Neurological Sciences
https://doi.org/10.1007/s10072-024-07419-6

ORIGINAL ARTICLE

JAM2 variants can be more common in primary familial brain calcification (PFBC) cases than those appear; may be due to a founder mutation

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Received: 13 November 2023 / Accepted: 20 February 2024

Abstract

Introduction Mutations in *JAM2* have been linked to ~ 2% of primary familial brain calcification (PFBC) cases. PFBC is a rare neurological disorder characterized by excessive calcium deposition in the brain. It causes movement disorders and psychiatric problems. Six other genes were identified as causing PFBC. However, the genetic basis of ~ 50% of PFBC cases remains unknown. This study presented the results of a comprehensive analysis of five unrelated Iranian PFBC families.

Methods Clinical and paraclinical features of all patients were recorded. Whole-exome sequencing (WES) was done on the DNAs of probands. Data was analyzed, and haplotypes were determined.

Results WES identified two homozygous variants in *JAM2* across four families: a novel variant, c.426dup:p.Ser143Leufs*23, in one family and a known mutation, c.685C > T:p.Arg229*, in the remaining three families. Haplotype analysis using six intragenic single-nucleotide polymorphisms (SNPs) in *JAM2* revealed an identical haplotype in probands who carried the same mutation, whereas two other probands presented diverse haplotypes.

Conclusion Based on our results, p.Arg229* may be a founder mutation in the Iranian population. The variant has been detected in two out of seven other reported *JAM2*-related families who may originate from the Middle East and exhibit an identical haplotype. Even though this particular mutation may not be classified as a founder mutation, it does appear to be a hotspot, given that it has been observed in 45% of the 11 *JAM2*-associated families. Our study expanded the clinical features and mutation spectrum of *JAM2* and revealed that mutations in *JAM2* may be more common than previously reported.

Keywords Primary familial brain calcification (PFBC) · FAHR disease · *JAM2* · Whole-exome sequencing (WES) · Founder mutation

Introduction

JAM2 is a gene that encodes junctional adhesion molecule 2, a member of the junctional adhesion molecule family, which plays an important role in the regulation of cell polarity and maintaining homeostasis in the central nervous system. The protein is expressed in cell types related to a neurovascular unit and is involved in cell–cell adhesion and endothelium permeability [1]. Recently, mutations in *JAM2* have been linked to an autosomal recessive form of primary familial brain calcification (PFBC; traditionally named FAHR by Karl Theodor Fahr in 1930), a rare neurological disorder characterized by extensive calcium deposition in the different parts of the brain, particularly the basal ganglia, thalami, and cerebellar nuclei [1, 2]. The symptoms of PFBC are highly variable and can affect patients on a spectrum ranging from being asymptomatic to severely impacted [3, 4]. The

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Published online: 05 March 2024

main features of PFBC are movement disorders and psychiatric or behavioral problems. The symptoms usually begin in mid-adulthood and worsen over time. Most affected individuals suffer from parkinsonism (bradykinesia, rigidity, and tremor). Psychiatric and behavioral problems, including difficulty in concentration, memory loss, personality changes, and psychosis with diverse severity and ages of onset, occur in 20 to 30% of PFBC cases. Affected individuals may also have dysphagia, dysarthria, headaches, episodes of extreme dizziness (vertigo), seizures, or urinary problems [4].

Genetically, PFBC is a rare inherited or sporadic neurodegenerative disease with a prevalence of less than 1/1,000,000 [5]. To date, mutations in seven genes have been identified in PFBC patients [4]: *SLC20A2* [6], *PDGFB* [7], *PDGFRB* [8], and *XPR1* [9] with an autosomal dominant (AD) pattern of inheritance and *MYORG* [10], *JAM2* [1, 2], and *CMPK2* [11] with an autosomal recessive (AR) pattern of inheritance. Despite the identification of these genes, the genetic basis of ~ 50% of the PFBC families and the precise pathophysiological mechanisms leading to idiopathic calcium deposition in brain structures are poorly understood [10]. Literature review have revealed that the most common and rare PFBC-related genes include *SLC20A2* (~ 60% of cases) and *JAM2* (~ 2% of cases), respectively [4]. Mutations in *JAM2* have only been reported twice in 2020 [1, 2], and no mutations in this gene have been reported since then. The frequency of mutations in other genes has been estimated ~ 13% in *MYORG*, ~ 13% in *PDGFB*, ~ 6% in *PDGFRB*, and ~ 6% in *XPR1* [4]. Mutations in *CMPK2* have been recently reported only in two families and those need to be confirmed [11].

Until now, a genetic examination of this particular disorder in Iran has not been conducted. Consequently, for the first time, we present a comprehensive account of the clinical and paraclinical characteristics as well as the outcomes of genetic analysis of five Iranian families affected by PFBC. This study will be the third report of PFBC families with *JAM2* mutations and suggests that the prevalence of mutations in this gene may be higher than expected particularly within societies with a high rate of consanguineous marriages. Moreover, it seems that patients with AR-PFBC have more severe symptoms than those with AD-PFBC [1], highlighting the need for more research and attention on AR-PFBC in these societies. Additionally, our study expands the spectrum of *JAM2* variants and provides the evidence of a probable founder mutation or even a hot spot codon in *JAM2*.

Materials and methods

This study was conducted according to the Declaration of Helsinki and approved by the ethics committee of the University of Social Welfare and Rehabilitation Sciences (USWR; IR.USWR.REC.1402.078) in Iran. Informed consent form was signed by the patients or their parents.

Subjects

Five unrelated PFBC families were referred to Genetics Research Center (GRC) at the USWR for genetic analysis from the Department of Neurology of Hazrat Rasool Hospital affiliated to the Iran University of Medical Sciences. Among those, the autosomal recessive pattern of inheritance was considered for two multi-affected families, PFBC3 and PFBC10, as affected siblings were born of a consanguineous marriages (Fig. 1). The disease appeared to be sporadic in three other families, although consanguineous marriage was observed in the parents of two families, PFBC2 and PFBC7 (Fig. 1).

Genetic analysis

DNA was isolated from the peripheral blood of probands and their family members by the salting-out protocol. Whole-exome sequencing by SureSelect V6-Post enrichment kit and Illumina HiSeq 4000 platform (Illumina, CA, USA) was performed for each proband. Different bioinformatics tools including Burrows-Wheeler Aligner (BWA), Picard, SAMTools, and Genome Analysis Toolkit (GATK) were applied to routine WES analysis. Sequence alignment was done against human reference genome UCSC NCBI37/hg19. Variant filtering was performed and all exonic, exonic splice, and splice site variants with a reported minor allele frequency (MAF) of less than 0.01 in the public genomic databases were considered [12]. Thereafter, based on recessive mode of inheritance in the PFBC3 and PFBC10 families, we considered the homozygous variants that were presented in the DNA of probands. In other probands, all homozygous and heterozygous variants were considered. The remaining variants were assessed to identify a disease-causing variant within any known PFBC or other neurodegenerative disease-causing genes. Finally, several *in silico* tools [13] were used for prediction of the probable effects of the candidate variants on the encoded proteins and variants were classified based on the American College of Medical Genetics (ACMG) criteria [14].

