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Short Report

Three Novel Mutations in Iranian Patients with Tay-Sachs Disease

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ABSTRACT

Background: Tay-Sachs disease (TSD), or GM2 gangliosidosis, is a lethal autosomal recessive neurodegenerative disorder, which is caused by a deficiency of beta-hexosaminidase A (*HEXA*), resulting in lysosomal accumulation of GM2 ganglioside. The aim of this study was to identify the TSD-causing mutations in an Iranian population. **Methods:** In this study, we examined 31 patients for TSD-causing mutations using PCR, followed by restriction enzyme digestion. **Results:** Molecular genetics analysis of DNA from 23 patients of TSD revealed mutations that has been previously reported, including four-base duplications c.1274_1277dupTATC in exon 11 and IVS2+1G>A, deletion TTAGGCAAGGGC in exon 10 as well as a few novel mutations, including C331G, which altered Gln>Glu in *HEXB*, A>G, T>C, and p.R510X in exon 14, which predicted a termination codon or nonsense mutation. **Conclusion:** In conclusion, with the discovery of these novel mutations, the genotypic spectrum of Iranian patients with TSD disease has been extended and could facilitate definition of disease-related mutations. *Iran. Biomed. J. 18 (2): 114-119, 2014*

Keywords: Tay-Sachs disease, β- hexosaminidase A, β- hexosaminidase B

INTRODUCTION

Deficiency of the lysosomal enzyme, β-hexosaminidase (*HEX*), leads to a heterogeneous group of recessive disorders. *HEXA* and *HEXB* are two isoenzymes of *HEX*. Tay-Sachs disease (MIM ID # 272800) is an autosomal recessive disorder, which results from a deficiency of *HEXA* activity [1, 2]. However, deficiencies of both *HEXA* and *HEXB* activities result in Sandhoff disease. β-N-acetyl *HEXA* is a heterodimer protein, which includes one α subunit and one β subunit, which are encoded by the *HEXA* (MIM 606869) and *HEXB* genes, respectively [3]. TSD is caused by mutations in the *HEXA* gene, thus leads to intralysosomal storage of its natural substrate (ganglioside GM2) [3, 4], primarily in neurocytes. TSD is a heterogeneous disease, in which the prototype of Tay-Sachs (infantile form) results from a complete absence of enzyme activity. This form manifests until the age of 3-5 months with the onset of hypotonia, decreasing attentiveness, developmental arrest by 83%, low muscle tone, blindness, macular cherry-red spots (typical ophthalmology feature) due to lipid-laden ganglion cells, intractable seizures, and rapid neurological deterioration, which leads to death in early childhood by the age of 5 [1, 5, 6]. Juvenile and adult subtypes of Tay-Sachs are less severe and extremely variable with slow progression due to the presence of some residual enzyme activities [5] and characterized by ataxia, dementia, cerebella dysfunction, dystopia, atypical motor neuron disease, and the psychiatric symptoms of depression and anxiety [5, 7]. Tay-Sachs disorder occurs at high frequency in Ashkenazi Jewish individuals due to a shared genetic background, with an incidence of 1 in 2,500 to 3,900 live births compared to 1 in 320,000 in the general population [8, 9]. Additionally, over 130 mutations in the *HEXA* gene have been already reported to cause TSD [9]. Study of Tay-Sachs in Ashkenazi patients showed that there are three mutations in this ethnic

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Table 1. DNA primers for identification of mutations in Tay-Sachs disease

