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A novel homozygous truncating variant in *PPFIBP1* further delineates *PPFIBP1*-associated neurodevelopmental disorder

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Abstract

Neurodevelopmental disorders (NDDs) are classified as a group of disorders affecting function and development of the brain and having wide clinical variability.

Herein, we describe two affected individuals segregating a recessive NDD. The affected individuals exhibited phenotypes such as global developmental delay (GDD), intellectual disability (ID), microcephaly and speech delay. Whole-exome sequencing (WES) followed by bidirectional Sanger sequencing techniques identified a homozygous nonsense variant (c.466C > T; p.Gln156*) in the *PPFIBP1* gene (NM_003622.4) that segregated with the disease phenotype. Further, to elucidate the effect of the variant on protein structure, 3D protein

Abbreviations: GDD, global developmental delay; ID, intellectual disability; NIPT, noninvasive prenatal testing; PGTA, preimplantation genetic testing for aneuploidies; PPFIBP1, protein-tyrosine phosphatase receptor-type F polypeptide-interacting protein-binding protein 1; SAM, sterile alpha motif; WES, whole-exome sequencing.

Ahmed Waqas and Romana Liaqat contributed equally.

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modelling was performed for the mutant and normal protein that suggested substantial reduction of the mutant protein.
Our data support the evidence that *PPFIBP1* has a pivotal role in neurodevelopment in humans, and loss-of-function variants cause clinically variable neurodevelopmental phenotypes.

KEYWORDS
GDD, ID, microcephaly, NDD, *PPFIBP1*, speech delay

1 | INTRODUCTION

The human brain is highly complex, and during development, distinctive cell types differentiate, proliferate, migrate and integrate into a unified circuitry. However, studying the development and proper functioning of the brain is extremely difficult, and to critically understand how the brain components work in normal development and function is very important (Nøstvik et al., 2021; Scala et al., 2022). High-throughput sequencing and genetic data analysis allowed capturing variants, causing atypical or subtle phenotypes, including less penetrant and hypomorphic alleles (Nishikawa et al., 2022). Thus, disease-causing variants in genes are being linked to phenotypes (Huguet et al., 2013; Royer-Bertrand et al., 2021).

Recently, the *PPFIBP1* gene (Protein Tyrosine Phosphatase Receptor Type F Polypeptide-Interacting Protein-Binding Protein 1) variants encoding the liprin-β1 protein have been associated with mild-severe developmental delay (DD), epilepsy (early-onset) and progressive microcephaly in 14 patients from 10 unrelated families (Rosenhahn et al., 2022). Liprins interact with members of the leukocyte common antigen related (LAR) family of transmembrane protein tyrosine phosphatases, which are important for *Drosophila* axon guidance and development. In addition, features such as spasticity, short stature, muscular hypertonia, feeding difficulties, failure to thrive, impaired vision/hearing and cardiovascular anomalies were also revealed. Liprin-β1 has been identified as a binding partner of liprin-α proteins that play a role in synapse formation, synaptic transmissions, intracellular transport, cell motility and protein assembly (Spangler & Hoogenraad, 2007; Wei et al., 2011; Wong et al., 2018). However, the precise biological function of liprin-β1 linked with neurodevelopmental disorders (NDDs) is not properly understood.

Herein, we report a proband with global developmental delay (GDD), intellectual disability (ID), microcephaly and speech delay and characterised the disorder using whole-exome sequencing (WES), which revealed potential pathogenic homozygous variants in the *PPFIBP1* gene.

2 | MATERIALS AND METHODS

2.1 | Ethical appraisal

Ethical approval to conduct the current study was obtained from University of Management and Technology (UMT's) institutional review board (IRB) following Helsinki protocols. Written informed consent for the molecular testing and permission to publish the case reported data were obtained from the father/guardian of the affected individuals.

2.2 | Genomic DNA extraction

Pedigree was constructed carefully and peripheral blood samples of the affected and normal individuals were collected in ethylenediaminetetraacetic acid (EDTA)-containing Vacutainer. Genomic DNA was extracted using a GenElute™ Blood Genomic DNA Kit (Sigma-Aldrich, USA), and the extracted DNA was quantified using a Nanodrop 1000 Spectrophotometer (Thermo Scientific, Wilmington, USA; Umair et al., 2016).