The candidate disease-causing variants in the *JAM2* gene (NM_021219), c.685C > T:p.Arg229* in exon 6 and c.426dup:p.Ser143Leufs*23 in exon 5, were confirmed in the respective probands and evaluated in family members by polymerase chain reaction (PCR) and Sanger sequencing by ABI Big Dye terminator chemistry and an ABI3130 genetic analyzer instrument (Applied Biosystems, Foster City, CA). The sequences of primers are available in supplementary file S1.

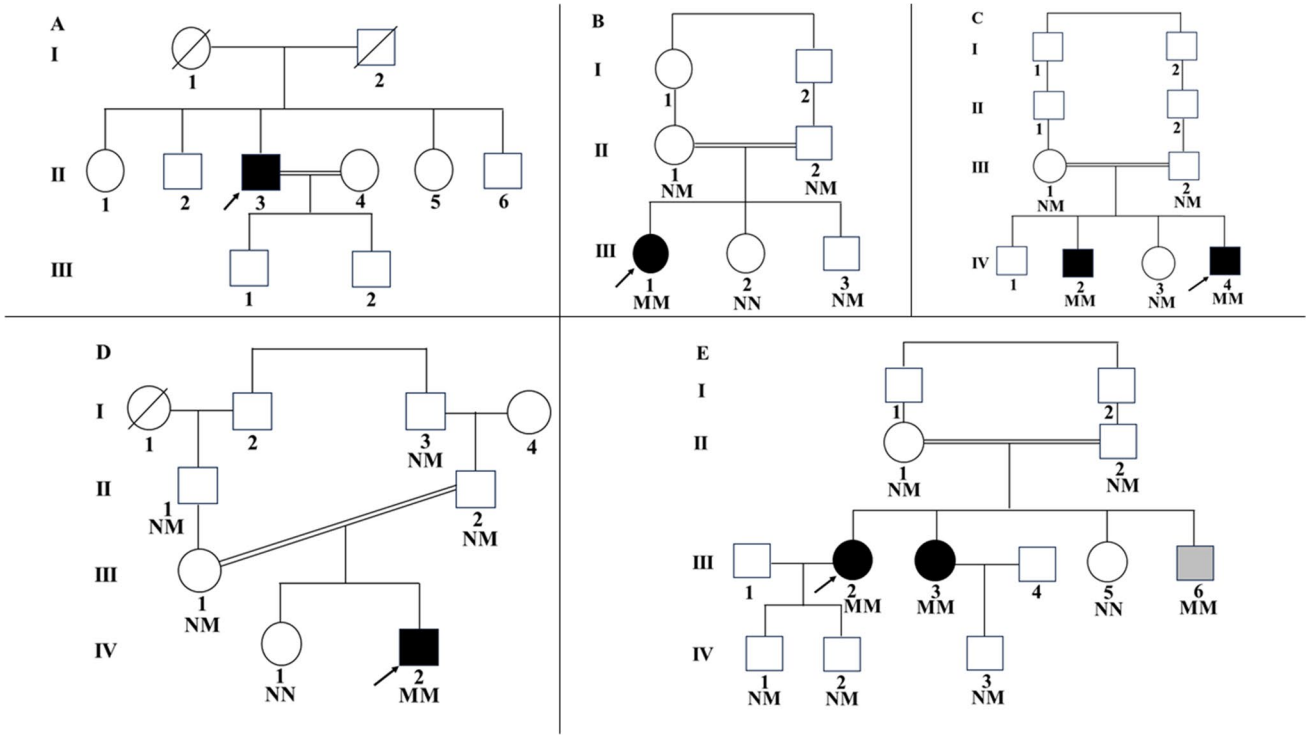


Fig. 1 The Iranian PFBC pedigrees: **A** PFBC1, **B** PFBC2, **C** PFBC3, **D** PFBC7, **E** PFBC10. The genotypes of the candidate variants for each family are shown when individuals were assessed. Arrows show probands. Unfilled circles and squares, normal individuals; black circles and squares indicate PFBC patients. Gray square indicates an affected individual with homozygous genotype who has not checked by a neurologist. Abbreviations: M mutated allele, N normal allele

Copy number variations (CNVs) were assessed in seven known PFBC genes as well as in other genes by analyzing of WES data using GermlineCNVCaller and Annotation and Ranking of Human Structural Variations (AnnotSV; https://lbgi.fr/AnnotSV/).

Given that four out of five probands were offspring of consanguineous marriages, the identification of runs of homozygosity (ROHs) was performed using AutoMap software. The software can perform homozygosity mapping using VCF file from WES [15]. The shared homozygous blocks of chromosome 21, on which the *JAM2* gene is located, of all probands were determined.

Haplotypes were also determined for all probands including the probands who carried p.Arg229* variant in the *JAM2* gene. Six intragenic single-nucleotide polymorphisms (SNPs) located within *JAM2* were identified in exome data of the probands. These SNPs were utilized to define a haplotype associated with the putative disease-causing variant c.685C > T:p.Arg229*.

Results

Clinical and paraclinical presentations

Clinical manifestations of all patients are summarized in Table 1. Details of clinical and radiological data of these individuals are meticulously documented herein.

PFBC1 family

The proband (PFBC1-II3) was a 60-year-old man who was born to non-consanguineous parents (Fig. 1A). His first symptoms were dyskinetic movements in shoulder and leg and tremor like movements of fingers, which appeared at the end of the age of 56. He referred to a neurologist (MR) when he was 57-year-old and had developed abnormal movements in his right upper and lower limbs. He suffered

Table 1 Detailed clinical features of Iranian PFBC patients with/without *JAM2* mutations

References		This study							
Patients ID		PFBC1-II3*	PFBC2-III1*	PFBC3-IV2	PFBC3-IV4*	PFBC7-IV2*	PFBC10-III2*	PFBC10-III3	PFBC10-III6
Variant in cDNA level		No mutation in PFBC-related genes	c.426dup	c.685C > T	c.685C > T	c.685C > T	c. 685C > T	c. 685C > T	c.685C > T
Variant in protein level		No mutation in PFBC-related known genes	p.Ser-143Leufs*23	p.Arg229*	p.Arg229*	p.Arg229*	p.Arg229*	p.Arg229*	p.Arg229*
Zygosity		–	Hom	Hom	Hom	Hom	Hom	Hom	Hom
Gender		Male	Female	Male	Male	Male	Female	Female	Male
Consanguinity		−	+	+	+	+	+	+	+
Nationality		Iranian	Iranian	Iranian	Iranian	Iranian	Iranian	Iranian	Iranian
Onset symptoms		Dyskinesia and tremor	Seizures	BMD	Limbs stiffness	Seizure	Non-specific manifestations like headache	Seizure	Seizure
Age at onset (y)		56	4	24	9	Childhood	22	3	3
Present age (y)		60	41	48	39	38	39	37	32
Movement disorders	Hypophonia	−	−	+	−	−	−	−	−
	Reduce arm swing	+	+	−	+	+	+	−	−
	Abnormal gait	Now unable to walk	+	+	+	−	+	+	−
	Parkinsonism	+	+	+	+	+	+	+	−
	Hypomimia	+	−	+	−	−	−	−	−
	Tremor	+	−	−	−	−	−	−	−
	Dystonia	+	+	−	−	−	−	−	−
Cerebellar signs	Shuffling gait with freezing	−	−	+	−	−	−	−	−
	Eye-related disorders	−	+	+	−	−	−	−	−
	Limbs dysmetria	−	+	−	−	−	−	−	−
	Slurred speech	+	+	+	+	−	+	+	−
Pyramidal signs	Brisk DTR	−	−	−	+	−	−	−	−
	Babinski sign	−	+	−	+	−	−	−	−
Psychiatric manifestations	Agitation	−	−	−	−	+	+	+	+
	Hallucination	−	−	−	−	+	−	−	−
	OCD	−	−	−	+	+	−	−	+
	BMD	−	−	+	−	−	−	−	−
	Depression	+	+	−	+	−	−	−	−
	Irritability	+	−	−	−	+	−	−	−
	Anxiety	−	+	−	−	+	+	+	+
Cognitive function	Memory dysfunction	−	−	+	−	−	+	−	−
	Cognitive decline	−	−	−	−	−	−	−	−

