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A homozygous founder variant in *PDE2A* causes paroxysmal dyskinesia with intellectual disability

Hammad Yousaf[1] | Shagufta Rehmat[2] | Muhammad Jameel[1,2] | Rabab Ibrahim[3] | Sohana Nadeem Hashmi[3] | Ehtisham Ul Haq Makhdoom[4] | Justyna Iwaszkiewicz[5] | Saadia Maryam Saadi[1] | Muhammad Tariq[1] | Shahid M. Baig[1,3] | Mathias Toft[6,7] | Ambrin Fatima[3] | Zafar Iqbal[7]

[1]National Institute for Biotechnology and Genetic Engineering College, Pakistan Institute of Engineering and Applied Sciences (NIBGE-C, PIEAS), Faisalabad, Pakistan

[2]Center for Regenerative Medicine and Stem Cell Research (CRM), The Aga Khan University, Karachi, Pakistan

[3]Department of Biological and Biomedical Sciences, The Aga Khan University, Karachi, Pakistan

[4]Neurochemicalbiology and Genetics Laboratory (NGL), Department of Physiology, Faculty of Life Sciences, Government College University, Faisalabad, Pakistan

[5]Molecular Modeling Group, SIB Swiss Institute of Bioinformatics, Lausanne, Switzerland

[6]Institute of Clinical Medicine, University of Oslo, Oslo, Norway

[7]Department of Neurology, Oslo University Hospital, Oslo, Norway

Correspondence
Zafar Iqbal, Department of Neurology, Oslo University Hospital, P.O. Box 4950 Nydalen, N-0424 Oslo, Norway.
Email: zafar.iqbal@ous-research.no; iqbal.z.phd@gmail.com

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Abstract

Intellectual developmental disorder with paroxysmal dyskinesia or seizures (IDDPADS, OMIM#619150) is an ultra-rare childhood-onset autosomal recessive movement disorder manifesting paroxysmal dyskinesia, global developmental delay, impaired cognition, progressive psychomotor deterioration and/or drug-refractory seizures. We investigated three consanguineous Pakistani families with six affected individuals presenting overlapping phenotypes partially consistent with the reported characteristics of IDDPADS. Whole exome sequencing identified a novel missense variant in *Phosphodiesterase 2A* (*PDE2A*): NM_002599.4: c.1514T > C p.(Phe505Ser) that segregated with the disease status of individuals in these families. Retrospectively, we performed haplotype analysis that revealed a 3.16 Mb shared haplotype at 11q13.4 among three families suggesting a founder effect in this region. Moreover, we also observed abnormal mitochondrial morphology in patient fibroblasts compared to controls. Belonging to diverse age groups (13 years-60 years), patients presented paroxysmal dyskinesia, developmental delay, cognitive abnormalities, speech impairment, and drug-refractory seizures with variable onset of disease (as early as 3 months of age to 7 years). Together with the previous reports, we observed that intellectual disability, progressive psychomotor deterioration, and drug-refractory seizures are consistent outcomes of the disease. However, permanent choreodystonia showed variability. We also noticed that the later onset of paroxysmal dyskinesia manifests severe attacks in terms of duration. Being the first report from Pakistan, we add to the clinical and mutation spectrum of *PDE2A*-related recessive disease raising the total number of patients from six to 12 and variants from five to six. Together, with our findings, the role of *PDE2A* is strengthened in critical physio-neurological processes.

KEYWORDS
consanguinity, founder mutation, IDDPADS, Pakistani population, *PDE2A*, WES

1 | INTRODUCTION

Paroxysmal dyskinesias are a group of neurogenetic disorders characterized by episodes of abnormal involuntary movements having specific triggers, with variable age of onset and durations.[1] These disorders involve recurrent episodes of ballism, chorea, or dystonia. Other conditions such as periodic paralysis, ataxia, tremor, neuromyotonia, myoclonus, or epilepsy can also occur in association with paroxysmal dyskinesia.[2] Related to paroxysmal dyskinesias, IDDPADS (OMIM# 619150) is an ultra-rare autosomal recessive disease caused by biallelic variants in the phosphodiesterase 2A (*PDE2A*) featuring early onset paroxysmal dyskinesia, psychomotor developmental delay and variable incidence of epileptic seizures.[3]

PDE2A is located on chromosome 11q13.4 and encodes a cyclic guanosine monophosphate (cGMP)-dependent 3′,5′-cyclic phosphodiesterase. This protein has specificity for both intracellular second messengers, cGMP, and cyclic adenosine monophosphate (cAMP), which are the key regulators for many crucial brain processes such as neurotransmitter specification, neuronal connectivity, axon guidance, and cell-cycle regulation.[4–6]

So far, only six patients with biallelic variants in *PDE2A* are reported. In 2018, Salpietro et al., reported a 12 year old patient from the Canary Islands harboring a homozygous missense variant presenting developmental delay, intellectual disability (ID), chronic choreic movements, fluctuating dyskinesia, and dystonic posturing.[7] Three patients were reported by Doummar et al., in 2020 featuring paroxysmal dyskinesia, impaired cognition, and psychomotor development.[3] In the same year, Haider et al., reported two patients of Iraqi descent with homozygous splice site change presenting seizures in both patients. However, only one had ataxia and none had dyskinesia or chorea.[8]

Here, we investigated three Pakistani families with six affected individuals born to cousin marriages. Patients aged from 13 to 60 years, presented paroxysmal dyskinesia, developmental delay, cognitive abnormalities, speech impairment, and seizures with variable disease onset. Whole exome sequencing (WES) was employed for genetic characterization that identified a novel variant in *PDE2A* (NM_002599.4: c.1514 T > C p.(Phe505Ser)). The patient's clinical presentation and abnormal mitochondrial morphology in fibroblasts complement the established phenotype of IDDPADS. Moreover, haplotype analysis suggested the founder effect of the identified variant.

