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ORIGINAL ARTICLE

Expanding the allelic spectrum of *ELOVL4*-related autosomal recessive neuro-ichthyosis

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Abstract

Background: Very long-chain fatty acids (VLCFAs) composed of more than 20 carbon atoms are essential in the biosynthesis of cell membranes in the brain, skin, and retina. VLCFAs are elongated beyond 28 carbon atoms by ELOVL4 enzyme. Variants in *ELOVL4* are associated with three Mendelian disorders: autosomal dominant (AD) Stargardt-like macular dystrophy type 3, AD spinocerebellar ataxia, and autosomal recessive disorder congenital ichthyosis, spastic quadriplegia and impaired intellectual development (ISQMR). Only seven subjects from five unrelated families with ISQMR have been described, all of which have biallelic single-nucleotide variants.

Methods: We performed clinical exome sequencing on probands from four unrelated families with neuro-ichthyosis.

Results: We identified three novel homozygous *ELOVL4* variants. Two of the families originated from the same Saudi tribe and had the exact homozygous exonic deletion in *ELOVL4*, while the third and fourth probands had two different novel homozygous missense variants. Seven out of the eight affected subjects had profound developmental delay, epilepsy, axial hypotonia, peripheral hypertonia, and ichthyosis. Delayed myelination and corpus callosum hypoplasia were seen in two of five subjects with brain magnetic rosonance imaging and cerebral atrophy in three.

Conclusion: Our study expands the allelic spectrum of *ELOVL4*-related ISQMR. The detection of the same exonic deletion in two unrelated Saudi family from same tribe suggests a tribal founder mutation.

KEYWORDS

autosomal recessive, copy number variant, *ELOVL4*, neuro-ichthyosis

1 | INTRODUCTION

Neurocutaneous syndromes are a heterogeneous group of disorders characterized by skin and central nervous system involvement and are mainly genetic in nature (Fischer & Bourrat, 2020). One particular group of these disorders are neuro-ichthyoses in which there is dryness and scaling of skin with variable neurological manifestations (Rizzo et al., 2012). Over the past decade and with the application of next-generation sequencing, the genetic basis of neuro-ichthyoses has been increasing recognized and a variety of pathways including those involved in lipid metabolism, glycoprotein synthesis, or intracellular vesicle trafficking have been implicated (Rizzo et al., 2012).

Very long-chain fatty acids (VLCFAs) are fatty acids with more than 22 carbon atoms. VLCFAs can be divided into saturated, monounsaturated, and polyunsaturated fatty acids (Leonard et al., 2004), all of which are important for maintaining the structure and function of cell membranes (Schneiter et al., 2004). VLCFAs play a unique role in the brain, retina, and skin (Agbaga, 2016). They are synthesized by a multiprotein elongation system referred to as the ELOVL group (Beaudoin et al., 2009). Elongation of very long-chain fatty acids-4 (*ELOVL4)* is a member of the ELOVL group that is responsible for fatty acid elongation and is the only member that catalyzes production of VLCFAs and very long-chain polyunsaturated fatty acids (VLC-PUFAs) of 28 carbon atoms and more, with the highest expression of *ELOVL4* in the brain, retina, and skin (Deák et al., 2019; Vasireddy et al., 2007).

Until now, pathogenic variants in *ELOVL4* have been associated with three different Mendelian disorders with variable neurological, ophthalmological, and cutaneous manifestations and different modes of inheritance (Deák et al., 2019). The first disease is Stargardt-like macular dystrophy type 3 (STGD3) (MIM #600110), an autosomal dominant (AD) disorder caused by heterozygous pathogenic variants in *ELOVL4* that presents with isolated juvenile visual loss (Agbaga et al., 2008). The second is an AD disorder caused by heterozygous pathogenic variants in *ELOVL4* resulting in spinocerebellar ataxia 34 with erythrokeratodermia (SCA34) (MIM#133190) which consists of ataxia, incoordination, visual problems, dysarthria, and skin involvement (Bourassa et al., 2015; Sullivan et al., 2019). The third and most rare disorder associated with *ELOVL4* is ichthyosis, spastic quadriplegia, and impaired intellectual development (ISQMR; MIM #614457) which has an autosomal recessive (AR) inheritance (Aldahmesh et al., 2011; Diociaiuti et al., 2021; Mir et al., 2014). Patients with ISQMR exhibit features of ichthyosis, seizures, intellectual disability, and spasticity (Rizzo et al., 2012), although isolated congenital ichthyosis without neurological involvement has also been described in one family (Mir et al., 2014).

To date, only seven cases with ISQMR from five unrelated families have been described; five of these cases share almost the same clinical features of profound developmental delay, seizures, and congenital ichthyosis (Aldahmesh et al., 2011; Diociaiuti et al., 2021; Mir et al., 2014).

Here, four new families with ISQMR were reported, including two families originating from the same Saudi tribe with a novel homozygous exonic deletion in *ELOVL4*, possibly representing a founder allele. Our cases expand the

genotypic spectrum of ISQMR and highlight the contribution of small copy number variants (CNVs) in the pathogenesis of rare Mendelian disorders.

2 | METHODS

Informed consent for publication of relevant findings and clinical photographs was obtained from the legal guardians of the affected individuals from all families. The research study was approved by the Research and Ethical Committee of the Ministry of Health in Kuwait and King Faisal Specialist Hospital and Research Center and was conducted according to the ethical principles of Declaration of Helsinki. The proband from Family 4 was previously published with limited clinical information (Monies et al., 2017).

2.1 | Genetic testing

Proband clinical exome sequencing (cES) was performed on the proband from all families. cES of the index cases from Families 1 and 2 was performed at an accredited diagnostic company (CENTOGENE GmbH), and cES for the index of Family 3 and 4 was performed in a local Clinical Laboratory Improvement Amendments (CLIA)-certified genetic laboratory. All variants are described according to transcript NM_022726.3.

DNA was extracted from ethylenediamine tetracetic acid blood or from dried blood spots (DBSs) on filter cards (CentoCard®) with QIASymphony using a magnetic bead-based method (Qiagen), with an acceptance criterion of minimum 3 ng/μL. Genomic DNA is enzymatically fragmented, and target regions are enriched using DNA capture probes. These regions include approximately 41 Mb of the human coding exome (targeting >98% of the coding RefSeq from the human genome build GRCh37/hg19), as well as the mitochondrial genome. The generated library is sequenced on an Illumina platform to obtain at least 20× coverage depth for >98% of the targeted bases. An in-house bioinformatics pipeline, including read alignment to GRCh37/hg19 genome assembly and revised Cambridge Reference Sequence (rCRS) of the Human Mitochondrial DNA (NC_012920), variant calling, annotation, and comprehensive variant filtering, is applied. All variants with minor allele frequency (MAF) of less than 1% in gnomAD database, and disease-causing variants reported in HGMD®, in ClinVar or in CentoMD® are evaluated. The investigation for relevant variants is focused on coding exons and flanking ±10 intronic nucleotides of genes with clear gene-phenotype evidence (based on OMIM® information). All potential patterns for mode of inheritance are considered. In addition, family history and clinical information are used to evaluate identified variants with respect to their pathogenicity and disease causality. Variants are categorized into five classes (pathogenic, likely pathogenic, variant of unknown significance, likely benign, and benign) along American College of Medical Genetics guidelines for the classification of variants. The CNV detection software has a sensitivity of more than 95% for all homozygous/hemizygous and mitochondrial deletions, as well as heterozygous deletions/duplications and homozygous/hemizygous duplications spanning at least three consecutive exons.