Table 1 (continued)

References		This study							
Patients ID		PFBC1-II3*	PFBC2-III1*	PFBC3-IV2	PFBC3-IV4*	PFBC7-IV2*	PFBC10-III2*	PFBC10-III3	PFBC10-III6
Other	Afford his ADL	−	+	+	+	+	+	+	+
	DM	+	−	−	−	−	−	−	−
	Seizure	−	+	−	−	+	−	+	+
	Urinary incontinence	−	+	−	+	−	+	−	−
	Dizziness	−	+	−	−	−	+	+	+
	Dysphagia	−	−	+	−	−	−	−	−
	Stooped posture	−	−	+	−	−	−	−	−
	Difficulty in arising from chair	−	−	+	−	−	−	−	−
	Fatigue	−	−	−	+	−	−	−	−
	Anorexia	−	−	−	+	−	−	−	+
	Slight hearing loss	−	−	−	+	−	−	−	−
	Headache	−	−	−	+	−	+	+	+
	Respiratory problems	−	−	−	−	+	−	−	−
	Ulcerative colitis	−	−	−	−	+	−	−	−
	Vomiting	−	−	−	−	−	+	+	+
Brain calcification	Bilateral basal ganglia	−	+	−	+	+	+	+	−
	Thalami	+	+	−	+	+	+	+	−
	Lentiform nuclei	+	−	−	−	−	−	−	−
	Dentate nuclei	−	+	+	−	+	+	+	−
	Subcortical white matter	−	+	+	+	+	+	+	−
	Striatum	−	−	+	−	−	−	−	−
	Cerebellum	+	−	−	+	−	−	−	−
	Midbrain	−	−	−	−	+	+	+	−
	Caudates	+	−	−	−	−	−	−	−

Hom homozygous, *y* year, *OCD* obsessive–compulsive disorder, *BMD* bipolar mood disorder, *ADL* activities of daily living, *DM* diabetes mellitus, *DTR* deep tendon reflexes

*Proband

from slurred speech, depressed and irritable moods, and diabetes mellitus (DM). Neurological examination showed oromandibular dystonia (OMD), severe dystonic movements of right hand with tremor, and mild hypomimia. There was no history of seizures. Now, in 60 years old, he is unable to perform activities of daily living (ADL) (Table 1). All of the biochemical examinations including parathyroid hormone and serum calcium and phosphate were within the standard ranges (Supplementary file S2). Computed tomography (CT) scan of the brain revealed calcification of caudates, lentiform nuclei, thalami, and cerebellum (his total brain calcification score, TCS, was 2 according to Nicolas et al.) [8] (Supplementary figure S1, A and B).

PFBC2 family

The proband (PFBC2-III1) is a 41-year-old female who was born to consanguineous parents (Fig. 1B).

According to the explanations provided by her mother, she had seizures at the age of four which were partially controlled by phenobarbital and she has been free of seizures since the age of 12 years. She also experienced slowly progressive speech disturbance and abnormal gait at the age of 6 years. Despite these symptoms, she managed to successfully complete an undergraduate program, perform daily tasks, and secure employment at a reputable organization. Neurological examination at the age of 36 years revealed slurred speech, bilateral symmetrical bradykinesia and rigidity in the upper and lower limbs (parkinsonism), oromandibular dystonia and dystonic posture of hands, and Babinski sign. Arm swing was reduced during walking and she was not able to walk in tandem. Finger-to-nose test was disturbed. Her memory was normal at age of examination (Table 1). Her optic disk was normal but vertical saccades were limited. Biochemical tests including parathyroid hormone and serum calcium and phosphate were within the normal range (Supplementary file S2). Her brain CT scan revealed heavy calcification in bilateral basal ganglia, thalami, dentate nuclei, and subcortical white matter (Fig. 2). The total calcification score was 4.

PFBC3 family

The proband (PFBC3-IV4) was a 39-year-old man born to consanguineous parents (Fig. 1C). He had a history of limbs stiffness since the age of nine. His academic performance during his school period was good and accompanied by an absence of any record of psychological or psychiatric abnormalities. However, he was diagnosed as a PFBC case approximately 7 years earlier, at the age of 32 when he complained of slurred speech and sometimes urinary incontinence. There was no recorded of seizure in the patient's medical history. He successfully achieved a bachelor's degree and demonstrated the capacity to execute routine activities on a regular basis. At age of 35, he had depressed mood and slowness of movements, fatigue, and anorexia, as well as mental slowness and symptoms indicative of obsessive–compulsive disorder (OCD). In addition, he manifested a mild hearing impairment and headache. Neurological examination at the age of 35 years revealed slurred speech, brisk deep tendon reflexes, and a positive Babinski sign. Eye movements were normal. There was no dysmetria on finger to nose test and no hypokinesia. Arm swing was reduced. Gait was nearly normal (Table 1). Parathyroid hormone and serum calcium and phosphate were within the normal range (Supplementary file S2). Brain CT scan revealed heavy calcification in bilateral basal ganglia, thalami, subcortical white matter, and cerebellum (Supplementary figure S1, C). His total calcification score was 3.

His elder brother (PFBC3-IV2) was 48-year-old. At the age of 24, he was diagnosed with bipolar mood disorder (BMD). He had severe dysarthria and dysphagia, slowness of movements, gait freezing, and memory dysfunction. There was no history of seizures. Neurological examination at the age of 44 years revealed hypophonia, hypomimia, hypokinesia, rigidity, difficulty in arising from chair, and shuffling gait with severe freezing. He had a stooped posture (Table 1 and Video 1). Saccadic eye movements were slow. The biochemical assessments, which included measurements of parathyroid hormone, serum calcium, and serum phosphate, revealed results that were within the normal range (Supplementary file S2). His brain CT scans revealed heavy calcification in striatum, dentate nuclei, and subcortical white matter (Supplementary figure S1, D). His total calcification score was 4. He received levodopa for hypokinesia and rigidity which was effective, though he developed levodopa-induced dyskinesia.