2.3 | WES and data analysis

The DNA sample of the affected individual (II-3) was subjected to WES by using HiSeq 2500 systems (Illumina) as described previously (Umair et al., 2020; Umair, Khan, et al., 2021; Umair, Palander, et al., 2021). The sequences were enriched by using SureSelect XT Human All Exon kit (Agilent Technologies). The sequence reads were aligned with the reference human genome assembly hg19 (GRCh38 [hg38]) and to the transcript GenBank NM_003622.4 (Ensembl: ENST00000228425.11), representing the transcript with the highest expression across all tissues. Software asset

management (SAM) tools, PINDEL and Exome Depth were used for variant calling.

2.4 | Sanger sequencing

Cosegregation analysis of the prioritised variants was performed by using the standard Sanger sequencing method (Umair, Khan, et al., 2021; Younus et al., 2019). DNA sequences flanking the prioritised variants were downloaded from the Ensembl Genome Browser, and Polymerase Chain Reaction (PCR) primers were designed using Primer 3 software (Massadeh et al., 2019).

2.5 | Pathogenicity index

To evaluate the pathogenicity of the variants, various online protein prediction tools were used, including MutationTaster, EIGEN, PROVEAN, CADD, BayesDel noAF, FATHMM-MKL and others (Khan et al., 2022). The frequency of the identified candidate variant was further cross-checked with in-house 2000 + exomes, ExAC, 1000 Genomes and gnomAD. Conservation of the mutated amino acid was checked across different species using NCBI Homologene (Asiri et al., 2020).

2.6 | 3D protein modelling

The amino acid sequence (1005 aa, including the sterile alpha motif, SAM 1–3 domain) of the PPFIBP1-encoded protein was obtained in FASTA format from the UniProt Knowledgebase database (Q86W92). The crystal structure of the human PPFIBP1 protein (Liprin-beta-1) was retrieved from the AlphaFold Protein Structure Database (AlphaFold DB), and the Swiss-Model was used to create the structure of the mutated protein. The protein structure from the AlphaFold DB was used as a template to yield the mutated protein structure. The model was then run through the Ramachandran plot server and ERRAT (Nayab et al., 2021; Waqas et al., 2022).

3 | RESULTS

3.1 | Clinical assessment

The affected individuals (II-2 and II-3) were born to a consanguineous Pakistani couple with no previous family history of such disease (Figure 1a). The proband was a product of normal delivery with a normal birth weight (3.75 and 36.5 kg). The affected individual II-2 at 11 years exhibited the following growth parameters: height 86 cm (<1 percentile, −8.4 SD), weight 10.5 kg (<1 percentile, −5.1 SD) and head circumference (HC) of 44 cm (<1 percentile, −6.9 SD), and similarly, the affected individual II-3 at 9 years exhibited the following growth parameters: height 98.5 cm (<1 percentile, −5.8 SD), weight 13.6 kg (<1 percentile, −4.3 SD) and head circumference (HC) of 46 cm (<1 percentile, −5.1 SD). The affected individuals showed ID, GDD, microcephaly and speech delay.

Parents recognised the first concern at the age of 3–4 months. The parents observed difficulty in feeding and choking while swallowing. The affected individual (II-2) was not able to hold his or her head and turning, crawling and walking were delayed. Currently, both the affected individuals revealed hallmark features of the core phenotype of ID and GDD. The biochemical analysis was unremarkable. The estimation of organic acids, urine amino acids (aminoaciduria), guanidinoacetate, creatine and purine/pyrimidine were under normal limits. Carbamazepine was recommended for seizures and has shown positive effects.

The brain MRI revealed bilateral posterior peritrigonal, periventricular white matter volume loss with increased T2 and FLAIR signal intensity with extension along the right corona radiata and slightly irregular ventricular walls. It is associated with minimal thinning of the corpus callosum posteriorly with no signal changes. The cortex showed signal intensity, intraventricular extra-axial cerebrospinal fluid (CSF) spaces and extracranial visualised soft tissues, and the cerebellum and posterior fossa were unremarkable (Figure 1b). Clinical comparison of the patients reported recently and reported here is summarised in Table 1.