Quantitative polymerase chain reaction (QPCR) reactions were performed on individuals from Family 1 (proband [Subject S1] and parents) and Family 2 (proband [Subject S2], affected siblings [Subjects S3 and S4], and parents) using the SensiMix™ SYBR® No-ROX Kit (Bioline), on LightCycler® 480 II (Roche) system. Gene-specific primers encompassing coding exons 1 and 2 (or part of it) of *ELOVL4* based on transcript NM_022726.3. The primer design was as follows:

Exon 1 forward (5′ → 3′) primer: GTGTCCTAAACGTAGTGTCCAC
Exon 1 reverse (5′ → 3′) primer: GAAGTCAGCGGCTTTACCTG
Exon 2 forward (5′ → 3′) primer: AATTGGCCTCTGATGCAGTC
Exon 2 reverse (5′ → 3′) primer: AAAGGTTCTCGGTCCTTCATCC

Additionally, multiple gene-specific primers encompassing coding exons 1 and 2 of *ELOVL4* as well as the upstream region were designed for long-range PCR to determine the breakpoint junction. The design was as follows:

Exon 1 forward primer 1: caccacgcctacctcatctt
Exon 1 reverse primer 1: gaggggaggccttaacattc
Upstream forward primer: ttgctttgttcattccatgtat
Upstream reverse primer: gaagaatgggtgttctgtgg
Exon 2 forward primer: tgggactcaaaggacagtga
Exon 2 reverse primer: caatgatggttttacacattctca
Exon 1 forward primer 2: ttcatgcaattatatgttgatttgtt
Exon 1 reverse primer 2: ggagatgtgggagcacagg
Upstream forward primer 2: tgtgaaagggcaggaacaat
Upstream reverse primer 2: tcgacccatgaacaaatcaa

2.2 | Biochemical tests

Very long-chain fatty acid-containing lysophosphatidylcholine (LysoPC) analysis was performed on DBSs from

the proband from Family 3 (subject S5) and proband of Family 4 (Subject S7) according to the method previously described after adaptation of the multiple reaction monitorings to also measure the abundance C28:0-LysoPC in addition to C26:0-LysoPC (Jaspers et al., 2020). The C28:0-lysoPC concentration was calculated using the deuterium-labeled C26:0-LysoPC internal standard assuming identical response.

2.3 | Clinical reports

2.3.1 | Family 1 (Subject S1)

The proband is a 2-year-old Saudi boy who is the first child of a non-consanguineous couple from the same tribe (Figure 1a). He was delivered by cesarean section with a birth weight of 3.180 kg following an uneventful pregnancy. Activity, pulse, grimace, appearance and respiration scores were 7 and 9 at 1 and 5 min, respectively. He was admitted to the neonatal intensive care unit (NICU) for 1 week after the team noticed he had shiny, dry, and scaly skin. He was diagnosed with ichthyosis and prescribed topical emollients by a dermatologist. An echocardiogram was done as part of screening for associated congenital anomalies which showed patent ductus arteriosus (PDA) and atrial septal defect (ASD) secundum. At the age of 4 months, his mother brought him to medical attention due to poor feeding and episodes of cyanosis. He was admitted to the hospital in Saudi Arabia and was noted to be dehydrated. Workup showed evidence of hypernatremia (sodium level of 151 mmol/L). He was diagnosed with gastroesophageal reflux disease (GERD) and was discharged after 4 days following rehydration with intravenous fluids and initiation of proton pump inhibitors. Follow-up echocardiogram during hospitalization was normal. The episodes of cyanosis continued and were associated with tonic stiffening of limbs for 10–15 s. He was later diagnosed with seizures and started on phenobarbitone.

At 13 months of age, he presented with uncontrolled seizures in the form of recurrent episodes of tonic stiffening lasting for 10–15 s, and levetiracetam was added to his antiepileptic medication regimen. Thorough general and neurological examinations were performed, and he

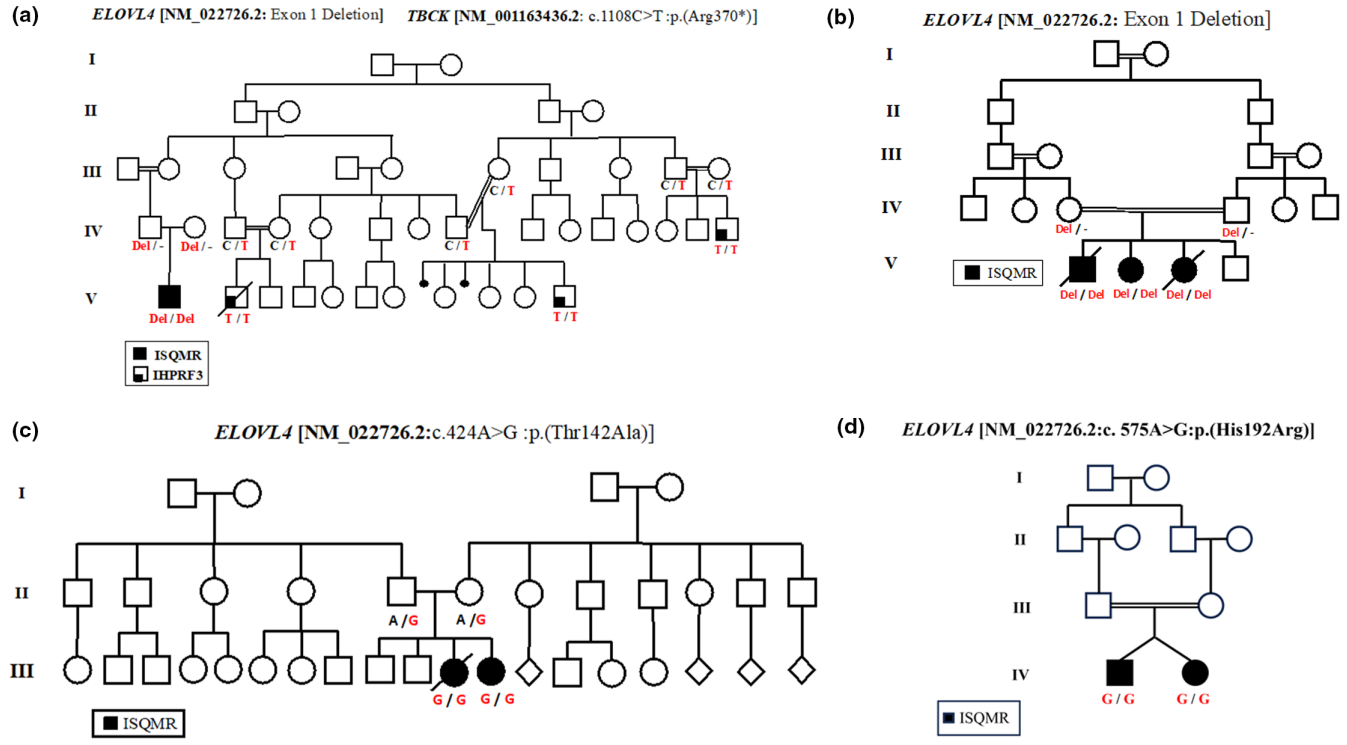

FIGURE 1 Pedigrees of families 1–4. (a) Extended 5-generation pedigree of the proband of Family 1. Note that two autosomal recessive neurodevelopmental disorders have been detected in this family and confirmed to be caused by two different genes accounting for the intrafamilial variability. (b) Extended 5-generation pedigree of Family 2. Note the single loop of consanguinity as the parents are second-degree cousins. The black-colored square and circles depict the affected children in the family. (c) A 3-generation pedigree of Family 3 showing the proband's extended family members, her two healthy older brothers, and her youngest affected sister. The proband and her younger affected sister are depicted with black-colored circle. (d) A 4-generation pedigree of Family 4 showing the proband and his affected twin sibling which are products of a first-degree cousin marriage. The proband and his affected twin sister are depicted in black-colored squares.