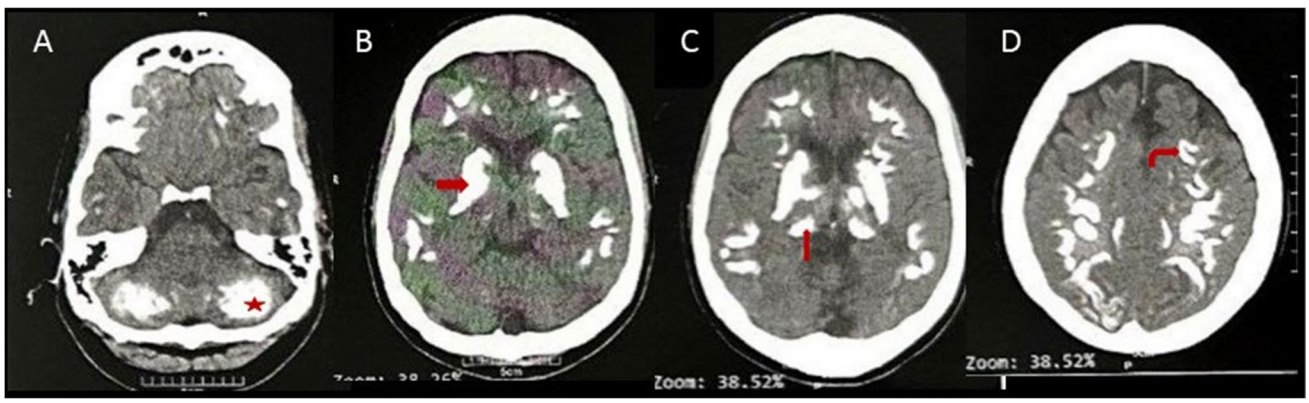


Fig. 2 Brain CT scan of PFBC2-III1 case, showing calcification of dentate nuclei (**A**, red star), striatum (**B**, horizontal red arrow), thalami (**C**, vertical red arrow), and subcortical white matter (**D**, curved arrow)

PFBC7 family

The proband (PFBC7-IV2) was a 38-year-old male born to consanguineous parents (Fig. 1D).

He underwent normal physical development. At the age 15, he presented rigidity in his left hand. He had history of seizure in childhood and taking phenobarbital for 12 years. He showed irritability, agitation, hallucination, anxiety, and OCD. In addition, he suffered from ulcerative colitis and respiratory problems. In 34-year-old, he was diagnosed as PFBC case. Despite these challenges, he was able to attain a master's degree and perform secretarial work in his business. Neurological examination revealed very mild hypokinesia with reduced left arm swing on walking (Table 1). Biochemical tests of patients showed that levels of parathyroid hormone, serum calcium, and serum phosphate were all within the normal range (Supplementary file S2). His brain CT scan showed severe calcifications in the bilateral basal ganglia, thalamus, dentate nuclei, subcortical white matter, and midbrain (Supplementary figure S1, E and F). His total calcification score was 4.

PFBC10 family

The proband (PFBC10-III2) was a 39-year-old female born to parents who were consanguineous (Fig. 1E). She had no history of seizure but had experienced a range of non-specific symptoms, including anxiety, agitation, headache, dizziness, memory dysfunction, vomiting, and urinary inconsistency since the age of 22. Neurological examination revealed slurred speech, orofacial dystonia, bradykinesia, and hypokinesia and reduced left arm swing during walking (Table 1 and Video 2). All the biochemical tests fell within the expected range of values, thereby indicating that no abnormalities were detected (Supplementary file S2). Her brain CT scan revealed severe calcifications in the bilateral basal ganglia, thalamus, dentate nuclei, subcortical white matter, and midbrain (Supplementary figure S1, G and H). Her brain MRI showed hyperintensity of dentate nuclei and basal ganglia on T1 sequences (Supplementary figure S2) and hypointensity in the same areas on T2/FLAIR sequences. The total calcification score of this case was 5.

Her sister (PFBC10-III3) was a 37-year-old female (Fig. 1E). She has a history of seizure in 3-year-old. She had also experienced anxiety, agitation, headache, and dizziness. Neurological examination revealed slurred speech, orofacial dyskinesia, and slowness of movements but no prominent gait impairments (Table 1). All biochemical tests were within the normal range (Supplementary file S2). Her brain CT scan was the same PFBC10-III2.

Her brother (PFBC10-III6) was a 32-year-old male (Fig. 1E). He also had a history of seizure in 3-year-old. He had experienced anxiety, agitation, headache, dizziness, and OCD. He complained of vomiting and the decrease in appetite. He has never seen a neurologist for neurological examination.

Genetic findings

WES results of all probands indicated high quality of sequencing: mean depth of 60 × and coverages more than 10 × and 20 × in ~ 97% and 93% of target sequences, respectively. Filtering of WES data resulted in the detection of the disease-causing variants in four probands out of five; interestingly, all of them carried the variants in the *JAM2* gene. Probands of families PFBC3, PFBC7, and PFBC10 shared the same known homozygous variant: c.685C > T:p.Arg229* (rs781261918) while the proband of PFBC2 family carried a novel homozygous variant in *JAM2*: c.426dup:p.Ser143Leufs*23 (Supplementary files S3 and S4). No candidate disease-causing variant was detected in the proband of PFBC1 family. MAF of c.685C > T variant was 0.000021 in GnomAD and it was not detected in exome data of 1000 unrelated healthy Iranian individuals including Iranome (http://iranome.com/) and the in-house exome data of 200 unrelated individuals. Thus, it was considered as a very rare variant. The Combined Annotation Dependent Depletion (CADD)_phred score of this variant was 42. On the basis of ACMG criteria, c.685C > T was categorized as pathogenic. The second variant, c.426dup, was a novel variant that not detected in the public genomic databases and in Iranian control individuals and predicted as likely pathogenic by ACMG.

Based on using GermlineCNVCaller, no pathogenic CNV was detected as a disease-causing variant in our probands.

The identified variants c.685C > T and c.426dup were co-segregated respectively with the disease status in available members of families "PFBC3, PFBC7, and PFBC10" and "PFBC2"; affected individuals of each family carried the variants in the homozygous state, while unaffected individuals harbored at least one normal allele (Fig. 1 and Supplementary file S3).

Following the observation of the mutation in the *JAM2* gene and the consanguinity of parents in four families, ROH analysis identified a shared homozygous region, ~ 4.5 Mb on chromosome 21, in all affected individuals harboring the *JAM2* variants.

Haplotype analysis of all probands based on six intragenic SNPs in the *JAM2* gene in their exomes revealed the same haplotype in three probands who carried the c.685C > T variant, whereas two other probands (PFBC1 with no genetic result and PFBC2 with a novel variant in *JAM2*) presented two different haplotypes (Table 2). Based on our results, the c.685C > T:p.Arg229* variant in *JAM2* may be a founder mutation in the Iranian population.

