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SHORT COMMUNICATION

A novel biallelic variant further delineates *PRDX3*-related autosomal recessive cerebellar ataxia

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Abstract

Cerebellar ataxias (CAs) comprise a rare group of neurological disorders characterized by extensive phenotypic and genetic heterogeneity. In the last several years, our understanding of the CA etiology has increased significantly and resulted in the discoveries of numerous ataxia-associated genes. Herein, we describe a single affected individual from a consanguineous family segregating a recessive neurodevelopmental disorder. The proband showed features such as global developmental delay, cerebellar atrophy, hypotonia, speech issues, dystonia, and profound hearing impairment. Whole-exome sequencing and Sanger sequencing revealed a biallelic nonsense variant (c.496A > T; p.Lys166*) in the exon 5 of the *PRDX3* gene that segregated perfectly within the family. This is the third report that associates the *PRDX3* gene variant with cerebellar ataxia. In addition, associated hearing impairment further delineates the PRDX3 associated gene phenotypes.

Keywords PRDX3 · Whole-exome sequencing · Novel variant · Nonsense variant · Cerebellar ataxia · Profound deafness

Introduction

Hereditary cerebellar ataxias are a genetically and clinically heterogeneous group of neurodegenerative disorders characterized by progressive gait incoordination, cerebellum damage, and associated with poor speech, hands, and eye coordination [1, 2]. Current, global epidemiological studies on ataxia have estimated an overall ataxia occurrence rate of 26/100,000 in children, and the dominant and recessive forms of hereditary cerebral ataxia occurrence rate is 2.7/100,000 and 3.3/100,000 respectively [3]. It is difficult to estimate the frequency of specific etiologies among CA patients due to differences in ethnicity, age, and family history.

Cerebellar ataxias (CAs) are a heterogeneous group of neurological disorders characterized by impaired coordination of limb and eye movements and dysarthria. The primary

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pathology in CAs is progressive cerebellar atrophy; however, in most cases, the phenotype is complex and involves multiple neurological deficits. Due to significant phenotypic and genetic heterogeneity, the diagnostic work-up in CAs remains a challenge [4].

Recently, five subjects affected by cerebellar ataxia were associated with biallelic variants in the *PRDX-3*gene, encoding the peroxiredoxin (PRDX 3) [5]. PRDX3 belongs to a superfamily of peroxidase that functions as a protective antioxidant enzyme, which is exclusively localized to the mitochondria [5]. PRDX3 catalyzing the reduction of hydrogen peroxide (H_2O_2) and plays a key role against oxidative stress [6]. Upon reaction with H_2O_2, cysteine residues in the PRDX3 are oxidized, forming an intermolecular disulfide bond with a cysteine from another subunit. The peroxiredoxin family members can protect cells from oxidative stress and promote cell survival [7]. Increased levels of ROS (reactive oxygen species) are harmful that can cause mitochondrial dysfunction, DNA damage, protein, and lipid oxidation that might lead to apoptosis [5].

Here, we report a single proband, clinically diagnosed with a neurodevelopmental disorder. In addition, WES identified a novel biallelic nonsense variant in the *PRDX3* gene located on chromosome 10q26.11.

Materials and methods

Study and ethical approval

The study was approved by the Institutional Review Board (IRB) of UMT and followed the Helsinki protocols. Written informed consent for the publication of related data was obtained from the parents. Pedigree was constructed, and patents were interviewed (Fig. 1A).

Clinical examination

The affected individual was clinically evaluated at the local government hospital, and associated biochemical tests and MRI were performed. In addition, informed consent for publication of this case report and conducting research study was obtained from the parents.

Karyotype analysis

Using peripheral blood lymphocytes, karyotype analysis was performed for the affected individual (II-1) using the G-banding technique [8].

Genomic DNA extraction and quantification

According to the manufacturer's instructions, DNA was extracted from the fresh blood of all the available individuals using the QIAamp DNA kit. NanoDrop™ spectrophotometer was used for the DNA quantification.

Whole-exome sequencing (WES)

WES was commercially performed for the single affected family member (II-1), using an Illumina platform (Illumina, San Diego, USA). Approximately 38 Mb of the Consensus Coding Sequence (CCS) was enriched from fragmented genomic DNA using > 340,000 probes designed against the human genome. Libraries were finally sequenced with an average coverage depth of 80 × –100 ×.

