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Original Article

Mutation spectrum and clinical features of *MYORG* in Iranian patients with Primary Familial Brain Calcification (PFBC)

Parsa Soleimani Affiliationids : Aff1

Mana Khojasteh Affiliationids : Aff1

Aida Ghasemi Affiliationids : Aff2

Ali Heshmati Affiliationids : Aff3

Mohammad Rohani

Email : mohammadroohani@gmail.com

Affiliationids : Aff4, Correspondingaffiliationid : Aff4

Afagh Alavi

Email : afaghalavi@gmail.com

Email : af.alavi@uswr.ac.ir

Affiliationids : Aff1 Aff2, Correspondingaffiliationid : Aff1

Aff1 Genetics Research Center, University of Social Welfare and Rehabilitation Sciences, Tehran, Iran

Aff2 Neuromuscular Research Center, Tehran University of Medical Sciences, Tehran, Iran

Aff3 School of Biology, University College of Science, University of Tehran, Tehran, Iran

Aff4 Department of Neurology, The Five Senses Health Institute, University of Medical Sciences, Tehran, Iran

Received: 15 September 2024 / Accepted: 5 March 2025

Abstract

Introduction

AQ1 Mutations in myogenesis regulating glycosidase (*MYORG*), are resulted in autosomal recessive (AR) form of Primary Familial Brain Calcification (PFBC) which is a rare neurodegenerative disease. PFBC is characterized by symmetric brain calcifications, particularly in the thalami, cerebellum, basal ganglia, and subcortical white matter. To date, eight genes have been linked with PFBC, however, currently about half of people with PFBC remain without a genetic diagnosis. Among these genes, *MYORG*, *JAM2*, *CMPK2*, and *NAA60* are associated to an AR-PFBC. Within AR-PFBCs, the frequency of mutations in *MYORG* and *JAM2* is 13% and 2%, respectively. In this study, we present a comprehensive clinical and genetic analysis of a group of Iranian PFBC patients.

AQ2 Methods

Clinical and paraclinical assessments of all patients were done. Whole-exome sequencing was performed for all probands. Candidate variants confirmed and checked in their family members.

Results

Four homozygous variants in *MYORG* across four families were identified: two novel variants, c.1727G > A;p.Arg576His and c.1687del;p.The563Glnfs*191, in two families and two known mutations, c.176G > A;p.Gly59Asp and c.1092_1097del;p.Phe365_Asp366del in the remaining two families. A potential SNV/CNV in the PFBC-related genes that causes disease was not detected in one proband.

Conclusion

Our study expanded the clinical features and mutation spectrum of *MYORG* and emphasizes to genetic heterogeneity in different populations. While *SLC20A2* mutations are the common cause of PFBC in other populations, *MYORG* and *JAM2* mutations seem to be the main cause of this disease in Iran. This issue could prove to be advantageous in the process of gene prioritization for screening within this specific population.

Keyword

Primary familial brain calcification (PFBC)
FAHR disease
MYORG
Whole-exome sequencing (WES)

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1007/s10072-025-08105-x.

Introduction

MYORG, formerly identified as *KIAA1161* or *NET37*, is a gene that encodes a 714-amino acid protein, named myogenesis regulating glycosidase, MYORG, belonging to the glycosyl hydrolase 31 family (1). This gene was expressed in skeletal muscles, colon, small intestine, liver, as well as in astrocytes and may regulate the processes of protein glycosylation in the endoplasmic reticulum of these brain-resident cells (1–4). In 2018, the first evidence linking *MYORG* mutations to Primary Familial Brain Calcification (PFBC), a rare neurodegenerative disease also known as Fahr's disease, was provided in a Chinese cohort (1). This connection has been confirmed in other autosomal recessive (AR)-PFBC cases from a variety of ethnic backgrounds by further investigations (1, 5–12). PFBC is characterized by widespread and symmetric brain calcifications, particularly in the basal ganglia, thalami, cerebellum, and subcortical white matter (12, 13). Affected individuals typically maintain good health throughout childhood and young adulthood, with symptoms mostly emerging in their forties and fifties; however, childhood onset has also been reported in PFBC (14, 15). They may present a gradually progressive movement disorder such as parkinsonism, ataxia and dystonia, as well as mild cognitive impairment along with neuropsychiatric manifestations including alterations in personality or behavior, which can progress to psychosis or dementia. Additionally, seizures, headaches, vertigo, and urinary urgency or incontinence may occur. It should be noted that PFBC has a wide range of clinical presentation from being completely asymptomatic (these cases are being found by a brain imaging which has been taken for other reasons such as mild headache or just a checkup) to more severe with a combination of ataxia, parkinsonism, chorea, spasticity, and psychiatric symptoms (16).

There are currently eight genes associated with PFBC (14, 17). Among those, *SLC20A2* (18), *PDGFB* (19), *PDGFRB* (19), and *XPR1* (20) are associated with an autosomal dominant (AD)-form of the disease, while *MYORG* (1), *JAM2* (15), *CMPK2* (21) and a recently reported gene named *NAA60* (14) are linked to an autosomal recessive (AR)-type of PFBC (10). It should be noted that although the role of *CMPK2* has not yet been confirmed as a "pure" PFBC gene, it appears to resulted in brain calcifications through mitochondrial impairment (21).

A review of the literature found that whereas 15% of cases have biallelic gene mutations (an AR inheritance), over 85% of PFBC patients have mutations in genes with a

Among this 15%, the frequency of mutations in the *MYORG* and *JAM2* genes is ~ 13% and ~ 2%, respectively (22) and mutations in the *CMPK2* and *NAA60* have been rarely reported (14, 21). Despite the identification of these genes, at present, approximately half of PFBC patients lack a pathogenic variant in these identified genes (14, 23), indicating that additional genes may still be involved in PFBC.

On the other hand, literatures represent different PFBC gene variants may be associated with different patterns of brain calcification, variable expressivity/clinical heterogeneity, and incomplete penetrance (12, 22).

