ELISA) was used to assess the serum level of EPO using a human EPO ELISA Pack (Glory Science Co., China) according to the manufacturer's instructions. TaqMan single nucleotide polymorphism (SNP) genotyping test was used for ACE rs1799752 SNP genotyping by Step One real-time polymerase chain reaction (PCR) (Biosystems, USA). A total reaction volume of 25 µl was prepared using 12.5 µl TaqMan® Genotyping master mix and 1.25 µl specific TaqMan® SNP genotyping test) with its forward and reverse primers and two TaqMan® MGB tests; one VIC® color for the first allele, one FAM™ color for the second allele , and 5 µl (20 ng) of DNA. The temperature was raised to 95 for 10 minutes and then by 40 amplification cycles. Each cycle included denaturation at 95 for 15 seconds, annealing of primers, and then extension at 60 for 60 seconds. PCR outcomes were analyzed using TaqMan® Genotyper™ Program. ERI is defined as the weekly adjusted EPO dose (U/kg/week) divided by the HB level (g/dl), and it is calculated to investigate the resistance to EPO treatment. The patients enrolled in this study received alpha EPO (epoetin alpha, Pharco, Egypt) 4,000 IU twice weekly according to this formula (11):

$$\text{ERI} = \frac{\text{dose of ESA (IU/ wk)}}{\text{HB (g /dl)} \times \text{body weight (kg)}}.$$

Statistical Package for the Social Sciences computer program version 25 was used to assess the study's data. The quantitative results were presented as means ± SD and median. Qualitative results were presented as numbers and percentages. Tests of significance such as the chi-square test, independent *t*-test, and one-way ANOVA test were utilized for comparing the different parameters in the allocated participants. A value of $p < 0.05$ was chosen as the significance level in all the statistical tests in our consideration. Also, bivariate correlation analysis was done among clinical and laboratory parameters. For correlation analyses, r (Pearson's correlation coefficient) was used ($r < 0.2$ means negligible correlation, 0.2-0.4 means mild correlation, 0.4-0.6 means moderate correlation, and r = 0.6-0.8 means high correlation, and >0.8 means excellent correlation).

Results

In this study, ERI was not significantly associated with diverse *ACE* genotype groups (*p*-value between DD &II = 0.184; *p*-value between DD &II = 0.837; *p*-value between ID &II = 0.184) (Figure 1). There was a non-critical contrast between *ACE* genotypes and utilizing ACEI treatment or not (*p*-value = 0.153) (Table 1). ERI

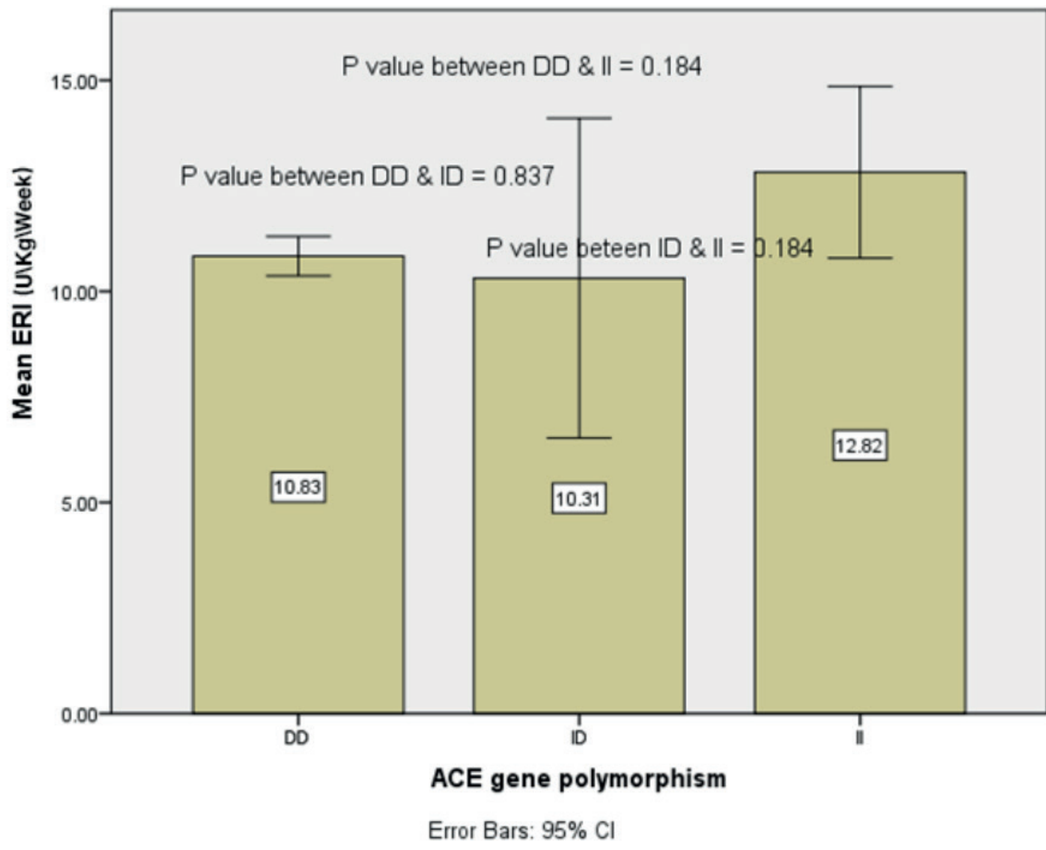


Figure 1. *Relation between ERI with ACE gene polymorphism.*

Table 1. *ACE inhibitor and ACE gene polymorphism cross-tabulation.*

			ACE gene polymorphism		Total	*p*-value
			DD	ID + II		
ACE inhibitor	(Non-using ACEI)	Number of participants	65	17	82	0.153 NS
		(% within *ACE* gene polymorphism)	89.0%	100.0%	91.1%	
	(Using ACEI)	Number of participants	8	0	8	
		(% within *ACE* gene polymorphism)	11.0%	0.0%	8.9%	
Total		Number of participants	73	17	90	
		(% within *ACE* gene polymorphism)	100.0%	100.0%	100.0%	

and ferritin have a significant proportional relationship (p-value < 0.0001) (Table 2 and Figure 2). The relationship between ERI and PTH was found non-significant (p-value = 0.085) (Figure 3). There was a non-significant correlation between EPO and PTH (p-value = 0.554, r = 0.063) (Figure 4). The relationship was significant upon using ACEI therapy (p-value 0.012, r = –0.825) (Table 3). There was a non-significant correlation between EPO and length of dialysis (p-value = 0.582, r = 0.059) (Figure 5). Further, the genotype distribution of *ACE* rs1799752 showed no deviation from Hardy Weinberg Equilibrium ($p = 0.242 > 0.05$).

Discussion

EPO lack is the leading cause of anemia due to chronic kidney diseases (CKD). Upon treatment with EPO stimulators, few dialysis patients presented with manifest resistance to ESA, which may increase the mortality hazards due to kidney infections (15). ESAs are commonly utilized to treat anemia related to CKD. ESA resistance or hypo-responsiveness is defined as patients who don't accomplish the specified HB levels despite the higher-than-normal doses of ESAs or who require progressively higher ESA doses to preserve a specific HB level (16). To slow down the lethal results of EPO resistance, observation programs should maintain the nutritional supplements (iron and folate stores), minimize oxidative stress-induced hemolysis, treat hyperparathyroidism, dodge catheter disease, and improve urea clearance (17). Our study revealed that ERI was not significantly associated with the *ACE* genotype, which agrees with a previous report where no notable impact of the *ACE* I/D polymorphism was on EPO resistance (18). Moreover, a recent study reported

Table 2. Relationship between ERI and ferritin level.

Item	Group A ERI 15 (30%)	Group B ERI 25 (50%)	Group C ERI 10 (20%)	*p*-value
Ferritin (ng/ml)	56.13 ± 8.64	113 ± 16.83	169.4 ± 17.46	<0.0001* significant

p-value compares the three groups $p < 0.05$ statistically significant.
(Group A) ERI = <9 U/kg/week/g/100 ml.
(Group B) ERI = 9-12 U/kg/week/g/100 ml (Group C) ERI = >12U/kg/week/g/100 ml.

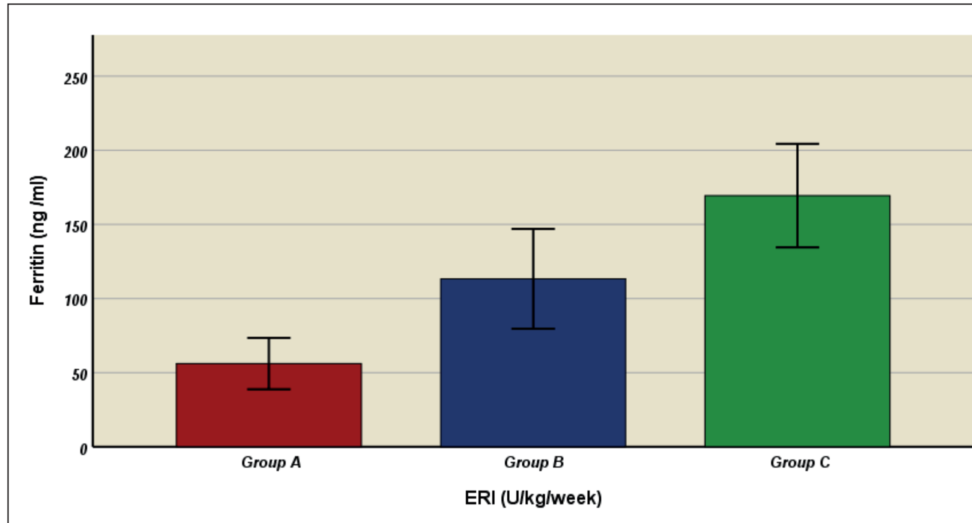


Figure 2. *Relationship between ERI and serum ferritin level using one-way ANOVA test (Group A); ERI ≤9 U/kg/week/g/100 ml; Group B ERI = 9-12 U/kg/week/g/100 ml; Group C ERI ≥12 U/kg/week/g/100 ml; p < 0.05 was set for statistically significant results; Error bars showed ± 2 SD).*

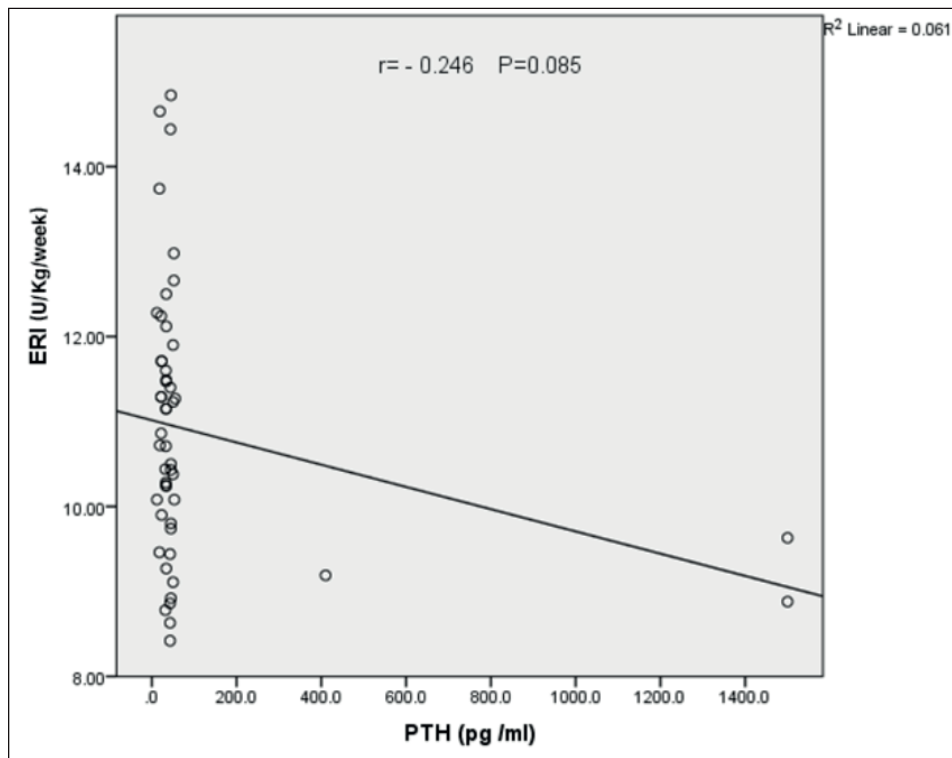


Figure 3. *Correlation between ERI and PTH level (p < 0.05 denotes statistically significant values).*

a decreased EPO resistance in patients with the D/D genotype compared to other genotypes (14). Our data found that the ID genotype had less EPO resistance, and II had more EPO resistance. Among hemodialysis patients in Korea, the D/D genotype had less EPO resistance than other genotypes (19). The *ACE* DD genotype lowers the

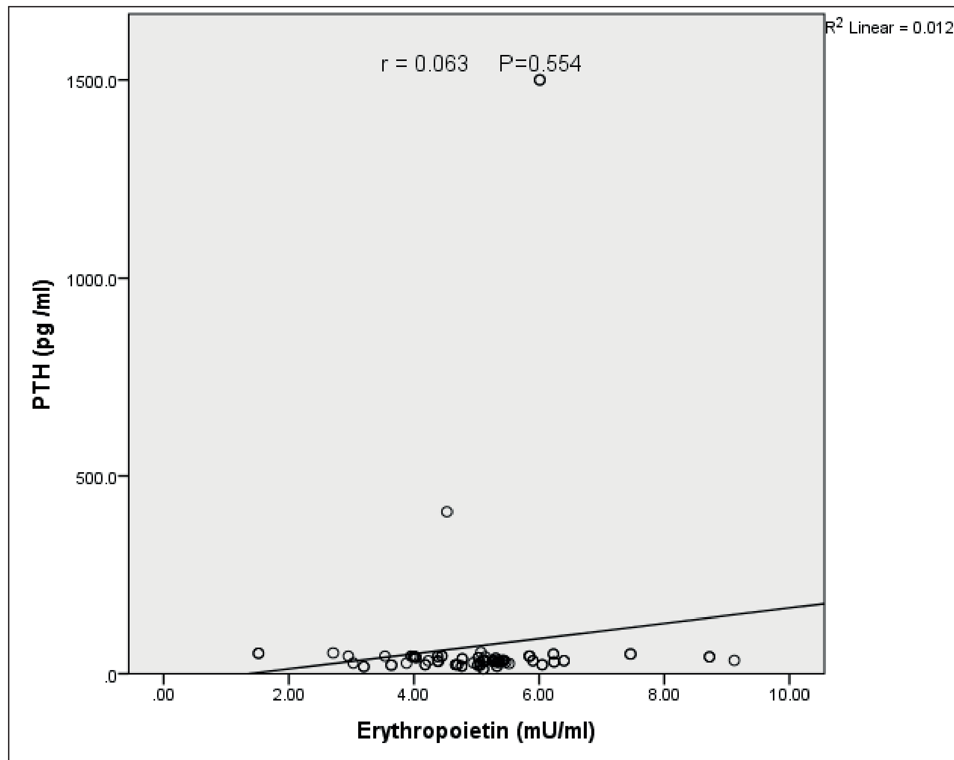


Figure 4. *Correlation between EPO and PTH (p < 0.05 denotes statistically significant values).*

Table 3. *Correlation between EPO and PTH in patients using ACEI versus patients not using ACEI (p < 0.05 denotes statistically significant values).*

Correlation between EPO and PTH	Correlation coefficient	*p*-value
Overall	0.063	0.544
Not using ACE inhibitors	0.161	0.149
Using ACE inhibitors	-0.825	0.012

EPO requirement due to the differing allele frequencies between races. The dispersion of patients with ACEI treatment among genotype bunches within the referred ponders may well be potential clarifications for our disparity as (6 out of 45 hemodialysis patients with DD genotype used ACEI therapy; 8 from the total of 73 DD participants used ACEI therapy (Figure 1, Table 1) as ACEIs piece post transplantation erythrocytosis. ACEIs did not essentially alter the erythropoietic reaction to rHUEPO (20). Patients on Continuous Ambulatory Peritoneal Dialysis reported that the *ACE* II/ID genotypes appear to be related to decreasing EPO responses (21,22). A significant correlation existed between ERI and serum ferritin level ($p = 0.0001$). High ferritin level was associated with high ERI, which agreed with the findings on patients whose ERI had the highest ferritin values (23), an indicator of active inflammatory status. Chronic aggravation and cytokines can decrease anemia by decreasing the life span of erythrocytes, enhancing erythroid antecedent's apoptosis, and specifically repressing the proliferation of erythrocytes progenitors (24,25) (Table 2 and Figure 2). Our study reported a non-significant correlation between PTH and ERI ($p = 0.085$). Similarly, there were no critical differences in ERI between the subgroups of patients classified according to the serum levels of parathyroid hormones (26) (Figure 3). The correlation between levels of PTH and EPO was insignificant ($p = 0.554$ (Figure 4). Also, there was a significant correlation between PTH and EPO upon using ACEIs (Table 3). Similarly, patients with ESA responsiveness had more cruel PTH levels than ESA hypo responders (27). In another cohort study in hemodialysis patients, there was a modest but significant relationship between higher PTH levels and decreased erythropoiesis (28). Decreased ESA responsiveness was related to more prominent serum PTH and alkaline phosphatase levels (29). However, the most impressive ESA responsiveness was associated with moderate to low PTH levels (150-300 pg./ml) and low-normal alkaline phosphatase value. Moreover, higher PTH levels were independently related to decreased ESA responsiveness. Hyperparathyroidism may specifically cause ESA hypo-responsiveness by reducing the endogenous EPO, diminishing bone marrow erythroid cells, and shortening the erythrocyte's life span. That impacts the effects of renal osteodystrophy on bone marrow fibrosis as confirmed by the increase in reestablished bone marrow space and increments in serum EPO levels after parathyroidectomy. Subsequently, ESA hypo responsiveness could be adjusted through treatment with active vitamin D (calcitriol) and/or

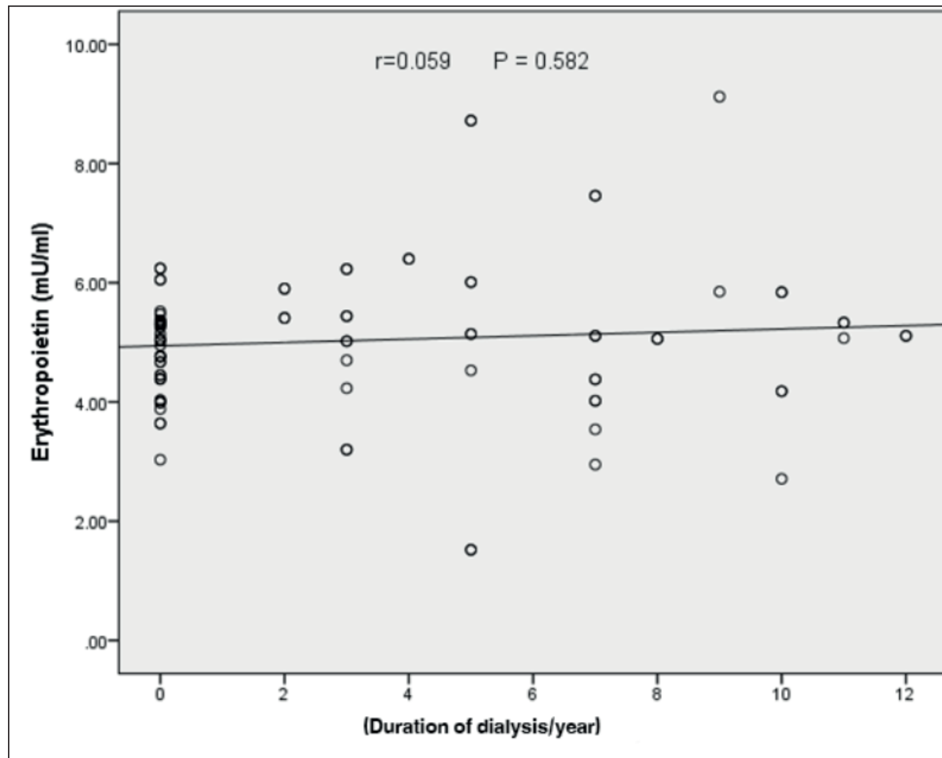


***Figure 5** Correlation between EPO level and the duration of dialysis p < 0.05 denotes statistically significant values. Correlation between EPO level and the duration of dialysis p < 0.05 denotes statistically significant values.*

calcimimetics, which diminish PTH emission and improve the high-turnover bone infection manifested as decreased serum alkaline phosphatase levels. Moreover, there is a significant correlation between the duration of dialysis and EPO level ($p = 0.582$) (Figure 5). Also, there is a significant correlation between the time of dialysis and EPO requirements in different *ACE* genotypes (21). Moreover, there is a substantial relationship between the impacts of intravenous and subcutaneous EPO and the duration of dialysis in hemodialysis patients ($p = 0.187$) (30). Erythropoiesis occurs within the bone marrow, where endogenous or exogenous EPO acts upon erythroid precursors, which undergo maturation into reticulocytes and erythrocytes. This process includes a few cytokines (IL-3, IL-12, insulin-like growth factor-1) and granulocyte-monocytecolony–stimulating growth factor, which stimulates cell multiplication. In contrast, other cytokines, e.g., IL-1, IL-6, tumor necrotic factor alpha, and interferon-gamma, can block this process. The last-mentioned cytokines may also be included in inflammation, intense or unremitting disease conditions, and malignancy. Any of these circumstances may favor erythropoiesis resistance. Moreover, comparing patients under distinctive circumstances and assessing the impact of distinctive medications on a single patient may cause this discrepancy (26).

Conclusion

Considering the non-critical connection between ERI and our components, it is vital to enhance the treatment of anemic patients with CKD to recognize the potential causes of resistance and ponder other variables for resistance before proposing an expanded EPO -stimulating agent administration.

List of Abbreviations

SPSS	Statistical Package for the Social Sciences
ACE	Angiotensin converting enzyme
ACEI	Angiotensin converting enzyme inhibitors
ARB	Angiotensin receptor blocker
EPO	Erythropoietin
ERI	Erythropoietin resistance index
ESA	Erythropoietin stimulating agents
PTH	Parathyroid hormone
rHuEPO	Recombinant human erythropoietin

Funding
None.

Declaration of conflicting interests
The authors of this article have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

Consent to participate
Informed consent was obtained from the patients.

Ethical approval
This study was approved by the Institutional Research Board of the Sohag Faculty of Medicine. Dated : December 2019, Approval Number: med: 23-02-2020.

Author details

Nagwa S. Ahmed[1], Aida A Mahmoud[1], Nayel Abd Elhamed Zaki[2], Amera A.Genedy[1*]

1. Medical Biochemistry Department, Faculty of Medicine, Sohag University, Sohag, Egypt
2. Department of Internal Medicine and Cardiology, Faculty of Medicine, Sohag University, Sohag, Egypt

References

1. Malik J, Kim AR, Tyre KA, Cherukuri AR, Palis J. Erythropoietin critically regulates the terminal maturation of murine and human primitive erythroblasts. Haematologica. 2013;98(11):1778–87. https://doi.org/10.3324/haematol.2013.087361
2. Suzuki N, Hirano I, Pan X, Minegishi N, Yamamoto M. Erythropoietin production in neuroepithelial and neural crest cells during primitive erythropoiesis. Nat Common. 2013;4:2902. https://doi.org/10.1038/ncomms3902
3. Hirano I, Suzuki N. The neural crest as the first production site of the erythroid growth factor erythropoietin. Front Cell Dev Biol. 2019;12(7):105. https://doi.org/10.3389/fcell.2019.00105
4. Palis J. Primitive and definitive erythropoiesis in mammals. Front Physiol. 2014;5:3. https://doi.org/10.3389/fphys.2014.00003
5. Palis J, Koniski A. Functional analysis of erythroid progenitors by colony-forming assays. Methods Mol Biol. 2018;1698:117–32. https://doi.org/10.1007/978-1-4939-7428-3_7
6. Suresh S, Rajvanshi PK, Noguchi CT. The many facets of erythropoietin physiologic and metabolic response. Front Physiol. 2020;17(10):1534. https://doi.org/10.3389/fphys.2019.01534
7. Goldberg MA, Dunning SP, Bunn HF. Regulation of the erythropoietin gene: evidence that the oxygen sensor is a heme protein. Science. 1988;242(4884):1412–5. https://doi.org/10.1126/science.2849206
8. Jelkmann WE, Fandrey J, Frede S, Pagel H. Inhibition of erythropoietin production by cytokines. Implications for the anaemia involved in inflammatory states. Ann N Y Acad Sci. 1994;718:300–9. https://doi.org/10.1111/j.1749-6632.1994.tb55728.x
9. Krantz SB. Pathogenesis and treatment of the anemia of chronic disease. Am J Med Sci. 1994;307(5):353–9. https://doi.org/10.1097/00000441-199405000-00009
10. Alves MT, Vilaça SS, das Carvalho M, Fernandes AP, Dusse LM, Gomes KB. Resistance of dialyzed patients to erythropoietin. Rev Bras Hematol Hemoter. 2015;37(3):190–7. https://doi.org/10.1016/j.bjhh.2015.02.001
11. Santos EJF, Hortegal EV, Serra HO, Lages JS, Salgado-Filho N, Dos Santos AM. Alpha erythropoietin resistance in hemodialysis patients with chronic kidney disease: a longitudinal study. Braz J Med Biol Res. 2018;51(7):e7288. https://doi.org/10.1590/1414-431x20187288
12. Petrov A. Erythropoietin resistance in patients undergoing dialysis. IOSR Journal of Dental and Medical Sciences (IOSR-JDMS). 2020;19(3):56–9.
13. Samavat S, Nafar M, Khoshdel A, Alipour-Abedi B. Factors contributing to erythropoietin hypo responsiveness among hemodialysis patients: a cross-sectional multicenter study. Nephro Urol Mon. 2017;9(3):e45003. https://doi.org/10.5812/numonthly.45003
14. Kiss Z, Ambrus C, Kulcsár I, Szegedi J, Kiss I. Effect of angiotensin-converting enzyme gene insertion/deletion polymorphism and angiotensin-converting enzyme inhibition on erythropoiesis in patients on haemodialysis. J Renin Angiotensin Aldosterone Syst. 2015;16(4):1021–7. https://doi.org/10.1177/1470320314535276
15. Santos EJF, Dias RSC, Lima JFB, Salgado Filho N, Miranda Dos Santos A. Erythropoietin resistance in patients with chronic kidney disease: current perspectives. Int J Nephrol Renovasc Dis. 2020;13:231–7. https://doi.org/10.2147/IJNRD.S239151
16. Sibbel SP, Koro CE, Brunelli SM, Cobitz AR. Characterization of chronic and acute ESA hypo response: a retrospective cohort study of hemodialysis patients. BMC Nephrol. 2015;16:144. https://doi.org/10.1186/s12882-015-0138-x
17. Bamgbola OF. Pattern of resistance to erythropoietin-stimulating agents in chronic kidney disease. Kidney Int. 2011;80(5):464–74. https://doi.org/10.1038/ki.2011.179
18. Hatano M, Yoshida T, Mimuro T, Kimata N, Tsuchiya K, Sanaka T, et al. The effects of ACE inhibitor treatment and ACE gene polymorphism on erythropoiesis in chronic hemodialysis patients. Nihon Jinzo Gakkai Shi. 2000;42(8):632–9.
19. Jeong KH, Lee TW, Ihm CG, Lee SH, Moon JY. Polymorphisms in two genes, IL-1B and ACE, are associated with erythropoietin resistance in Korean patients on maintenance hemodialysis. Exp Mol Med. 2008;40(2):161–6. https://doi.org/10.3858/emm.2008.40.2.161
20. Saudan P, Halabi G, Perneger T, Wasserfallen JB, Wauters JP, Martin PY, et al. ACE inhibitors or angiotensin II receptor blockers in dialyzed patients and erythropoietin resistance. J Nephrol. 2006;19(1):91–6.
21. Varagunam M, McCloskey DJ, Sinnott PJ, Raftery MJ, Yaqoob MM. Angiotensin-converting enzyme gene polymorphism and erythropoietin requirement. Perit Dial Int. 2003;23(2):111–5. https://doi.org/10.1177/089686080302300203
22. Sharples EJ, Varagunam M, Sinnott PJ, McCloskey DJ, Raftery MJ, Yaqoob MM. The effect of pro inflammatory cytokine gene and angiotensin-converting enzyme polymorphisms on erythropoietin requirements in patients on continuous ambulatory peritoneal dialysis. Perit Dial Int. 2006;26(1):64–8. https://doi.org/10.1177/089686080602600110
23. Petrulienė K, Žiginskienė E, Kuzminskis V, Nedzelskienė I, Bumblytė IA. Hepcidin serum levels and resistance to recombinant human erythropoietin therapy in hemodialysis patients. Medicina (Kaunas). 2017;53(2):90–100. https://doi.org/10.1016/j.medici.2017.03.001
24. Canavesi E, Alfieri C, Pelusi S, Valenti L. Hepcidin and HFE protein: iron metabolism as a target for the anemia of chronic kidney disease. World J Nephrol. 2012;1(6):166–76. https://doi.org/10.5527/wjn.v1.i6.166
25. Valenti L, Messa P, Pelusi S, Campostrini N, Girelli D. Hepcidin levels in chronic hemodialysis patients: a critical evaluation. Clin Chem Lab Med. 2014;52(5):613–9. https://doi.org/10.1515/cclm-2013-0769
26. López-Gómez JM, Portolés JM, Aljama P. Factors that condition the response to erythropoietin in patients on hemodialysis and their relation to mortality. Kidney Int Suppl. 2008;111:S75–81. https://doi.org/10.1038/ki.2008.523

27. Rao DS, Shih MS, Mohini R. Effect of serum parathyroid hormone and bone marrow fibrosis on the response to erythropoietin in uremia. N Engl J Med. 1993;328(3):171–5. https://doi.org/10.1056/NEJM199301213280304
28. Gaweda AE, Goldsmith LJ, Brier ME, Aronoff GR. Iron, inflammation, dialysis adequacy, nutritional status, and hyperparathyroidism modify erythropoietic response. Clin J Am Soc Nephrol. 2010;5(4):576–81. https://doi.org/10.2215/CJN.04710709
29. Kalantar-Zadeh K, Lee GH, Miller JE, Streja E, Jing J, Robertson JA, et al. Predictors of hypo responsiveness to erythropoiesis-stimulating agents in hemodialysis patients. Am J Kidney Dis. 2009;53(5):823–34. https://doi.org/10.1053/j.ajkd.2008.12.040
30. Ghasemi F, Abdi A, Salari N, Tohidi MR, Faraji A. Comparing the effects of intravenous and subcutaneous erythropoietin on blood indices in hemodialysis patients. Sci Rep. 2019;9(1):2284. https://doi.org/10.1038/s41598-018-38193-z

ORIGINAL ARTICLE

Uncovering the genetic basis of hyperphosphatasia with impaired intellectual development syndrome type 2: identification of a novel biallelic nonsense mutation in *PIGO* gene

Anam Nayab[1], Shagufta Andleeb[2], Shah Zeb[3,4], Hafiza Yasmin Manzoor[5], Zamrud Zehri[6], Arif Mahmood[7], Hammal Khan[8], Muhammad Umair[9*], Ahmed Waqas[2*]

ABSTRACT

Background: Glycosylphosphatidylinositol (GPI) is a glycolipid containing phosphatidylinositol related to the protein surfaces by covalent attachment. Inherited GPI deficiencies have various phenotypic chrematistics, which range from intellectual disability to dysmorphic features, epilepsy, and other severe anomalies.

Methods: Molecular diagnosis was performed using whole exome sequencing (WES) followed by Sanger sequencing.

Results: WES revealed a novel homozygous nonsense variant (c.250C>T; *p*.Gln84Ter) in the exon 2 of the phosphatidylinositol glycan anchor biosynthesis class Ogene that might explain the disease phenotype in the patient.

Conclusion: This study will help in proper genetic counselling of the family and help in genotype-phenotype correlation in the future.

Keywords: GPI, WES, missense variant, PIGO, ID, novel variant.

Introduction

Glycosylphosphatidylinositol (GPI) is a glycolipid containing phosphatidylinositol that covalently attaches proteins to the plasma membrane (cell surface). In forming GPI-anchored proteins (GPI-APs), almost 26-30 genes are involved (1,2). The GPI-APs group include different receptors, enzymes having hydrolytic nature, adhesion molecules, immune system-associated proteins, and complement regulatory proteins (1,2). Disease-causing variants have been identified in various components of the GPI-anchored-synthesis pathway, thus causing diverse phenotypes referred to as congenital disorders of glycosylation (1). Inherited GPI deficiencies include features such as epilepsy, ID, dysmorphic facial features, and multiple organ anomalies depending on the gene involved and the position of the identified variant. Pathogenic sequence variants in different genes have been reported in the GPI biosynthesis, such as the phosphatidylinositol glycan anchor biosynthesis class O (*PIGO*), *PIGV*, *PIGW*, *PGAP2*, *PGAP3*, and the *PIGY* reported to cause hyperphosphatasia with mental retardation syndrome (HPMRS; MIM # 614749; Table 1) also known as

Correspondence to: Muhammad Umair
*Department of Life Sciences, School of Science, University of Management and Technology (UMT), Lahore, Pakistan.
Email: m_umair@umt.edu.pk
Correspondence to: Ahmed Waqas
*Department of Zoology, Division of Science and Technology, University of Education, Lahore, Pakistan.
Email: ahmed.waqas@ue.edu.pk
Full list of author information is available at the end of the article.
Received: 09 January 2023 | **Accepted:** 01 March 2023

This is an open access article distributed in accordance with the Creative Commons Attribution (CC BY 4.0) license: https://creativecommons.org/licenses/by/4.0/) which permits any use, Share — copy and redistribute the material in any medium or format, Adapt — remix, transform, and build upon the material for any purpose, as long as the authors and the original source are properly cited. © The Author(s).

Table 1. *Genes associated with HPMRS and their clinical comparison.*

OMIM number	# 239300	# 616809	# 614749	# 614207	# 616025	# 615716
Disorder name	Hyperphosphatasia with impaired intellectual development syndrome 1; HPMRS1	Hyperphosphatasia with impaired intellectual development syndrome 6; HPMRS6	Hyperphosphatasia with impaired intellectual development syndrome 2; HPMRS2	Hyperphosphatasia with impaired intellectual development syndrome 3; HPMRS3	GPI biosynthesis defect 11; GPIBD11	Hyperphosphatasia with impaired intellectual development syndrome 4; HPMRS4
Gene	*PIGV* - 610274	*PIGY* - 610662	*PIGO* - 614730	*PGAP2* - 615187	*PIGW* - 610275	*PGAP3* - 611801
Inheritance (6/6)	- AR	- AR	- AR	- AR	- AR	- AR
Growth (3/6)	N.A.	- Poor growth	- Poor growth	N.A.	N.A.	- Poor growth
Head & neck (6/6)	N.A.	- Microcephaly	- Microcephaly	- Microcephaly	N.A.	- Microcephaly - Large anterior fontanel
	Face - Midface hypoplasia - Prognathism	*Face* - Bitemporal narrowing	N.A.	N.A.	N.A.	N.A.
	Hearing impairment	Thickened helices	Hearing impairment	Hearing impairment	N.A.	- Hearing loss
	Eyes - Hypertelorism - Upslanting palpebral fissures - Long palpebral fissures - Arched eyebrows	*Eyes* - Congenital cataracts - Cerebral visual impairment - Deep-set eyes - Long palpebral fissures - Strabismus	*Eyes* - Hypertelorism - Long palpebral fissures - Upslanting palpebral fissures	N.A	N.A	*Eyes* - Hypertelorism - Upslanting palpebral fissures - Epicanthal folds
	Nose - Broad nasal bridge - Broad nasal tip - Short nose	*Nose* - Depressed nasal bridge - Upturned nares - Bulbous nasal tip	*Nose* - Short nose - Broad nasal bridge - Broad nasal tip	*Nose* - Broad nasal bridge - Broad nasal tip - Short nose	*Nose* - Broad nasal bridge	*Nose* - Broad nasal bridge - Broad nasal tip
	Mouth - Cleft palate (rare) - Short philtrum - Tented mouth	*Mouth* - High-arched palate - Wide mouth	*Mouth* - Tented mouth - Cleft palate	*Mouth* - Cleft palate - Tented upper lip	*Mouth* - Tented upper lip - Large tongue	*Mouth* - Cleft palate - Bruxism -Abnormal dentition
Cardiovascular (3/6)	*Heart* - Cardiac defects - Ventral septal defect (rare)	N.A.	*Heart* - Heart defects - Atrial septal defect	N.A.	N.A.	*Heart* - Congenital heart defects (in 1 family)

Continued

OMIM number	# 239300	# 616809	# 614749	# 614207	# 616025	# 615716
Skeletal (4/6)	- Plagiocephaly	- Joint contractures - Osteopenia - Hip dysplasia - Proximal limb shortening	- Plagiocephaly - Coronal synostosis	N.A.	N.A.	N.A.
	- Hypoplastic toes - Bilateral adducted forefoot (rare)	N.A	Brachytelephalangy - Broad halluces	N.A	N.A	- Pes equinovarus
Neurologic (6/6)	*Central Nervous System* - Hypotonia - Seizures - Mental retardation, severe - Athetoid and dystonic hand movements - Moderate cortical atrophy - Delayed myelinization - Speech delay - No speech development	*Central Nervous System* - Delayed psychomotor development - Delayed speech - Developmental regression - Seizures, intractable - Truncal hypotonia	*Central Nervous System* - Delayed psychomotor development, moderate to severe - Delayed speech and language development - Hypotonia - Seizures - Enlarged ventricles	*Central Nervous System* - Delayed psychomotor development - Mental retardation, severe - Intellectual disability, mild - Hypotonia - Poor or absent speech - Seizures - Disordered sleep pattern - Cerebral atrophy	*Central Nervous System* - Delayed psychomotor development - Intellectual disability - Seizures, variable types - Poor or absent speech - Abnormal EEG	*Central Nervous System* - Delayed psychomotor development, severe - Inability to walk - Lack of speech development - Seizures, generalized - Seizures, myoclonic - Involuntary movements - Hypoplastic corpus callosum - Hypoplastic cerebellum with absent vermis - Cerebellar vermis hypoplasia
Laboratory abnormalities (6/6)	- Elevated alkaline phosphatase (varies from 1.3-20 times the age-adjusted upper limit of normal) - Hyperphosphatasia	- Increased serum creatine kinase - Increased alkaline phosphatase - Decreased expression of GPI-APs on fibroblasts	- Increased serum alkaline phosphatase - Hyperphosphatasia	- Increased serum alkaline phosphatase - Hyperphosphatemia	- Increased serum alkaline phosphatase - Decreased expression of GPI -anchored membrane proteins	- Increased serum alkaline phosphatase

Mabry syndrome (2-5). Herein, we report a proband (the first case from the Pakistani population) with epileptic encephalopathy caused by a novel disease-causing variant in the *PIGO* gene.

Subjects and Methods

For the present study, a family with an autosomal recessive (AR) inheritance pattern was recruited from the Khyber Pakhtunkhwa province of Pakistan (Figure 1A). The patient was evaluated by taking a medical history and performing biochemical tests at a local government hospital. Consent in written form was obtained from the participants for the genetic analysis in compliance with the Helsinki Declaration. The University of Education, Lahore, Pakistan's Institutional Review Board approved the current study. Blood samples were collected and processed further for DNA extraction and quantification using standard methods (6). WES was performed using DNA from the proband (IV-1). WES and variants filtering steps were performed as described earlier (7). Standard-screening principles were used to search for different functional variants associated with the patient phenotype (8). The genes already reported in the Online Mendelian Inheritance in Man and literature (PUBMED) were given priority. Prioritized disease-causing variants were Sanger sequenced for segregation analysis (9,10). The pathogenic nature of the identified variant was calculated using different tools. The Exome Aggregation Consortium (ExAC) and genomAD were searched to see if the variant was reported in the general population. Amino acid conservation was determined using NCBI-HomoloGene.

Protein modelling

The structure sequence of PIGO full length was retrieved from the Protein Data Bank. The protein modelling was executed according to the previously outlined methodology (11,12). Figures were made using the Py-Molecule molecular viewer (https://pymol.org/) (Figure 2A and B) (13).

Results

Clinical description

The proband (boy: IV-1) was the first child of a healthy Pakistani Pashto-speaking family. Pregnancy was unremarkable, and he was born with vaginal delivery having an average birth weight. Shortly after birth, features such as feeding difficulties, severe axial hypotonia, and muscular dystrophy were observed. He could not recognize his parents and did not establish eye contact presenting the features of global developmental delay (GDD). The proband also showed frequent seizures and drooling. He is on several antiepileptic drugs like phenobarbitone and topiramate. Recurrent episodes with pneumonia were observed in the second year of life, which led to respiratory insufficiency, and a gastrostomy tube was used to fulfil the feeding difficulties. Serum alkaline phosphatase was unremarkable; however, slightly in the upper ranges, i.e., 235, 247, 263, and 257 U/l (Normal range: 96-297 U/l). His younger brother is healthy with no epileptic or any other complications.

Molecular investigation

WES was performed as described earlier (14). Screening and filtering different homozygous and compound heterozygous variants manifested a novel homozygous stop gain variant (c.250C>T; *p*.Gln84Ter) in the exon 2 of *PIGO* (NM_032634.4) located on chromosome 9p13.3-9p13.3. The variant was also screened in ExAC, genomAD, and 145 control exomes (Figure 1B), and the variant was not observed in the homozygous state using both databases. The Gln84 amino acid was also conserved across different species (Figure 1C).

Protein modelling

3D modelling of the mutated PIGO and wild-type PIGO was performed (15). Their structural comparison showed that the mutated PIGO protein would result in a more minor, non-functional protein that loses its main domains. Thus, the mutated PIGO will not perform a proper function.

