

CASE REPORT

Identification of a novel *HEXB* Mutation in an Iranian Family with suspected patient to GM2-gangliosidosis

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Abstract

Sandhoff disease is one of the GM2-gangliosidosis which is caused by a mutation in the *HEXB* preventing the breakdown of GM2-ganglioside. We report a novel *HEXB* variant in a family with a history of a dead girl with Sandhoff disease which was not found in controls.

KEYWORDS

GM2-gangliosidosis, *HEXA*, *HEXB*, sandhoff, β -hexosaminidase

1 | INTRODUCTION

The GM2-gangliosidosis are autosomal recessive metabolic diseases caused by a reduced ability to metabolize the GM2-ganglioside and result in ganglioside accumulation especially in the central nervous system.^{1,2} The disorders show a

spectrum of clinical severity. Generally, the earlier the age of onset of clinical symptoms is associated with the more severe form of the disease.³ The GM2-gangliosidosis are caused by mutations in the genes encoding the subunit α (*HEXA* gene) or subunit β (*HEXB* gene) of heterodimeric β -hexosaminidase A (*HEX A*), or its cofactor (the GM2 activator

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protein).^{4,5} The lysosomal hydrolase, beta-hexosaminidase, has two isoenzymes: hexosaminidase A and hexosaminidase B. Hexosaminidase A is composed of α and β subunits, whereas hexosaminidase B is only composed of β subunits.^{6,7} Sandhoff disease⁸ (OMIMNo. 268800) caused by mutations of *HEXB* gene that contains 14 exons and spans about 40 kb of DNA on chromosome 5q13⁹ in which there is a deficiency of both lysosomal hydrolase β -hexosaminidase A and β -hexosaminidase B. This deficiency causes accumulation of GM2-ganglioside especially in the neuronal cells.^{4,10} In regard to the age at the onset of symptoms, three types of Sandhoff disease are distinguishable: infantile (classical Sandhoff disease), juvenile, and adult groups of patients.^{2,11,12} Sandhoff disease has several symptoms which may include motor weakness, hyperacusis, blindness, mental deterioration, cherry-red spot in the macula, seizures, and optic atrophy.¹³ Tay-Sachs disease (TSD) is a fatal neurodegenerative disorder manifesting the same ophthalmic symptoms found in Sandhoff disease, such as optic atrophy and a cherry-red spot in the macula.^{14,15} It is caused by mutations within the *HEXA* gene on chromosome 15q 23-24.¹ The aim of this study was to investigate and identify the plausible disease-causing mutation in an Iranian family with the dead suspected infant having GM2-gangliosidosis.

2 | METHODS

2.1 | Subjects

The family of this study had a history of an affected girl based on the clinical documents and family history was strongly suspected of Sandhoff or Tay-Sachs disease. The parents were healthy and first cousin. They had 3 children: two normal boys and only a girl who was born via normal delivery without any pre/postnatal insults with good Apgar score and normal birth weight and head circumference.

She was completely normal until the age of 9 months (based on her parents' claims), but thereafter they noticed she has a problem as she lost her motor skills such as turning over, sitting, and crawling. Later, she developed exaggerated startle reactions to loud noises and experienced seizures. At the age of 2 years, observing cherry-red spots by an eye specialist in her confirms the diagnosis of GM2-gangliosidosis. Despite taking different antiepileptic drugs, her seizure never gets controlled completely. After several times of hospitalization due to severe infections and fever, she died at the age of 3 years due to respiratory problems.

In this case-control study, we had two groups including parents of a case with suspected GM2-gangliosidosis and 100