2 | METHODS

2.1 | Research subjects and clinical evaluation

We studied three multigenerational families from Attock, a district in the province Punjab of Pakistan. All families belong to the same tribe, locally called as "Malyar." The probands were clinically assessed by the neurophysicians at nearby tertiary care hospitals and remained without a definitive diagnosis. These families were identified through our local sources and were visited at their homes. Clinical history was taken after a detailed interview of the parents and consulting medical records. After taking informed consent, peripheral blood was obtained from all available individuals (parents, affected individuals, and healthy siblings). Genomic DNA was extracted from all available individuals using standard protocols.[9]

2.2 | Whole exome sequencing and data analysis

WES from DNA of four individuals (Family A-IV:2; Family B-V:1; Family C-V:1 and V:5) was performed at the Novogene Co., Ltd (Cambridge, UK). In brief, Agilent SureSelect Human All Exome V6 (Agilent Technologies, Santa Clara, CA, USA) was used to capture the whole exome and subsequent paired-end (PE150) sequencing was performed on an Illumina platform, NovaSeq 6000 (Illumina, Santa Clara, CA, USA). The detailed methodology for WES and variant prioritization has been described elsewhere.[10] Functional annotation of the variants was carried out by Annotate Variation (ANNOVAR).[11] Variant filtering was carried out by FILTUS.[12] We also considered the presence or absence of homozygotes in the control population and the phenotypic relevance of the candidate genes implicated in human diseases with patient phenotypes (paroxysmal dyskinesia being the key phenotype).

Standard protocol for variant interpretation developed by the American College of Medical Genetics and Genomics (ACMG) was followed to interpret the sequence variants.[13] The candidate variant was confirmed, and segregation analysis was performed by Sanger sequencing. PCR primers and conditions used for variant verification are available on request.

2.3 | Haplotype analysis

We used annotated variant files of the four WES-analyzed individuals, to inspect the possible common haplotype around the genomic region on the long arm of chromosome 11, encompassing three identified homozygous variants located in the genes: *LRTOMT*, *PDE2A*, and *C2CD3*. We considered exonic SNPs in the target region to construct the haplotype.

2.4 | Intolerance to variation landscape and amino acid conservation analysis

Intolerance to variation at each position in human PDE2A was retrieved from MetaDome.[14] PDE2A sequence across different species was aligned using Clustal Omega at EMBL-EBI.[15] UniProt identifiers of the sequences from different species are as follows: *Homo sapiens*: O00408-1; *Pan troglodytes*: A0A6D2W1U4; *Loxodonta africana*: G3TBE3; *Equus caballus*: F6UGJ6; *Mus musculus*: A0A1B0GRJ9; *Rattus norvegicus*: F8WFW5; *Bos taurus*: A0A3Q1MZ56; *Felis catus*: A0A337S7P8; *Oryctolagus cuniculus*: G1U4Q4.

2.5 | Primary skin fibroblasts

Skin biopsies were derived from patient IV:3 from Family A, his father (III-1), and an unrelated healthy individual as control. Primary skin fibroblasts were derived from these biopsies and were grown in Dulbecco's Modified Eagle Medium (DMEM) media supplemented with 1% penicillin–streptomycin, 1% glutamine, and 20% Fetal Bovine Serum (FBS) in sterile conditions at 37°C incubator with a supply of 5% CO_2.

2.6 | Mitochondrial morphology analysis

Fifty thousand cells were seeded on poly-ornithine (PLO) coated glass coverslips (12 mm) in 12 well culture plates. After 24 h, cells were washed with 1X PBS and incubated with 100 nM Mito Red (Sigma: 53271) for 45 min at 37°C. After incubation, cells were fixed using 4% paraformaldehyde in 1x PBS, washed three times in PBS for 5 min each, and stained with 25 μM DAPI for 10 min. Coverslips were washed with 1x PBS and mounted for imaging. Images were taken using Zeiss AxioImager microscope at 20x resolution (N.A. 0.45). For analysis, more than 100 cells in each replicate (the experiment was run in triplicate) were counted using the cell counter plugin of ImageJ software (https://fiji.sc/; Fiji version 1.52p). The percentage of cells displaying aberrant mitochondrial morphology was calculated in each replicate.

2.7 | In silico modeling of PDE2A variants

For the analysis of the PDE2A mutations, the crystal structure stored under 3IBJ code[16] in the Protein Data Base, and AlphaFold2 derived protein model from AlphaFold2 Database[17,18] was used. The model and crystal structure of PDE2A as well as mutations were visualized using UCSF Chimera software.[19] The FoldX software and AlphaFold2 model were used to assess the impact of mutations on protein stability.[20]

2.8 | Statistical analysis

Statistical analysis was carried out with ordinary one-way ANOVA followed by Tukey's multiple comparisons test using Prism3 software. Data are shown as mean ± standard error of the mean represented by error bars.

