Genotype, phenotype and in silico pathogenicity analysis of HEXB mutations: Panel based sequencing for differential diagnosis of gangliosidosis

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Objectives: Gangliosidosis is an inherited metabolic disorder causing neurodegeneration and motor regression. Preventive diagnosis is the first choice for the affected families due to lack of straightforward therapy. Genetic studies could confirm the diagnosis and help families for carrier screening and prenatal diagnosis. An update of HEXB gene variants concerning genotype, phenotype and in silico analysis are presented.

Patients and Methods: Panel based next generation sequencing and direct sequencing of four cases were performed to confirm the clinical diagnosis and for reproductive planning. Bioinformatic analyses of the HEXB mutation database were also performed.

Results: Direct sequencing of HEXA and HEXB genes showed recurrent homozygous variants at c.509G > A (p.Arg170Gln) and c.850C > T (p.Arg284Ter), respectively. A novel variant at c.416T > A (p.Leu139Gln) was identified in the GLB1 gene. Panel based next generation sequencing was performed for an undiagnosed patient whose variant in HEXB mutation database showed 97% consistency in silico genotype analysis with the phenotype. Bioinformatic analysis of the novel variants predicted to be disease causing. In silico structural and functional analysis of the novel variants showed structural effect of HEXB and functional effect of GLB1 variants which would provide fast analysis of novel variants.

Conclusions: Panel based studies could be performed for overlapping symptomatic patients. Consequently, genetic testing would help affected families for patients’ management, carrier detection, and family planning.

1. Introduction

Gangliosides are main components of the neuronal plasma membrane. Six major gangliosides have been identified; GM1 and GM2 gangliosidoses are major fatal neurodegenerative diseases due to defects in ganglioside catabolism. The clinical manifestation of gangliosidosis correlates with the different substrates that are stored and not catabolized e.g. GM1 and GM2 gangliosidases; GM1-gangliosidosis has different substrates including GM1 gangliosidase, oligosaccharides and keratan sulfate. GM2 ganglioside is the substrate for B-hexosaminidase A, which is deficient in Tay-Sachs disease, but only Hex A deficiency is seen in Tay-Sachs disease. Sandhoff disease inherited with autosomal recessive inheritance is caused by defect in lysosomal B-hexosaminidase A, composed of α chain, β chain and GM2-activator proteins [2,3]. Symptoms of these two disorders may overlap with GM1 gangliosidosis which can make the diagnosis difficult. There are different phenotypes for gangliosidosis based on biochemical findings and age of onset; infantile (acute form; < 0.1% activity), late infantile and juvenile (subacute form; 0.5% activity) and adult (chronic; 2–4% activity) forms [4]. Mutations in the individual proteins of the B-hexosaminidase enzyme complex cause different levels of activity and structural changes.

Sandhoff disease is caused by mutations in HEXB gene, which encodes the beta chain, located on 5q13 chromosome, encompassing 14 exons, spanning 2 Kb mRNA and encoding 556 amino acids. HEXA encodes the α chain, located on 15q23-q24, which mutations clinically cause Tay-Sachs disease [2,5]. These two genes have approximately
60% similarity in function and structure [5,6,7]. In addition, GM2A gene, the other component of hexosaminidase A complex, acts as substrate specific co-factor causing AB-variant phenotype. Mutations in any of the three related gene products of B-hexosaminidase A protein lead to GM2 accumulation in neuronal lysosomes and cause fatal neurodegeneration and apoptosis of neurons [8].

To date, 182 mutations for HEXA, 105 for HEXB gene and 9 for GM2A have been reported in the Human Gene Mutation Database (HGMD) to cause GM2 gangliosidosis (www.hgmd.org). Also, 211 mutations have been reported for GLB1 gene in HGMD.

We report a case suspected of having gangliosidosis with unspecific biochemical and enzymatic findings, and patients clinically diagnosed with Sandhoff disease, Tay-Sachs disease, and GM1-gangliosidosis. Molecular genetic diagnosis was established by panel based next generation sequencing for the suspected case and by direct sequencing for clinically diagnosed patients. In silico structural and functional analyses were performed to evaluate novel variants and to predict pathogenicity. In addition, we provide a literature review of the spectrum of HEXB gene mutations described in Sandhoff disease performed up to December 2017.

2. Patients and methods

2.1. Genetic testing

We studied four patients from Iranian population. Informed consent was obtained and DNA for genetic testing was extracted from peripheral blood using standard protocols. Coding regions and exon-intron boundaries were enriched using NimbleGen kit (NimbleGen, Roche, Basel, Switzerland). Sequencing analysis was performed for first case by targeted-next generation sequencing (NGS) on an Illumina, Hiseq2000 (Illumina, San Diego, California, USA). Reads were aligned using Burrows-Wheeler Aligner (BWA) on reference genome (hg19) [9]. Variants should have been annotated; annotation was performed by SAMTools [10]. Gangliosidosis genes [11] including 4 genes (HEXB, HEXA, GM2A and GLB1) were used as a pane for analysis. For finding rare variants, they were filtered based on their frequency (minor allele frequency < 0.01) in 1000 Genome and dbSNP [12] (https://www.ncbi.nlm.nih.gov/projects/SNP/). Variants were validated based on sequencing analysis and segregation analysis of the patients. Coverage of target region with at least depth of 30X was approximately 99%.