Exon	Primer sequence (5'→3')	Amplicon size (bp)
1.	Forward: 5'GTCTGGGCTCTGCTCCTTTAC 3' Reverse: 5'CAAGAGCGTGGGTTTCAGTC3'	698
2	Forward: 5'GGCAGCATGGATTTGAGGAG3' Reverse: 5'CAGCGAGCACCTGGGATATA 3'	297
3	Forward: 5'AATAGGTCATGTGCTTGGGAG3' Reverse: 5'GGATCTAAGGCGGCAAAGTTTT3'	289
4,5	Forward: 5'ATGGGTACAATGAAGTAAAGCAC3' Reverse: 5'TCCCCTGTTCCAAACTACACA3'	766
6	Forward: 5'GAGCCAAGATCATGCCACTG3' Reverse: 5'AACTAGGGGACACTGAGGGT3'	798
7	Forward: 5'GCTGTCAAATATCAAATGCAAGC3' Reverse: 5'GGGTGACAGAACAAGACTCC3'	295
8	Forward: 5'CAAAGAGGCAAAGAGACAGG 3' Reverse: 5'GTAGAGATGTGGTTTCAC3'	537
9	Forward: 5'GGTGGTAAGGTAAAGAAAGCCA3' Reverse: 5'GGCAAAGGATGTAGAGAAAATGT 3'	412
10	Forward: 5'GGCACCTCTCAAAATGCAAGA3' Reverse: 5'ACCCACAACACTTGCCAA 3'	818
12,13	Forward: 5'TGCCTCTGTGTATAAGCTTTGA3' Reverse: 5'TGGAGTTCTAAGTTACACCAACA3'	585
14	Forward: 5'GCTGCAGGATGGTCGAGTAA3' Reverse: 5'GATGCCAGGCCTCTAAATGT3'	599

TABLE 1 Primers for amplification of *HEXB* exons

TABLE 2 Primers for amplification of *HEXA* exons

Exon	Primer sequence (5'→3')	Amplicon size (bp)
1	Forward: 5'TGGCCGGTATTTACTGCTCT3' Forward: 5'CATAGGGCGTCTCTGGAGC3'	600
2	Forward: 5'CTTTCCTTTACCACAGGC3' Forward: 5'CTCCAGGCCGAGCATCAG3'	300
3	Forward: 5'CAAAGGCATTGGGAGCATCG3' Forward: 5'TCCACCAACACCAACCTTCC3'	526
4	Forward: 5'AGTGGCTTCCTAATATCCCCT3' Forward: 5'AATCTACCAAACCAGTTTTCCAC3'	384
5	Forward: 5'AAGAATCCTGGGAGAGTTGTC3' Forward: 5'CTCTTAAGTGTGAAGAAGGCCT3'	236
6	Forward: 5'CTGAGAGCTCGCCCAACAT3' Forward: 5GCCACAGCCAGATTTCAGACA3'	244
7	Forward: 5'AGTCTGTGGGCATTTTGTAGT3' Forward: 5'TATGCCACTTCCATGAGCCA3'	299
8	Forward: 5'GTGAGTGCCTAAGTGTGCCT3' Forward: 5'AGACAATTCTGTGCCAGGG3'	591
9,10	Forward: 5'CAGGTGACTAATCCCCAGGC3' Forward: 5'ACTGCTGGTGGCTTCTTCTC3'	560
11,12	Forward: 5'GGAATCTCCTCAGCTTTGTGT3' Forward: 5'TGGGATTGGGTCTCTAAGGGA3'	598
13	Forward: 5'TTGCTCAAGACCCAGCACAA3' Forward: 5'CCCACAGCTTGCTTACCTCA3'	531
14	Forward: 5'TTCATGGACAGGACAGCACCC3' Forward: 5'AATACTTTGCCCCACCCAG3'	515

controls from population-matched Iranian subjects who had no disease in their family. After informed consent has been obtained from all participants, blood samples were collected. The experiments were approved by the Ethics Committee of Shahrekord University.

2.2 | DNA genotyping

Genomic DNA from peripheral blood of the parent and 100 control samples was extracted using the Diatome kit (Isogen Laboratory Russia) according to the vendor's recommended protocol. For all exons of the *HEXA* and *HEXB* gene, oligonucleotide primers were designed using primer3.0 software (Tables 1 and 2); the accuracy and efficiency of the designed primers were verified with the BLAST server (Basic Local Alignment Search Tool) (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>).

