



# Exome sequencing in four families with neurodevelopmental disorders: genotype–phenotype correlation and identification of novel disease-causing variants in *VPS13B* and *RELN*

Tehseen Ullah Khan Afridi<sup>1</sup> · Ambrin Fatima<sup>2</sup> · Humayoon Shafique Satti<sup>1</sup> · Zaineb Akram<sup>1</sup> · Imran Khan Yousafzai<sup>1</sup> · Wajahat Bin Naeem<sup>1</sup> · Nasreen Fatima<sup>1</sup> · Asmat Ali<sup>2</sup> · Zafar Iqbal<sup>3</sup> · Ayaz Khan<sup>4</sup> · Muhammad Shahzad<sup>5</sup> · Chunyu Liu<sup>6</sup> · Mathias Toft<sup>3,7</sup> · Feng Zhang<sup>8</sup> · Muhammad Tariq<sup>4</sup> · Erica E. Davis<sup>9,10</sup> · Tahir N. Khan<sup>1,9,10</sup>

Received: 19 November 2023 / Accepted: 30 April 2024

© The Author(s), under exclusive licence to Springer-Verlag GmbH Germany, part of Springer Nature 2024

## Abstract

Neurodevelopmental disorders (NDDs) are a clinically and genetically heterogeneous group of early-onset pediatric disorders that affect the structure and/or function of the central or peripheral nervous system. Achieving a precise molecular diagnosis for NDDs may be challenging due to the diverse genetic underpinnings and clinical variability. In the current study, we investigated the underlying genetic cause(s) of NDDs in four unrelated Pakistani families. Using exome sequencing (ES) as a diagnostic approach, we identified disease-causing variants in established NDD-associated genes in all families, including one hitherto unreported variant in *RELN* and three recurrent variants in *VPS13B*, *DEGSI*, and *SPG11*. Overall, our study highlights the potential of ES as a tool for clinical diagnosis.

**Keywords** Exome sequencing · Neurodevelopmental disorders · Mutation screening

## Introduction

NDDs are a clinically and genetically heterogeneous group of prenatal or neonatal onset pediatric conditions that impair the brain's structure and/or function, typically resulting from

disruption of tightly regulated developmental processes (Khan et al. 2019; Mitani et al. 2021). NDDs arise from the disruption of crucial neurodevelopmental mechanisms or pathways and are associated frequently with abnormalities in neurogenesis, glial and neuronal proliferation, neuronal migration, synapse formation, and myelination (Homberg

Communicated by Shaohua Fan.

✉ Erica E. Davis  
EriDavis@luriechildrens.org

✉ Tahir N. Khan  
tkhan@luriechildrens.org

<sup>1</sup> Department of Biological Sciences, National University of Medical Sciences, Rawalpindi 46000, Pakistan

<sup>2</sup> Department of Biological and Biomedical Sciences, The Aga Khan University, Karachi 74800, Pakistan

<sup>3</sup> Department of Neurology, Oslo University Hospital, Oslo, Norway

<sup>4</sup> National Institute for Biotechnology and Genetic Engineering College, Pakistan Institute of Engineering and Applied Sciences (NIBGE-C, PIEAS), Faisalabad, Pakistan

<sup>5</sup> Department of Neurosurgery, District Headquarter Hospital, Kohat, Pakistan

<sup>6</sup> International Peace Maternity and Child Health Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai 200030, China

<sup>7</sup> Institute of Clinical Medicine, University of Oslo, Oslo, Norway

<sup>8</sup> Institute of Medical Genetics and Genomics, Fudan University, Shanghai 200438, China

<sup>9</sup> Advanced Center for Translational and Genetic Medicine, Stanley Manne Children's Research Institute, Ann & Robert H. Lurie Children's Hospital of Chicago, Chicago, IL, USA

<sup>10</sup> Department of Pediatrics and Department of Cell and Developmental Biology, Feinberg School of Medicine, Northwestern University, Chicago, IL, USA

et al. 2016). Subsequent impaired cognitive function may affect behavior, motor skills, learning, reasoning, memory, problem-solving, decision-making, communication, and attention, culminating in cognitive, communicative, behavioral, and psychomotor impairments (Bogaert et al. 2023; Cardoso et al. 2019; Morris-Rosendahl and Crocq 2020). Intellectual disability (ID) in mild to severe forms are the most predominant types of NDD and affects 1–3% of the world's population, while its prevalence is twofold in the developing world (Riazuddin et al. 2017). Common manifestations of NDDs include developmental delay (DD), specific learning and motor disorders, communication disorders, autism spectrum disorders, attention deficit hyperactivity disorder, movement disorders, and epilepsy (Morris-Rosendahl and Crocq 2020; Parenti et al. 2020). Up to 50% of NDD cases are estimated to have a genetic etiology (Servetti et al. 2021). In the last decade, the widespread integration of next-generation sequencing approaches, most notably ES, into the diagnostic process has increased the number of affected individuals who receive a molecular diagnosis (Farwell et al. 2015; Lee et al. 2014; Wright et al. 2023; Yang et al. 2013, 2014). With an average diagnostic yield of 30–50%, ES has emerged as a key diagnostic tool in clinical and research environments and is considered a first-tier (Clark et al. 2018; Srivastava et al. 2019) diagnostic approach. This approach is particularly helpful when investigating patient-parent trios or related affected or unaffected individuals, followed by confirmation and co-segregation analysis of candidate variants within the family through Sanger sequencing, further enhancing the diagnostic process. ES offers a relatively fast, inexpensive, and precise molecular diagnosis in affected individuals with overlapping, unexplained, heterogeneous disorders impacting the central and peripheral nervous system.

This study aimed to identify the genetic etiology of NDDs in four unrelated Pakistani families using ES as a first-tier diagnostic approach. We identified disease-causing variants in established NDD-associated genes in all four families, including one previously unreported variant in *RELN* and three recurrent variants in *VPS13B*, *DEGS1*, and *SPG11*.