Discussion

In this study, we report an affected child having GDD, severe epileptic seizures, ID, and little elevation of ALP. We performed WES and identified a biallelic stop gain variant (c.250C>T; p.Gln84Ter) residing in the transmembrane domain of the protein (Figure 1B) of the PIGO, thus expanding the clinical and variant spectrum of *PIGO*-related pathogenesis. To date, disease-causing variants in the *PIGO* gene have only been reported in a few studies, including seven females and two males from six families (1-5,16). If the mRNA molecule coding for a protein avoids degradation through the nonsense-mediated decay pathway, the resulting truncated protein may exhibit a distinct structural formation that deviates from the full-length version of the protein, which can lead to improper functioning and potential cellular dysfunction.

Kuki et al. (17) and Nakamura et al. (3) reported patients that possessed missense variants in the alkaline phosphatase domain (core domain). They showed more progressive and severe phenotypes than the affected individuals reported by Krawitz et al. (2) and in the present study. The mild neurodevelopmental feature in our patient can be associated with the location of the variant identified in the transmembrane domain. Thus, the position of a variant in the protein might play a role in the diverse phenotypic presentation. Similarly, Nakamura et al. (3) reported that severe phenotypes might be associated with the location of variants identified in the specific PIGO domain, such as the core domain. These observations might lead toward genotype-phenotype correlation associated with *PIGO*-pathogenesis. However, more substantial evidence and functional analysis are required to elucidate phenotype–genotype correlations and to prove such a hypothesis. Neurological dysfunction in the affected individual

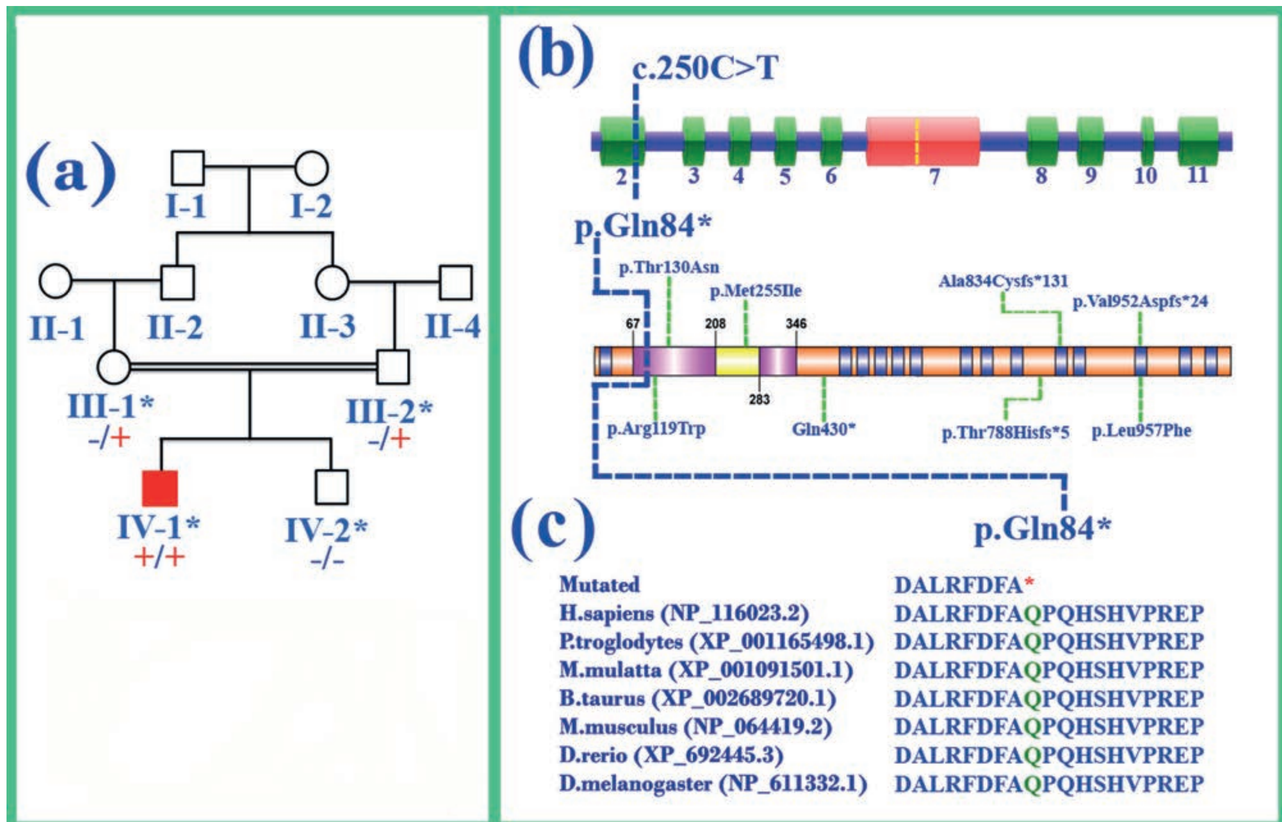


Figure 1. *(A) Pedigree of the present family along with genotype. (B) Surface expression of CD16 antigen. (C) Exons and domains of the PIGO and location of the identified variant. (D) Conservation of Gln84 across different species.*

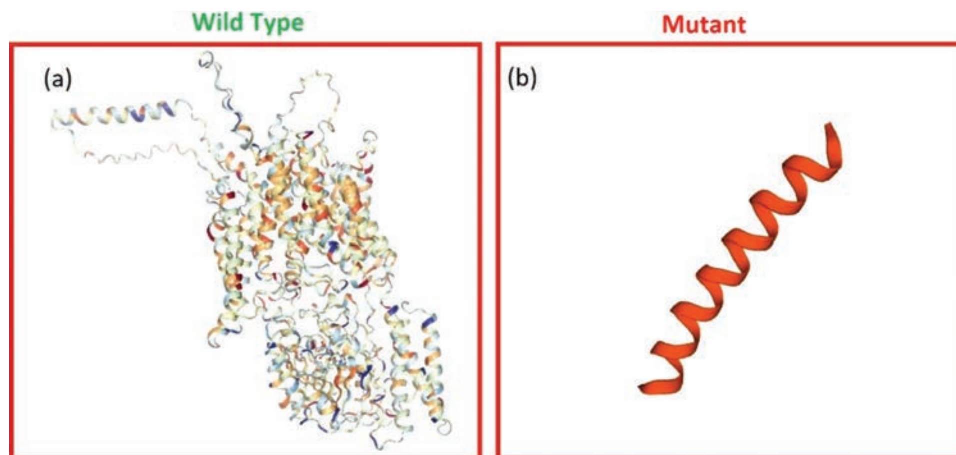


Figure 2. *Comparison of Wild Type and Mutant Protein Structures. (A) Representation of the wild-type protein structure. (B) Representation of the mutant protein structure. The structural differences between the wild type and mutant proteins include loss of 3/4th amino acid residues.*

reported here and in the patients said previously (4,17) is complex and thus cannot be related to alkaline phosphatase impairment.

Recent research and technological advancement have compelled scientists to think outside the box and develop a better understanding of neurodevelopmental disorders and their etiological bases (18-20) Given the complex nature of such disorders, any theoretical model designed to explain the disease pathogenesis will depend on advanced functional studies involving novel disease-gene identification, cohort studies, and available studies using animal models (21-24). Identification of such variants will help build a database that might lead to future therapeutic interventions and help conduct clinical trials (25,26). We revealed that homozygous loss-of-function variants in *PIGO* cause hyperphosphatemia with impaired intellectual development syndrome-2. Furthermore, novel variant identification for rare genetic disorders and making a database will help add such variants to the newborn screening program. In addition, preimplantation genetic testing for aneuploidies, noninvasive prenatal testing, and PGT-M can be employed for parents wishing to have future pregnancies (27-30). Identification of

additional families and functional studies are required to understand the cellular role of PIGO associated with neurodegeneration.

Conclusion

In conclusion, we have detected a novel homozygous variant in the *PIGO* gene in an affected individual having mild epileptic encephalopathy, along with slightly increased serum alkaline phosphatase levels and decreased CD16 expression but normal CD59 and CD24 expression. Furthermore, we suggest a genotype-phenotype correlation concerning the association between the location of the identified variant in the transmembrane domain and milder clinical phenotypes.

List of Abbreviations

ExAC The Exome Aggregation Consortium

PIGO Phosphatidylinositol glycan anchor biosynthesis class O

Funding

None.

Declaration of conflicting interests

The authors of this article have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

Consent for publication

The consent for publication for this case was obtained from the parents.

Ethical approval

Ethical approval was granted by the Institutional Research Board of the University of Education, Lahore, Pakistan (UE/S&T/2019/222).

Author details

Anam Nayab[1], Shagufta Andleeb[2], Shah Zeb[3,4], Hafiza Yasmin Manzoor[5], Zamrud Zehri[6], Arif Mahmood[7], Hammal Khan[8], Muhammad Umair[9], Ahmed Waqas[2]

1. Department of Biotechnology, Fatima Jinnah Women's University, Rawalpindi, Pakistan
2. Department of Zoology, Division of Science and Technology, University of Education, Lahore, Pakistan
3. Institute for Advanced Study, Shenzhen University, Shenzhen, People's Republic of China
4. College of Physics and Optoelectronics Engineering, Shenzhen University, Shenzhen, People's Republic of China
5. Department of laboratory, Carle Foundation Hospital, Urbana, IL, USA
6. Department of Gynecology and Obstetrics, Civil Hospital Quetta, Quetta, Pakistan
7. Center for Medical Genetics and Hunan Key Laboratory of Medical Genetics, School of Life Sciences, Central South University, Changsha, China
8. Department of Biosciences, COMSATS University Islamabad, Islamabad, Pakistan
9. Department of Life Sciences, School of Science, University of Management and Technology (UMT), Lahore, Pakistan

References

1. Kinoshita T, Fujita M, Maeda Y. Biosynthesis, remodelling and functions of mammalian GPI-anchored proteins: recent progress. J Biochem. 2008;144:287–94. https://doi.org/10.1093/jb/mvn090
2. Krawitz PM, Murakami Y, Hecht J, Krüger U, Holder SE, Mortier GR, et al. Mutations in PIGO, a member of the GPI-anchor-synthesis pathway, cause hyperphoshyperphosphatasia with mental retardation. Am J Hum Genet. 2012;91:146–51. https://doi.org/10.1016/j.ajhg.2012.05.004
3. Nakamura K, Osaka H, Murakami Y, Anzai R, Nishiyama K, Kodera H, et al. PIGO mutations in intractable epilepsy and severe developmental delay with mild elevation of alkaline phosphatase levels. Epilepsia. 2014;55(2):e13–7. https://doi.org/10.1111/epi.12508
4. Zehavi Y, von Renesse A, Daniel-Spiegel E, Sapir Y, Zalman L, Chervinsky I, et al. Homozygous PIGO mutation associated with severe infantile epileptic encephalopathy and corpus callosum hypoplasia, but normal alkaline phosphatase levels. Metab Brain Dis. 2017. https://doi.org/10.1007/s11011-017-0109-y
5. Tanigawa J, Mimatsu H, Mizuno S, Okamoto N, Fukushi D, Tominaga K, et al. Phenotype-genotype correlations of PIGO deficiency with variable phenotypes from infantile lethality to mild learning difficulties. Hum Mutat. 2017;38(7):805–15. https://doi.org/10.1002/humu.23219
6. Umair M, Rafique A, Ullah A, Ahmad F, Ali RH, Nasir A, et al. Novel homozygous sequence variants in the GDF5 gene underlie acromesomelic dysplasia type-grebe in consanguineous families. Congenit Anom (Kyoto). 2017;57(2):45–51. https://doi.org/10.1111/cga.12187
7. Hayat A, Hussain S, Bilal M, Kausar M, Almuzzaini B, Abbas S, et al. Biallelic variants in four genes underlying recessive osteogenesis imperfecta. Eur J Med Genet. 2020;63(8):103954. https://doi.org/10.1016/j.ejmg.2020.103954
8. Younus M, Ahmad F, Malik E, Bilal M, Kausar M, Abbas S, et al. SGCD homozygous nonsense mutation (p.Arg97*) causing limb-girdle muscular dystrophy type 2F (LGMD2F) in a consanguineous family, a case report. Front Genet. 2019;9:727. https://doi.org/10.3389/fgene.2018.00727
9. Umair M, Bilal M, Ali RH, Alhaddad B, Ahmad F, Abdullah, et al. Whole-exome sequencing revealed a nonsense mutation in STKLD1 causing non-syndromic pre-axial polydactyly type A affecting only the upper limb. Clin Genet. 2019;96(2):134–39. https://doi.org/10.1111/cge.13547
10. Ullah A, Umair M, Majeed AI, Abdullah, Jan A, Ahmad W. A novel homozygous sequence variant in GLI1 underlies first case of autosomal recessive pre-axial polydactyly. Clin Genet. 2019;95(4):540–1. https://doi.org/10.1111/cge.13495
11. Umair M, Palander O, Bilal M, Almuzzaini B, Alam Q, Ahmad F, et al. Biallelic variant in DACH1, encoding dachshund homolog 1, defines a novel candidate locus for recessive postaxial polydactyly type A. Genomics. 2021;113(4):2495–502. https://doi.org/10.1016/j.ygeno.2021.05.015
12. Umair M, Khan A, Hayat A, Abbas S, Asiri A, Younus M, et al. Biallelic missense mutation in the ECEL1 underlies distal arthrogryposis type 5 (DA5D). Front Pediatr. 2019;7:343. https://doi.org/10.3389/fped.2019.00343
13. Ahmad F, Ahmed I, Nasir A, Umair M, Muhammad D, Shahzad S, et al. A disease-causing novel missense mutation in the ST14 gene underlies autosomal recessive ichthyosis

with hypotrichosis syndrome in a consanguineous family. Eur J Dermatol. 2018;28(2):209–16. https://doi.org/10.1684/ejd.2017.3210

14. Umair M, Ballow M, Asiri A, Alyafee Y, al Tuwaijri A, Alhamoudi KM, et al. EMC10 homozygous variant identified in a family with global developmental delay, mild intellectual disability, and speech delay. Clin Genet. 2020;98(6):555–61. https://doi.org/10.1111/cge.13842
15. Nayab A, Alam Q, Alzahrani OR, Khan R, Sarfaraz S, Albaz AA, et al. Targeted exome sequencing identified a novel frameshift variant in the PGAM2 gene causing glycogen storage disease type X. Eur J Med Gent. 2021;64(9):104283. https://doi.org/10.1016/j.ejmg.2021.104283
16. Xue J, Li H, Zhang Y, Yang Z. Clinical and genetic analysis of two Chinese infants with Mabry syndrome. Brain Dev. 2016;38(9):807–18. https://doi.org/10.1016/j.braindev.2016.04.008
17. Kuki I, Takahashi Y, Okazaki S, Kawawaki H, Ehara E, Inoue N, et al. Vitamin B6-responsive epilepsy due to inherited GPI deficiency. Neurology. 2013;81:1467–469. https://doi.org/10.1212/WNL.0b013e3182a8411a
18. Nøstvik M, Kateta SM, Schönewolf-Greulich B, Afenjar A, Barth M, Boschann F, et al. Clinical and molecular delineation of PUS3 associated neurodevelopmental disorders. Clin Genet. 2021;100(5):628–33. https://doi.org/10.1111/cge.14051
19. Royer-Bertrand B, Jequier Gygax M, Cisarova K, Rosenfeld J, Bassetti JA, Moldovan O, et al. De novo variants in CACNA1E found in patients with intellectual disability, developmental regression and social cognition deficit but no seizures. Mol Autism 2021;12:69. https://doi.org/10.1186/s13229-021-00473-3
20. Khan A, Molitor A, Mayeur S, Zhang G, Rinaldi B, Lannes B, et al. A homozygous missense variant in PPP1R1B/DARPP-32 is associated with generalized dystonia. Mov Disord. 2021;37(2):365–74. https://doi.org/10.1002/mds.28861
21. Waqas A, Abbas S, Nayab A, Shaheen S, Latif M, Rafeeq MM, et al. Biallelic variant in the tRNA methyltransferase domain of the AlkB homolog 8 causes syndromic intellectual disability. Front Genet. 2022;13:878274. https://doi.org/10.3389/fgene.2022.878274
22. Scala M, Drouot N, MacLennan SC, Wessels MW, Krygier M, Pavinato L, et al. Truncating variants in De novo truncating NOVA2 variants affect alternative splicing and lead to heterogeneous neurodevelopmental phenotypes. Hum Mutat. 2022; 43(9):1299–313. https://doi.org/10.1002/humu.24414
23. Umair M, Khan MF, Aldrees M, Nashabat M, Alhamoudi KM, Bilal M, et al. Mutated VWA8 is associated with developmental delay, microcephaly, scoliosis and play a novel role in early development and skeletal morphogenesis in Zebrafish. Front Cell Dev Biol. 2021;9:736960. https://doi.org/10.3389/fcell.2021.736960
24. Meng T, Chen X, He Z, Huang H, Lin S, Liu K, et al. ATP9A deficiency causes ADHD and aberrant endosomal recycling via modulating RAB5 and RAB11 activity. Mol Psychiatry. 2023;6:1–13. https://doi.org/10.1038/s41380-022-01940-w
25. Alfadhel M, Nashabat M, Saleh M, Elamin M, Alfares A, Othaim AA, et al. Long-term effectiveness of carglumic acid in patients with propionic acidemia (PA) or methylmalonic acidemia (MMA): a randomized clinical trial. Orphanet J Rare Dis. 2021;16(1):422. https://doi.org/10.1186/s13023-021-02032-8
26. Alfadhel M, Umair M, Almuzzaini B, Alsaif S, AlMohaimeed SA, Almashary MA, et al. Targeted SLC19A3 gene sequencing of 3000 Saudi newborn: a pilot study toward newborn screening. Ann Clin Transl Neurol. 2019;6(10):2097–2103. doi: 10.1002/acn3.50898. https://doi.org/10.1002/acn3.50898
27. Alyafee Y, Al Tuwaijri A, Alam Q, Umair M, Haddad S, Alharbi M, et al. Next generation sequencing based non-invasive prenatal testing (NIPT): first report from Saudi Arabia. Front Genet. 2021a;12:630787. https://doi.org/10.3389/fgene.2021.630787
28. Alyafee Y, Alam Q, Tuwaijri AA, Umair M, Haddad S, Alharbi M, et al. Next-generation sequencing-based pre-implantation genetic testing for aneuploidy (PGT-A): first report from Saudi arabia. Genes. 2021b;12:461. https://doi.org/10.3390/genes12040461
29. Alyafee Y, Al Tuwaijri A, Umair M, Alharbi M, Haddad S, Ballow M, et al. Non-invasive prenatal testing for autosomal recessive disorders: a new promising approach. Front Genet 2022. https://doi.org/10.3389/fgene.2022.1047474
30. Ullah A, Gull A, Umair M, Irfanullah, Ahmad W. Homozygous sequence variants in the WNT10B gene underlie split hand/foot malformation. Genet Mol Biol. 2018;41(1):1–8. doi: 10.1590/1678-4685-GMB-2016-0162.

ORIGINAL ARTICLE

A biallelic variant in IQCE predisposed to cause non-syndromic post-axial polydactyly type A

Muhammad Bilal[1*], Muhammad Raheel[2], Gul Hassan[3], Shah Zeb[4,5], Arif Mahmood[6], Zamrud Zehri[7], Hafiza Yasmin Manzoor[8], Muhammad Umair[9*]

ABSTRACT

Background: Polydactyly or hexadactyly is a familiar limb defect that either occurs as an isolated entity (non-syndromic) or is associated with severe (syndromic) morphological phenotypes. Generally, it appears due to a defect in the anteroposterior patterning during limb development.

Methods: Here, we present a proband having non-syndromic post-axial polydactyly (PAP) evaluated using whole exome sequencing followed by Sanger sequencing. Furthermore, 3D protein modeling was executed for the normal and mutated IQ domain-containing protein E (*IQCE*) gene.

Results: WES analysis revealed an already reported bi-allelic variant (c.395-1 G>A) in the *IQCE* gene, previously associated with PAP 7. Furthermore, 3D modeling revealed significant fluctuations in the IQCE protein secondary structure, thus affecting downstream signaling.

Conclusion: The work presented validated the significant role of the *IQCE* gene in the development and patterning of human limbs.

Keywords: PAPA, *IQCE*, reported variant, Pakistani population, 3D modeling, WES.

Introduction

Polydactyly is characterized as the presence of well-formed extra digits in upper or lower limbs (1). It can be an isolated deformity (non-syndromic) or associated with a complex progressive syndrome (syndromic). The syndromic condition exhibits severe phenotypic complications, including disorders such as Laurin-sand row syndrome, Acrocallosal syndrome, split-hand foot malformation, Bardet-Biedl Syndrome, and complex ciliatory diseases (2-5). Polydactyly is classified into three categories, which include postaxial polydactyly (PAP), pre-axial polydactyly and complex polydactyly. PAP and preaxial polydactyly is further divided into two subgroups: type A, with fully developed bone in the extra digit or type B, which is non-function in the form of the skin tag, with or without nail (6-8). PAP type A and B are the most prevalent type of polydactyly. To date, eleven genes have been associated with non-syndromic polydactyly [glioma-associated oncogene family zinc finger 1 (*GLI3*), *SHH*, *STKLD1*, *MIPOL1*, *GLI1*, *ZNF141*, IQ domain-containing protein E (*IQCE*), *FAM92A*, *KIAA0825*, *DACH1*, *PITX*] (6-13) (Tables 1 and 2). Abnormalities of human hands and feet occur frequently in the general population. The development of human limbs is regulated by a series of complex cellular pathways including hedgehog (HH), WNT, and bone morphogenetic proteins. Deficiency of any regulator in such pathways leads to diverse types of limb deformities and other syndromic

Correspondence to: Muhammad Bilal
*Department of Biochemistry, Faculty of Biological Sciences, Quaid-i-Azam University, Islamabad, Pakistan.
Email: mbilal@bs.qau.edu.pk
Correspondence to: Muhammad Umair
*Department of Life Sciences, School of Science, University of Management and Technology (UMT), Lahore, Pakistan.
Email: m_umair@umt.edu.pk
Full list of author information is available at the end of the article.
Received: 12 January 2023 | **Accepted:** 12 March 2023

This is an open access article distributed in accordance with the Creative Commons Attribution (CC BY 4.0) license: https://creativecommons.org/licenses/by/4.0/) which permits any use, Share — copy and redistribute the material in any medium or format, Adapt — remix, transform, and build upon the material for any purpose, as long as the authors and the original source are properly cited. © The Author(s).

skeletal deformities (14). Polydactyly is one of such deformities that results due to defects in anterior-posterior patterning of the limb development (14). In the era of advanced technologies, WES has been very successful in clinical exome analysis, solving many cases, and identifying novel candidate genes. WES has both low cost and quick meth for molecular analysis of genetic disorders (15). In the present study, we have investigated a proband exhibiting non-syndromic PAPA phenotype segregating in autosomal recessive mode. Using WES, we identified a previously reported bi-allelic variant in the intron five of the *IQCE* gene that might be associated with the polydactyly condition in our patient.

Subjects and Methods

For the present study, a family with an AR inheritance pattern was recruited from the Khyber Pakhtunkhwa province of Pakistan (Figure 1A). The proband (II-1) was evaluated by taking a medical history and performing biochemical tests at a local government hospital. Consent in written form was obtained from the participants for the genetic analysis, and the University of Management and Technology (UMT), Lahore, Pakistan Institutional Review Board approved the study in compliance with the Helsinki Declaration. Blood samples were collected and processed further for DNA extraction and quantification using standard methods (16,17). WES was performed using DNA from the proband (IV-1). WES and variants filtering steps were performed as described earlier (18-21). Standard-screening principles were used to search for different functional variants associated with the patient phenotype (22). The genes already reported in the Online Mendelian Inheritance in Man (OMIM) (Table 1) and literature (PUBMED) were given priority. Prioritized disease-causing variants were Sanger sequenced for segregation analysis (23,24). The pathogenic nature of the identified variant was calculated using different tools. ExAC, in-house 175 exomes and genomAD were searched to see if the variant is reported in the general population (25,26). Conservation of amino acid was determined using HomoloGene (National Center for Biotechnology Information). The partial amino acid sequence of IQCE, the encoding protein, was retrieved from the UniProt database with accession number P78357-1. The IQCE model was examined/evaluated and, after that, selected according to the obtained evaluation score provided by I-TASSER and MODELLER (27,28).

Results

Clinical examination

The proband (age 4 years) is a boy born to consanguineous parents that revealed bilateral PAP in hands and feet (Figure 1B). The extra digits were well-developed. Samples were attained from all the available family members. Hand/digit photographs were provided by the index (II-2). Hands and feet X-rays revealed underdeveloped carpals, metacarpals, and similarly underdeveloped tarsals and mete tarsals. No associated abnormality was observed, such as kidney stones, eye deformity, obesity, or hypogonadism. Syndactyly, facial dysmorphism and nail deformity was not observed. Physical examination demonstrated that the other finger originated from the fifth metacarpal. Later, the extra digits were removed surgically.

Molecular analysis

Using WES, we identified a previously reported splice site variant in the *IQCE* gene associated with PAPA-AR. The identified variant is a bi-allelic splice acceptor site variant (c.395-1G>A) in the intron 5 of the *IQCE* (NM_152558.5) located on chromosome 7p22.3-7p22.3 (Figure 1C). The variant was Sanger sequenced and segregated using standard protocols (Figure 1C). The variant was not observed in a homozygous state in an internal database, ExAC, gnomAD, and was predicted deleterious by several tools.

3D structure prediction

Using homology modeling, three-dimensional models of wild type and mutated IQCE protein (p.Gly132Valfs*22) were predicted and assessed using online structure analysis tools (Figure 2A and B). 3D protein modeling showed substantial changes and reduction of key

Table 1. *Genes associated with non-syndromic PAP.*

Genes	Disease	Inheritance	Locus	OMIM
GLI3	PAPA1	AD	7p14.1	174200
Unknown	PAPA2	AD	13q21-q32	602085
Unknown	PAPA3	AD	19p13.2-p13.1	607324
Unknown	PAPA4	AD	7q22	608562
Unknown	PAPA5	AR	13q13.3-q21.2	263450
ZNF141	PAPA6	AR	4p16.3	615226
IQCE	PAPA7	AR	7p22.3	617642
GLI1	PAPA8	AR	12q13.3	618123
FAM92A	PAPA9	AR	8q22.1	618219
KIAA0825	PAPA10	AR	5q15	618498
DACH1	PAPA11	AR	13q2133	603803

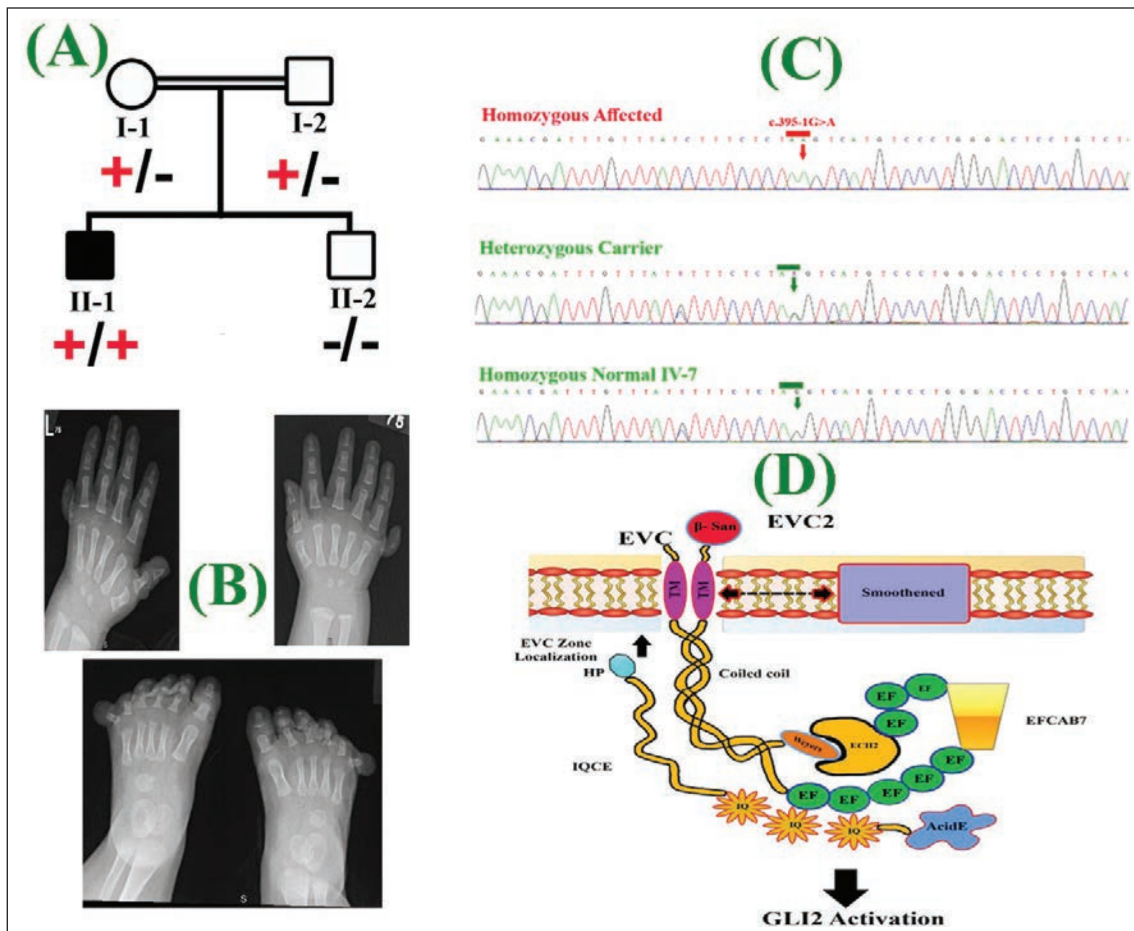


Figure 1. *(A) Pedigree of the family showing AR pattern of inheritance. (B) Xrays of the proband (II-1). (C) Sanger electrograms of the affected, carrier and wildtype. (D) Schematic representation of IQCE with EFCAB7 that intern interacts with EVC/EVC2 proteins that ultimately regulate the HH signalling pathway responsible for limb patterning and development.*

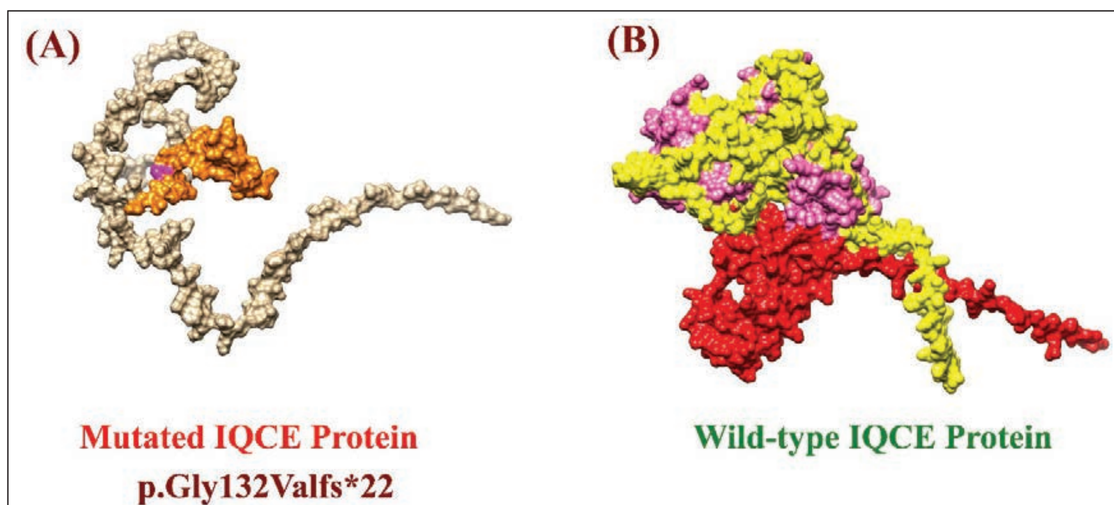


Figure 2. *IQCE protein modelling. (A) IQCE^{Mutated} structure showing the complete reduction. (B) IQCE$^{Wild\text{-}type}$ structure.*

domains in the mutated IQCE protein secondary structure compared to the wild-type IQCE.

Discussion

Herein, we used clinical and molecular methods to characterize a proband having bilateral non-syndromic PAPA without syndactyly. However, previously the same variant has been associated with PAPA-restricted lower limbs only. Later, it was confirmed that variants in *IQCE* cause PAPA in both upper and lower limbs (29). WES data analysis revealed an already reported bi-allelic variant (c.395-1G>A) in the *IQCE* gene. Variants in

Table 2. *PAPA types and associated clinical phenotypes.*

Title	PAPA1	PAPA6	PAPA7	PAPA8	PAPA9	PAPA10	PAPA11
Gene	*GLI3* - 165240	*ZNF141* - 194648	*IQCE* - 617631	*GLI1* - 165220	*CIBAR1* - 617273	*KIAA0825* - 617266	*DACH1- 603803*
Inheritance	Autosomal dominant	Autosomal recessive	Autosomal recessive	Autosomal recessive	Autosomal recessive	Autosomal recessive	Autosomal recessive
Skeleton	***Hands*** - PAP(bilateral, sometimes extra digits are well formed and articulated) - Preaxial polydactyly (bilateral or unilateral) - Triphalangeal thumb - Syndactyly - Broad thumbs ***Feet*** - PAP - Preaxial polydactyly - Syndactyly	***Hands*** - Fifth finger duplication, well-formed - Broad fifth finger, unilateral or bilateral - Deviation of fifth finger, radial or ulnar, to variable degree - Duplicated distal phalanx of fifth finger - Small central phalanx of fifth finger (in some patients) ***Feet*** - Fifth toe duplication, well-formed	***Hands*** -PAP, bilateral - Brachydactyly ***Feet*** - PAP, unilateral or bilateral - Thick, broad, 2-headed fifth metatarsal - Cutaneous 2-3 toe syndactyly - Brachymetatarsia of fifth toe	***Hands*** - PAP - No duplication of metacarpals ***Feet*** - PAP	***Hands*** - PAP ***Feet*** - PAP	***Hands*** -PAPtype A -PAP, type B (rare) ***Feet*** -PAP, type A	***Hands*** -PAP, type A ***Feet*** -PAP, type A

IQCE have been previously associated with PAPA7, and the identified variant has been validated using minigene splice assay (8), showing deletion of G nucleotide from exon 6. This deletion results in frameshift and premature stop codon (p.Gly132Valfs*22) that might lead to small IQCE protein or mRNA nonsense-mediated decay.

Using patients' fibroblasts, the RNA expression analysis revealed that IQCE pathogenesis results in the dysregulation of several genes associated with the HH-signaling pathway. Furthermore, knock-out zebrafish trials revealed astonishing phenotypes associated with cilia dysregulation, such as left-right asymmetry, body curvature issues, misdirected cilia in the pronephric duct, kidney cysts, and retinal defects (29). Thus, suggesting the key role of IQCE in the ciliatory development and regulating genes associated with the HH pathway. As disease-causing variants in the Ellis-Van Creveld (EVC) and EVC2 are associated with EVC syndrome in humans. One of the phenotypes in EVC patients is PAP. The disorder is characterized by severe skeletal deformities, including PAP, cardiac anomalies, and facial dimorphism (3). EVC/EVC2 makes a complex with the smoothened, frizzled class receptor (SMO) and interacts with IQCE/EFCAB7 at the base of primary cilia associated with the activation of GLI2, which further causes HH signaling activation (Figure 1D). Thus, EVC is mostly caused by impaired HH signaling pathways. EVC/EVC2 inactivation does not affect the SMO phosphorylation or ciliary accumulation; however, it affects the GLI ciliary activation and localization. This suggests a key role of IQCE in the downstream HH signaling cascades (30). The discovery of cilia involvement has improved our in-depth knowledge regarding the HH signaling pathway. Still, we lack a precise understanding of how these newly identified players/genes, such as *FAM92A*, *KIA0825*, and *DACH1*, interact and how these key proteins are associated with cilia trafficking and how their dis-regulation leads to abnormal limb patterning.

Polydactyly in humans is a genetically and phenotypically heterogeneous disorder, as polydactyly is linked with syndromic and non-syndromic phenotypes. Syndromic types constitute 496 disorders searched in OMIM, including severe disorders such as Split-Hand/Foot Malformation, EVC, neurodevelopmental disorders, syndactyly, and many more (18,31-33). However, non-syndromic types are few, but they help us understand the prevalence of the variants in a population and help us understand the pathophysiology of the disorder in detail. Thus, identifying novel genes implicated in congenital limb abnormalities is important to understand limb development in humans and help manage associated syndromic disorders. In addition, proper genetic counseling of the family having severe skeletal disorders might help eradicate the disorder in future poignancies. In addition, introducing the newborn screening program in a developing country like Pakistan will be the first step in screening some severe genetic disorders. Parenteral diagnosis can play a major role in reducing the burden of such severe disorders (34,35). This can be accomplished by prenatal genetic testing for monogenetic disorders (PGT-M). PGT and in vitro fertilization are options for parents wishing to have future pregnancies (36,37). In conclusion, we have presented an association of a splice site variant in *IQCE* with an isolated PAP in humans. This information will help researchers understand the intricate signaling cascades needed for proper limb orientation and development and will also help them prevent the pathogenesis of limb deformities.

List of Abbreviations

GLI	Glioma-associated oncogene family zinc finger 1
HH	Hedgehog
IQCE	IQ domain-containing protein E
OMIM	Online Mendelian Inheritance in Man
PAPA	Postaxial polydactyly type A
SMO	Smoothened, frizzled class receptor

Funding

None.

Declaration of conflicting interests

The authors of this article have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

Ethical approval

Ethical approval was granted by the Institutional Research Board of the UMT, Lahore, Pakistan.

Author details

Muhammad Bilal[1], Muhammad Raheel[2], Gul Hassan[3], Shah Zeb[4,5], Arif Mahmood[6], Zamrud Zehri[7], Hafiza Yasmin Manzoor[8], Muhammad Umair[9]

1. Department of Biochemistry, Faculty of Biological Sciences, Quaid-i-Azam University, Islamabad, Pakistan
2. Department of Microbiology, Shah Abdul Latif University, Khairpur, Pakistan
3. Department of Biochemistry, Shah Abdul Latif University, Khairpur, Pakistan
4. Institute for Advanced Study, Shenzhen University, Shenzhen, People's Republic of China
5. College of Physics and Optoelectronics Engineering, Shenzhen University, Shenzhen, People's Republic of China
6. Center for Medical Genetics and Hunan Key Laboratory of Medical Genetics, School of Life Sciences, Central South University, Changsha, China
7. Shaheed Nawab Ghous Bakhsh Raisani Memorial Hospital, Mastung, Pakistan
8. Department of Laboratory, Carle Foundation Hospital, Urbana, IL, USA
9. Department of Life Sciences, School of Science, University of Management and Technology (UMT), Lahore, Pakistan

References

1. Ahmad Z, Liaqat R, Palander O, Bilal M, Zeb S, Ahmad F, et al. Genetic overview of post-axial polydactyly: updated classification. Clin Genet. 2023;103(1):3–15. https://doi.org/10.1111/cge.14224
2. Ullah A, Ali RH, Majeed AI, Liaqat K, Shah PW, Khan B, et al. A novel insertion and deletion mutation in the BHLHA9 underlies polydactyly and mesoaxial synostotic syndactyly with phalangeal reduction. Eur J Med Genet. 2019;62(4):278–81. https://doi.org/10.1016/j.ejmg.2018.08.005
3. Umair M, Seidel H, Ahmed I, Ullah A, Haack TB, Alhaddad B, et al. Ellis-van Creveld syndrome and profound deafness resulted by sequence variants in the EVC/EVC2

and TMC1 genes. J Genet. 2017;96(6):1005–14. https://doi.org/10.1007/s12041-017-0868-6

4. Alfadhel M, Umair M, Almuzzaini B, Asiri A, Al Tuwaijri A, Alhamoudi K, et al. Identification of the TTC26 Splice variant in a novel complex ciliopathy syndrome with biliary, renal, neurological, and skeletal manifestations. Mol Syndromol. 2021;12(3):133–40. https://doi.org/10.1159/000513829

5. Ullah A, Khalid M, Umair M, Khan SA, Bilal M, Khan S, et al. Novel sequence variants in the MKKS gene cause Bardet-Biedl syndrome with intra- and inter-familial variable phenotypes. Congenit Anom (Kyoto). 2018;58(5):173–5.

6. Umair M, Palander O, Bilal M, Almuzzaini B, Alam Q, Ahmad F, et al. Biallelic variant in DACH1, encoding Dachshund homolog 1, defines a novel candidate locus for recessive post-axial polydactyly type A. Genomics. 2021;113(4):2495–502. https://doi.org/10.1016/j.ygeno.2021.05.015

7. Umair M, Ahmad F, Bilal M, Ahmad W, Alfadhel M. Clinical genetics of polydactyly: an updated review. Front Genet. 2018;9:447. https://doi.org/10.3389/fgene.2018.00447

8. Umair M, Shah K, Alhaddad B, Haack TB, Graf E, Strom TM, et al. Exome sequencing revealed a splice site variant in the IQCE gene underlying post-axial polydactyly type A restricted to lower limb. Eur J Hum Genet. 2017;25:960–5. https://doi.org/10.1038/ejhg.2017.83

9. Hayat A, Umair M, Abbas S, Rauf A, Ahmad F, Ullah S, et al. Identification of a novel biallelic missense variant in the KIAA0825 underlies post-axial polydactyly type A. Genomics. 2020;112(4):2729–33. https://doi.org/10.1016/j.ygeno.2020.03.006

10. Umair M, Wasif N, Albalawi AM, Ramzan K, Alfadhel M, Ahmad W, et al. Exome sequencing revealed a novel loss-of-function variant in the GLI3 transcriptional activator 2 domain underlies non-syndromic post-axial polydactyly. Mol Genet Genomic Med. 2019;7(7):e00627. https://doi.org/10.1002/mgg3.627

11. Umair M, Ahmad F, Ahmad S, Alam Q, Rehan M, Alqosaibi AI, et al. A novel homozygous missense mutation in the zinc finger DNA binding domain of GLI1 causes recessive post-axial polydactyly. Front Genet. 2021;12:746949. https://doi.org/10.3389/fgene.2021.746949

12. Ullah A, Umair M, Majeed AI, Abdullah, Jan A, Ahmad W. A novel homozygous sequence variant in GLI1 underlies first case of autosomal recessive preaxial polydactyly. Clin Genet. 2019;95:540–1. https://doi.org/10.1111/cge.13495

13. Bilal M, Ahmad W. A frameshift variant in KIAA0825 causes postaxial polydactyly. Mol Syndromol. 2021;12:20–4. https://doi.org/10.1159/000512062

14. Umair M, Ahamd F, Bilal M, Asiri A, Younus M, Khan A. A comprehensive review of genetic skeletal disorders reported from Pakistan: a brief commentary. Meta Gene. 2019;20:100559. https://doi.org/10.1016/j.mgene.2019.100559

15. Umair M, Ahmad F, Ullah A. Whole exome sequencing as a diagnostic tool for genetic disorders in Pakistan. Pak J Med Res. 2018;57 (2):90–1.