3.2 | Molecular diagnosis

After WES, data analysis and variant filtration were performed using standard methods (Younus et al., 2019). Variants in genes already known to cause syndromic or nonsyndromic ID were shortlisted for downstream cosegregation analysis with Sanger sequencing standard protocols as described earlier (Rafeeq et al., 2022; Umair, Khan, et al., 2021; Umair, Palander, et al., 2021; Figure 1a). A homozygous nonsense variant (c.466C > T; p.Gln156*) in the Exon 6 of the *PPFIBP1* gene (NM_003622.4; NP_003613.3) was identified that cosegregated with the disease phenotype in the family (Figure 2a,b). The identified mutation is located in a highly conserved coiled coil domain of the PPFIB1 (Figure 2c). The conservation of the amino acid (Gln156) in the different PPFIBP1 orthologs revealed high conservation across different species (Figure 2c). Furthermore,

the mutation is located in the first half of the protein, which might cause mRNA decay and loss of function (LOF).

The identified variant (c.466C > T; p.Gln156*) was not identified in the homozygous state in different online databases, such as gnomAD, ExAC and dbSNP, and in the 1000 Genomes Project or in-house exome database (Figure 3). The variant was classified as pathogenic (Class 1), according to American College of Medical Genetics (ACMG) guidelines.

3.3 | 3D protein modelling

The AlphaFold DB provided a crystal structure of the human PPFIBP1 protein (Liprin-beta-1) with a high level of precision. The 30 alpha helices in the wild-type human PPFIBP1 protein (Liprin-beta-1) also include three SAMs (SAM domains; SAM1, SAM2 2 and SAM3). These alpha helices are located at positions 2–20, 44–61, 64–73,76–91, 99–288, 300–346, 644–646, 649–658, 662–666, 668–670, 675–677, 683–689, 695–709, 715–718,721-730,734–742, 747–751, 755–760, 766–781, 801–806, 809–818, 825–828, 835–840, 846–852, 860–886, 953–955, 957–960, 963–987, 990–992, 994–997.

The mutation caused a change in the glutamine (Q) codon to a stop codon at amino acid number 156. As a result, the altered structure is projected to end at the 5th alpha helix. The SAM domains are projected to be absent from the altered structure (SAM1–3). The SAM domains are known to interact with proteins in a variety of ways. Different assessment programmes evaluated the final refined model. Three-dimensional models of wild-type and mutant PPFIBP1 protein (p.Q15*) were predicted and assessed using online structural analysis tools using homology modelling. According to the