The data analysis and interpretation were performed using an end-to-end in-house bioinformatics pipeline with applications including base calling, filtering of low quality reads, and alignment of reads to the GRCh37/hg19 (http://genome.ucsc.edu/) genome assembly. The final VCF file was analyzed using Illumina basespace online analysis tool (https://login.illumina.com/) [9].

Variant scoring and prioritization

Primary filtering was performed using standard Illumina basespace, such as filtering out low-quality reads and possible artifacts, and subsequent variant annotation was performed. In addition, all disease-causing variants reported in the ClinVar, HGMD®, PubMed, and variants with minor allele frequency (MAF) of > 1% in the gnomAD/ExAC database were given predilection.

As pedigree showed autosomal recessive inheritance pattern, thus variant filtration steps also focused on the mode of inheritance. In addition, coding exons along with flanking + / – 20 intronic base pairs were given priority. All other pertinent inheritance patterns were considered; however, recessive pattern was given preference. The patient clinical description was evaluated to perform genotype–phenotype correlations. Several online in silico prediction tools were used to score the variant's pathogenicity, such as Varsome, SIFT, and MutationTaster. Conservation of the amino acid across different species was checked using Homologene online tool.

Sanger sequencing

Sanger segregation was performed for all the available family members as described previously [10, 11]. Primer pairs