16. Mahmood A, Samad A, Shah AA, Wadood A, Alkathiri A, Alshehri MA, et al. A novel biallelic variant in the popeye domain-containing protein 1 (POPDC1) underlies limb girdle muscle dystrophy type 25. Clin Genet. 2022. https://doi.org/10.1111/cge.14238

17. Umair M, Rafique A, Ullah A, Ahmad F, Ali RH, Nasir A, et al. Novel homozygous sequence variants in the GDF5 gene underlie acromesomelic dysplasia type-grebe (AMDG) in consanguineous families. Congenit Anom (Kyoto). 2016;57(2):45–51.

18. Waqas A, Nayab A, Shaheen S, Abbas S, Latif M, Rafeeq MM, et al. Case report: biallelic variant in the tRNA methyltransferase domain of the AlkB homolog 8 causes syndromic intellectual disability. Front Genet. 2022;13:878274. https://doi.org/10.3389/fgene.2022.878274

19. Umair M, Ballow M, Asiri A, Alyafee Y, Al Tuwaijri A, Alhamoudi KM, et al. EMC10 homozygous variant identified in a family with global developmental delay, mild intellectual disability, and speech delay. Clin Genet. 2020;98(6):555–61. https://doi.org/10.1111/cge.13842

20. Waqas A, Liaqat R, Shaheen S, Khan AZ, Mujahid, Habib AH, et al. A novel homozygous truncating variant in PPFIBP1 further delineates PPFIBP1-associated neurodevelopmental disorder. Int J Dev Neurosci. 2022. https://doi.org/10.1002/jdn.10247

21. Ahmad F, Ahmed I, Alam Q, Ahmad T, Khan A, Ahmad I, et al. Variants in the PNPLA1 gene in families with autosomal ecessive congenital ichthyosis reveal clinical significance. Mol Syndromol. 2021;12(6):351–61. https://doi.org/10.1159/000516943

22. Khan A, Bruno LP, Alomar F, Umair M, Pinto AM, Khan AA, et al. SPTBN5, encoding the βV-spectrin protein, leads to a syndrome of intellectual disability, developmental delay, and seizures. Front Mol Neurosci. 2022;15:877258. https://doi.org/10.3389/fnmol.2022.877258

23. Khan A, Wang R, Han S, Umair M, Alshabeeb MA, Ansar M, et al. A novel homozygous nonsense mutation p.Cys366* in the WNT10B gene underlying split-hand/split foot malformation in a consanguineous Pakistani family. Front Pediatr. 2020;7:526. https://doi.org/10.3389/fped.2019.00526

24. Khan S, Rawlins LE, Harlalka GV, Umair M, Ullah A, Shahzad S, et al. Homozygous variants in the HEXB and MBOAT7 genes underlie neurological diseases in consanguineous families. BMC Med Genet. 2019;20(1):199. https://doi.org/10.1186/s12881-019-0907-7

25. Khan A, Miao Z, Umair M, Ullah A, Alshabeeb MA, Bilal M, et al. Two cases of recessive intellectual disability caused by NDST1 and METTL23 variants. Genes (Basel). 2020;11(9):1021. https://doi.org/10.3390/genes11091021

26. Almatrafi A, Umair M, Eldardear A, Al-Luqmani M, Hashmi JA, et al. A homozygous missense variant in the homeobox domain of the NKX6-2 results in progressive spastic ataxia type 8 associated with lower limb weakness and neurological manifestations. J Gene Med. 2020;22(8):e3196. https://doi.org/10.1002/jgm.3196

27. Nayab A, Alam Q, Alzahrani OR, Khan R, Sarfaraz S, Albaz AA, et al. Targeted exome sequencing identified a novel frameshift variant in the PGAM2 gene causing glycogen storage disease type X. Eur J Med Genet. 2021;64(9):104283. https://doi.org/10.1016/j.ejmg.2021.104283

28. Ahmad F, Ahmed I, Nasir A, Umair M, Shahzad S, Muhammad D, et al. A disease-causing novel missense mutation in the ST14 gene underlies autosomal recessive ichthyosis with hypotrichosis syndrome in a

consanguineous family. Eur J Dermatol. 2018;28(2):209–16. https://doi.org/10.1684/ejd.2017.3210

29. Estrada-Cuzcano A, Etard C, Delvallée C, Stoetzel C, Schaefer E, Scheidecker S, et al. Novel IQCE variations confirm its role in post-axial polydactyly and cause ciliary defect phenotype in zebrafish. Hum Mutat. 2020;41(1):240–54. https://doi.org/10.1002/humu.23924
30. Pusapati GV, Hughes CE, Dorn KV, Zhang D, Sugianto P, Aravind L, et al. EFCAB7 and IQCE regulate hedgehog signaling by tethering the EVC-EVC2 complex to the base of primary cilia. Dev Cell. 2014;28(5):483–96. https://doi.org/10.1016/j.devcel.2014.01.021
31. Umair M, Hayat A. Non-syndromic split-hand/foot malformation: recent classification. Mol Syndromol. 2020;10(5):243–54. https://doi.org/10.1159/000502784
32. Ullah A, Gul A, Umair M, Irfanullah, Ahmad F, Aziz A, et al. Homozygous sequence variants in the WNT10B gene underlie split hand/foot malformation. Genet Mol Biol. 2018;41(1):1–8. https://doi.org/10.1590/1678-4685-gmb-2016-0162
33. Umair M, Ahmad F, Bilal M, Abbas S. Syndactyly genes and classification: a mini review. JBCGenetics. 2018;1(1):10–8. https://doi.org/10.24911/JBCGenetics/183-1532177257
34. Alfadhel M, Umair M, Almuzzaini B, Alsaif S, AlMohaimeed SA, Almashary MA, et al. Targeted SLC19A3 gene sequencing of 3000 Saudi newborn: a pilot study toward newborn screening. Ann Clin Transl Neurol. 2019;6(10):2097–103. https://doi.org/10.1002/acn3.50898
35. Alyafee Y, Alam Q, Altuwaijri A, Umair M, Haddad S, Alharbi M, et al. Next-generation sequencing-based pre-implantation genetic testing for aneuploidy (PGT-A): first report from Saudi Arabia. Genes (Basel) 2021;12(4):461. https://doi.org/10.3390/genes12040461
36. Alyafee Y, Al Tuwaijri A, Alam Q, Umair M, Haddad S, Alharbi M, et al. Next generation sequencing based non-invasive prenatal testing (NIPT): first report from Saudi Arabia. Front Genet. 2021;12:630787. https://doi.org/10.3389/fgene.2021.630787
37. Alyafee Y, Al Tuwaijri A, Umair M, Alharbi M, Haddad S, Ballow M. Non-invasive prenatal testing for autosomal recessive disorders: a new promising approach. Front Genet. 2022;13:1047474. https://doi.org/10.3389/fgene.2022.1047474

Journal of Biochemical and Clinical Genetics

ORIGINAL ARTICLE

Opinion of geneticist regarding performing preimplantation genetic testing for monogenic disorder for variants of unknown significance

Reema Alduaiji[1], Laila Alqahtani[1], Reema Alqadiri[1], Lena Alotaibi[1], Mostafa Abolfotouh[2], Majid Alfadhel[1,2,3*]

ABSTRACT

Background: Preimplantation genetic testing (PGT) is used to identify a pathogenic variant in embryos created through *in vitro* fertilization. A "variant of uncertain significance" (VOUS) is a genetic variant discovered through genetic testing but with unknown clinical significance. The primary goal is to gauge geneticists' perspectives on performing PGT-M for VOUS in Saudi Arabia, which results in the development of recommendations from higher authorities regarding the criteria of PGT-M in clinical practice.

Methods: After reviewing the literature, a cross-sectional study was conducted employing questionnaire developed using survey monkey. The reliability of the questionnaire was assessed in terms of internal consistency and Cronbach's alpha-assessed test-retest.

Results: In particular, a total of 96 Saudis and non-Saudis, male and female geneticists, agreed to participate in the study. Out of the 96 geneticists, 56 (59.6%) were female. Most participants were of Saudi origin, with a percentage of (76.6%). The most important finding of this study is that 64% of geneticists opposed performing PGT-M for VOUS. The outcome that 94.5% of geneticists concurred that PGT-M is poorly understood was another noteworthy finding.

Conclusion: Future research with a larger sample size is required for performing PGT-M for VOUS, which will help in developing guidelines for PGT-M in Saudi Arabia.

Keywords: PGT-M, VOUS, a variant of uncertain significance, preimplantation genetic testing, variant, Saudi Arabia, geneticists.

Introduction

When one or both of the parents have a known genetic abnormality and are at high risk of inheriting it from their offspring, Preimplantation genetic testing for monogenic disorders (PGT-M) or its previous term preimplantation genetic diagnosis (PGD) is one of the options to prevent the recurrence of the disease in future pregnancies (1). PGT-M is a technique that identifies a pathogenic variant in the early developing embryos created through *in vitro* fertilization before pregnancy (1). The idea behind PGT-M is to prevent those couples from having another affected child with a similar genetic condition, increasing the chance of a successful pregnancy (1). The use of PGT-M is limited worldwide due to a lack of expertise in the field, insufficient guidelines, and ethical dilemmas. A recent study in the USA demonstrated that many laboratories have limitations because of ethical considerations regarding PGT-M (2). In Saudi Arabia, the first report of next-generation sequencing (NGS)-based PGT for aneuploidy was published in March 2021, which indicates that PGT-M is rarely used. To perform

Correspondence to: Majid Alfadhel
*College of Medicine, King Saud Bin Abdulaziz University for Health Sciences (KSAU-HS), Ministry of National Guard Health Affairs (MNG-HA), Riyadh, Saudi Arabia.
Email: dralfadhelm@gmail.com
Full list of author information is available at the end of the article.

Received: 20 February 2023 | **Accepted:** 07 March 2023

OPEN ACCESS
This is an open access article distributed in accordance with the Creative Commons Attribution (CC BY 4.0) license: https://creativecommons.org/licenses/by/4.0/) which permits any use, Share — copy and redistribute the material in any medium or format, Adapt — remix, transform, and build upon the material for any purpose, as long as the authors and the original source are properly cited. © The Author(s).

PGT-M, *in vitro* fertilization, the IVF technique is used to create embryos. Cleavage-stage embryo biopsy is the most common approach to extract a single blastomere from a developing embryo to remove an intact cell with a careful approach not to affect the remaining embryo. Then, DNA will be extracted from this single blastomere and checked for the specific mutation (1-4). Numerous reasons support an indication of PGT-M. For example, a previously affected pregnancy, a genetic condition in the couple, and advanced maternal age (5). A "variant of uncertain significance" (VOUS) is a genetic variant discovered through genetic testing but whose relevance is unknown. According to the American College of Medical Genetics recommendations, genetic variants are classified into five categories based on the quantity and quality of evidence required to categorize the variant as pathogenic, likely pathogenic, VOUS, likely benign, or benign. Suppose a variant is classified as a VOUS. In that case, it signifies insufficient information to identify whether or not the variant is linked to disease at the time of interpretation (5,6). In perinatal genetics, several molecular genetic testing could be used in addition to PGT-M to prevent the recurrence of the diseases in a future pregnancy. For example, chromosomal microarray (CMA) and non-invasive perinatal testing (NIPT), which are based on NGS technology, allow the detection of chromosomal abnormalities either through amniocentesis or chorionic villus sampling in case of CMA or maternal blood in case of NIPT (7,8). Protecting human life is one of the main basic principles in Islam, in other words, encouraging the prevention of any predictable diseases. The government of Saudi Arabia is based on Islamic Sharia. Therefore, the government of Saudi Arabia supports any procedure that helps in starting a healthy family through PGT-M, which aims to prevent any known familial mutation that would affect a fetus (7). There is an ethical dilemma regarding using PGT-M genetic testing in VOUS by clinicians. An ongoing ethical debate about recommending whether to perform PGT-M for VOUS suspected cases has been occurring in the geneticist community (9). This study aims to assess the opinion of geneticists around Saudi Arabia in performing PGT-M for VOUS and help formulate recommendations from high authorities regarding criteria for performing PGT-M in clinical practice for VOUS.

Subjects and Methods

A cross-sectional questionnaire-based study was conducted involving hospitals across Saudi Arabia (Figure 1). The research committee of King Abdullah International Medical Research Center (KAIMRC), Riyadh, Saudi Arabia, approved all procedures and were performed in accordance with the ethical standards. Concerning the physical distancing strategy and minimizing face-to-face interaction, we developed an outline questionnaire via Survey Monkey (https://www.surveymonkey.com) that limits one-time participation per unique internet protocol (IP) address. This questionnaire was sent to a sample of Saudi Arabian geneticists via Social media groups. The inclusion criteria were Saudi and non-Saudis, male and female geneticist physicians who agreed to participate in the study. Exclusion criteria were lack of access to the internet, inability to complete an online survey, and non-geneticist physicians. Ninety-six participants agreed to participate and responded with a complete questionnaire. After reviewing the literature, a self-administered questionnaire was designed to have a validated tool (10). The survey was distributed to the participants, who are geneticists from all over Saudi Arabia hospitals. To assess the opinion of geneticists and develop a guideline regarding PGD for VOUS about preimplantation genetic diagnosis, it was a self-administered questionnaire-based survey in English as a soft copy done in Saudi Arabia. Moreover, it would increase awareness about existing problems and ethical issues related to VOUS in the field of PGD. The reliability of the questionnaire was assessed in terms of internal consistency. Cronbach's

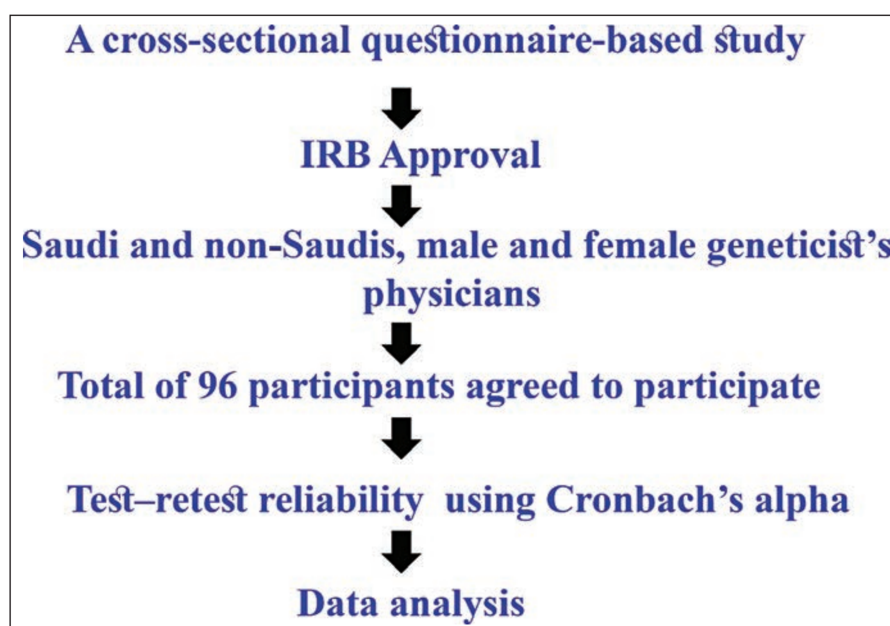


Figure 1. *Flow sheet diagram showing the methodology steps.*

alpha was computed. Test–retest reliability was also assessed using Cronbach's alpha Construct validity of the checklist was assessed using expert opinion, and the final version was approved accordingly after pilot testing. The independent variables were the demographics of the respondents, such as age, experience level, and genetic specialty. Other independent variables were taken to test the geneticist's knowledge level and opinion regarding VOUS and PGT-M. Using a scale of 1 to 3, 1 = Disagree, 2 = Neutral, 3 = Agree. Data entry and analysis were carried out using the statistical program Statistical Package for the Social Sciences version 24. Descriptive analysis was done for categorical variables such as genetic specialty and opinions of geneticists towards PGT-M and VOUS were reported as percentages and frequencies in tables. In addition, tables represented numerical variables such as age and years of experience as percentages and frequencies.

Results

In our study, a survey that asses the opinion of geneticists on performing PGT-M for VOUS was conducted on 96 participants. Most participants were of Saudi ethnicity, with a percentage (76.6%). The participating geneticists consisted of 59.6% females and 40.4% males. As shown in Table 1, 84.8% answered the class of VOUS correctly. The main dilemma is needing more knowledge and evidence regarding performing PGT-M for monogenic diseases for VOUS. Of the 96 geneticists (86), 94.5% agreed with this statement. One of the key findings was that (55) 64.7% of the geneticists were against performing PGT-M for VOUS. However, the majority of the geneticist (75), 82.5%, will perform PGT-M only based on the parents' opinion. In addition, we found (65) 82.3% of the geneticist agreed with the right of patients to choose whether or not to be informed of their PGT-M carrier status.

Moreover, (90) 96.8% of the geneticist agreed on the need for informed consent to perform PGT-M for VOUS. On the other hand, (48) 52.2% agreed that it is only needed if the parents insist on performing it. All geneticists agreed with counseling couples on the ethical issues and psychological stresses of PGT-M. When performing PGT-M for VOUS, (92) 98.9% agreed that they need to include in genetic counseling the risk of having a baby with genetic disorders whether they do PGT-M or not. Furthermore, (85) 96% of the geneticists believed that patients of reproductive age and their families who are at risk should be counseled on IVF-PGT-M as early as possible to maximize their chances of conception. To prevent the transmission of genetic abnormality from a genetic mutation carrier to their offspring, carrier parents can choose to perform IVF-PGT-M, supported by (86) 95.6% of the geneticists. Additionally, (72) 83.7% of the geneticists were in favor that carriers of genetic mutations preferred PGT-M to prenatal testing to decide on terminating the pregnancy or not. Moreover, (69) 88.1% of geneticists supported the claim of children born after PGT-M seem as healthy as children delivered after natural conception or other forms of conception such as IVF or ICSI treatments (Table 2).

Table 1. Demographic data in numbers and percentages.

	No	Percentage
Male	38	(40.4)
Female	56	(59.6)
Saudi	72	(76.6)
Non Saudi	22	(23.4)
Experience (in years)		
<10	48	(51.1)
10-<20	30	(31.9)
20-<30	13	(13.8)
>30	3	(3.2)

Discussion

The most remarkable finding in this study is that the opinion of geneticists was significantly against performing PGT-M for VOUS. Ultimately, this led to the geneticists deciding to go with the parents' opinion on whether or not to perform PGT-M. Detailed genetic counseling regarding performing PGT-M for VOUS and its expected psychological stresses is necessary for the parents. Ultimately, this led to the geneticist's decision to go with the parents' opinion on whether or not to perform PGT-M. In addition, geneticists have agreed on the need to mention that whether or not PGT-M is performed, it will not guarantee a genetic disorder-free embryo. A study suggests that the parents should be counseled about the ethical issues, psychosocial stress, procedural limitations, possible results, and its application before performing PGT-M for VOUS (11). Moreover, PGT-M has many advantages over other prenatal testing methods. One is that it helps carriers of genetic mutation avoid the difficult decision of terminating an affected pregnancy or giving birth to a sick child. Another advantage of PGT-M, in the opinion of most geneticists, is that children born using this method seem as healthy as children born to other forms of conception. Another study suggests a great advantage of PGT-M, specifically its ability to detect chromosomal aneuploidy in the embryos and transfer normal chromosomal embryos in order to achieve a healthy and normal pregnancy (3). Furthermore, almost all geneticists agreed on the need for informed consent to perform PGT-M for VOUS. The usage of PGT-M has been widely popular over the past three decades. PGT-M was developed for various genetic conditions and severe disorders, and there are three major disease groups which PGT-M is used for. The first group is sex-linked disorders, such as Rett Syndrome. The second is single gene defects and genetic mutations such as BRCA-1. In addition, the third group PGT-M can help in diagnosing chromosomal disorders (12). Moreover, the PGT-M laboratory must be appropriately insured against the possibility of a misdiagnosis. Even though it is censorious that PGT-M is performed using tests that have been verified and tailored for the couple, there have been multiple incidents of misdiagnosis. Misdiagnosis can occur due to sample-specific factors, such as chromosomal mosaicism in the embryo. It could be a

Table 2. Responses to perception of geneticists towards PGD in numbers and percentages.

Statements	AG	NS	DA
1. PGD is an early form of genetic testing and, when combined with IVF, enables gestation of only unaffected embryos.	83 (91.2)	5 (5.5)	3 (3.3)
2. There is lack of knowledge and evidence regarding performing PGD for monogenic diseases (PGT-M) for variants of unknown significance (VOUS).	86 (94.5)	4 (4.4)	1 (1.1)
3. I will not perform PGD for monogenic diseases (PGT-M) for variants of unknown significance (VOUS).	55 (64.7)	25 (29.4)	5 (5.9)
4. Informed consent is needed to perform PGD for monogenic diseases (PGT-M) for variants of unknown significance (VOUS).	90 (96.8)	2 (2.2)	1 (1.1)
5. I will go with the opinion of the parents, after detailed genetic counseling to perform PGD for monogenic diseases (PGT-M) for variants of unknown significance (VOUS).	75 (82.5)	10 (11.0)	6 (6.6)
6. Patients at risk of a genetic disorder have the right to choose not to know of their carrier status. Non-disclosure PGD or exclusion PGD can enable this while offering the option of conceiving mutation-free children who will not go through similar emotional turmoil.	65 (82.3)	10 (12.7)	4 (5.1)
7. Informed consent is needed only in case the parents insist on doing PGT-M.	48 (52.8)	21 (23.1)	22 (24.2)
8. Couples should be counseled on ethical issues relevant to their PGD and the expected psychological stresses during the decision-making process and the IVF–PGD treatment	92 (100)		
9. When performing PGT-M for VOUS you need to include in detailed genetic counseling there is a risk of having a baby with genetic disorders whether you do PGT-M or not	92 (98.9)	1 (1.1)	
10. Patients who are at risk and/or family members at reproductive age should be counseled on IVF–PGD as an option to conceive healthy children, as early as possible to maximize their chances of conception	85 (96)	2 (2.3)	1 (1.1)
11. Most carriers of genetic mutations opt for PGD over prenatal testing to avoid facing the difficult decision of whether or not to terminate an affected pregnancy or to give birth to a sick child	72 (83.7)	9 (10.5)	5 (5.8)
12. A patient carrying a known genetic mutation or chromosomal abnormality can choose to use IVF–PGD to prevent transmission of the genetic abnormality to their offspring and future generations	86 (95.6)	4 (4.4)	
13. Thousands of children born after PGD seem as healthy as those delivered after natural conception or after IVF and/or ICSI treatments only for infertility	69 (88.1)	7 (9.0)	2 (2.6)

technique-specific issue, such as maternal or paternal contamination or allele dropout (13,14). On the other hand, many are unaffected by the method. System failures could include mistakes in labeling and misidentification of tagged samples. The most crucial factor is abstaining from and eliminating human error or system failure. Proper genetic counseling for the affected family is essential for rare hereditary diseases. In addition, the best approach for treating such a condition, which has no treatment, is parenteral genetic screening/diagnosis (15,16). Similarly, in Saudi Arabia, newborn screening (NBS) of infants between 24 and 72 hours of birth can avoid disability and possibly even death by checking for conditions advised by the national Newborn Screening Committee. The NBS program aims to identify infants born with specific genetic, metabolic, and functional abnormalities (17). Future treatment studies may be aided by identifying genes-variants linked to a given condition from a particular population (18,19). The main limitation of this study was the literature gap, particularly in PGT-M for VOUS. Therefore, this presents the need for further development in research about PGT-M for VOUS in the field of genetics.

Conclusion

This study showed that 64% of geneticists were against performing PGT-M for VOUS. Moreover, most agreed that there is a lack of knowledge about PGT-M due to a lack of guild lines and a lack of research regarding PGT-M. Therefore, future research is needed regarding ethical considerations of PGT-M and its implication. It will help higher authorities develop the guild lines for PGT-M regarding VOUS usage in clinical practice in Saudi Arabia. Furthermore, improving the psychosocial impact on the couple performing PGT-M for VOUS is crucial.

List of Abbreviation

CMA Chromosomal microarray
IVF In vitro fertilization
KAIMRC King Abdullah International Medical Research Center
NIPT Non-invasive perinatal testing
PGD Preimplantation genetic diagnosis
PGT Preimplantation genetic testing
PGT-M Preimplantation genetic testing for monogenic disorders
VOUS Variant of uncertain significance

Acknowledgments
We thank all participants who took the time and contributed valuable contributions to this project.

Funding
None.

Declaration of conflicting interests
The authors of this article have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

Ethical approval
The study was approved by the Institutional Review Board of KAIMRC, Riyadh, Saudi Arabia (Memo Ref. No. IRBC/0999/ 21).

Author details
Reema Alduaiji[1], Laila Alqahtani[1], Reema Alqadiri[1], Lena Alotaibi[1], Mostafa Abolfotouh[2], Majid Alfadhel[1,2,3]
1. College of Medicine, King Saud Bin Abdulaziz University for Health Sciences (KSAU-HS), Ministry of National Guard Health Affairs (MNG-HA), Riyadh, Saudi Arabia
2. Genetics and Precision Medicine Department, King Abdullah Specialized Children Hospital, King Abdulaziz Medical City, Ministry of National Guard Health Affairs (MNG-HA), Riyadh, Saudi Arabia
3. Medical Genomics Research Department, King Abdullah International Medical Research Center (KAIMRC), King Saud Bin Abdulaziz University for Health Sciences (KSAU-HS), Ministry of National Guard Health Affairs (MNG-HA), Riyadh, Saudi Arabia

References

1. Dhanjal S, Kakourou G, Mamas T, Saleh N, Doshi A, Gotts S, et al. Preimplantation genetic diagnosis for retinoblastoma predisposition. Br J Ophthalmol. 2007;91(8):1090–1. https://doi.org/10.1136/bjo.2006.108597
2. Porto A, Gaber Caffrey R, Crowley-Matoka M, Spencer S, Li M, Propst L. Offering preimplantation genetic testing for monogenic disorders (PGT-M) for conditions with reduced penetrance or variants of uncertain significance: ethical insight from U.S. laboratory genetic counselors. J Genet Couns. 2022;31(1):261–8. https://doi.org/10.1002/jgc4.1482
3. Alyafee Y, Alam Q, Tuwaijri AA, Umair M, Haddad S, Alharbi M, et al. Next-generation sequencing-based pre-implantation genetic testing for aneuploidy (PGT-A): first report from Saudi Arabia. Genes (Basel). 2021;12(4):461. https://doi.org/10.3390/genes12040461
4. Huang CC, Chang LJ, Tsai YY, Hung CC, Fang MY, Su YN, et al(2013). A feasible strategy of preimplantation genetic diagnosis for carriers with chromosomal translocation: using blastocyst biopsy and array comparative genomic hybridization. J Formosan Med Assoc = Taiwan has been,112(9), 537–44. https://doi.org/10.1016/j.jfma.2013.02.010
5. Chang LJ, Chen SU, Tsai YY, Hung CC, Fang MY, Su YN, et al. An update of preimplantation genetic diagnosis in gene diseases, chromosomal translocation, and aneuploidy screening. Clin Exp Reprod Med. 2011;38(3):126–34. https://doi.org/10.5653/cerm.2011.38.3.126
6. Blueprint Genetics. VUS - the most maligned result in genetic testing. [cited 2020 Oct 20]. Available from: https://blueprintgenetics.com/resources/vus-the-most-maligned-result-in-genetic-testing/
7. Nigro V, Piluso G. Next generation sequencing (NGS) strategies for the genetic testing of myopathies. Acta Myol. 2012;31(3):196–200.
8. Levy B, Wapner R. Prenatal diagnosis by chromosomal microarray analysis. Fertil Steril. 2018;109(2):201–12. https://doi.org/10.1016/j.fertnstert.2018.01.005
9. Nussbaum R, McInnes R, Willard H, Hamosh A. Thompson JS, Thompson MW. Thompson & Thompson genetics in medicine. Philadelphia, PA: Elsevier/Saunders; 2016.
10. Sultan H, Harper JC. Legalization and Islamic bioethical perspectives on prenatal diagnosis and advanced uses of pre implantation genetic diagnosis in Saudi Arabia. J Clin Res Bioeth. 2013;01S1:2. https://doi.org/10.4172/2155-9627.S1-003
11. Angell RR, Xian J, Keith J. Chromosome anomalies in human oocytes in relation to age. Hum Reprod. 1993;8(7):1047–54. https://doi.org/10.1093/oxfordjournals.humrep.a138190
12. Rotshenker-Olshinka K, Srebnik Moshe N, Weiss O, Shaviv S, Freireich O, Segel R, et al. Preimplantation genetic testing (PGT) for copy number variants of uncertain significance (CNV- VUS) in the genomic era: to do or not to do?. J Assist Reprod Genet. 2021;38(3):719–25. https://doi.org/10.1007/s10815-020-02055-3
13. SenGupta SB, Dhanjal S, Harper JC. Quality control standards in PGD and PGS. Reprod Biomed Online. 2016;32(3):263–70. https://doi.org/10.1016/j.rbmo.2015.11.020
14. Wilton L, Thornhill A, Traeger-Synodinos J, Sermon KD, Harper JC. The causes of misdiagnosis and adverse outcomes in PGD. Hum Reprod. 2009;24(5):1221–8. https://doi.org/10.1093/humrep/den488
15. Alyafee Y, Al Tuwaijri A, Alam Q, Umair M, Haddad S, Alharbi M, et al. Next generation sequencing based non-invasive prenatal testing (NIPT): first report from Saudi Arabia. Front Genet. 2021;12:630787. https://doi.org/10.3389/fgene.2021.630787
16. Alyafee Y, Al Tuwaijri A, Umair M, Alharbi M, Haddad S, Ballow M, et al. Non-invasive prenatal testing for autosomal recessive disorders: a new promising approach. Front Genet. 2022;13:1047474. https://doi.org/10.3389/fgene.2022.1047474
17. Alfadhel M, Umair M, Almuzzaini B, Alsaif S, AlMohaimeed SA, Almashary MA, et al. Targeted SLC19A3 gene sequencing of 3000 Saudi newborn: a pilot study toward newborn screening. Ann Clin Transl Neurol. 2019;6(10):2097–103. https://doi.org/10.1002/acn3.50898
18. Alfadhel M, Nashabat M, Saleh M, Elamin M, Alfares A, Al Othaim A, et al. Long-term effectiveness of carglumic acid in patients with propionic acidemia (PA) and methylmalonic acidemia (MMA): a randomized clinical trial. Orphanet J Rare Dis. 2021;16(1):422. https://doi.org/10.1186/s13023-021-02032-8
19. Alfadhel M, Abadel B, Almaghthawi H, Umair M, Rahbeeni Z, Faqeih E, et al. HMG-CoA lyase deficiency: a retrospective study of 62 Saudi patients. Front Genet. 2022;13:880464. https://doi.org/10.3389/fgene.2022.880464

REVIEW ARTICLE

The landscape of acid sphingomyelinase deficiency in a new therapeutic era: insights from experts in the Gulf region

Moeenaldeen AlSayed[1], Fatma Al-Jasmi[2,3], Tawfeg Ben Omran[4], Fathiya Al-Murshedi[5], Rawda Sunbul[6], Nadia Al-Hashmi[7], Talal Al-Enazi[8]

ABSTRACT

Acid sphingomyelinase deficiency (ASMD) is an autosomal-recessive progressive multiorgan metabolic disorder due to pathogenic variants in the sphingomyelin phosphodiesterase 1 gene. It can lead to death in early childhood in its most severe form. According to previous registries, the birth prevalence of ASMD is nearly 0.4-0.6 per 100,000 live births. The diagnosis of ASMD is usually delayed or missed due to the wide variability of clinical manifestations of the disease. Until recently, the management of ASMD patients was based on symptomatic treatments and supportive care; however, the introduction of enzyme replacement therapy (ERT) has revolutionized the management landscape of ASMD. ERT with a recombinant human Acid Sphingomyelinase Enzyme administered intravenously demonstrated a significant improvement in the non-neuronopathic type of ASMD in phase 2/3 trials. In June 2022, the European Medical Agency granted the ERT, olipudase alfa, marketing authorization. The prevalence of inherited metabolic disorders, including lysosomal storage diseases, is relatively higher in the Arab world than in the rest of the world due to the high consanguinity rate. In this study, we aim to review the current landscape of ASMD in the Gulf Cooperation Council countries and gather insights from experts regarding the roadmap to diagnosis, prevalence, and management approaches of ASMD in the region.

Keywords: Acid sphingomyelinase deficiency, lysosomal storage diseases, enzyme replacement therapy, Niemann-Pick disease, acid sphingomyelinase enzyme.

Introduction

Acid sphingomyelinase deficiency (ASMD), also known as Niemann-Pick disease (NPD), is historically divided into two main phenotypes: Niemann-Pick disease type A (NPD-A, OMIM 257200) – a rapidly progressing and fatal neuronopathic disorder, and Niemann-Pick disease type B (NPD-B, OMIM 607616) – a chronic non-neuronopathic, slowly progressive, visceral disorder (1). An intermediate neurovisceral phenotype, called NPD A/B, with intermediate symptoms between NPD-A and NPD-B, is also reported in the literature. The condition is characterized by a progressive, debilitating course that affects multiple organs and can lead to death in early childhood in its most severe form (2). According to the previous registries, the birth prevalence of ASMD is nearly 0.4-0.6 per 100,000 live births (3). ASMD is caused by pathogenic variants in the sphingomyelin phosphodiesterase 1 (*SMPD1*) gene, leading to variable degrees of deficiency of lysosomal acid sphingomyelinase (ASM) enzyme in all tissues. Subsequently, sphingomyelin and other lipids accumulate in the mononuclear phagocytic system and hepatocytes, with secondary impairment in tissue function, organomegaly, and multiple organ failure (4).

ASMD is broadly classified into three phenotypes that vary in the course and clinical presentation. Infantile neurovisceral is the most severe phenotype of ASMD (NPD-A), characterized by progressive neurodegeneration, hepatosplenomegaly, and lung infiltration in early infancy due to severe ASM deficiency (5). While in chronic neurovisceral ASMD (NPD-A/B), the neurological involvement occurs slower than in infantile neurovisceral, leading to better survival. Lastly,

Correspondence to: Moeenaldeen AlSayed
*King Faisal Specialist Hospital & Research Center, Riyadh, Saudi Arabia.
Email: moeen@kfshrc.edu.sa
Full list of author information is available at the end of the article.
Received: 17 January 2023 | **Accepted:** 07 November 2023

OPEN ACCESS This is an open access article distributed in accordance with the Creative Commons Attribution (CC BY 4.0) license: https://creativecommons.org/licenses/by/4.0/) which permits any use, Share — copy and redistribute the material in any medium or format, Adapt — remix, transform, and build upon the material for any purpose, as long as the authors and the original source are properly cited. © The Author(s).

chronic visceral ASMD (NPD-B) is characterized by a variable age of onset and slow progression resulting in multisystem affection in the absence of neurological impairment (6). Therefore, the diagnosis of ASMD is usually delayed or missed (particularly in chronic visceral form) due to the wide variation in the clinical presentation of the disease. The diagnosis is confirmed by reduced or absent ASM enzyme activity measured in peripheral blood leukocytes and cultured skin fibroblasts. Dried blood spots (DBS) can be used to measure ASM enzyme activity as a practical screening tool. However, low activity should be confirmed by measurement in peripheral blood leukocytes or cultured skin fibroblasts or/and the presence of a pathogenic variant in both alleles in the *SMPD1* gene (6,7).

Despite the progressive nature of ASMD, symptomatic treatment and supportive care remained the only available management options for affected patients. However, the introduction of enzyme replacement therapy (ERT) has significantly changed the management landscape of the disease (8). Currently, ERT with a recombinant human ASM Enzyme administered intravenously demonstrated a significant improvement in the non-neurological manifestations of ASMD in phase 2/3 trials (8). In addition, ERT showed its safety in adults with chronic ASMD (9). A recent phase 1/2 study demonstrated ERT's tolerability and disease-modifying effects on children with chronic ASMD (10). Based on such results, ERT has been granted breakthrough therapy designation submission by the Food and Drug Administration. In June 2022, the European Medical Agency granted the ERT, olipudase alfa, marketing authorization. Thus, experts from the present report stated that the results of ERT are promising, and its anticipated introduction to clinical practice is likely to advance the management of ASMD. The observed improvements in the ASMD outcomes in clinical trials are expected to be translated into clinical benefits in the real-world setting and advance the treatment landscape of ASMD management. However, multinational collaboration with a larger pool of patients and longer follow-ups are necessary to confirm ERT's long-term clinical benefits. The prevalence of rare metabolic disorders, such as lysosomal storage diseases (LSDs), is presumed to be comparatively higher in the Arab world than in the rest of the world due to the high rate of consanguineous marriages (11). In this report, we aim to review the current landscape of ASMD in the Gulf Cooperation Council (GCC) countries and gather insights from experts regarding the roadmap to diagnosis, prevalence, and management approaches of ASMD in the region.

Methodology

On Friday, Nov 12, 2021, an experts' meeting was held to gather the insights and recommendations of the GCC's experts concerning the current prevalence of ASMD in the region and the unmet medical needs in terms of disease diagnosis and management. The panel consisted of seven experts with comprehensive experience managing rare metabolic diseases. It was affiliated with academic or healthcare institutions within the GCC countries: Kingdom of Saudi Arabia (KSA), Qatar, Oman, and the United Arab Emirates (UAE). The panel represented current practices in different healthcare sectors across the GCC countries. During the preparation of this manuscript, we conducted a comprehensive literature search to retrieve the current evidence for ASMD epidemiology, diagnosis, and management from the following databases: Medline via PubMed, Scopus, Embase, ScienceDirect, ClinicalKey, Cochrane library, and the known guideline organizations and societies guidelines websites, such as the International Niemann-Pick Disease Registry project (www.inpdr.org). The online search covered these databases from their inception till June 2022.

Epidemiology of ASMD

Although ASMD is a pan-ethnic disorder and affects both sexes similarly, the accurate prevalence is unknown. Globally, the estimated birth prevalence is 4-6 per 1,000,000 live births, while the global prevalence is estimated to be 1 in every 250,000 individuals (12). Nonetheless, it is suggested that these figures underestimate the actual burden of ASMD since there are many undiagnosed cases, in addition to the lack of awareness about the disease in the medical community, especially in primary healthcare settings (3,7). Furthermore, the current body of evidence shows differences in the incidence of ASMD according to ethnicity; the highest birth prevalence is found in the Ashkenazi Jewish community, estimated to be 2:3 per 100,000 births for NPD-A (1). Besides, geographical disparities were also noted concerning the epidemiology of ASMD. In South America, the incidence of ASMD was approximately 1 per 500,000 live births (13), compared to 1 per 250,000 live births in Europe (14). In addition, the age of presentation of ASMD varies according to its subtypes, while the incidence of the disease shows slight female predominance (15).

Rare genetic disorders are presumably more prevalent amongst the Arab population than in other parts of the world. Previous reports hypothesized that the high rate of consanguinity (up to 60%) is a significant contributing factor to the comparatively high prevalence of genetic disorders in the Arab world (16).

Only a few reports investigated the incidence and prevalence of ASMD in the Arab world; nonetheless, they showed a comparatively higher prevalence of ASMD than in other parts of the world. Moammar et al. (17) retrospectively retrieved the data of 165,130 live births in the Eastern region of KSA over 25 years. A total of eight cases with NPD type A were identified, given an incidence rate of 5 per 100,000 live births. The overall incidence of LSDs was 44 per 100,000 live births. In another report from the Eastern region of KSA that covered the period from 1983 to 2016, the prevalence of NPD type A/B was 3.31 per 100,000 live births (18). According to Alfadhel et al. (19) the incidence of LSDs was 37 per 100,000 live births at a tertiary center in KSA over 13 years, while one case with NPD type B was identified out of 110,601 live births. In the UAE, Al-Jasmi et al. (20) reviewed LSD cases at the metabolic referral centers to determine the birth prevalence of LSDs and associated genetic variants. The birth prevalence of NPD type B was 0.25

per 100,000, while there were no reported cases of NPD type A. The incidence of LSDs in Oman was reported to be 1 per 2,318 live births (21). In a 2021 systematic review of genetic disorders in Tunisia, the prevalence of ASMD type B was reported to be 1 per 200,000 live births (22). From the above studies, we can conclude that the incidence of ASMD in Arab countries appears to be higher than in western countries.

However, the reports mentioned above were limited to one geographical area and lacked generalizability to the GCC countries' entire population. Besides, most of the studies were retrospective and carried the limitations of misclassification and ascertainment bias. Thus, there is a need for a regional study with multi-center collaboration to truly reflect the actual incidence and prevalence of ASMD in the GCC countries. Besides, a national newborn screening program is required to precisely reflect the current burden of ASMD and other LSDs in the GCC countries.