Genotyping of all the exons was carried out using polymerase chain reaction (PCR) and direct sequencing methods. The reactions were performed in 25 μ L volume containing 2 μ L genomic DNA, 2.5 μ L (10 \times) PCR buffer, 0.75 μ L MgCl₂ (50 mmol/L), 0.5 μ L of four dNTPs (40 mmol/L),

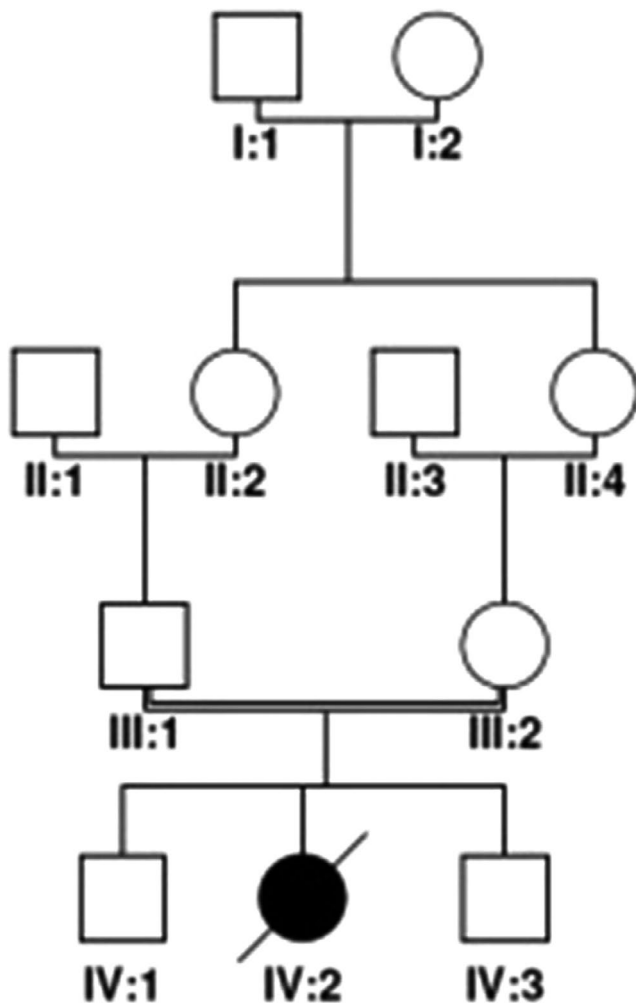
0.5 μ L of each primers (10 μ mol/L) (Tables 1 and 2), and 0.25 μ L Taq DNA polymerase (5 U/ μ L) (KBC, IRAN). Amplification was achieved by incubation in a BIOER DNA Thermal Cycler (TC-XP-G) for an initial denaturation of 5 minutes at 95°C, succeeded by 35 cycles of denaturation at 95°C for 40 seconds, annealing at 56°C for 40 seconds, and extension at 72°C for 30 seconds followed by final extension of 10 minutes at 72°C. Before sequencing analysis, PCR amplicons were assayed for size and purity by separation on 1% agarose gel, stained by DNA green viewer (Afratoos, Iran).

2.3 | Bioinformatics analyses

To predict the possible effect(s) of the plausible mutation on protein function, we used online server SIFT (Sorting Intolerant From Tolerant) (<http://sift.bii.a-star.edu.sg/index.html>),¹⁶ PROVEAN (Protein Variation Effect Analyzer) (<http://provean.jcvi.org>),¹⁷ PolyPhen 2.0 (<http://genetics.bwh.harvard.edu/pph2>),¹⁸ I-Mutant 3.0 (<http://gpcr2.biocomp.unibo.it/cgi/predictors/I-Mutant3.0/IMutant3.0.cgi>),¹⁹ and PHD-SNP (<http://gpcr.biocomp.unibo.it/cgi/predictors/PhD-SNP/PhD-SNP.cgi>)²⁰ as stated in Table 3. The results