3 | RESULTS

3.1 | Clinical manifestations and history

3.1.1 | Family A

The four-generation consanguineous family (Figure 1A) has two affected male siblings, IV:2 and IV:3, who were aged 17 and 15 years, respectively. Patients presented moderate ID, dysarthria, and frequent drug-refractory generalized tonic clonic seizures (Table 1). The head circumference (HC) of individuals IV:2 and IV:3 was 54.5 and 54 cm, respectively. A typical dyskinesia attack lasts between 10 and 15 min for individual IV:2 and occurs 15–30 times a day. Individual IV:3 experiences 10–20 paroxysmal dyskinesia attacks per day showing non-rhythmic chorea-dystonic moments of all four limbs (online resource; Video S1 IV:3), each lasting for 2–3 min. The patients were developmentally delayed and achieved sitting at 2 years, unaided standing at around 4 years, and have normal gait except for intermittent sudden flexion at the knee joint (online resource; Video S2 IV:2). Moreover, IV:3 also presented dextrocardia in addition to the features mentioned above.

3.1.2 | Family B

This family consists of three affected individuals from two related loops (Figure 1A). One patient, who reportedly featured a similar phenotype as the living patients, had died. The affected individuals V:1 (online resource; Video S3) and III:2 were enrolled at the age of 13.5 and 60 years, respectively. The proband in this family (V:1) was born to consanguineous parents at term with uneventful obstetrics and gynecology (OBGY) history. Interestingly, only one patient, III:2, showed permanent choreodystonia (Table 1). Both patients are unable to communicate and have inefficient social interactions. Due to cognitive impairment, they are unable to take care of personal hygiene and feeding. Both patients in this family have recurrent epileptic seizures, with frequent foaming, occurring daily. Individual V:1 electroencephalogram (EEG) was conducted during wakefulness and is unremarkable (Table 1).

3.1.3 | Family C

In family C, there are two related consanguineous branches with one affected individual in each branch (Figure 1A). Individual V:1 was aged 22 years with a HC of 55.5 cm at the time of investigation. He was born at term with uneventful gestational and birth history. He displayed neurodevelopmental disease through paroxysmal dyskinesia (beginning at 3 months of age), moderate ID, choreoathetoid movements, and delayed developmental milestones (Table 1). He is bedridden and dependent for daily needs like feeding. Progressive psychomotor deterioration has been noticed as he was able to walk till 15 years of age and lost ambulation since then. At the time of ascertainment, he could stand and walk with support only. The second patient in this family (V:5) was aged 31 years with HC of 54.5 cm at the time of enrolment., He was born at term with uneventful OBGY history. His clinical presentation is similar to the other patient with few disparities (Table 1). In contrast to the patient V:1, he manifests permanent choreodystonia that started at 8 months of age. He cannot take care of himself and experiences tonic clonic seizures daily, associated with backward propulsion (Table 1).