Table 2 Proposed haplotype associated with of *JAM2* variant NM_021219:c.685C > T:p.Arg229* in three Iranian PFBC probands (and two other PFBC probands)

rs# of variant	Chromosome 21 position (hg 19/ GRCh37.p13)	Nucleotide change	Position	MAF in 1000G	MAF in GnomAD-exome-ALL	MAF in GnomAD-genome-ALL	MAF in Iranome	Effect on protein
rs76277076	27017535	A>G	intronic	0.15	-	0.0762	-	-
rs9981246	27043972	C>G	intronic	0.62	-	0.6748	-	-
rs57503516	27043998	T>G	intronic	0.15	-	0.0865	-	-
rs3787616	27051285	A>T	intronic	0.23	-	0.2051	-	-
rs8133602	27056250	A>G	exonic	0.13	0.1085	0.0799	0.1187	p.Val41Val
rs781261918	**27074569**	**C>T**	**exonic**	**-**	**1.63E-05**	**3.23E-05**	**-**	**p.Arg229***

Genotype in FAHR3	Genotype in FAHR7	Genotype in FAHR10	Proposed haplotype of FAHR3	Proposed haplotype of FAHR7	Proposed haplotype of FAHR10	Genotype in FAHR1	Proposed haplotype of FAHR1	Genotype in FAHR2	Proposed haplotype of FAHR2
GG	GG	GG	G	G	G	AA	A	AA	A
GG	GG	GG	G	G	G	GG	G	CC	C
GG	GG	GG	G	G	G	TT	T	TT	T
TT	TT	TT	T	T	T	AA	A	AA	A
GG	GG	GG	G	G	G	AA	A	AA	A
TT	**TT**	**TT**	**T**	**T**	**T**	CC	C	CC	C

The bold black border box shows the genotypes and haplotypes of the probands who harbored the c.685C > T:p.Arg229* mutation

MAF minor allele frequency

Discussion

The junctional adhesion molecule 2 (JAM2), or alternatively referred to as JAM-B [16], is a protein of 298 amino acids that is encoded by the *JAM2* gene on chromosome 21q21.3. This transmembrane protein is expressed by endothelial and lymphatic cells [17]. It is localized at the tight junctions of both epithelial and endothelial cells and serves as a mediator for heterotypic cell–cell interactions [1, 16]. JAM2 is classified as a member of the immunoglobulin superfamily (IgSF) molecules and characterized by two immunoglobulin (Ig) domains, namely, the Ig-like C2-type and Ig-like V-type domains, that are situated in its extracellular region [17] (Fig. 3).

Bi-allelic mutations in *JAM2* lead to a recessive form of primary familial brain calcification [1, 2]; mutations in *JAM2* have only been documented twice in seven families in the year 2020 [1, 2]. So, those are ultra-rare causes of PFBC. It has been estimated that mutations in this gene account for approximately 2% of PFBC patients [4]. Here, we presented two disease-causing variants in *JAM2*, a known mutation p.Arg229* and a novel variant p.Ser143Leufs*23, that were identified among the four unrelated Iranian PFBC families studied. Our report presented the third set of identified cases of *JAM2*-related PFBC. Therefore, it appears that mutations in *JAM2* could potentially be more prevalent than previously documented. P.Arg229* was common among our patients and observed in the probands of three pedigrees (60%; PFBC3, PFBC7, and PFBC10). All p.Arg229* mutated alleles identified were associated with the same haplotype (Table 2), and this haplotype was not observed among the chromosomes of two other probands (PFBC1 and PFBC2) and 100 control individuals; the probands of the PFBC1 and PFBC2 families presented two distinct haplotypes (Table 2). The linkage of all observed p.Arg229* mutated alleles with the same haplotype and the rarity of that haplotype among Iranian control

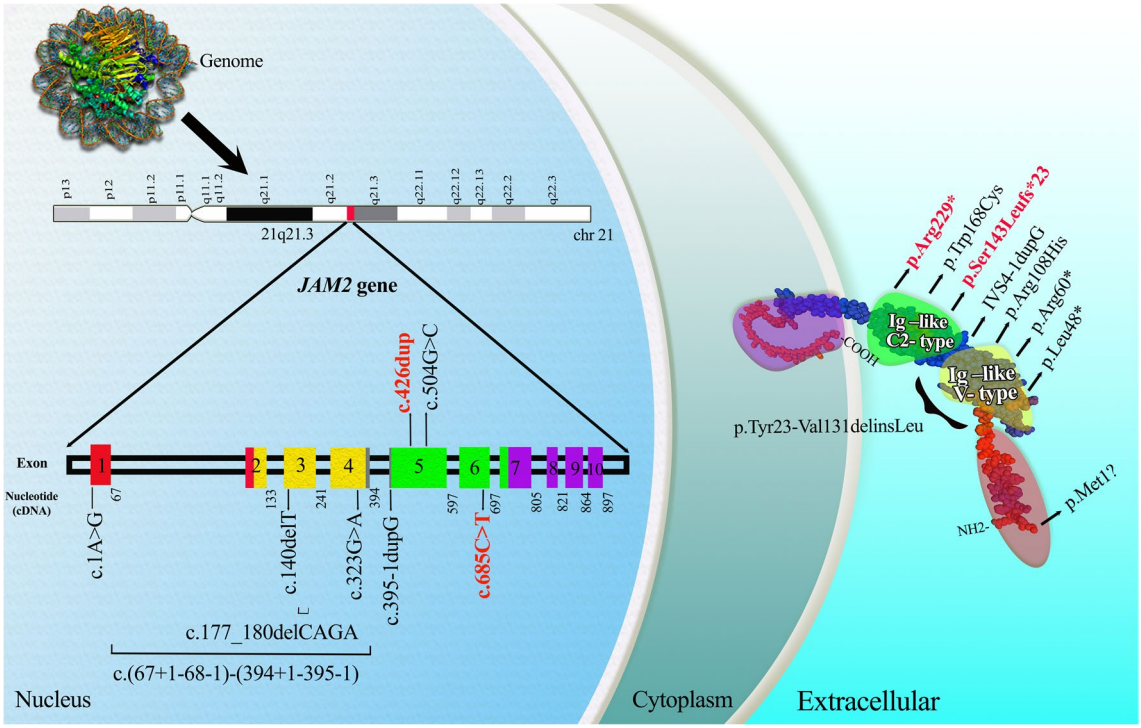


Fig. 3 Schematic view of the *JAM2* gene, its corresponding 3D protein structure and reported variants. The color-coded regions within the exons aid in understanding the different functional domains of the protein, and the highlighted variants emphasize their significance in the context of JAM2-related conditions. The *JAM2* gene is located on the 21q21.3 chromosomal region and consists of 10 exons. Each exon encodes specific functional regions of the protein, as illustrated by the color-coded areas. The N-terminal region of the protein is encoded by the exon 1 to a part of exon 2, which marked by red color, the Ig-like V-type domain of the protein is encoded by a part of exon 2 to about end of exon 4, which marked by yellow color, the Ig-like C2-type domain of the protein is encoded by about beginning of exon 5 to a part of exon 7 which colored by green, and the C-terminal region of the protein is encoded by a part of exon 7 to 10 which is purple in the figure. The JAM2 protein segments and domains are highlighted based on the exon colors. Also, the previously identified variants in both the *JAM2* gene and protein are marked in black, while the variants discovered in our patients are indicated in red