Experts' opinion

The panel emphasized the lack of national registries and reliable newborn screening programs that can accurately estimate the incidence of ASMD in the GCC countries. Experts from Qatar stated that they have 12 patients with confirmed ASMD in their institution's database (four type A, two type A/B, and six type B). All of them are Qatari, and there are three siblings among them. At the same time, experts from Oman reported three patients with ASMD type B. In UAE, there are four patients with ASMD type B and one with ASMD type A. Concerning KSA. The advisors reported nine confirmed ASMD patients in KSA; two cases have ASMD type A, while the remaining seven have ASMD type B. The experts agreed on the scarcity of published literature that assesses the prevalence of ASMD in the GCC region. They confirmed that many ASMD patients might be undiagnosed; hence, the currently limited figures underestimate the true prevalence of ASMD in the region. Several factors account for this underestimated figure, including the lack of national registries for ASMD in the GCC countries, the lack of newborn screening programs for LSDs in many GCC countries, and limited awareness of the presentation of ASMD among healthcare providers.

Pathogenesis of ASMD and Associated Genetic Variants

ASMD is genetically inherited and results primarily from bi-allelic variants in the *SMPD1* gene presented in the chromosome 11 Band (11p15.4), encoding for ASM enzyme (23), responsible for catalytic hydrolysis of sphingomyelin to ceramide and phosphocholine inside the Lysosomes, which in turn leads to deficiency or impairment of ASM enzyme activity (24). As a result, progressive accumulation of sphingomyelin and other lipids occurs in the brain and other organs, such as the spleen, liver, lung, and bone marrow. The abnormal increase in sphingomyelin in different cells results in damage to related tissues and multiple organ involvement (6). In pathological examinations, the presence of foam cells, which are large lipid-laden cells, in the examined tissues of the affected organ is suggestive of ASMD (6).

ASMD is classified into three phenotypes (A, A/B, B) according to the disease's clinical manifestations, neurological degeneration, and severity. *SMPD1* gene variants reflect the severity of the disease, which in turn correlates with ASM residual enzyme activity (4).

Over 720 distinct known pathogenic variants causing ASMD have been described on the *SMPD1* gene, including frameshift, missense, nonsense, and frame deletions. These variants differ according to geographical variations (24), and the genotype-phenotype correlation is known for some variants (25). To illustrate, three known variants in the SMPD1 gene (R496L, L302P, and fsP330) are commonly present in Ashkenazi Jewish ancestry and represent over 90% of the ASMD patients in this population. The three variants are associated with ASMD phenotype A (26). The *SMPD1* variant (A359D) in Chilean patients was associated with ASMD phenotype B (14). ASMD phenotype A/B is associated with p.W393G and p.Q294K genetic variations (27,28)

In North Africa, (R610del) variant is the most prevalent *SMPD1* variant correlated with a mild form of ASMD phenotype B without neurological impairment (29,30). Moreover, the two alleles (677delT and R608) correlate with severe progressive ASMD phenotype A in Israeli Arabs and ASMD phenotype B in northern Africa, respectively (28,30).

Presentation and Subtypes

Type A

The most severe form of ASMD is NPD-A, also known as infantile neurovisceral ASMD (5). ASM Enzyme activity in this type is deficient to non-existent (31). Early infancy symptoms include failure to thrive, hypotonia, neurodegeneration, dysphagia, hepatosplenomegaly, and pulmonary involvement (2). On the other hand, there is no cardiac or musculoskeletal involvement. Based on the severity of the disease, psychomotor development may generally proceed for several months after birth before plateauing between the ages of 6 and 15 months and regressing. Death usually occurs before 3 years and is caused by respiratory failure due to infection (2).

Type A/B

Compared to infantile neurovisceral ASMD, NPD A/B, also known as chronic neurovisceral ASMD, has a slower progression of neurological symptoms and a longer life expectancy (28,32). It is an intermediate form of ASMD with childhood onset and is characterized by learning disabilities, psychiatric symptoms, extrapyramidal signs, peripheral neuropathy, and ataxia (5). In addition, it presents macular halo, diarrhea, abnormal liver function tests, portal hypertension, liver fibrosis, and hepatosplenomegaly (2). Thrombocytopenia and bleeding tendency are the predominant hematologic signs in these patients (5). Reported cardiac and pulmonary manifestations include early-onset coronary artery disease, mixed dyslipidemia, cardiac valve disease,

interstitial lung disease, abnormal pulmonary function tests, and radiological findings (33-35). Previous reports showed that patients with chronic visceral ASMD showed pulmonary nodules, reticular or reticulonodular patterns, and, eventually, honeycombing (35,36). Moreover, growth restriction, delayed bone maturation, reduced bone density, and bone and joint pain are commonly seen (37). Premature death can occur during childhood or adulthood due to respiratory and liver disease (38,39).

Type B

NPD-B, or chronic visceral ASMD, occurs at any time from infancy to adulthood and is marked by a gradual progression of multisystem disease symptoms without neurodegeneration (4,40). NPD-B is associated with an average life span; however, disease complications such as bleeding, liver failure, and respiratory failure can induce premature death (38,39). Hepatosplenomegaly is the most frequent first symptom in early childhood; however, moderate illness may not be detected until maturity (5). Delays in puberty, tiredness, bone pain, osteopenia, thrombocytopenia, and anemia are common clinical findings in children (37). Pulmonary infections and interstitial lung disease are prevalent, and pulmonary function can deteriorate with time (35,4). Mixed dyslipidemia is observed early in the disease course, and some individuals develop coronary artery disease (34,41). Hepatic fibrosis, ranging from mild to severe, is common, and the worsening of liver disease can lead to early death in some patients (42,43). Progressive portal hypertension and sphingomyelin deposition can lead to progressive splenomegaly (5).

There is considerable overlap in the clinical presentation between ASMD and Gaucher disease. Gaucher disease is the most common LSD. It is an autosomal recessive disorder due to a deficiency of the lysosomal enzyme, known as glucocerebrosidase, resulting in the progressive accumulation of glucosylceramide. The birth incidence is estimated at 1 per 40,000 to 1 per 60,000 live births. Pathogenesis of Gaucher disease is related to genetic variations in the *GBA1* gene. Clinical presentations of Gaucher disease include; hepatosplenomegaly with or without hypersplenism, thrombocytopenia, bony lesions, and osteopenia, in addition to neurological manifestations in type 2 and 3. These symptoms are also common with ASMD. Therefore, differentiation between the two disorders should be integrated as an essential component of the diagnostic algorithm of ASMD (44,45). Thus, parallel testing of ASM and acid β-glucosidase (the deficient enzyme in Gaucher disease) may be recommended to distinguish between the two diseases.

Experts' opinionThe panel agreed that the distribution of the ASMD subtypes in the GCC countries is similar to the global figures. The presentation of the patients with ASMD from the experts' institutions is reported in Table 1.

Natural History

The findings of the natural history studies are summarized in Table 2.

Hepatosplenomegaly and liver function

Wasserstein et al. (40) assessed 29 patients with NPD-B and reported that among patients with NPD-B, the mean volume of the spleen was 12.7 multiples of normal (MN), and the mean volume of the liver was 1.91 MN. In addition, two patients were subjected to splenectomy due to hypersplenism. Similar to these findings, McGovern et al. (46) , who included 59 NPD-B patients in their study, found that 73% and 78% of the patients had hepatomegaly and splenomegaly, respectively, and presented with a mean volume of spleen of 11.1 ± 5.7 and a mean liver volume of 1.9 MN ± 0.7. Four patients had a total splenectomy, and one had a partial splenectomy. They highlighted that spleen volume was negatively correlated with white blood cell count ($r = -0.47$; $p < 0.001$), hemoglobin ($r = -0.33$; $p = 0.02$), height Z-score ($r = -0.51$; $p = 0.0001$), high-density lipoprotein (HDL) ($r = -0.62$; $p < 0.001$), and positively correlated with triglyceride level ($r = 0.55$; $p < 0.001$) and liver volume ($r = 0.76$; $p < 0.001$). The liver volume correlated positively with the serum level of aspartate transaminase (AST) ($r = 0.64$; $p < 0.001$) and alanine transaminase (ALT) ($r = 0.60$; $p < 0.001$) (46).

Another study by McGovern et al. (38,47) showed that all patients with NPD-B had splenomegaly, 7.7% underwent total splenectomy, 1.9% underwent partial splenectomy, and 8.7% of the patients had liver diseases such as liver failure and cirrhosis. They proposed that splenectomy is an independent risk factor for mortality in patients with NPD-B.

According to Hollak et al. (4) all patients with NPD-A had hepatosplenomegaly. In contrast, in the NPD-B group, all patients had splenomegaly, 90% had hepatomegaly, and one patient underwent splenectomy due to severe cytopenia. Cassiman et al. (39) showed that 82.6% of patients with NPD-B and A/B had liver dysfunction, 91.4% had hepatomegaly, and 96.6% had splenomegaly. Likewise, Cox et al. (48) reported that hepatosplenomegaly was more frequent in patients with NPD-A (92%), followed by NPD-A/B (83%) and NPD-B (80%).

Regarding the liver function test, patients with NPD-B were noted to have elevated transaminase (ALT and AST) levels with normal bilirubin levels, except for one patient who died due to liver dysfunction. They observed that bilirubin levels tended to be higher in adults compared with children ($p = 0.01$) (40). In around half of the patients in the McGovern study (46), ALT and AST were increased, whereas total bilirubin was raised in a third. Similar findings were reported by Hollak et al. (4) who found that 2/3 and 19/20 patients with NPD-A and NPD-B, respectively, had elevated levels of liver enzymes, which was more apparent in young patients and those with severe illness.

Lipid abnormalities

Wasserstein et al. (40) showed that the majority of NPD-B patients had low levels of HDL cholesterol, borderline to high triglycerides (TG), low-density lipoprotein (LDL) cholesterol, and total cholesterol (TC). The worst lipid profile was observed in males and adults. In the study

Table 1. *Clinical presentation of the patients with ASMD from the GCC countries.*

Country	No.	Category	No.	Age	Clinical presentation of the patients with ASMD	Outcome
Qatar	12	ASMD A	4	Not reported	Not reported	All died
		ASMD A/B	2	6 years 7 years	Both have neurological manifestations (developmental delay and seizures).	Alive
		ASMD B	6	30 years old 23-years old The remaining are children	The 30-year-old female has a mild disease. She usually develops thrombocytopenia and anemia, mainly during pregnancy, and she has hepatosplenomegaly, and high cholesterol, mainly managed by diet. Her brother is 23 years old; he has the same features. Other patients have no reported features.	Alive
Oman	3	ASMD B	3	11 years old 17 years old 7 years old	***First case's manifestations:*** At the age of 4 months, hepatosplenomegaly was incidentally diagnosed with a chest infection during admission. Currently, he has microcephaly, a learning disability, and hyperactivity. No ataxia, no focal neurological signs, and no autoregression. Diagnosed with interstitial lung disease with low Diffusing capacity for carbon monoxide (DL_{CO}). Required Continuous positive airway pressure at night. He has recurrent epistaxis, elevated transaminases, and mild hyperlipidemia He has macular changes with electroretinography evidence of maculopathy Short stature and kyphosis that is slowly worsening with time. Homozygous with *SMPD1* variant: p.Ala481Val ***Second case's manifestations:*** A 17-year-old female Incidental finding of hepatosplenomegaly at the age of 2 years. She has a history of recurrent epistaxis. Otherwise, she has normal growth and development Normal PFTsLaboratory findings include mild thrombocytopenia, elevated transaminases, and a normal lipid profile. Homozygous for *SMPD1* variant p.Ala415Val ***Third case's manifestations:*** The patient is almost 7 years old and was born in 2015 He presented at the age of 5 months with abdominal distention and was found to have massive hepatosplenomegaly with anemia and elevated liver enzymes, mainly AST. He has no other significant clinical symptoms and is currently stable. He is homozygous with *SMPD1* variant: p.Leu382Phe	Alive

Continued

Country	No.	Category	No.	Age	Clinical presentation of the patients with ASMD	Outcome
UAE	4	ASMD B	4	Adults	They have dyslipidemia and hepatosplenomegaly. One of the patients has mild thrombocytopenia. They have very minimal lung disease.	Alive
	1	ASMD A	1	8 months	The patient presented with a recurrent respiratory infection, global developmental delay On examination, the patient failed to thrive, hypotonia, hepatosplenomegaly, hydrocele, and reducible inguinal hernia. Eye examination showed bilateral cherry red spot. A liver biopsy showed foamy cellular changes and microvesicular steatosis. Diagnosis confirmed with low enzyme activity and *SMPD*1 homozygous (c.762delG)	Lost to follow up
KSA	9	ASMD A	2	1.5 years old. Few months age-old	***The first:*** The patient has massive hepatosplenomegaly. He also has a developmental delay, nystagmus, and bilateral cherry-red spots. Additionally, he has seizure attacks that are not controlled with multiple antiepileptic medications. He was in ICU for a long time. ***The second:*** He presented with developmental delay. He had recurrent hyperactive airway disease and CNS manifestations. Later, he needed recurrent admissions and died because of recurrent chest infections.	Alive, and the second died.
		ASMD B	7	2.5 years old Two patients were 7 years old.	One patient has no CNS manifestations; he has massive hepatosplenomegaly, dyslipidemia, low cell count, complex heart disease (truncus arteriosus, large patent ductus arteriosus, significant ventricular septal defect). One patient and his sister have hepatosplenomegaly and ascites with hyperlipidemia. In two patients, one has abdominal distension, gastroenteritis, and hepatosplenomegaly, while the other is asymptomatic. Two patients had slow disease progression. However, one has a more severe disease.	Alive

Table 2. *Main findings of the studies reporting the natural history of ASMD.*

Variables	Cox et al. (48) (*n* = 100)	McGovern et al. (2) (*n* = 10)	McGovern et al. (46) (*n* = 59)	McGovern et al. (38) (*n* = 103)	Wasserstein et al. (40) (*n* = 29)	Hollak et al. (4) (*n* = 25)
Country	Brazil, Canada, USA	USA	Brazil, France, Germany, Italy, USA	USA	USA	The Netherlands and Belgium
Phenotype, No.						
Infantile neurovisceral	13	10	0	0	0	4
Chronic neurovisceral	6	0	0	8	0	6
Chronic visceral	81	0	59	95	29	15
Features	HS, GI disorders, respiratory disorders, infections	HS, GI symptoms, respiratory symptoms	HS, respiratory infections, ILD, bleeding	HS, TCP, bleeding, ILD, liver disease	HS, TCP, atherogenic lipid profile, pulmonary disease	HS, ILD, TCP
Natural history						
Infantile neurovisceral	--	NR	NR	NR	NA	--
Chronic neurovisceral	--				NA	Gradual decrease in platelet count in some patients
Chronic visceral	Reduction in platelet counts, WBC Increase total bilirubin				Progressive hypersplenism worsening atherogenic profile gradual deterioration in pulmonary function decrease in platelet decrease in WBC counts	Gradual decrease in platelet count Decreased bone marrow fat fractions in chronic visceral disease
Mortality						
Infantile neurovisceral	76.9%	100.0%	NA	NA	NA	100.0%
Chronic neurovisceral	0.0%	NA	NA	87.5%	NA	0.0%
Chronic visceral	2.5%	NA	NR	11.6%	10.3%	33.3%

GI: gastrointestinal; HS: hepatosplenomegaly; ILD: interstitial lung disease; NA: not applicable; NR: not reported; TCP: thrombocytopenia; WBC: white blood cell

of McGovern et al. (46) lipid profile abnormalities were common in NPD-B patients; low HDL-C (74%), high TC (41%), high TG (62%), and high LDL-C (46%). On the other hand, Hollak et al. (4) study that included a group of patients with NPD-A, A/B, and B reported that 12/16 patients had low HDL-C values at baseline; however, throughout follow-up, HDL-C levels steadily fell in two patients and remained steady in the others. LDL cholesterol levels were lower in five cases and higher in one. Adults and children, intermediate and attenuated phenotypes, and sex were shown to have no significant differences.

Moreover, Wasserstein et al. (40) mentioned that statins, exercise, and dietary modifications could improve TG, TC, and LDL-C serum levels. However, these interventions should be applied with caution, as patients with severe NPD-B are associated with deterioration in transaminase levels and pulmonary function after administering statins. Moreover, the risk of coronary artery disease is substantially higher in this group of patients, as the serum levels of HDL-C tended to be low in all age groups and did not respond to the previously mentioned interventions.

Pulmonary studies

The lung is a target organ of the disease and contributes to morbidity and mortality in patients with ASMD (38). Accumulation of foamy macrophages, interstitial fibrosis, and endogenous lipid pneumonia are the most common findings in lung biopsies in adults with NPD-B (49). The pulmonary function tests (PFTs) results indicated a restricted pattern of lung involvement with impaired diffusing capacity, which is consistent with interstitial lung disease. Many NPD-B patients are asymptomatic; however, those more seriously affected may have cyanosis, recurring respiratory infections, shortness of breath, cough, clubbing, and rhonchi (40). In the study of McGovern et al. (46,47) interstitial lung disease and pulmonary dysfunction were reported in 66% of the patients with NPD-B. An increase in the number of patients with abnormal PFT over time, and the fact that older patients with NPD-B were associated with lower mean PFT values compared to younger patients, support the progressive nature of the pulmonary disease in this group (40). In the study of Hollak et al. (4) there is no data on pulmonary function for NPD-A patients; however, variable degrees of restrictive lung disease and impaired CO-diffusion were found in the 21 NPD-B patients. Pulmonary involvement was reported to be higher in patients with NPD-B versus. NPD-A/B (79.2% *vs.* 65.0%), according to Cassiman et al. (39). In NPD-B patients, pulmonary dysfunction is linked to a higher risk of respiratory infections, leading to respiratory failure (39). Recurrent respiratory tract infections and persistent lung function deterioration can worsen the quality of life (48,50-41).

Cardiac studies

McGovern et al. (46) showed that 28% of patients with NPD-B had ECG abnormalities, including conduction abnormalities, left ventricular hypertrophy, and sinus bradycardia. Moreover, two patients had a history of myocardial infarction (MI). Regarding echocardiography, half of the patients showed abnormalities such as pulmonary hypertension, moderate to severe aortic regurgitation, mild ventricular dysfunction, and mild mitral valve regurgitation. The same group of investigators published another report that showed a much lower prevalence (8.7%) of cardiac diseases in patients with NPD-B, in the form of valvular heart disease and coronary artery disease (38). In contrast to pulmonary, cardiac involvement was more frequent in NPD-A/B patients than in NPD-B (62.5% *vs.* 39.2%) respectively (39).

Hematologic indices

Platelets and leukocyte count decrease as patients age, indicating the natural course of hematologic problems, although hemoglobin concentration remained constant. The diminishing cell numbers are most likely attributed to hypersplenism. Infection, particularly in the respiratory system, and bleeding episodes are two clinical implications of progressive leukopenia and thrombocytopenia (40). In patients with NPD-A, 4/5 patients had anemia; however, thrombocytopenia was less common in this group of patients. While in the NPD-B, 15/18 patients had thrombocytopenia, and 6/18 patients had anemia, mainly reported in intermediate phenotype and young patients (4). Hematologic manifestations were reported to be more prevalent in NPD-B patients than in NPD-A/B (Anemia 69.2% *vs.* 57.1%; thrombocytopenia 74.1% *vs.* 50%), respectively (44).

Genotype/phenotype correlations

Homozygosity for missense variants such as P330R, P323A, and R608 was linked to mild disease. On the other hand, moderate and severe patients were presented with a combination of heterozygotes for R608 and another variant, indicating that the other missense genetic variations, such as H425K, W391G, R600H, R441X, R496L, P475L, and H567L, are more hazardous (40). Similarly, McGovern et al. (46) reported that the most frequent variant in NPD-B patients was R608 (48%), and the most common type of genetic lesion was missense (59%). They also mentioned that the R608 variant is associated with non-neuronopathic and milder disease (38). Another report found that the most common variants were p.R610del, p.R476W, p.R498L, p.R476W, p.M1T, and p.Q294K (47).

The p.Arg610del variant was detected in 61.9% of individuals with NPD-B in the study of Hollak et al. (4) and 62% in Lidove et al. (52). This genetic variation was consistently linked to NPD-B disease in both compound heterozygous variant and homozygous forms; in this study, 12 of the 25 patients were of Dutch or Belgian ancestry, 7 patients were Turkish, 2 from Morocco, 2 from Tunisia, 1 from Iraq, and 1 of Surinamese descent (4). In the Cassiman study (39), which included a literature review of 85 patients, the most common variant in patients with NPD-B was p.R610del and p.A359D, while in NPD-A/B, the most common variant was p.Q274R. The mild p.L163P variant was found in two patients

with NPD-B, confirming the variant's mild nature. Two sisters were homozygous for the p.R230C variant (4), previously found in a group of NPD-B patients (53). Both had a severe illness history with substantial lung disease but no neurological signs. Both died early, the first from respiratory failure and the second post hematopoietic stem cell transplantation (4).

Other variants were also reported to be linked with NPD-B disease, including p.L551P, p.C91H, and p.L103P variants, while in NPD-A, p.Y539H, p.P477L, p.F465S, p.G29DfsX48, and p.S250R variants were reported. Regarding patients with NPD-A/B, the most reported variants were p.R230C, p.L105P, p.C91H, p.R610del, p.L474QfsX20, and p.V557IfsX19 variants (4).

Neurological and ophthalmological findings

According to McGovern et al. (46) all patients with NPD-B had normal mental development, coordination, muscle strength, sensation, deep tendon reflexes, and cranial nerve function. Peripheral neuropathy was reported in 11%, 25% had cherry-red spots, and 8.4% had cognitive impairment. In another report by McGovern et al. (38) neurological diseases in the form of sensory dyspraxia, learning disabilities, petit mal seizures, and ataxia were recorded in 12.6% of NPD-B patients. Cherry red spots were recorded in 1 out of 4 patients with NPD-A and 1 out of 21 patients with NPD-B in the study of Hollak et al. (4). Furthermore, they reported that severe neurological manifestations also led to early death in 4 out of 5 patients with NPD-A. In patients with NPD-B, 4 out of 21 patients had neurological manifestations, including mental retardation, Parkinson's disease, peripheral facial palsy, multiple sclerosis, and delayed psychomotor development (4). It was reported that neurologic and ophthalmic involvement was significantly higher in patients with NPD-A/B than in NPD-B patients (68.8% *vs.* 33.3%; $p = 0.032$ and 55.6% *vs.* 15.7%; $p > 0.0001$, respectively). Also, patients 18 years old or younger at the time of death or liver transplant had more significant neurological symptoms ($p = 0.048$) (39).

Mortality

In the study of Wasserstein et al. (40) three patients died from ages 9 to 18 years due to liver failure, traumatic subdural hematoma, and long-term complications post a failed bone marrow transplant. Four patients with NPD-A died due to severe neurological manifestations, subdural hematoma, malignant edema, and pneumonia, according to Hollak et al. (4) Five NPD-B patients died, three due to progressive pulmonary disease related to ASMD, one of malignancy and one of unknown cause. The McGovern et al. study reported 18 deaths in patients with NPD-B. Respiratory failure/Pneumonia ($n = 5$) were the leading causes of mortality. Three patients died of liver failure, while three others died of complications post-bone marrow transplant. Subdural bleeding, multi-organ failure, low-output heart failure, liver cancer, splenic vein tear, and postoperative bleeding were among the mortality reasons in one patient each (38). In a retrospective analysis of 100 patients, 12 patients were deceased; ten patients with NPD-A/B died at a mean age of 2.4 years from hepatic failure ($n = 1$), pneumonia ($n = 3$), respiratory failure ($n = 2$), lung disorder ($n = 1$), or unknown causes ($n = 3$). Two patients with NPD-B died from hepatic failure at 2 years of age and respiratory failure at 42 years of age (48). In a recent study, eight patients died from reasons linked to ASMD morbidities. Three patients aged 20, 35, and 71 died from pneumonia. While two patients aged 16 and 65 died from liver failure or cancer, respectively. One patient died due to a tear in the splenic vein at 14. The other two individuals died at 17 and 56 years old due to portal hypertension with esophageal varices and multiorgan failure. Pneumonia was by far the most prevalent cause of mortality (47).

Quality of life assessment

Based on parental reports, diminished quality of life in pediatrics with NPD-B was demonstrated in the following domains: parental impact–emotional, general health perceptions, mental health, and physical functioning, assessed by CHQ-PF50. In adults, the 36-Item Short Form Health Survey questionnaire (SF-36) subscale showed no significant impact of the disease on mental health, role-emotional, social functioning, vitality, bodily pain, role-physical, and physical functioning (46). According to Cox et al. (48) who assessed 100 patients with ASMD, 5% worked part-time due to disability, 16% did not work due to disability, and 27% did not work for other reasons. Early diagnosis and appropriate management are essential for reducing the risk of complications and improving quality of life (52).

Experts' opinion

The panel highlighted that lung involvement in ASMD is usually underdiagnosed, and the percentage of lung involvement in ASMD is considerable and should not be passed unnoticed. Pulmonary involvement amongst ASMD patients from the GCC countries usually includes tachypnoea, shortness of breath, recurrent hyperactive airway disease, and recurrent respiratory infection. On the other hand, the experts stated that most ASMD patients present with hepatosplenomegaly and dyslipidemia. On the other hand, ophthalmological findings were mainly cherry red spots in patients with ASMD type A.

Typically, ASMD patients in the GCC seek medical advice from a general pediatrician or gastroenterologist before seeing a healthcare provider specializing in rare disorders. The lack of awareness about ASMD among general pediatricians and gastroenterologists usually leads to a significant delay in diagnosing ASMD. In a considerable proportion of patients from the GCC region, several years elapse between symptom onset and diagnosis, which can be as long as 3-10 years.

The panel highlighted the need to increase awareness about ASMD symptoms to help healthcare providers prompt early diagnosis and improve patients' quality of life. Moreover, the experts recommended establishing more centers of excellence in the GCC region to help with early and proper diagnosis of rare diseases like ASMD. In addition, enhanced access to diagnostic centers and specialized services will help reduce the diagnostic delay and improve the outcomes of ASMD in the region.

A diagnostic care pathway for ASMD aiming to reduce referral time should be developed and implemented to positively impact the diagnostic journey for this disorder. Educational programs targeting general pediatricians, hematologists, pulmonologists, and gastroenterologists should be conducted.

Diagnostic Approaches

Enzyme activity testing in ASMD

When there is a suspicion of ASMD, an enzyme assay for ASM activity should always be done first, followed by gene sequencing once the biochemical diagnosis has been established (5). To differentiate ASMD from Gaucher disease, glucocerebrosidase activity should be measured simultaneously (54). The presence of ASMD should be demonstrated by the lack of considerably reduced enzyme activity below the cut-off values, considering the numerous unique genetic variants of unknown significance (1). Other clinical and laboratory findings, such as lipid-laden foam cells and mixed dyslipidemia, while highly suggestive of ASMD, do not replace the need for confirmation by enzyme test results. In strong clinical suspicion for ASMD, direct molecular testing for known pathogenic mutations or familial mutations can be performed to confirm the diagnosis. However, if molecular analysis reveals variants of uncertain significance, an enzyme assay is essential to fully confirm the diagnosis.

DBS, fibroblasts, and isolated peripheral blood can all be used to evaluate ASM enzyme activity (55). Whole blood is typically required by laboratories that take samples from worldwide, allowing the same sample to be used for second-tier testing (5). DBS that are particularly stable may be utilized; however, DBS testing has limits, including the impact of anemia and recent transfusions on findings (56,57). Because of its increased analytical range and more accurate assessment of enzyme activity in the lower detection ranges, tandem mass spectrometry (MS/MS) is preferred for assaying ASM activity over fluorometric assays, as well as radio-labeled native sphingomyelin substrate due to the need for radiochemical licenses (58). With MS/MS tests, there is improved discrimination between unaffected and affected patients, according to fluorometry comparisons (59).

Pathogenic variant

Gene sequencing is indicated following confirmation of the diagnosis by demonstrating diminished ASM activity (5). More than 720 genetic variations have been identified in the (*SMPD1*) gene. Splicing genetic variations, small and large insertions, deletions, and splicing abnormalities often cause little or no residual ASM activity and are more likely to cause severe ASMD phenotype (5). Chronic ASMD is more likely to come from missense and other lesions (such as in-frame codon deletions) that maintain high residual activity (e.g., >5% of wild-type ASM activity, depending on the cell and test method) (60). In the Ashkenazi Jewish community, three genetic variations, p.P333Sfs, p.L304P, and p.R498L, account for more than 90% of NPD-A/B (61).

The most frequent variant in individuals with NPD-B is p.R610, which is seen in 20% of all North African, Western European, and North American patients (62). When homoallelic or heteroallelic, p.R610 is linked with a larger quantity of residual enzyme activity and is considered neuroprotective (5). A 10-year study of 29 individuals with NPD-B found that homozygosity variants of p.P332R, p.P325A, and p.R610del are linked to attenuated illness (40). L549P, fsP189, and L137P account for approximately 75% of the alleles in Turkish patients, and K576N and H421Y variants account for approximately 85% of the alleles in Saudi Arabian patients (70). In Portuguese/Brazilian patients, F480L, R474W, R441X, and S379P variants are common, while in Scottish/English patients, the most common variant is A196P (70). Further studies are required to identify new *SMPD1* variants and their phenotypic correlations.

Biomarkers and follow-up

Once an ASMD diagnosis has been obtained, biomarker testing may benefit disease monitoring. The presence of elevated levels of one or more markers is never enough to diagnose ASMD, even though they might help determine disease severity. As ERT becomes accessible, ASMD biomarkers may become more critical for monitoring therapy responses (5). For example, in NPD-B, plasma chitotriosidase, a biomarker of macrophage activation, is significantly elevated (63). Even though chitotriosidase plasma levels in ASMD are lower than those seen in individuals with Gaucher disease, chitotriosidase might be a viable diagnostic for chronic ASMD (53,64). However, up to 6% of people have a recessively inherited chitotriosidase deficiency, and genetic variations in the chitotriosidase gene that affect test results can lead to inaccurate interpretations of chitotriosidase plasma levels (65,66).

CCL18 levels in the blood are also elevated in individuals with ASMD and Gaucher disease, which can be used as a surrogate measure for disease activity (63,67). When patients with chitotriosidase deficiency, this biomarker becomes particularly useful. In various LSDs, plasma glycosphingolipids are indicators of sphingolipidosis. Glucosyl sphingosine, for example, is a specific and sensitive biomarker for Gaucher's illness that is not elevated in other LSDs (68).

Lysosphingomyelin levels are higher in the DBS and plasma of individuals with chronic ASMD, indicating that it might be used as a biomarker; nevertheless, more research is needed (69).

Diagnostic algorithm

An algorithm for diagnosing ASMD presenting in childhood has been developed based on the common clinical manifestations of ASMD, differential diagnoses, and diagnostic testing approaches. If the patient presents with splenomegaly with or without hepatomegaly, in addition to one or more features suggestive of ASMD such as low HDL-C, hypotonia, developmental delay, and cherry red spots, in that case, he/she should be subjected to ASM enzyme activity. If the activity is low, *SMPD1* gene sequencing should be performed; if not, repeat the

enzyme assay using MS/MS. Gene sequencing results can be used for genotype-phenotype correlation. In case of an unknown phenotype, a clinical assessment should be performed. To detect NDP-B in adults, the same approach should be followed; however, the suggestive features of ASMD are mainly pathologic fracture, interstitial lung disease, and low HDL-C.

Gaucher disease differential diagnosis

Gaucher disease is an autosomal recessive inherited disorder of metabolism that results from a low activity or absence of the glucocerebrosidase enzyme (70). Because individuals with Gaucher disease and NPD have comparable and overlapping clinical symptoms, a thorough laboratory workup examining both diseases simultaneously is critical (5). Type 1 GD is a non-neuronopathic form, and type 2 and 3 are neuronopathic. Type 1 GD and NPD-B symptoms are similar, including failure to thrive, bone marrow involvement, irritability, cytopenia, and hepatosplenomegaly (71). Enzyme assays are used to get a definitive diagnosis for both disorders. Molecular testing can help to verify diagnoses, screen family members, and predict the disease's prognosis (70). It was reported that one patient, out of a total of five patients, with suspected Gaucher had an ASMD and was diagnosed with ASMD type A/B; therefore, it is recommended to test suspected patients for both Gaucher and ASMD simultaneously (72).

Experts' opinion

The experts highlighted the comparatively high incidence of rare metabolic disorders in the GCC region, including ASMD. Thus, patients suspected of ASMD should be immediately referred for ASM enzyme activity assay and genetic counseling. However, they stated that the limited access to genetic testing and enzyme assays in the GCC countries are significant challenges that can delay the diagnosis of ASMD patients; however, the landscape is improving with the introduction of faster and more reliable diagnostic methods such as next-generation sequencing. Additional challenges in diagnosing ASMD include the non-specific and highly variable disease features, prohibitive cost of genomic testing, and lack of awareness amongst healthcare practitioners regarding the overlapping features between ASMD and Gaucher disease.

Therefore, the panel also advocated the development of a unified and comprehensive diagnostic algorithm that can aid in the timely identification and diagnosis of patients with suspected ASMD. This algorithm should advocate ASM enzyme activity in children with splenomegaly, with or without hepatomegaly, and one suggestive feature of ASMD, such as low HDL-C, high cholesterol, high triglyceride, thrombocytopenia, and anemia. If the activity is low, *SMPD1* gene sequencing should be performed; if not, repeat the enzyme assay using MS/MS.

Management of ASMD

In the current literature, there is no known curative therapy for managing ASMD. Only symptomatic treatments and supportive care are available to improve the quality of life for ASMD patients (6). First, evaluations of the ASMD-diagnosed individuals should be performed to define the severity and extent of the disease so that the management plan can be designed accordingly (73). Management of ASMD manifestations requires an interdisciplinary approach, as it needs coordination between different healthcare physicians (74). Splenectomy may be performed for patients suffering from severe splenomegaly with hypersplenism (75,76). However, some studies proposed that splenectomy is an independent risk factor for mortality in patients with NPD-B (38,47) and should be avoided as much as possible. In addition, liver transplantation is considered for patients with liver failure (1). In extensive lung disease, oxygen supplements and the administration of vaccines against respiratory pathogens to decrease the risk of pulmonary infections are advised (6). Other symptomatic treatments may be prescribed, such as lipid-lowering drugs, multivitamins, mineral supplements, and growth hormone therapy. Besides, psychotherapy has an essential role in management (73). In contrast, the safety concern regarding bone marrow transplantation (BMT) and total lung lavage largely outweighs their benefits for ASMD patients (1).

BMT was proposed in ASMD patients with equivocal results. Previous results showed that BMT might improve neurological involvement; nonetheless, with a high incidence of severe complications of the transplant procedure (77). To date, BMT has been extensively studied in animal models, with limited data on humans and a lack of clinically-relevant outcomes (78). Besides, BMT did not prevent disease progression in a patient with ASMD type A, despite early transplantation (79)

ERT is an intravenously administrated recombinant human ASM to treat non-neurological manifestations of ASMD that is still under clinical development (9). ERT eliminates the progressively accumulated lipid substances resulting from the building-up of sphingomyelin, which improves the symptoms and outcome of the disease (9,80). McGovern et al. (80) performed a phase I, open-label, and non-randomized clinical trial. Only 11 adult ASMD type B patients from one center were enrolled. The safety of the intravenous administration of ERT, with a dose ranging from 0.03 to 1.0 mg/kg, was assessed. The involved patients were followed up after 2 and 4 weeks. The authors found that a dose of more than 0.6 mg/kg is more likely to induce drug side effects and adverse drug reactions (ADRs) the first time of drug administration. Therefore, a gradual increase in the dose is recommended to reduce ERT adverse reactions.

A phase 1b, open-label trial enrolled five adult ASMD type B patients from the US and UK to examine the safety and efficacy of ERT. The patients received an escalated drug dose (0.1 to 3.0 mg/kg) every 2 weeks for 26 weeks. The study proved that a gradual increase in within-patient ERT dose is effective and tolerable in managing ASMD patients without neurological impairment (9).

Recently, the results of the ASCEND trial, an international, phase 2/3, placebo-controlled trial, were released. The trial recruited 36 patients with ASMD type B and

followed for 52 weeks. The results showed a significant improvement in lung functions by 22%, compared to 3% in the placebo arm. Besides, the spleen size decreased by 39.5% at the end of the study, compared with a 0.5% increase in the placebo arm. ERT showed an acceptable safety profile, with only five patients experiencing severe adverse events that were not treatment-related, and none of the patients discontinued treatment (81).

Moreover, an international phase 2, open-label trial (ASCEND-Peds/NCT02292654) recruited 20 pediatric patients (aged from 1.5 to 17.5 years) with ASMD type B or A/B from six regions "Brazil, France, Germany, Italy, United Kingdom, and the United States." ERT was administered intravenously, and the dose was gradually elevated within-patient. The drug significantly improved the disease symptoms and tolerability during the 64 weeks of the trial; the average improvement in splenomegaly and hepatomegaly was more than 40%, and the average lung diffusing capacity improved by nearly 33%. Significant improvements were also noted in liver enzymes and growth parameters. ERT showed an acceptable safety profile, with only three patients experiencing severe ADRs (10).

Based on such results, ERT has been granted breakthrough therapy designation submission by the Food and Drug Administration. In June 2022, the European Medical Agency granted the ERT, olipudase alfa, marketing authorization.

Experts' opinion

The experts stated that the results of ERT are promising, and its anticipated introduction to the clinical practice is likely to positively impact the treatment landscape of ASMD. Patients with non-neurological manifestations are more likely to benefit from ERT. Early initiation of therapy in the presymptomatic or early symptomatic stages will also be a key factor in the long-term success of treatment. Thus, future studies should focus on identifying the criteria of the patients with a high probability of achieving response to ERT, the types of required monitoring during treatment, and real-world experience from the GCC region.

In terms of the GCC region's needs, the experts confirmed that establishing a national registry for ASMD would support the substantial role of ERT for ASMD in the region. There is a need for national and institutional guidelines for patient referral to ensure optimal patient management. Also, there is a need for standardized multidisciplinary ASMD management and unified treatment protocol in the GCC region.

Conclusion and Future Directions

The suspected comparatively high incidence of ASMD in the GCC countries demonstrated that developing a comprehensive diagnostic and management approach for such a debilitating condition is a significant unmet need in the region. The current literature shows controversy, and further studies are still required and should be tailored to meet local and regional needs. Therefore, an urgent need to create a practical diagnostic and management algorithm that can significantly reduce the diagnostic delay of the ASMD, improve the prognosis of the patients, and limit the impairment in the quality of life. The current experts' view highlighted several unmet needs in the ASMD landscape in the region. There is a scarcity of published literature that assesses the burden of ASMD in the GCC region. The experts emphasized the lack of a multidisciplinary approach to managing ASMD in the region. Besides, the lack of awareness about ASMD among general pediatricians or gastroenterologists usually leads to a significant delay in diagnosing ASMD. Thus, there is an urgent need to increase awareness about ASMD symptoms and more centers of excellence in the GCC region to help with early and proper diagnosis of ASMD cases.

Acknowledgments

Dr. Gehad El Ashal and Dr. Ahmed Salah Hussein from RAY-CRO provided medical writing assistance and editorial support for this manuscript. Also, we would like to thank Dr. Omar M. Hussein and Dr. Reham Elgarhy from RAY-CRO for their valuable efforts exerted on this project. Medical writing assistance and publication were funded with logistical support from Sanofi.

Funding

None.

Declaration of conflicting interests

The authors of this article have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

Consent to participate

Not applicable.

Ethical approval

Not applicable.

