Novel Mutations in Sandhoff Disease: A Molecular Analysis among Iranian Cohort of Infantile Patients

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Abstract

Background: Sandhoff disease is an autosomal recessive disorder caused by β-hexosaminidase deficiency and accumulation of GM2 ganglioside resulting in progressive motor neuron manifestations and death from respiratory failure and infections in infantiles. Pathogenic mutations in HEXB gene were observed which leads to enzyme activity reduction and interruption of normal metabolic cycle of GM2 ganglioside in sandhoff patients.

Methods: Six infantile index patients with typical biochemical and clinical picture of the disease were studied at the molecular level. After DNA extraction and amplification, probands and their parents, were evaluated by direct sequencing of amplicons.

Results: We identified 7 different mutations among which 4 were novel. The most prevalent finding (50%) among our population was a 16 kb deletion including the promoter and exons 1-5. The other findings included c.1552delG and c.410G>A, c.362 A>G, c.550delT, c.1597C>T, c.1752delTG.

Conclusion: We conclude that Cys137Tyr and R533C mutations may be pathogenic because of changing amino acid and locating at the conserved region and also they have not been observed in hundred controls. Besides, four mutations including: Cys137Tyr, c.1552delG, c.1597C>T and c.550delT fulfilled almost criteria for pathogenic mutation.

Keywords: Sandhoff disease, HEXB, Motor neuron disorder, Infantile patients, Hexosaminidase activity

Introduction

Sandhoff Disease is an autosomal recessive lysosomal storage disorder which is caused by β-hexosaminidase (HEX) deficiency. There are two catalytic forms of HEX namely HEXA, a heterodimer of α and β subunit, and HEXB which consist of two β subunits. Sandhoff Disease is caused by mutations in HEXB isoenzyme and appears as a wide spectrum of clinical manifestations with four categories of infantile, late infantile, juvenile and adult groups of patients, each group have their own characteristics in severity and age at the onset of disease (1).

There are several symptoms which may include motor weakness, sharp reaction to noise, blindness, mental deterioration, cherry-red spots in the eyes, seizures. Other symptoms may include frequent pneumonia, enlarged liver and spleen. Increased lower limb reflexes were the most common physical finding. Diagnosis was on the basis of hexosaminidase deficiency. Some studies showed that residual enzyme activity did not correlate with the clinical picture (2).

The responsible gene is located on 5q13 and includes 14 exons. Several mutations have been detected in HEXB so far which cause infantile

form of the disorder; the most common among them is a 16 kb deletion at the 5' end of the gene (3). In this study, we describe a group of infantile probands and their families with some novel and previously reported SNPs.

Materials and Methods

Study Population

We included 6 Iranian infantile index patients (4 males and 2 females) from 6 non-related pedigrees in our study who were suffering from infantile Sandhoff's Disease. The diagnosis was also enzymatically confirmed after thorough clinical exams and history taking. The molecular diagnostic investigation was done for both index patients and their parents. One hundred healthy individuals, who had no disease in their family, were used as controls.

Hex activity

Total Hex α and Hex β activities were measured in plasma and leukocytes according to O'Brein and also Wegner and William in reference diagnostic biochemistry laboratory (4, 5).

Preparation of genomic DNA

Genomic DNA was extracted from fresh blood samples of all patients using FlexiGene DNA Kit (QIAGEN). The extracted genomic DNA was solved in 100μl ddH2O and stored in -20°C.

DNA Amplification and Direct Sequencing:

Both exonic and exon/intron junctions of genomic DNA were amplified by PCR using specific pairs of primers (Table 1).

Table 1: Primers for amplification of HEXB exons

EXON	Primer sequence	Tm	Amplicon size
1	Forward:5'GGCAGACCGGGCGGAAAGCAG3'[a] Reverse: 5'TGCGCAGTGGGGTGGTGAGGG3'[a]	65	425bp
2	Forward:5'AGGAGTTAACTACAATGTTACTAG3'[a] Reverse: 5'AATAGGAATCATAAACTC3'[a]	53	400bp
3	Forward:5'AAATGAGGAACACAGAAGACCA3' Reverse: 5'TGTTCAATGGAAATCATTTTGG3'	55	466bp
4,5	Forward:5'TTTATCATCTCAATTTGTTGATTT3'[a] Reverse: 5'AAAGGAGACATCTTCAGA3'[a]	55	523bp
6	Forward:5'ATGGATTGTATATGATATCTGCAG3'[a] Reverse: 5'CTTGTAATGAAACTATACC3'[a]	52	214bp
7	Forward:5'ACAATTTCCAGGATCAAATCTACG3'[a] Reverse: 5'GGTGACAGAACAAGACTCCA3'[a]	60	261bp
8	Forward:5'TGGGAAACAAAGAGGCAAAG3' Reverse: 5'GCTGGGATTATAGGCGTGAG3'	60	458bp
9	Forward:5'ATGGAGGAGAAAGGTGGTAAGG3' Reverse: 5'TGCCTTCCTATATTTCAACAGC3'	60	535bp
10	Forward:5'TCCCAACTTGAGCTGCTTCT3' Reverse: 5'GACTTGCAAATTCCCAGTTG3'	57	301bp
11	Forward:5'ATTTTTCTTGGGGCAACTGG3' Reverse: 5'GGAGCCATCACTGAAAATGG3'	60	854bp
12,13, 14	Forward:5'TAAAGATGGAGGAAACAAATCTTG3'[a] Reverse: 5'TTGTGTTTCTTTTGTGTCCTTAA3'[a]	60	1030bp