Direct sequencing of HEXB, HEXA gene and GLB1 gene was performed for clinically diagnosed patients (#2-4).

2.2. Case presentation

2.2.1. Case 1

A 10 month old boy referred to Children’s Hospital Center with nystagmus, weakness and wasting of limb muscles. He was born after a full term pregnancy with head circumference of 35 cm and birth weight of 3.450 Kg from a first cousin couple. His older brother had no related clinical symptoms. No other affected family member was seen.

The clinical onset of symptoms began at 5 months of age with nystagmus and hypotonia but parents did not seek pediatrician consultation until 10 months old. He had developmental delay since the early months of life and regressed in later months. Speech was initially delayed, then became absent as he aged. In addition regression of motor skills and cognition was noticed.

Physical examination revealed head circumference of 46 cm, dysmorphic features, hypoacusis, startle reaction to loud noise and no organomegaly. He had uprolling of eyes and tonic contraction of limbs. Sonography of the abdomen was also normal. Neurological examination showed hypotonia of limbs. Ophthalmlogical examination showed presence of bilateral cherry-red spots. Auditory brainstem response (ABR) was normal. Cerebral computed tomography (CT) scanning showed a bilateral thalamic hyperdensity with hypodensity of the white matter. Magnetic resonance imaging (MRI) revealed increased signal intensity on T1-weighted images in thalamus and hypointense on T2-weighted images.

Enzyme analysis showed deficiency of hexosaminidase B-HexA and HexB in serum. Beta galactosidase activity was lower (< 0.017) than normal range (0.017–0.048 unit nmol/mg). Serum alanine aminotransferase (ALT or glutamic pyruvic transaminase = SGPT) was 126 (normal level: 7-57 U/L) and aspartate aminotransferase (AST or glutamic oxaloacetic transaminase = SGOT) level was 146 (normal range: 5-40U/L). Clinical data suggested gangliosidosis.

2.2.2. Case 2

A couple referred for prenatal diagnosis having a child clinically diagnosed with Sandhoff disease. Their child was a 21 month old boy having nystagmus, muscle weakness, problems in walking and delayed motor skills. He was the first child of this healthy consanguineous couple, although they had similar disease in the mother’s cousin. The onset of the disease began at 6 months. He had cognitive, speech and motor delay and regression. On physical examination hepatosplenomegaly, hypotonia and limb spasticity were noted. He lost the ability to perform tasks and decreased eye movements. Ophthalmologic examination showed bilateral cherry red spots. HPLC biochemical analysis of amino acids was normal. Tandem mass spectrometry (MS/MS) showed no significant deficiency of fatty acids and amino acids. Liver function tests (LFT) were normal. Enzyme assay of the activity of hexaminidase A and Hex B revealed deficiency; consequently, the clinical diagnosis of Sandhoff disease was made at the age one year old.

2.2.3. Case 3

A one year old girl with neurologic regression in the first year of life referred to genetic laboratory for molecular testing of GLB1 gene. She was the first child of healthy consanguineous parents. She had developmental delay beginning at 3 months old. She also had hearing problems. She presented hypotonia and hepatosplenomegaly. β-galactosidase activity was very low.

2.2.4. Case 4

A one year and 9 months old girl referred to Children’s Hospital Center. She was born as the first child of consanguineous parents. She had normal growth and development until 6 months of age. Motor regression appeared by this age. Ophthalmology examination showed right sided strabismus. She was also diagnosed with Chronic Rhinosinusitis. Mucopolysaccharide urine analysis of MPSI, MPSII, MPSVI and GM1 analysis were in normal range. Tandem mass spectrometry (MS/MS) analysis of the specific metabolic enzymes was normal. She developed hyperacusis but no organomegaly was noted, therefore, she was clinically suspected to have GM2 gangliosidosis. Enzyme assay revealed deficiency of Hex A while Hex AB and Hex B were normal. She was clinically diagnosed with Tay-Sachs disease.

2.3. In silico structural and functional analyses of HEXB and GLB1 novel variants

Position of each identified variant was determined based on HEXB gene reference sequence: NP_000512.1 and NM_000521.3. In silico analysis was performed for all the reported variants to determine pathogenicity of the variants by MutationTaster [13], SIFT [14], and PROVEAN (Protein Variation Effect Analyzer) [15]. CADD (Combined Annotation Dependent Depletion) was software used to characterize the pathogenicity of variants in the studied cases [16].

Structural analysis was based on Phyre2 and I-TASSER servers. Structural analysis was based on protein homology/analogy recognition engine v2.0 (Phyre2) [17] to determine the structure and function of the variants in protein [18]. Iterative threading assembly refinement (I-TASSER) server was also applied for protein structure and function predictions [19]. The protein sequence of hexaminidase B and B-
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