was also noted at that time to have profound global developmental delay (GDD), microcephaly, and failure to thrive. At 13 months of age, he was unable to support his neck, turn, or sit independently. He could not fix or follow and had no social smile. He was unable to recognize his parents and had never produced any sounds. His anthropometric measurements were as follows: head circumference of 45.5 cm (2nd %tile for age), 6.7 kg (<1st %tile for age) of weight, and 79 cm (<1st %tile for age) of height. He also had strabismus and mild dysmorphic features including epicanthal folds and triangular face. His skin was dry, scaly, and erythematous, and he had seborrheic dermatitis on his scalp (Figure 2Ai–iii). Neurological examination showed severe axial hypotonia with poor head control, peripheral hypertonia with fisted hands, contractures, and scissoring of his legs. His reflexes were brisk throughout. Additionally, he had abdominal distension, but there was no evidence of organomegaly to suggest a storage disease.

From age 13 months to 2 years, he had recurrent hospitalization and pediatric intensive care unit admissions due to acute exacerbation of asthma and aspiration pneumonias with severe respiratory distress. His skin changes and seborrheic dermatitis on his scalp never resolved. Family history was significant for two second cousins with profound developmental delay and hypotonia (Figure 1a) and a previous diagnosis of infantile hypotonia with psychomotor retardation and characteristic facies type 3 (MIM# 616900) due to a likely pathogenic homozygous variant

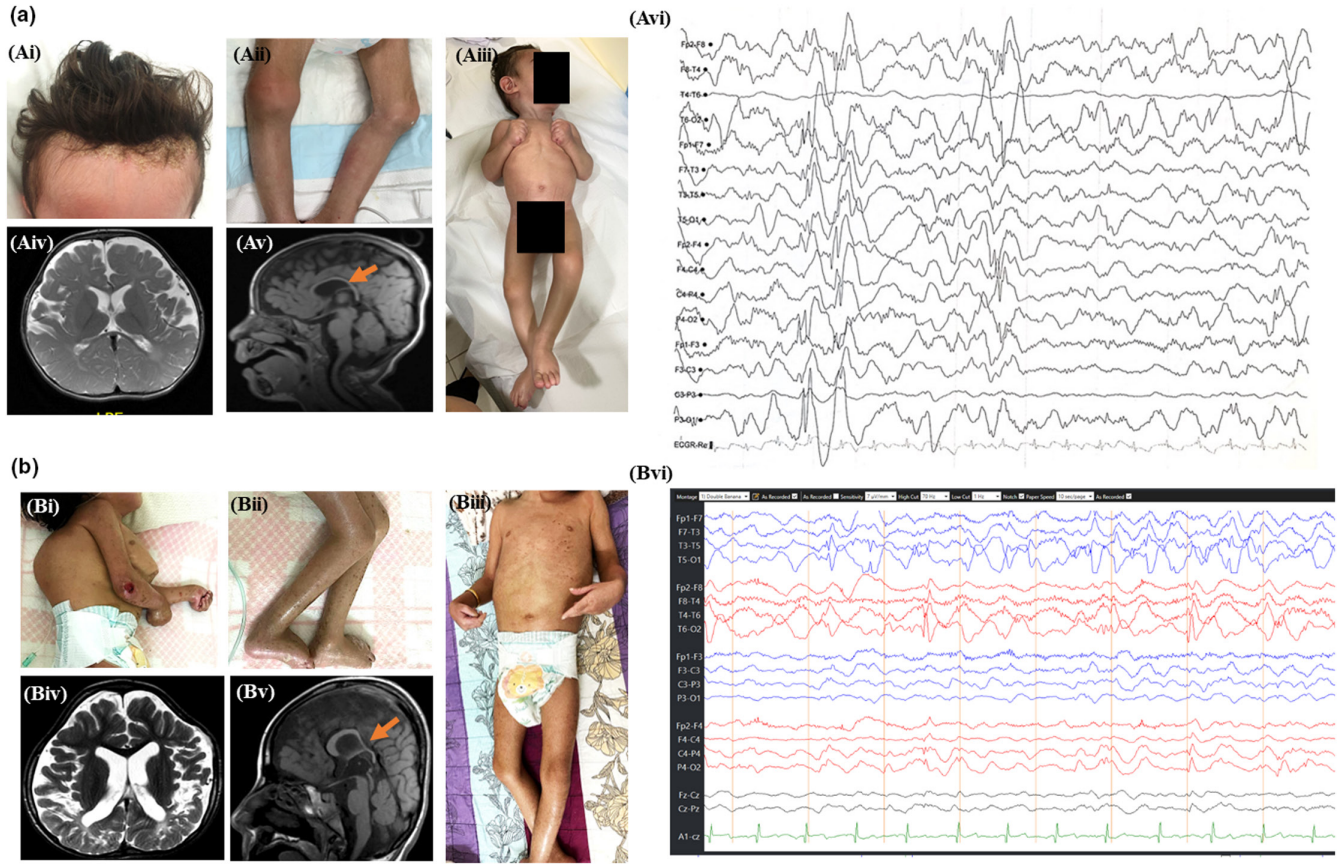

FIGURE 2 Photos and magnetic resonance imaging (MRI) and electroencephalogram (EEG) images of patients from Families 1 and 2. (Ai–Aiii) Photographs of proband of Family 1 (Subject S1) showing seborrheic dermatitis of the scalp, limb contractures, and ichthyosis. (Aiv–Av) MRI brain (axial T2-weighted and sagittal T1-weighted images) of proband of Family 1 (Subject S1) showing diffuse cerebral volume loss, delayed myelination for age, and thin corpus callosum (orange arrow). (Avi A) screenshot of the proband's (Family 1/Subject S1) EEG 10-second epoch (bipolar montage; sensitivity 15 μV/mm) showing slow background and multifocal epileptiform activity. (Bi–Bii) Photograph of proband's first sister of Family 2 (V-2, Subject S3) showing chest deformity, ulcerative skin lesions, dry shiny skin in the lower limbs with skin peeling, and contractures. (Biii) A photograph of the proband's second sister (V-3) of Family 2 (Subject S4) showing dry shiny skin. (Biv–Bv) MRI brain (axial T2-weighted and sagittal T1-weighted images) of proband of Family 2 (V-1, Subject S2) shows ulegyria, volume loss, and abnormal hyperintensity in the parietooccipital white matter and of the depths of the sulci, diffuse atrophy, and marked thinning of the body and splenium of the corpus callosum (orange arrow). No atrophy of the cerebellum or brainstem. (Bvi) A screenshot of the proband's first sister's (Subject S3's) EEG 10-second epoch (bipolar montage; sensitivity 7 μV/mm) showing bilateral independent posterior spike slow wave discharges seen bilaterally but more on the right side. There is also poorly formed and poorly organized background. Findings are suggestive of epileptic encephalopathy with epileptiform discharges more abundant in the right hemisphere.

in *TBCK* [NM_001163436.2: c.1108C>T: p.(Arg370*)]; however, these two cousins lacked the skin findings and had a less severe course of illness. *TBCK* encodes a TBC1 domain-containing kinase (TBCK). The patient had extensive investigations and workup which included normal plasma amino acids and urine organic acids levels. VLCFA was ordered but was not available. His awake and asleep electroencephalogram (EEG) recording at 9 months of age was abnormal with diffuse delta background slowing and frequent multifocal epileptiform discharges over the left frontocentral, left occipital, right frontocentral, and right temporooccipital regions. Brain magnetic resonance imaging (MRI) was performed at 9 months of age and showed diffuse cerebral atrophy and thin corpus callosum with no evidence of structural abnormalities (Figure 2Aiv–v). He was also referred twice for detailed ophthalmological exam, visual evoked potential (VEP), and electro-retinography, but none were performed due to lack of follow-up.