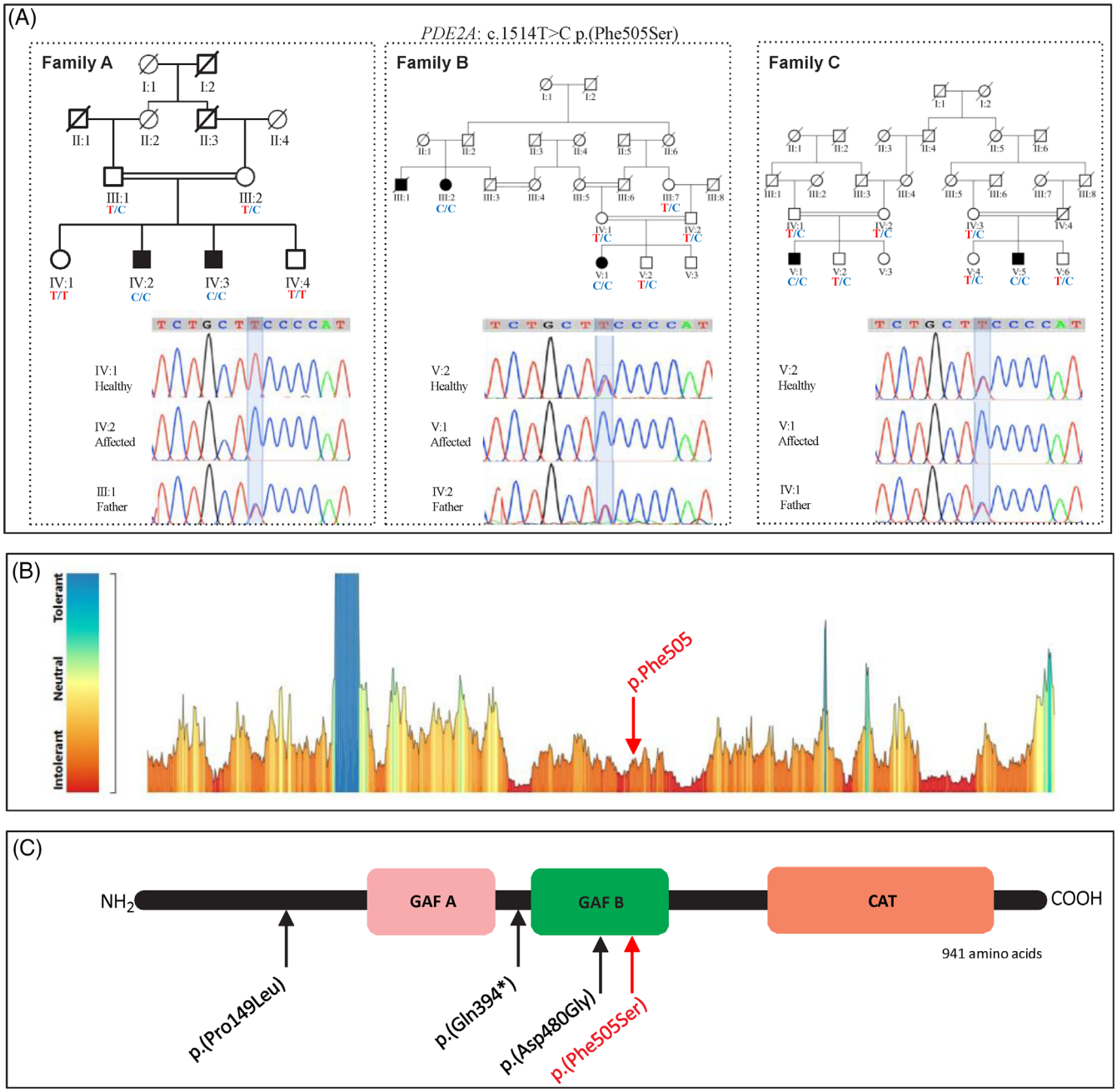

FIGURE 1 A founder homozygous missense variant in *PDE2A* is present in three consanguineous families. (A) Pedigrees of the three Pakistani families with IDDPADS in this study. Filled boxes and circles represent affected males and females, respectively. Double line indicate consanguinity. *PDE2A* genotype at position NM_002599.4: c.1514T > C is indicated for each individual as T/T (wild-type), T/C (heterozygous), and C/C (homozygous variant). Representative chromatograms from each family are also given. (B) Intolerance to variation landscape of 941 amino acid long PDE2A. Note that the p. Phe505 affected by the variant identified in this study is predicted to be intolerant to change. (C) Schematic domain structure of PDE2A and location of the disease-causing variants across the domains. The variant in red is identified in this study. Two of the five reported variants affect splice sites (c.1922 + 5G > A and c.323 + 1G > A) and are not shown. [Colour figure can be viewed at wileyonlinelibrary.com]

3.2 | Whole exome sequencing identifies a likely pathogenic founder variant in *PDE2A*

We acquired good quality WES data with at least 93% base calls having Phred-scaled quality score greater than 30 (Q30), and on an average 86.4% and 96.8% of the bases were covered with the depth >50x and >20x, respectively, in the target region.

Based on overlapping clinical features of the affected individuals in three families, first cousin relatedness of their parents, and families belonging to the same tribe, we hypothesized an autosomal recessive mode of inheritance and investigated common rare homozygous variants among them. Our filtering criteria revealed only three shared homozygous variants among the WES-analyzed patients of three families: GenBank: NM_001145307.5: c.438-2A > C in *LRTOMT*,

TABLE 1 Clinical features of individuals with homozygous variants in *PDE2A*.

	Family A		Family B		Family C		Doummar et al., 2020[3]			Salpietro et al., 2018[7]	Haider et al., 2020[8]	
	IV:2	IV:3	V:1	III:2	V:1	V:5	Patient 1	Patient 2	Patient 3	Patient 1	III:1	III:3
Origin	Pakistan						Moroccan		Caucasian	Canary Island	Iraq	
Gender	M	M	F	F	M	M	F	M	M	M	F	F
Age (years)	17	15	13.5	60	22	31	9	15	26	12	14	10
Variation in *PDE2A* (NM_002599.4)	c.1514 T > C p.(Phe505Ser)	c.1514 T > C p.(Phe505Ser)	c.1514 T > C p.(Phe505Ser)	c.1514 T > C p.(Phe505Ser)	c.1514 T > C p.(Phe505Ser)	c.1514 T > C p.(Phe505Ser)	c.1180C > T p.(Gln394*)	c.1180C > T p.(Gln394*)	c.1922 + 5G > A p.(Ala618Valfs56); c.446C > T p.(Pro149Leu)	c.1439A > G p.(Asp480Gly)	c.323 + 1G > A	c.323 + 1G > A
Characteristics of paroxysmal dyskinesia												
Age at onset	7 years	5 years	3.5 years	3 years	3 months	8 months	17 months	None	7 years	2 years	None	None
Triggers	Crowds, Noise, light etc.	Crowds, Noise, light etc.	Social interaction	Random	Social interaction	Random	Sudden movements	NA	Emotional stress, sudden sensorial stimuli	Emotional stress, sudden movement	None	None
Frequency and duration	15–30/day 10–15 min	10–20/day 2–3 min	10–20/day <1 min	10–20/day <1 min	10–20/day <1 min	10–20/day <1 min	>100/day <1 min	NA <1 min	30–50/day 2–5 min	>1000/day <1 min	None	None
Chronic involvement												
Permanent choreodystonia (age of onset)	No	No	No	Yes (3 years)	No	Yes (8 months)	Yes (2.5 years)	No	No	Yes (7 years)	No	No
Hypotonia	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	NA	NA	NA
Intellectual disability	Moderate	Moderate	Moderate	Severe	Moderate	Mild	Moderate to severe	Moderate	Moderate	Severe	Learning difficulties reported	Learning difficulties reported
Developmental milestones												
Age at sitting	2 years	2 years	1.5 years	Bed ridden	2 years	2.5 years	9 months	NA	NA	NA	Delayed	Delayed
Standing	Normal	Normal	Normal	Bed ridden	With support	With support	Normal	NA	NA	NA	Delayed	Delayed
Walking	Normal	Normal	Normal	Bed ridden	Normal till 15 years; bedridden now	With support	With support	NA	Started at 15 months	NA	Started at 18 months	Started at 18 months. Has ataxia since 8 years of age
Speech	Dysarthria	Dysarthria	Dysarthria	Dysarthria	Dysarthria	Dysarthria	Can speak few words	NA	Delayed	Language difficulties reported	Delayed	Delayed
Progressive psychomotor deterioration	Yes	Yes	NA	NA	Yes	Yes	Yes	NA	Yes	Yes	Yes	Yes
Seizures/epilepsy												
Epileptic seizures	Yes	Yes	Yes	Yes	Yes	Yes	Unclear	Yes	No	No (1 febrile seizure at 2 years)	Yes	Yes
Type of seizure	Tonic clonic/ Generalized	Tonic clonic/ Generalized	Upper limb only	NA	Myoclonic pattern/Focal	Tonic clonic/ Generalized	NA	Upper limb only	NA	NA	NA	NA
Foaming	Yes	Yes	Yes	Yes	No	No	NA	NA	NA	NA	NA	NA
Treatment with anticonvulsants and any improvement	Yes. Not effective after 1 week	Yes. Not effective after 1 week	Yes. NA	Yes, No significant effect	Yes. NA	Yes. NA	Yes, No significant effect	Yes. Not effective after 1 month	Yes, No significant effect	NA	Partial improvement	Partial improvement