## Materials and methods

### Research participants and sample collection

This study was approved by the Institutional Review Board and Ethical Committee at the National University of Medical Sciences, Rawalpindi, Pakistan, and the Department of Biological and Biomedical Sciences, The Aga Khan University, Karachi, Pakistan to ensure adherence to ethical guidelines. Four Pakistani families, including three consanguineous and one non-consanguineous, were

ascertained, with all families having at least one member exhibiting NDD phenotypes likely of genetic origin. We obtained written informed consent from each proband or their legal guardians in accordance with the Declaration of Helsinki. Detailed clinical histories were taken for each affected individual and peripheral blood samples were collected from all consented affected and unaffected family members. Genomic DNA extraction from blood samples was performed using the GeneJET Genomic DNA Purification Kit (Thermo Scientific).

### Exome sequencing, variant filtration, and in silico analyses

We performed paired-end ES using genomic DNA of at least one affected individual from each family. Genomic DNA was fragmented and target enriched for exome capture using the Twist Human Core Exome library preparation kit (Twist Biosciences) following the manufacturer's protocol. The captured libraries were then sequenced on the Illumina Novaseq 6000 platform, generating 150 bp paired-end reads. This achieved a mean depth of 68–84× across individuals, with 87–90% coding region coverage at a minimum of 20 reads. The sequencing yielded FASTQ files that underwent downstream bioinformatics analysis for variant calling and filtration. No copy number variants analysis was performed in these families.

Quality control (QC) of raw paired-end ES reads in FASTQ format was first evaluated with FastQC. Based on the QC results, the low-quality reads and adapter sequences were trimmed (or filtered out) using the fastp tool (Chen et al. 2018). Trimmed and filtered reads underwent a second round of QC with FastQC to evaluate the effectiveness of fastp preprocessing. The trimmed and filtered reads were then aligned to the hg19 human reference genome with BWA-MEM (Li 2013) resulting in a sequence alignment/map (SAM) file. The aligned sequences were processed with Picard tools to generate a sorted and deduplicated binary alignment map (BAM) file. Germline single nucleotide variants (SNVs) and indels were called and processed with the Genome Analysis Toolkit (GATK4) (DePristo et al. 2011; McKenna et al. 2010) following the GATK Best Practices workflow (Van der Auwera et al. 2013) resulting in a variant call format (VCF). Variants were annotated by wANNOVAR (Yang and Wang 2015) to add functional and interpretive information from databases including Gene Ontology, Genome Aggregation Database (gnomAD), Exome Aggregation Consortium (ExAC), 1000 Genomes, NHLBI-ESP, dbSNP, SIFT, PolyPhen-2, MutationTaster, and CADD as well as clinical associations from ClinVar and GWAS catalog.

Reads with a minimum of 10× coverage were retained for further analysis. Variants were filtered to remove

reported SNVs with minor allele frequencies (MAFs) > 0.01 in public databases including dbSNP, 1000 Genomes, gnomAD, ExAC, and TOPMed BRAVO. Exonic or splice variants with MAFs < 0.01 consistent with the inheritance patterns of disease and previous association with NDDs were prioritized as candidates. These candidate variants underwent bidirectional Sanger sequencing validation and segregation analysis in DNA from all available consented family members using BigDye Terminator v3.1 on an ABI 3730 platform (List of primers is available in Supplementary Table 1). Bioinformatics pipeline results were validated using VarSome Clinical (v11.6). Variants were classified and interpreted according to the guidelines of the American College of Medical Genetics and Genomics (ACMG) (Richards et al. 2015).

## Results

### Family 1

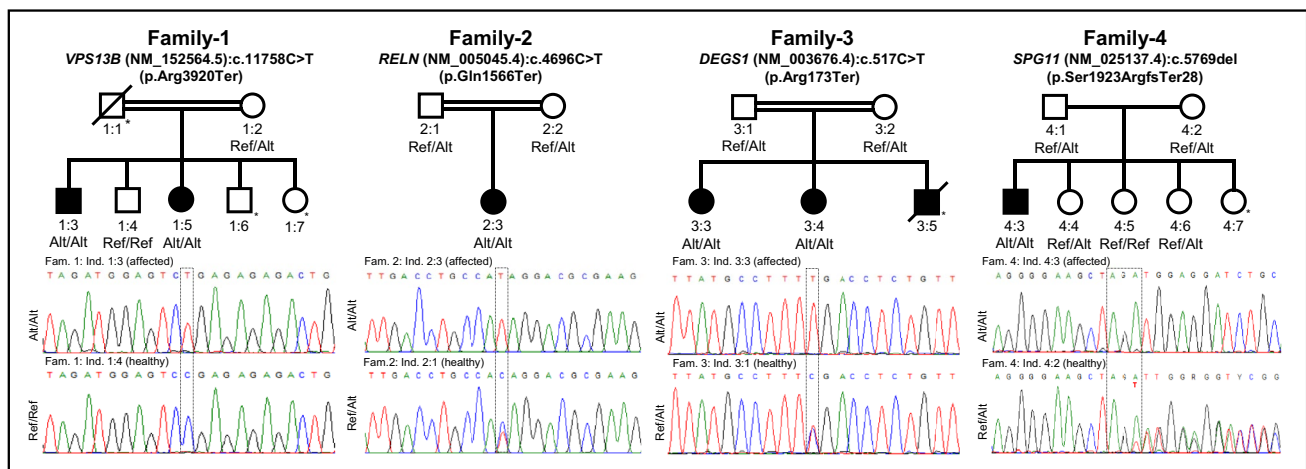
#### Clinical findings

A consanguineous Pakistani family with two affected siblings 1:3 and 1:5 (Fig. 1) exhibited a severe neurodevelopmental condition characterized by ID, DD, speech and language delay, and microcephaly. Both affected individuals were delivered full-term by spontaneous vaginal delivery with no reported pregnancy complications. Individual 1:3 (male, age 17 years at last clinical assessment) had seizure onset at 6 months after a fever, lasting 1 year and requiring

hospitalization. Hands and feet contractures were noticed at around 6 months of age. He has microcephaly (−5.44 SD), severe ID, DD, speech delay, musculoskeletal anomalies, muscle spasticity, hypotonia, hand tremors, short stature, bed-confined, inability to self-feed, crawl, and stand with support by age 3 years, and is only able to speak a few words by the age of 16 years. No myopia or retinopathy was observed in this patient. The individual exhibited sociable behavior, typical facial dysmorphism, and joint hyperextensibility. Sensation was normal in his right toe but absent in his left toe. He tried to respond to words but was unable to speak. Additionally, he had a disturbed sleeping pattern. Individual 1:5 (female, age 20 years at last clinical assessment) has microcephaly (−4.10 SD), severe ID, DD, and speech delay. By the age of 5 years, she was only able to speak 1–3 words. She exhibited sociable and cheerful behavior, although occasional episodes of aggression were observed. She had low vision, night blindness, joint hypermobility, and typical facial characteristics. She had a normal sensation in her toes. She tried to respond to some words but was unable to speak. She demonstrated independent self-feeding and eating skills. Detailed clinical features are described in Supplementary Table 2.