individuals suggest that p.Arg229* is a founder mutation in this population. ROH analysis showed that the mutation was located within a relatively large homozygous block, ~ 4.5 Mb, on chromosome 21 of all *JAM2*-related cases. Interestingly, this variant has been previously detected in two out of seven (~ 29%) other reported *JAM2*-related PFBC families [1, 2]. These families were from the traveler communities in England and Northern Ireland [2]. They may originate from Iran or other Middle Eastern countries and carry the same haplotype. But, due to a lack of access to their WES data, haplotype analysis of these families [2] was not possible. Even though this particular mutation may not be classified as a founder mutation, it is noteworthy that the codon seems to be a hotspot codon, because it has been observed in five out of the eleven (45%) *JAM2*-related PFBC families. P.Arg229* is located within the Ig-like C2-type domain of JAM2 (Fig. 3), and Schottlaender et al. have shown that the mutation resulted in a reduction of *JAM2* mRNA expression levels and the absence of JAM2 protein in the fibroblasts of the proband who harbored this mutation [2]. The aforementioned observations confirmed the loss-of-function characteristic inherent of the mutation. Furthermore, the presence of this specific mutation among our patients serves as further affirmation of its pathogenic nature. Another identified *JAM2* variant in the Iranian patients was c.426dup, which has not been documented before and was exclusively observed in the PFBC2 family. The variant results in the disruption of the normal reading frame of the gene, thereby causing a premature termination of 23 codons downstream, p.Ser143Leufs*23, and consequently produces a non-functional protein. The pathogenic variant including SNV or CNV within the seven established PFBC genes

Table 3 Detailed clinical features of other reported *JAM2*-related cases

References		Zhidong Cen et al. (2020) [1]				Lucia V. Schottlaender et al. (2020) [2]						
Patients ID		F1-II:2*	F1-II:3	F2-II:2*	F3-II:1*	F1-II:2*	F2-III:2*	F2-III:3	F2-III:4	F2-III:5	F3-II:1*	F4-II:3*
Variant in cDNA level		c.140delT	c.140delT	c.1A > G	c.504G > C, c.(67 + 1_68-1)_(394 + 1_395-1)	c.685C > T	c.685C > T	c.685C > T	c.685C > T	c.685C > T	c.395-1dupG, c.323G > A	c.177_180delCAGA
Variant in protein level		p.Leu48*	p.Leu48*	p.Met1?	p.Trp168Cys, p.Tyr23-Val-131delinsLeu	p.Arg229*	p.Arg229*	p.Arg229*	p.Arg229*	p.Arg229*	IVS4-1dupG, p.Arg108His	p.Arg60*
Zygosity		Hom	Hom	Hom	Com Het	Hom	Hom	Hom	Hom	Hom	Com Het	Hom
Gender		Male	Female	Female	Female	Male	Male	Female	Male	Male	Male	Female
Consanguinity		+	+	+	−	+	+	+	+	+	−	+
Nationality		NR	NR	NR	NR	Traveler communities in England	Traveler communities in Northern Ireland	Traveler communities in Northern Ireland	Traveler communities in Northern Ireland	Traveler communities in Northern Ireland	United States	Turkish
Onset symptoms		Parkinsonism, slurred speech	Parkinsonism, slurred speech (seizures[a])	Numbness of the feet[a] dizziness[a] (syncope[a])	Parkinsonism, slurred speech	Cerebellar ataxia, cognitive decline	Cognitive decline, depression	Walking difficulty	Depression, dysarthria	Depression, dysarthria	ASD	Seizures (18 months)
Age at onset (y)		20	23 (8 months[a])	37[a] (27[a])	38	Childhood	Late 20 s	Late 30 s	Teenage	Teenage	Childhood	Early childhood
Age at examination (y)		37	35	37	50	24	41	39	40	49	15	7
Movement disorders	Hypophonia	NR	NR	NR	NR	NR	+	+	NR	NR	NR	NR
	Abnormal gait	+	+	NR	+	NR	NR	+	+	+	NR	NR
	Parkinsonism	+	+	NR	+	+	+	+	+	+	NR	NR
	Hypomimia	NR	NR	NR	NR	NR	+	+	NR	NR	NR	NR
	Tremor	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
	Dystonia	+	+	NR	NR	+	+	+	NR	NR	NR	NR
Cerebellar signs	Eye-related disorders	NR	NR	NR	NR	+	+	+	+	+	+	NR
	Ataxia	NR	NR	NR	NR	+	+	+	+	+	+	NR
	Slurred speech	+	+	NR	+	+	NR	+	+	+	NR	NR

Table 3 (continued)

References		Zhidong Cen et al. (2020) [1]				Lucia V. Schottlaender et al. (2020) [2]						
Patients ID		F1-II:2*	F1-II:3	F2-II:2*	F3-II:1*	F1-II:2*	F2-III:2*	F2-III:3	F2-III:4	F2-III:5	F3-II:1*	F4-II:3*
Pyramidal symptoms	Absence of a postural reflex	+	+	NR	+	NR	NR	NR	NR	NR	NR	NR
	Brisk DTR	NR	NR	NR	NR	+	+	+	+	+	NR	NR
	Increased tone	NR	NR	NR	NR	+	+	+	+	+	NR	NR
	Positive glabellar tap	NR	NR	NR	NR	NR	+	+	NR	NR	NR	NR
	Grasp reflex	NR	NR	NR	NR	NR	+	+	NR	NR	NR	NR
	Brisk jaw jerk	NR	NR	NR	NR	NR	+	+	NR	NR	NR	NR
	Babinski sign	NR	NR	NR	NR	+	+	+	+	+	NR	NR
Psychiatric manifestations	Depression	NR	NR	NR	NR	NR	+	+	+	+	NR	NR
	Behavioral problems	NR	NR	NR	NR	+	+	+	NR	NR	NR	NR
	ASD	NR	NR	NR	NR	NR	NR	NR	NR	NR	+	NR
	Hyperactivity	NR	NR	NR	NR	NR	NR	NR	NR	NR	+	NR
Cognitive function	Memory dysfunction	−**	NR	NR	NR	NR	+	+	+	+	+***	NR
	Cognitive decline	−**	NR	NR	NR	+	NR	NR	+	+	+***	NR
Other	Seizure	NR	+	NR	NR	+	NR	NR	NR	NR	NR	+
	Dysphagia	NR	NR	NR	NR	+	+	+	NR	NR	NR	NR
	Ophthalmoplegia	NR	NR	NR	NR	+	NR	NR	NR	NR	NR	NR
	PEG inserted in advance stage	NR	NR	NR	NR	+	+	NR	NR	NR	NR	NR
	Developmental delay	NR	NR	NR	NR	NR	NR	NR	NR	NR	+	NR
	Syncope	NR	NR	+	NR	NR	NR	NR	NR	NR	NR	NR
	Coordination problems	NR	NR	NR	NR	NR	NR	NR	NR	NR	+	NR

Table 3 (continued)