Exon	Forward	Reverse	Product size (bp)	TM (°C)
E1	CGTGATTCGCCGATAAGTCA	TCCGACTCACCTGTGAGGTA	352	59.6
E2	TGTGAGCTGAGGGCTAGAGC	CCAGGCCATCCAGAGTTACA	250	60.0
E3	CATGAGGTAGGTGGTGCTTTG	TTGCAGTGAGCAGGGACTGG	453	62.0
E4	GCTACATTGAGAACCTTCCA	ACAGTGATTCCAAACAGA	313	55.6
E5	TAAGAATCCTGGGAGAGTTG	GGTTACCAGAGTGTCCAGGA	207	57.0
E6	TGAGAGCTGAGGCAGGTGAA	AACTGGCTGGTTAGGATGAG	229	60.0
E7	GCATCTTCTACTCTGCTAGC	AAGCTTCACTCTGAGCATAA	252	55.6
E8	GACACTCATATGGGGTTTTC	GAGTAAGCAACTGATCAGGC	260	55.6
E9	CAGGCATTAGGCTTTCAGGA	GGCCTGACTCGGTATGGAAA	223	59.6
E10	CAGTCTAGAACCCATCAGAG	ACTGCTGGTGGCTTCTTCTC	172	59.6
E11	ACTGCCATTTGACCTTTT	CCATCCTGTGCCCCAACCCA	267	57.0
E12	GAAACAACTTAGCTGGGGTG	TCCTGCTCTCAGGCCCAAC	240	58.7
E13	TGTGGATGTCCAGCACCTTT	CTCAGCAACTCACAGCGGAA	270	61.2
E14	TGACTGGTGTGAAAAGTGTTGCTG	AGGGAGGTGGATGAGTATGC	690	61.2

group: a 4-bp insertion in exon 11 of the *HEXA* gene (c.1274_1277dupTATC, 81%), a splicing mutation (c.1421+1G4C IVS12+1G4C, 15%), and a later-onset mutation (c.805G4A, 2%) [10]. This limited number of founder mutations has led to the design of a prevention program (carrier screening), which has successfully reduced the occurrence of TSD in the Ashkenazi population [10, 11]. In addition to Ashkenazi Jews, TSD has been described in non-Ashkenazi populations. In the Middle East, TSD has been reported in Arab, Iraqi, Turkish populations [12]. Studies of *HEXA* mutations in Saudi Arabian populations showed two nonsense mutations, including one novel mutation in exon 14 (c.1528C>T [p.R510X]) and one known mutation (c.78G>A [p.W26X]) [8] as well as one known missense mutation (1510G>A [p.R504H]) [13-16]. In the Iraqi Jewish population, a transition c.1351 C>G was found [17] in exon 12, which resulted in the change of Leucine to Valine (Val) at position 451. However, one missense mutation c.1A>G (p.MIV) and one nonsense mutation c.1177C>T (p.R393X) were found in infantile Tay-Sachs disease in the Persian population [18]. In this study, we examined the TSD patients in order to identify the novel TSD-causing mutations in the *HEXA* and *HEXB* genes in an Iranian population.

MATERIALS AND METHODS

Human subjects. Thirty one patients who had received clinical and biochemical diagnoses (deficiency of HEXA activity) of TSD, were referred to the Medical Genetics Lab in Tehran, Iran for molecular analysis. All patients were informed of the aims of the study and gave their informed consent to the genetics analysis.

Molecular analysis of HEXA. The genomic DNA (DNA fast, QIAGEN, Cat. No. 51204) was isolated from 31 TSD patients using peripheral blood leukocytes and chorionic villus sampling according to the manufacturer's protocol. The exons, exon-intron boundaries and at least 20 bp of flanking intronic sequences of the *HEXA* gene (α and β subunits) were PCR amplified in 14 fragments using primer pairs (Table 1).

RESULTS

Mutation analysis of the *HEXA* gene was performed on genomic DNA from the submitted specimen using sequence analysis of coding exons and corresponding intron/exon boundaries. The results revealed heterozygous mutations in patient 1 (c.986+3A>G) and patients 6 and 10 (c.170G>A in exon 5), heterozygote mutation in patients 7 and 8 (IVS2+1 G>A), patient 11 (c.393, R>X), patients 13 and 14 (deletion [Del] TTAGGCAAGGGC in exon 10), and patient 21 (c.368, Lys>stop) as well as non-pathogenic mutations in patients 4 (A->G in exon 13 [coding-region] and G->A in exon 14 [non-coding region]) in *HEXA* gene (Table 2).

Patient 3 with homozygote mutation (Del TTAGGCAAGGGC in exon 10), patient 9 with homozygote Del TTCC (c.631-634), patients 15 and 16 with homozygote mutation (IVS2+1 G>A and c.1 T>C, respectively), patient 17 with homozygote Del TCT in exon 9, patients 19 and 20 with homozygote insertion (TATC) in exon 11, patients 25 (c.393/R>X) and 29 (c.37 TAC>stop in exon 1) with homozygote mutations were affected for TSD (Table 2).