#### Genetic findings

ES in both affected siblings (1:3 and 1:5) and subsequent bioinformatic filtering identified ten variants (Supplementary Table 3) including a homozygous variant (NM\_152564.5):c.11758C>T (p.Arg3920Ter) in *VPS13B* shared by both affected individuals (Fig. 1; Table 1).



**Fig. 1** Pedigrees and genetic findings in families with NDDs. Top bold text shows the name and identified variant in each family. The double horizontal lines in pedigrees indicate consanguinity; squares and circles symbolize males and females, respectively. Filled symbols indicate affected individuals; unfilled symbols indicate healthy individuals; diagonal line, deceased. The genotypes for identified variants

are indicated below each symbol where DNA was available. Electropherograms of affected (upper panel) and healthy (lower panel) individuals below each pedigree highlight the precise location (dotted rectangle) of variants in the DNA sequence. Asterisks in the pedigree indicate family members whose DNA was unavailable. Ref, reference allele; Alt, alternative allele

**Table 1** List of potential disease-causing variants identified by ES in this study

Family	Gene	Variant (HGVS)	Putative function	CADD Score	SIFT	PolyPhen-2		Mutation taster	gnomAD v4.0.0		MAF SAS	ACMG classification <sup>a</sup>		Reference
						HDIV	HVAR		Allele counts (Het/Wt/Hom)	MAF global		ACMG criteria	ACMG class	
1	<i>VPS13B</i> (NM_152564.5)	c.11758C>T (p.Arg3920Ter)	nonsense	47	NA	NA	NA	D	8/1613930/0	0.000004957	-	PVS1, PP5, PM2	Pathogenic	This study; VCV000550808.37
2	<i>RELN</i> (NM_005045.4)	c.4696C>T (p.Gln1566Ter)	nonsense	40	NA	NA	NA	D	-	-	-	PVS1, PM2	Likely Pathogenic	This study
3	<i>DEGS1</i> (NM_003676.4)	c.517C>T (p.Arg173Ter)	nonsense	37	NA	NA	NA	A	12/1614060/0	0.000007435	0.00001098	PVS1, PP5, PM2	Pathogenic	Pant et al. (2019)
4	<i>SPG11</i> (NM_025137.4)	c.5769del (p.Ser1923ArgfsTer28)	frameshift	-	NA	NA	NA	NA	41/1614052/0	0.00002540	0.0004501	PVS1, PP5, PM2	Pathogenic	Stevanin et al. (2008)

A minus sign (-) sign denotes that no variant (or frequency) is found in gnomAD; Het, heterozygous; Wt, wild type; Hom, presence of a variant in the homozygous state; MAF, minor allele frequency SAS, South Asian; D, disease-causing; A, disease-causing automatic; NA, not available or applicable. PVS1, null variant in a gene where loss-of-function is a known mechanism of disease; PP5, Reputable source recently reports the variant as pathogenic; PM2, Absent from controls (or at extremely low frequency if recessive) in Exome Sequencing Project, 1000 Genomes Project, ExAC, or gnomAD

<sup>a</sup>Candidate variants were classified and interpreted utilizing VarSome (Kopanos et al. 2019) and InterVar (Li and Wang 2017) following the ACMG guidelines (Richards et al. 2015)

The identified variant is rare in public databases (MAF of 0.000004957 in gnomAD v4.0.0). We prioritized the *VPS13B* variant due to previous association of the gene with autosomal recessive Cohen syndrome (Kolehmainen et al. 2003) overlapping with the clinical presentation of affected individuals in Family 1. Bi-directional Sanger sequencing validated the segregation of the *VPS13B* variant with disease status across the family. This variant has not been reported in the literature although ClinVar submissions (VCV000550808.37) classifies this variant as pathogenic/likely pathogenic with no information available on phenotype.

## Family 2

### Clinical findings

A consanguineous Pakistani family with one affected female individual (2:3) aged two years (at last clinical assessment) was ascertained from the Sindh province (Pakistan). She was born at full term through a C-section. She has shown delayed developmental milestones, including delays in sitting, babbling, walking, and learning difficulties. She has strabismus in both eyes, stiffness in both the upper and lower limbs, and muscle atrophy in both limbs. She has been assessed clinically for her aggressive behavior. Over time, the disease symptoms have exhibited a progressive and deteriorating trend. She experiences recurring seizures, typically once a month. Presently, she faces challenges in thriving, is confined to a bedridden state, and necessitates assistance for fundamental self-care activities and feeding.

### Genetic findings

In Family 2 proband-only ES identified multiple variants (Supplementary Table 3) including a previously unreported homozygous nonsense variant in *RELN* (NM\_005045.4):c.4696C>T (p.Gln1566Ter) that segregated with disease in the trio (Fig. 1; Table 1). *RELN* is associated with autosomal recessive Lissencephaly 2 (Norman-Roberts type; OMIM 257320) (Hong et al. 2000), and lateral temporal lobe epilepsy-7 (OMIM 616436) (Dazzo et al. 2015). Lissencephaly, sometimes known as “smooth brain” is a severe neurodevelopmental condition in which neuronal migration is impeded, resulting in a thicker cerebral cortex with a smoothed-out, simplified fold pattern. Lissencephaly is characterized by significant hippocampal and cerebellar abnormalities (Hong et al. 2000). Due to previous association of *RELN* variants with the disease and its concordance with the proband’s clinical presentation, we prioritized this variant. The identified variant is hitherto unreported and is absent in gnomAD v4.0.0 and in our

in-house healthy control database, and it is classified as Likely pathogenic (Table 1).