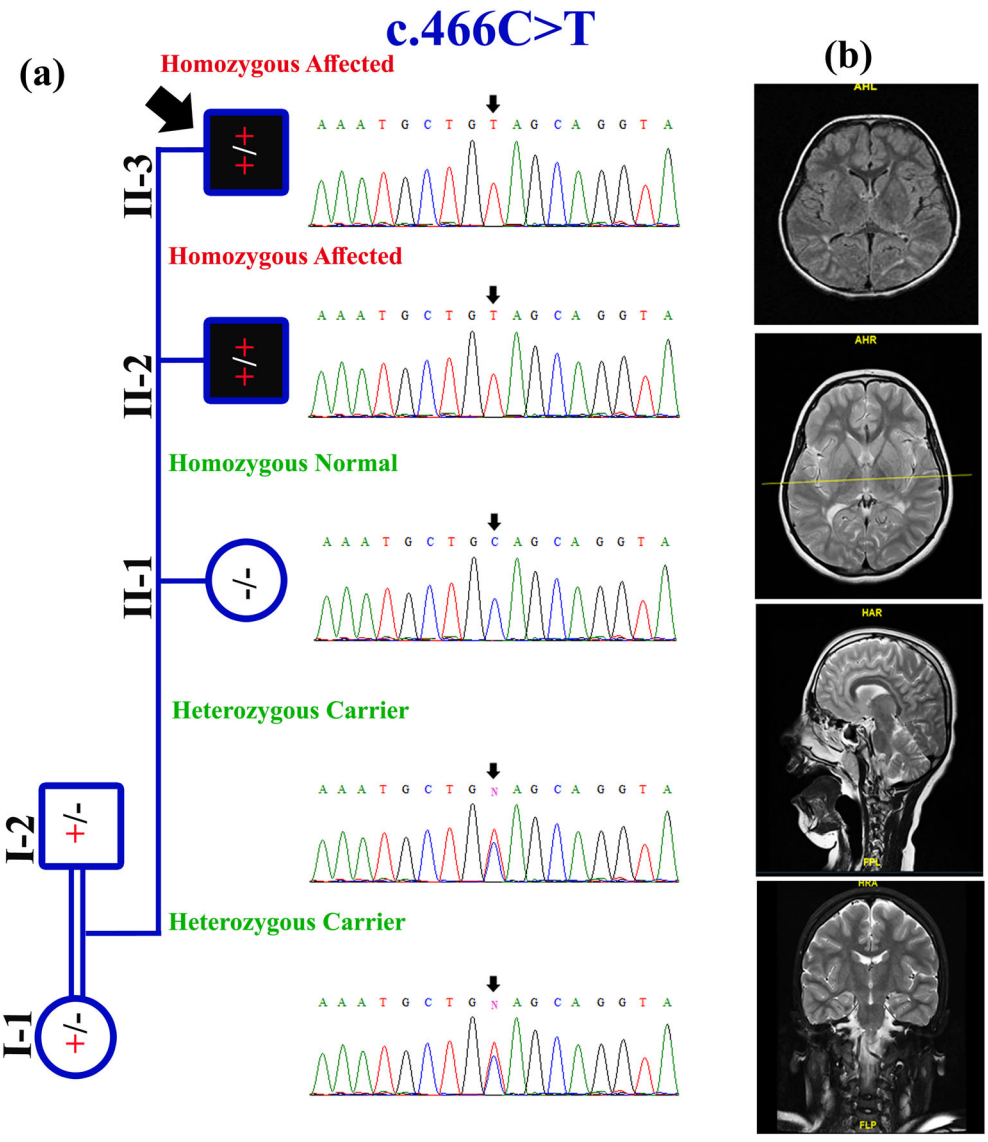

FIGURE 1 (a) Pedigree of the family showing recessive inheritance and sanger electrograms along with each individual showing segregation of the identified variant in all available family members. Black arrow represents the individual for whom MRI was performed. (b) MRI images of affected individual (II-3) showing abnormal MRI (periventricular white matter volume loss)

TABLE 1 Clinical phenotypic comparison of patients reported in literature with the index reported in the present study

	Rosenhahn et al., 2022							
Clinical description Sex	**Ind. 1 Male**	**Ind. 2 Female**	**Ind. 3 Male**	**Ind. 4 Male**	**Ind. 5 Male**	**Ind. 6 Male**	**Ind. 7 Female**	**Ind. 8 Female**
Development	Moderate ID, normal motor development	Profound DD, unable to sit	Profound DD, unable to sit	Profound DD, unable to sit	Profound DD, unable to sit	Profound DD, unable to sit	Profound DD, unable to sit	Profound DD, unable to sit
Speech	Delayed	No speech	No speech	No speech	No speech	No speech	No speech	No speech
Seizure types	Focal seizure	Focal, generalised tonic	Epileptic spasms, focal, tonic	Epileptic spasms,	Epileptic spasms, focal, multifocal	Epileptic spasms, focal, generalised tonic	Focal, myoclonic	Focal, myoclonic, tonic
MRI	Normal	Paucity of the WM, ventriculomegaly, hypoplastic CC, Blakes's pouch cyst	Periventricular leukomalacia, metopic synostosis	Moderate hyperintensity of periventricular white matter, mild ventriculomegaly	Ventriculomegaly, abnormal signal intensity of the WM, bilateral temporal and left occipital pachygyria	Ventriculomegaly, paucity of the WM, bilateral parietal and occipital pachygyria.	Not done	Ventriculomegaly on CT
Neurological findings	None	Spastic tetraplegia, nystagmus	Spastic tetraplegia	Spastic tetraplegia	Spastic tetraplegia	Spastic diplegia, hyperreflexia	Spastic tetraplegia	Hypertonia of the limbs, dystonia
Microcephaly	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Growth	Small for gestational age (SGA)	Short stature, low weight	SGA, low weight	SGA, low weight	SGA, low weight	SGA, short stature	SGA, short stature, low weight	Short stature
CHD	No	No	Yes	Yes	Yes	Yes	No	No
Hearing	Normal	Left hypoacousis	Impaired	Impaired	Impaired	Not tested	Normal	Normal
Ocular features	Normal	Bilateral papillary pallor, no eye contact	Poor fixation	Normal	Normal	Haemorrhagic retinitis, chronic retinal detachment	NA	Normal
Variant (NM_003622.4)	c.1146 + 1G > A	c.2654del	c.1368_1369del	c.1368_1369del	c.1368_1369del	c.1368_1369del	c.2413C > T	c.2413C > T
Protein change	p?,	p.(Tyr885Leufs* 4),	p.(Glu456Aspfs* 3),	p.(Glu456Aspfs* 3),	p.(Glu456Aspfs* 3),	p.(Glu456Aspfs* 3),	p.(Arg805*),	p.(Arg805*),
Zygosity	Homo	Homo	Homo	Homo	Homo	Homo	Homo	Homo