Table 2. Mutations detected in 31 cases for Tay-Sachs in an Iranian population.

Patient number	Disease Status	DNA Change/Mutation	Gene/Exonic location	Status
1	-	c.986+3A>G	*HEXA*	Het
2	?	C331G Gln>Glu	*HEXB*	Hom
3	*	DelTTAGGCAAGGGC C.365	Exon 10/*HEXA*	Hom
4	-	G>A(4326265) G>A(43426711)	*HEXA*/Exon 14 Non-coding region	Hom
		A>G Glu>Glu	Exon 13/coding region Homozygote	Hom
5	?	G70A, G76A and G45A	Exon 14/*HEXA*	Het/novel mutation
		T713G	Exon 3/*HEXB*	Het
6	-	c.170 G>A	Exon 5/*HEXA*	Het
7	-	IVS2+1 G>A	*HEXA*	Het
8	-	IVS2+1 G>A	*HEXA*	Het
9	*	c.631-634/ Del TTCC	*HEXA*	Hom
10	-	c.170 G>A	Exon 5/*HEXA*	Het
11	-	c.1177, Arg393>X C>T	Exon 11/*HEXA*	Het
12	?	DelG713	Exon 14 Non coding region / *HEXB* (β subunit)	Het
13	-	Del TTAGGCAAGGGC	Exon 10/*HEXA*	Het
14	-	Del TTAGGCAAGGGC	Exon 10/*HEXA*	Het
15	*	IVS2+1 G>A	*HEXA*	Hom
16	*	c.1 T>C	*HEXA*	Hom
17	*	DelTCT	Exon 9/*HEXA*	Hom
18	?	T to C R510 Stop	Exon 14/*HEXA*	Het
		Del G	Exon 3/*HEXA*	Het
19	*	c.1278 Insertion TATC	Exon 11/*HEXA*	Hom
20	*	c.1278 Insertion TATC	Exon 11/*HEXA*	Hom
21	-	c.368 Lys>stop	Exon 11/*HEXA*	Het
22	?	A>G c.436 I>V	Exon 11/*HEXA*	Hom
23	?	A 175 G	*HEXA*	Hom
24	?	InsG	*HEXA* (β Subunit),Intron5	
25	*	c.1177, R393>X	*HEXA*	Hom
26	?	G80A	*HEXA*/(β Subunit),Intron15	Hom
		G458A	*HEXA*/(β Subunit),Intron15	Het
		G744A/I207V	*HEXA*/(α Subunit),Exon 5	Het
27	?	InsG	*HEXA*/(β Subunit),Intron5	Hom
28	?	c.436 A>G Iso>Val	Exon 11/ *HEXA*	Hom
29	affected	c.37 C>G TAC>stop	Exon 1/*HEXA*	Hom
30	?	Del A	Exon 3/*HEXA*	Het/Novel Mutation
31	?	Del A	Exon 3/*HEXA*	Het/Novel Mutation

Het: Heterozygote; Hom: Homozygote; (-): not affected; (?): Unclear; (*): affected

The disease status for patients 2, 5, 12, 18, 22, 23, 24, 26, 27, 28, 30, and 31 are unclear, i.e. the pathogenicity of these mutations is unclear. In patient 2, a homozygous mutation (C331G) was observed in the *HEXB* gene, which resulted in amino acid (Gln>Glu) substitution. In patient 5, heterozygous novel mutations, including G70A, G76A, and G45A in *HEXA* exon 14 and T713G in *HEXB* exon 3 were found. Also, in patient 12, a heterozygous mutation (DelG713) in *HEXB* exon 14 in a non-coding region was identified. This mutation had not been reported in any literature before. In patient 18, two novel heterozygous mutations in *HEXA*, including a T-to-C polymorphism and a DelG were identified in exon 14 and 3, respectively, but the pathogenicity of these mutations in this patient was unclear. In patient 22, polymorphism c.436I>V in *HEXA* exon 11 was found. In patient 26, three mutations were observed: a G80A homozygous mutation in *HEXA* (β subunit) intron 15, a G458A heterozygous mutation in *HEXA* (β subunit) intron 15, and a heterozygous mutation G744A in exon 5, which resulted in I207V. In patients 24 and 27, homozygous mutations (InsG in intron 5 of the *HEXA* β subunit) were found. In patients 30 and 31, two mutation (DelA in exon 3), which had not been previously reported in other populations, were found (Table 2).