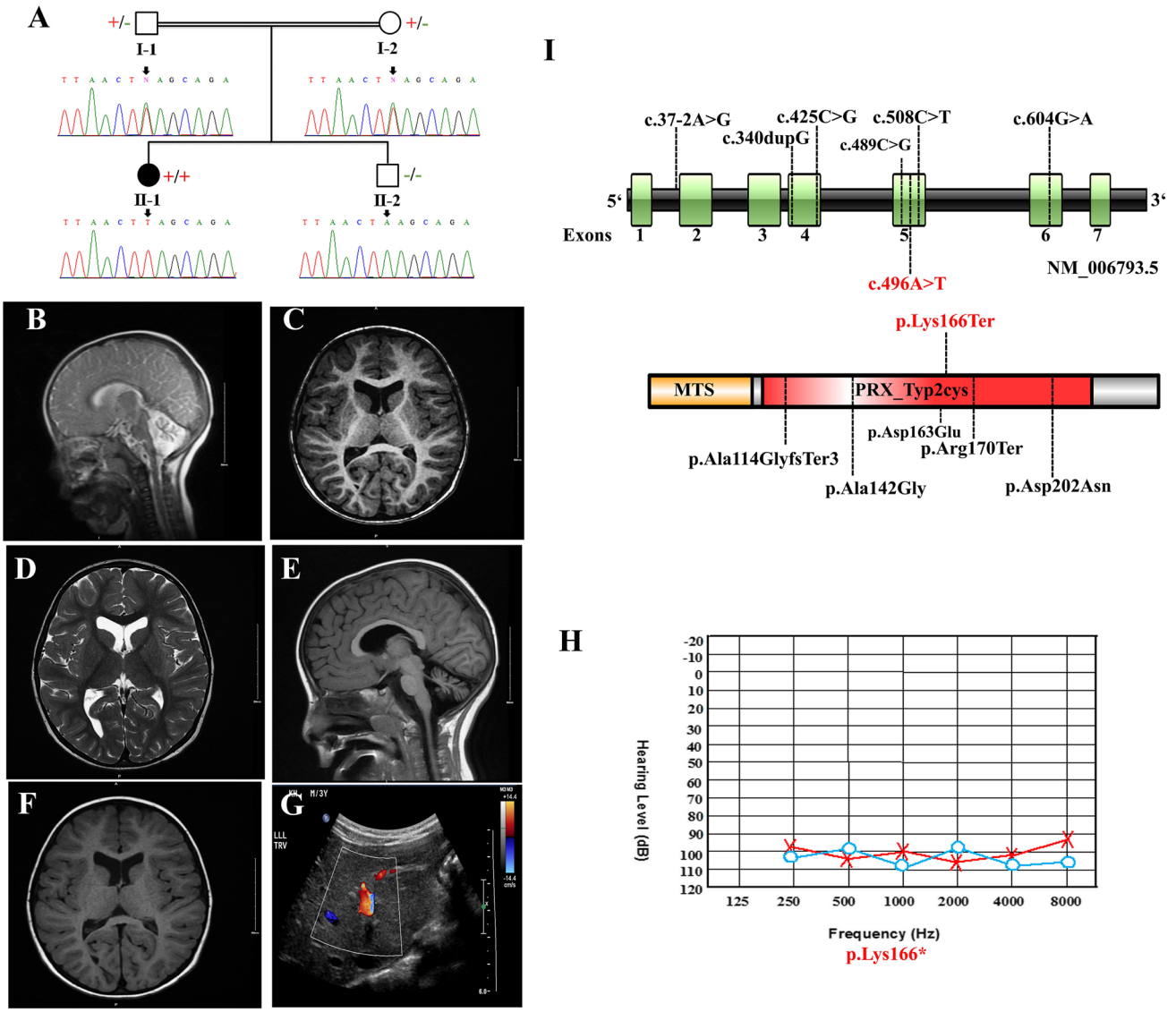

Fig. 1 **A** Pedigree of the family showing autosomal recessive inheritance. Sanger electrograms are shown beneath each member suggesting segregation of the identified variant (variant (c.496A > T; p.Lys166*) in all available family members. **B**, **C**, **E** Brain MRI showing midline sagittal showed atrophic vermis and cerebellar atrophy. **D** Suggesting unilateral white matter demyelination. **F** T2 Flair shows diffused normal signal intensity affecting the cortex of both the cerebellar hemispheres. **G** Abdominal ultrasound showing unremarkable results. **H** Air conduction thresholds for the index revealed profound bilateral profound hearing impairment. Circles with smooth connecting lines represent the right ear and cross with dotted connecting lines, the left ear. **I** Schematic representation of the *PRDX3* gene and protein domains. The variant identified in the present study is presented in red

were designed using Primer3 online tool (http://bioinfo.ut.ee/primer3-0.4.0/). Primers will be provided upon request.

Results

Clinical evaluation

The index (II-1) evaluated in the present study belonged to a consanguineous Pakistani family (Fig. 1A). The II-1 presented at birth with moderate hypotonia, developmental delay, and hearing impairment. Her motor skills involving sitting, crawling, walking, and speaking was significantly delayed, as she started sitting at 14 months, standing with help at 3.5 years. Currently, she is 15 years old and unable to walk without support and is bound to a wheelchair. Socially, she cannot speak, which might be due to hearing impairment. Limb spasticity and dystonia resulted in joint contractures, dyskinetic limb movements, subtle twitching of the perioral muscles, and mild superimposed myoclonic jerks in the limbs. Her growth parameters at 12 years include height 103.6 cm (< 1 percentile (− 6.4 SD)), weight 16 kg

(< 1 percentile (− 4.1 SD)), and head circumference 48.6 cm (< 1 percentile (− 3.8 SD)). All biochemical investigations and ECGs were unremarkable.

Detailed brain MRI of the index revealed atrophic cerebellum. The images showed cortical thinning and loss of underlying white matter, thus leading to enlarged fissures in the vermis and cerebellar hemisphere (T2 Flair, Fig. 1B–F). The T1 Flair midline sagittal MRI showed an atrophic vermis, while the brain stem volume seems normal (Fig. 1B, E). Thus, the MRI is evidence of degenerative cerebellar disorder. The midbrain appears within normal limits, and no acute intracranial hemorrhage or infarction was observed. The visualized orbits and paranasal sinuses appear within normal limits. The abdominal ultrasound study was unremarkable, apart from two focal hyperechoic liver lesions that could represent focal fatty infiltration versus hemangioma (Fig. 1G). The affected individual's audiograms showed profound bilateral hearing impairment (HI) across all the frequencies (Fig. 1H). Detailed clinical comparison of our patient and those reported previously have been summarized in Table 1.