Abbreviations: CC, corpus callosum; CHD, Congenital heart defects; CT, computed tomography; DD, developmental delay, ID, intellectual disability; OFC, occipitofrontal circumference; SGA, small for gestational age; WM, white matter.

TABLE 1 (Continued)

	Rosenhahn et al., 2022						
Clinical description **Sex**	**Ind. 9** **Male**	**Ind. 10** **Male**	**Ind. 11** **Female**	**Ind. 13** **Female**	**Ind. 14** **Male**	**Ind. 15** **Male**	**Present study** **Male**
Development	Profound DD, sat independently at 6y	Profound DD, no head support	Severe DD delay but can stand and walk	Profound DD, unable to sit	Severe DD, can sit but not walk	Severe DD, motor delay	Severe DD
Speech	No speech	No speech	No speech	No speech	No speech	No speech	No speech
Seizure types	Generalised tonic clonic, myoclonic	Generalised tonic clonic, myoclonic,	Epileptic spasms, focal with apnoea, myoclonic	Generalised tonic clonic	Epileptic spasms	Focal myoclonic, epileptic spasms	Focal myoclonic
MRI	Ventriculomegaly, cortical atrophy, demyelination of periventricular WM, thin CC, cerebellar vermian hypoplasia	Asymmetrical ventriculomegaly, cortical atrophy, demyelination of periventricular WM, thin CC, cerebellar vermian hypoplasia	Thin CC, periventricular dysmyelination, possibly reduction of the WM.	Ailateral parietal pachygyria, periventricular heterotopia, ventriculomegaly, hyperintensity and paucity of the WM	Abnormal	Abnormal myelination of the periventricular WM and at corona radiata and centrum semiovale, hypoplastic CC, mild ventriculomegaly	Bilateral peritrigonal, periventricular white matter volume loss
Neurological findings	Hypertonia of the limbs	Spasticity, rigidity, dystonic movement	Hypotonia	Hypotonia, nystagmus	Spastic tetraplegia, no sphincter control	Hypotonia, nystagmus	Spasticity, Hypotonia
Microcephaly	Yes	Yes	Yes	No, but low OFC	Yes	Yes	Yes
Growth	Short stature, low weight	Short stature, low weight	Normal	SGA	SGA	Normal	Short stature, low weight
CHD	No	Yes	No	Yes	No	No	No
Hearing	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Ocular features	Optic atrophy, followed light	Optic atrophy, could not follow light	Normal	Normal, but poor fixation	Blindness	Right ptosis, left iris coloboma, diffuse chorioretinal degeneration	Normal
Variant (NM_003622.4)	c.1468C > T, p.(Gln490*)	c.1468C > T	c.403C > T	c.1417_1427del	c.1300C > T	c.2629C > T	c.466C > T;
Protein change	p.(Gln490*),	p.(Gln490*),	p.(Arg135*),	p.(Ala473Lysfs* 20),	p.(Gln434*),	p.(Arg877*),	p.Gln156*
Zygosity	Homo	Homo	Homo	Homo	Homo	Homo	Homo

Abbreviations: CC, corpus callosum; CHD, Congenital heart defects; CT, computed tomography; DD, developmental delay, ID, intellectual disability; OFC, occipitofrontal circumference; SGA, small for gestational age; WM, white matter.