### Family 3

#### Clinical findings

A consanguineous Pakistani family with second-cousin parents has three affected members (Fig. 1) exhibiting a severe NDD marked by global developmental delay (GDD), speech delay, hypotonia, and microcephaly. Proband 3:3 was a 7-year-old (at last clinical assessment) female who presented GDD, ID, severe microcephaly ( $-6.05$  SD), spasticity, hypotonia, lack of speech, motor delay, inability to sit/walk/self-feed, and limb contractures. Her mother reported she did not cry at birth and exhibited transient neonatal hyperkinesia that later resolved. She was born full-term through a cesarean section. Her brain magnetic resonance imaging (MRI) noted moderate to severe brain atrophy. Proband 3:4 was a 14-year-old (at last clinical assessment) female who exhibited GDD, ID, microcephaly ( $-6.17$  SD), spasticity, hypotonia, lack of expressive speech, motor delay, limb contractures, and scoliosis. She was born full-term through spontaneous vaginal delivery. Proband 3:5 was a deceased 9-year-old (at last clinical assessment) male who presented with GDD, ID, hypotonia, lack of speech, inability to sit/walk/self-feed, childhood milestone regression, and limb contractures. He was also born full-term via spontaneous vaginal delivery.

#### Genetic findings

Trio-ES of an affected individual (3:3) and both parents (3:1 and 3:2) identified a homozygous recurrent variant (NM\_003676.4):c.517C>T (p.Arg173Ter) in *DEGS1* (Fig. 1; Table 1). The variant causes a premature translational stop signal (p.Arg173Ter) and is predicted to result in nonsense-mediated decay (NMD). *DEGS1* is associated with autosomal recessive leukodystrophy, hypomyelinating, 18 (HLD18, OMIM 618404). HLD18 is hallmarked by an onset of GDD in early infancy. In the most severe cases, affected individuals have impaired psychomotor development, which includes the inability to sit or walk independently, aphasia or poor speech, dystonia, spasticity, and seizures may occur in some cases (Pant et al. 2019).

Segregation analysis showed that the *DEGS1* variant segregates in a recessive inheritance pattern in the family, homozygous in affected individuals, and heterozygous in unaffected parents. A complete list of additional variants identified after bioinformatic filtering is provided in Supplementary Table 3.

### Family 4

#### Clinical findings

A 19-year-old male proband (4:3) from a non-consanguineous pedigree (Fig. 1) presented with a progressive learning disability, task-specific tremors, and gait abnormality beginning in late childhood. He achieved normal early developmental milestones. At the age of 12 years, intention tremors started in his right arm before progressing to involve the left arm bilaterally by the age of 15 years. He also developed progressive gait abnormalities and leg spasticity without lower limb tremors. Cognitive decline became evident impairing his academic performance. Clinical exam showed bilateral arm tremors on intent, leg spasticity, and normal muscle strength. Spiral drawing testing supported essential tremor. He has four unaffected siblings and has no family history of a similar neurological condition. Brain MRI at age of 16 years and nerve conduction studies and electromyography at age 18 years were normal. Extensive biochemical testing, including thyroid function, copper studies, ceruloplasmin, and alpha-fetoprotein were unremarkable. Despite a 7-year long clinical testing history, no definitive diagnosis was achieved by the age of 19 years.

#### Genetic findings

Parent-proband Trio-ES identified a recurrent homozygous frameshift deletion in *SPG11* (NM\_025137.4):c.5769del (p.Ser1923ArgfsTer28) (Fig. 1; Table 1) and a missense variant in *PRKAG3* (Supplementary Table 3). Loss of *SPG11* function is an established disease mechanism in autosomal recessive *SPG11*-related neurological conditions (Stevanin et al. 2007) overlapping with the phenotypes of proband. Hereditary spastic paraplegias (HSPs) are a heterogeneous group of neurodegenerative disorders characterized by progressive weakness and spasticity of the lower limbs associated with corticospinal axon degeneration.

The *SPG11* variant has been reported previously as pathogenic in individuals with HSP (Kara et al. 2016; Paisan-Ruiz et al. 2008; Riazuddin et al. 2017; Schneider et al. 2012; Stevanin et al. 2008; Wakil et al. 2012; Zulfiqar et al. 2019). The variant segregates with disease in the pedigree; it is homozygous in the affected individual, heterozygous in the unaffected parents, and heterozygous or wild type in siblings (Fig. 1).

### Discussion

Identification of a disease-causing gene(s) is a key step towards understanding the pathophysiological mechanisms of NDDs which enables precise molecular diagnosis, genetic

counseling about recurrence risk, prenatal testing, disease management, and potential development of personalized treatments (Blesson and Cohen 2020; Parenti et al. 2020; Wright et al. 2018). Furthermore, definitive molecular diagnosis may prevent unnecessary testing, provide prognostic information, facilitate access to support services, end the diagnostic odyssey, and improve quality of life by providing psychological benefits (Carter et al. 2023). Despite advancements in diagnostic genetic testing, such as ES, achieving a precise molecular diagnosis for NDDs can be challenging due to diverse genetic underpinnings and clinical variability, where more than half of individuals may not get a definitive molecular diagnosis (Wojcik et al. 2023), face misdiagnosis, or receive multiple diagnoses, leading to a diagnostic odyssey.

ES has become a common clinical diagnostic tool for NDDs, with a reported diagnostic yield of ~30–50% (Clark et al. 2018; Srivastava et al. 2019), given its ability to provide a rapid, cost-effective, and highly accurate molecular diagnosis for patients with overlapping, unexplained, heterogeneous NDDs, we utilized ES in four unrelated NDD families for genetic diagnosis.

In Family 1, we identified a rare homozygous variant *VPS13B* (NM\_152564.5):c.11758C>T (p.Arg3920Ter) as a likely disease cause. Pathogenic variants in *VPS13B* that segregate in an autosomal recessive inheritance pattern are associated with Cohen syndrome (El Chehadeh et al. 2010; Kolehmainen et al. 2003). There was marked intra-familial phenotypic variability among both affected individuals. Overlapping clinical characteristics among both affected individuals, such as ID, DD, speech delay, and microcephaly are consistent with Cohen syndrome.