The only comprehensive survey that was conducted in Iran for PFBC began in 2023. In that study, we clinically and genetically focused on five families afflicted by PFBC and showed that four of these families (80%) had mutations in the *JAM2* gene. So, we suggested that *JAM2* mutations may be more common than they appear, particularly within populations with a high rate of consanguineous marriages. We also suggested that the high rate of *JAM2* mutations in our cases may be due to the occurrence of a founder mutation (24).

In this study, we present a comprehensive examination AQ3 of both clinical and paraclinical (computed tomography scan (CT scan), magnetic resonance imaging (MRI) and laboratory tests) attributes alongside outcomes derived from genetic analysis within a second group of Iranian PFBC patients, and suggest that the mutation in the *MYORG* gene appears to exhibit a frequency comparable to that of the mutation observed in *JAM2*.

Materials and methods

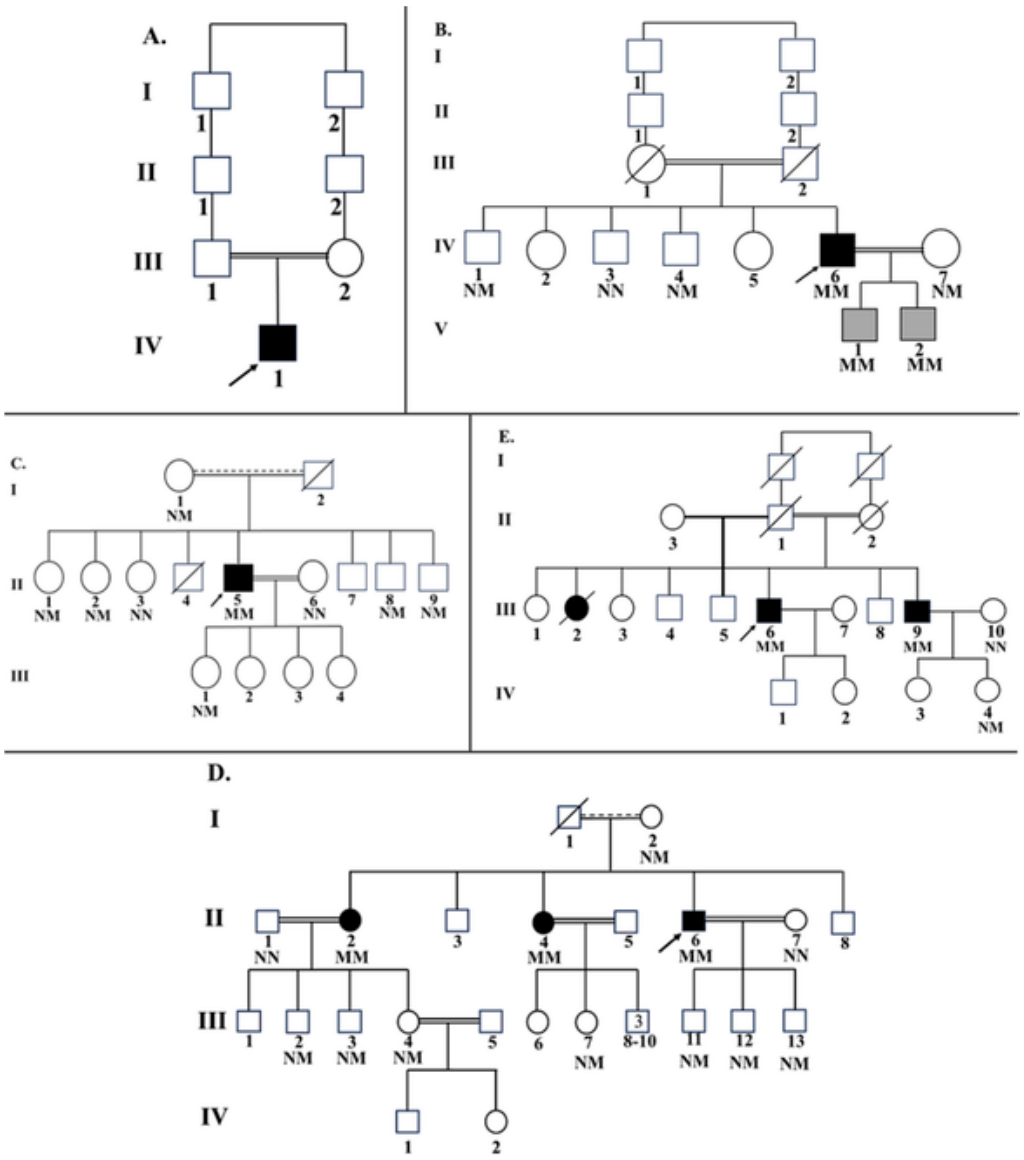
This study was authorized by the University of Social Welfare and Rehabilitation Sciences' ethics committee (USWR; IR.USWR.REC.1402.003) in Iran and carried out in accordance with the Declaration of Helsinki. The patients or their parents signed the informed consent form.

Subjects

Five unrelated PFBC families were referred to the Genetics Research Center (GRC) at the USWR for genetic analysis by the Department of Neurology of Hazrat Rassoul Hospital, affiliated with the Iran University of Medical Sciences. Because the afflicted siblings were born of consanguineous marriages, two multi-affected families, PFBC8 and PFBC9, were among those for whom the autosomal recessive pattern of inheritance was taken into consideration (Fig. 1). In three other families, the condition seemed to be sporadic; nonetheless, consanguineous marriage was AQ4 noted in the parents of those (Fig. 1).

Fig. 1

Five Iranian PFBC pedigrees: (A) PFBC4 (B) PFBC5 (C) PFBC6 (D) PFBC8 (E) PFBC9. 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 Squares indicate individuals with homozygous variants in *MYORG* who are asymptomatic. They had not undergone a neurological examination. Abbreviations: M, mutated allele; N, normal allele



A neurologist and movement disorder specialist did the neurologic examination for all cases. For cognitive assessment we did mini mental state exam (MMSE) with scores from 0 to 30 and considered scores lower than 25 as cognitive impairment.

We also performed the neurologic examination with special focus on finding (hypokinetic parkinsonism) and hyperkinetic movements (i.e. dystonia) and ataxia.