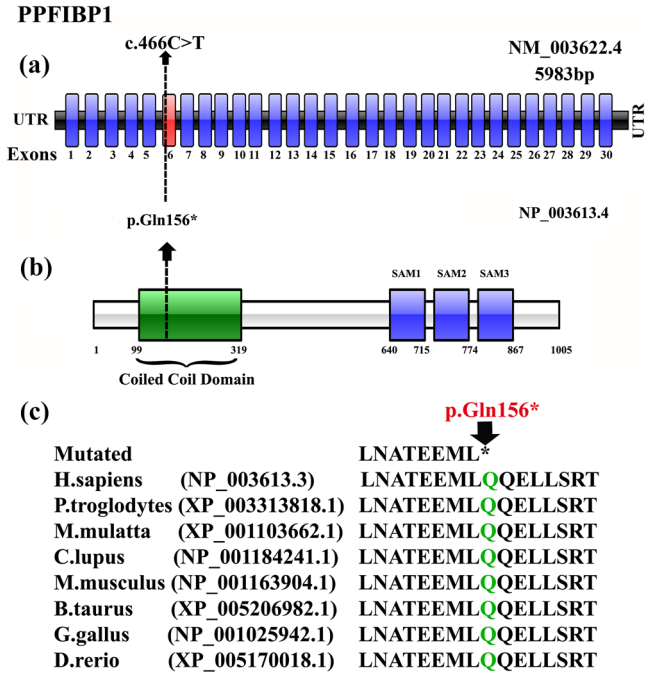

FIGURE 2 (a) Schematic representation of the *PPFIB1* exons (30 exons) and the position of variant identified in the present study (Exon 6). (b) Schematic representation of the PPFIB1 protein domains and position of identified mutation in the coiled coil domain. (c) Partial amino acid sequence of the PPFIB1 protein and showing conservation of the Gln156 across several species

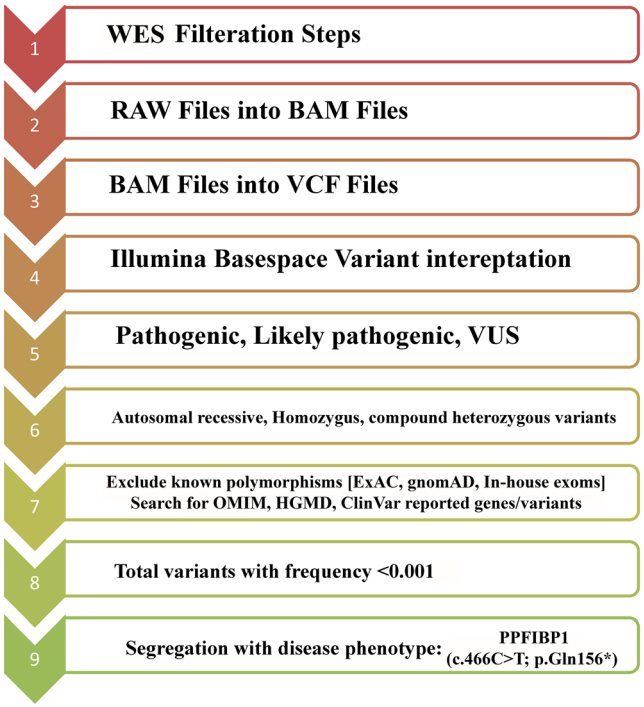

FIGURE 3 Schematic representation of whole-exome sequencing (WES) variant filtration flow sheet

Ramachandran plot, about 87% and 99% of residues in the wild-type and mutant structures are in permissible torsion angle zones, respectively. The ERRAT protein structure was used to validate the 3D structures of both wild-type and mutant PPFIBP1. ERRAT generated an overall quality factor of 91% for the wild-type structure model and 95% for the mutant structure model (Figure 4).