*VPS13B* is a peripheral Golgi membrane protein that plays a crucial role in important cellular processes such as intracellular protein transport, sorting, vesicle trafficking, and maintaining Golgi integrity. It possesses functional motifs and putative transmembrane domains (Duplomb et al. 2014; Kolehmainen et al. 2003; Seifert et al. 2011). The C terminus of *VPS13B* localizes protein to the Golgi apparatus (Seifert et al. 2011). The identified variant p.Arg3920Ter introduces a premature stop codon in the C-terminus, resulting in a truncated protein lacking the final 78 amino acids that encompass the Golgi localization domain. Previous reports have identified disease-causing variants p.Ser3945GlnfsTer22, p.Asn3954Lysfs\*60, p.Pro3944LeufsTer41 and (NM\_152564.5):c.6657+1G>A, farther towards the C-terminus (El Chehadeh et al. 2010; Kolehmainen et al. 2004). Disruption of C-terminus may lead to defects in proper subcellular targeting of *VPS13B* to the Golgi apparatus (Seifert et al. 2011). This results in pathogenic defects in Golgi structure and function. Therefore, truncation of the C-terminal domain provides a

molecular mechanism linking the p.Arg3920Ter variant to pathogenesis associated with Golgi dysfunction.

In family 2, we identified a hitherto unreported homozygous nonsense variant p.Gln1566Ter in *RELN*, leading to a premature stop codon that likely results in loss of Reelin protein. Reelin is an essential protein for brain growth and physiological activities. Functional analysis in a *reeler* loss of function mouse model reported that *RELN* leads to neurological symptoms with main features including ataxia, tremors, and a distinctive ‘reeling’ gait (Di Donato et al. 2022). Furthermore, multiple genome-wide association studies have also established a genetic link between *RELN* and various psychiatric disorders, including schizophrenia and autism spectrum disorders (Ishii et al. 2016). Biallelic variants in *RELN* are associated with a form of lissencephaly with cerebellar hypoplasia (Di Donato et al. 2022).

In Family 3, we identified a recurrent pathogenic variant (p.Arg173Ter) in *DEGSI* associated previously with autosomal recessive HLD18 (Dolgin et al. 2019; Pant et al. 2019). *DEGSI* encodes a  $\Delta 4$ -dihydroceramide desaturase, an endoplasmic reticulum lipid desaturase that catalyzes the final step in the de novo ceramide (Cer) biosynthesis pathway. Pathogenic variants in *DEGSI* lead to dihydroceramide (DhCer) accumulation and DhCer/Cer imbalance which may be toxic for myelinating oligodendrocytes and disruptive for myelin biogenesis and neuronal health (Pant et al. 2019). Loss-of-*DEGSI* function is a known mechanism of disease (Pant et al. 2019). The phenotypes of our patients are consistent with those reported previously, adding further evidence to the pathogenicity of the *DEGSI* variants.

Family 4 exemplifies a diagnostic odyssey resolved by genetic diagnosis. In this family a recurrent homozygous *SPG11* (NM\_025137.4):c.5769del (p.Ser1923ArgfsTer28) variant segregated with disease. *SPG11* is associated with autosomal recessive Spastic paraplegia 11 (OMIM 604360), caused by loss of function mechanism. *SPG11* encodes spatacsin, a protein with a role in the maintenance and/or growth of neuronal axons and intracellular cargo trafficking (Pérez-Brangulí et al. 2014). The identified homozygous variant was reported previously as disease-causing in individuals with HSP (Kara et al. 2016; Paisan-Ruiz et al. 2008; Riazuddin et al. 2017; Schneider et al. 2012; Stevanin et al. 2008; Wakil et al. 2012; Zulfiqar et al. 2019) and clinical presentation of our patient was concordant.

In conclusion, we report four pedigrees with likely disease-causing variants in established NDD-associated genes, facilitated by large family structures ascertained in this study. This study demonstrates the utility of ES as a diagnostic tool for heterogeneous neurological disorders. Our study expands the clinical and mutational spectrum of NDDs.

## Web resources

Genome Aggregation Database (gnomAD), <https://gnomad.broadinstitute.org/>  
 TOPMed Bravo <https://bravo.sph.umich.edu/>  
 1000 Genomes Project, <https://www.internationalgenome.org/>  
 The Human Gene Mutation Database (HGMD), <http://www.hgmd.cf.ac.uk>  
 VarSome <https://varsome.com/>  
 InterVar <https://wintervar.wglab.org/>  
 ClinVar <https://www.ncbi.nlm.nih.gov/clinvar/>  
 OMIM (Online Mendelian Inheritance in Man) <https://www.omim.org/>  
 wANNOVAR <https://wannovar.wglab.org/>

**Supplementary Information** The online version contains supplementary material available at <https://doi.org/10.1007/s00438-024-02149-y>.

**Acknowledgements** The authors are grateful to the individuals and their families for taking part in this study. EED is the Ann Marie and Francis Klocke, MD Research Scholar.

**Funding** This work was supported by grants from the Higher Education Commission of Pakistan NRP 20-12107 to TNK and NRP 20-16914 to AF. Z.I. was supported by the South-Eastern Norway Regional Health Authority.

**Data availability** The datasets generated during the current study are available from the corresponding author upon reasonable request.

## Declarations

**Conflict of interest** The authors have no conflicts of interest to disclose related to the present study.

## References

- Blesson A, Cohen JS (2020) Genetic counseling in neurodevelopmental disorders. *Cold Spring Harb Perspect Med* 10(4):a036533. <https://doi.org/10.1101/cshperspect.a036533>
- Bogaert E, Garde A, Gautier T, Rooney K, Duffourd Y, LeBlanc P, van Reempts E, Tran Mau-Them F, Wentzensen IM, Au KS, Richardson K, Northrup H, Gatinois V, Geneviève D, Louie RJ, Lyons MJ, Laulund LW, Brasch-Andersen C, Maxel Juul T et al (2023) SRSF1 haploinsufficiency is responsible for a syndromic developmental disorder associated with intellectual disability. *Am J Hum Genet* 110(5):790–808. <https://doi.org/10.1016/j.ajhg.2023.03.016>
- Cardoso AR, Lopes-Marques M, Silva RM, Serrano C, Amorim A, Prata MJ, Azevedo L (2019) Essential genetic findings in neurodevelopmental disorders. *Hum Genomics* 13(1):31. <https://doi.org/10.1186/s40246-019-0216-4>
- Carter MT, Srour M, Au PB, Buhás D, Dyack S, Eaton A, Inbar-Feigenberg M, Howley H, Kawamura A, Lewis SME, McCready E, Nelson TN, Vallance H (2023) Genetic and metabolic investigations for neurodevelopmental disorders: position statement of the