Molecular studies

Using whole-exome sequencing (WES), we identified a biallelic nonsense variant (c.496A > T; p.Lys166*) in the exon 5 of the *PRDX3*gene (NM_006793.5) located on chromosome 10q26.11. WES, Sanger sequencing, and variant filtration steps were similar to those described previously [9]. The novel biallelic variant (c.496A > T; p.Lys166*) in the *PRDX3* gene (Fig. 1I) was the best candidate according to the phenotypic presentation and recently published cases [5]. The variant was confirmed in the siblings and parents using standard Sanger sequencing (Fig. 1A). The identified variant is located in the PRX_Type 2-cys domain of the PRDX3 protein (Fig. 1I). The novel nonsense variant (p.Lys166*) might

Table 1 Clinical comparison of our case with previous reports

	Rebelo et al. [5]					Martínez-Rubio et al. [12]	Umair et al
Family	1	2	3	4	5	1	Present study
Age at study	36 y	36 y	55 y	32 y	40 y	19 months	15by
Sex	Male	Male	Female	Male	Male	Male	Female
Ethnicity	Kurdish	Kurdish	German	Indian	French	Spain	Pakistani
Consanguinity	Unknown	Yes	Unknown	Yes	No	Yes	Yes
Mode of inheritance	Simplex Homozygous	Simplex Homozygous	Simplex Homozygous	Simplex Homozygous	Simplex Compound heterozygous	Simplex Homozygous	Autosomal recessive Homozygous
Variant cDNA position	c.604G > A	c.604G > A	c.340dupG	c.508C > T	c.425C > G, c.37-2AA > G	c.489C > G	c.496A > T
Protein change	p.Asp202Asn	p.Asp202Asn	p.Ala114GlyfsTer3	p.Arg170*	p.Ala142Gly, p.Ser12fs	p.Asp163Glu	p.Lys166*
Gait ataxia	+	+	+	+	+	+	+
Upper limb ataxia	+	+	+	+	+	+	+
Dysarthria	+	+	+	+	+	+	+
SARA score (age)	13 (30), 14 (35)	11 (30), 13.5 (36)	21.5 (54)	8.5 (31)	14 (36), 14 (37), 15 (39)	19 (5), 40 (6.5)	10.5 (12)
Age at last exam	35	36	54	31	39	6.5	12
Oculomotor signs observed	+	+	+	+	+	+	+
Cerebellar dysarthria	+	+	+	+	+	+	+
Muscle weakness	-	-	-	-	Ptosis	-	-
Cognitive impairment	-	-	-	-	Learning disability in childhood	-	Learning disability
Hearing impairment	-	-	-	-	-	-	+ Profound
MRI (cerebellar atrophy)	+	+	+	+	+	+	+

cause loss of function (LOF), indicating that PRDX3 failure might lead to cerebellar deformities in humans. Other variants identified in the WES filtration process are summarized in supplementary table 1.

Pathogenicity score

The identified variant was declared disease causing by majority of the online available tool. The variant was also not observed in public databases such as gnomAD, ExAC, and in-house exomes.

Discussion

In the present study, we clinically and genetically evaluated a single proband (female, II-1, 15 years old) exhibiting variable phenotypic severity highlighted by displaying features such as GDD, cerebellar atrophy, hypotonia, speech issues, dystonia, and profound hearing impairment. She was born to healthy consanguineous parents and harbored a biallelic disease causing-variant in the *PRDX3* gene with early disease onset, exhibiting features such as global developmental delay and cerebellar atrophy hypotonia, speech issues, and profound hearing impairment.

Rebelo et al. [5] reported five patients showing cerebellar dysarthria and oculomotor signs, which is also evident in our patient. In this report, learning difficulty was observed in only one patient, which is also apparent in our index. Recently, Martínez-Rubio et al. [12] reported a 19-month proband suffering from cerebellar atrophy, peripheral neuropathy, loss of autonomous ambulation, dysmetria, dysarthria, tremor, and abnormal ocular pursuit movements. At age 23, MRI revealed cerebellar atrophy (52–61% decrease) [12]. Similarly, one of the patient reported by Rebelo et al. [5] revealed a homozygous variant (c.508C > T; p.Arg170*), which is located in near proximity to our variant (c.496A > T; p.Lys166*). Interestingly, our patient had learning difficulties and hearing impairment that was not observed in the patient reported previously. The slight delineation of the phenotype might be due to the nature and position of mutation.