Local neurology panel at the Kuwait Medical Genetic Centre was negative. The panel included *SNAP29* but did not include *TBCK* nor other genes associated with neuroichthyosis such as *ALDH3A2*, *AP1B1*, *ELOVL4*, *DOLK*, and *SUMF1*. A chromosomal microarray (CMA) with both microarray-based comparative genomic hybridization (aCGH) and single-nucleotide polymorphism (SNP) performed showed three long contiguous stretches of homozygosity greater than 10 Mb on chromosomes 6, 12, and 14 [arr [hg19] 6p13q14.1 (71,076,993–82,660,403), 12q22q23 (95,579,058–106,514,145), and 14q13.1q21.2 (34,449,999–45,157,248)] indicating parental consanguinity. Given the clinical presentation which was different from that in his second cousins with a homozygous *TBCK* variant, the severity of his condition, the possibility of dual molecular or alternative diagnoses, and the implication for future pregnancies, a decision was made to proceed with exome sequencing.

2.3.2 | Family 2 (Subjects S2-S4)

The proband (V-1, Subject S2) in this family is a Saudi male who died at 6 years of age and who originated from the same tribe as proband from Family 1 but was apparently unrelated. His parents are first-degree cousins, and he had three younger siblings including two similarly affected sisters and one healthy brother (Figure 1b). He was born prematurely at 32 weeks of gestation, and the pregnancy was complicated by oligohydramnios and meconium-stained amniotic fluid. He was admitted to the NICU for 3 months and was mechanically ventilated for acute respiratory distress syndrome. After he was discharged, he had multiple hospital admissions due to recurrent chest infections, aspiration pneumonia, and during one admission, he had a cardiac arrest. During his multiple hospitalizations, he was found to have spastic quadriplegia, profound GDD, drug-responsive epilepsy (controlled with valproic acid), and generalized ichthyosis, and the possibility of Sjogren–Larsson syndrome was raised. He was also diagnosed with GERD after repeated vomiting and failure to thrive and underwent fundoplication and gastric tube placement for feeding.

At 4 years of age, he presented to the hospital for severe respiratory distress and was transferred to intensive care unit for respiratory support. His workup included an echocardiogram and abdominal ultrasounds which were normal. MRI brain showed generalized cerebral atrophy and marked thinning of the body and splenium of the corpus callosum (Figure 2Biv–v). His abdominal computerized tomography scan showed mild splenomegaly, multiple bilateral renal stones with hydroureteronephrosis and perinephritic inflammatory changes suggestive of chronic inflammation, bilateral atelectasis, and bronchiectasis, incidentally, noted bilateral inguinal hernia and significant muscle wasting and right hip dislocation. He never had a detailed ophthalmological exam, nor VEP or electroretinogram (ERG). Plasma amino acids, urine organic acids, and carnitine and acylcarnitine levels were unremarkable. He had a skin biopsy performed on his right upper arm as part of workup for ichthyosis which showed epidermal hyperkeratosis, hypergranulosis, acanthosis, spongiosis, and nonspecific dermal inflammation with no evidence of porokeratosis, granuloma annulare, psoriasis, or fungal infection. The overall findings were thought to be nonspecific but possibly suggestive of Netherton syndrome. He became ventilator-dependent and was transferred to the pediatric long stay unit and managed with rehabilitation care for 2 years. He was assigned "do not resuscitate status" and eventually passed away in the hospital at 6 years of age.

The first affected sibling (V-2, Subject S3) is a female who is now 5 years old with GDD and drug-responsive epilepsy. Her seizures are controlled with levetiracetam and carbamazepine. She has the same cutaneous features of the proband in the form of skin dryness and scaling (Figure 2Bi–ii). Her neurological examination shows inability to fix or follow, generalized hypotonia, and exaggerated deep tendon reflexes. Her routine EEG demonstrates poorly organized slow background activity that is mainly in the delta-theta range with repeated electro-decrements bilaterally. She never had detailed eye exam, VEP, or ERG.

The second and youngest affected sibling (V-3, Subject S4) is a 4-year-old female with severe GDD, axial hypotonia, peripheral hypertonia, drug-responsive epilepsy, and congenital ichthyosis (Figure 2Biii). Her seizures are controlled with levetiracetam. Her skin findings were noticed

soon after birth which manifested as colloid membrane that progressed to ichthyosis. Her development is profoundly delayed as she cannot produce any sounds or hold her head up, and she has limited social interaction. Her awake and sleep routine EEG showed poorly organized background rhythm in the delta-theta range with mild asymmetry on the right. She also had frequent bursts and runs of 2 Hz spikes and slow wave activity bilateral raising the concern for epileptic encephalopathy. Detailed ophthalmological exam, VEP, and ERG were never performed. The child died at 4 years of age due to a respiratory infection that led to respiratory failure.

2.3.3 | Family 3 (Subjects S5-S6)

The proband in this family (Subject S5) is a Pakistani female who died at 6 years of age. Her parents are unrelated but come from the same small town (Figure 1c). She has two healthy brothers and one similarly affected younger sister. She was born at 38 weeks following an uncomplicated pregnancy via emergency cesarean section due to fetal deceleration. Prenatal ultrasound was normal with normal amniotic fluid volume. After delivery, the proband had extremely dry, scaly erythematous skin which improved with emollients. She developed her first seizure at the age of 3 months after vaccination. She had drug-resistant epilepsy with daily seizures and was diagnosed with early-onset epileptic encephalopathy with multiple seizure types as well as GDD. She had failure to thrive as her weight, height, and head circumference were all on the 0.4th percentile for age. She received her feeds by a gastrostomy tube. She had coarse dysmorphic features with synophrys, long eye lashes, and pectus excavatum. The cutaneous features included dry, scaly skin, and severely erythematous skin lesions. She had abnormal neurological examination with axial hypotonia. Her ophthalmological examination demonstrated cone-rod macular dystrophy and possible optic disc atrophy associated with severe visual impairment. Her brain MRI at 1 year of age was normal. Her EEG at 5 years of age showed hypsarrhythmia.

The proband's affected younger sister (Subject S6) is a 2-month-old girl who was born at term by spontaneous vaginal delivery. There was no history of colloid membrane or skin shedding at birth, but skin dryness and skin thickening were noted. She was evaluated at 2 months of age in the genetics clinic at which time her examination was normal apart from the skin ichthyosis. She also had normal developmental milestones up to her current age of 2 months as she was able to smile and track. Her neurological examination was also normal. EEG and brain MRI were not performed, given she had normal neurological examination with no history of seizures. She never had ERG or VEP, given no concern about her vision at 2 months of age.