[a] previously introduced by Narkis (6)

The reactions were performed in 50 μl volume containing 0.2-0.5 μg genomic DNA, 10mM Tris-HCL(pH 8.3), 50mM KCL, 1.5 mM MgCL2, 0.2 mM of four dNTPs, 0.5 μg of each primers, and 2 units of Taq DNA polymerase (Cinnagen, IRAN). Amplification was achieved by incubation in a DNA Thermal Cycler (Techne) for an initial denaturation of 5min at 95°C, succeeded by 32 cycles of denaturation at 95°C for 40s, annealing at 52-65°C for 40s and extension at 72°C for 30s followed by final extension of 10 min at 72°C. PCR amplicons were

then detected in 1.5% agarose gel, stained by ethidium bromide. Subsequently all amplicons were sequenced by a 3730XL ABI sequencer (Macrogen, Korea).whenever a mutation was detected in an index patient, the parents were also investigated to confirm the finding.

Results

We had 6 index patients with mean age of 16 and 14 months at the onset of disease (4 males and 2 females). Among them four patients (2 males and 2 females) died during the period of study. The average life time of patients was 44 months for males and 42 months for females and the mean enzyme activity also for males and females was 10.9 and 6.6 percent, respectively (Table 2).

We identified 7 different mutations of which 4 were novel. The most prevalent finding (50%) among our population was 16 kb deletion including promoter and exon 1-5 (Fig. 1), which is also the most common molecular finding in other studies of Infantile Sandhoff Disease. The other findings include c.1552delG and c.410G>A (Fig. 2), c.362 A>G, c.550delT (Fig. 3), c.1597C>T, and c.1752delTG.

None of above mutations was found in 100 healthy controls.

Table 2: Clinical, biochemical and molecular specifications of patients affected by infantile sandhoff disease