TABLE 3 In silico approaches available as online tools

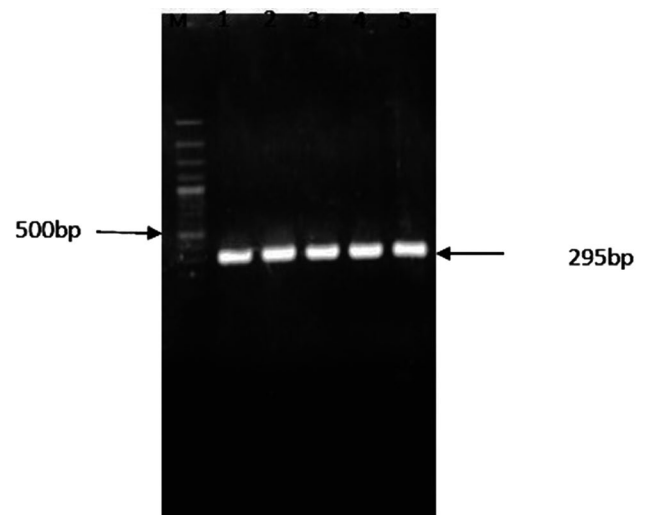
Server	Feature	Reference
SIFT	Sequence homology-based tool detect damaging single amino acid substitutions. The amino acid substitution is predicted damaging when the score is ≤ 0.05 and tolerated if the score is >0.05	Magesh & Doss (2014); Ng & Henikoff ¹⁶
PolyPhen 2.0	Sequence and structure-based method that predicts the influence of amino acid substitution on the structure and function of proteins. The output levels of probably damaging and possibly damaging were classified as deleterious (≤ 0.5) and the benign level being classified as tolerated (≥ 0.5).	Adzhubei et al ⁽²¹⁾ ; Ramensky et al ¹⁸
I-Mutant 3.0	For the automatic prediction of protein stability changes caused by single amino acid substitution. output result: $DDG < -0.5$: Large Decrease of Stability $DDG > 0.5$: Large Increase of Stability $-0.5 \leq DDG \leq 0.5$: Neutral Stability	Capriotti et al ¹⁹
PROVEAN	Estimates whether a protein variant affects protein function. A protein variant is predicted to be neutral or deleterious (default is -2.5)	Choi et al ¹⁷ ; Manickam, Ramanan, Singh, & Talwar ⁽²²⁾
PhD-SNP	SVM based on evolutionary information predictor of human deleterious single-nucleotide polymorphisms	Capriotti et al ²⁰ ; Magesh & Doss ⁽²³⁾
HOPE Project	An online web server for studying the structural features of native protein and the variant models.	Venselaar, te Beek, Kuipers, Hekkelman, & Vriend ⁽²⁴⁾

**FIGURE 1** Family pedigree with GM2 gangliosidosis; drawing with Cyrillic software

obtained from the different algorithms were clustered to increase the accuracy of the predictions.

3 | RESULTS

The patient's family pedigree is shown in Figure 1. The control group included an equal number of men and women. First of all, since the symptoms exhibited by the dead girl were more likely to be signs of sandhoff disease, therefore, we started from *HEXB* gene which has 14 exons. Second, it was

**FIGURE 2** Electrophoresis of the 295 bp PCR products of exon 7 of the *HEXB* gene on an agarose gel. Lanes 1 and 2: the parents' of the dead infant (CT); Lanes 3, 4, and 5 are controls (CC). GeneRuler 100 bp DNA ladder from Fermentas (Catalog number SM0321) was used as the DNA ladder