2.3.4 | Family 4 (Subjects S7-S8)

This proband (Subject S7) from this family was previously published as subject 16-2552 (Monies et al., 2017). He was 8 months old at last examination and was a product of a consanguineous marriage and part of a twin pregnancy. He was born preterm at 34 weeks of gestation and was admitted to the NICU for 20 days for respiratory support. He had ichthyosis since birth. He started having seizures at the age of 6 months, and they were generalized tonic–clonic in nature and were eventually controlled with two anticonvulsant medication (levetiracetam and phenobarbital). With time, he was noted to have GDD. He underwent corrective surgery of bilateral inguinal hernia at 6 months of age. He had recurrent hospital admissions for acute exacerbation of asthma, pneumonia, or bronchiolitis. He had GERD and feeding difficulties and receives his feeds by nasogastric tube (NGT). His examination at the age of 8 months showed failure to thrive; his weight was 3.65 kg (<1st %tile for his age), height was 54 cm, and head circumference was 39 cm (<3rd %tile for his age). He has fair skin and blond-gray thin hair, and his skin was scaly and dry. His neurological examination showed hypotonia and spasticity in all limbs. He was inattentive and had poor fixing and following. His fundus examination revealed bitemporal pallor of the optic disks mostly due to central lesion suggestive of optic atrophy. Complete blood count, renal profile, hepatic profile, and random glucose were normal. He also had normal urine creatinine (5180 μmol/L), urine creatinine LC (7380 μmol/L), urine guanidinoacetate (1071 μmol/L), and urine creatine/creatinine LC ratio (0.702). DBSs acylcarnitine profile and urine gas chromatography-mass spectrometry (GC–MS) were also unremarkable. Biotinidase level was slightly low at 3.8 nmol/min/mL. Barium swallow showed repeated episodes of tracheal aspiration with no cough reflex, otherwise normal upper gastrointestinal barium swallow study. EEG was abnormal with abundant epileptic discharges arising from the posterior head region with disorganized background. A myoclonic jerk of the upper extremity was captured during the routine EEG and was associated with attenuation of background. Echocardiography was normal, and abdominal ultrasound showed mild diffuse increased liver parenchymal echogenicity with borderline size to mild enlargement and no focal lesions. MRI brain done at the age of 8 months was normal. Chromosomal microarray analysis (CGH array) showed no definitely pathogenic CNVs.

The proband's twin (Subject S8) in a female that has similar phenotypic features except the MRI brain shows moderate diffuse brain atrophy predominantly in frontal lobe.

3 | RESULTS

For Family 1, proband cES (on subject S1) did not identify any causative single-nucleotide variants (SNVs). CNV analysis from unphased exome data identified a novel homozygous deletion in the first exon of approximately 649 bp, in *ELOVL4* [Chr6:80656887–80657532 (hg19): NM_022726.3] (Figure 3). The detected CNV in *ELOVL4* was not present in publicly available control databases (Atherosclerosis Risk in Communities, ARIC; Grand Opportunity Exome Sequencing Project, GO-ESP; 1000 Genomes Project; and the genome aggregation database, gnomAD) or in CENTOGENE bio/databank (600,000 exomes) in either the homozygous or heterozygous state. QPCR confirmed the homozygosity for exon 1 deletion in the proband from Family 1 and heterozygosity in both parents consistent with Mendelian expectations. *ELOVL4* falls within one of three long regions of homozygosity (>10 Mb) detected by SNP array [6p13q14.1 (71,076,993–82,660,403)]. Interestingly, review of the array CGH data showed *ELOVL4* is covered with six probes, and a small homozygous 5.14 Kb CNV in *ELOVL4* was observed but was below the threshold for reporting (Figure 3). Both microarray and exome data showed normal copy number of exons 2–6 of *ELOVL4* (Figure 3), suggesting that the first break point of this deletion could be located in intron 1 (15.5 Kb) of *ELOVL4*. Additionally, in the microarray data, a probe upstream *ELOVL4* showed normal copy number 25.6 Kb away from the deletion. This indicates that the second break point is within the 25.6-Kb intergenic region. Both of these regions contain a number of repeat sequences. One of the suggested mechanisms for the formation of such a small deletion is from non-allelic homologous recombination (NAHR). Low-copy repeats sequence trigger recombination and result in copy number variation (McDonald-McGinn et al., 2015). Sequences that trigger NAHR may be present in the two areas where the suspected break points fall. Several attempts to map the

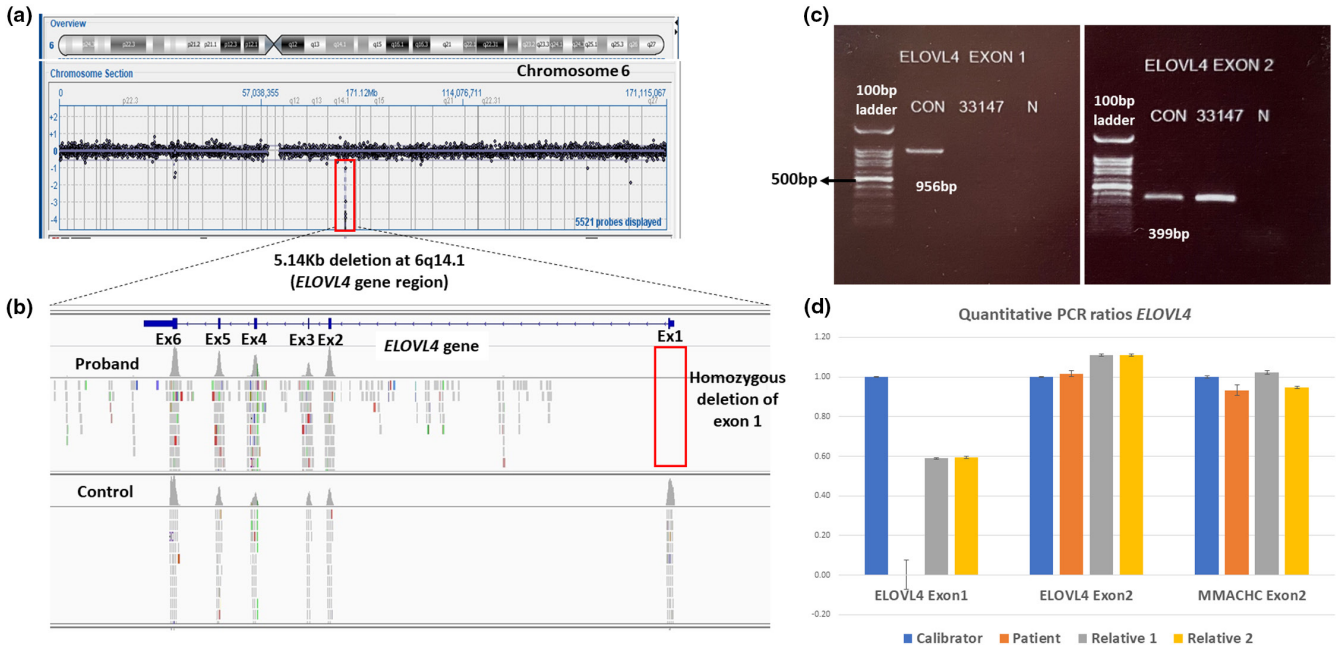

FIGURE 3 Genomic data from proband of Family 1 (Subject S1) including array CGH and BAM files around the deletion and PCR confirmation. (a) A microarray result of 5.14 Kb deletion (Chr6:80652752–80657887) involving exon 1 of *ELOVL4* in the proband from Family 1 (Subject S1) is shown. The image was obtained from microarray *CytoSure® Interpret Software*, *Oxford Gene Technology*. (b) Whole-exome sequencing of the same proband from Family 1 (Subject S1) which show no coverage of exon 1 of *ELOVL4* indicating there is homozygous deletion of exon 1. As for the control samples of the same region, the coverage of exon 1 was observed. The image was obtained from Integrative Genomics Viewer (IGV) bam files of the proband and a control. (c) Polymerase chain reaction (PCR) was used to amplify both exon 1 and 2 of *ELOVL4*. (Left side) No amplification of exon 1 in the proband from Family 1 (33147) was detected; however, there is amplification in control (product size: 956 bp). (Right side) Amplification of exon 2 of *ELOVL4* was successful in both control and proband from Family 1 (33147) (product size:399 bp). (d) Real-time PCR of proband from Family 1 (Subject S1) targeting *ELOVL4* exon 1, *ELOVL4* exon 2, and reference gene *MMACHC* exon 2 is shown. The graph shows the ratio of the quantitative PCR, patient in orange, control sample in blue, and two relative samples (unaffected parents).

breakpoint junction with long-range PCR using primers described in the methods failed to amplify the region. The size of the regions where the suspected break points fall are large and contain a number of repeat sequences, making the process of mapping the break points challenging.