References		Zhidong Cen et al. (2020) [1]				Lucia V. Schottlaender et al. (2020) [2]						
Patients ID		F1-II:2*	F1-II:3	F2-II:2*	F3-II:1*	F1-II:2*	F2-III:2*	F2-III:3	F2-III:4	F2-III:5	F3-II:1*	F4-II:3*
Brain calcification	Bilateral basal ganglia	+	+	+	+	+	+	+	+	+	+	+
	Thalami	+	+	+	+	+	+	+	+	+	NR	NR
	Dentate nuclei	+	+	+	+	NR	NR	NR	NR	NR	NR	+
	Subcortical white matter	+	+	+	+	NR	NR	NR	NR	NR	NR	NR
	Cerebellum	NR	NR	NR	NR	+	+	+	+	+	NR	+
	Midbrain	+	+	NR	+	NR	NR	NR	NR	NR	NR	NR
	Vermis	+	+	NR	NR	NR	NR	NR	NR	NR	NR	NR
	Cortex	+	+	+	+	NR	NR	NR	NR	NR	+	NR
	Pons	NR	+	NR	NR	NR	NR	NR	NR	NR	NR	NR
	Medulla	NR	+	NR	NR	NR	NR	NR	NR	NR	NR	NR
	Deep gray matter	NR	NR	NR	NR	+	+	+	+	+	NR	NR

Hom homozygous, *Com Het* compound heterozygous, *y* year, *PEG* percutaneous endoscopic gastrostomy, *DTR* deep tendon reflexes, *ASD* autism spectrum disorder, *NR* not reported

*Proband

**It was not directly mentioned in the article, but according to their education and academic performance, their cognitive function was considered normal

***Decline in academic performance

[a]The relationships between PFBC and symptoms or onset ages were uncertain

was not detected in the PFBC1 family. This indicates the presence of additional genes contributing to the manifestation of this disease and suggests that the genetic analysis of many PFBC families from different societies is essential in order to identify these novel genes.

In the clinical and paraclinical assessments of our cases, several noteworthy points were observed, which are outlined below.

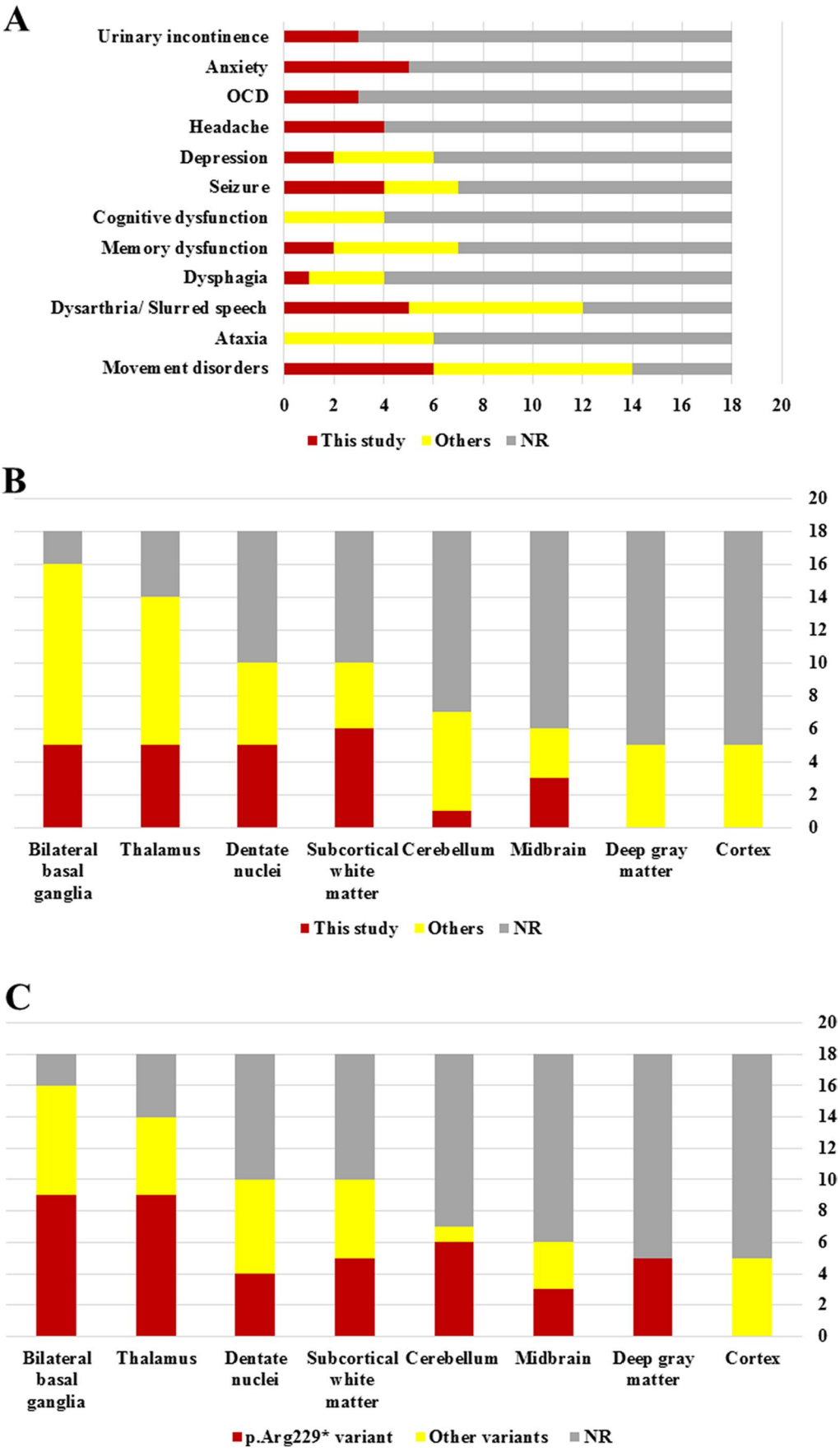
Clinical assessments

It was observed that the age at onset (AAO) of all seven *JAM2*-related cases in our study was below 25 years (a range from 3 to 24 years, with a mean AAO of 10.1 ± 8.3). Conversely, the initial symptoms of a non-*JAM2*-related proband, PFBC1, were noted to have manifested at the age of 56 (Table 1). AAO of 11 previous reported *JAM2*-related cases observed across 7 families was found to be below 38 (a range from childhood to 38 years) (Table 3) [1, 2]. This observation highlights that the AAO appears to be decreased in patients with *JAM2* variants—similar to *PDGFB*-related cases with a median AAO of 30 years—compared to those with *SLC20A2*, *XPR1*, *PDGFRB*, and *MYORG* variants. The median AAO of cases who carry mutations in these genes has been reported at 47, 44, 48, and 46 years, respectively [18, 19].