Genetic analysis

Using the salting-out procedure, DNA was extracted from the peripheral blood of probands and their relatives. For every proband, whole-exome sequencing (WES) was carried out using the Illumina HiSeq 4000 platform (Illumina, CA, USA) and the SureSelect V6-Post enrichment kit. The human reference genome, UCSC NCBI37/hg19, was used for sequence alignment. Data was analysed using various bioinformatics tools, such as Picard, Burrows-Wheeler Aligner (BWA), SAMTools, and Genome Analysis Toolkit (GATK) as previously reported (24). The variant filtering was done as follows: 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 taken into consideration (24). After that, in PFBC8 and PFBC9 probands, with recessive mode of inheritance, homozygous and compound heterozygous variants were considered. In other families where there seemed to be only one affected individual, all heterozygous, homozygous and compound heterozygous variants were taken into consideration. The remaining variants were evaluated to find a disease-causing variant in any known PFBC or other neurological disease-causing genes. The American College of Medical Genetics (ACMG) criteria (25) was utilized to classify the variants after a number of in silico tools (24) were employed to predict the likely impact of the candidate variants on the encoded proteins.

The putative disease-causing variants were verified in the corresponding probands and assessed in the family members by polymerase chain reaction (PCR) and Sanger sequencing utilizing an ABI Big Dye terminator chemistry and an ABI3130 genetic analyzer device (Applied Biosystems, Foster City, CA). The sequences of primers are available upon request.

In addition to single AQ5 nucleotide variants (SNVs) analysis, copy number variations (CNVs) were also assessed using GermlineCNVCaller and Annotation and Ranking of Human Structural Variations (AnnotSV; https://lbgi.fr/AnnotSV/).

Results

Clinical and paraclinical presentations

Table 1 provides an overview of each patient's clinical features. A thorough documentation of the clinical and paraclinical data of these patients is provided here.

Table 1

Detailed clinical features of Iranian PFBC patients with/without *MYORG* variants (NM_020702.5)

Family ID	PFBC4	PFBC5			PFBC6	PFBC8			PFBC9	
Patients ID	IV1*	IV6*	V1	V2	II5*	II2	II4	II6*	III6*	III9
Variant in cDNA level	No mutation in PFBC-related genes	c.176G > A			c.1727G > A	c.1687del			c.1092_1097del	
Variant in protein level	-	p.Gly59Asp			p.Arg576His	p.Thr563Glnfs*191			p.Phe365_Asp366del	
Hom: Homozygouse, y: year, OCD: Obsessive–compulsive disorder, DTR: Deep tendon reflexes, *: proband, NA: Not available, GH31: Glycoside hydrolase family 31, ACMG: American College of Medical Genetics,										
** Based on Franklin: https://franklin.genoox.com/										
# If we add more evidence like the results of co-segregation analysis (PP1), this variant will be predicted as a pathogenic variant										
$ Although ACMG criteria considered two variants as VUS, our segregation analysis and literature search classified them as likely pathogenic										

Family ID		PFBC4	PFBC5			PFBC6	PFBC8			PFBC9	
Patients ID		IV1*	IV6*	V1	V2	II5*	II2	II4	II6*	III6*	III9
ACMG classification **		-	VUS (PM2)[$]			Likely pathogenic (PP3, PM2)	Likely pathogenic (PVS1, PM2)[#]			VUS (PM2, PM4)[$]	
Known/Novel		-	Known (Chelban et al. 2020)			Novel	Novel			Known (Yao et al. 2018)	
Protein domain		-	Transmembrane segment			GH31 domain	GH31 domain			GH31 domain	
Zygosity		-	Hom			Hom	Hom			Hom	
Gender		Male	Male	Male	Male	Male	Female	Female	Male	Male	Male
Consanguinity		+	+	+		+	+			+	
Nationality		Iranian	Iranian			Iranian	Iranian			Iranian	
First symptoms		Progressive gait disturbance and frequent falls	Frequent falls	Apparently asymptomatic	Apparently asymptomatic	Gait disturbance, frequent falls and dysarthria	Dysarthria and challenges in mobility	Dysarthria, abnormal gait and dysphasia	Irratibility, urinary dysfunction, and movement difficulties	Slowness of movement, dysphasia and memory dysfunction	Slowness of movement and dysarthria
Age at onset (y)		10	45	NA	NA	34	62	58	47	41	25
Age at examination (y)		18	53	NA	NA	38	68	62	55	44	35
Present age (y)		23	57	28	15	45	68	62	60	46	37
Movement & Cerebellar disorders	Dysarthria	+	+	NA	NA	+	+	+	+	+	+
	Parkinsonism	+	-	NA	NA	+	+	+	+	+	+
	Tremor	-	-	NA	NA	-	-	-	-	-	-
	Spasticity	+	+	NA	NA	+	-	-	-	+	+
	Gait disturbance	+	+	NA	NA	+	+	+	+	+	+
	Nystagmus	-	-	NA	NA	-	-	-	-	-	-
	Gait ataxia	-	-	NA	NA	+	+	+	+	-	-
	Limb dysmetria	-	+	NA	NA	+	-	-	+	-	-
Psychiatric manifestations	Agitation	+	+	+	-	+	+	-	+	+	+
	Hallucination	-	-	-	-	-	+	-	+	+	-
	OCD	+	-	-	-	+	+	-	+	+	+
	Depression	+	+	-	-	+	+	+	+	+	-
	Irritability	+	-	+	-	+	+	-	+	+	+
	Anxiety	+	+	+	-	+	+	+	+	-	+
Cognitive function	Memory dysfunction	+	-	-	-	-	-	-	-	+	-
	Cognitive decline	+	-	-	-	-	-	-	-	+	-
	Seizure	-	+	-	-	-	-	-	-	-	+
	Urinary incontinence	-	-	-	-	+	+	+	+	+	+
	Dizziness	-	+	-	-	-	-	-	-	+	+
	Dysphagia	-	+	-	-	+	+	+	+	+	+
	Difficulty in arising from chair	-	-	-	-	+	+	+	+	+	-
	Fatigue	+	-	-	-	+	+	-	+	+	+
	Headache	-	-	+	-	+	-	-	+	-	+
Brain calcification	Thalami	+	+	NA	NA	+	+	+	+	+	-
	Dentate nuclei	+	+	NA	NA	+	+	+	+	+	+
	Subcortical white matter	+	+	NA	NA	+	+	+	+	+	+
	Basal gangelia	+	+	NA	NA	+	+	+	+	+	+
	Striatum	+	+	NA	NA	+	+	+	+	+	+