- Canadian College of Medical Geneticists (CCMG). *J Med Genet* 60(6):523–532. <https://doi.org/10.1136/jmg-2022-108962>
- Chen S, Zhou Y, Chen Y, Gu J (2018) fastp: an ultra-fast all-in-one FASTQ preprocessor. *Bioinformatics* 34(17):i884–i890. <https://doi.org/10.1093/bioinformatics/bty560>
- Clark MM, Stark Z, Farnaes L, Tan TY, White SM, Dimmock D, King-smore SF (2018) Meta-analysis of the diagnostic and clinical utility of genome and exome sequencing and chromosomal microarray in children with suspected genetic diseases. *NPJ Genom Med* 3:16. <https://doi.org/10.1038/s41525-018-0053-8>
- Dazzo E, Fanciulli M, Serioli E, Minervini G, Pulitano P, Binelli S, Di Bonaventura C, Luisi C, Pasini E, Striano S, Striano P, Coppola G, Chiavegato A, Radovic S, Spadotto A, Uzau S, La Neve A, Giallonardo AT, Mecarelli O et al (2015) Heterozygous reelin mutations cause autosomal-dominant lateral temporal epilepsy. *Am J Hum Genet* 96(6):992–1000. <https://doi.org/10.1016/j.ajhg.2015.04.020>
- DePristo MA, Banks E, Poplin R, Garimella KV, Maguire JR, Hartl C, Philippakis AA, del Angel G, Rivas MA, Hanna M, McKenna A, Fennell TJ, Kernysky AM, Sivachenko AY, Cibulskis K, Gabriel SB, Altshuler D, Daly MJ (2011) A framework for variation discovery and genotyping using next-generation DNA sequencing data. *Nature genetics* 43(5):491–498. <https://doi.org/10.1038/ng.806>
- Di Donato N, Guerrini R, Billington CJ, Barkovich AJ, Dinkel P, Freri E, Heide M, Gershon ES, Gertler TS, Hopkin RJ, Jacob S, Keedy SK, Kooshavar D, Lockhart PJ, Lohmann DR, Mahmoud IG, Parrini E, Schrock E, Severi G et al (2022) Monoallelic and biallelic mutations in RELN underlie a graded series of neurodevelopmental disorders. *Brain* 145(9):3274–3287. <https://doi.org/10.1093/brain/awac164>
- Dolgin V, Straussberg R, Xu R, Mileva I, Yogev Y, Khoury R, Konen O, Barhum Y, Zvulunov A, Mao C, Birk OS (2019) DEGS1 variant causes neurological disorder. *Eur J Hum Genet* 27(11):1668–1676. <https://doi.org/10.1038/s41431-019-0444-z>
- Duplomb L, Duvet S, Picot D, Jegou G, El Chehadeh-Djebbar S, Marle N, Gigot N, Aral B, Carmignac V, Thevenon J, Lopez E, Riviere JB, Klein A, Philippe C, Droin N, Blair E, Girodon F, Donadieu J, Bellanne-Chantelot C et al (2014) Cohen syndrome is associated with major glycosylation defects. *Hum Mol Genet* 23(9):2391–2399. <https://doi.org/10.1093/hmg/ddt630>
- El Chehadeh S, Aral B, Gigot N, Thauvin-Robinet C, Donzel A, Delrue MA, Lacombe D, David A, Burglen L, Philip N, Moncla A, Cormier-Daire V, Rio M, Edery P, Verloes A, Bonneau D, Afenjar A, Jacquette A, Heron D et al (2010) Search for the best indicators for the presence of a VPS13B gene mutation and confirmation of diagnostic criteria in a series of 34 patients genotyped for suspected Cohen syndrome. *J Med Genet* 47(8):549–553. <https://doi.org/10.1136/jmg.2009.075028>
- Farwell KD, Shahmirzadi L, El-Khechen D, Powis Z, Chao EC, Tippin Davis B, Baxter RM, Zeng W, Mroske C, Parra MC, Gandomi SK, Lu I, Li X, Lu H, Lu HM, Salvador D, Ruble D, Lao M, Fischbach S et al (2015) Enhanced utility of family-centered diagnostic exome sequencing with inheritance model-based analysis: results from 500 unselected families with undiagnosed genetic conditions. *Genet Med* 17(7):578–586. <https://doi.org/10.1038/gim.2014.154>
- Homberg JR, Kyzar EJ, Scattoni ML, Norton WH, Pittman J, Gaikwad S, Nguyen M, Poudel MK, Ullmann JF, Diamond DM (2016) Genetic and environmental modulation of neurodevelopmental disorders: translational insights from labs to beds. *Brain Res Bull* 125:79–91
- Hong SE, Shugart YY, Huang DT, Shahwan SA, Grant PE, Hourihane JO, Martin ND, Walsh CA (2000) Autosomal recessive lissencephaly with cerebellar hypoplasia is associated with