The most common symptoms of the disease in our *JAM2*-related patients were movement disorders, slurred speech, and anxiety which were observed in 6, 5, and 5 cases, respectively (Fig. 4A). In contrast, the most common features in 11 other documented cases were movement disorders, slurred speech, and cognitive dysfunction, which were observed in 8, 7, and 4 cases, respectively (Fig. 4A). While cognitive dysfunction and ataxia were exclusively observed in previously reported patients, OCD, anxiety, headache, and urinary incontinence were exclusively observed in our *JAM2*-related cases. Overall, movement disorders and slurred speech were the most prevalent features in all 18 identified cases, which were observed in 14 (77.8%) and 12 (66.7%) cases, respectively [1, 2] (Fig. 4A). So, based on the aforementioned observations, clinical heterogeneity was detected among patients who harbor mutations in *JAM2*, and this heterogeneity was noted in cases with a particular mutation, even among different affected individuals of the same family. For instance, in the case of PFBC3-IV2, bipolar mood disorder (BMD) was observed as the initial symptom, whereas his affected sibling (PFBC3-IV4) has yet to present any manifestation of BMD at the age of 39, and his initial symptom was rigidity (Table 1). Similarly, in the family PFBC10, two affected siblings (III3 and III6) exhibited seizures at the age of 3, whereas the affected individual III2 has not displayed any signs of seizures until reaching the age of 39 (Table 1). Remarkably, unlike his siblings, the affected individual III6 has not exhibited any movement disorders so far. Such clinical heterogeneities have been indisputably observed in other *JAM2*-related patients who have been documented (Table 1 and 3 and Fig. 4A) [1, 2]. The existence of such heterogeneities, even within affected individuals of the same family harboring a particular mutation, can imply the potential involvement of other genetic, epigenetic, and environmental factors. Clinical heterogeneities have also been noted in non-*JAM2*-related PFBC patients [18]. While many of these patients share similar typical symptoms, including parkinsonism, speech disturbance, anxiety, depression, and seizures, a study of the literature reveals that some features appear to be unique to patients who carry certain gene mutations. For instance, among *JAM2*/*PDGFRB*/*XPR1*-related patients, chorea has not been documented so far. Even among the typical symptoms of the disease, the frequency of these symptoms appears to vary in patients harboring mutations in distinct genes. Speech disturbance, which is noted in 66.7% of individuals with *JAM2* (Fig. 4A), has been observed in 14.1%, 6.7%, 8.3%, 28.6%, and 78.3% of patients with a mutation in the *SLC20A2*, *PDGFB*, *PDGFRB*, *XPR1*, and *MYORG* genes, respectively [18, 19]. It seems that speech disturbance may exhibit a higher prevalence in the autosomal recessive forms of the disease. Furthermore, dystonia, which is observed in 6/18 (~ 33.3%) of the *JAM2*-related cases (Table 1), is relatively uncommon in PFBC patients who have mutations in other genes; it has been reported in 13.6% of *SLC20A2*, 9.1% of *PDGFB*, 4.8% of *XPR1*, and 6.7% of *MYORG*-related cases but not found in *PDGFRB*-related patients. Alternatively, *JAM2*-related cases appear to have less cognitive dysfunction compared to other PFBC cases. It appears that patients with *MYORG* mutations have cognitive deficits more frequently (~ 43%) than those with mutations in other genes [18]. Or headache, which has been noted more commonly in patients with *PDGFB* mutations (~ 42%), was observed in 4 of the 18 (22%) *JAM2*-related patients overall [18].

Paraclinical assessments

Brain calcification with a relatively similar pattern was detected in different parts of brains of the *JAM2*-related patients, including basal ganglia, thalamus, dentate nucleus, subcortical white matter, cerebellum, and midbrain, although some differences were also observed. Notably, our patients did not display cortex involvement whereas these particular involvements were reported in five previous *JAM2*-related cases each (Fig. 4B). It is plausible that these variations are attributable to the duration of time that has elapsed since the onset of the disease.


A
Urinary incontinence
Anxiety
OCD
Headache
Depression
Seizure
Cognitive dysfunction
Memory dysfunction
Dysphagia
Dysarthria/ Slurred speech
Ataxia
Movement disorders
0 2 4 6 8 10 12 14 16 18 20
This study
Others
NR
B
20 18 16 14 12 10 8 6 4 2 0
Bilateral basal ganglia
Thalamus
Dentate nuclei
Subcortical white matter
Cerebellum
Midbrain
Deep gray matter
Cortex
This study
Others
NR
C
20 18 16 14 12 10 8 6 4 2 0
Bilateral basal ganglia
Thalamus
Dentate nuclei
Subcortical white matter
Cerebellum
Midbrain
Deep gray matter
Cortex
p.Arg229* variant
Other variants
NR

◂**Fig. 4** **A** Distribution of the clinical features among all 18 *JAM2*-related cases including our cases. **B** The pattern of calcification in CT-scan of all 18 *JAM2*-related cases. **C** The pattern of calcification in CT-scan of all 11 cases harboring the p.Arg229* mutation comparing seven cases with other *JAM2* variants. OCD obsessive–compulsive disorder, NR not reported

Interestingly, the comparison of the *JAM2*-related patients with or without p.Arg229* mutation revealed the involvement of deep gray matter is exclusively observed in the *JAM2*-related patients who carried the p.Arg229* mutation whereas cortex calcification just detected in *JAM2*-related patients with other mutations in *JAM2* (Fig. 4C). However, it should be noted that the findings are limited by the restricted sample size of these patients. As such, these results cannot be considered inconclusive and a larger cohort of patients harboring *JAM2* mutations should be examined to determine whether there is a genotype–phenotype correlation. Additionally, the pattern of brain calcification may vary over time and be age-related.

On the other hand, when *JAM2*-related patients are compared to patients with mutations in other genes, there is a noticeable variation in the pattern and quantity of calcification. Compared to patients with autosomal dominant PFBC genes, those with biallelic mutations in *MYORG* and *JAM2* (autosomal recessive forms of PFBC) typically had more extensive calcified regions [18, 19]. Calcification of basal ganglia, which was observed in 89% of *JAM2*-related cases, has been reported in 80% of *SLC20A2*, 100% of *PDGFB* and *PDGFRB*, 71% of *XPR1*, and 88% of *MYORG*-related cases. It appears that regardless of the type of mutant gene, basal ganglia involvement is observed in the majority of PFBC patients [18]. However, thalamus involvement was more common in *JAM2*-related patients (77.8%) than non-*JAM2*-related cases; in patients with *SLC20A2*, *PDGFB*, *PDGFRB*, *XPR1*, and *MYORG* mutations, this involvement has been observed in 50%, 40%, 25%, 48%, and 78%, respectively. Or, PFBC patients with a mutation other than a mutation in the *JAM2* gene, especially *MYORG*, seem to have cerebellar involvement more frequently than those with *JAM2*-related cases (38.9% in *JAM2* and 45%, 51%, 67%, 62%, and 85% in *SLC20A2*, *PDGFB*, *PDGFRB*, *XPR1*, and *MYORG*-related cases, respectively) [18].

To summarize, we reported four additional *JAM2*-related families in the current study. Three of them had the same mutation, which may have been a founder mutation. However, these patients showed clinical heterogeneity. Our research widened the clinical characteristics and mutation spectrum of *JAM2* and showed that *JAM2* mutations might be more frequent than previously thought. Furthermore, we were unable to find the disease-causing variant in one case, indicating the increased genetic heterogeneity of PFBC.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10072-024-07419-6.

Funding The project was funded by the University of Social Welfare and Rehabilitation Sciences (Grant number: 3030; 2023).

Data availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical approval This research was performed by the Declaration of Helsinki and with the approval of the ethics board of the University of Social Welfare and Rehabilitation Sciences (IR.USWR.REC.1402.078) in Iran.

Informed consent Informed consent was obtained from all subjects involved in the study as mentioned in the manuscript "Informed consent form was signed by the patients or their parents".

Conflict of interest The authors declare no competing interests.

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