Hom: Homozygouse, y: year, OCD: Obsessive–compulsive disorder, DTR: Deep tendon reflexes, *: proband, NA: Not available, GH31: Glycoside hydrolase family 31, ACMG: American College of Medical Genetics,

** Based on Franklin: https://franklin.genoox.com/

If we add more evidence like the results of co-segregation analysis (PP1), this variant will be predicted as a pathogenic variant

$ Although ACMG criteria considered two variants as VUS, our segregation analysis and literature search classified them as likely pathogenic

PFBC4 family

The patient (PFBC4-IV1) was a 23-year-old man born to consanguineous parents and was the only child in the family (Fig. 1A). The onset of the symptoms was at the age of 10 years old, with progressive gait disturbance and frequent falls. At the age of 18 years, he had an ischemic stroke with right hemiparesis and Broca's aphasia, which improved to some extent with speech therapy and physiotherapy. During these years, he also had memory dysfunction, agitation, obsessive–compulsive disorder (OCD), depression, anxiety, fatigue, and anorexia. On neurologic examination, he had dysarthria and spastic right hemiparesis (due to the previous stroke). Additionally, the right hand remains numb and immobile. Biochemical evaluations, including tests for parathyroid hormone, serum calcium, and serum phosphate levels, showed results that fell within the standard range (Supplementary File S1).

Brain MRI revealed an old infarction in the left middle cerebral artery (MCA) territory, also evidence of brain calcification (T1 hyperintensity) in caudate nuclei, putamina, thalami, and dentate nuclei, which were confirmed with brain CT scan.

PFBC5 family

The proband (PFBC5-IV6) was a 57-year-old male individual born to consanguineous parents who were distant relatives; “third cousins” (Fig. 1B). The proband experienced a progressive imbalance and dysarthria for the past 12 years, with a history of seizures at the age of 46. At 53 years old, he sought consultation with a neurologist due to the development of ataxia and gait disturbance. He had depressed mood and anxiety. Neurological examination revealed additional symptoms, including dysphagia, brisk deep tendon reflexes (DTRs), and flexor plantar reflexes. Eye movements were normal, and on finger-to-nose test, he had dysmetria. There was no prominent hypokinesia. Tandem gait was normal. The proband also presented with a mild hearing impairment. Biochemical assessments, encompassing measurements of parathyroid hormone, serum calcium, and serum phosphate, yielded results within the normal range (Supplementary File S1). Brain MRI revealed pronounced calcification in the thalami, dentate nuclei, and striatum (hyperintensity on T1 weighted sequences). Furthermore, a brain CT scan demonstrated multiple distinct calcifications throughout the brain, compatible with brain MRI findings.

His son (PFBC5-V1) was a 28-year-old man (Fig. 1B). He experienced symptoms of anxiety, agitation, and headaches. However, he had not undergone precise neurological examination by a specialist.

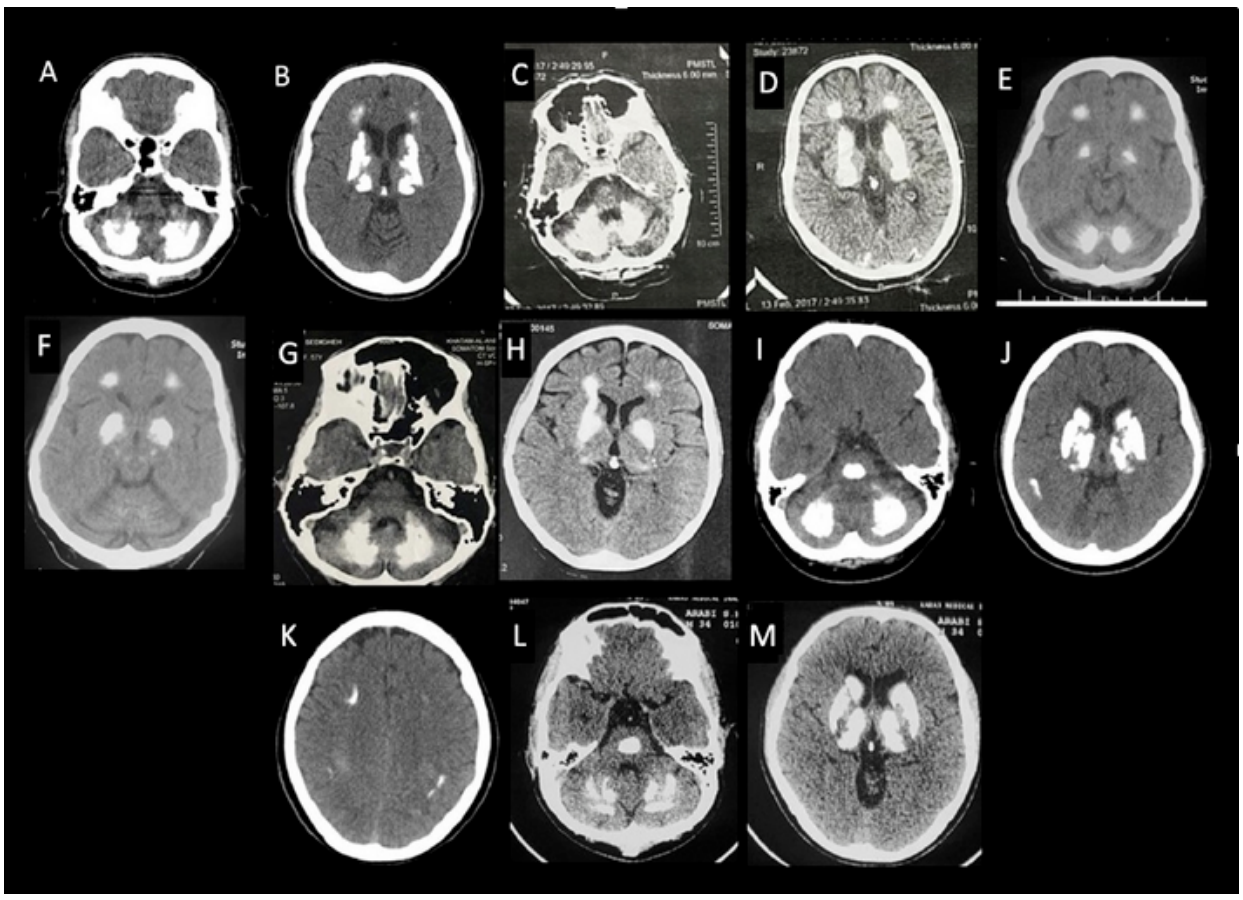
Likewise, his other son (PFBC5-V2) was a 15-year-old male. According to his family reports, he did not exhibit any particular symptoms until this age and had not undergone a neurological examination.