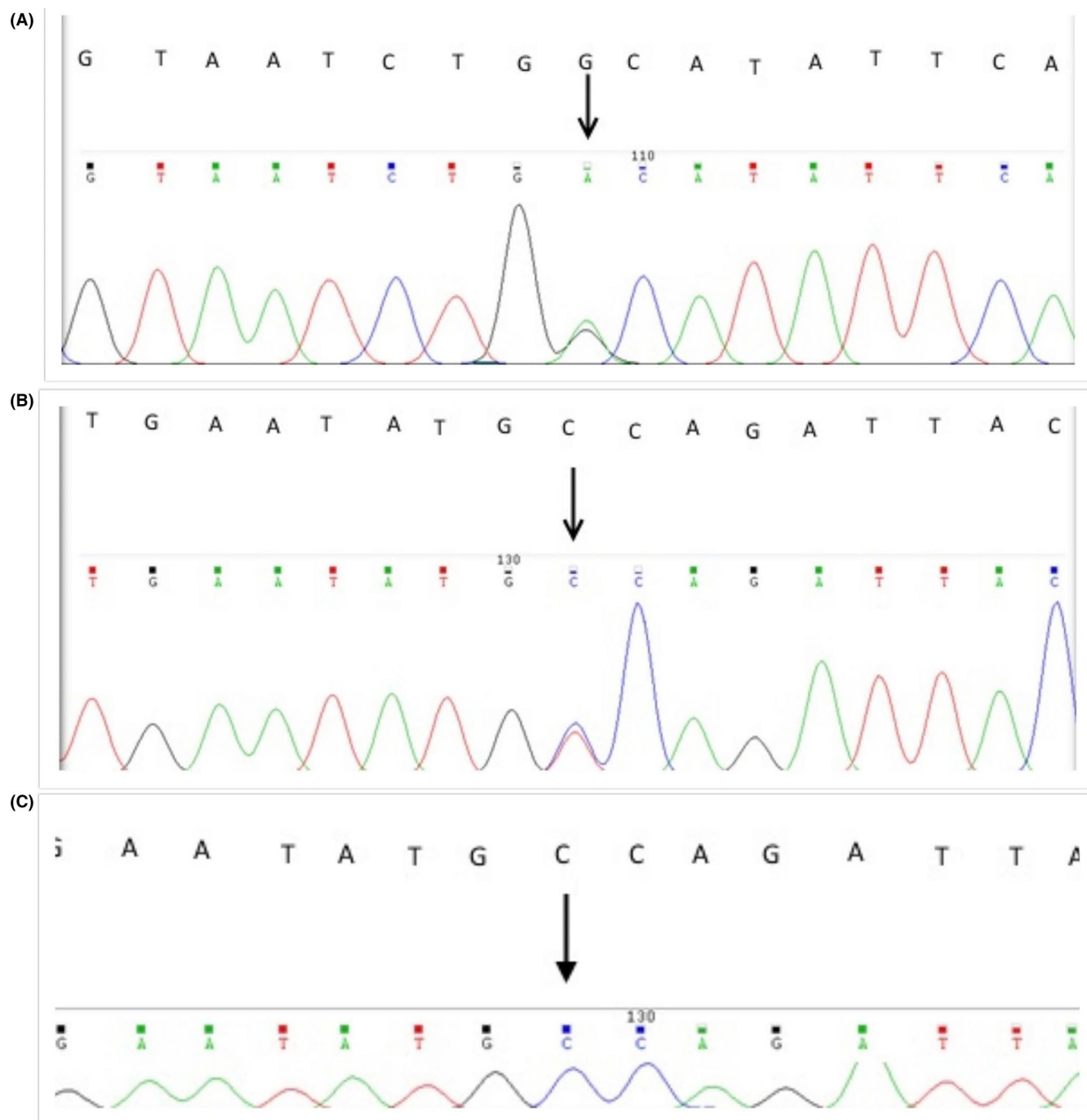


FIGURE 3 Results of the sequencing of exon 7 *HEXB* gene. a, b in the carriers (CT). Failure to match two sequences is due to the fact that one of the sequences is from the coding strand and the other from the noncoding strand. c DNA sequencing of exon 7 of the *HEXB* gene in the control sample (CC)

better to sequence firstly the exons which were more likely affected by mutation (s) and impact protein function that in turn lead to a severe form of the disease. Third, by considering the *HEXB* gene mutations in the GM2-gangliosidosis database (<http://data.mch.mcgill.ca/gm2-gangliosidosis>), we found that among the 14 exons of the gene, 4 exons including 7, 11, 13, and 14 have more mutations to take into account for

further analysis. Fourth, the literature review has also revealed that among these 4 exons, mutations in exon 7 is more related to the acute form of GM2 gangliosidosis which is a devastating disease.⁵ Fifth, investigation in the three-dimensional structure of beta-hexosaminidase β -subunit (*HEXB*) showed that exon 7 has less distance with the enzyme catalytic site and actually it is located in the vicinity of the enzyme active

TABLE 4 Outputs of in silico analysis

Functional consequence	SIFT	Score	PROVEAN	Score	PolyPhen-2	Score	I-Mutant3.0	Score	Stability	PhD SNP
A278V	Damaging	0.001	Deleterious	-3.74	Probably Damaging	1.000	Large decrease RI:5	-0.25	Disease	