TABLE 1 (Continued)

	Family A		Family B		Family C		Doummar et al., 2020[3]			Salpietro et al., 2018[7]	Haider et al., 2020[8]	
	IV:2	IV:3	V:1	III:2	V:1	V:5	Patient 1	Patient 2	Patient 3	Patient 1	III:1	III:3
Sudden falls	No	No	Yes	Bed ridden	Cannot stand without support	Cannot stand without support	NA	NA	Yes	Yes	NA	NA
Backward propulsion	No	No	NA	NA	Yes	Yes	Yes	NA	NA	Yes	NA	NA
Other information	NA	NA	EEG: Unremarkable	NA	EEG: At 20 years; myoclonic patterned focal seizures Brain MRI: Normal	NA	Brain MRI: Normal	Brain MRI: Normal	Brain MRI: Normal	NA	Brain CT: Dilatation of lateral ventricles	Brain MRI: Normal

Abbreviations: CT, computed tomography; EEG, electroencephalogram; F, female; M, male; MRI, magnetic resonance imaging; NA, not available.

NM_002599.4: c.1514 T > C p.(Phe505Ser) in *PDE2A*, and NM_001286577.2: c.704C > T p.(Pro235Leu) in *C2CD3*. The variants located in *LRTOMT* and *C2CD3* were further filtered out because they are classified as benign by the ClinVar and ACMG criteria respectively and are present in homozygous state in the gnomAD database (Table S1). Since *PDE2A* is known to cause IDDPADS with clinical manifestation overlapping with probands, we considered p.(Phe505-Ser) as the only plausible shared variant to explain the disease phenotype. This variant is absent in the gnomAD database [accessed December 5, 2022], has CADD score of 29.2 (CADD model GRCh37-v1.6),[21] and is categorized as likely-pathogenic by the ACMG criteria (i.e., PM1, PM2, PP1, PP3, and PP4). Importantly, segregation analysis in all available persons of three families revealed complete segregation of the *PDE2A* variant with the phenotype (Figure S1). The genotype of all the tested individuals is mentioned (Figure 1A).

All the other unshared rare homozygous variants identified in each family are listed (Table S1). None of the other variants seem relevant to the presented phenotypes in all three families. These variants were categorized as benign, likely-benign, and VUS, except a reported pathogenic variant[22] in Family A, GenBank: NM_017570: c.2608dupC p.(His870Profs*92) in *OPLAH*, known to cause 5-oxoprolinase deficiency (OMIM# 260005); a benign biochemical condition. We considered it as an incidental finding and completely irrelevant to the observed clinical phenotype in Family A (Table S1).

Retrospectively, using the annotated variant files of the four WES-analyzed (Family A-IV:2; Family B-V:1; Family C-V:1 and V:5) individuals of three families, we inspected the region flanking the identified homozygous *LRTOMT* and *C2CD3* variants on chromosome 11. Our analysis revealed a shared haplotype stretch of at least 3.16 Mb among all four patients at chr11:71249386–74 419 378 [hg19] (Figure S2A).

3.3 | p.Asp480Gly and p.Phe505Ser are predicted to affect the structural stability of PDE2A

The Asp480Gly and Phe505Ser mutation sites are found on the GAF B domain (Figure 1C), that binds to the allosteric activator of PDE2A, that is, cGMP. The residue Asp480 side chain is located on a loop on the outer part of the GAF B domain and forms a hydrogen bond with a backbone of Tyr483 (Figure 2). The stability loss upon Asp480Gly mutation was estimated by FoldX as 1.78 kcal/mol.