PFBC6 family

The proband (PFBC6-II5) was a 45-year-old male born to consanguineous parents (Fig. 1C). His first symptoms were slowness of movement, gait disturbance, frequent falls, and dysarthria since the age of 34 years. These symptoms gradually got worse over time. Urinary frequency, swallowing problems, and excessive daytime sleepiness were mentioned as other complaints. He had depressed mood, anxiety, agitation, and other symptoms indicative of OCD. He had no history of seizures. Neurological examination at the age of 38 years revealed normal fundoscopy and normal eye movements, dysarthria, hypokinesia, rigidity and spasticity of upper and lower limbs, brisk DTRs, and negative Babinski sign. The finger to nose test yielded dysmetria. He had a wide-based stance and ataxic gait with impaired tandem walking. His cognitive function was normal at the age of examination. It is worth noting that the use of Amantadine (100 mg BID) had a positive effect on his balance and movement. All the biochemical tests fell within the expected range of values, thereby indicating that no abnormalities were detected (Supplementary File S1). His brain CT scan revealed severe calcifications in the bilateral basal ganglia, thalami, dentate nuclei and subcortical white matter (Fig. 2A and 2B).

Fig. 2

Brain CT scans of cases PFBC6-II5 (A & B), PFBC8-II6 (C & D), PFBC8-II2 (E & F), PFBC8-II4 (G & H), PFBC9-III6 (I, J & K) and PFBC9-III9 (L & M). All cases who carried a homozygous *MYORG* variant show calcification of dentate nuclei and striatum (putamina and caudate nuclei)



PFBC8 family

The proband (PFBC8-II6) was a 60-year-old male born to apparently consanguineous parents (Fig. 1D). The symptoms were started at the age of 47 years old, includes irritability, urinary incontinence, impaired handwriting, restlessness, and movement difficulties such as parkinsonism and shuffling gait. These symptoms have persisted for three years, leading to a complete loss of mobility and speech. Additionally, the individual experiences difficulty swallowing, agitation, hallucination, depression, headache, OCD, and drooling. On neurologic examination, he had a masked face with drooling, slow saccadic eye movements, and hypophonic speech.

There was hypokinesia and rigidity of arms and legs. He was not able to arise from chair, stand or walk. The biochemical evaluations, which included measuring parathyroid hormone, serum calcium, and serum phosphate, demonstrated results that were within the expected range (Supplementary File S1).

Brain CT scan was abnormal due to striatal, thalamic and dentate calcifications (Fig. 2C and 2D).

His sister (PFBC8-II2) was 68 years old (Fig. 1D). She experienced the initial symptoms six years prior at the age of 62, which were predominantly characterized by difficulty in speaking and challenges in mobility, particularly in climbing stairs and performing daily tasks. History of seizure was negative. Additionally, she exhibited symptoms of OCD and depression, alongside urinary incontinence and a tendency to drop objects from her hands. On neurologic examination she had mild dysarthria, normal eye movements and optic discs and other cranial nerves. She had mild hypokinesia and rigidity of limbs. Finger to nose test did not reveal dysmetria. She had a mildly wide-based stance and shuffling gait. She was not able to do heel-to-toe walking. The biochemical examinations, encompassing measurements of parathyroid hormone, serum calcium, and serum phosphate, indicated values within the normal range.

Brain CT scan showed calcification in basal ganglia, thalami, dentate nuclei and subcortical white matters (Fig. 2E and 2F).

His second sister (PFBC8-II4) was 62 years old (Fig. 1D). Her first symptoms were at the age of 57, including walking difficulties (slowness and imbalance along with occasional falls), hypophonia characterized by a low-tone voice, and dysphasia. Neurological examination reveals symmetrical hypokinesia and rigidity, a shuffling gait, and impaired tandem gait. Eye movements and finger to nose tests were normal. The patient has a history of hypothyroidism. The biochemical assessments, encompassing measurements of parathyroid hormone, serum calcium, and serum phosphate, demonstrated values within the accepted physiological range.

Brain CT scan indicated the presence of calcifications within the basal ganglia, striatum, thalami, and dentate nuclei (Fig. 2G and 2H).

PFBC9 family

The proband (PFBC9-III6) was a 46-year-old male born to consanguineous parents (Fig. 1E). His first symptoms were slowness of movement, dysphasia and memory dysfunction, at the age of 41. He exhibits a masked face and hypophonic speech with hypokinesia and rigidity of extremities and shuffling gait. DTRs were brisk and finger to nose test was normal. Levodopa had a positive effect on improving slowness of movements and also led to levodopa induced dyskinesia. The biochemical evaluations, which involved testing for parathyroid hormone, serum calcium, and serum phosphate, indicated levels that were within normal range (Supplementary File S1).

Brain CT scan was similar to other cases with pathologic calcification of deep brain and cerebellar nuclei and subcortical white matters (Fig. 2I, 2J and 2K).