| Number	Sex	Major clinical manifestations	Enzyme activity (%)	Age at the onset of disease(Month)	Age of death(month)	cDNA mutation	Mutation effect	Exon	reference
1	M	Proximal dominant Muscular weakness	19	20	–	c.1552delG	p.D518fsX12	13	This study
						c.410G>A	p. C137Y	2	This study
						c.1752delTG	*5-*6delTG	3'UTR	(7)
2	M	Motor neuron disoredres, muscle atrophy, ataxia, myoclonus, dyslexia, incontinece	5.3	12	39	c.362 A>G	p.K 121 R	2	(8)
						c.550delT	p. S183fsX23	4	This study
3	F	Motor neuron diosdre, ataxia, cachexia, muscle atrophy, incontinence	6.1	14	41	16 kb deletion	16 kb incl. promoter+ex. 1-5	promoter+ex. 1-5	(9)
4	M	Muscular atrophy, weakness	12.5	17	–	c.1597C>T	p.R533C	13	This study
5	F	Motor neuron disorders, ataxia, muscle atrophy, dyslexia	7.2	14	43	16 kb deletion	16 kb incl. promoter+ex. 1-5	promoter+ex. 1-5	(9)
6	M	Motor neuron disorders, ataxia, cachexia, mascular atrophy	6.8	16	49	16 kb deletion	16 kb incl. promoter+ex. 1-5	promoter+ex. 1-5	(9)
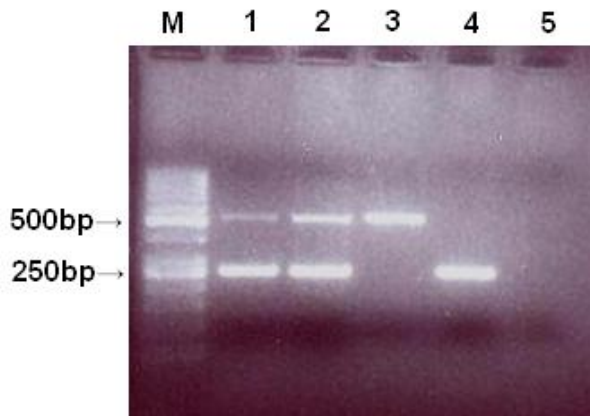
Fig. 1: Amplicons electrophoretic results of Exon 1-5 (exon 1, 2 and 3 in separate tubes and exon 4, 5 in same tube). Here only exon 4 and 5 depicted. M) marker 50bp, 1, 2) two normal samples with both exon 4, 5 HEXB and internal control primers, 3) normal sample with primers for exon 4, 5, 4) proband's PCR product with both inernal and exon 4,5 primers, 5) proband's PCR product with only exon 4, 5 primers
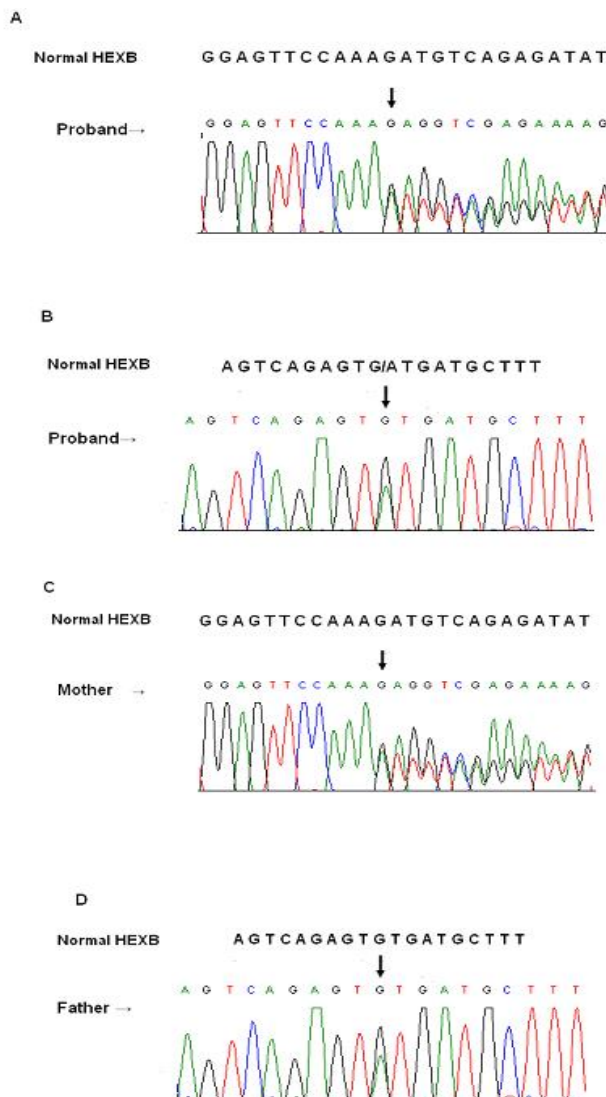

Fig. 2: DNA sequencing of HEXB gene demonstrated 2 novel mutations in exon 13 (A) and 2 (B) in proband number 1. One of these mutations was maternal(C) and the other was paternal (D)
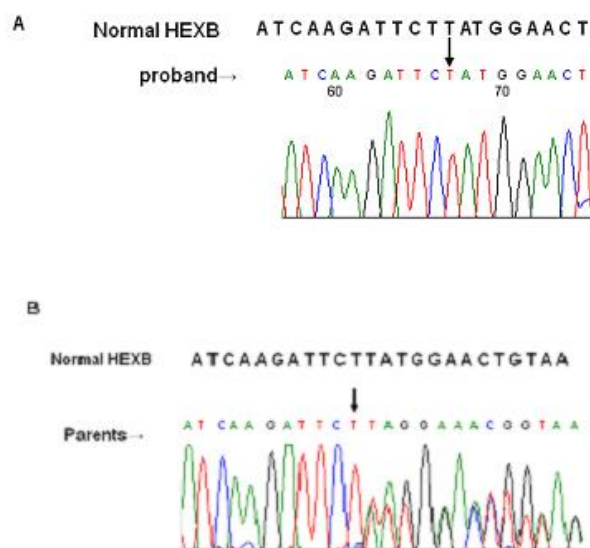
Fig. 3: Direct DNA sequence analysis of HEXB gene showed a homozygote mutation c.183del T (c: 550del T) in exon 4 (leading to stop codon in codon 207, c: 619Term in exon5) in proband number 2(A) and his parents (B)
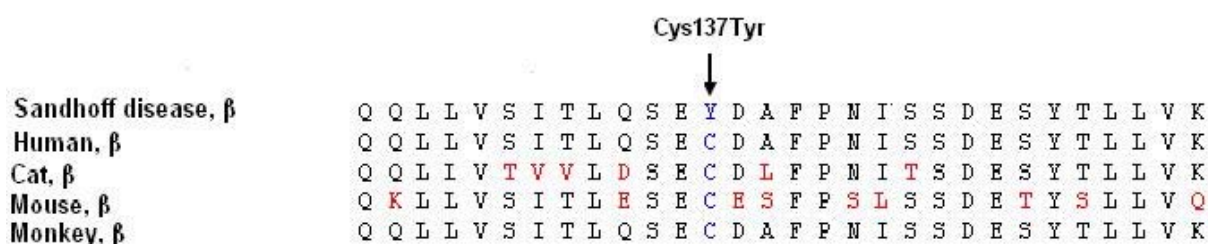