- human RELN mutations. *Nat Genet* 26(1):93–96. <https://doi.org/10.1038/79246>
- Ishii K, Kubo KI, Nakajima K (2016) Reelin and Neuropsychiatric Disorders. *Front Cell Neurosci* 10:229. <https://doi.org/10.3389/fncel.2016.00229>
- Kara E, Tucci A, Manzoni C, Lynch DS, Elpidorou M, Bettencourt C, Chelban V, Manole A, Hamed SA, Haridy NA, Federoff M, Preza E, Hughes D, Pittman A, Jaunmuktane Z, Brandner S, Xiromerisiou G, Wiethoff S, Schottlaender L et al (2016) Genetic and phenotypic characterization of complex hereditary spastic paraplegia. *Brain* 139(Pt 7):1904–1918. <https://doi.org/10.1093/brain/aww111>
- Khan TN, Khan K, Sadeghpour A, Reynolds H, Perilla Y, McDonald MT, Gallentine WB, Baig SM, Davis EE, Katsanis N (2019) Mutations in NCAPG2 cause a severe neurodevelopmental syndrome that expands the phenotypic spectrum of condensinopathies. *Am J Hum Genet* 104(1):94–111. <https://doi.org/10.1016/j.ajhg.2018.11.017>
- Kolehmainen J, Black GC, Saarinen A, Chandler K, Clayton-Smith J, Träskelin AL, Perveen R, Kivitie-Kallio S, Norio R, Warburg M, Fryns JP, de la Chapelle A, Lehesjoki AE (2003) Cohen syndrome is caused by mutations in a novel gene, COH1, encoding a transmembrane protein with a presumed role in vesicle-mediated sorting and intracellular protein transport. *Am J Hum Genet* 72(6):1359–1369. <https://doi.org/10.1086/375454>
- Kolehmainen J, Wilkinson R, Lehesjoki AE, Chandler K, Kivitie-Kallio S, Clayton-Smith J, Traskelin AL, Waris L, Saarinen A, Khan J, Gross-Tsur V, Traboulsi EI, Warburg M, Fryns JP, Norio R, Black GC, Manson FD (2004) Delineation of Cohen syndrome following a large-scale genotype-phenotype screen. *Am J Hum Genet* 75(1):122–127. <https://doi.org/10.1086/422197>
- Kopanos C, Tsiolkas V, Kouris A, Chapple CE, Albarca Aguilera M, Meyer R, Massouras A (2019) VarSome: the human genomic variant search engine. *Bioinformatics* 35(11):1978–1980. <https://doi.org/10.1093/bioinformatics/bty897>
- Lee H, Deignan JL, Dorrani N, Strom SP, Kantarci S, Quintero-Rivera F, Das K, Toy T, Harry B, Yourshaw M, Fox M, Fogel BL, Martinez-Agosto JA, Wong DA, Chang VY, Shieh PB, Palmer CG, Dipple KM, Grody WW et al (2014) Clinical exome sequencing for genetic identification of rare Mendelian disorders. *JAMA* 312(18):1880–1887. <https://doi.org/10.1001/jama.2014.14604>
- Li H (2013) Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM. arXiv preprint [arXiv:1303.3997](https://arxiv.org/abs/1303.3997)
- Li Q, Wang K (2017) InterVar: clinical interpretation of genetic variants by the 2015 ACMG-AMP guidelines. *Am J Hum Genet* 100(2):267–280. <https://doi.org/10.1016/j.ajhg.2017.01.004>
- Mitani T, Isikay S, Gezdirici A, Gulec EY, Punetha J, Fatih JM, Herman I, Akay G, Du H, Calame DG, Ayaz A, Tos T, Yesil G, Aydin H, Geckinli B, Elcioglu N, Candan S, Sezer O, Erdem HB et al (2021) High prevalence of multilocus pathogenic variation in neurodevelopmental disorders in the Turkish population. *Am J Hum Genet* 108(10):1981–2005. <https://doi.org/10.1016/j.ajhg.2021.08.009>
- McKenna A, Hanna M, Banks E, Sivachenko A, Cibulskis K, Kernytzky A, Garimella K, Altshuler D, Gabriel S, Daly M, DePristo MA (2010) The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. *Genome research* 20(9):1297–1303. <https://doi.org/10.1101/gr.107524.110>
- Morris-Rosendahl DJ, Crocq MA (2020) Neurodevelopmental disorders—the history and future of a diagnostic concept. *Dialogues Clin Neurosci* 22(1):65–72. <https://doi.org/10.31887/DCNS.2020.22.1/macroqc>
- Paisan-Ruiz C, Dogu O, Yilmaz A, Houlden H, Singleton A (2008) SPG11 mutations are common in familial cases of complicated hereditary spastic paraplegia. *Neurology* 70(16 Pt 2):1384–1389. <https://doi.org/10.1212/01.wnl.0000294327.66106.3d>
- Pant DC, Dorboz I, Schluter A, Fourcade S, Launay N, Joya J, Aguilera-Albesa S, Yoldi ME, Casanovas C, Willis MJ, Ruiz M, Ville D, Lesca G, Siquier-Pernet K, Desguerre I, Yan H, Wang J, Burmeister M, Brady L et al (2019) Loss of the sphingolipid desaturase DEGS1 causes hypomyelinating leukodystrophy. *J Clin Invest* 129(3):1240–1256. <https://doi.org/10.1172/jci123959>
- Parenti I, Rabaneda LG, Schoen H, Novarino G (2020) Neurodevelopmental disorders: from genetics to functional pathways. *Trends Neurosci* 43(8):608–621. <https://doi.org/10.1016/j.tins.2020.05.004>
- Pérez-Brangulí F, Mishra HK, Prots I, Havlicek S, Kohl Z, Saul D, Rummel C, Dorca-Arevalo J, Regensburger M, Graef D, Sock E, Blasi J, Groemer TW, Schlötzer-Schrehardt U, Winkler J, Winner B (2014) Dysfunction of spatacsin leads to axonal pathology in SPG11-linked hereditary spastic paraplegia. *Human Mole Genet* 23(18):4859–4874. <https://doi.org/10.1093/hmg/ddu200>
- Riazuddin S, Hussain M, Razaq A, Iqbal Z, Shahzad M, Polla DL, Song Y, van Beusekom E, Khan AA, Tomas-Roca L, Rashid M, Zahoor MY, Wissink-Lindhout WM, Basra MAR, Ansar M, Agha Z, van Heeswijk K, Rasheed F, Van de Vorst M et al (2017) Exome sequencing of Pakistani consanguineous families identifies 30 novel candidate genes for recessive intellectual disability. *Mol Psychiatry* 22(11):1604–1614. <https://doi.org/10.1038/mp.2016.109>
- Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, Grody WW, Hegde M, Lyon E, Spector E, Voelkerding K, Rehm HL (2015) Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med* 17(5):405–424. <https://doi.org/10.1038/gim.2015.30>
- Schneider SA, Mummery CJ, Mehrabian M, Houlden H, Bain PG (2012) SPG11 Presenting with tremor. *Tremor Other Hyperkinet Mov* (n Y). <https://doi.org/10.7916/d82b8wrr>
- Seifert W, Kuhnisch J, Maritzen T, Horn D, Haucke V, Hennies HC (2011) Cohen syndrome-associated protein, COH1, is a novel, giant Golgi matrix protein required for Golgi integrity. *J Biol Chem* 286(43):37665–37675. <https://doi.org/10.1074/jbc.M111.267971>
- Servetti M, Pisciotto L, Tassano E, Cerminara M, Nobili L, Boeri S, Rosti G, Lerone M, Divizia MT, Ronchetto P, Puliti A (2021) Neurodevelopmental disorders in patients with complex phenotypes and potential complex genetic basis involving non-coding genes, and double CNVs. *Front Genet* 12:732002. <https://doi.org/10.3389/fgene.2021.732002>
- Srivastava S, Love-Nichols JA, Dies KA, Ledbetter DH, Martin CL, Chung WK, Firth HV, Frazier T, Hansen RL, Prock L, Brunner H, Hoang N, Scherer SW, Sahin M, Miller DT (2019) Meta-analysis and multidisciplinary consensus statement: exome sequencing is a first-tier clinical diagnostic test for individuals with neurodevelopmental disorders. *Genet Med* 21(11):2413–2421. <https://doi.org/10.1038/s41436-019-0554-6>
- Stevanin G, Santorelli FM, Azzedine H, Coutinho P, Chomilier J, Denora PS, Martin E, Ouvrard-Hernandez AM, Tessa A, Bouslam N, Lossos A, Charles P, Loureiro JL, Elleuch N, Confavreux C, Cruz VT, Ruberg M, Leguern E, Grid D et al (2007) Mutations in SPG11, encoding spatacsin, are a major cause of spastic paraplegia with thin corpus callosum. *Nat Genet* 39(3):366–372. <https://doi.org/10.1038/ng1980>
- Stevanin G, Azzedine H, Denora P, Boukhris A, Tazir M, Lossos A, Rosa AL, Lerer I, Hamri A, Alegria P, Loureiro J, Tada M, Hannequin D, Anheim M, Goizet C, Gonzalez-Martinez V, Le Ber I, Forlani S, Iwabuchi K et al (2008) Mutations in SPG11 are frequent in autosomal recessive spastic paraplegia with thin corpus