For Family 2, cES was carried out in the proband (Subject S2) and did not identify any potential SNVs to explain the phenotype. CNV analysis from unphased exome data identified the same novel homozygous *ELOVL4* deletion detected in the proband from Family 1 [Chr6:80656887–80657532 (hg19): NM_022726.3]. Subsequent QPCR analysis for the suspected deletion confirmed homozygosity in the proband from Family 2 as well as his affected sisters (Subjects S3–S4) and heterozygosity in his parents co-segregating with the disease. VLCFA (by GC–MS) in the youngest affected sister was normal: behenic acid (C22:0) 83 μmol/L, lignoceric acid (C24:0) 55.9 μmol/L, cerotic acid (C26:0) 0.5 μmol/L, C24/C22 ratio 0.67, C26/C22 ratio 0.01, phytanic acid 1.46 μmol/L, and pristanic acid <0.14 μmol/L.

In Family 3, cES on the proband (Subject S5) identified a novel homozygous missense variant c.424A>G; p.(Thr142Ala) in *ELOVL4*. This variant is ultrarare and is not present in gnomAD or in CENTOGENE bio/databank. Segregation study showed that the parents were heterozygous and youngest affected sister (Subject S6) was also homozygous for the variant conforming with Mendelian expectations for AR. In silico predictions were categorized as damaging (Polyphen, MutationTaster, SIFT, with a high CADD). C26:0-lysoPC analysis in fibroblasts showed slightly elevated levels of 16 pmol/μgram protein (ref. 2–14). D3-C22 loading in fibroblasts to ascertain both VLCFA β-oxidation capacity and elongation was normal with a D3-C26 level (elongation) of 0.29 (ref. 0.15–0.51) and D3-C16/D3-C22 ratio (β-oxidation) of 1.17 (ref. 0.88–1.93). Additional LysoPC testing in DBSs from the proband of Family 3 (Subject S5) was performed to measure C26:0- and C28:0-lysoPC for the proband, which showed normal C26:0-lysoPC levels but low C28:0-lysoPC levels and a clearly lowered C28/26 ratio when compared to controls (Figure 4).

In Family 4, cES in the proband (Subject S7) identified a novel homozygous missense variant [NM_022726:c.575A>G:p.(His192Arg)] in *ELOVL4*. C26:0- and C28:0-LysoPC as well as the ratio showed a similar pattern as observed for subject S5 (Figure 4).

4 | DISCUSSION

We report four unrelated families with novel homozygous *ELOVL4* variants and ISQMR. Three of these families originated from Saudia Arabia, and two of them from the same Saudi tribe and carried a novel homozygous exonic deletion in the first coding exon of *ELOVL4*, while the probands from the third and fourth family were

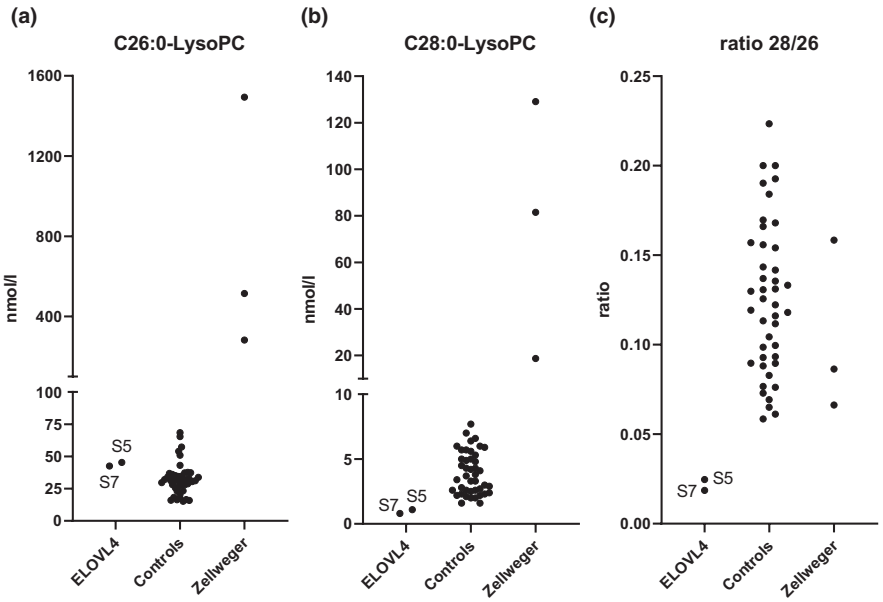

FIGURE 4 Very long-chain fatty acid-containing LysoPC levels (C26:0, C28:0) in subject S5 (Family 3) and subjects S7 (Family 4) with ISQMR compared to control and subjects with Zellweger syndrome (ZS). (a) Measurements of C26:0-LysoPC in dried blood spots in subjects S5 (Family 3 proband) and S7 (Family 4 proband) show that the C26:0-LysoPC level is normal in both. (b) Measurements of C28:0-LysoPC in dried blood spots in subjects S5 (Family 3 proband) and S7 (Family 4 proband) show that the C28:0-LysoPC level is reduced in both. (c) Measurement of C28/26 ratios in dried blood spots in subjects S5 (Family 3 proband) and S7 (Family 4 proband) show that C28/C26 ratio is reduced in both subjects compared to controls and subjects with ZS.

homozygous for a novel missense variant. Only five families with *ELOVL4* SNVs have been reported to date, and no patients with a homozygous CNV involving *ELOVL4* have been published so far (Pubmed, HGMD, Decipher).

Table 1 summarizes the demographic, variant information, and investigations in all subjects with biallelic *ELOVL4* variants reported to date. Table 2 summarizes the clinical features observed in all subjects with biallelic *ELOVL4* variants reported to date. Our subjects have cutaneous, neurological, and ophthalmological features, consistent with the features in previously reported patients.

The cutaneous features mostly resemble those seen in the seven previously reported cases with *ELOVL4* variants (Aldahmesh et al., 2011; Diociaiuti et al., 2021; Mir et al., 2014). Three out of the seven published cases first presented with colloid membrane soon after birth. A colloid membrane was also observed after birth in two out of six subjects in our study (Family 2), while this information was not available in one family (Family 1). All our subjects had skin signs from early infancy, presenting during the neonatal period with thick, dry, and scaly skin suggestive of ichthyosis. All the reported cases with biallelic *ELOVL4* variants to date have had the same cutaneous findings of dry and scaly ichthyotic skin. Erythema of the skin has also been noted in five out of the eight subjects in our study. The proband from Family 1 also has seborrheic dermatitis of the scalp that persisted until the current age of 2 years which had not been described in any of the previously reported cases.