The Phe505 is located on the domain's central beta-strand and is surrounded by hydrophobic residues: Ile475, Ala518, Phe540, and Ale537 (Figure 2). The mutation to polar serine will likely impair the local structure. According to FoldX program, the average loss of protein stability in F505S variant, compared with the wild-type protein is 4.45 kcal/mol.

Both variants cause some loss of structural stability of the cGMP binding domain, which might perturb the binding of the allosteric activator of PDE2A and the function of the enzyme.

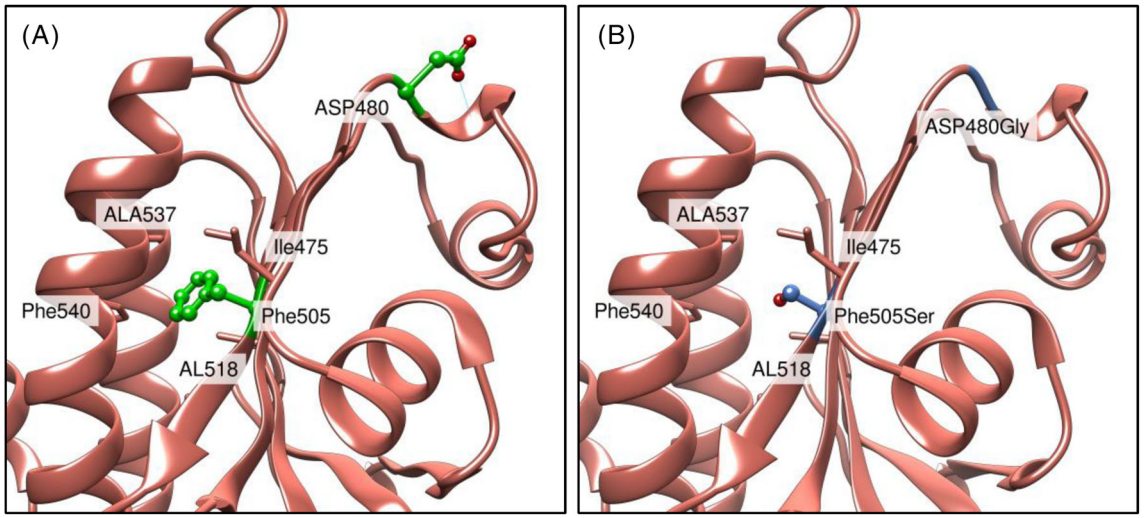

FIGURE 2 Molecular modeling of PDE2A: p.Asp480Gly and p.Phe505Ser (A) Wild-type PDE2A sequence model showing Asp480 (green) side chain forms a hydrogen bond with a backbone of Tyr483. Phe505 (green) is located on the domain's central beta-strand, interacting with hydrophobic residues: Ile475, Ala518, Phe540, and Ale537. (B) Mutated Gly480 (blue) is unable to form the hydrogen bond present in the wild-type protein, while the replacement of large aromatic Phe side chain by a smaller and polar Ser in position 505 (blue) will cause an empty space in the core of the protein probably leading to destabilization of the local structure. [Colour figure can be viewed at wileyonlinelibrary.com]