4 | DISCUSSION

In the present study, we investigated a single family having two affected individuals having ID, GDD and microcephaly and speech abnormality. WES identified a homozygous nonsense variant (c.466C > T; Gln156*) in the Exon 6 of the *PPFIBP1* gene located on chromosome 12p11.23-p11.22, consisting of a total 30 exons, and encodes a 1005 amino acid protein in humans (Figure 2a,b). The identified mutation is located in the Exon 6 of the *PPFIBP1* gene, which changes Gln to a premature stop codon. As the mutation is present in the first half of the protein, it might result in nonsense-mediated mRNA decay. Features exhibited by the patient under investigation in the present study overlap with the already reported ones (Rosenhahn et al., 2022). Some of the features that were not common among the reported patients include ocular defects that were only observed in eight (53%; 8/16) individuals, and hearing impairment was observed in four individuals (26%; 04/16). These features were also not observed in our proband. Common features amounting to 85% of the affected individuals include developmental delay, speech issues, microcephaly and short stature.

PPFIBP1 consists of a total 30 exons encoding a PPFIA binding protein 1 (1005 amino acid), also known as liprin-β1. Liprin-β1 belongs to the liprin protein family characterised by a highly conserved N-terminal coiled coil region and three adjacent C-terminal SAM domains forming multiple protein binding surfaces and is thus associated with protein–protein interaction and cell signalling (Wei et al., 2011). SAM domains are a family of protein interaction modules present in a wide variety of proteins.

In mammals, the liprin family are associated with major scaffold proteins involved in synapse formation and synaptic signalling. It is also associated with the axonal transport processes via the assembly of protein complexes and a presynaptic scaffolding protein essential for neurodevelopment (Astigarraga et al., 2010; Becker et al., 2020; Spangler & Hoogenraad, 2007; Xie et al., 2021). The nonsense variant c. 466 C > T is found in Exon 6 of PPFIBP1, causing stop codons for the