DISCUSSION

In recent decades, early stage detection and carrier screening programs for specific inherited disorders (which occur more frequently within a particular group in the general population) have effectively reduced the occurrence of a disease. High carrier frequency in a target population for a recessive genetic disorder is the prerequisite for the establishment of a carrier screening program. In addition to the frequency of a carrier in a target population, investigation of the carrier frequency in a similar population from different countries is also important, since migration between countries lead to the establishment of populations with mix origins.

Previously, Haghighi *et al.* [19] reported two mutations, including a missense mutation (c.1A4G [p.MIV]), which altered the initiation methionine to a Val, and one nonsense mutation (c.1177C4T [p.R393X] in exon 11) in 3 patients in an Iranian population. Approximately 20% of the Ashkenazi carriers harbored a splice junction defect, while almost 80% bore a 4-bp insertion, TATC, in exon 11 of the *HEXA* gene with Tay-Sachs disease [20]. Additionally, this mutation was accounted as the major mutation detected in non-Jewish populations at a frequency of 30% [21], while it was accounted for approximately 6% (patients 19 and 20) in our study. The G to A transition in exon 5 in a CpG dinucleotide, which resulted in Arg170 Gln, was found in ~6% of mutations (patients 6 and 10 with heterozygous mutation) in this study. This mutation is a disease-causing mutation inactivating the α subunit of the *HEXA* gene and was previously reported in Japanese infants with TSD [22] and in Moroccan Jewish populations [23, 24].

A 12-bp Del (TTAGGCAAGGGC) in exon 10 of the α subunit of *HEXA* was reported in patient 3 (homozygote) and non-affected patients 13 and 14 (heterozygote). This mutation was reported previously in TSD patients in a Turkish population. In patient 25, a c.1177C>T in exon 11 caused nonsense mutation p.R393X (heterozygous, not affected). This mutation was initially identified in French infants with TSD [25], and later in Turkish ones [26]. Two patients were found with mutations in exon 11 (c.436, A>G), which caused a change in Isoleucine (Iso)>Val. This mutation had been reported as a polymorphism in African-Americans and Ethiopian Jews [27]. Iso and Val are hydrophobic amino acids. Analysis of protein structure showed that the substitution of Iso>Val caused an increase in stability of the protein structure. In addition to this mutation, in patient 18, a heterozygous mutation (T>C) in exon 14 of *HEXA* caused a nonsense mutation (p.R510X), where homozygosity for this mutation predicts the production of premature termination of enzyme. Generally, nonsense and frame-shift mutations result in the reduction of mRNA in the *HEXA* gene. Diminished amount of mRNA has been reported in several mutations in the *HEXA* gene: Tyr180Stop in exon 5 of *HEXA* in Moroccan Jews, and Arg137 stop codon in exon 3 [23]. In patients 30 and 31, DelA in exon 3 (heterozygote) was observed, which this homozygous Del might be able to cause the disease. In addition, DelG in exon 3 (heterozygous mutation), which was not reported before, was found in patient18.

In addition to the mutations in α subunit of *HEXA*, three mutations were identified in the β subunit of the *HEXA* gene in non-coding regions. Patient 26 showed two heterozygous mutations (G80A and G458A) in Intron 15 of the β subunits, and patient 27 showed a heterozygous mutation (insertion G) in intron 5. Since the clinical significance of novel mutations is unknown, further investigation is required to determine the role of novel TSD-causing mutations. Among the novel mutations found in this study, two mutations were found in *HEXB*: a homozygote mutation (c.331) in patient 2, which resulted in alteration of Gln>Glu, where the pathogenicity of this mutation is under investigation as well as DelG713 in the subunit of non-coding region exon 14 of *HEXB*, which had not been identified before [9]. Due to the lack of an effective therapy for TSD, current efforts have focused on carrier screening programs to identify the TSD risk among Iranian population. This research may help in the understanding of the disease mechanism and may open up new experimental and therapeutic opportunities of TSD for diagnostic testing and also for future investigations.

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