His younger brother (PFBC9-III9) was 37 years old (Fig. 1E). He exhibited initial symptoms of slowness of movement and dysarthria, which manifested at the age of 25, alongside a history of seizures in childhood. Subsequently, he developed an abnormal gait, spasticity, and shuffling gait with freezing, agitation, OCD, irritability, anxiety, difficulty affording his activities of daily living, urinary incontinence, dizziness, dysphagia, and fatigue. The biochemical assessments, covering tests for parathyroid hormone, serum calcium, and serum phosphate, showed results that were within the standard range.

Brain CT scan revealed calcifications in the thalami, dentate nuclei, striatum, Pons, and subcortical white matter (Fig. 2L and 2M).

Genetic findings

A mean depth of 60X and coverages greater than 10X and 20X in about 96% and 94% of target sequences, respectively, were noted in the WES data, indicating high quality of WES.

Four out of the five probands carried the disease-causing mutations identified through filtering of the WES data; remarkably, they were all carrying the variants in the *MYORG* gene. No additional variant in other PFBC-related genes was detected during WES data analysis. The probands of families PFBC5, PFBC6, PFBC8, and PFBC9 carried homozygous variants c.176G > A;p.Gly59Asp, c.1727G > A;p.Arg576His, c.1687del;p.The563Glnfs*191, and c.1092_1097del; p.Phe365_Asp366del (NM_020702.5), respectively (Supplementary file S2). MAF of all variants have been shown in Table 2. None of them was found in homozygous state in public genomic databases. Variants c.176G > A and c.1092_1097del were classified as variant of uncertain significance (VUS), whereas variants c.1727G > A and c.1687del were categorized as likely pathogenic in accordance with the criteria established by the ACMG (Table 1). The detected variants exhibited co-segregation with the disease status in the accessible members of their corresponding families; afflicted individuals within each family carried the variants in a homozygous state, whilst those who were not affected maintained a minimum of one normal allele (Fig. 1 and Supplementary file S2). Although ACMG criteria considered two variants as VUS, our segregation analysis and literature search classified them as likely pathogenic.

Table 2

Minor allele frequency of all *MYORG* variants (NM_020702.5) in this study in the gnomAD and Iranome databases

							Variant in		ACMG		Allele Count in gnomAD v4.0.0				
Family ID	Chr	Start	End	Ref	Alt	Zygosity	c.DNA	Protein	prediction	rs #	Exomes	Genomes	Total	# of homozygotes	Al Fr
PFBC5	chr9	34,372,766	34,372,766	C	T	hom	c.176G > A	p.Gly59Asp	VUS #	rs778863651	27	1	28	0	0.0
PFBC6	chr9	34,371,215	34,371,215	C	T	hom	c.1727G > A	p.Arg576His	LP	rs866335788	5	0	5	0	0.0
PFBC8	chr9	34,371,255	34,371,255	T	-	hom	c.1687del	p.Thr563Glnfs*191	LP	-	0	0	0	0	0
PFBC9	chr9	34,371,845	34,371,850	TCGAAG	-	hom	c.1092_1097del	p.Phe365_Asp366del	VUS #	-	0	0	0	0	0

Chr: chromosome, hom: homozygous, Alt: alternative allele, Ref: reference, VUS: variant of uncertain significance, LP: Likely Pathogenic, Iranome: http://iranome.com/

ACMG: American College of Medical Genetics

#: Although ACMG criteria considered these variants as VUS, our segregation analysis and literature search classified them as likely pathogenic

Based on WES data analysis, a potential variant in the PFBC-related genes that causes disease was not found in the proband of PFBC4 family. GermlineCNVCaller did not detect any pathogenic CNV in our probands including PFBC4 family.

Discussion

The first evidence of a connection between *MYORG* mutations and PFBC was identified in six Chinese families in 2018 (1). More research has validated this connection in more AR-PFBC cases from diverse ethnic backgrounds (26–29). These investigations revealed that, whereas *MYORG* mutations are not a common cause of PFBC (30), they are now responsible for approximately 13% of cases and are the most common cause of AR-PFBC (12).

The *MYORG* gene encodes a member of the glycoside hydrolase (GH) 31 (GH31) family, MYORG, which is thought to be involved in the organization and mobility of cells. This protein has multiple conserved domains: a short N-terminal cytoplasmic domain (aa 1–58), a single transmembrane segment (aa 59–79), the glycoside hydrolase family 31 domain (GH31; a common glycosidase domain) (aa 311–714), and a domain of unknown function (aa 80–310; may be involved in protein–protein interactions) (4, 31). Previous assumptions suggested that MYORG might be an active α-glucosidase (31); however, investigations in this area are still ongoing. Undoubtfully, understanding the function, substrate, and variants of MYORG would facilitate the identification of molecular pathways that underlie the onset and progress of PFBC, in addition to offering prospects for the creation of novel therapeutics or the modification of already-

approved ones. Such research can help explain how distinct gene variants may cause identical neuroimaging findings but varied age at onset (AAO) and clinical features, as well as provide light on the underlying mechanisms of the disease.

In this regard, we identified four variants, two known and two novels, in *MYORG* and presented the details of clinical manifestations of the cases that harbor these variants.

Although the age of onset of PFBC is considered to be in the fourth or fifth decades of life, it varies greatly. According to Balck et al., the mean AAO of *MYORG*-related PFBC cases (46 years) is considerably greater than that of *PDGFB* (30 years) and *JAM2* (23 years)-related PFBC patients, and it is comparable to that of *SLC20A2* (47 years), *XPR1* (44 years), and *PDGFRB* (48 years)-related PFBC patients (12). Also, among the patients with mutations in the newly discovered PFBC gene, *NAA60*, four patients had AAOs in their early or late twenties, whereas the remaining six cases had AAOs at birth (14). So, evidently, the mean AAO in PFBC cases associated with *MYORG* exceeds that observed in cases with *NAA60* mutations. It appears that, within the patients diagnosed with AR-PFBC, those harboring a genetic variant in *MYORG* exhibit the highest average AAO. In validation of this assertion, the mean AAO of symptomatic patients related to *MYORG* in the present study (~ 44.57 ± 12.9 years), aligned with previously documented cases (~ 46 years), exhibiting a higher age compared to the AAO observed in our prior study involving *JAM2*-related cases (10.1 ± 8.3 years)(24).