Fig.4: Comparison of β-hexosaminidase protein sequences around the C 137 Y mutation site between proband and normal human (13), Cat (14), mouse (15), monkey (http://www.ncbi.nlm.nig.gov) and fish (http://www.ncbi.nlm.nig.gov)

Discussion

In this study we report the clinical and molecular characterization of a group of non-related Iranian patients with Infantile Sandhoff's Disease (SD). The studied populations were also screened for biochemical abnormalities such as HEX decrease activity which revealed more decrease in female patients than males. It should be notified that during completion of our investigation 5 infants including 3 males and 2 females died due to the severity of their disease and associated infections.

We have found a compound heterozygosity in patient number one for HEXB. His clinical signs and symptoms commenced at 20 months of life

and resulted in Infantile Sandhoff Disease with motor neuron symptoms such as dyslexia and dysphagia which ends up to ataxia and finally bedbound state. HEXA and HEXB and also HEXA+B activity was low (with mean HEXB activity of 20%) in the patient but other serum biochemistry indices were within normal range. His main clinical sign was proximal dominant muscular weakness especially in his upper limbs. Also the patient had two other affected brothers of 7 and 5 years old respectively with similar clinical signs and symptoms. Molecular analyses of the genomic DNA revealed two heterozygote mutations as paternal novel Cys137Tyr and ma-

ternal De novo c.1552delG. The first mutation is one of the six cystein residues which form a disulfide bond with Cys 91 (10) that is well conserved and these disulfides bonds can be important for enzymatic activity (11, 12). Moreover it was not reported as a common polymorphism in this disease; hence the pathogenic possibility for changes in the sequence is highly suspected (Fig. 4).

Other studies have shown that the TG deletion (delTG) in the 3' untranslated region (3'-UTR) of the HEXB gene, 7 bp upstream from the polyadenylation signal, is part of a conserved sequence motif present in the 3'-UTR of many eukaryotic mRNAs. Analysis of the HEXB mRNA from leukocytes of patients with delTG heterozygotes has revealed reduced mRNA levels and reduced enzymatic activity (7); this condition had also happened in our patient which was paternal too.

The second change in this patient inherited from his mother causes a frameshift in exon 13 with a premature stop codon which totally changes the protein. Moreover this mutation can affect protein translation which is deleterious if accompanied by common del TG polymorphism and causes 30% reduction in enzyme activity according to (16). Some authors though believe that the change might be also found in normal controls and sometimes considered as a common polymorphism (7).

The second patient, on the other hand, had a more complex clinical picture with vast motor neuron findings, and involvement of both limbs in proximal and distal muscular atrophy. The patient had severe ataxia which aggravated during the progress of disease within a 24 months period of time. He also had developed obvious occasional tonic clonic convulsions in the right hemifacial accompanied by dyslexia without intelligent quotient (IQ) reduction. There were urinary and fecal incontinence among the dominant findings during the last 12 months of the patient's life. Molecular findings included novel c.550delT which resulted in a frameshift change of p. S183fsX23 and led to prominent change in

protein structure. We found that the patient was homozygote for the mutation and both parents were heterozygote.

The forth proband in this study was a 20-months-old male with muscular atrophy who has been also analyzed for mutations in HEXB and the molecular finding was a De novo c.1597C>T which resulted in p.R533C. This change may have an effect on conserved region of the gene which could be then pathogenic according to Yoshizawa (17).

There have been other reports showing that R533H (18) is located at the conserved region of the gene and considered as a pathogenic mutation; however, this novel mutation is highly suspected for its pathogenic potential.

The frequency of newly found mutations among the Iranian cohort was 50% which may show diverse and different molecular characterization of the Iranian infants suffering from disease.

We conclude that four mutations including: Cys137Tyr, c.1552delG, c.1597C>T and c.550delT fulfilled almost criteria for pathogenic mutation such as: a) changing amino acid b) conserved location and c) not found in healthy controls. Functional studies need to confirm results.

Ethical considerations

Ethical issues (Including plagiarism, Informed Consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc) have been completely observed by the authors.

Acknowledgments

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