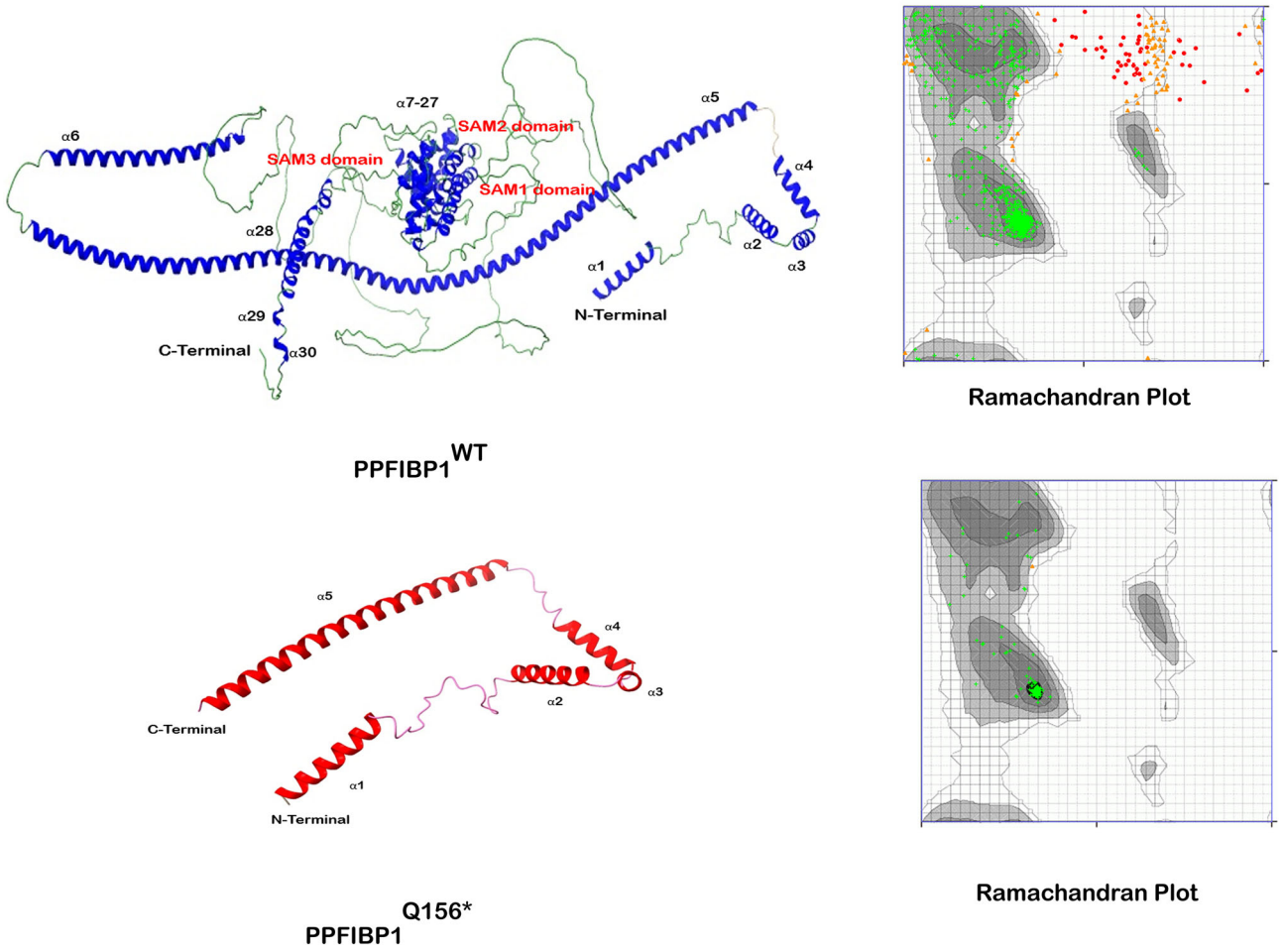

FIGURE 4 3D comparison of the PPFIB1^{Gln156*} and PPFIB1$^{wild\text{-}type}$ protein, showing complete loss of secondary structure in case of mutant PPFIB1

concerned protein. The Gly156* in the SAM domain of PPFIBP1 destabilising both the second SAM domain and the interaction between the second and third SAM domain. Therefore, this variant is expected to severely disturb the topology of the SAM domain region and its function in protein–protein interactions. Given the hypothesis that liprin-β1 acts as a core scaffold to mediate protein assembly in the presynaptic zone, the ability to precisely interact with other proteins would appear to be critical for protein function.

As recently reported, PPFIBP1 is a strong candidate gene for neurodevelopmental disorder based on the 16 patients' published data, which reported having profound developmental delay, early-onset epilepsy and progressive microcephaly (Rosenhahn et al., 2022). Features reported previously overlap the clinical phenotypes reported in our patients. However, epilepsy and periventricular calcifications were not observed in our patients. Our case adds the molecular and phenotypic frame of the PPFIBP1-related NDD and suggests that there exists a significant phenotypic variability among patients having different variants. This also suggests the involvement of undetermined genetic, epigenetic and environmental factors modulating the variable expression of the mutated *PPFIBP1* gene.

Taken together, we showed that homozygous loss-of-function variants in *PPFIBP1* could be added to the growing list of gene variants known to NDD. Furthermore, identification of new candidate genes can be added to the newborn screening programme, and methods such as preimplantation genetic testing for aneuploidies (PGT-A) and noninvasive prenatal testing (NIPT) can be employed for parents wishing to have future pregnancies (Alfadhel et al., 2019; Alyafee, Al Tuwaijri, et al., 2021; Alyafee, Alam, et al., 2021; Alyafee et al., 2022). Additional research is required to understand the cellular response to the loss of *PPFIBP1* and how it results in altered neurodevelopment.

In conclusion, we provide further evidence that the homozygous variant in the *PPFIBP1* gene causes NDD in humans. However, to uncover the exact mechanism, further research is required.

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CONFLICTS OF INTEREST

None declared.

ETHICS STATEMENT

The study was approved by the research committee of UMT, Lahore, Pakistan. The parents of the patient provided a written informed consent for the publication of the case. Written informed consent was obtained from the patient (father).

AUTHOR CONTRIBUTIONS

Nawaz S, Amjad Khan A wrote the first draft, data analysis and experimentation. Waqas A performed clinical evaluation and data analysis. Abbas S, Muhammad Umair M supervised the study, edited the final MS and performed protein modelling.

DATA AVAILABILITY STATEMENT

Data will be provided by the corresponding author upon potential requests.

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