Aside from variability of AAO, *MYORG*-related cases exhibit clinical heterogeneity and decreased penetrance. Put another way, a literature review indicates that these patients exhibit clinical heterogeneity despite sharing common symptoms such as speech disturbance, hyperkinetic movement disorders, Parkinsonism, appendicular and truncal cerebellar abnormalities, and cognitive deficits. It should even be noted that these common manifestations are not present among all patients, as evidenced by the occurrence of speech disruption in 78%, cognitive deficits in 43%, ataxia in 36.7%, and Parkinsonism in 26.7% of *MYORG*-related cases (12). Other symptoms such as chorea, dystonia, or seizures have been reported only in a very small percentage of cases—5%, 6.7%, and 3.3%, respectively (12). In our *MYORG*-related PFBC cases (excluding the two asymptomatic cases), the corresponding percentages of symptoms were 100%, 14.3%, 57.1%, 85.7%, 0, 0, and 28.5%, respectively. Therefore, although the predominant feature observed in *MYORG*-related cases, including this study, is a notable speech problem (6, 12), clinical heterogeneity is even observed in patients with a specific mutation. For example, the PFBC5-IV6 case who carried known variant c.176G > A, presented some similarities and differences comparison with another case with the same variant (5). PFBC5-IV6 case and case 1 reported by Chelban et al. exhibit typical PFBC features, including dysarthria, gait disturbances, and dystonia, along with calcifications in the basal ganglia and subcortical white matter. However, our case displayed additional symptoms such as seizures, dysphagia, and anxiety, while Chelban et al.'s case manifested supranuclear gaze palsy. Calcifications in the cerebellar folia were noted only in Chelban et al.'s case (5).

Such clinical heterogeneity was also observed even across different affected members of the same family. For example, in our study, dysarthria was found to be the early symptom in the case of PFBC8-II2, whereas irritability was found to be the earliest symptom in her afflicted brother (PFBC8-II6) (Table 1). Comparably, in the family PFBC9, the proband (III6) showed signs of hallucination, and depression, but the patient III9 did not show any of those symptoms until he was 37 years old (Table 1). Such heterogeneities can suggest the possible involvement of other genetic, epigenetic, and environmental factors.

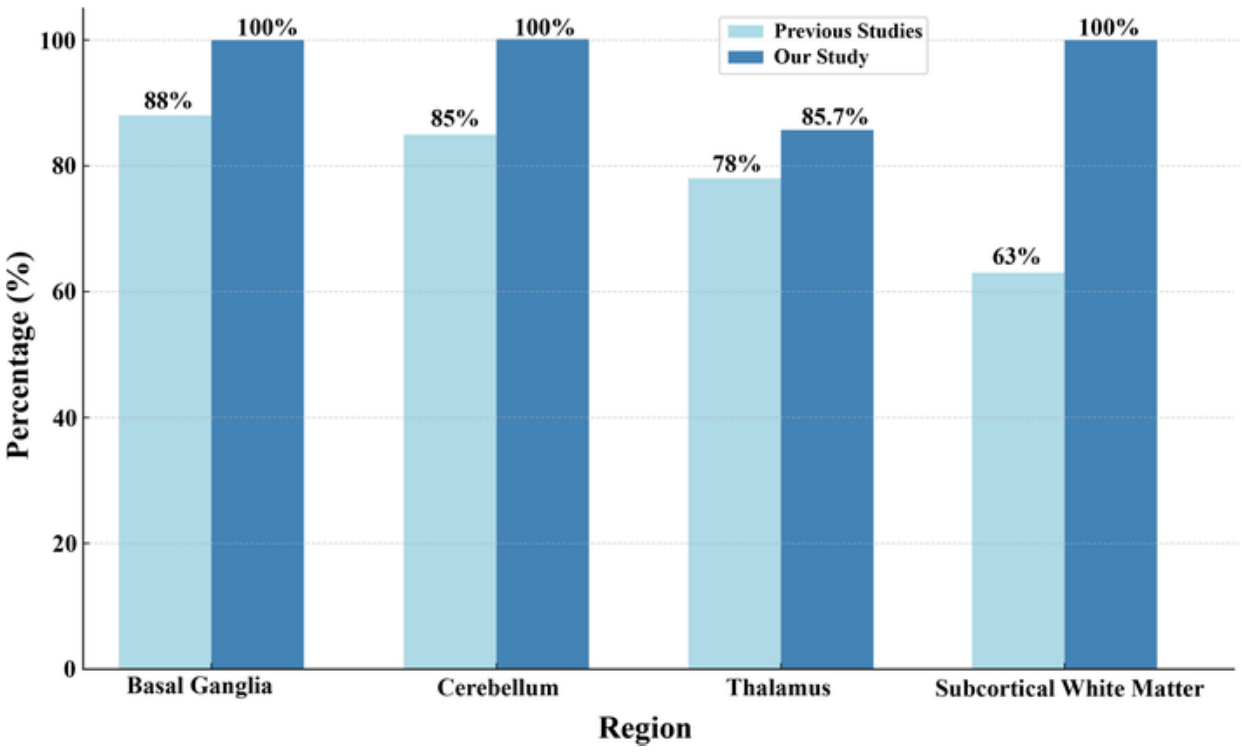
Another clinical finding observed in our study was the occurrence of a stroke in the PFBC4-IV1 patient (who displayed no mutations in known PFBC-related genes) at the age of 18. Although the association between ischemic stroke and PFBC remains to be definitively established, it has been previously reported (32–35). Various mechanisms have been proposed that link PFBC to ischemic stroke, including arterial calcification that may arise from impaired neuronal calcium-phosphorus metabolism, in addition to hypoperfusion. The accumulation of calcium within the walls of cerebral vessels in PFBC may also increase inflammatory responses, thereby facilitating the disruption of the blood–brain barrier (BBB) (32, 33, 35).