Author details

Moeenaldeen AlSayed[1], Fatma Al-Jasmi[2,3], Tawfeg Ben Omran[4], Fathiya Al-Murshedi[5], Rawda Sunbul[6], Nadia Al-Hashmi[7], Talal Al-Enazi[8]

1. King Faisal Specialist Hospital & Research Center, Riyadh, Saudi Arabia
2. Department of Genetics & Genomics, United Arab Emirates University, Al Ain, United Arab Emirates
3. Tawam Hospital, Al Ain, United Arab Emirates
4. Division of Genetic and Genomic Medicine, Sidra Medicine and Hamad Medical Corporation, Ar-Rayyan, Qatar
5. Genetic and Developmental Medicine Clinic, Department of Genetics, Sultan Qaboos University Hospital, Seeb, Oman
6. Qatif Central Hospital, Qatif, Saudi Arabia
7. Royal Hospital, Muscat, Oman
8. Prince Sultan Military Medical City, Riyadh, Saudi Arabia

References

1. Schuchman EH, Wasserstein MP. Types A and B Niemann-Pick disease. Best Pract Res Clin Endocrinol Metab. 2015;29(2):237–47. https://doi.org/10.1016/j.beem.2014.10.002

2. McGovern MM, Aron A, Brodie SE, Desnick RJ, Wasserstein MP. Natural history of type A Niemann-Pick disease: possible endpoints for therapeutic trials. Neurology. 2006;66(2):228–32. https://doi.org/10.1212/01.wnl.0000194208.08904.0c
3. Kingma SD, Bodamer OA, Wijburg FA. Epidemiology and diagnosis of lysosomal storage disorders; challenges of screening. Best Pract Res Clin Endocrinol Metab. 2015;29(2):145–7. https://doi.org/10.1016/j.beem.2014.08.004
4. Hollak CE, de Sonnaville ES, Cassiman D, Linthorst GE, Groener JE, Morava E, et al. Acid sphingomyelinase (Asm) deficiency patients in the Netherlands and Belgium: disease spectrum and natural course in attenuated patients. Mol Genet Metab. 2012;107(3):526–33. https://doi.org/10.1016/j.ymgme.2012.06.015
5. McGovern MM, Dionisi-Vici C, Giugliani R, Hwu P, Lidove O, Lukacs Z, et al. Consensus recommendation for a diagnostic guideline for acid sphingomyelinase deficiency. Genet Med. 2017;19(9):967. https://doi.org/10.1038/gim.2017.7
6. McGovern MM, Avetisyan R, Sanson BJ, Lidove O. Disease manifestations and burden of illness in patients with acid sphingomyelinase deficiency (ASMD). Orphanet J Rare Dis. 2017;12(1):41. https://doi.org/10.1186/s13023-017-0572-x
7. He X, Chen F, Dagan A, Gatt S, Schuchman EH. A fluorescence-based, high-performance liquid chromatographic assay to determine acid sphingomyelinase activity and diagnose types A and B Niemann-Pick disease [Internet]. Anal Biochem. 2003;314(1):116–20. https://doi.org/10.1016/S0003-2697(02)00629-2
8. Thurberg BL, Diaz GA, Lachmann RH, Schiano T, Wasserstein MP, Ji AJ, et al. Long-term efficacy of olipudase alfa in adults with acid sphingomyelinase deficiency (ASMD): further clearance of hepatic sphingomyelin is associated with additional improvements in pro- and anti-atherogenic lipid profiles after 42 months of treatment. Mol Genet Metab. 2020;131(1–2):245–52. https://doi.org/10.1016/j.ymgme.2020.06.010
9. Wasserstein MP, Jones SA, Soran H, Diaz GA, Lippa N, Thurberg BL, et al. Successful within-patient dose escalation of olipudase alfa in acid sphingomyelinase deficiency. Mol Genet Metab. 2015;116(1–2):88–97. https://doi.org/10.1016/j.ymgme.2015.05.013
10. Diaz GA, Jones SA, Scarpa M, Mengel KE, Giugliani R, Guffon N, et al. One-year results of a clinical trial of olipudase alfa enzyme replacement therapy in pediatric patients with acid sphingomyelinase deficiency. Genet Med. 2021;23(8):1543. https://doi.org/10.1038/s41436-021-01156-3
11. Ozand PT, Gascon G, al Aqeel A, Roberts G, Dhalla M, Subramanyam SB. Prevalence of different types of lysosomal storage diseases in Saudi Arabia. J Inherit Metab Dis. 1990;13(6):849–61. https://doi.org/10.1007/BF01800209
12. Meikle PJ, Hopwood JJ, Clague AE, Carey WF. Prevalence of lysosomal storage disorders. JAMA. 1999;281(3):249–54. https://doi.org/10.1001/jama.281.3.249
13. Acuña M, Martínez P, Moraga C, He X, Moraga M, Hunter B, et al. Epidemiological, clinical and biochemical characterization of the p.(Ala359Asp) SMPD1 variant causing Niemann-Pick disease type B. Eur J Hum Genet. 2016;24(2):208. https://doi.org/10.1038/ejhg.2015.89
14. Orphanet: Niemann Pick disease type A [Internet]. [cited 2022 Jan 9]. Available from: https://www.orpha.net/consor/cgi-bin/OC_Exp.php?Lng=GB&Expert=77292
15. Pokrzywinski R, Hareendran A, Nalysnyk L, Cowie S, Crowe J, Hopkin J, et al. Impact and burden of acid sphingomyelinase deficiency from a patient and caregiver perspective. Sci Rep. 2021;11(1):1–13. https://doi.org/10.1038/s41598-021-99921-6
16. Al-Gazali L, Hamamy H, Al-Arrayad S. Genetic disorders in the Arab world. BMJ Br Med J. 2006;333(7573):831. https://doi.org/10.1136/bmj.38982.704931.AE
17. Moammar H, Cheriyan G, Mathew R, Al-Sannaa N. Incidence and patterns of inborn errors of metabolism in the Eastern Province of Saudi Arabia, 1983-2008. Ann Saudi Med. 2010;30(4):271–7. https://doi.org/10.4103/0256-4947.65254
18. Al-Sannaa NA, Al-Abdulwahed HY, Al-Ghamdi MS, Abbas Al-Sannaa N, Geneticist C, Hopkins Aramco J. Lysosomal storage disorders (LSDs): the prevalence in the Eastern Province of Saudi Arabia. Int J Neurol Dis. 2017;1(2):38–043.
19. Alfadhel M, Benmeakel M, Hossain MA, Al Mutairi F, Al Othaim A, Alfares AA, et al. Thirteen year retrospective review of the spectrum of inborn errors of metabolism presenting in a tertiary center in Saudi Arabia. Orphanet J Rare Dis. 2016;11(1):126. https://doi.org/10.1186/s13023-016-0510-3
20. Al-Jasmi FA, Tawfig N, Berniah A, Ali BR, Taleb M, Hertecant JL, et al. Prevalence and novel mutations of lysosomal storage disorders in United Arab Emirates: LSD in UAE. JIMD Rep [Internet]. 2013 [cited 2022 Jan 10];10:1. Available from: /pmc/articles/PMC3755583/
21. Joshi SN, Hashim J, Venugopalan P. Pattern of inborn errors of metabolism in an Omani population of the Arabian Peninsula. Ann Trop Paediatr. 2022;22(1):93–6. https://doi.org/10.1179/027249302125000238
22. Mezzi N, Messaoud O, Mkaouar R, Zitouna N, Romdhane S, Abdessalem G, et al. Spectrum of genetic diseases in Tunisia: current situation and main milestones achieved. Genes (Basel) [Internet]. 2021 Nov 1 [cited 2022 Jan 9];12(11). Available from: /pmc/articles/PMC8617973/
23. Zampieri S, Filocamo M, Pianta A, Lualdi S, Gort L, Coll MJ, et al. SMPD1 utation update: database and comprehensive analysis of published and novel variants [Internet]. Hum Mutat. 2016;37(2):139–47. https://doi.org/10.1002/humu.22923
24. Pastores GM, Lau HA. Niemann-Pick disease. Encycl Neurol Sci [Internet]. 2021;590–1. https://doi.org/10.1016/B978-0-12-385157-4.00102-0
25. Zhang H, Wang Y, Gong Z, Li X, Qiu W, Han L, et al. Identification of a distinct mutation spectrum in the SMPD1 gene of Chinese patients with acid sphingomyelinase-deficient Niemann-Pick disease. Orphanet J Rare Dis. 2013;8(1):15. https://doi.org/10.1186/1750-1172-8-15
26. Levran O, Desnick RJ, Schuchman EH. Type A Niemann-Pick disease: a frameshift mutation in the acid sphingomyelinase gene (fsP330) occurs in Ashkenazi Jewish patients. Hum Mutat. 1993;2(4):317–9. https://doi.org/10.1002/humu.1380020414
27. Mihaylova V, Hantke J, Sinigerska I, Cherninkova S, Raicheva M, Bouwer S, et al. Highly variable neural involvement in sphingomyelinase-deficient Niemann-Pick disease caused by an ancestral Gypsy mutation. Brain.

2007;130(Pt 4):1050–61. https://doi.org/10.1093/brain/awm026

28. Wasserstein MP, Aron A, Brodie SE, Simonaro C, Desnick RJ, McGovern MM. Acid sphingomyelinase deficiency: prevalence and characterization of an intermediate phenotype of Niemann-Pick disease. J Pediatr. 2006;149(4):554–9. https://doi.org/10.1016/j.jpeds.2006.06.034
29. Vanier MT, Ferlinz K, Rousson R, Duthel S, Louisot P, Sandhoff K, et al. Deletion of arginine (608) in acid sphingomyelinase is the prevalent mutation among Niemann-Pick disease type B patients from northern Africa. Hum Genet. 1993;92(4):325–30. https://doi.org/10.1007/BF01247328
30. Gluck I, Zeigler M, Bargal R, Schiff E, Bach G. Niemann Pick disease type A in Israeli Arabs: 677delT, a common novel single mutation. Hum Mutat. 1998;12(2):136. https://doi.org/10.1002/(SICI)1098-1004(1998)12:2<136::AID-HUMU12>3.3.CO;2-P
31. Thurberg BL. Autopsy pathology of infantile neurovisceral ASMD (Niemann-Pick disease type A): clinicopathologic correlations of a case report. Mol Genet Metab Rep. 2020;24:100626. https://doi.org/10.1016/j.ymgmr.2020.100626
32. Pavlů-Pereira H, Asfaw B, Poupctová H, Ledvinová J, Sikora J, Vanier MT, et al. Acid sphingomyelinase deficiency. Phenotype variability with prevalence of intermediate phenotype in a series of twenty-five Czech and Slovak patients. A multi-approach study. J Inherit Metab Dis. 2005;28(2):203–27. https://doi.org/10.1007/s10545-005-5671-5
33. McGovern MM, Pohl-Worgall T, Deckelbaum RJ, Simpson W, Mendelson D, Desnick RJ, et al. Lipid abnormalities in children with types A and B Niemann Pick disease. J Pediatr. 2004;145(1):77–81. https://doi.org/10.1016/j.jpeds.2004.02.048
34. Ishii H, Takahashi T, Toyono M, Tamura M, Harada K, Yoshida M, et al. Acid sphingomyelinase deficiency: cardiac dysfunction and characteristic findings of the coronary arteries. J Inherit Metab Dis. 2006;29(1):232–4. https://doi.org/10.1007/s10545-006-0226-y
35. Mendelson DS, Wasserstein MP, Desnick RJ, Glass R, Simpson W, Skloot G, et al. Type B Niemann-Pick disease: findings at chest radiography, thin-section CT, and pulmonary function testing. Radiology. 2006;238(1):339–45. https://doi.org/10.1148/radiol.2381041696
36. von Ranke FM, Pereira Freitas HM, Mançano AD, Rodrigues RS, Hochhegger B, Escuissato D, et al. Pulmonary involvement in Niemann-Pick disease: a state-of-the-art review [Internet]. Lung. 2016;194(4):511–8. https://doi.org/10.1007/s00408-016-9893-0
37. Wasserstein M, Godbold J, McGovern MM. Skeletal manifestations in pediatric and adult patients with Niemann Pick disease type B. J Inherit Metab Dis. 2013;36(1):123–7. https://doi.org/10.1007/s10545-012-9503-0
38. McGovern MM, Lippa N, Bagiella E, Schuchman EH, Desnick RJ, Wasserstein MP. Morbidity and mortality in type B Niemann-Pick disease. Genet Med. 2013;15(8):618–23. https://doi.org/10.1038/gim.2013.4
39. Cassiman D, Packman S, Bembi B, Turkia HB, Al-Sayed M, Schiff M, et al. Cause of death in patients with chronic visceral and chronic neurovisceral acid sphingomyelinase deficiency (Niemann-Pick disease type B and B variant): literature review and report of new cases. Mol Genet Metab. 2016;118(3):206–13. https://doi.org/10.1016/j.ymgme.2016.05.001
40. Wasserstein MP, Desnick RJ, Schuchman EH, Hossain S, Wallenstein S, Lamm C, et al. The natural history of type B Niemann-Pick disease: results from a 10-year longitudinal study. Pediatrics. 2004;114(6):e672–7. https://doi.org/10.1542/peds.2004-0887
41. Orchard EA, Kempson D, Lindsay DC. Niemann-Pick disease type B with severe coronary artery disease and early recurrence after coronary artery bypass grafting. J Clin Lipidol. 2009;3(1):57–8. https://doi.org/10.1016/j.jacl.2008.11.011
42. Labrune P, Bedossa P, Huguet P, Roset F, Vanier MT, Odievre M. Fatal liver failure in two children with Niemann-Pick disease type B. J Pediatr Gastroenterol Nutr. 1991;13(1):104–9. https://doi.org/10.1097/00005176-199107000-00020
43. Thurberg BL, Wasserstein MP, Schiano T, O'Brien F, Richards S, Cox GF, et al. Liver and skin histopathology in adults with acid sphingomyelinase deficiency (Niemann-Pick disease type B). Am J Surg Pathol. 2012;36(8):1234–46. https://doi.org/10.1097/PAS.0b013e31825793ff
44. Stirnemann J, Vigan M, Hamroun D, Heraoui D, Rossi-Semerano L, Berger MG, et al. The French Gaucher's disease registry: clinical characteristics, complications and treatment of 562 patients. Orphanet J Rare Dis. 2012;7(1):77. https://doi.org/10.1186/1750-1172-7-77
45. Stirnemann JÔ, Belmatoug N, Camou F, Serratrice C, Froissart R, Caillaud C, et al. A review of Gaucher disease pathophysiology, clinical presentation and treatments. Int J Mol Sci [Internet]. 2017 Feb 17 [cited 2022 Jan 19];18(2):441. Available from: /pmc/articles/PMC5343975/
46. McGovern MM, Wasserstein MP, Giugliani R, Bembi B, Vanier MT, Mengel E, et al. A prospective, cross-sectional survey study of the natural history of Niemann-Pick disease type B. Pediatrics. 2008;122(2):e341–9. https://doi.org/10.1542/peds.2007-3016
47. McGovern MM, Wasserstein MP, Bembi B, Giugliani R, Mengel KE, Vanier MT, et al. Prospective study of the natural history of chronic acid sphingomyelinase deficiency in children and adults: eleven years of observation. Orphanet J Rare Dis. 2021;16(1):212. https://doi.org/10.1186/s13023-021-01842-0
48. Cox GF, Clarke LA, Giugliani R, McGovern MM. Burden of illness in acid sphingomyelinase deficiency: a retrospective chart review of 100 patients. JIMD Rep. 2018;41(41):119–29. https://doi.org/10.1007/8904_2018_120
49. Nicholson AG, Florio R, Hansell DM, Bois RM, Wells AU, Hughes P, et al. Pulmonary involvement by Niemann-Pick disease. A report of six cases. Histopathology. 2006;48(5):596–603. https://doi.org/10.1111/j.1365-2559.2006.02355.x
50. Iaselli F, Rea G, Cappabianca S, Fabozzi G, Montemarano M, Vitale C, et al. Adult-onset pulmonary involvement in Niemann-Pick disease type B. Monaldi Arch Chest Dis. 2011;75(4):235–40. https://doi.org/10.4081/monaldi.2011.211
51. Freitas HM, Mançano AD, Rodrigues RS, Hochhegger B, Torres PP, Escuissato D, et al. Niemann-Pick disease type B: HRCT assessment of pulmonary involvement. J Bras

Pneumol. 2017;43(6):451–5. https://doi.org/10.1590/s1806-37562017000000062

52. Lidove O, Belmatoug N, Froissart R, Lavigne C, Durieu I, Mazodier K, et al. Acid sphingomyelinase deficiency (Niemann-Pick disease type B) in adulthood: a retrospective multicentric study of 28 adult cases. Rev Med Interne. 2017;38(5):291–9.
53. Brinkman J, Wijburg FA, Hollak CE, Groener JE, Verhoek M, Scheij S, et al. Plasma chitotriosidase and CCL18: early biochemical surrogate markers in type B Niemann-Pick disease. J Inherit Metab Dis. 2005;28(1):13–20. https://doi.org/10.1007/s10545-005-4416-9
54. Oliva P, Mechtler TP, Schwarz M, Balendran S, Cobos PN, Lukacs Z, et al. Differential diagnosis of Niemann-Pick disease types A and B in cases of suspected Gaucher disease. Mol Genet Metab. 2020;129(2):S121. https://doi.org/10.1016/j.ymgme.2019.11.314
55. Gal AE, Brady RO, Hibbert SR, Pentchev PG. A practical chromogenic procedure for the detection of homozygotes and heterozygous carriers of Niemann-Pick disease. N Engl J Med. 1975;293(13):632–6. https://doi.org/10.1056/NEJM197509252931304
56. Kuchar L, Sikora J, Gulinello ME, Poupetova H, Lugowska A, Malinova V, et al. Quantitation of plasmatic lysosphingomyelin and lysosphingomyelin-509 for differential screening of Niemann-Pick A/B and C diseases. Anal Biochem. 2017;525:73–7. https://doi.org/10.1016/j.ab.2017.02.019
57. Gelb MH, Turecek F, Scott CR, Chamoles NA. Direct multiplex assay of enzymes in dried blood spots by tandem mass spectrometry for the newborn screening of lysosomal storage disorders. J Inherit Metab Dis. 2006;29(2–3):397–404. https://doi.org/10.1007/s10545-006-0265-4
58. Legnini E, Orsini JJ, Mühl A, Johnson B, Dajnoki A, Bodamer OA. Analysis of acid sphingomyelinase activity in dried blood spots using tandem mass spectrometry. Ann Lab Med. 2012;32(5):319–23. https://doi.org/10.3343/alm.2012.32.5.319
59. Gelb MH, Ronald Scott C, Turecek F, Liao HC. Comparison of tandem mass spectrometry to fluorimetry for newborn screening of LSDs. Mol Genet Metab Rep. 2017;12:80. https://doi.org/10.1016/j.ymgmr.2017.05.004
60. Lipiński P, Kuchar L, Zakharova EY, Baydakova GV, Ługowska A, Tylki-Szymańska A. Chronic visceral acid sphingomyelinase deficiency (Niemann-Pick disease type B) in 16 polish patients: long-term follow-up. Orphanet J Rare Dis. 2019;14(1):55. https://doi.org/10.1186/s13023-019-1029-1
61. Wasserstein MS. Acid sphingomyelinase deficiency. In: Pagon R, Adamm M, Ardinger H, et al., editors. GeneReviews. Seattle, DC: University of Washington; 2015.
62. Simonaro CM, Desnick RJ, McGovern MM, Wasserstein MP, Schuchman EH. The demographics and distribution of type B Niemann-Pick disease: novel mutations lead to new genotype/phenotype correlations. Am J Hum Genet. 2002;71(6):1413–9. https://doi.org/10.1086/345074
63. Sheth JJ, Sheth FJ, Oza NJ, Gambhir PS, Dave UP, Shah RC. Plasma chitotriosidase activity in children with lysosomal storage disorders. Indian J Pediatr. 2010;77(2):203–5. https://doi.org/10.1007/s12098-009-0249-0
64. Sikora J, Pavlu-Pereira H, Elleder M, Roelofs H, Wevers RA. Seven novel acid sphingomyelinase gene mutations in Niemann-Pick type A and B patients. Ann Hum Genet. 2003;67(Pt 1):63–70. https://doi.org/10.1046/j.1469-1809.2003.00009.x
65. Boot RG, Renkema GH, Verhoek M, Strijland A, Bliek J, de Meulemeester TM, et al. The human chitotriosidase gene. Nature of inherited enzyme deficiency. J Biol Chem. 1998;273(40):25680–5. https://doi.org/10.1074/jbc.273.40.25680
66. Bussink AP, Verhoek M, Vreede J, Ghauharali-van der Vlugt K, Donker-Koopman WE, Sprenger RR, et al. Common G102S polymorphism in chitotriosidase differentially affects activity towards 4-methylumbelliferyl substrates. FEBS J. 2009;276(19):5678–88. https://doi.org/10.1111/j.1742-4658.2009.07259.x
67. Deegan PB, Moran MT, McFarlane I, Schofield JP, Boot RG, Aerts JM, et al. Clinical evaluation of chemokine and enzymatic biomarkers of Gaucher disease. Blood Cells Mol Dis. 2005;35(2):259–67. https://doi.org/10.1016/j.bcmd.2005.05.005
68. Murugesan V, Chuang WL, Liu J, Lischuk A, Kacena K, Lin H, et al. Glucosylsphingosine is a key biomarker of Gaucher disease. Am J Hematol. 2016;91(11):1082–9. https://doi.org/10.1002/ajh.24491
69. Chuang WL, Pacheco J, Cooper S, McGovern MM, Cox GF, Keutzer J, et al. Lyso-sphingomyelin is elevated in dried blood spots of Niemann-Pick B patients. Mol Genet Metab. 2014;111(2):209–11. https://doi.org/10.1016/j.ymgme.2013.11.012
70. Nagral A. Gaucher disease. J Clin Exp Hepatol. 2014;4(1):37–50. https://doi.org/10.1016/j.jceh.2014.02.005
71. Puri RD, Kapoor S, Kishnani PS, Dalal A, Gupta N, Muranjan M, et al. Gaucher disease task force. Diagnosis and management of Gaucher disease in India - consensus guidelines of the Gaucher disease task force of the society for Indian academy of medical genetics and the Indian academy of pediatrics. Indian Pediatr. 2018;55(2):143–53. https://doi.org/10.1007/s13312-018-1249-9
72. Oliva P, Schwarz M, Wiesinger T, Scott J, Sansen S, Mechtler T, et al. The incidence of acid sphingomyelinase deficiency (ASMD) in cases of suspected Gaucher disease, genotype-phenotype correlation together with Lyso-SPM biomarker. Mol Genet Metab. 2021;132(2):S79. https://doi.org/10.1016/j.ymgme.2020.12.186
73. Wasserstein MP, Schuchman EH. Acid sphingomyelinase deficiency. GeneReviews® [Internet]. 2021 Feb 25 [cited 2022 Jan 10]; Available from: https://www.ncbi.nlm.nih.gov/books/NBK1370/
74. Pastores GM, Hughes DA. Non-neuronopathic lysosomal storage disorders: disease spectrum and treatments [Internet]. Best Pract Res Clin Endocrinol Metab. 2015;29(2):173–82. https://doi.org/10.1016/j.beem.2014.08.005
75. Chen Z, Chen Z, Wu S, Wang X. Niemann-Pick disease resulting in spontaneous splenic rupture in an adult: report of a case. Surg Today. 2008;38(5):473–5. https://doi.org/10.1007/s00595-007-3641-2
76. Simões RG, Maia H. Niemann-Pick type B in adulthood. Case Rep. 2015;2015:bcr2014208286. https://doi.org/10.1136/bcr-2014-208286

77. Victor S, Coulter JB, Besley GT, Ellis I, Desnick RJ, Schuchman EH, et al. Niemann-Pick disease: sixteen-year follow-up of allogeneic bone marrow transplantation in a type B variant [Internet]. J Inherit Metab Dis. 2003;26(8):775–85. https://doi.org/10.1023/B:BOLI.0000009950.81514.c8
78. Schuchman EH, Desnick RJ. Types a and B Niemann-pick disease. Mol Genet Metab. 2017;120(1–2):27–33. https://doi.org/10.1016/j.ymgme.2016.12.008
79. Morel CF, Gassas A, Doyle J, Clarke JT. Unsuccessful treatment attempt: cord blood stem cell transplantation in a patient with Niemann-Pick disease type A. J Inherit Metab Dis. 2007;30(6):987. https://doi.org/10.1007/s10545-007-0700-1
80. McGovern MM, Wasserstein MP, Kirmse B, Duvall WL, Schiano T, Thurberg BL, et al. Novel first-dose adverse drug reactions during a phase I trial of olipudase alfa (recombinant human acid sphingomyelinase) in adults with Niemann-Pick disease type B (acid sphingomyelinase deficiency). Genet Med. 2016;18(1):34–40. https://doi.org/10.1038/gim.2015.24
81. Wasserstein M, Arash-Kaps L, Barbato A, Gallagher R, Giugliani R, Guelbert N, et al. One-year results of the placebo-controlled ASCEND trial of olipudase alfa enzyme replacement therapy in adults with chronic acid sphingomyelinase deficiency. Mol Genet Metab. 2021;132:S64–5. https://doi.org/10.1016/S1096-7192(21)00178-5

REVIEW ARTICLE

Genetic advances in skeletal disorders: an overview

Safdar Abbas[1*†], Hammal Khan[2,3], Qamre Alam[4], Arif Mahmood[5], Muhammad Umair[6*†]

ABSTRACT

Genetic skeletal disorders (GSDs) are a large group of rare heterogeneous disorders characterized by abnormal development, remodeling, and growth of the human skeleton's cartilage and bones. GSDs have a high spectrum of phenotypes that range from disproportionate short stature (dwarfism) in childhood to osteoarthritis in old age. According to the latest nosology classification of skeletal dysplasias, 461 disorders under 42 groups are classified according to specific radiographic, clinical, and molecular standards. In addition, correct molecular diagnosis for these rare GSDs is important for genetic and psychological counseling and treatment. GSDs are also associated with many syndromic forms that affect other parts such as hearing, vision, neurological, pulmonary, renal, or cardiac function. This review highlights the importance of GSDs and details a few selected disorders and their management strategies.

Keywords: GSDs, osteogenesis imperfecta, chondrodysplasias; polydactyly, syndactyly, acromesomelic dysplasia, SHMF, diagnosis; genetics; management.

Introduction

The word skeleton is derived from the Greek "skeletos," meaning "dried up", and it is composed of bone and cartilage. It is a complex organ, which consists of 206 bones and divided into 6 ossicles, 74 axial, and 126 appendicular bones (1). The human skeleton is divided into axial and appendicular skeleton. The shoulder girdle, lower and upper limbs, and pelvic girdle constitute the appendicular skeleton. The axial skeleton includes the skull, associated bones, spinal column, and rib cage. The axial skeleton attaches the appendicular skeleton to the body through the pelvic and pectoral girdles (2). The main component of the skeleton is the bone. It is the main reservoir for accumulating minerals like calcium and phosphorous (1,3). It consists of three types of cells, osteocytes, osteoblasts, and osteoclasts (4). Skeletal disorders resolute during the embryonic development, such as their location, shape, growth, and differentiation rate. A process called intramembranous ossification, from where mesenchymal cells (MSCs) differentiate directly into osteoblasts, while the majority of skeletal elements are formed by endochondral ossification, such as the lateral halves of the clavicle and parts of the skull (3,4). The latter process starts with forming a cartilaginous template, which is eventually replaced by the bone. This requires co-regulation of differentiation of the cell types specific for chondrocytes and osteoblasts, respectively (5,6). During embryogenesis, the human skeleton originates from three different sites, such as the MSCs, which are responsible for the appendicular skeleton. The paraxial mesoderm originates in the axial skeleton, and the cranial neural crest gives rise to craniofacial bones (7). Special signaling pathways control skeletal development during embryonic stages for appropriate propagation and sharing of sclerotomes and lateral plate mesoderm (LPM) cranial neural crest by involving several genes to accumulate mesenchymal aggregate (8). During embryonic development, three germ layers, including ectoderm, endoderm, and mesoderm, transform into derivatives, including an ectoderm-derived neural tube, mesoderm-derived notochord, and LPM (9). The neural tube desquamates the neural crests. The cells differentiate into various types, such as neuronal cells and melanocytes. The LPM gives rise to

Correspondence to: Safdar Abbas
*Department of Biological Science, Dartmouth College, Hanover, NH.
Email: Safdar.Abbas@dartmouth.edu
Correspondence to: Muhammad Umair
*Department of Life Sciences, School of Science, University of Management and Technology [UMT], Lahore, Pakistan.
Email: m_umair@umt.edu.pk
†Authors contributed equally.
Full list of author information is available at the end of the article.
Received: 26 December 2022 | **Accepted:** 29 January 2023

This is an open access article distributed in accordance with the Creative Commons Attribution (CC BY 4.0) license: https://creativecommons.org/licenses/by/4.0/) which permits any use, Share — copy and redistribute the material in any medium or format, Adapt — remix, transform, and build upon the material for any purpose, as long as the authors and the original source are properly cited. © The Author(s).

the limb skeletal structure (appendicular skeleton), the sternum (axial skeleton), and nonskeletal elements. The paraxial mesoderm gives rise to somites, forming most axial skeleton, such as ribs and vertebrae (10).

Skeletal patterning

During endochondral bone formation, the limb skeleton builds up from cartilage anlagen. It begins when chondrocytes progenitors, which then develop into a cartilage template that is eventually substituted by bones. The crucial step in establishing limb skeletal "patterning" occurs throughout the cartilaginous anlagen (11). Skeletal patterning, condensation, and differentiation of MSCs into chondrocytes (cartilage formation), osteoblasts (bone formation), osteoclasts, and bone remodeling is under the tight control of several cytokines, growth factors, and intercellular signaling pathways including Wingless/Integrated (*Wnt*), Sonic hedgehog (*SHH*), Indian hedgehog, fibroblast growth factor (*FGF*), Notch, TGF-β, and bone morphogenetic protein (BMPs). Genetic variations in cytokines and growth factors lead to inherited skeletal disorders (11,12). During the skeletal patterning and remodeling, the size, shape, number, and skeletal primordial are defined in correct relationship to one another. Different skeletal elements like axial, craniofacial, and appendicular skeleton are formed during the skeletal patterning process in an organized manner (13).

Limb development

The development of limbs in vertebrates is controlled by genetic processes, which are impenetrable and still not fully understood. Experimental studies of the molecular genetics of human limb development theorize and manipulate the different genetic interactions and their concerned pathways. There are three principal zones for the development of limbs: the proximal stylopod, zeugopod, and distal autopod (14).

Initiation of limbs

The limb bud originates from the edge of the embryo, that is, LPM covered by a layer of ectoderm. It has lineages for all types of limb tissues except muscles. Muscle progenitors initiate from somites and rapidly migrate to the embryonic limb buds. The skeletal element elaborates when the tissue progenitors differentiate, and the limb bud grows toward the distal side. In the limb bud, when it grows toward the distal side, the varied tissue progenitors differentiate and establish the elaborated pattern of skeletal elements (15,16).

The *HOX* gene plays a fundamental role during embryonic development, generates morphological diversity with the body axis, and genetically determines the position of the limb buds (17,18). When the limb's position is decided, a series of interactions between epithelial-to-mesenchymal and the LPM, the ectoderm, is established. In this event, the establishment of the apical ectodermal ridge (AER), an epithelial thickened structure from limb ectoderm occurs, which facilitates the distal margins of the limbs bud to its posterior tip from its anterior side and is dorso-ventrally (DV) located along the border of the limb bud (14,18). Several studies have revealed that a number of molecules expressed in specific domains, either in dorsal or ventral ectoderm, are involved in limb developmental processes such as *WNT7A*, Radical fringe, Engrailed-1 *(EN-1)*, and *TGF*-β*/BMP* (19).

Limbs patterning

After the development of limb buds, the undifferentiated mesenchyme is targeted by a series of signals to determine the morphology of skeletal elements. The AER, the zone of polarizing activity (ZPA), and the dorsal ectoderm play a key role in controlling the limbs proximal to distal outgrowth, anterior-posterior (AP) patterning, and establishing DV polarity, respectively (15). The limb buds have mesoderm cells, homogenous masses covered with a layer of ectoderm. The initial mark of patterning is a thin epithelial thickening at the limb bud proximal tip, known as the AER (14). The AER is important for proximal to distal patterning, and it is revealed that when the AER in chick wings is removed, the wings become truncated (20). AER promotes the proliferation of mesodermal cells and stops apoptotic events by providing *FGF* signaling to the mesoderm cells (21). *FGF10* expression, which is required for maintaining FGF8 expression in the limb mesenchyme, is induced by the AER. Thus, *FGF8* and *FGF10* establish an epithelial-mesenchymal positive feedback loop during limb growth (22).

During limb development, abnormalities in AER maintenance lead to abnormal phenotypes, including split-hand/foot syndromes resulting from *TP63* mutations, whose expression is vital for AER maintenance (23). A cell colony termed the ZPA is localized in the posterior limb bud mesenchyme, showing posture activity. The molecular basis of ZPA was discovered when *SHH* was proposed to be the diffusible morphogen responsible for polarizing activity (24). Another gene reported was Glioma-associated oncogene 3 (*GLI3*), which has two isoforms, one with active full-length *GLI3* (*GLI3F*) and repressor truncated *GLI3* (*GLI3R*). *SHH* signaling promotes the expression of *GLI3F* in the posterior mesenchyme, while the absence of *SHH* signaling leads to the production of *GLI3R* (25). The importance of *GLI3* and *SHH* during vertebral limb growth was discovered in the mouse by gene inactivation; *SHH*_mutant mice had only one rudimentary digit while all other digits were absent.

On the other hand, *GLI3* mutant mice show polydactyly. *SHH* and GLI3 mutations in human leads to different limb anomalies, including preaxial or postaxial polydactyly (PAP) or even severe conditions like acheriopodia (26). *SHH* plays a key role in AP patterning, maintains limb bud proliferation, and expands the digit-forming field (25). *SHH*, *GLI3*, and other regulators promote digit numbers and identity. In this context, a BMP signaling gradient was also suggested as a mediator, while genetic analysis of mice did not prove its role. It was shown that patterning information in chicks is stored in the interdigital mesenchyme. Signals to the growing phalanges are directed from the interdigital mesenchyme,

which provides them with information necessary for reaching their final length (27).

DV patterning is mediated by LIM homeobox transcription factor-1 (*LMX-1*) in the dorsal mesenchyme with subsequent expression of *WNT-7A* in the dorsal ectoderm and EN-1 in the ventral ectoderm. In the ventral ectoderm, EN-1 inhibits the *WNT7A* expression (28). Acting as a morphogen, *WNT7A* diffuses to the dorsal mesoderm and induces expression of the *LMX1B* (transcriptional factor). In the limb bud mesenchyme, *LMX1B* is considered a key regulator of dorsal patterning. *LMX1B* mutation in human result in a syndrome (Focal segmental glomerulosclerosis 10; MIM 256020) characterized by a defect in DV patterning of the limb, which is known as a nail-patella syndrome [MIM 161200] (29).

Signaling pathways involved in limb patterning

This is instinctive that AER, dorsal ectoderm, and ZPA are the centers of signaling and have strong coordination in their functions, as AER removal results in cell death in the underlying mesenchyme and leads to loss of *SHH* expression. Interestingly, *FGF4* could reimburse this function of the AER. Similarly, SHH actively controls *FGF4* expression in the AER. Thus both (*SHH* and *FGF4*) molecules form a positive feedback loop. This feedback loop is the best example of a signal relay in epithelial-to-mesenchymal communication: *SHH* actively controls the Gremlin 1 (Grem1) expression, a BMP inhibitor. Taken together, *GREM1* inhibits BMP action, which has a negative effect on the AER (30). *WNT7A* is required to replace the removed ectoderm, while *SHH* is expressed in the dorsal mesoderm. In mammals, this function is highly conserved. There is a decrease in *SHH* expression by the inactivation of *WNT7A*, and the posterior digit is lost (19).

Genetic skeletal disorders (GSDs)

GSDs arise from complex skeletal development, growth, and homeostasis disturbances caused by gene mutations. These disorders represent a challenge in diagnosis and treatment due to their rarity and verity (31). GSDs are clinically and genetically heterogeneous groups of disorders affecting bone and cartilage growth (32). It has significant effects on muscles, tendons, and ligaments. The overall incidence of skeletal dysplasia is 1/500 to 1/1,000 live birth. It is mainly associated with abnormalities of the linear skeleton and results from somatic mosaicism, teratogen exposure, and imprinting errors (33). Mutation in metabolism signaling pathways or in the synthesis of structural proteins, degradation of macromolecules, receptors, growth factors, or transcription factors may cause skeletal dysplasia (34).

Classification of skeletal disorders

Nomenclature and classification of Osteochondrodysplasias termed as “taxonomy.” The dysostoses have been incorporated into the nomenclature, also called “nosology” (35). From 1977 to 1997, several efforts were made to classify the nosology (skeletal dysplasia). Categorizing different skeletal disorders based on clinical diagnosis, metabolism, and radiology was challenging. The list of genetic disorders mentioned in nosology helps to diagnose and delineate variants or newly recognized genetic disorders (36). The latest classification was performed in 2019, “Nosology and classification of GSDs: 2019 revision” (37). They classified the known GSDs into 461 disorders, organized into 42 groups. The classification was based on the involvement of 337 different genes in establishing molecular pathways, genetic, and radiographic criteria, and the role of biochemical was defined as the cause of these disorders.

The growth and development of the limbs involve several genetic pathways, and disruption of these genetic pathways leads to various anomalies in size, shape, and structure of the limbs, collectively known as congenital limb deformities. Limb deformities involve an odd number of digits in hands and feet, anomalous separation of the digits, or deviation of central rays of the autopods. The congenital limb malformations rate is 1 per 500 to 1 in 1,000 live births for upper limbs (37). Based on the clinical radiological manifestations, many congenital limb deformities have been discussed here, including osteogenesis imperfect (OI), Acromesomelic Dysplasia (AMD), split hand foot malformations (SHFM), Bardet–Biedl syndrome (BBS), and polydactyly. Such reviews might be helpful for clinicians and researchers to get an overview regarding rare GSDs that might help in genetic screening of the culprit gene involved and correct molecular diagnosis.

Osteogenesis imperfecta

(OI; MIM 166200) is a rare skeletal dysplasia characterized by growth deficiency, reduced bone mass, and fragility (38). The term “OI” refers to imperfect bone formation and disorder of the connective tissue matrix. The condition’s hallmark lies in bone fragility and easy fractures caused by decreased bone mass (38,39). OI patients may have short stature, blue sclerae, joint laxity, scoliosis, skeletal deformity, and dentinogenesis imperfect, which are the secondary clinical features of OI (39,40).

OI is also known as “brittle bone disease,” as it is a genetically and clinically heterogeneous heritable connective tissue disorder resulting from defects in type I collagen biosynthesis (41). Defects of collagen type I include abnormalities of primary collagen structure, unusual folding, abnormal post-translational modification, and matrix incorporation (41,42). The affection range is spread from mild osteopenia to moderate and severe forms, including limb deformity and lethal cases (42). According to the definition, mutations in one of the two genes, i.e., *COL1A1* and *COL1A2*, are responsible for OI as a heritable disorder. *COL1A1* and *COL1A2* encode the two chains, proα-1(I) and proα-2(I), respectively, of type I pro-collagen. In bones, type I collagen is most abundantly present; normally, collagen is composed of alpha chains (42). Overall, the incidence of OI ranges from 1 in 15,000–20,000 births, and autosomal dominant inheritance is the major source of causation. Phenotype depends on the type of mutation present; thus,

a genotype–phenotype relation does exist up to a certain level (43).

About 90% of the patients have OI, which is dominantly inherited. The set of genes involved in *COL1A1* or *COL1A2* encodes the α-1 and α-2 chains of type I collagen (44). Due to mutations in these genes, the structure or the amount of type I collagen is altered, leading to the severe skeletal phenotype that ranges from subclinical to lethal (44).

Consanguineous marriages are the major reason for autosomal recessive (AR) OI, which accounts for only 10%. Recent advances in molecular diagnosis have increased the number of novel candidate genes associated with recessive OI (Table 1). Genes responsible for AR OI usually encode proteins responsible for the assembly of the triple helix, chaperoning of the type I pro-collagen hetero trimer (42,45). Only three genes have been associated with dominant OI, accounting for 90% of the disease, while 17 recessive OI have been characterized. Those account for only 10% of the disease pathogenesis (45).

Acromesomelic dysplasias

AMD are severe skeletal dysplasias that constitute disproportionate shortening of skeletal elements, mainly affecting the hands and feet [distal segments] and the forearms and forelegs [middle segments] of the appendicular skeleton. Three main types of AMD have been reported in the literature (46; Table 2).

AMD type 1 (Maroteaux)

Acromesomelic dysplasia maroteaux (AMDM), also known as AMDM (MIM 602875) caused by homozygous or compound heterozygous variants in the *NRP2* gene located on chromosome 9p13.3. Associated clinical features include disproportionate short stature, bowed forearms, increased lumbar lordosis, short tubular bones, metaphyseal flaring of long bones, flattened midface, short, broad digits, bowing of the radius, short nails, and other associated phenotypes (46). It has been observed that the individual's carrier for the mutation is shorter than normal (47). Natriuretic peptide-natriuretic peptide receptor B (NPR-B) acts as a receptor for the C-type natriuretic peptide, which performs the function of an autocrine regulator in several different human tissues. The structure of NPR-B constitutes a ligand-binding domain [extracellular; 23-441 amino acids (a.a)], a transmembrane domain [hydrophobic; 442-512 a.a], protein kinase homology domain [intracellular; 512-826 a.a], and nucleotide cyclase domain [826-1046] (47; Figure 1).

Table 1. *OI classification.*

Phenotype	Inheritance	MIM number	Gene/Locus	Location
OI, type I	AD	166200	*COL1A1*	17q21.33
OI, type II	AD	166210	*COL1A2*	7q21.3
OI, type III	AD	259420	*COL1A2*	7q21.3
OI, type IV	AD	166220	*COL1A2*	7q21.3
OI, type V	AD	610967	*IFITM5*	11p15.5
OI, type VI	AR	613982	*SERPINF1*	17p13.3
OI, type VII	AR	610682	*CRTAP*	3p22.3
OI, type VIII	AR	610915	*P3H1*	1p34.2
OI, type IX	AR	259440	*PPIB*	15q22.31
OI, type X	AR	613848	*SERPINH1*	11q13.5
OI, type XI	AR	610968	*FKBP10*	17q21.2
OI, type XII	AR	613849	*SP7*	12q13.13
OI, type XIII	AR	614856	*BMP1*	8p21.3
OI, type XIV	AR	615066	*TMEM38B*	9q31.2
OI, type XV	AR	615220	*WNT1*	12q13.12
OI, type XVI	AR	616229	*CREB3L1*	11p11.2
OI, type XVII	AR	616507	*SPARC*	5q33.1
OI, type XVIII	AR	617952	*TENT5A*	6q14.1
OI, type XIX	XLR	301014	*MBTPS2*	Xp22.12
OI, type XX	AR	618644	*MESD*	15q25.1
OI, type XXI	AR	619131	*KDELR2*	7p22.1
OI, type XXII	AR	619795	*CCDC134*	22q13.2

Abbreviations: AR, autosomal recessive; AD, autosomal dominant; XLR, X-linked recessive.

Table 2. AMD classification.

Phenotype	Inheritance	MIM number	Gene/Locus	Location
AMD 1, Maroteaux type	AR	602875	*NPR2*	9p13.3
AMD 2A	AR	200700	*GDF5*	20q11.22
AMD 2B	AR	228900	*GDF5*	20q11.22
AMD 2C, Hunter-Thompson type	AR	201250	*GDF5*	20q11.22
AMD 3	AR	609441	*BMPR1B*	4q22.3
AMD 4	AR	619636	*PRKG2*	4q21.21

Abbreviations: AR, autosomal recessive.