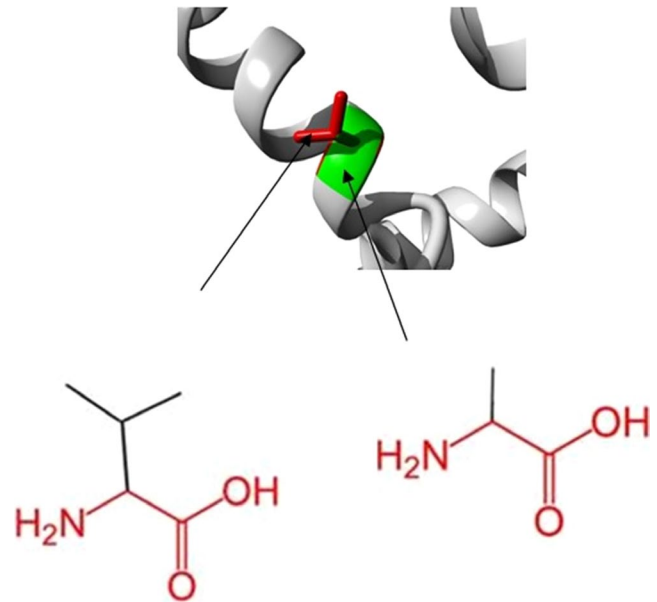


FIGURE 4 Deep view of the superimposed structure of wild-type and mutant residue at position 278. The main protein core is shown in gray color while the wild-type and mutated residues are shown in green and red color, respectively, and protein position 278 changed from Alanine to Valine. <http://www.cmbi.ru.nl/hope/>

site.^{25,26} Considering all the above reasons (first to fifth), we decided to begin our analysis from exon 7.

PCR was performed for exon 7 of *HEXB* gene for all the samples including the parent's of the dead girl and the controls (Figure 2). Sequence analysis of exon 7 of *HEXB* revealed heterozygote C → T substitution in both parent of the dead suspected girl having GM2-gangliosidosis. Sequences of the parent (Figure 3A, B) and one of the controls have been shown in Figure 3C.

After mutation was found in exon 7 *HEXB*, other exons of *HEXB* and *HEXA* were also sequenced, but no mutation was detected in any of the other *HEXB* exons and *HEXA* exons.

C → T substitution resulted in amino acid change at 278 position leading to Ala to Val substitution. This alteration was detected to be deleterious and damaging with in silico tools (SIFT, PROVEAN, Polyphen-2, I-Mutant3.0, PHD-SNP) (Table 4). Project Hope (<http://www.cmbi.ru.nl/hope/>) revealed that the wild-type and mutant amino acids differ in their size. The wild-type residue was buried in the core of the protein. The mutant residue is bigger and probably will not fit (Figure 4).

Inspecting the position of the mutation in the NCBI database (<https://www.ncbi.nlm.nih.gov/>) revealed that c.833 C>T was not a reported single nucleotide polymorphism. The results of this survey are shown in (Figure 5). Furthermore, the position of c.833 C>T mutation corresponded to the nucleotide number 74713567 and its flanking SNPs is shown in Figure 6.

Region	Chr. position	mRNA pos	dbSNP rs# cluster id	Heterozygosity	Validation	MAF	Allele origin	3D	Linkout	Function	dbSNP allele	Protein residue	Codon pos	Amino acid pos	PubMed
										contig reference	G	Val [V]	1	271	
	74713548	931	rs759209123	0.000						missense	T	Cys [C]	1	272	
										contig reference	C	Arg [R]	1	272	
	74713549	932	rs533645939	0.000						missense	A	His [H]	2	272	
										contig reference	G	Arg [R]	2	272	
	74713551	934	rs753066549	0.000						missense	G	Val [V]	1	273	
										contig reference	A	Met [M]	1	273	
	74713558	941	rs532333261	0.001		0.0004				missense	C	Thr [T]	2	275	
										contig reference	T	Ile [I]	2	275	
	74713575	958	rs138914144	0.000				Yes		nonsense	T		1	281	
										contig reference	C	Arg [R]	1	281	
	74713576	959	rs751678693	0.000						missense	A	Gln [Q]	2	281	
										contig reference	G	Arg [R]	2	281	
	74713589	972	rs781190762	0.000						synonymous	A	Val [V]	3	285	
										contig reference	C	Val [V]	3	285	
	74713596	979	rs750645495	0.000						missense	C	Gln [Q]	1	288	
										contig reference	G	Glu [E]	1	288	
	74713601	984	rs756316442	0.000						missense	A	Leu [L]	3	289	
										contig reference	T	Phe [F]	3	289	
	74713607	990	rs778593630	0.000						synonymous	T	Thr [T]	3	291	