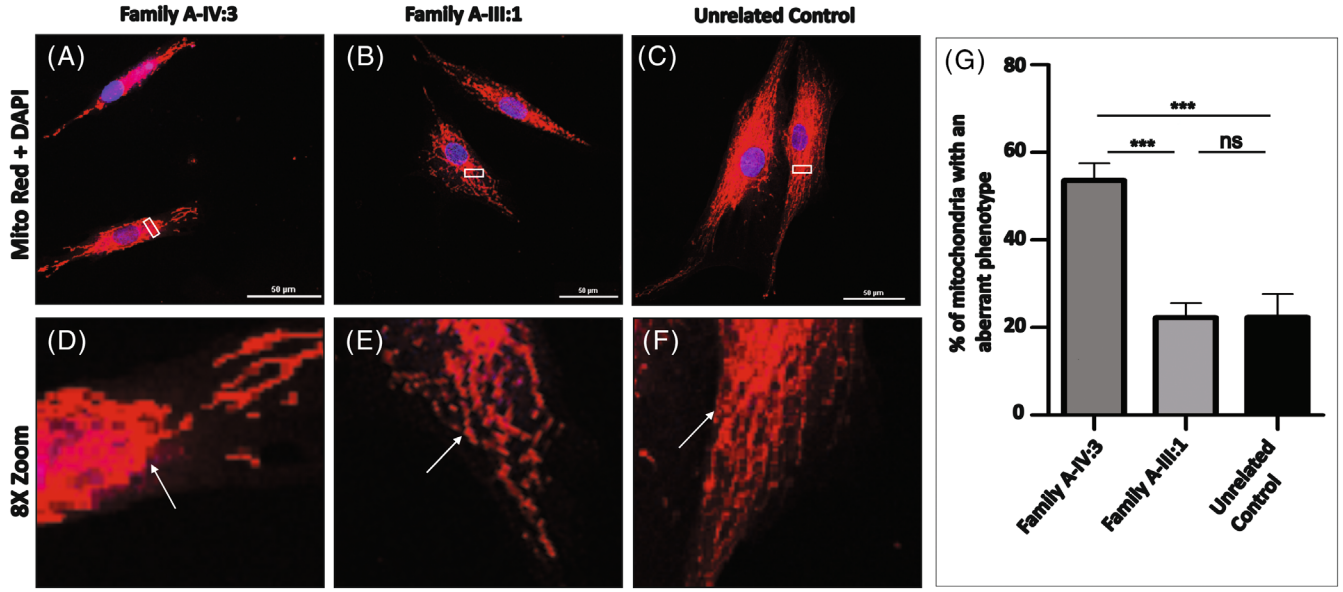

FIGURE 3 Biallelic *PDE2A*: c1514T > C bearing fibroblasts from patient show aberrant mitochondrial morphology. (A–C) Representative confocal images of fibroblasts labeled with Mito Red (red) and DAPI (blue). Boxes on Mito Red + DAPI images correspond to the 8× magnified insets below each panel (D–F). Arrow shows mitochondrial morphology in patient and control individuals at 8× zoom. The patient's fibroblasts displayed an atypical mitochondrial morphology with thicker and dotted mitochondrial filaments as compared to regular elongated morphology observed in controls. (G) Quantification shows significant aberrant mitochondrial morphology in patient cells (IV:3) compared to healthy father (III:1) and unrelated control. Cells with abnormal mitochondrial networks were counted in three independent experiments, each encompassing 100 cells, and calculated the mean of the three experiments. The significance was determined using ordinary one-way ANOVA followed by Tukey's multiple comparisons test. ***$p < 0.001$; Error bars indicate standard error of the mean. Scale bars in panels A–C: 50 μm, with equal sizing across panels. [Colour figure can be viewed at wileyonlinelibrary.com]

3.4 | Mitochondrial morphology is significantly aberrant in primary fibroblasts

Since *PDE2A* dysfunction is shown to affect mitochondrial morphology,[3] we further investigated whether *PDE2A*: c.1514 T > C results in any aberrations. Mitochondrial morphology in fibroblasts from patient IV:3 (Family A) with the homozygous *PDE2A*: c.1514 T > C variant was abnormal having thicker and more irregular mitochondrial filaments in comparison to the healthy heterozygous father (III:1) and the unrelated control. Furthermore, the mitochondrial

morphology of the heterozygous father was similar to the unrelated control (Figure 3).

4 | DISCUSSION

Biallelic variants in *PDE2A* are known to cause IDDPADS, an ultra-rare disease for which only six patients have been reported so far.[3,7,8] WES revealed a novel homozygous missense variant in *PDE2A*: p.(Phe505Ser) as a potential causative variant that segregated with the disease in three Pakistani families presenting paroxysmal dyskinesia, developmental delay, cognitive abnormalities, speech impairment and seizures with onset as early as 3 months in Family C to 7 years in Family A. The candidacy of *PDE2A* variant was supported by; (1) phenotypic match of the patients with IDDPADS (Table 1), (2) resulting abnormal mitochondrial morphology in patient fibroblasts (Figure 3), (3) the identified variant alters 'intolerant to change' amino acid as shown in the intolerance landscape generated through MetaDome[14] (Figure 1B), (4) variant affecting GAF (c**G**MP-specific phosphodiesterases, **a**denylyl cyclases and the bacterial transcription factor **F**hlA) B domain of PDE2A predicted to result in structural instability (Figure 1C, Figure 2) where cGMP binds allosterically and modulate enzymatic activity,[23] (5) the evolutionary conservation of the phenylalanine at position 505 and adjacent amino acids across various species (Figure S2B), (6) *PDE2A* is highly constrained for missense variants as reflected by a *z* score of 4.06.[24]

The clinical features of the patients in this study complement the already reported IDDPADS characteristics[3] such as early age onset of dyskinesia, occurring multiple times every day, drug-refractory seizures, ID, and motor delay. The earliest age of onset reported in the literature is 17 months.[3] We observed a trend in the duration of paroxysmal dyskinesia attacks with age of onset; later onset is associated with longer duration. In Family A, the age of onset is 5 (IV:3) and 7 years (IV:2) and both individuals experience 10–30 attacks per day, with longer durations (2–15 min) compared to the other two families wherein the onset is at 3.5 years or less with every attack lasting less than 1 min (Table 1). Consistently, Doummar et al., 2020[3] reported paroxysmal dyskinesia in patient 3 starting at 7 years, occurring 30–50 times per day with each attack lasting between 2–5 min. Of the 12 patients with biallelic *PDE2A* variants, only four display permanent choreodystonia (2/6 in this study and 2/6 in the previous patients),[3,7,8] reflecting that this is an inconsistent outcome of the disease. In line with the published data,[3,7,8] ID has been witnessed in all patients. Strikingly, all six patients in this study show seizures, which were only reported in 3/6 individuals previously.[3,7,8] Together with the previous reports, we noticed progressive psychomotor deterioration in all patients for which the data is available. Literature shows the intrafamilial variability in *PDE2A*-related recessive disease,[3,7] which has been observed in this study also. In Family C, individual V:1 exhibit focal seizures with a myoclonic pattern, while V:5 shows generalized tonic clonic seizures. Similarly, permanent choreodystonia in only one of the two related affected individuals in families B and C is worth mentioning. It is important to note that the phenotype of the patients studied by Haider et al.,[8] is not consistent with other reports[3,7] including the current study where paroxysmal dyskinesia is one of the predominant features (Table 1). Since the identified variant affects the splice site, the authors reasoned that residual "normal" splicing of the *PDE2A* transcript might be contributing to the milder phenotype matching atypical Rett syndrome.