Our index also showed profound hearing impairment (Fig. 1H) and dystonia that was not previously observed, thus expanding the clinical spectrum of this new genetic condition. This confirms that our patient displayed more severe clinical presentation as compared to the previously reported cases. As only five cases have been documented in the literature [5], a proper genotype–phenotype correlation was challenging to perform.

It is interesting to note that the two patients (1 and 2) reported by Rebelo et al. [5] despite having the same age (36 years) and the homozygous for the same variant (c.604G > A; p.Asp202Asn) displayed variable onset of the disease and variable phenotypes. Patient 1 showed saccadic pursuit and hypermetric saccades, while patient 2 had hypermetric saccades and gaze-evoked nystagmus. In addition, patient 1 had myoclonus of the trunk, arms, and legs, while patient 2 did not present such features. Despite having the same variant, these variations in the clinical presentation might be contributed by genetic modifiers.

In addition, Rebelo et al. [5] revealed decreased levels of maximal mitochondrial respiration and spare respiratory capacity in patient fibroblasts, indicating that mitochondria can be compromised under conditions (and in tissues) of high energy demand. The impaired mitochondrial metabolism in combination with the accumulation of increased levels of H_2O_2could be considered a possible pathological disease mechanism in our patients [5]. This is supported by other studies that have reported induced oxidative stress and mitochondrial dysfunction upon *PRDX3*knockdown [13]. Earlier results also showed decreased glutathione peroxidase activity in all PRDX3 fibroblasts relative to control fibroblasts. This indicated that loss of PRDX3 interferes with the glutathione peroxidase pathway, probably due to crosstalk between electrons that flow between the two pathways [14]. Likewise, it has been reported that the knockdown of PRDX6 (peroxiredoxin) also decreases glutathione peroxidase activity in lung cancer cells [15]. PRDX3 downregulation in cerebellar medulloblastoma causes a significant decrease in cell viability accompanied by increased accumulation of H_2O_2, supporting oxidative stress as the underlying pathological mechanism [4]. KO of *Drosophila*Prx3 resulted in decreased lifespan, increased susceptibility to oxidative stress, and induced brain degeneration. Moreover, ubiquitous loss of Prx3 in Drosophila resulted in motor behavior defects, characterized by aberrant locomotion trajectories [5].

The human PRDX3 consists of two main domains: the mitochondrial targeting sequence (MTS) and the PRX_Type 2-cys domain (Fig. 1I). Notably, the variant (p.Lys166*), identified in our patient is located in the PRX_Type 2-cys domain, thus might result in a partial non-functional PRDX3 protein, which might be subjected to nonsense-mediated decay (NMD). PRDX3 forms a dodecamer ring complex containing 12 identical PRDX3 subunits, as depicted by the 3D structure protein model (Fig. 1J). Structural analysis revealed that this nonsense mutation (PRDX3Lys166*) results in an incomplete and non-functional protein subunit (Fig. 1J), affecting the protein complex assembly.

PRDX3 is localized in the mitochondria, the primary source of free radicals generated by electron leakage from the respiratory chains during oxidative phosphorylation. Ablation of *prdx3*in mice has been shown to cause decreased physical strength, decreased mitochondrial copy number in the skeletal muscles, and loss of hippocampus cells due to oxidative stress and mitochondrial impairment [15,16].

In conclusion, we identified a peculiar case with a pathogenic variant in the *PRDX3* that further proves an essential yet unexplored role of PRDX3 in causing cerebellar ataxia and associated malformations.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10048-022-00701-9.

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Author contribution Rafeeq MM and Umair M drafted the manuscript and performed and analyzed the data. Bilal M and Habib AH performed protein modeling. Waqas A, Sain ZM, and Alam MZ performed clinical analysis. Ali RH and Umair M performed data analysis and reviewed the final manuscript.

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

Declarations

Ethics approval and consent to participate The study was approved by the Institutional Research Committee. Written informed consent was obtained from the patients.

Consent for publication The parents of the patients provided written informed consent for publication of the case details and analysis.

Competing interests The authors declare no competing interests.

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