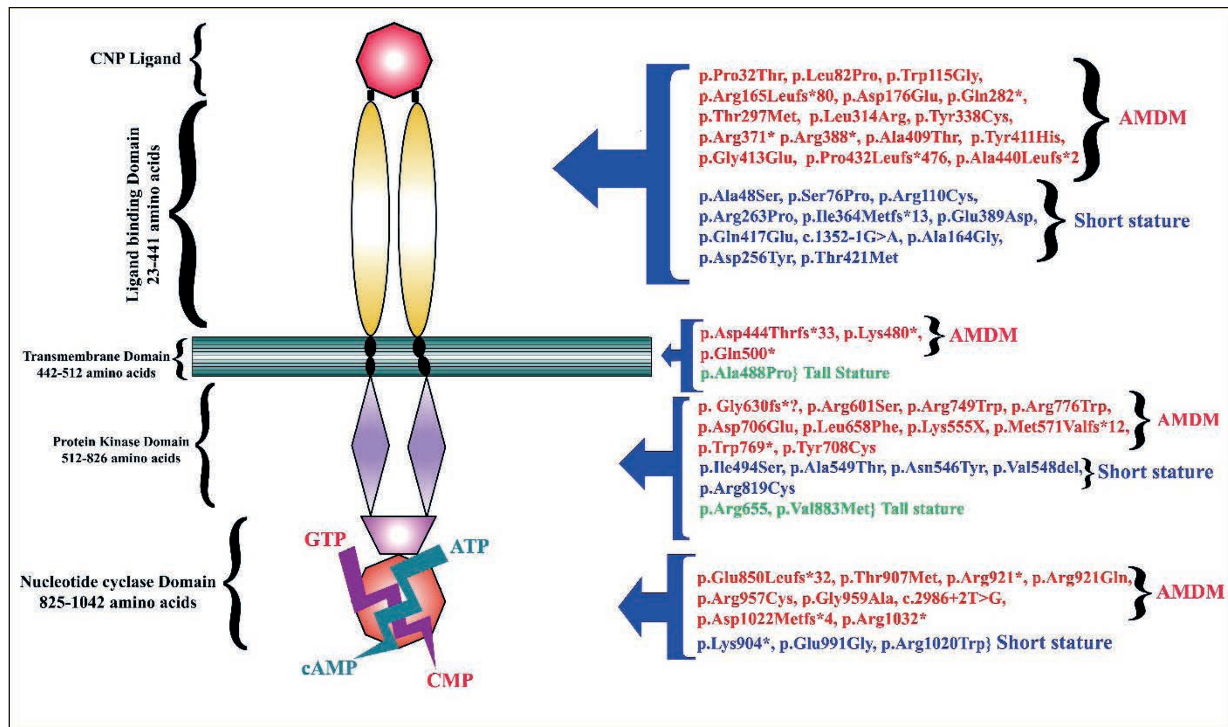


Figure 1. *Structure of NPR2 and position of previously reported variants in different domains.*

AMD type 2 (AMD2)

AMD2 (OMIM) is a recessively inherited distinct limb developmental disorder. AMDG is further divided into three categories such as AMD 2A, also known as Grebe dysplasia (AMDG; OMIM 200700), AMD 2B, also known as Du pan syndrome (OMIM 228900) and AMD 2C also known as Hunter-Thompson type (OMIM 201250). All the AMD2 types are caused by homozygous or compound heterozygous variants in the *GDF5* gene located on chromosome 20q11.22 (48-50).

AMD type 3 (AMD3)

AMD with genital or without anomalies, also known as "Demirhan type," have overlapping features with Grebe, Hunter-Thompson, and DuPan syndrome (fibular hypoplasia and complex brachydactyly) patients (OMIM 609441). Demirhan *et al.* (51) reported a homozygous mutation in *BMPR1B* in a patient having severe AMD and ovarian dysfunction. Later, Ullah *et al.* (48) reported a novel homozygous missense variant (c.1190T > G, p.Met397Arg) in the *BMPR1B* gene associated with AMD Hunter-Thompson type. BMPR1B acts as the major receptor for CDMP1, which play a major role in the signaling process for the bone morphogenbetic pathway, suggesting a critical role in skeletal development, digit patterning, chondrocyte differentiation, and joint development (48).

Split hand-foot malformation

SHFM is an extremely rare limb developmental abnormality that results in improper patterning and development of both upper and lower limbs. It results in the development of deep median clefts unilaterally or bilaterally in hands and feet, resulting in aplasia or hypoplasia of the digits. SHFM can occur as a part of a complex syndrome or as an isolated entity, and the phenotypic presentation can range from mild-severe phenotypes depending on the gene/variant involved (Figure 2), (52).

Nonsyndromic SHFM is further characterized into eight different types in humans. These include four types

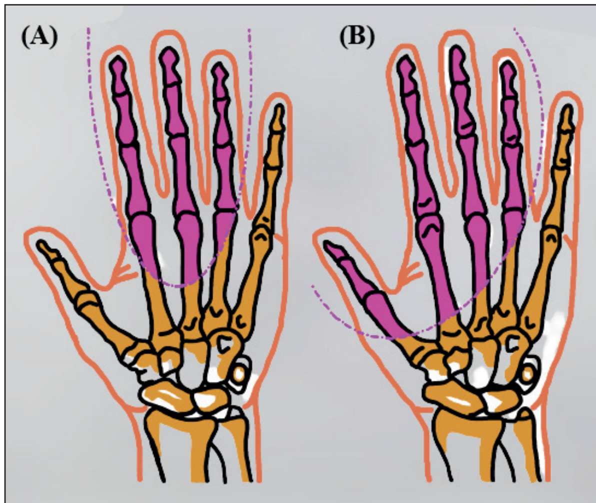


Figure 2. *A: Exhibiting type of SHFM in which fingers colored in pink are missing. B: Manifesting monodactyly in which all fingers are missing except little finger.*

inherited in an autosomal dominant fashion, caused by heterozygous variants in the concerned gene, including SHFM1 (*DLX5* gene, *DLX6* gene), SHFM3 (SHFM3 locus 10q24), SHFM4 (*TP63* gene), SHFM5 (SHFM5 locus 2q31), three types inherited in AR fashion caused by homozygous variants including *SHFM6* Wnt Family Member 10B (WNT10B gene), SHFM7 (*ZAK* gene, SHFM8 (*EPS15L1* gene) and only one type associated with X-linked SHFM (SHFM2 (SHFM2 locus Xq26) (52-58) (Table 3). To date, disease-causing variants have been associated with only 6 genes causing SHFM, including *TP63, DLX5, DLX5, WNT10B, ZAK, and EPS15L1* (52) (Table 3).

SHFM type 1

SHFM1 (OMIM 183600) results in the deep median clefts, lack of the central digital rays, and complex syndactyly, mapping to 7q21.3. Microdeletion and variants in the *DLX5* and *DLX6* have been associated with SHFM1 (53,59).

SHFM type 2

SHFM2 (OMIM 313350) is inherited in an X-linked fashion, mapped to chromosome Xq26.3. It is characterized by bilateral lobster-claw deformity, metacarpal hypoplasia, partial syndactyly, and phalangeal hypoplasia involving both upper and lower limbs (52).

SHFM type 3

SHFM3 (OMIM 246560), characterized by maxillary hypoplasia, hearing loss, cleft palate, ectrodactyly, phalangeal hypoplasia, and intellectual disability, was observed in some patients. SHFM3 was mapped to human chromosome 10q24 (60).

SHFM type 4

SHFM4 (OMIM 605289) exhibits variable features such as lobster-claw anomaly, monodactyly, syndactyly, missing phalanges, and triphalangeal thumb. Affected individuals in different families and even within the same family have been observed to have reduced penetrance. SHFM4 is associated with disease-causing variants in the *TP63* gene located on chromosome 3q28. *TP63* is a transcription factor that encodes the p53 family of transcription factors. The p53 family proteins have several domains, including an N-terminal transactivation domain, a central DNA-binding domain, and an oligomerization domain (57).

SHFM type 5

SHFM5 (OMIM 606708) has an autosomal dominant inheritance mode. It was mapped on chromosome 2 with a genetic address of 2q31 (52). It includes closely related genes *DLX1* and *DLX2* with no pathogenic variants yet reported (61). No associated genes have been identified so far for SHFM5.

SHFM type 6

SHFM6 (OMIM 225300) is inherited in an AR fashion, characterized by hallmark features such as ectrodactyly, split foot, split hands, complex syndactyly, polydactyly (some patients), and other variable phenotypes. SHFM6 is mapped at chromosome 12q13.11-q13, and disease-causing variants in *WNT10B* have been associated with the phenotype (55-57). Wnt Family Member 10B (*WNT10B*) gene is a member of the *WNT* gene family, and its protein signaling is a molecular switch that governs adipogenesis. WNT signaling is involved in the translocation of β-catenin to the nucleus and binds to several transcription factors, resulting in the regulation of osteoblastogenesis (52,61).

SHFM type 7

SHFM7 is characterized by split-foot malformation, cutaneous syndactyly, digit duplication, and sensorineural hearing impairment. Disease-causing variants in the *ZAK* gene have been associated with SHFM7, located on chromosome 2q31.1 (58). ZAK is a serine-threonine kinase that belongs to the MAPKKK family of signal transduction molecules. It has been observed that Zak is a positive mediator of cell hypertrophy in cultured rat cardiac myocytes and mediates TGF-beta-induced cardiac hypertrophy via a TGF-beta-ZAK-MKK7-ANF signaling pathway (62).

SHFM type 8

SHFM8 (OMIM 616826) is characterized by features such as mild SHFM, cutaneous syndactyly, aplasia, and hypoplasia of carpals and metacarpals. Epidermal Growth Factor Receptor Pathway Substrate 15 Like 1 (*EPS15L1*) is involved in the endocytosis of integrin beta-1 (ITGB1) and transferrin receptor; internalization of ITGB1 as DAB2-dependent cargo. Disease-causing variants in the *EPS15L1* gene have been associated with SHFM8, located on chromosome 19p13.11 (54). Only a single family with two affected individuals has been associated with SHFM8 having a homozygous frameshift variant (c.409delA) in exon 7 of the *EPS15L1* (54).

Table 3. SHFM current classification.

SHFM type	Locus	OMIM	Causative gene/Molecular mechanism	Chromosomal localization	Inheritance
Isolated SHFM	SHFM1	183600	Mutations in *DLX5* and *DLX6*	7q21.2-q21.3	AD
	SHFM1D	220600	Suspected dysregulation of *DLX5* and *DLX6*	7q21.2–q21.3	AD
	SHFM2	313350	Unknown	Xq26	XL
	SHFM3	246560	Microduplications involving *BTRC*, *POLL*, and *FBXW4*	10q24	AD
	SHFM4	605289	*TP63* mutations	3q28	AD
	SHFM5	606708	Suspected dysregulation of *HOXD* cluster	2q31	AD
	SHFM6	225300	*WNT10B* mutations	12q13.12	AR
	SHFM7	616890	*ZAK* mutations	2q31.1	AR
	SHFM8	616826	*EPS15L1* microdeletions/mutations	19p13.11	AR

Abbreviations: AR, autosomal recessive; AD, autosomal dominant; XLR, X-linked recessive.

Bardet-Biedl syndrome

BBS is a multi-systemic recessive syndrome characterized by hallmark features such as intellectual disability, obesity, renal anomalies, retinal cone-rod dystrophy, hexadaxtyly, and hypogenitalism. If the affected individual presents four out of the six significant features or three major features and two minor features, he is classified as having BBS. Other associated features include cardiovascular anomalies, hearing loss, oral/ dental abnormalities, neurodevelopmental abnormalities, metabolic defects, and diabetes mellitus (63).

BBS exhibits extensive clinical heterogeneity, and the occurrence of digenic and transgenic inheritance has been observed. BBS has been associated with disease-causing mutations in 23 different genes mapped on different chromosomes, including *BBS1* (OMIM 209901), *BBS2* (OMIM 606151), *BBS3 (ARL6; O*MIM 608845), *BBS4* (OMIM 600374), *BBS5* (OMIM 603650), *BBS6* (*MKKS*; OMIM 604896), *BBS7* (OMIM607590), *BBS8* (*TTC8*) (OMIM 608132), *BBS9* (OMIM 607968), *BBS10* (OMIM 610148), *BBS11* (*TRIM32*; OMIM 602290), *BBS12* (OMIM 610683), BBS13 (*MKS1*; OMIM 609883), BBS14 (*CEP290*; OMIM 610142), BBS15 (*C2ORF86*; OMIM 613580), BBS16 (*SDCCAG8* (OMIM 613524), BBS17 (*LZTFL1*; OMIM 606568)**,** BBS18 (*BBIP1*; OMIM 615995)**,** BBS19 (*IFT27*; OMIM 615996)**,** BBS20 (*IFT172*; OMIM 619471)**,** BBS21 (*CFAP418*; OMIM 617406)**,** BBS22 (*IFT74*; OMIM 617119), and BBS23 (*CEP19*; OMIM615586) (64,65) (Table 4).

BBS is a genetically heterogeneous syndrome with overlapping clinical features with other ciliopathies disorders. Thus, molecular testing through a ciliopathy gene panel or whole exome sequencing (WES) is the correct method for proper molecular diagnosis. To date, no successful therapy has been suggested for BBS, as the disorder is multi-systemic. Thus several organs are affected; therefore, the patient requires multidisciplinary care, proper coordinated management, and extensive therapeutic interventions (65,66).

Polydactyly

Polydactyly, also termed hexadactyly, is the development of supernumerary digits or toes. Polydactyly is an inherited condition and one of the most common inherited digital anomalies, manifesting in various forms. It might range from complete duplication of a limb or limb part to complete duplication of a digit. Polydactyly can occur as an isolated entity or be associated with a complex syndrome (syndromic forms; 67).

Nonsyndromic polydactyly is further divided into three types, (a) preaxial polydactyly (PPD), having an extra digit at the side of the thumb or great toe, (b) PAP, with extra digits at the side of the 5th finger or toe and (c) complex polydactyly, where the extra digit originates from the middles of the hand (67-69).

PPD is further divided into four types. Type 1 is characterized by an extra digit with the first finger, polydactyly of the triphalangeal first digit is included in type 2, type 3 is polydactyly of the second digit. In contrast, type 4 is polysyndactyly (67,70).

PAP is classified into type A, and type B. In type A, the extra digit is fully developed, with fully developed bone (both functional or nonfunctional), and in type B, where the extra digit is not well formed and occurs in the form of a nonfunctional skin tag (67,66) (Figure 3).

Nonsyndromic (isolated) polydactyly segregates in autosomal dominant and recessive fashion. PPD is further classified into four types such as PPD1, caused by variants in the *GLI* gene located on chromosome 12q13.3 (OMIM165220) (71,72), PPD2 caused due by mutations in the *LMBR1* gene located on 7q36.3 (OMIM174500), PPD3, whose locus is not mapped up till now (OMIM174600), PPD4 inherited in dominant fashion and *GLI3* associated mutations have been linked to the disease phenotypes (73). Triphalangeal thumb, type I, caused by variants in the *LMBR1* gene located on 7q36.3 (OMIM174500), and PPD5 inherited in AR fashion and homozygous mutation in *STKLD1* has

Table 4. BBS classification.

Phenotype	Inheritance	MIM number	Gene/Locus	Location
BBS 1	AR	209900	*BBS1*	11q13.2
BBS 1	AR	209900	*CCDC28B*	1p35.2
BBS 1	AR	209900	*ARL6*	3q11.2
BBS 2	AR	615981	*BBS2*	16q13
BBS 3	AR	600151	*ARL6*	3q11.2
BBS 4	AR	615982	*BBS4*	15q24.1
BBS 5	AR	615983	*BBS5*	2q31.1
BBS 6	AR	605231	*MKKS*	20p12.2
BBS 7	AR	615984	*BBS7*	4q27
BBS 8	AR	615985	*TTC8*	14q31.3
BBS 9	AR	615986	*PTHB1*	7p14.3
BBS 10	AR	615987	*BBS10*	12q21.2
BBS 11	AR	615988	*TRIM32*	9q33.1
BBS 12	AR	615989	*BBS12*	4q27
BBS 13	AR	615990	*MKS1*	17q22
BBS 14	AR	615991	*CEP290*	12q21.32
BBS 14	AR	615991	*TMEM67*	8q22.1
BBS 15	AR	615992	*WDPCP*	2p15
BBS 16	AR	615993	*SDCCAG8*	1q43-q44
BBS 17	AR	615994	*LZTFL1*	3p21.31
BBS 18	AR	615995	*BBIP1*	10q25.2
BBS 19	AR	615996	*IFT27*	22q12.3
BBS 20	AR	619471	*IFT172*	2p23.3
BBS 21	AR	617406	*CFAP418*	8q22.1
BBS 22	AR	617119	*IFT74*	9p21.2
BBS 23	AR	615586	*CEP19*	3q29

Abbreviations: AR, autosomal recessive.

been associated with the disease phenotype located on chromosome 9q34.2 (OMIM618530) (67).

PAP is associated with 11 genes/loci located on different human chromosomes (Table 5). PAP1 mapped on chromosome 7p13 with *GLI3* gene mutations (74), PAP2 having a chromosomal address of 13q21-q32 (no gene identified), PAPA3 with characteristics of PAP-A/B mapped on chromosome 19p13.1-13.2 (no gene identified) and PAPA4 have an autosomal dominant inheritance with PAP-A/B phenotypes and partial cutaneous syndactyly mapped on chromosome 7q21-q34 (no gene identified). PAPA5 was mapped in a large Pakistani family having AR on chromosome 13q13.3-q21.2 (no gene identified). PAPA6 has AR inheritance, and the associated gene is *ZNF141*, located on chromosome 4p16.3. A disease-causing variant in the *IQCE* gene has been associated with PAPA7, located on chromosome 7p22.3 (70). A disease-causing variant in the *GLI1* gene has been associated with PAPA8 with *EVC* overlapping features on chromosome 12q13.3 (75). PAPA9 having recessive inheritance has been associated with *FAM92A* gene variants. A disease-causing variant in the *KIAA0825* gene has been associated with PAPA10, located on chromosome 5q15 (76). Similarly, PAPA11

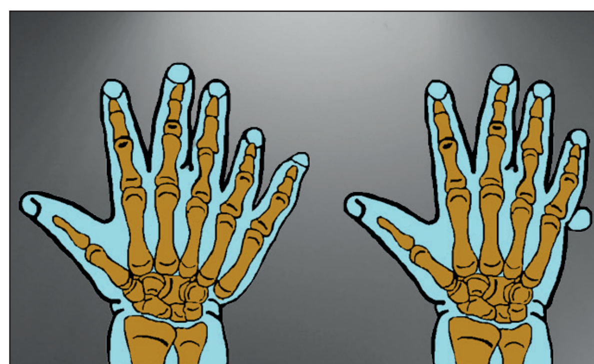


Figure 3. *A: Type of fully developed polydactyly with metacarpal bone. B: Manifesting features of polydactyly type B.*

has been associated with homozygous *DACH1* variants on chromosome 13q21.33 (77).

If we type the mesh "Polydactyly" in OMIM (https://omim.org/), we receive "496" entries, thus showing its involvement in many different disorders. These syndromic polydactyly disorders/syndromes present diverse phenotypes and are very severe. Polydactyly can

Table 5. Polydactyly classification.

Disease	Genes	Inheritance	Locus	OMIM
PPD1	*GLI*	AR	12q13.3	165220
PPD2	*LMBR1*	AD	7q36.3	174500
PPD3	*PPD3*	AD	**U**	174600
PPD4	*GLI3*	AD	7p14.1	174200
PPD5	*STKLD1*	AR	9q34.2	618530
Triphalangeal thumb, type I	*LMBR1*	AD	7q36.3	174500
PAPA1	*GLI3*	AD, AR	7p14.1	174200
PAPA2	*U*	AD	13q21-q32	602085
PAPA3	*U*	AD	19p13.1-p13.2	607324
PAPA4	*U*	AD	7q21-q34	608562
PAPA5	*U*	AR	13q13.3-q21.2	263450
PAPA6	*ZNF141*	AR	4p16.3	615226
PAPA7	*IQCE*	AR	7p22.3	617642
PAPA8	*GLI1*	AR	12q13.3	165220
PAPA9	*FAM92A*	AR	8q22.1	617273
PAPA10	*KIAA0825*	AR	5q15	617266
PAPA11	*DACH1*	AR	13q21.33	603803

Abbreviations: AR, autosomal recessive; AD, autosomal dominant; U, unknown.

be identified at the early stage using ultrasound, which might give time for the clinicians to start management strategies for the severe syndromic form of the disorder (68,78,79).

Diagnosis and genetic counseling

GSDs are diagnosed mainly by the radiological features in association with either targeted gene, panel sequencing, or next-generation sequencing (NGS), either WES or whole genome sequencing. Phenotypic appearance and radiographic analysis of the affected individuals can be the first step of diagnosis; however, for a multi-systemic disorder such as BBS, molecular diagnosis is required to identify the culprit genetic variant. Once mutation in the specific gene is identified, carrier testing and proper genetic counseling of the family can be performed (80,81).

Discussion

GSDs are characterized by inconsistent growth, severe bone malformations, and distortion of individual bones or groups of bones that results in either nonsyndromic (isolated) or as a part of a complex syndrome (syndromic form) (81). Disruption of specific developmental pathways results in GSDs that can be either due to disruption of the intricate processes of growth, development, and/or homeostasis of the skeletal system. With the advent of the latest NGS technology and the development of new machines, the molecular diagnosis of GSDs is now accurate, quick, and cost-effective (82).

Many GSDs are very severe and result in the death of the affected individuals. Thus, genetic counseling, newborn screening, and molecular diagnosis are necessary (2). Genetic screening can be either targeted gene sequencing, panel gene sequencing in case there are more genes associated with a particular disorder, or WES of two or three individuals from each family. Recently, molecular diagnostic techniques such as prenatal genetic testing (PGT), especially pre-natal genetic screening for monogenetic disorders (PGT-M), served an excellent deal in the future management of monogenetic disorders (83,84). PGT, in association with *in vitro* fertilization, is an option for parents wishing to have future pregnancies (85). Disorders such as frontonasal dysplasias can be dealt with plastic surgeries so that the affected individuals can live a normal life (86-88). However, proper disease management and therapeutic interventions are only possible if the concerned clinicians receive a correct molecular diagnosis. In such a scenario, knowledge about the molecular etiology and pathophysiology of the disorder is a must to implement and draw future therapeutic interventions.

A total of 437 different genes are involved in causing 461 different GSDs, making it a complex heterogeneous group of disorders, thus making diagnosis difficult. Monogenetic disorders are best to study as loss of function in these genes presents a perfect model and help us to track down the proper gene function and associated pathways. Studying rare genetic disorders and the pathogenic mutations involved provide insight into different preventive measures and diagnostic applications and, finally, helps in therapeutic strategies. Furthermore, the number of increased patients associated with a particular disorder can be subjected to clinical trials using Food and Drug Administration-approved drugs (89).

As a result, large-scale DNA sequencing using NGS is mostly performed, which might help researchers to

diagnose easily, which is a prerequisite for accurate genetic counseling (90). Establishing a proper medical policy is vital, which would significantly reduce the risk of misdiagnosis and improve/develop a treatment for GSDs. A strong network and collaboration among international scientists from different institutes should be established to find an ultimate treatment for GSDs.

Conclusion

In developing countries, proper genetic testing and establishing newborn screening are still a great issue, and thus rare GSDs receive less attention. In such countries, a database development should be developed to save the data for patients with such severe conditions. Furthermore, there should be a register for GSDs that might provide information about the prevalent mutation in the community and tribe. Due to the unitability of such resources and documentation, this creates diagnostics issues for clinicians and researchers.

In such a situation, a systematic bibliographic study of GSDs might help to estimate the prevalence or occurrence of GSDs in a community and pinpoint hotshot variants. Knowledge regarding the pathophysiologic nature of the disorders, the disease mechanism, unrevealing the biomarkers, and the disease pathway is mandatory to proceed with gene therapy.

List of Abbreviations

AMD	Acromesomelic dysplasias
BBS	Bardet–Biedl syndrome
GSD	Genetic skeletal disorders
OI	Osteogenesis Imperfecta
PAP	Postaxial polydactyly
PPD	Preaxial polydactyly

Funding

None.

Declaration of conflicting interests

The authors of this article have no affiliations with or involvement in any organization or entity with any financial interest or nonfinancial interest in the subject matter or materials discussed in this manuscript.

Consent to participate

Not applicable.

Ethical approval

Not applicable.

Author details

Safdar Abbas[1], Hammal Khan[2,3], Qamre Alam[4], Arif Mahmood[5], Muhammad Umair[6]

1. Department of Biological Science, Dartmouth College, Hanover, NH
2. Department of Biochemistry, Faculty of Biological Sciences, Quaid-i-Azam University, Islamabad, Pakistan
3. Department of Biosciences, COMSATS University, Islamabad, Pakistan
4. Molecular Genomics and Precision Medicine, ExpressMed Laboratories, Zinj, Bahrain
5. Center for Medical Genetics and Hunan Key Laboratory of Medical Genetics, School of Life Sciences, Central South University, Changsha, China
6. Department of Life Sciences, School of Science, University of Management and Technology [UMT], Lahore, Pakistan

References

1. Krakow D, Rimoin DL. The skeletal dysplasias. Genet Med. 2010;12(6):327–41. https://doi.org/10.1097/GIM.0b013e3181daae9b.
2. Umair M, Younus M, Shafiq S, Nayab A, Alfadhel M. Clinical genetics of spondylocostal dysostosis: a mini-review. Front Genet. 2022;13:996364. https://doi: 10.3389/fgene.2022.996364.
3. Anderson BW, Ekblad J, Bordoni B. Anatomy, appendicular skeleton. Treasure Island, FL: StatPearls Publishing; 2022. Available from: https://www.ncbi.nlm.nih.gov/books/NBK535397/
4. Matsushita Y, Ono W, Ono N. Skeletal stem cells for bone development and repair: diversity matters. Curr Osteoporos Rep. 2020;18(3):189–98. https://doi: 10.1007/s11914-020-00572-9.
5. Knight MN, Hankenson KD. Mesenchymal stem cells in bone regeneration. Adv Wound Care (New Rochelle). 2013;2(6):306–16. https://doi: 10.1089/wound.2012.0420.
6. Hartmann C. Skeletal development--Wnts are in control. Mol Cells. 2007;24(2):177–84.
7. Tani S, Chung UI, Ohba S, Hojo H. Understanding paraxial mesoderm development and sclerotome specification for skeletal repair. Exp Mol Med. 2020;52(8):1166–77. https://doi: 10.1038/s12276-020-0482-1.
8. Kidwai F, Mui BWH, Arora D, Iqbal K, Hockaday M, de Castro Diaz LF, et al. Lineage-specific differentiation of osteogenic progenitors from pluripotent stem cells reveals the FGF1-RUNX2 association in neural crest-derived osteoprogenitors. Stem Cells. 2020;38(9):1107–23. https://doi: 10.1002/stem.3206.
9. Elshazzly M, Lopez MJ, Reddy V, Caban O. Embryology, central nervous system. Treasure Island, FL: StatPearls Publishing; 2022. https://www.ncbi.nlm.nih.gov/books/NBK526024/.
10. Nagashima H, Sugahara F, Watanabe K, Shibata M, Chiba A, Sato N. Developmental origin of the clavicle, and its implications for the evolution of the neck and the paired appendages in vertebrates. J Anat. 2016;229(4):536–48. https://doi: 10.1111/joa.12502.
11. Long F, Ornitz DM. Development of the endochondral skeleton. Cold Spring Harb Perspect Biol. 2013;5(1):a008334. https://doi: 10.1101/cshperspect.a008334.
12. Wu M, Chen G, Li YP. TGF-β and BMP signaling in osteoblast, skeletal development, and bone formation, homeostasis and disease. Bone Res. 2016;4:16009. https://doi: 10.1038/boneres.2016.9.
13. Berendsen AD, Olsen BR. Bone development. Bone. 2015;80:14–8. https://doi: 10.1016/j.bone.2015.04.035
14. Yang Y, Kozin SH. Cell signaling regulation of vertebrate limb growth and patterning. J Bone Joint Surg Am. 2009;91(Suppl 4):76–80. https://doi: 10.2106/JBJS.I.00079.
15. Tickle C. How the embryo makes a limb: determination, polarity and identity. J Anat. 2015;227(4):418–30. https://doi: 10.1111/joa.12361.
16. Newton AH, Williams SM, Major AT, Smith CA. Cell lineage specification and signalling pathway use during

development of the lateral plate mesoderm and forelimb mesenchyme. Development. 2022;149(18):dev200702. https://doi: 10.1242/dev.200702.

17. Pineault KM, Wellik DM. Hox genes and limb musculoskeletal development. Curr Osteoporos Rep. 2014;12(4):420–7. https://doi: 10.1007/s11914-014-0241-0.
18. Seifert A, Werheid DF, Knapp SM, Tobiasch E. Role of Hox genes in stem cell differentiation. World J Stem Cells. 2015;7(3):583–95. https://doi: 10.4252/wjsc.v7.i3.583.
19. Barrow JR, Thomas KR, Boussadia-Zahui O, Moore R, Kemler R, Capecchi MR, et al. Ectodermal Wnt3/beta-catenin signaling is required for the establishment and maintenance of the apical ectodermal ridge. Genes Dev. 2003;17(3):394–409. https://doi: 10.1101/gad.1044903.
20. Lu P, Yu Y, Perdue Y, Werb Z. The apical ectodermal ridge is a timer for generating distal limb progenitors. Development. 2008;135(8):1395–405. https://doi: 10.1242/dev.018945.
21. Pownall ME, Isaacs HV. FGF signalling in vertebrate development. In: Limb development. San Rafael, CA: Morgan & Claypool Life Sciences; 2010. Available from: https://www.ncbi.nlm.nih.gov/books/NBK53162/
22. Jin L, Wu J, Bellusci S, Zhang JS. Fibroblast growth factor 10 and vertebrate limb development. Front Genet. 2019;9:705. https://doi: 10.3389/fgene.2018.00705.
23. Villanueva C, Jacobson-Dickman E, Xu C, Manouvrier S, Dwyer AA, Sykiotis GP, et al. Congenital hypogonadotropic hypogonadism with split hand/foot malformation: a clinical entity with a high frequency of FGFR1 mutations. Genet Med. 2015;17(8):651–9. https://doi: 10.1038/gim.2014.166
24. Tickle C, Towers M. Sonic Hedgehog signaling in limb development. Front Cell Dev Biol. 2017;5:14. https://doi: 10.3389/fcell.2017.00014.
25. Chaudhry P, Singh M, Triche TJ, Guzman M, Merchant AA. GLI3 repressor determines Hedgehog pathway activation and is required for response to SMO antagonist glasdegib in AML. Blood. 2017;129(26):3465–75. https://doi: 10.1182/blood-2016-05-718585.
26. Hong M, Schachter KA, Jiang G, Krauss RS. Neogenin regulates Sonic Hedgehog pathway activity during digit patterning. Dev Dyn. 2012;241(3):627–37. https://doi: 10.1002/dvdy.23745.
27. Watson BA, Feenstra JM, Van Arsdale JM, Rai-Bhatti KS, Kim DJH, Coggins AS, et al. LHX2 mediates the FGF-to-SHH regulatory loop during limb development. J Dev Biol. 2018;6(2):13. https://doi: 10.3390/jdb6020013.
28. Geetha-Loganathan P, Nimmagadda S, Scaal M. Wnt signaling in limb organogenesis. Organogenesis. 2008;4(2):109–15. https://doi: 10.4161/org.4.2.5857.
29. Feenstra JM, Kanaya K, Pira CU, Hoffman SE, Eppey RJ, Oberg KC. Detection of genes regulated by Lmx1b during limb dorsalization. Dev Growth Differ. 2012;54(4):451–62. https://doi: 10.1111/j.1440-169X.2012.01331.x.
30. Galloway JL, Tabin CJ. Classic limb patterning models and the work of Dennis Summerbell. Development. 2008;135(16):2683–7. https://doi: 10.1242/dev.021188.
31. Kornak U, Mundlos S. Genetic disorders of the skeleton: a developmental approach. Am J Hum Genet. 2003;73(3):447–74. https://doi: 10.1086/377110.
32. Wagner MW, Poretti A, Benson JE, Huisman TA. Neuroimaging findings in pediatric genetic skeletal disorders: a review. J Neuroimaging. 2017;27(2):162–209. https://doi: 10.1111/jon.12413.
33. Krakow D. Skeletal dysplasias. Clin Perinatol. 2015;42(2):301–19, viii. https://doi: 10.1016/j.clp.2015.03.003.
34. Piróg KA, Briggs MD. Skeletal dysplasias associated with mild myopathy-a clinical and molecular review. J Biomed Biotechnol. 2010;2010:686457. https://doi: 10.1155/2010/686457.
35. Bonafe L, Cormier-Daire V, Hall C, Lachman R, Mortier G, Mundlos S, et al. Nosology and classification of genetic skeletal disorders: 2015 revision. Am J Med Genet A. 2015;167A(12):2869–92. https://doi: 10.1002/ajmg.a.37365.
36. Warman ML, Cormier-Daire V, Hall C, Krakow D, Lachman R, LeMerrer M, et al. Nosology and classification of genetic skeletal disorders: 2010 revision. Am J Med Genet A. 2011;155A(5):943–68. https://doi: 10.1002/ajmg.a.33909.
37. Mortier GR, Cohn DH, Cormier-Daire V, Hall C, Krakow D, Mundlos S, et al. Nosology and classification of genetic skeletal disorders: 2019 revision. Am J Med Genet A. 2019;179(12):2393–419. https://doi: 10.1002/ajmg.a.61366.
38. Subramanian S, Viswanathan VK. Osteogenesis imperfecta. Treasure Island, FL: StatPearls Publishing; 2022. Available from: https://www.ncbi.nlm.nih.gov/books/NBK536957/.
39. Botor M, Fus-Kujawa A, Uroczynska M, Stepien KL, Galicka A, Gawron K, et al. Osteogenesis imperfecta: current and prospective therapies. Biomolecules. 2021;11(10):1493. https://doi: 10.3390/biom11101493.
40. Umair M, Hassan A, Jan A, Ahmad F, Imran M, Samman MI, et al. Homozygous sequence variants in the FKBP10 gene underlie osteogenesis imperfecta in consanguineous families. J Hum Genet. 2016;61(3):207–13. https://doi: 10.1038/jhg.2015.129.
41. Van Dijk FS, Sillence DO. Osteogenesis imperfecta: clinical diagnosis, nomenclature and severity assessment. Am J Med Genet A. 2014;164A(6):1470–81.
42. van Dijk FS, Cobben JM, Pals G. Osteogenesis imperfecta, normal collagen folding, and lack of cyclophilin B. N Engl J Med. 2010;362(20):1940–1; author reply 1941–2. https://doi: 10.1056/NEJMc1002797.
43. Umair M, Alhaddad B, Rafique A, Jan A, Haack TB, Graf E, et al. Exome sequencing reveals a novel homozygous splice site variant in the WNT1 gene underlying osteogenesis imperfecta type 3. Pediatr Res. 2017;82(5):753–8. https://doi: 10.1038/pr.2017.149.
44. Maioli M, Gnoli M, Boarini M, Tremosini M, Zambrano A, Pedrini E, et al. Genotype-phenotype correlation study in 364 osteogenesis imperfecta Italian patients. Eur J Hum Genet. 2019;27(7):1090–100. https://doi: 10.1038/s41431-019-0373-x.
45. Hayat A, Hussain S, Bilal M, Kausar M, Almuzzaini B, Abbas S, et al. Biallelic variants in four genes underlying recessive osteogenesis imperfect. Eur J Med Genet. 2020;63(8):103954. https://doi: 10.1016/j.ejmg.2020.103954.
46. Irfanullah, Umair M, Khan S, Ahmad W. Homozygous sequence variants in the NPR2 gene underlying acromesomelic dysplasia maroteaux type (AMDM) in consanguineous families. Ann Hum Genet. 2015;79(4):238–44. https://doi: 10.1111/ahg.12116.

47. Khan S, Basit S, Khan MA, Muhammad N, Ahmad W. Genetics of human isolated acromesomelic dysplasia. Eur J Med Genet. 2016;59(4):198–203. https://doi: 10.1016/j.ejmg.2016.02.011.
48. Ullah A, Umair M, Muhammad D, Bilal M, Lee K, Leal SM, et al. A novel homozygous variant in BMPR1B underlies acromesomelic dysplasia Hunter-Thompson type. Ann Hum Genet. 2018;82(3):129–34. https://doi: 10.1111/ahg.12233.
49. Umair M, Rafique A, Ullah A, Ahmad F, Ali RH, Nasir A, et al. Novel homozygous sequence variants in the GDF5 gene underlie acromesomelic dysplasia type-grebe in consanguineous families. Congenit Anom (Kyoto). 2017;57(2):45–51.
50. Ullah A, Umair M, Hussain S, Jan A, Ahmad W. Sequence variants in GDF5 and TRPS1 underlie brachydactyly and tricho-rhino-phalangeal syndrome type III. Pediatr Int. 2018;60(3):304–6. https://doi: 10.1111/ped.13473.
51. Demirhan O, Türkmen S, Schwabe GC, Soyupak S, Akgül E, Tastemir D, et al. A homozygous BMPR1B mutation causes a new subtype of acromesomelic chondrodysplasia with genital anomalies. J Med Genet. 2005;42(4):314–7. https://doi: 10.1136/jmg.2004.023564.
52. Umair M, Hayat A. Nonsyndromic split-hand/foot malformation: recent classification. Mol Syndromol. 2020;10(5):243–54. https://doi: 10.1159/000502784.
53. Ullah A, Hammid A, Umair M, Ahmad W. A novel heterozygous intragenic sequence variant in DLX6 probably underlies first case of autosomal dominant split-hand/foot malformation type 1. Mol Syndromol. 2017;8(2):79–84. https://doi: 10.1159/000453350.
54. Umair M, Ullah A, Abbas S, Ahmad F, Basit S, Ahmad W. First direct evidence of involvement of a homozygous loss-of-function variant in the EPS15L1 gene underlying split-hand/split-foot malformation. Clin Genet. 2018;93(3):699–702. https://doi: 10.1111/cge.13152.
55. Ullah A, Gul A, Umair M, Irfanullah, Ahmad F, Aziz A, et al. Homozygous sequence variants in the WNT10B gene underlie split hand/foot malformation. Genet Mol Biol. 2018;41(1):1–8. https://doi: 10.1590/1678-4685-GMB-2016-0162.
56. Khan A, Wang R, Han S, Umair M, Alshabeeb MA, Ansar M, et al. A novel homozygous nonsense mutation p.Cys366* in the WNT10B gene underlying split-hand/split foot malformation in a consanguineous Pakistani family. Front Pediatr. 2020;7:526. https://doi: 10.3389/fped.2019.00526.
57. Bilal M, Hayat A, Umair M, Ullah A, Khawaja S, Malik E, et al. Sequence variants in the WNT10B and TP63 genes underlying isolated split-hand/split-foot malformation. Genet Test Mol Biomarkers. 2020;24(9):600–7. https://doi: 10.1089/gtmb.2020.0024
58. Spielmann M, Kakar N, Tayebi N, Leettola C, Nürnberg G, Sowada N, et al. Exome sequencing and CRISPR/cas genome editing identify mutations of ZAK as a cause of limb defects in humans and mice. Genome Res. 2016;26:183–91.
59. Shamseldin HE, Faden MA, Alashram W, Alkuraya FS. Identification of a novel DLX5 mutation in a family with autosomal recessive split hand and foot malformation. J Med Genet. 2012;49:16–20.
60. de Mollerat XJ, Gurrieri F, Morgan CT, Sangiorgi E, Everman DB, Gaspari P, et al. A genomic rearrangement resulting in a tandem duplication is associated with split hand-split foot malformation 3 (SHFM3) at 10q24. Hum Molec Genet. 2003;12:1959–71.
61. Elliott AM, Evans JA. Genotype-phenotype correlations in mapped split hand foot malformation (SHFM) patients. Am J Med Genet A. 2006;140(13):1419–27. https://doi: 10.1002/ajmg.a.31244.
62. Huang CY, Kuo WW, Chueh PJ, Tseng CT, Chou MY, Yang JJ. Transforming growth factor-beta induces the expression of ANF and hypertrophic growth in cultured cardiomyoblast cells through ZAK. Biochem Biophys Res Commun. 2004;324:424–31.
63. Khan SA, Muhammad N, Khan MA, Kamal A, Rehman ZU, Khan S. Genetics of human Bardet-Biedl syndrome, an updates. Clin Genet. 2016;90(1):3–15. https://doi: 10.1111/cge.12737.
64. Ullah A, Khalid M, Umair M, Khan SA, Bilal M, Khan S, et al. Novel sequence variants in the MKKS gene cause Bardet-Biedl syndrome with intra- and inter-familial variable phenotypes. Congenit Anom (Kyoto). 2018;58(5):173–5. https://doi: 10.1111/cga.12264.
65. Ullah A, Umair M, Yousaf M, Khan SA, Nazim-Ud-Din M, Shah K, et al. Sequence variants in four genes underlying Bardet-Biedl syndrome in consanguineous families. Mol Vis. 2017;23:482–94.
66. Umair M, Seidel H, Ahmed I, Ullah A, Haack TB, Alhaddad B, et al. Ellis-van creveld syndrome and profound deafness resulted by sequence variants in the EVC/EVC2 and TMC1 genes. J Genet. 2017;96(6):1005–14. https://doi: 10.1007/s12041-017-0868-6.
67. Umair M, Ahmad F, Bilal M, Ahmad W, Alfadhel M. Clinical genetics of polydactyly: an updated review. Front Genet. 2018;9:447. https://doi: 10.3389/fgene.2018.00447.
68. Ullah A, Ali RH, Majeed AI, Liaqat K, Shah PW, Khan B, et al. A novel insertion and deletion mutation in the BHLHA9 underlies polydactyly and mesoaxial synostotic syndactyly with phalangeal reduction. Eur J Med Genet. 2019;62(4):278–81. https://doi: 10.1016/j.ejmg.2018.08.005.
69. Ahmad Z, Liaqat R, Palander O, Bilal M, Zeb S, Ahmad F, et al. Genetic overview of postaxial polydactyly: updated classification. Clin Genet. 2023;103(1):3–15. https://doi: 10.1111/cge.14224.
70. Umair M, Shah K, Alhaddad B, Haack TB, Graf E, Strom TM, et al. Exome sequencing revealed a splice site variant in the IQCE gene underlying postaxial polydactyly type A restricted to lower limb. Eur J Hum Genet. 2017;25(8):960–5. https://doi: 10.1038/ejhg.2017.83.
71. Ullah A, Umair M, Majeed AI, Abdullah, Jan A, Ahmad W. A novel homozygous sequence variant in GLI1 underlies first case of autosomal recessive preaxial polydactyly. Clin Genet. 2019;95(4):540–1. https://doi: 10.1111/cge.13495.
72. Bakar A, Ullah A, Bibi N, Khan H, Ur Rahman A, Ahmad W, et al. A novel homozygous variant in the GLI1 underlies postaxial polydactyly in a large consanguineous family with intra familial variable phenotypes. Eur J Med Genet. 2022;65(10):104599.
73. Khan H, Ahmed S, Nawaz S, Ahmad W, Rafiq MA. Greig cephalopolysyndactyly syndrome: phenotypic variability associated with variants in two different domains of GLI3. Klin Pädiatr. 2021;233(02):53–8.
74. Umair M, Bilal M, Ali RH, Alhaddad B, Ahmad F, Abdullah, et al. Whole-exome sequencing revealed a nonsense mutation in STKLD1 causing nonsyndromic preaxial

polydactyly type A affecting only upper limb. Clin Genet. 2019;96(2):134–9. https://doi: 10.1111/cge.13547.