- callosum, cognitive decline and lower motor neuron degeneration. *Brain* 131(Pt 3):772–784. <https://doi.org/10.1093/brain/awm293>
- Van der Auwera GA, Carneiro MO, Hartl C, Poplin R, Del Angel G, Levy-Moonshine A, Jordan T, Shakir K, Roazen D, Thibault J, Banks E, Garimella KV, Altshuler D, Gabriel S, DePristo MA (2013) From FastQ data to high confidence variant calls: the Genome Analysis Toolkit best practices pipeline. *Curr Protoc Bioinformatics*. <https://doi.org/10.1002/0471250953.bi11110s43>
- Wakil SM, Murad HN, Baz BM, Hagos ST, Al-Amr RA, Al-Yamani SA, Al-Wadaee SM, Meyer BF, Bohlega SA (2012) Autosomal recessive hereditary spastic paraplegia with thin corpus callosum among Saudis. *Neurosciences (riyadh)* 17(1):48–52
- Wojcik, M. H., Reuter, C. M., Marwaha, S., Mahmoud, M., Duyzend, M. H., Barseghyan, H., Yuan, B., Boone, P. M., Groopman, E. E., Délot, E. C., Jain, D., Sanchis-Juan, A., Starita, L. M., Talkowski, M., Montgomery, S. B., Bamshad, M. J., Chong, J. X., Wheeler, M. T., Berger, S. I., et al. (2023). Beyond the exome: what's next in diagnostic testing for Mendelian conditions. *The American Journal of Human Genetics*, 110(8), 1229–1248. <https://doi.org/10.1016/j.ajhg.2023.06.009>
- Wright CF, FitzPatrick DR, Firth HV (2018) Paediatric genomics: diagnosing rare disease in children. *Nat Rev Genet* 19(5):253–268. <https://doi.org/10.1038/nrg.2017.116>
- Wright CF, Campbell P, Eberhardt RY, Aitken S, Perrett D, Brent S, Danecek P, Gardner EJ, Chundru VK, Lindsay SJ, Andrews K, Hampstead J, Kaplanis J, Samocha KE, Middleton A, Foreman J, Hobson RJ, Parker MJ, Martin HC et al (2023) Genomic diagnosis of rare pediatric disease in the United Kingdom and Ireland. *N Engl J Med* 388(17):1559–1571. <https://doi.org/10.1056/NEJMo a2209046>
- Yang H, Wang K (2015) Genomic variant annotation and prioritization with ANNOVAR and wANNOVAR. *Nat Protoc* 10(10):1556–1566. <https://doi.org/10.1038/nprot.2015.105>
- Yang Y, Muzny DM, Reid JG, Bainbridge MN, Willis A, Ward PA, Braxton A, Beuten J, Xia F, Niu Z, Hardison M, Person R, Bekheirnia MR, Leduc MS, Kirby A, Pham P, Scull J, Wang M, Ding Y et al (2013) Clinical whole-exome sequencing for the diagnosis of mendelian disorders. *N Engl J Med* 369(16):1502–1511. <https://doi.org/10.1056/NEJMo a1306555>
- Yang Y, Muzny DM, Xia F, Niu Z, Person R, Ding Y, Ward P, Braxton A, Wang M, Buhay C, Veeraraghavan N, Hawes A, Chiang T, Leduc M, Beuten J, Zhang J, He W, Scull J, Willis A et al (2014) Molecular findings among patients referred for clinical whole-exome sequencing. *JAMA* 312(18):1870–1879. <https://doi.org/10.1001/jama.2014.14601>
- Zulfqar S, Tariq M, Ali Z, Fatima A, Klar J, Abdullah U, Ali A, Ramzan S, He S, Zhang J, Khan A, Shah S, Khan S, Makhdoom EH, Schuster J, Dahl N, Baig SM (2019) Whole exome sequencing identifies novel variant underlying hereditary spastic paraplegia in consanguineous Pakistani families. *J Clin Neurosci* 67:19–23. <https://doi.org/10.1016/j.jocn.2019.06.039>

**Publisher's Note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Springer Nature or its licensor (e.g. a society or other partner) holds exclusive rights to this article under a publishing agreement with the author(s) or other rightsholder(s); author self-archiving of the accepted manuscript version of this article is solely governed by the terms of such publishing agreement and applicable law.