In addition, majority of our subjects had similar findings such as failure to thrive (7/8), profound GDD (7/8), epilepsy (7/8), axial hypotonia (6/7), and peripheral hypertonia (5/6), which were reported in all but two cases with biallelic *ELOVL4* variants. The two cases were from same family (Family 7 in Tables 1 and 2) and had skin manifestations but lacked the additional neurological findings (Mir et al., 2014). This intrafamilial variability is striking but was not seen in the multiplex family (Family 2) in this report. However, Subject S6 from Family 3 has a normal neurological examination until now but will be closely followed up to monitor for any new neurological manifestation. Epilepsy was drug-resistant in minority of our subjects (2/7), and epileptiform activity was observed in 4/5 of our subjects who had an EEG. A study on homozygous knock in mice for the STGD3-causing *ELOVL4* mutations, with skin-specific rescue of wild-type *ELOVL4* expression to avoid neonatal lethality due to dehydration, showed that the mice had seizures postnatally. These mice had evidence of spontaneous epileptiform activity in the hippocampus with aberrant neurotransmission due to dysregulated presynaptic fusion and accelerated neurotransmitter release (Hopiavuori et al., 2018). Although none of the subjects in this study had ataxia, a rat model of *ELOVL4*-related SCA34 showed impaired

TABLE 1 Summary of the demographic data, variant information, and investigations of all subjects with biallelic *ELOVL4* variants to date.

		This report						
	Family #	**Family 1**	**Family 2**			**Family 3**		**Family 4**
	Subject #	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7***
Demographics	Ethnicity	Saudi				Pakistani		Saudi
	Gender	M	M	F	F	F	F	M
	Living status (age)	Alive (2 yr)	Died (6 yr)	Alive (5 yr)	Died (4 yr)	Died (6 yr)	Alive	Alive (8 mo)
	Consanguinity	+	+			−		+
	Affected sibling	−	+	+	+	+	+	+
		CNV				**SNV**		
Variant Information (NM_022726.2)	Variant type	Exonic deletion (ex 1)				Missense		Missense
	Zygosity	hmz				hmz		hmz
	c.DNA	Break points undetermined				c.424A>G		c.575A>G
	Aminoacid	N/A				p.(Thr142Ala)		p.(His192Arg)
EEG findings	Slow background	+	N/A	+	+	+	N/A	+
	Epileptiform activity	+ (multifocal)	N/A	−	++ (SSW)	+ (Hypsarrhythmia)	N/A	+
Brain MRI findings	Delayed myelination	+	+	N/A	N/A	−	N/A	−
	Cerebral atrophy	+	+	N/A	N/A	−	N/A	−
	Thin CC	+	+	N/A	N/A	−	N/A	−

Abbreviations: CC, corpus callosum; CNV, copy number variant; Ex, exon; F, female; Hmz, homozygous; M, male; mo, months; N/A, not available; SNV, single-nucleotide variant; SSW, spike and slow wave activity; yr, years, * previous published in Monies et al. (2017)

synaptic plasticity and dysregulation of the cerebellar network which may possibly underline some of the neurological features observed in subjects with ISQMR. Six out of 12 patients reported to date, including our patients, complained of GERD. Swallowing difficulty was also relatively common in six out of the 11 subjects (55%) necessitating nasogastric, orogastric, or gastric tube feeding. Only one of our eight subjects had inguinal hernia which had been previously noted in another subject with ISQMR (Aldahmesh et al., 2011). Dental abnormalities were commonly observed (5/7 cases to date), in the form of delayed teeth eruption (3/7) or tooth loss secondary to gingivitis (1/7), or teeth erosions (1/7). These findings are unexpected, given the low expression of *ELOVL4* in teeth or gum.

Five of eight subjects in our study had brain imaging showing brain atrophy (3/5), delayed myelination (2/5), and thin corpus callosum (2/5), all of which had been observed on brain imaging in three subjects previously reported (Aldahmesh et al., 2011; Diociaiuti et al., 2021; Mir et al., 2014).

Eye findings were uncommon overall, although one major limitation in our current study is the lack of detailed ophthalmological assessment. Retinal screening and VEP were not performed in seven out of the eight subjects in our study. The only subject (proband from Family 3) with detailed eye examination was found to have cone-rod dystrophy. The proband in Family 1 has myopia and amblyopia as well as a Saudi patient previously reported (Aldahmesh et al., 2011). The proband from Family 4 has optic atrophy similar to subject 15 from Family 10 (Diociaiuti et al., 2021). The two cases from Family 7 in Tables 1 and 2, who only manifested with cutaneous findings, also had abnormal macular changes in their ophthalmological examination like the proband from Family 3 in our study (Mir et al., 2014). Eye findings in the form of macular changes and/or myopia were reported in two parents to date (Aldahmesh et al., 2011; Mir et al., 2014). This is unsurprising given the high expression of *ELOVL4* in the retina, particularly the photoreceptors and association with Stargardt disease (Agbaga et al., 2010). None of the parents in our four families are known to have had visual problems, though they have not had formal eye examination.

ELOVL4 is the only ELOVL member responsible for the elongation of VLCFA and VLC-PUFA beyond 28 carbon (≥C28) atoms (Deák et al., 2019; Tvrdik et al., 2000). VLCFA levels were not available on any of the previously published subjects (Aldahmesh et al., 2011; Diociaiuti et al., 2021; Mir et al., 2014). In this study, VLCFA levels were measured for three subjects using GC–MS (Family 2/S3, Family 3/S5 and Family 4/S7) and were normal apart from slightly elevated C26:0 in two of the three subjects. However, C28:0-lyso PC levels of the probands from two

	Aldahmesh et al. (2011)		Mir et al. (2014)			Diociaiuti et al. (2021)		
	Family 5	Family 6	Family 7			Family 8	Family 9	
S8	S9	S10	S11	S12	S13	S14	S15	Total
	Saudi	Indian	Pakistani			Italian		
M	M	M	M	M	F	M	M	
Alive (8 mo)	Alive (6 yr)	Died (2 yr)	Alive (24 yr)	Died (17 yr)	Alive (22 yr)	Alive (2 yr)	Alive (3 yr)	
	+	+	+			+	−	8/9
+	−	+ (died at 6 mo)	+	+	+	−	−	11/15
	Nonsense hmz c.646C>T p.(Arg216*)	Frameshift hmz c.689delT p.(Ile230Metfs*22)	Nonsense hmz c.78C>G p.(Tyr26*)			Frameshift hmz c.435dup p.(Ile146Tyrsfs*29)	missense hmz c.487T>C p.(Cys163Arg)	
N/A	N/A	N/A	N/A	N/A	N/A	+	+	7/7
N/A	N/A	+	N/A	N/A	N/A	+ (Hypsarrhythmia)	+ (b/l posterior SSW)	7/8
−	+	N/A	N/A	N/A	N/A	+	+	5/8
+	+	N/A	N/A	N/A	N/A	+	+	6/8
−	+	N/A	N/A	N/A	N/A	+	+	5/8

TABLE 2 Summary of the clinical features of all subjects with biallelic *ELOVL4* variants to date.