The brain is the most complex human organ, working in a very intricate coordinated manner. Spatiotemporal maintenance of faithful coordination and cell–cell communication is crucial for the proper functioning of the nervous system. Important players in these processes are cAMP and cGMP second messenger signaling systems that transduce various extracellular signals to the effector systems within a single cell. The synthesis and degradation of these molecules need to be tightly regulated. Phosphodiesterases (PDE) are members of a family of proteins that includes 11 subfamilies with different substrate specificities that catalyze the hydrolysis of the 3′ phosphate bond of cAMP and cGMP to generate respective monophosphates.[25] Different substrate specificities, either cAMP, cGMP, or both; CNS distribution; and subcellular localization, enables these enzymes to ensure that these two molecules trigger unique responses consequent to common stimulus in different cell types, thus fine-tuning neuronal activity.[26] Comprising of two adjacent GAF domains; A and B, followed by a c-terminal catalytic domain that determines substrate specificity,[27] PDE2A is a dual substrate (cAMP and cGMP) hydrolytic enzyme that is activated by cGMP.[28] It is reported to be expressed in multiple tissues with the brain having the highest expression[29] where it is particularly localized in the cortex, hippocampus, and striatum.[30,23,27] Till date, five disease-causing variants have been reported in *PDE2A*. The missense variant p.(Asp480Gly) in the study by Salpietro et al.,[7] affects the GAF B domain of the protein like p.(Phe505Ser) identified in this study. The nonsense variant p.(Gln394*) in two siblings reported by Doummar et al., locates between the GAF A and GAF B domains while the third patient has a missense change p.(Pro149Leu) that lies upstream of the GAF A domain (Figure 1C). The second allele in this patient is a splice site variant (c.1922 + 5G > A) that skips exon 22 leading to a frameshift.[3] Similarly, Haider et al., also reported a splice site (c.323 + 1G > A) variant in two siblings.[8]

PDE2A has three isoforms (PDE2A1, PDE2A2, PDE2A3) that differ in their amino termini enabling different intracellular localization.[31] PDE2A1 is cytosolic, PDE2A2 localizes to mitochondria while PDE2A3 is largely expressed in the plasma membrane.[32] In mitochondria, PDE2A2 regulates the respiratory chain through cAMP/cGMP-dependent signaling regulation[33] and phosphorylation of Dynamin-related protein 1 (DRP1) modulating mitochondrial fission and survival.[32] The abnormal mitochondrial morphology (thicker and irregular mitochondrial filaments) in mutant fibroblasts points to a disturbed fission/fusion balance likely due to the PDE2A variant. In agreement with previous reports,[3,32] our findings further endorse the role of PDE2A in maintaining mitochondrial integrity and function.

Identifying the same disease-causing *PDE2A* variant in three families compelled us to suspect a mutational hotspot or a founder effect. The latter possibility is supported by the fact that these families belong to the same tribe (locally called as "Malyar") and the geographical location of Pakistan. Most importantly, the identification of a

shared haplotype in exome-sequenced affected individuals (Figure S2A) further strengthens the founder status of the PDE2A: p.(Phe505Ser) and suggests a common ancestor of these three families.

5 | CONCLUSION

We present three Pakistani families with a founder missense variant in *PDE2A*, thus adding to the mutation spectrum of the disease. Collating the clinical presentation of the patients reported so far, we noticed some discrepancies in disease outcomes that emphasize the need for comprehensive studies aimed at establishing genotype–phenotype correlation. Being the largest study to date on IDDPADS, this report will hold a key value in establishing consensus on the clinical outcome of *PDE2A*-related recessive disease. We also recommend screening for *PDE2A* c.1514T > C in families of matched ethnic group with suspected IDDPADS.

AUTHOR CONTRIBUTIONS

Hammad Yousaf, Shagufta Rehmat, Shahid M. Baig, Mathias Toft, Ambrin Fatima, and Zafar Iqbal contributed to the conception and design of the study. Hammad Yousaf, Shagufta Rehmat, Ambrin Fatima, and Zafar Iqbal acquired data. Hammad Yousaf, Shagufta Rehmat, Rabab Ibrahim, Sohana Nadeem Hashmi, Muhammad Tariq, Ambrin Fatima, and Zafar Iqbal contributed to drafting/revision of the manuscript. Justyna Iwaszkiewicz performed molecular modeling. Ambrin Fatima and Zafar Iqbal acquired funding. Hammad Yousaf, Muhammad Jameel, Saadia Maryam Saadi, and Ehtisham Ul Haq Makhdoom provided research subjects. All authors read the manuscript and approved the submitted version.

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CONFLICT OF INTEREST STATEMENT

The authors report no disclosures relevant to the manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

ETHICS STATEMENT

This study was carried out in accordance with the protocols of the Declaration of Helsinki protocols and was approved by the institutional review board of National Institute for Biotechnology and Genetic Engineering, Faisalabad, Pakistan. Written informed consent was obtained from all participants or parents prior to enrollment.

ORCID

Muhammad Tariq https://orcid.org/0000-0002-5334-403X

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SUPPORTING INFORMATION

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