Regarding the penetrance of PFBC, it should be noted that the penetrance is not 100% and it seems that forms with autosomal recessive inheritance have more penetrance than forms with autosomal dominant inheritance. The highest penetrance was reported in patients with mutations in the *NAA60I* (a fully penetrant), that followed by *JAM2* and *MYORG* genes (85%) and the lowest penetrance in patients with mutations in the *PDGFRB* gene (46%) (12). If we assume that the individual PFBC5-V1, who is asymptomatic and manifests non-specific symptoms, is in the early stages of the disease, and individual PFBC5-V2 who is asymptomatic at the age of 15 due to his young age and could have the condition later in life, then the penetrance in our patients will be 100%. But it should be noted that if these individuals remain asymptomatic, the penetrance will be 77.8%.

Comparison of calcification in patients with mutations in different PFBC-related genes reveals that individuals harboring biallelic variants in *MYORG* and *JAM2* exhibit a higher extent of calcified regions compared to those with mutations in AD-PFBC genes (12). The distinctive features of PFBC linked to *MYORG* mutations include the presence of calcification in the brainstem, particularly evident in the pons region, along with diverse degrees of cerebellar atrophy (6). In our study, dentate nucleus, which is a part of the cerebellar nuclei, and pons involvements were detected in all and one (PFBC9-III9) *MYORG*-related cases, respectively. Nevertheless, the patterns of brain calcification identified in *MYORG*–related cases, including our cases, display similarities and differences among cases even in patients with a specific mutation, even across different affected members of the same family (1, 5). In our cases, calcification of dentate nucleus, striatum, and subcortical white matter were observed in all symptomatic *MYORG*-related cases (100%) whereas, involvement of thalami was detected in 6 out of 7 symptomatic cases (85.7%)(Fig. 3). Although the degree of calcification observed in these brain regions in alternative research was less than that found in our subjects (12).

Fig. 3

(A) The pattern of calcification in CT-scan of *MYORG*-related cases in our patients (blue) and other reported cases (gray)



In total, these findings highlight the complexity of genotype–phenotype correlations in PFBC. Further exploration is warranted to elucidate the underlying mechanisms driving these variable clinical and paraclinical manifestations.

Genetically, in this study, we identified causative variants in the *MYORG* gene across four out of five families using whole-exome sequencing. Our findings revealed two missense variants c.176G > A;p.Gly59Asp and c.1727G > A;p.Arg576His, a frameshift variant c.1687del;p.Thr563Glnfs*191 and an in-frame deletion of c.1092_1097del;p.Phe365_Asp366del which were co-segregated with the disease status in the families, strongly suggesting their pathogenicity. Variants of c.1687del;p.Thr563Glnfs*191 and c.1727G > A;p.Arg576His, which are located in the GH31 domain are both novel variants and expand the mutational spectrum of *MYORG*. The c.1687del variant causes a frameshift and disruption in the GH31 domain and it probably affects the glucosidase function of MYORG. Regarding the c.1727G > A variant, a mutation involving nucleotide G at position 1727 has been previously documented in the literature (4, 29). Tsai et al. and Zhao et al. have indicated that nucleotide G at position 1727 has been substituted with nucleotide C, resulting in a change at the protein level where Proline at position 576 is replaced by Arginine (p.Arg576Pro). In contrast, our analysis reveals that nucleotide G at position 1727 has undergone substitution with nucleotide A, which consequently leads to the alteration of Arginine at position 576 to Histidine (p.Arg576His). Consequently, we considered this missense variant as a novel mutation that probably affects the glucosidase function of MYORG.

Among two known variants, c.176G > A;p.Gly59Asp (5) located in the transmembrane segment of the protein, and it seems the substitution of an Aspartic acid (negatively charged) with a Glycine (without charged) might affect the localization of the protein in membrane. C.1092_1097del;p.Phe365_Asp366del (1, 29) also situated in the GH31 domain of protein which probably disrupts the protein structure and glucosidase function of MYORG. As in previous studies, most of our variants are located in the catalytic domain of GH31. This domain, a prevalent glycosidase domain, plays a crucial role in the breakdown of complex carbohydrates and seems to be responsible for the galactosidase activity of this protein (4). All variants were located in conserved amino acid positions. The importance of this finding lies in the fact that conserved regions of proteins are often critical to their structure and function, implying that mutations in these regions are more likely to have detrimental effects on protein activity and stability. This highlights the potential pathogenic significance of these mutations and emphasizes the necessity for further functional studies to understand their impact on the *MYORG* gene and associated disease mechanisms.

In conclusion, it is noteworthy that through the examination of these five families alongside the preceding five families, pathogenic variants were detected in 8 families (four families, 40%, exhibited mutations in the *JAM2* gene, while the remaining four, 40%, displayed mutations in the *MYORG* gene). Notably, no variants in genes following a dominant pattern of inheritance, particularly mutations within the *SLC20A2* gene, have been noted within the Iranian population. Therefore, although in other populations, the frequency of mutations in *MYORG* and *JAM2* genes has been reported as 13% and 2%, respectively, it seems that this frequency is much higher in the Iranian population (22, 24). This observation may reflect the influence of consanguinity, which is prevalent in this demographic, as a contributing factor to the higher frequency of AR-form of PFBC. Such findings could prove advantageous in the process of gene prioritization for screening within this specific population. Conversely, within two families out of 10 studied families, the causative variants for the disease remained undisclosed, implying the involvement of novel genes in the disease pathogenesis and underscoring the heightened genetic heterogeneity within this disorder. These results highlight the importance of expanding genetic studies to include larger cohorts from underrepresented populations and diverse geographic regions to validate these observations and further elucidate the genetic landscape of the disorder.

Publisher's Note

Funding

The project was funded by the University of Social Welfare and Rehabilitation Sciences (Grant number: 2888; 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 (USWR; IR.USWR.REC.1402.003) in Iran. AQ6

Conflicts of interest

All authors claim absence of financial interests and absence of conflicts of interest.

Supplementary Information

Below is the link to the electronic supplementary material.

ESM 1

(MOV 21.6 MB)

ESM 2

(MOV 15.5 MB)

ESM 3

(XLSX 15.5 MB)

ESM 4

(DOCX 2.66 MB)

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