75. Umair M, Wasif N, Albalawi AM, Ramzan K, Alfadhel M, Ahmad W, et al. Exome sequencing revealed a novel loss-of-function variant in the GLI3 transcriptional activator 2 domain underlies nonsyndromic postaxial polydactyly. Mol Genet Genomic Med. 2019;7(7):e00627. https://doi: 10.1002/mgg3.627.

76. Umair M, Ahmad F, Ahmad S, Alam Q, Rehan M, Alqosaibi AI, et al. A novel homozygous missense mutation in the zinc finger DNA binding domain of GLI1 causes recessive post-axial polydactyly. Front Genet. 2021;12:746949. https://doi: 10.3389/fgene.2021.746949.

77. Hayat A, Umair M, Abbas S, Rauf A, Ahmad F, Ullah S, et al. Identification of a novel biallelic missense variant in the KIAA0825 underlies postaxial polydactyly type A. genomics. 2020;112(4):2729–33. https://doi: 10.1016/j.ygeno.2020.03.006.

78. Umair M, Palander O, Bilal M, Almuzzaini B, Alam Q, Ahmad F, et al. Biallelic variant in DACH1, encoding dachshund homolog 1, defines a novel candidate locus for recessive postaxial polydactyly type A. genomics. 2021;113(4):2495–502. https://doi: 10.1016/j.ygeno.2021.05.015.

79. Alfadhel M, Umair M, Almuzzaini B, Asiri A, Al Tuwaijri A, Alhamoudi K, et al. Identification of the TTC26 splice variant in a novel complex ciliopathy syndrome with biliary, renal, neurological, and skeletal manifestations. Mol Syndromol. 2021;12(3):133–40. https://doi: 10.1159/000513829.

80. Waqas A, Nayab A, Shaheen S, Abbas S, Latif M, Rafeeq MM, et al. Case report: biallelic variant in the tRNA methyltransferase domain of the AlkB homolog 8 causes syndromic intellectual disability. Front Genet. 2022;13:878274. https://doi: 10.3389/fgene.2022.878274.

81. Umair M, Ahmad F, Ullah A. Whole exome sequencing as a diagnostic tool for genetic disorders in Pakistan. Pak J Med. 2018;57(2):91–2.

82. Umair M, Ahamd F, Bilal M, Asiri A, Younus M, Khan A. A comprehensive review of genetic skeletal disorders reported from Pakistan: a brief commentary. Meta Gene. 2019;20:100559. https://https://doi.org/10.1016/j.mgene.2019.100559.

83. Alfadhel M, Umair M, Almuzzaini B, Alsaif S, AlMohaimeed SA, Almashary MA, et al. Targeted SLC19A3 gene sequencing of 3000 Saudi newborn: a pilot study toward newborn screening. Ann Clin Transl Neurol. 2019;6(10):2097–103. https://doi: 10.1002/acn3.50898.

84. Alyafee Y, Alam Q, Altuwaijri A, Umair M, Haddad S, Alharbi M, et al. Next-generation sequencing-based pre-implantation genetic testing for aneuploidy (PGT-A): first report from Saudi Arabia. Genes (Basel). 2021;12(4):461. https://doi: 10.3390/genes12040461

85. Alyafee Y, Al Tuwaijri A, Alam Q, Umair M, Haddad S. Next generation sequencing based non-invasive prenatal testing (NIPT): first report from Saudi Arabia. Front Genet. 2021;12:630787. https://doi: 10.3389/fgene.2021.630787.

86. Alyafee Y, Al Tuwaijri A, Umair M, Alharbi M, Haddad S, Ballow M. Non-invasive prenatal testing for autosomal recessive disorders: a new promising approach. Front Genet. 2022;13:1047474. https://doi: 10.3389/fgene.2022.1047474.

87. Ullah A, Umair M, E-Kalsoom U, Shahzad S, Basit S, Ahmad W. Exome sequencing revealed a novel nonsense variant in ALX3 gene underlying frontorhiny. J Hum Genet. 2018;63(1):97–100. https://doi: 10.1038/s10038-017-0358-y.

88. Ullah A, Kalsoom UE, Umair M, John P, Ansar M, Basit S, et al. Exome sequencing revealed a novel splice site variant in the ALX1 gene underlying frontonasal dysplasia. Clin Genet. 2017;91(3):494–8. https://doi: 10.1111/cge.12822.

89. Umair M, Ahmad F, Bilal M, Arshad M. Frontonasal dysplasia: a review. JBCGenetics. 2018;1(2):66–76. https://doi:10.24911/JBCGenetics/183-1530765389

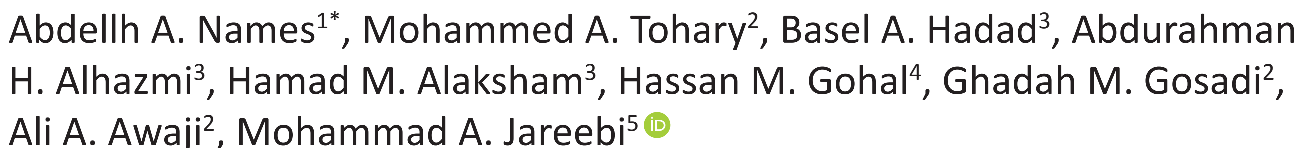
90. Alfadhel M, Nashabat M, Saleh M, Elamin M, Alfares A, Al Othaim A, et al. Long-term effectiveness of carglumic acid in patients with propionic acidemia (PA) and methylmalonic acidemia (MMA): a randomized clinical trial. Orphanet J Rare Dis. 2021;16(1):422. https://doi: 10.1186/s13023-021-02032-8.

Abdellh A. Names et al. JBC Genetics. 2023;6(1):070-074
https://doi.org/10.24911/JBCGenetics/183-1668575222

Journal of Biochemical and Clinical Genetics

CASE REPORT

Dilated cardiomyopathy associated with NRAP gene: a case series



Abdellh A. Names[1*], Mohammed A. Tohary[2], Basel A. Hadad[3], Abdurahman H. Alhazmi[3], Hamad M. Alaksham[3], Hassan M. Gohal[4], Ghadah M. Gosadi[2], Ali A. Awaji[2], Mohammad A. Jareebi[5]

ABSTRACT

Background: The genetic basis of dilated cardiomyopathy (DCM) is highly diverse, with over 100 known genes and several possibilities described. Nebulin-related-anchoring protein (NRAP) is an action-binding cytoskeletal protein that has a role in the myofibrillar assembly in the embryonic heart. It is primarily generated in striated and cardiac muscles.

Case Presentation: We described three cases of DCM that were related to *NRAP* gene mutations [NM_001261463.1: c.3568G > T; p. (Glu1190*)].

Conclusion: Our data imply that biallelic nonsense mutations in the NRAP might be a genetic risk factor with limited penetrance and induce DCM at various ages.

Keywords: NRAP gene, heart failure, cardiomyopathies, DCM, genetics.

Introduction

Dilated cardiomyopathy (DCM) is differentiated in the absence of other etiological causes by left ventricular hypertrophy and systolic dysfunction (1). Even within the same family, the presentation of DCM can range from asymptomatic to heart failure in advanced stages and sudden cardiac death. The estimated frequency of DCM in the general population is between 1:500 and 1:3,000 (2).

Despite the fact that the genetic basis of DCM is still poorly understood, multiple genes have been associated with it (3). These genes code for cytoskeletal, sarcomere, nuclear envelope, ion channel, and intercellular junction proteins (4). Diverse genes have been identified as dominant loci for the condition, which appears to be hereditary in over 20% of patients (5,6). Several of these genes code for cytoskeletal proteins, indicating that inadequate force transmission is among the major causes of DCM (7). For example, a mutation in the MYH7 gene, which encodes myosin heavy chain 7, a structural protein in the heart, has been associated with DCM. Similarly, a mutation in the TNNT2 gene, which encodes troponin T, a protein involved in muscle contraction, has been linked to DCM. Other genetic risk factors for DCM include mutation in the LMNA, TNNI3, and TTN genes. Nebulin-related anchoring protein (NRAP), which is involved in the cycle of sarcomeric contraction, is one of the most recent genetic findings linked to DCM.

Heart and striated muscle-expressed NRAP is the second-largest actin-binding cytoskeletal protein in the nebulin family (8). NRAP is expressed in myofibrillogenesis by myofibril precursors. NRAP participates in myofibrillar assembly during cardiomyocyte development in the fetal heart and connects terminal actin filaments to membrane complexes in the adult heart (9,10). Experimentally, NRAP expression was upregulated in both DCM animal models and DCM patients (11).

In this case series, three confirmed cases of DCM associated with *NRAP* mutations are presented.

Case Presentation

Case 1

A 5-year-old female presented to the emergency department with persistent vomiting, epigastric discomfort, and easy fatigability for the last one and a

Correspondence to: Abdellh A. Names
*Medical Student, Jazan University, Jazan, Saudi Arabia.
Email: abdualelah07@gmail.com
Full list of author information is available at the end of the article.
Received: 16 November 2022 | **Accepted:** 24 January 2023

OPEN ACCESS This is an open access article distributed in accordance with the Creative Commons Attribution (CC BY 4.0) license: https://creativecommons.org/licenses/by/4.0/) which permits any use, Share — copy and redistribute the material in any medium or format, Adapt — remix, transform, and build upon the material for any purpose, as long as the authors and the original source are properly cited. © The Author(s).

half months. She was managed at a local hospital and then referred to our hospital for further management. She is the second child of a consanguineous marriage. Her family history is unremarkable. On examination, an unwell child weighed 17 kg (50th-75th centile), height of 105 cm (25th-50th centile), and had a head circumference of 50 cm (50th centile). An abdominal examination revealed hepatomegaly. On cardiovascular examination, normal heart sounds and a pan-systolic murmur with a displaced apex beat were discovered. The rest of the systemic examination was unremarkable. Her initial blood investigations were within the normal limit. Electrocardiography showed normal sinus rhythm, right axis deviation, and left ventricular hypertrophy with inverted T waves in lateral leads. Echocardiography showed severe left ventricular dilation, moderate mitral regurgitation, and an ejection fraction of 15%. She was treated as a case of DCM and started on milrinone at 0.5 µg/kg/hour, but her condition deteriorated every day. During the hospital stay, she became unwell and was shifted to the pediatric intensive care unit (ICU), where she was intubated. She was getting full inotropic support, but she later had problems with several organs and died within a month of being admitted. A homozygous autosomal recessive likely pathogenic variant in the *NRAP* gene [NM_001261463.1: c.3568G>T; p. (Glu1190*)] results in a premature stop codon (Figure 1A).

Case 2

An 11-month-old male was referred from a peripheral hospital for pediatric ICU care with a history of shortness of breath and respiratory distress. He has an uneventful neonatal history and is developmentally normal. His parents were first cousins. He has a cousin who died at the age of three from cardiac problems. He was active and alert on examination, with a glasgow coma scale (GCS) of 15/15 and no dysmorphic features. weight is 9 kg (10th-25th centile) and his height is 74.5 cm (10th centile). Head circumference: 46 (25th centile). Chest examination revealed crepitation with normal vesicular breath. An abdominal exam shows hepatomegaly. A cardiovascular examination showed a pansystolic murmur. The rest of the systemic examination was unremarkable. Echocardiography showed a severely dilated left ventricle and atrium. He has severely depressed left ventricular systolic function, with an ejection fraction (EF) estimated at 15%-17%. Severe mitral regurgitation, moderate aortic insufficiency, normal sinus rhythm, right axis deviation, left ventricular hypertrophy, ST-segment alterations, T-wave inversion, and large Q waves in the lateral precordial leads were found in the ECG lab. Inflammatory markers and metabolic profiles were unremarkable. He was admitted to the pediatrics intensive care unit (PICU) and started on heart failure treatment. He was discharged home on LASIX PO 9 mg TID, CAPTOPRIL 3.25 mg PO BID, ASPIRIN 45 mg PO OD, and DIGOXIN 50 MCG PO BID. Propranolol 4.5 PO BID. On follow-up later, we received information that his condition deteriorated at home, which resulted in his sudden death. This whole exome sequence came back showing a homozygous autosomal recessive likely pathogenic variant was identified in the *NRAP* gene (NM_001261463.1: c.3568G>T; p.(Glu1190*) which creates a premature stop codon. Thus, the obtained result is consistent with a genetic diagnosis of NRAP-related cardiomyopathy (Figure 1B).

Case 3

A 4-year-old boy with a known case of DCM and sickle cell trait presented to the emergency department with generalized body swelling, cough, and decreased urine output. His parents were first cousins. His father died of a cardiac problem. He has a normal developmental history. He had previously been admitted to the PICU for the same complaint. On examination the following observations were made: he was afebrile, had a pulse of 130 bpm, blood pressure of 83/41 mmHg, had cold extremities, had capillary refills >2 seconds; GCS 15/15, no dysmorphic features, 11 kg (3rd centile), and height 92 cm (10th centile). Examination revealed abdominal distention and tender hepatomegaly. Chest examination showed bilateral crepitation with bronchovesicular breathing. He has a pansystolic murmur with a displaced apex beat on the background of normal S1 and S2. Electrocardiography showed normal sinus rhythm, right axis deviation, and left ventricular hypertrophy, with inverted T waves in the lateral lead. Echocardiography showed severe left ventricular depression with an 18% ejection fraction. He was admitted to the PICU and started on heart failure treatment. The full genetic workup-initiated report revealed an *NRAP* gene mutation and patient transfer to a high-level cardiac transplant center (Figure 1C).

Discussion

NRAP is essential for the assembly of thin filaments, actin cytoskeleton organization, and myofibril assembly in cardiomyocytes. NRAP attaches to the membrane, facilitating cardiac muscle contraction and relaxation via a complex of -integrins, talin, and vinculin. It is anticipated that these super repeats will give directionality to actin filaments (12).

We report the discovery of a novel variant in the *NRAP* gene in three patients from unrelated families with confirmed DCM. In all three cases, a homozygous likely pathogenic variant was recognized within the *NRAP* gene NM_001261463.1: c.3568G>T variant p. (Glu1190*). The OMIM phenotype has never been reported to have been linked to the pathogenic variants in the N-RAP gene. Again, based on a review of the clinical database, we found 172 variants in the *NRAP* gene. Of them, 22 reported variants in *NRAP* were classified as pathogenic and 2 as likely pathogenic. The rest are either benign or variants of uncertain significance. Nevertheless, numerous studies show that many patients with cardiomyopathy have the *NRAP* gene consisting of a loss-of-function variant. The three case reports align with the findings of various reports that associate homozygous mutations within the *NRAP* gene with cardiomyopathy (13,14).

However, only two homozygous LoF variations have been documented in the literature. Ahmed et al. (15) described a 13-month-old female with an *NRAP*-associated mutation, and the second instance was reported in a previously healthy 26-year-old woman (12). In our cases, all were younger than 6 years old, had a positive consanguinity

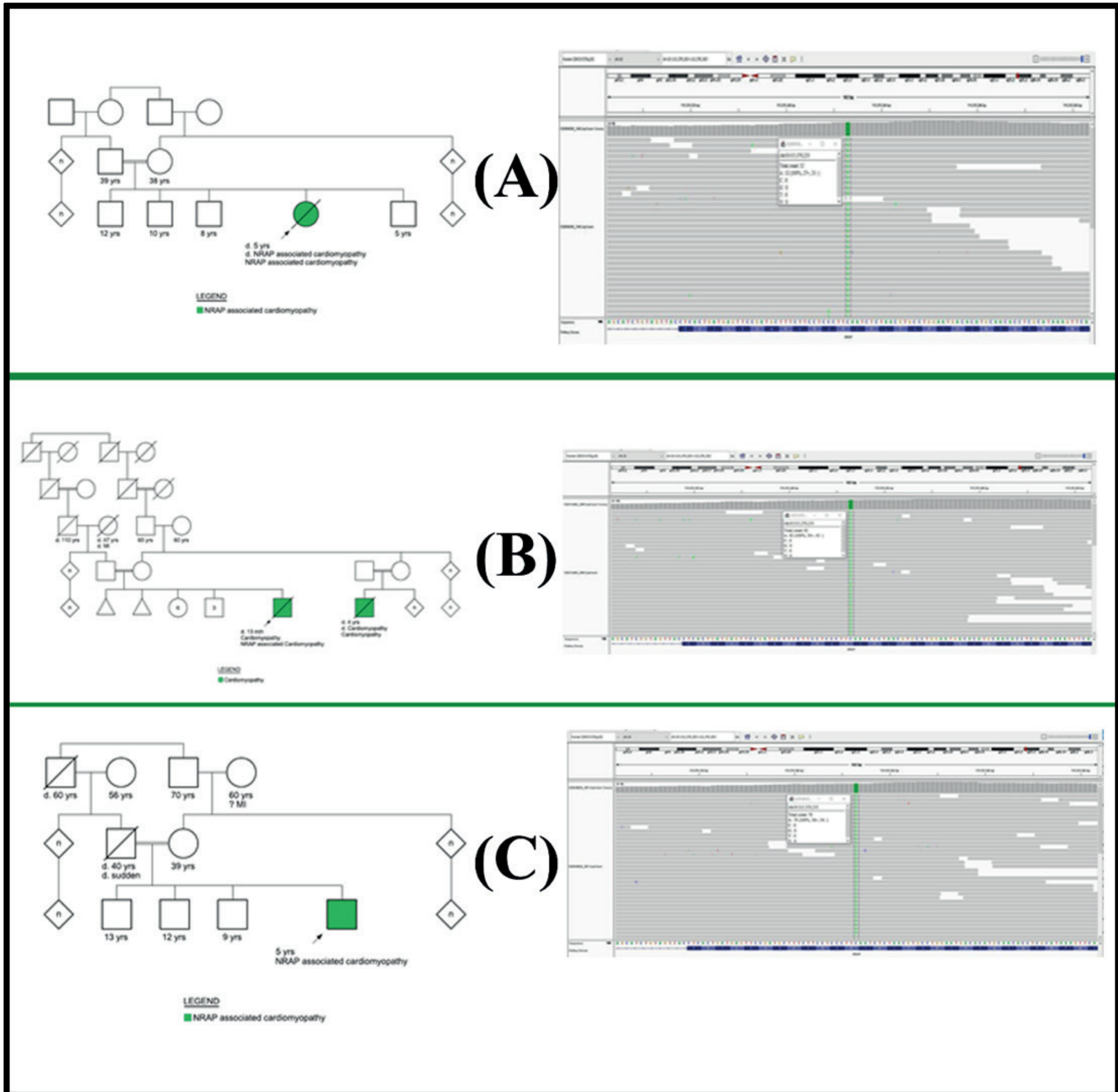


Figure 1. *(A) A family pedigree and a chromatograph of the mutations of Case 1 with NRAP-associated cardiomyopathy. (B) A family pedigree and a chromatograph of the mutations of Case 2 with NRAP-associated cardiomyopathy. (C) A family pedigree and a chromatograph of the mutations of Case 3 with NRAP-associated cardiomyopathy.*

test, and were reported to the emergency department with signs of heart failure. Ahmed et al. (15) documented a consanguineous pedigree in which the index patient was a 13-month-old girl diagnosed with DCM who presented with heart failure, easy fatigability, weakness, irritability, and shortness of breath. Her healthy 33-year-old father was found to be homozygous for the same frameshift variation detected in the proband, while her mother was heterozygous. This finding may explain the different ages of presentation. Unfortunately, we were not able to perform a genetic test for one of the parents of the affected child, who passed away suddenly from cardiac arrest.

One of our cases is an 11-month-old boy who supports that NRAP has a crucial role in enabling myofibrillogenesis within early childhood. The *NRAP* gene expressed in cardiac and striated muscles is known to be the second largest member of the actin-binding cytoskeletal proteins. Zhang et al. (16) indicated that the development of cardiomyocytes within the fetal heart engages N-RAP in the myofibrillar collection. It also connects terminal actin filaments towards an adult heart's membrane complexes to force transmission between the sarcomere and extracellular matrix. Homozygosity for rs530462185 was indicated even in the asymptomatic boy. This shows that the mutation of N-RAP in people can be tolerated due to the limited penetrance and must need other factors to cause an illness. The idea that the N-RAP is inadequate to cause disease is also supported by a study conducted by Bielecka-Dabrowa et al. (17) and D'Avila et al. (18); nevertheless, the deaths of the two patients in the case study indicate that a homozygous

gene in N-RAP was inherited genetically by the patients to cause cardiomyopathy (14). This was identified through the double-stranded DNA detection method, which was conducted against the human coding exome. Also, clinical data and family history information are essential as they help to assess the noted variants by referring to their causality and pathogenicity. In conclusion, the report presents three different cases of patients diagnosed with cardiomyopathy.

Conclusion

This case series confirms the clinical and natural history of this disease and its fatal prognosis. We proposed international registries to study *NRAP* gene-related DCM and collect larger cohorts that would help us characterize the disease and make genotype-phenotype correlations.

Funding
None.

Declaration of conflicting interests
The authors of this article have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

Consent for publication
Due permission was obtained from the parents of the patient to publish the cases.

Ethical approval
Ethical approval is not required at our institution to publish an anonymous case report.

Author details
Abdellh A. Names[1], Mohammed A. Tohary[2], Basel A. Hadad[3], Abdurahman H. Alhazmi[3], Hamad M. Alaksham[3], Hassan M. Gohal[4], Ghadah M. Gosadi[2], Ali A. Awaji[2], Mohammad A. Jareebi[5]

1. Medical Student, Jazan University, Jazan, Saudi Arabia
2. Consultant Medical Genetic and Metabolic Disorders, Prince Mohammed Bin Nasser Hospital, Jazan, Saudi Arabia
3. Consultant Pediatric Cardiologist, Prince Mohammed Bin Nasser Hospital, Jazan, Saudi Arabia
4. Consultant Pediatric Intensive Care, Prince Mohammed Bin Nasser Hospital, Jazan, Saudi Arabia
5. Lecturer, Jazan University, Jazan, Saudi Arabia

References

1. Elliott P, Andersson B, Arbustini E, Bilinska Z, Cecchi F, Charron P, et al. Classification of the cardiomyopathies: a position statement from the European Society of Cardiology Working Group on myocardial and pericardial diseases. Eur Heart J. 2008;29(2):270–6. https://doi.org/10.1093/eurheartj/ehm342
2. McNally EM, Golbus JR, Puckelwartz MJ. Genetic mutations and mechanisms in dilated cardiomyopathy. J Clin Invest. 2013;123(1):19–26. https://doi.org/10.1172/JCI62862
3. Vasilescu C, Ojala TH, Brilhante V, Ojanen S, Hinterding HM, Palin E, et al. Genetic basis of severe childhood-onset cardiomyopathies. J Am Coll Cardiol. 2018;72(19):2324–38. https://doi.org/10.1016/j.jacc.2018.08.2171
4. Hershberger RE, Morales A, Siegfried JD. Clinical and genetic issues in dilated cardiomyopathy: a review for genetics professionals. Genet Med. 2010;12(11):655–67. https://doi.org/10.1097/GIM.0b013e3181f2481f
5. Richard P, Villard E, Charron P, Isnard R. The genetic bases of cardiomyopathies. J Am Coll Cardiol. 2006;48(9):A79–A89. https://doi.org/10.1016/j.jacc.2006.09.014
6. McLaughlin HM, Funke BH. Chapter 17 - Molecular testing in inherited cardiomyopathies. In: Coleman WB, Tsongalis GJ, editors. Diagnostic molecular pathology. Academic Press; 2017. pp 213–20. https://doi.org/10.1016/B978-0-12-800886-7.00017-0
7. Schönberger J, Seidman CE. Many roads lead to a broken heart: the genetics of dilated cardiomyopathy. Am J Hum Genet. 2001;69(2):249–60. https://doi.org/10.1086/321978
8. Yuen M, Ottenheijm CAC. Nebulin: big protein with big responsibilities. J Muscle Res Cell Motil. 2020;41(1):103–24. https://doi.org/10.1007/s10974-019-09565-3
9. Lu S, Borst DE, Horowits R. Expression and alternative splicing of N-RAP during mouse skeletal muscle development. Cell Motil Cytoskeleton. 2008;65(12):945–54. https://doi.org/10.1002/cm.20317
10. Luo G, Herrera AH, Horowits R. Molecular interactions of N-RAP, a nebulin-related protein of striated muscle myotendon junctions and intercalated disks. Biochemistry. 1999;38(19):6135–43. https://doi.org/10.1021/bi982395t
11. Lu S, Crawford GL, Dore J, Anderson SA, Despres D, Horowits R. Cardiac-specific NRAP overexpression causes right ventricular dysfunction in mice. Exp Cell Res. 2011;317(8):1226–37. https://doi.org/10.1016/j.yexcr.2011.01.020
12. Truszkowska GT, Bilińska ZT, Muchowicz A, Pollak A, Biernacka A, Kozar-Kamińska K, et al. Homozygous truncating mutation in NRAP gene identified by whole exome sequencing in a patient with dilated cardiomyopathy. Sci Rep. 2017;7(1):3362. https://doi.org/10.1038/s41598-017-03189-8
13. Lu S, Horowits R. Role of nonmuscle myosin IIB and N-RAP in cell spreading and myofibril assembly in primary mouse cardiomyocytes. Cell Motil Cytoskeleton. 2008;65(9):747–61. https://doi.org/10.1002/cm.20299
14. Carroll SL, Herrera AH, Horowits R. Targeting and functional role of N-RAP, a nebulin-related LIM protein, during myofibril assembly in cultured chick cardiomyocytes. J Cell Sci. 2001;114(Pt 23):4229–38. https://doi.org/10.1242/jcs.114.23.4229
15. Ahmed HA, Al-ghamdi S, Al Mutairi FJJoB, Genetics C. Dilated cardiomyopathy in a child with truncating mutation in NRAP gene. 2019;1(2):77. https://doi.org/10.24911/JBCGenetics/183-1542267981
16. Zhang JQ, Elzey B, Williams G, Lu S, Law DJ, Horowits R. Ultrastructural and biochemical localization of N-RAP at the interface between myofibrils and intercalated disks in the mouse heart. Biochemistry. 2001;40(49):14898–906. https://doi.org/10.1021/bi0107445
17. Bielecka-Dabrowa A, von Haehling S, Aronow WS, Ahmed MI, Rysz J, Banach M. Heart failure biomarkers

in patients with dilated cardiomyopathy. Int J Cardiol. 2013;168(3):2404–10. https://doi.org/10.1016/j.ijcard.2013.01.157

18. D'Avila F, Meregalli M, Lupoli S, Barcella M, Orro A, De Santis F, et al. Exome sequencing identifies variants in two genes encoding the LIM-proteins NRAP and FHL1 in an Italian patient with BAG3 myofibrillar myopathy. J Muscle Res Cell Motil. 2016;37(3):101–15. https://doi.org/10.1007/s10974-016-9451-7

Journal of Biochemical and Clinical Genetics

CASE REPORT

Homozygous variant FOXE3 causes autosomal recessive anterior segment dysgenesis type 2: a case report

Zuha Alkhaldi[1], Moosa Allawati[1], Nadia Alhashmi[2*]

ABSTRACT

Background: Anterior segment dysgenesis (ASD) is a developmental condition that affects the frontal part of the eyes. Genetic mutations in *FORKHEAD BOX E3* can lead to a variety of ASD conditions.

Case Presentation: Here, we report a 2-year-old female patient with ASD type 2 autosomal recessive linked disease. Whole exome sequencing test was conducted and resulted in a missense mutation at position 120 altering arginine to proline. To our knowledge, this is the first case reported in Oman.

Conclusion: For patients with ASD, it is crucial to take the full family history and genetic work up to aid in the diagnosis and long-term management of the condition.

Keywords: Case report, anterior segment dysgenesis, ASD, *FOXE3*.

Background

The anterior segment refers to the front-most region of the eye and includes the cornea, iris, and lens. Anterior segment dysgenesis disorders (ASDs) are a heterogeneous group of developmental conditions affecting this part of the eyes. The features of ASD display extensive phenotypic and genotypic heterogeneity with overlapping clinical features; it includes glaucoma secondary to aqueous humor flow dysregulation from the anterior chamber which can lead to an increase in intraocular pressure (IOP). In addition, features of ASD include iris hypoplasia, an enlarged or reduced corneal diameter, corneal vascularization, and opacity, posterior embryotoxon, corectopia, polycoria, an abnormal iridocorneal angle, ectopia lentis, and anterior synechiae between the iris and posterior corneal surface (1).

ASD has been found to be caused by mutations in genes such as *PXDN (OMIM605158), FORKHEAD BOX E3 (FOXE3) (OMIM601094, NG_016192.1 RefSeqGene), CYP1B1 (OMIM601771), PITX2 (OMIM601542), FOXC1 (OMIM601090), PITX3 (OMIM602669), PAX6 (OMIM607108)*, and *CPAMD8* (*OMIM608841*) which contribute to a spectrum of ocular disorders (2). For instance, *FOXE3*, which is a transcription factor located at chromosome 1p33 and expressed in the lens has a role in the developmental closure of the lens vesicle along with the survival and proliferation of the lens epithelium (3). This gene has been linked with both recessive and dominant eye disease. The recessive mutations in *FOXE3* have been identified in multiple cases with aphakia, microphthalmia, and sclerocornea as major clinical features (4,5). Whereas congenital cataracts and/or anterior segment disease have been seen with the dominant mutations in the same gene (4).

Case Presentation

A 2-year-old girl was referred from Al-Nahdha Hospital (one of the main ophthalmology centers in the Sultanate of Oman) at the age of 7 months for evaluation of aphakia as her sibling has a similar ophthalmological issue.

The child was a term baby, born by spontaneous vaginal delivery with an uneventful postpartum period and normal development. The family history of the child is significant for related parents (consanguineous marriage) (Figure 1), her brother was diagnosed with sclerocornea, anterior segment dysgenesis, and congenital glaucoma

Correspondence to: Nadia Alhashmi
*Senior Consultant, Clinical & Biochemical Geneticist, Child Health Department, Royal Hospital, Muscat, Sultanate of Oman.
Email: nadiaalhashmi@gmail.com
Full list of author information is available at the end of the article.
Received: 12 December 2022 | **Accepted:** 17 January 2023

This is an open access article distributed in accordance with the Creative Commons Attribution (CC BY 4.0) license: https://creativecommons.org/licenses/by/4.0/) which permits any use, Share — copy and redistribute the material in any medium or format, Adapt — remix, transform, and build upon the material for any purpose, as long as the authors and the original source are properly cited. © The Author(s).

and was started on treatment. In addition, there was no family history of metabolic diseases.

The child was seen at Al-Nahdha Hospital initially at the age of 40 days when she was referred from a regional hospital for evaluation of white lesions in both eyes. The general physical examination was insignificant with normal skin and no skeletal manifestations. On ophthalmic examination, she was found to have a narrow palpebral fissure, micro-cornea, shallow anterior chamber, and diffuse opacification in both eyes; and IOP was recorded to be 27 mmHg. The child was managed by anti-glaucoma medications at that time, and she was posted for examination under anesthesia (EUA). EUA revealed microphthalmos of the right eye along with sclerocornea, microcornea, and corneal diffuse opacification, and left eye EUA showed corneal diffuse opacification and sclerocornea. The B-scan of both eyes showed aphakia. Cyclophotocoagulation (CPC) was later done for the left eye and was continued on the same anti-glaucoma medications.

When the child was seen at the age of 2 years, parents reported that the child could obey commands, say her name, walk around, and play with other children. She was examined in the clinic, and she was noticed to have white to gray-looking eyes (Figure 2), but dysmorphic features were absent. Additionally, her brother has the same condition and is on the same medications, but he also has esotropia and nystagmus.

Workup

As part of the workup to confirm her suspected condition, a deoxyribonucleic acid (DNA) sample was collected from the child and stored in the Omani National Genetic Center's lab, and whole exome sequencing (WES) was

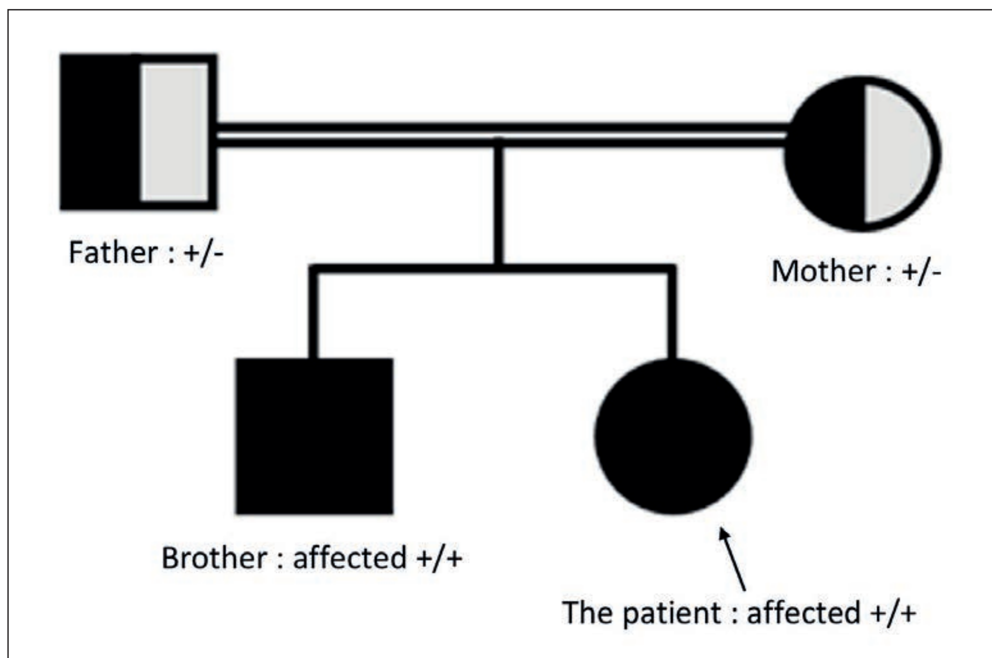


Figure 1. *Family pedigree.*

This figure shows the carrier parent in a consanguineous marriage with affected offspring (male and female).

Figure 2. *Clinical features of ASD in the patient.*

This figure displays white lesions in both eyes.

Table. 1. *Comparison of genotype and phenotype between previously reported cases of ASD.*

Publication	FOXE3 Mutation variant	Primary congenital Aphakia	Cataract	Sclero-cornea	Corneal opaci-ties	Megalo-cornea	Glaucoma	Sclero-malacia	Coloboma	Microph-thalmia	Aniridia	Retinal dysplasia
Our case 2022	c.359G>C (Arg-120Pro)	+		+			+					
Chen, 2017 (9)	c.307G>A (p.Glu-103Lys)		+									
Saboo, 2016 (9)	c.472G>C (p.Gl-y158Arg)	+		+						+		
Anjum et al. (5)	c.720C>A (p.Cys240X)	+										
Reis et al. (8)	c.244A>G (p.Met82Val)			+	+		+		+	+		
Iseri et al. (4)	c.244A>G (p.Met82Val)						+	+				
Valleix et al. (7)	c.720C>A (p.Cys240X)	+				+	+			+	+	+

Table 1 shows the differences between the clinical features of our reported case and the previously reported cases.

sent for the same sample. WES revealed the presence of a homozygous variant of uncertain significance class 3 in the *FOXE3* gene (OMIM: 601094) (6) which can possibly explain the phenotype of autosomal recessive ASD type 2.

The WES report also mentioned that the *FOXE3* variant causes an amino acid change from Arg to Pro at position 120. Furthermore, the report listed three conditions that could result secondary to pathogenic variance in this gene; the three conditions are autosomal recessive ASD type 2, autosomal recessive multiple types of cataract type 34, and autosomal dominant susceptibility to familial thoracic aortic aneurysm type 11.

WES was not sent for her brother as he was already known to have the disease; therefore, only a DNA sample was collected, and WES was not requested.

Ethical approval was not required but verbal consent was taken from the parents prior to writing this report.

Discussion

ASD is an umbrella term for a group of disorders affecting the anterior segment of eye development. ASD can be caused by genetic mutations, in our patient, *FOXE3* (*OMIM601094*) was found to be affected at position 120 changing amino acid from arginine to proline (NM_012186: c.359G>C; p. (Arg120Pro); missense with unknown significance).

The patient presented with an opacity of both eyes along with other features – identified by physical examination and EUA – at the age of 40 days and was diagnosed with ASD at the age of 2 years. Similarly, her brother was diagnosed with aphakia, sclerocornea, and congenital glaucoma, however, WES was not carried out for him.

In August 2006, it was first reported that *FOXE3* mutation can cause ocular manifestations; the study included three siblings of a consanguineous family who had features such as microphthalmia and sclerocornea secondary to proven nonsense mutation of *FOXE3* gene inherited in recessive inheritance pattern (7). In 2009, Iseri et al. (4) included two consanguineous pedigrees of Pakistani origin along with two further pedigrees with a total of four families with multiple affected subjects as part of a national developmental eye anomaly study based at Moorfields Eye Hospital, London, and Birmingham Children's Hospital, Birmingham, UK. The manifestations in the four families included anophthalmia, microphthalmia, and coloboma secondary to recessive *FOXE3* mutations identified by whole-genome single-nucleotide polymorphism arrays. In 2010, Reis et al. (8) found that *FOXE3* plays a significant role in autosomal recessive microphthalmia when they included five patients who had manifestations such as microphthalmia, aphakia, and glaucoma secondary to different types of mutations in the *FOXE3* gene; 4 out of the 5 patients belonged to a consanguineous family. And in the same year, Anjum et al. (5) reported a case of congenital primary aphakia inherited in the autosomal recessive manner in a consanguineous Pakistani family secondary to *FOXE3* mutation.

Thirty-three different variants of *FOXE3* mutations were discovered and reported over the past years, 23 of them were missense mutations, and 10 out of the 23 were found to be associated with eye disorders. These ten variants include c.232G>A (p.Ala78Thr), c.244A>G (p.Met82Val), c.269G>T (p.Arg90Leu), c.289A>G (p.Ile97Val), c.292T>C (p.Tyr98His),c.307G>A (p.Glu103Lys), c.310C>T (p.Arg104Cys), c.351C>G (p.Asn117Lys), c.358C>G (p.Arg120Gly), and c.472G>C (p.Gly158Arg) (9). Here, we report a patient with C.359G>C (Arg120Pro) and to our knowledge, this is the first case in Oman of a patient with type II ASD. Additionally, we believe this is the first case with the Arg120Pro missense variant compared to the updated *FOXE3* mutation report published in 2018 (9).

Additionally, the effect of this mutation on protein production was reported to have an unknown significance. However, recent animal studies have revealed the possible damaging effect of missense mutations which renders protein nonfunctional or with low affinity (8,9). Further WES analysis for the patient's brother is recommended to confirm a similar mutation.

As the patient was confirmed to have the condition, it is recommended that she should be followed up regularly with an ophthalmologist to manage her condition. The management will depend on the clinical manifestations of the condition which could involve medications, eye surgery, or corrective lenses for poor vision (10). The patient reported in this study was treated pharmacologically using anti-glaucoma medications and surgically by CPC.

Genetic counseling should be recommended for the family including carrier testing to confirm its hereditary nature. Additionally, pre-natal and pre-implantation diagnosis is necessary to avoid such a disease if the family wishes to get pregnant. This can be accomplished by prenatal genetic testing for monogenetic disorders. The family should be provided with social and emotional support to maintain the child's independence via visual rehabilitation, home assessment, and access to special education.

As the patient is young, we cannot comment on her future outcomes and whether she will develop complicated clinical features like her brother as WES was not carried out for him.

Conclusion

To conclude, ASD is a complex group of disorders that affects patients' vision and development. *FOXE3* gene was found to be affected in our patient resulting in a complex set of symptoms including narrow palpebral fissure, micro-cornea, shallow anterior chamber, and diffuse opacification. For children who are suspected to have ASD, taking a full family history and genetic workup might be necessary to assist in the diagnosis and long-term management of the patient and family.

List of Abbreviations

ASD Anterior segment dysgenesis
CPC Cyclophotocoagulation
DNA Deoxyribonucleic acid

EUA Examination under anesthesia
FOXE3 *FORKHEAD BOX E3*
IOP Intraocular pressure
WES Whole exome sequencing

Funding
This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Declaration of conflicting interests
The authors of this article have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

Consent for publication
Due permission was obtained from the parents of the patient to publish the case and the accompanying images.

Ethical approval
Ethics Approval was granted by Scientific Research Committee, Royal Hospital, Sultanate of Oman, Ministry of Health, via CR#2023/6, dated: 31 January, 2023.

Author contributions
All the authors listed in this article contributed to the acquisition of data from the patient's parents, drafting and writing the manuscript along with final approval of this version to be published.