FIGURE 5 Reported SNPs flanked the position of the Ala 278 *HEXB* in the NCBI database

4 | DISCUSSION

In this study, we reported a novel variant in an Iranian family with a history of a dead girl with Sandhoff disease which was not found in 100 controls.

The c.833C>T is a novel mutation in exon 7 of *HEXB* that causes a substitution of Alanine 278 with Valine. Both parents were heterozygous (CT) for this mutation, and all of the control samples were homozygous (CC) in this position.

SIFT, PROVEAN, PolyPhen, and PhD-SNP predicted that this mutation is causing disease and classified this mutation in a dangerous group of mutations. The I-mutant analysis showed that this mutation is reducing the stability of the

protein. We used 5 in silico servers because the accuracy of the in silico techniques for determining the effect (s) of mutation can be increased by combining different computational methods.²⁷

HOPE simulates the structural variation between mutant and wild-type residues. The wild-type residue is located in a region annotated in UniProt to form an α -helix. The mutation converts the wild-type residue to a residue that does not prefer α -helixes as a secondary structure (Figure 4).

Although Sandhoff disease is rare in the general population, there are several geographic regions with a high incidence of the disease including an inbred community of Metls (North American Indians), Lebanon, Creole population of



FIGURE 6 Nucleotide position 74713567 and its flanking SNPs

Argentina. Studies have revealed that mutations occur all along this gene.⁵ Very few studies have been done in Iran²⁸ on this group of diseases, and common mutations of the disease in the Iranian population are yet to be determined. Identification of common mutations in Iranian families appears essential especially for families who have a history of the disease, for genetic counseling, prenatal diagnosis, and prevention of neonatal with the disease.

HEXB gene is located on 5q13 and includes 14 exons.¹ Up to now, for *HEXB* gene 88 mutations in the human mutation database have been registered.²⁹ Several mutations have been detected in *HEXB* so far which causes an infantile form of the disorder; the most common mutation among them is a 16 kb deletion at the 5' end of the gene that no mRNA or β -protein produce.⁴ Some mutations have been found in the exon 7 of *HEXB* including a nonsense mutation, c.850C>T, which generated a stop codon at codon 284³⁰ in which all of the cell lines originated from the Kennedy Institute, Baltimore, MD; a 4-bp deletion in exon 7 (delCTTT) causes early stop codon at 273³¹ in Argentinean patients with Sandhoff disease and a single base-pair deletion in exon 7 that change the codon for Gly-258, GGA, and to GA²⁸ in the patient with Caucasian/Indian ancestry. Finally, A missense mutation, c.821T>A, which causes p.Val274Glu³³ has been reported in an Iranian child with juvenile Sandhoff disease.

5 | CONCLUSION

We concluded that c.833 C>T mutation carries out almost all criteria for pathogenic mutation such as changing amino acid at the conserved location and not found in nonpatient controls. Functional studies need to confirm the results.

ACKNOWLEDGMENTS

Not applicable.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

AUTHOR CONTRIBUTIONS

FMM: participated in the design and coordination of the research study, performed experiments, and analyzed the data; FA: participated in the design and coordination of the research study, performed experiments, analyzed the data, and wrote the initial manuscript; PN: participated in the design and coordination of the research study and revised the manuscript for submission; MG: participated in the design and coordination of the research study, provided some samples, assisted in the clinical analysis, performed some experiments, and analyzed the related data; MEB: participated in the design and coordination of the research study, supervised the study, wrote the initial manuscript, and

revised the manuscript for submission; all authors have seen and confirmed the final format of the manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The experiments were approved by the Ethics Committee of Shahrekord University.

CONSENT FOR PUBLICATION

Written informed consent was obtained from the patient parents' for publication of their individual details and accompanying images in this manuscript. The consent form is held by the authors.

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