		This report								Aldahmesh et al. (2011)		Mir et al. (2014)			Diociaiuti et al. (2021)		
	Family #	**Family 1**	**Family 2**			**Family 3**		**Family 4**		**Family 5**	**Family 6**	**Family 7**			**Family 8**	**Family 9**	
	Subject #	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**S10**	**S11**	**S12**	**S13**	**S14**	**S15**	**Total**
Skin findings	Colloid membrane	N/A	N/A	+	+	–	–	–	-	+	–	–	–	–	+	+	5/13
	Erythematous skin	+	+	+	+	+	–	–	–	–	+	+	+	+	+	+	11/15
	Ichthyosis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15/15
Eye findings	Macular changes	N/A	N/A	N/A	N/A	+ (CRD)	N/A	N/A	N/A	–	–	+	N/A	+	–	–	3/7
	Myopia and amblyopia	+	N/A	N/A	N/A	N/A	N/A	N/A	N/A	+	–	–	N/A	–	–	–	2/7
	Other eye findings	N/A	N/A	N/A	N/A	N/A	N/A	+ (optic atrophy)	N/A	–	+	–	N/A	–	–	+ (optic atrophy)	3/7
Neuro findings	Profound GDD	+	+	+	+	+	–	+	+	+	+	–	+	–	+	+	12/15
	Epilepsy	+ (R)	+ (C)	+(C)	+(C)	+	–	+ (C)	+ (C)	+	+	–	+	–	+	+	12/15
	Axial hypotonia	+	+	+	+	+	–	+	N/A	N/A	N/A	–	N/A	–	+	+	8/11
	Peripheral hypertonia	+	+	+	+	N/A	–	+	N/A	+	+	–	+	–	+	+	10/13
Other clinical findings	Dysmorphism	+	–	–	-	+	–	–	N/A	–	–	+	+	+	+	–	6/14
	FTT	+	+	+	+	+	–	+	+	+	+	–	+	–	+	+	12/15
	Asthma	+	–	N/A	N/A	–	N/A	+	N/A	+	–	–	–	–	+	+	5/11
	GERD	+	+	N/A	N/A	N/A	–	+	+	–	–	–	–	–	+	+	6/12
	Dysphagia	+ (NGT)	+ (GT)	N/A	N/A	+ (GT)	–	+(NGT)	N/A	–	–	–	N/A	–	+ (FJ)	+ (GT)	6/11
	Dental anomalies	+ (No teeth)	N/A	N/A	N/A	+	N/A	N/A	N/A	+ (teeth loss due to gingivitis)	+ (No teeth)	–	+	–	N/A	N/A	5/7

Abbreviations: C, controlled with antiepileptic drugs; CRD, cone-rode dystrophy; FJ, jejunostomy; FTT, failure to thrive; GDD, global developmental delay; GERD, gastroesophageal reflex disease; GT, gastric tube; N/A, not available; NGT, nasogastric tube; R, resistant to antiepileptic drugs.

families (Family 3/S5 and Family 4/S7) were at the lower end of the spectrum with a reduced C28/26 ratio in the patients' DBSs when compared to controls, which parallels the global VLCFA reduction (≥C28) including ceramide/glucosylceramide and free fatty acids in homozygous *Elovl4*$^{del/del}$ mice (Vasireddy et al., 2007). From this limited amount of measurements, the calculation of the C28:0-LysoPC over C26:0-lysoPC ratio in DBS appears to be a potential biomarker for this disorder but to confirm that this biomarker is indeed usable in clinical practice, more method development/validation should be performed and more *ELOVL4*-deficient subjects should be evaluated.

Molecular analyses in two unrelated families from same tribe showed a novel homozygous exonic deletion CNV, unlike the previously reported cases with ISQMR who had deleterious SNVs, yet leading to similar presentation (Aldahmesh et al., 2011; Diociaiuti et al., 2021; Mir et al., 2014). Evidence suggests that up to 8% of AR disorders are caused by homozygous CNVs (Yuan et al., 2020). The majority of homozygous CNVs resulting in AR conditions affect a single gene, and closer to half affect one exon as seen here (Yuan et al., 2020). Yet, small homozygous CNVs (<1000 kb) are often overlooked due to the limitation of CMA and aCGH in detecting small genetic and intragenic CNVs (<400 Kb). New bioinformatic algorithms have been proven sensitive in detecting small homozygous intragenic deletion (<50 Kb) from exome data (Duan et al., 2021; Gambin et al., 2017). Concurrent CMA and ES can optimize the diagnostic rate and allow more precise detection of small CNVs (Dharmadhikari et al., 2019). Retrospective review of CMA data in proband of Family 1 showed that the *ELOVL4* variant falls within a large run of homozygosity (ROH) and that there are six missing probes in aCGH data compared to control (Figure 3). The homozygous deletion was later confirmed by QPCR. It thus may be worth investigating such smaller CNVs detected on aCGH when it falls within an ROH interval, especially in highly consanguineous population in which the burden of AR disorders is high.

Interestingly, proband of Family 1 had cousins with a severe neurological disorder, yet; exome data proved the presence of two different molecular and neurological conditions within the same extended family (Figure 1a). Multi-locus pathogenic variation is a well-established cause for a blended phenotype in an affected individual and for intrafamilial variability such as in this extended pedigree (Karaca et al., 2018; Posey et al., 2017). This finding of two rare Mendelian AR disorders within the same extended family has implications not only on clinical diagnosis but also on counseling and family planning. Around 10% of consanguineous couples with an affected child have a second shared pathogenic or likely pathogenic variant leading to a second AR disease of moderate to profound severity (Mor-Shaked et al., 2021). Both parents in Family 1 were confirmed heterozygous carriers of *ELOVL4* variant, and testing for the *TBCK* variant was also strongly recommended in this couple undergoing preimplantation genetic diagnosis (PGD) so that both pathogenic variants are considered in planning to avoid the risk of having a child with a second, unrelated disorder.

The finding of two seemingly unrelated probands with the same *ELOVL4* variant in Families 1 and 2 from same tribe suggest that the *ELOVL4* variant is potentially a founder allele within their large tribe. Testing similarly affected individuals from the Middle East region for this exonic deletion is advisable.

In conclusion, we report four additional families harboring novel homozygous *ELOVL4* variants and ISQMR; two Saudi families from the same tribe harboring a novel homozygous exonic deletion, and two other families with novel missense variants, one from Pakistan and the other from Saudi Arabia. Our report improves the understanding of the phenotypic features of *ELOVL4*-related ISQMR, expands the genotypic spectrum of the disorder, and adds to the global effort of understanding this rare disease. The increasing clinical application of next-generation sequencing will likely lead to the identification of additional cases and allow for further delineation of the clinical and biochemical phenotype of *ELOVL4*-related ISQMR.

AUTHOR CONTRIBUTIONS

Fatima Al-abdulrazzaq and Dana Marafi contributed to drafting the manuscript and revising it critically for important intellectual content. Fatima Al-abdulrazzaq, Talal Alanzi, Dana Marafi, Ashraf H. Aboelanine, Amira Abdullah, Alice Gardham, Emma Wakeling, Harry G Leitch, Moeenaldeen AlSayed, Maha Abdulrahim, Amal Alwadani, and Laila Bastaki contributed to data acquisition, including clinical examination of patients, clinical images, sample collection, and genetic counseling. Haya H Al-Balool, Abdulaziz Aladwani, Antonio Romito, and Kapil Kampe performed the QPCR and breakpoint experiments and analyzed patient data and drafted the genetic reports. Frédéric M Vaz and Sasha Ferdinandusse performed and interpreted the biochemical analysis of VCLFA-containing LysoPCs. Aida Bertoli-Avella and Dana Marafi contributed to the analysis and interpretation of the data and critical review of the manuscript. All authors approved the final manuscript and agreed to be accountable for all aspects of the work.

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CONFLICT OF INTEREST STATEMENT

AR, KK, and AMBA are employees of CENTOGENE GmbH. Other authors have no potential conflicts to report.

DATA AVAILABILITY STATEMENT

All data included in this manuscript are available and can be de-identified and shared upon request to corresponding author.

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