Author details
Zuha Alkhaldi[1], Moosa Allawati[1], Nadia Alhashmi[2]
1. Medical Intern, Sultan Qaboos University, Muscat, Sultanate of Oman
2. Senior Consultant, Clinical & Biochemical Geneticist, Child Health Department, Royal Hospital, Muscat, Sultanate of Oman

References

1. Cheong SS, Hentschel L, Davidson Alice E, Gerrelli D, Davie R, Rizzo R, et al. Mutations in CPAMD8 cause a unique form of autosomal-recessive anterior segment dysgenesis. Am J Hum Genet. 2016;99:1338–52. https://doi.org/10.1016/j.ajhg.2016.09.022
2. Sowden JC. Molecular and developmental mechanisms of anterior segment dysgenesis [Internet]. Nature Eye [cited 2022 Dec 2]. Available from: https://www.nature.com/articles/6702852
3. Blixt A, Mahlapuu M, Aitola M, Pelto-Huikko M, Enerbäck S, Carlsson P. A forkhead gene, FoxE3, is essential for lens epithelial proliferation and closure of the lens vesicle. Genes Dev. 2000;14:245–54. https://doi.org/10.1101/gad.14.2.245
4. Iseri SU, Osborne RJ, Farrall M, Wyatt AW, Mirza G, Nürnberg G, et al. Seeing clearly: the dominant and recessive nature ofFOXE3in eye developmental anomalies. Hum Mutat. 2009;30:1378–86. https://doi.org/10.1002/humu.21079
5. Anjum I, Eiberg H, Baig SM, Tommerup N, Hansen L. A mutation in the FOXE3 gene causes congenital primary aphakia in an autosomal recessive consanguineous Pakistani family. Mol Vis. 2010;16:549–55.
6. Entry - *601094 - FORKHEAD BOX E3; FOXE3 - OMIM. Omim.org. 2016. Available from: https://www.omim.org/entry/601094
7. Valleix S, Niel F, Nedelec B, Algros MP, Schwartz C, Delbosc B, et al. Homozygous nonsense mutation in the FOXE3 gene as a cause of congenital primary aphakia in humans. Am J Hum Genet. 2006;79:358–64. https://doi.org/10.1086/505654
8. Reis LM, Tyler RC, Schneider A, Bardakjian T, Stoler JM, Melancon SB, et al. FOXE3plays a significant role in autosomal recessive microphthalmia. Am J Med Genet Part A. 2010;152A:582–90. https://doi.org/10.1002/ajmg.a.33257
9. Anand D, Agrawal SA, Slavotinek A, Lachke SA. Mutation update of transcription factor genesFOXE3,HSF4,MAF, andPITX3causing cataracts and other developmental ocular defects. Hum Mutat. 2018;39:471–94. https://doi.org/10.1002/humu.23395
10. Anterior segment dysgenesis: for professionals - gene vision. Gene Vision. 2020. Available from: https://gene.vision/knowledge-base/anterior-segment-dysgenesis-for-doctors

https://doi.org/10.24911/JBCGenetics/1183-1671616459

CASE REPORT

Coexistence of atopic dermatitis and thrombocytosis: diagnostic odyssey: a case report

Fuad Al Mutairi[1,2*], Meshal Alberreet[2,3], Lara Alkuhaimi[2,3], Khalid Aleisa[2,3], Rana Almana[2,3], Asma Awadalla[2,3]

ABSTRACT

Background: Atopic dermatitis (AD) is one of the most common diseases encountered in pediatric practice. Genetic factors play a role in the development of this condition. Topical corticosteroids are the cornerstone of AD management, but with potentially serious adverse events. Misuse of these medications is not uncommon.

Case Presentation: We describe a case of severe AD with inadvertent overuse of topical steroids. The patient presented with multisystem involvement and a cushingoid appearance. Laboratory tests showed thrombocytosis and abnormal liver function test, among other findings. A whole exome test showed mutations in two genes, a homozygous pathogenic variant c.317C>T p.(Pro106Leu) in the protooncogene, thrombopoietin receptor (*MPL*) gene (NM_005373.2) inherited from both parents and a de novo heterozygous c.139C>T p.(Arg47Cys) in the *CARD11* gene (NM_001324281.1), that explain her combined presentation.

Conclusion: The aim of this report is to share our experience with the diagnosis and treatment of a challenging case. This report also shows the association between the *MPL, CARD11* genes, and severe AD. In addition, this case is consistent with the published literature on systemic involvement in severe AD and variable response to routine management.

Keywords: Atopic dermatitis, *MPL*, thrombocytosis, *CARD11*, whole exome sequencing.

Background

Atopic dermatitis (AD) is one of the most common diseases in pediatric practice. AD is an inflammatory disease often associated with other IgE-mediated diseases. It can affect any age but usually manifests in infancy with dry skin, eczematous lesions, and, if chronic enough, lichenification (1). AD is the most common chronic skin disease. In a worldwide survey conducted in over 18 countries, the reported AD in children ranged from 13.5% to 41.9%, and in Saudi Arabia, it was as high as 41.7% (2). Genetic factors play a role in the development of AD. The probability of developing AD or other atopy is more than 50% and 80%, respectively, if one or both parents have atopy. The-loss of-function mutations within filaggrin (*FLG*) gene (**Fil**ament **Ag**gregating Prote**in**) have been identified in 30% of patients. *FLG* is a protein that acts as a natural moisturizing factor in the epidermis. This fact indicates the role of dryness in the pathological process of the disease. In addition, *FLG* helps to keep the outermost layer of skin, the corneocytes, together, forming an effective barrier against pathogenic microbes. Therefore, patients with AD have a higher risk of developing superimposed infections at the affected areas (3).

The various associations with AD make diagnosis difficult in some cases. Recent studies have shown associations between AD and a number of multi-organ and systemic disorders (e.g., cutaneous infections, sleep disorders, and cardiovascular risk factors) with combined effects of skin-barrier disruption, immune dysregulation, and iatrogenic such as corticosteroid use complications

Correspondence to: Fuad Al Mutairi
*Department of Genetics and Precision Medicine, King Abdullah Specialized Children Hospital, King Abdulaziz Medical City, Ministry of National Guard Health Affairs, Riyadh, Saudi Arabia.
Email: almutairifu@ngha.med.sa
Full list of author information is available at the end of the article.
Received: 21 December 2022 | **Accepted:** 29 January 2023

OPEN ACCESS This is an open access article distributed in accordance with the Creative Commons Attribution (CC BY 4.0) license: https://creativecommons.org/licenses/by/4.0/) which permits any use, Share — copy and redistribute the material in any medium or format, Adapt — remix, transform, and build upon the material for any purpose, as long as the authors and the original source are properly cited. © The Author(s).

(4). Fatty liver occurs concomitantly with AD in a significant percentage. An increased incidence of fatty liver has been reported in healthy non-obese children. The prevalence of fatty liver increased annually, reaching 12.5% in nonatopic children, 13.1% in patients with bronchial asthma, 13.7% in patients with allergic rhinitis, and 33.9% in patients with AD (4).

In some patients, a broad phenotypic spectrum or atypical presentation may be the result of monogenic disorders or co-occurrence of two suspected monogenic diseases (5). At present, the advanced genetic testing methods of next-generation sequencing (NGS) such as whole-exome sequencing (WES) and whole-genome sequencing play an important role in the diagnosis of various diseases. It is increasingly contributing to strategic diagnostic evaluation, especially in atypical clinical scenarios and in many cases solves the diagnostic odyssey (6).

Inherited loss-of-function mutations of the thrombopoietin receptor (also known as the protooncogene, thrombopoietin receptor (*MPL)* gene OMIM #159530) are frequently associated with severe thrombocytopenia and aplastic anemia. In contrast, a recent study observed a confirmed *MPL* mutation in 26 children with familial thrombocytosis in the Saudi population (7). While homozygous null mutations in *CARD11* (OMIM #617638) lead to immunodeficiency-11B with AD, heterozygous gain of function mutations lead to selective B-cell lymphoproliferative disorders (e.g., B-cell expansion with nuclear factor kappa B subunit 1 and T-cell anergy. In addition, the gene has also been identified as a risk locus for AD; however, there is no overlap in the function of these two genes (8).

In this paper, we report a case with severe AD and thrombocytosis. Using NGS we diagnosed her with two genetic mutations involving *MPL* and *CARD11* gene mutations. The case is thought to be unique because it is associated with the above genes in addition to the other systemic manifestations.

Case Presentation

A 12-month-old girl who had suffered from chronic eczema since 4 months of age was called to the emergency department after routine screening at a dermatology clinic because of abnormal laboratory results of severe thrombocytosis [1,206 × 109/l (150 ~ 450 × 109/l)]. The patient was delivered by cesarean section in breech presentation. Her birth weight was 3 kg. She was exclusively breastfed and received vaccinations at the proper time for up to 9 months. She has no known allergies to food or medications; however, the mother noticed a flare-up of lesions when she ate peanuts. She is the first-born child of consanguineous, healthy parents in their 30s. There is family a history of AD, allergic rhinitis, asthma, and an aunt who has breast cancer. The patient was treated with topical mometasone furoate 0.1% cream [group 4 potency] for eczematous lesions. However, it had been misapplied daily as a moisturizer all over her body for about 10 months.

On examination, the patient was noticed with cushingoid features, a swollen face, and fatty limbs. She was pale with a yellowish discoloration of the sclera. An eczematous rash extended over her face, hands, abdomen, and perioral and perianal areas (Figure 1). She has alopecia with sparse hair of abnormal brown color. She has stunted growth as her height was below the 3rd percentile while her body mass index was above the 95th percentile. Vital signs were within normal ranges for her age. The rest of the examination is unremarkable.

In addition to the severe uncontrolled eczema and steroid toxicity, she was diagnosed with severe malnutrition, as she was exclusively breastfed and consumed very little. Echocardiography ruled out cardiomyopathy secondary

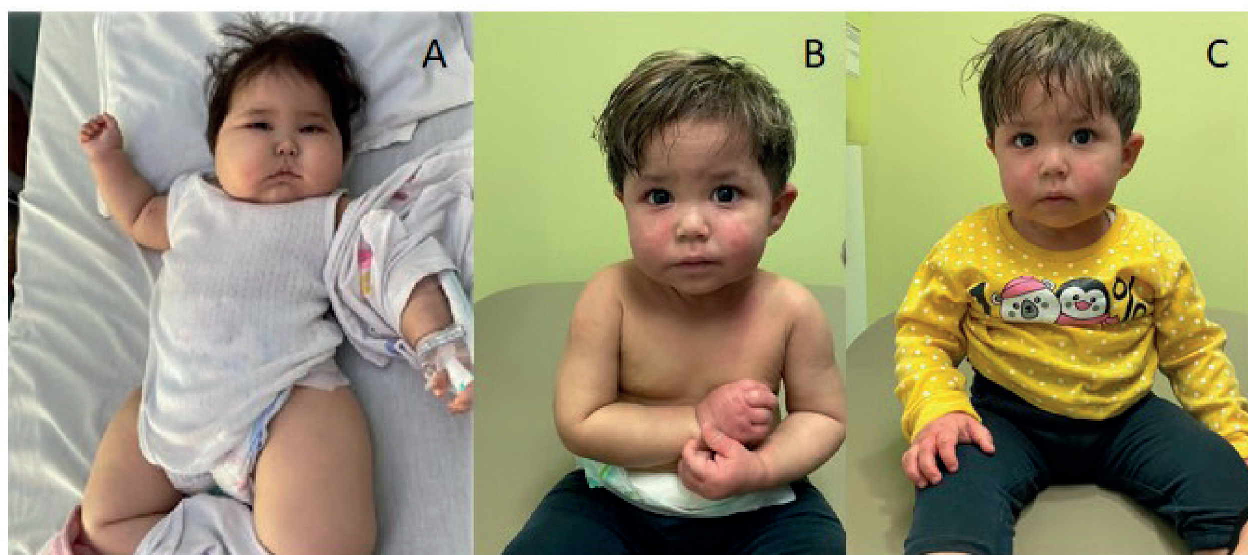


Figure 1. *(A) patient at presentation with typical cushingoid appearance, (B) patient with interval improvement of external cushingoid features at follow-up after 5 months, (C) note the erythematous eczematous rash over cheeks and hands.*

to malnutrition. The patient was started on nasogastric fed with caloric intake gradually increased to avoid refeeding syndrome. A *potent topical corticosteroid*, Mometasone Furoate, was replaced with 1% hydrocortisone and Protopic ointment, in addition to frequent application of moisturizing petrolatum. She was also treated with zinc sulfate, ferrous sulfate, multivitamin A, D, E, and K, and ursodeoxycholic acid and cholestyramine-l for cholestasis. The mother was informed in detail about the risk of adrenal suppression and the indication for a stress dose of hydrocortisone was explained to her. She was educated in detail about skin care and types of steroids.

Over the course of several months, out-patient department follow-up showed significant improvement in her nutritional, developmental, and skin condition. Her cholestasis resolved and ursodeoxycholic acid and cholestyramine were discontinued. Platelet levels never normalized, and she continued to have persistent thrombocytosis.

Method

Laboratory tests

Laboratory tests showed severe thrombocytosis, leukocytosis, and microcytic hypochromic anemia with reticulocytosis. Liver function tests revealed direct hyperbilirubinemia 119.1 (~8.6 umol/l) with high alkaline phosphtase 531 (156-369 U/l), and gamma-glutamyl transferase 65.8 (9-36 U/l) levels, hypoalbuminemia 20-22 (38-54 g/l). Cortisol levels, adrenocorticotropic hormone and lipid profile were in the acceptable range. No evidence of biochemical dysfunction of hypothalamic-pituitary-adrenal axis hormones was found. Examination for viral and autoimmune hepatitis was negative.

Ultrasound revealed hepatomegaly with evidence of fatty liver. Urinalysis suggested urinary tract infection and culture grew >100,000 cfu/ml *Escherichia coli*, with high C-reactive protein 55 (~8 mg/l), also culture of erosive eczematic lesions grew *Staphylococcus aureus*, but the result herpes simplex virus polymerase chain reaction result was negative, after which she received a treatment course with ciprofloxacin. Further testing for immunodeficiency disorders was performed, which showed a significantly elevated of total IgE level of 103 (<3.2 KU/l), whereas IgG 4.83 (7.51-15.60 g/l), IGA 0.97 (0.82-4.53 g/l), and IgM 0.41 (0.46-3.04 g/l) were normal. In addition, lymphocyte subset analysis showed slightly increased expression of CD25 in 8% (2%-5%) of total T cells; otherwise, expression of all other markers was within normal values consistent with patient age, including CD4 and natural killer cells. A hair biopsy was obtained and showed trichorrhexis nodosa.

Whole exome sequencing

The generated library is sequenced on an Illumina platform to achieve an average coverage depth of ~100×. Typically, ~97% of the target bases are covered >10×. An end-to-end in-house bioinformatic pipeline was used including base calling, alignment of reads to GRCh37/hg19 genome assembly, primary filtering out of low-quality reads and likely artifacts, and subsequent annotation of variants. The evaluation focuses on the coding exons as well as the flanking ±20 intronic bases. All inheritance patterns were considered. In addition, the provided family history and clinical information were used to evaluate the identified variants. The WES showed mutations in two genes, a homozygous pathogenic missense variantc.317C>T p.(Pro106Leu) in the *MPL* gene NM_005373.3, NP_005364.1 inherited from both parents, this variant has previously been described as disease causing for thrombocytosis. On other hand another de-novo heterozygous c.139C>T p.(Arg47Cys) missense variant of unknown significant was detected in the *CARD11* gene NM_032415.7, NP_115791.3. This mutation as along with other truncating variants was missing from a large-scale exome sequencing database such as Exome Aggregation Consortium, dbSNP/1,000 genome or Exome Sequencing Project, and the Genome Aggregation Database. In addition, this variant, along with other truncating variants, was absent from 2,000 ethnically matched controls in the local database. Both mutations were confirmed by Sanger in CAP-accredited laboratory.

Discussion

AD is known to be associated with a loss-of-function mutation in the *FLG* gene, leading to a decrease in FLG protein and consequent in barrier defect (3). Review of the literature revealed reports of successful use of 0.03% topical tacrolimus (TCs) in the treatment of certain types of dermatitis such as granuloma gluteale infantum and isolated lip dermatitis (atopic cheilitis) (9). Because the patient did not respond adequately and satisfactorily to the topical steroids previously used to control the flare-up of eczema, the patient was treated with TCs ointment 0.03% which resulted in significant improvement.

While IgE and T cells are the main mediators in the pathogenesis of AD, some studies have shown an opposed effect of mast cells. Hershko et al. (10) identified a protective role of mast cells against chronic eczema mediated by IL-2, which suppresses chronic allergic reactions. This finding may be related to the severe eczema observed in the reported case.

Caspase recruitment domain (CARD)-containing membrane-associated guanylate kinase proteins from a family of three scaffold proteins that are highly conserved in amino acid sequence, including CARD11/CARMA1 (CARMA1) (11). Recent evidence has shown that CARD proteins mediate the induction of an inflammatory response in keratinocytes and that mutations in the encoded genes segregate with familial transmission of chronic inflammatory diseases of human skin through the formation of a trimeric complex comprising BCL10 and MALT1 proteins (12). In recent decades, the formation of the CARD11-BCL10-MALT1 (CBM) signalosome complex has emerged as an essential step in the regulation of NF-KB in lymphoid immune cells (13). A *CARD11* gene mutation has been found to interact with BCL10 and promote NF-KB activation resulting in an autosomal dominant immunodeficiency type 11B with AD. Recent reports indicate that *CARD11* is also associated with

several immune-related diseases and traits, including multiple sclerosis, eczema, thrombocytopenia, and susceptibility to Kawasaki disease due to impaired B-cell development and B-cell function (9,14)

Much research is still needed to improve our understanding of the relationship between AD and many associated conditions. To date, there has been no report about the relationship with the *MPL* and *CARD11* gene. Although there is no specific management for these cases, patients are treated with supportive treatment, however, proper genetic counseling, the introduction of newborn screening programs and parenteral diagnosis can play a major role in reducing the burden of such severe disorders (15). This can be accomplished by prenatal genetic testing for monogenetic disorders (PGT-M). PGT and *in vitro* fertilization are options for parents wishing to have future pregnancies (16,17).

This report demonstrates the utility of early genetic testing in resolving diagnostic dilemmas and prompt treatment. As evident in this case, genetic testing has drawn attention to that fact that despite the typical clinical presentation of eczema, the patient has a genetic defect that would explain the persistent thrombocytosis. Whether or not these genetic defects are related to the failure to respond to high-potency steroids, even when inappropriately overdosed is still uncertain.

Conclusion

This case demonstrates the potential adverse effects of inappropriate use of TCs. Another lesson from this study is that genetic testing is appropriate in the setting of a systemic manifestation in addition to a chronic persistent AD. This report aims to sensitize pediatricians to the role of testing for underlying genetic pathologies in some disorders where typical treatment proves ineffective. Therefore, timely ordering of genetic testing is an essential step in patient care in such situations.

Acknowledgments

We are grateful to members of the family enrolled in this study. We acknowledge the copyediting performed research office at College of Public Health and Health Science King Saud Bin Abdulaziz University for Health Sciences, and Saudi Society of Medical Genetics

Funding

None.

Declaration of conflicting interests

The authors of this article have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

Consent for publication

Due permission was obtained from the parents/guardians of the patient to publish the cases and the accompanying images.

Ethical approval

Written informed consent was obtained from the individuals for the publication of any potentially identifiable images or data included in this article.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

Author contributions

F.M. and A.A. conceptualized the work. F.M. and M.A. drafted this manuscript. F.M., M.A., L.A., K.A., R.A., and A.A. revised this manuscript. F.M. and A.A. were involved in the clinical management of the patient and all authors read and approved the manuscript for submission.

Author details

Fuad Al Mutairi[1,2], Meshal Alberreet[2,3], Lara Alkuhaimi[2,3], Khalid Aleisa[2,3], Rana Almana[2,3], Asma Awadalla[2,3]

1. Department of Genetics and Precision Medicine, King Abdullah Specialized Children Hospital, King Abdulaziz Medical City, Ministry of National Guard Health Affairs, Riyadh, Saudi Arabia
2. Department of Pediatrics, King Abdullah Specialized Children Hospital, King Abdulaziz Medical City, Ministry of National Guard Health Affairs, Riyadh, Saudi Arabia
3. King Saud bin Abdulaziz University for Health Sciences, King Abdullah International Medical Research Centre, Ministry of National Guard Health Affairs, Riyadh, Saudi Arabia

References

1. Kolb L, Ferrer-Bruker SJ. Atopic dermatitis. Treasure Island, FL: StatPearls; 2022.
2. Alshamrani HM, Alsolami MA, Alshehri AM, Salman AK, Alharbi MW, Alzuhayri AJ, et al. Pattern of skin diseases in a university hospital in Jeddah, Saudi Arabia: age and sex distribution. Ann Saudi Med. 2019;39(1):22–8. https://doi.org/10.5144/0256-4947.2019.22
3. Sandilands A, Sutherland C, Irvine AD, McLean WH. Filaggrin in the frontline: role in skin barrier function and disease. J Cell Sci. 2009;122(Pt 9):1285–94. https://doi.org/10.1242/jcs.033969
4. Silverberg JI. Associations between atopic dermatitis and other disorders. F1000Res. 2018;7:303. https://doi.org/10.12688/f1000research.12975.1
5. Alfares A, Al-Khenaizan S, Al Mutairi F. Peeling skin syndrome associated with novel variant in FLG2 gene. Am J Med Genet A. 2017;173(12):3201–4. https://doi.org/10.1002/ajmg.a.38468
6. Alfares A, Alsubaie L, Aloraini T, Alaskar A, Althagafi A, Alahmad A, et al. What is the right sequencing approach? Solo VS extended family analysis in consanguineous populations. BMC Med Genomics. 2020;13(1):103. https://doi.org/10.1186/s12920-020-00743-8
7. Al-Harbi T, Al-Zahrani M, Al-Balwi M, Al-Hazmi A, Alsuhaibani A, Aljafn N, et al. Clinical course of myeloproliferative leukaemia virus oncogene (MPL) mutation-associated familial thrombocytosis: a review

of 64 paediatric and adult patients. Br J Haematol. 2021;194(5):893–8. https://doi.org/10.1111/bjh.17624

8. Greil J, Rausch T, Giese T, Bandapalli OR, Daniel V, Bekeredjian-Ding I, et al. Whole-exome sequencing links caspase recruitment domain 11 (CARD11) inactivation to severe combined immunodeficiency. J Allergy Clin Immunol. 2013;131(5):1376–83.e3. https://doi.org/10.1016/j.jaci.2013.02.012
9. Buchbinder D, Stinson JR, Nugent DJ, Heurtier L, Suarez F, Sukumar G, et al. Mild B-cell lymphocytosis in patients with a CARD11 C49Y mutation. J Allergy Clin Immunol. 2015;136(3):819–21.e1. https://doi.org/10.1016/j.jaci.2015.03.008
10. Hershko AY, Suzuki R, Charles N, Alvarez-Errico D, Sargent JL, Laurence A, et al. Mast cell interleukin-2 production contributes to suppression of chronic allergic dermatitis. Immunity. 2011;35(4):562–71. https://doi.org/10.1016/j.immuni.2011.07.013
11. Scudiero I, Vito P, Stilo R. The three CARMA sisters: so different, so similar: a portrait of the three CARMA proteins and their involvement in human disorders. J Cell Physiol. 2014;229(8):990–7. https://doi.org/10.1002/jcp.24543
12. Zotti T, Polvere I, Voccola S, Vito P, Stilo R. CARD14/CARMA2 signaling and its role in inflammatory skin disorders. Front Immunol. 2018;9:2167. https://doi.org/10.3389/fimmu.2018.02167
13. Jost PJ, Weiss S, Ferch U, Gross O, Mak TW, Peschel C, et al. Bcl10/Malt1 signaling is essential for TCR-induced NF-kappaB activation in thymocytes but dispensable for positive or negative selection. J Immunol. 2007;178(2):953–60. https://doi.org/10.4049/jimmunol.178.2.953
14. Snow AL, Xiao W, Stinson JR, Lu W, Chaigne-Delalande B, Zheng L, et al. Congenital B cell lymphocytosis explained by novel germline CARD11 mutations. J Exp Med. 2012;209(12):2247–61. https://doi.org/10.1084/jem.20120831
15. Alfadhel M, Umair M, Almuzzaini B, Alsaif S, AlMohaimeed SA, Almashary MA, et al. Targeted SLC19A3 gene sequencing of 3000 Saudi newborn: a pilot study toward newborn screening. Ann Clin Transl Neurol. 2019;6(10):2097–103. https://doi.org/10.1002/acn3.50898
16. Alyafee Y, Al Tuwaijri A, Alam Q, Umair M, Haddad S, Alharbi M, et al. Next generation sequencing based non-invasive prenatal testing (NIPT): first report from Saudi Arabia. Front Genet. 2021;12:630787. https://doi.org/10.3390/genes12040461
17. Alyafee Y, Alam Q, Tuwaijri AA, Umair M, Haddad S, Alharbi M, et al. Next-generation sequencing-based pre-implantation genetic testing for aneuploidy (PGT-A): first report from Saudi Arabia. Genes (Basel). 2021;12(4):461. https://doi.org/10.3390/genes12040461

Journal of Biochemical and Clinical Genetics

CASE REPORT

Progressive pseudorheumatoid dysplasia in an Omani family: a case report

Zuha Alkhaldi[1], Moosa Allawati[1], Nadia Alhashmi[2*]

ABSTRACT

Background: Progressive pseudorheumatoid dysplasia (PPRD) is an inherited autosomal recessive musculo-skeletal condition caused by mutations in the Cellular Communication Network Factor 6 (*CCN6*) gene. This causes a variety of clinical features such as short stature, genu varum, etc.

Case Presentation: This study reported cases of three patients from the same family who exhibited the clinical features of PPRD, and the condition was diagnosed through confirmatory genetic testing. The whole exome sequencing test results for the 15-year-old and 3-year-old males revealed a class-5 pathogenic homozygous mutation in the *CCN6* gene, resulting in both individuals being diagnosed with autosomal recessive PPRD. The results for the third patient had not come out yet.

Conclusion: For patients with PPRD, it is necessary to take the full family history and genetic testing that might help in the diagnosis and treatment of the condition.

Keywords: Case report, progressive pseudorheumatoid dysplasia, CCN6, PPRD, Oman.

Introduction

Progressive pseudorheumatoid dysplasia (PPRD) [Online Mendelian Inheritance in Man (OMIM) 208230] is a rare autosomal recessive genetic disorder that affects the articular cartilage, which protects both ends of bones. It involves progressive and non-inflammatory destruction of these cartilages, leading to symptoms such as pain and joint stiffness. PPRD usually starts during childhood after the age of 3 years and before the age of 8 years. Abnormal walking, weakness, fatigue, and joint stiffness are the first signs that can be detected in children. Additionally, camptodactyly, enlargement of finger joints, as well as reduced space between bones at the knee or hip joints, might develop over time due to this condition (1).

It has been estimated that PPRD occurs in about 1 per million people in UK but it appears to be more common in the Middle Eastern countries with no recorded data available on its exact prevalence rate. To diagnose PPRD, physicians use a combination of clinical examinations and investigations such as X-ray imaging along with laboratory tests. The condition could be underdiagnosed since it shares similar features to juvenile rheumatoid arthritis (1).

A mutation in Cellular Communication Network Factor 6 (*CCN6*) gene is responsible for causing PPRD. *CCN6* is a protein-coding gene located in 6q21 (OMIM 603400) (Gene ID 8838) which is produced from chondrocytes, and it is involved in the processes of bone growth and connective tissue maintenance (2). To explain the function of this gene furthermore, it encodes a member of the *WNT1* inducible signaling pathway (WISP) protein subfamily (3), Wnt1-inducible signaling protein 3 (WISP3), which encodes cysteine-rich secreted proteins that have important roles in cell growth and differentiation (4).

This study reported cases of three patients from the same family who exhibited the clinical features of the condition, and the condition was diagnosed through confirmatory genetic testing.

Case Presentation

In this report, we present the cases of three members from an Omani family with specific symptoms of PPRD .

Correspondence to: Nadia Alhashmi
*Senior Consultant, Clinical & Biochemical Geneticist, Child Health Department, Royal Hospital, Muscat, Sultanate of Oman.
Email: nadiaalhashmi@gmail.com
Full list of author information is available at the end of the article.
Received: 02 February 2023 | **Accepted:** 05 March 2023

This is an open access article distributed in accordance with the Creative Commons Attribution (CC BY 4.0) license: https://creativecommons.org/licenses/by/4.0/) which permits any use, Share — copy and redistribute the material in any medium or format, Adapt — remix, transform, and build upon the material for any purpose, as long as the authors and the original source are properly cited. © The Author(s).

First case was of a 4-year-old female child (III. 4), who was referred from Khoula Hospital (a well-known orthopedic hospital in Oman) to the genetic clinic in the National Genetic Centre of the Sultanate of Oman for evaluation of genu varum. The child was a term baby with a birth weight of 3 kg. She was born by spontaneous vaginal delivery with a good Appearance, Pulse, Grimace, Activity and Respiration score. The family history was significant as it was consanguineous marriage of the parents and a positive family history with the same presentation in her older brother and one cousin (III.3 and III.5) was noted. The child started to walk when she was 13 months old. At that time, the mother noticed that the child had bowing in both of her legs. She was found to have vitamin D deficiency and was discharged on supplements. The child was further referred to the orthopedics in Khoula hospital for the bowing of her legs. They started the treatment with special shoes that she should wear for a certain period. In September 2022, she underwent surgical correction of her legs. When the child was seen in November 2022, the patient was examined, and the features displayed PPRD (Figure 1).

Second case was of the eldest brother, who was 15 years old (III. 3), and was first presented at the age of 5 years with complaint of flat foot and interphalangeal joint swelling along with bowing of both of his legs. Correction surgery for his legs was done at Khoula Hospital at that time. He was taken to India for treatment and whole exome sequencing (WES) was done for him. The two other siblings were healthy with no symptoms.

The third case was of the 3-year-old male cousin (III. 5) who started to have similar symptoms like the previous family members such as interphalangeal joint swelling and bowing of the legs at the age of 2 years. His family history is significant for consanguineous marriage, and he has only one older sister, who was healthy without any symptoms (Figure 2).

The WES test results for the 15-year-old and 3-year-old males revealed a class 5 pathogenic homozygous

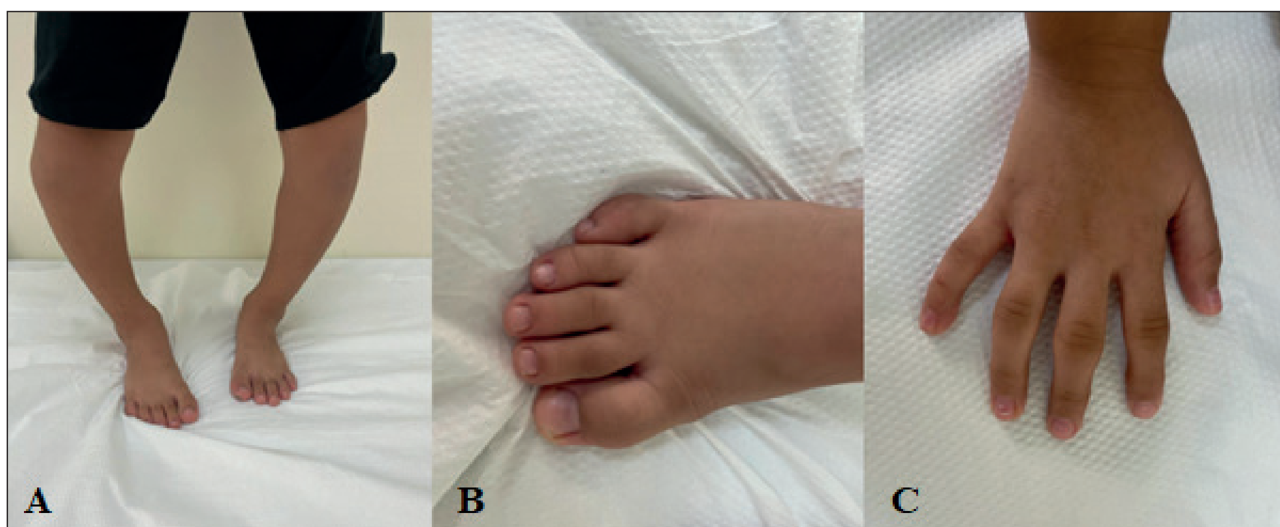


Figure 1. *This figure displays the features of PPRD. A: genu varum; B and C: interphalangeal swelling in the toes and fingers.*

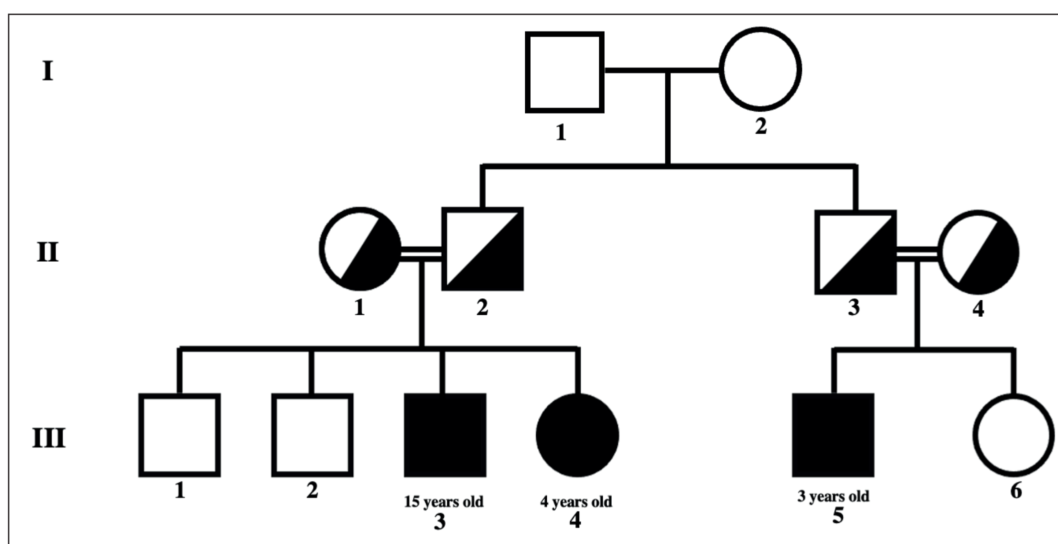


Figure 2. *This family pedigree chart of the whole family showing the affected patients.*

mutation in the *CCN6* gene, resulting in both individuals being diagnosed with autosomal recessive PPRD. In a molecular study, investigations of the patient's brother and first cousin showed similar mutation variants c.922_923del, p.(Ser308Leufs*12) which causes a shift in the reading frame starting at codon 308. The new reading frame ends in a stop codon 11 positions downstream, known to cause PPRD.

In addition, it was mentioned that PPRD presents as spondyloepiphyseal dysplasia tarda with progressive arthropathy and is described as a specific autosomal recessive subtype of spondyloepiphyseal dysplasia (SED). In addition, most patients would start showing symptoms before the age of 8 years and the symptoms would not appear in infancy. Bowing of the legs, muscle weakness, symmetric swelling of the proximal interphalangeal joints, motion range limitation, deformities, and progressive pain are the most common symptoms of PPRD. Some patients could develop some spinal manifestations like exaggerated lumbar lordosis, thoracic kyphosis, and scoliosis. However, extra-skeletal manifestations are usually not present in those patients, and they exhibit completely normal facial appearance and normal intelligence.

The 4-year-old female child presented in this case report is not diagnosed yet, the target mutation test for the CCN6 gene was sent and the results have not been out yet.

Discussion

PPRD is a rare genetic and skeletal disease affecting children. It was first described by Spranger et al. (5) as a progressive chondropathy, which mostly affects the articular cartilages and results in specific skeletal abnormalities.

In 2000, Mampaey et al. (6) reported a case of PPRD that involved spinal and articular features which were initially interpreted as juvenile rheumatoid arthritis and Scheuermann's disease but later excluded. In 2007, three cases of PPRD were reported in Morocco from the same family by Bennani et al. (7). The three reported cases (a 4-year-old girl, a 15-year-old boy and an uncle who was examined at the age of 4 years) had similar articular features. A Chinese study published in 2011 reported seven cases of PPRD from six different unrelated families all presenting with symptoms of PPRD and were diagnosed with the condition according to the clinical signs and symptoms, as well as radiographic imaging (8). In 2017, Sailani et al. (9) reported PPRD due to WISP3 mutation of in four patients from the same family. Alawbathani et al. (10) reported a case in 2017 of a 24-year-old Yemeni gentleman born to consanguineous parents and had features of PPRD (Table 1).

Comparing between the published variants in the previous studies and this study, the patients in this study had c.992_923delAG (p.Ser308Leufs*12) variant which causes a shift in the reading frame starting at codon 308 and was therefore found to cause PPRD. The case of the Yemeni patient reported by Alawbathani et al. (10), had homozygous frame shift mutation in WISP3 in exon 5; c.868_869delAG, p.Ser290Leufs*12, this protein change is a bit like the current study. Sailani et al. (9) found that there are around 71 pathogenic mutations

Table 1. *Comparison of clinical features between this case report and the previously published case reports.*

Study	Joint swelling	Genu varum	Flat foot	Arthritis/ arthralgia	Spinal manifestations (e.g., thoracic kyphosis, increased lumbar lordosis, scoliosis)	Stiffness / flexion contracture	Coxa valga	Short stature	Muscle weakness/ atrophy	Waddling gait or other gait abnormalities
Current study, 2023	X	X	X							X
Mampaey et al., 2000 (6)	X			X		X			X	X
Bennani et al., 2007 (7)	X			X	X	X	X			
Ye et al., 2011 (8)	X	X		X	X	X		X	X	X
Sailani et al., 2017 (9)	X				X	X				X
Alawbathani et al., 2017 (10)	X			X	X	X				X

of WISP3 distributed worldwide. The most common reported variant is c.156C>A (p.Cys52*) variant with 33 reported case distributed in Italy, France, Lebanon, Turkey, Germany, and India. The second most common variant is c.1010G>A (p.Cys337Tyr) with 18 reported cases located in India only (9). Therefore, the specific variant found in this study was not previously reported. This is the first reported case of PPRD in Oman, and this is the first case reported of this specific variant on an international level.

Although there is no standard treatment for PPRD, rehabilitation at an early age is a good approach to prevent disabling consequences. Previous studies showed that early rehabilitation could lead to pain-free walking and mobility in PPRD patients. In addition, surgical interventions such as arthroplasty and osteotomy are necessary in such cases to adjust bowing of the bones. Genetic testing and counseling are essential steps for families with PPRD to raise awareness about the disease and its implications on their lives (7).

Conclusion

PPRD is a life-altering medical condition, which could cause permanent deformity if left untreated, therefore, early diagnosis should always be sought by those who are suffering from any related symptoms associated with it, so they can get a proper treatment plan tailored for them accordingly.

Acknowledgments

National Genetic Centre of the Sultanate of Oman.

List of Abbreviations

CCN6 Cellular Communication Network Factor 6
PPRD Progressive pseudorheumatoid dysplasia
WES Whole exome sequencing
WISP3 Wnt1-inducible signaling protein 3

Funding
None.

Declaration of conflicting interests
The authors declare that there is no conflict of interest regarding the publication of this article.

Consent for publication
Informed consent was obtained from the parents of the patient to publish the case and the accompanying images.

Ethical approval
Ethical approval is not required at our institute for an anonymous case report.

Author details
Zuha Alkhaldi[1], Moosa Allawati[1], Nadia Alhashmi[2]

1. Medical Intern, Sultan Qaboos University, Muscat, Sultanate of Oman
2. Senior Consultant, Clinical & Biochemical Geneticist, Child Health Department, Royal Hospital, Muscat, Sultanate of Oman

References

1. Progressive pseudorheumatoid dysplasia: medlinePlus Genetics. Medlineplus.gov. 2013. Available from: https://medlineplus.gov/genetics/condition/progressive-pseudorheumatoid-dysplasia/#:~:text=Progressive%20pseudorheumatoid%20dysplasia%20(PPRD)%20is,between%20bones%20(articular%20cartilage).
2. CCN6 gene: MedlinePlus genetics. Medlineplus.gov. 2013. Available from: https://medlineplus.gov/genetics/gene/ccn6/
3. Database G. GeneCards - human genes. 2023. Available from: https://www.genecards.org/
4. Bork P. The modular architecture of a new family of growth regulators related to connective tissue growth factor. FEBS Lett. 1993;327(2):125–30. https://doi.org/10.1016/0014-5793(93)80155-N
5. Spranger J, Albert C, Schilling F, Bartsocas C, Stoss H. Progressive pseudorheumatoid arthritis of childhood (PPAC). Eur J Pediatr. 1983;140(1):34–40. https://doi.org/10.1007/BF00661902
6. Mampaey S, Vanhoenacker F, Boven K, Hul WV, De Schepper A. Progressive pseudorheumatoid dysplasia. Eur Radiol. 2000;10(11):1832–5. https://doi.org/10.1007/s003300000518
7. Bennani L, Amine B, Ichchou L, Lazrak N, Hajjaj-Hassouni N. Progressive pseudorheumatoid dysplasia: three cases in one family. Joint Bone Spine. 2007;74(4):393–5. https://doi.org/10.1016/j.jbspin.2006.11.014
8. Ye J, Zhang HW, Qiu WJ, Han LS, Zhang YF, Gong ZW, et al. Patients with progressive pseudorheumatoid dysplasia: from clinical diagnosis to molecular studies. Mol Med Rep. 2011; (1):190–5. https://doi.org/10.3892/mmr.2011.619
9. Sailani MR, Chappell J, Jingga I, Narasimha A, Zia A, Lynch JL, et al. WISP3 mutation associated with pseudorheumatoid dysplasia. Cold Spring Harb Mol Case Stud. 2017; 4(1):a001990. https://doi.org/10.1101/mcs.a001990
10. Alawbathani S, Kawalia A, Karakaya M, Altmüller J, Nürnberg P, Cirak S. Late diagnosis of a truncating wisp3 mutation entails a severe phenotype of progressive pseudorheumatoid dysplasia. Cold Spring Harb Mol Case Stud. 2017;4(1): a002139. https://doi.org/10.1101/mcs.a002139