

Tay-Sachs Disease: Two Novel Rare HEXA Mutations from Pakistan and Morocco

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Abstract

Background

Tay-Sachs disease (TSD) is a rare autosomal recessive genetic disorder characterized by progressive destruction of nerve cells in the brain and spinal cord. It is caused by genetic variations in the HEXA gene leading to a deficiency of β hexosaminidase A (HEXA) isoenzyme activity. This study aimed to identify causative gene variants in 3 unrelated consanguineous families presented with TSD from Pakistan and Morocco.

Methods Detailed clinical investigations were carried out on probands in 3 unrelated consanguineous families of Pakistani and Moroccan origin. Targeted gene sequencing and Whole Exome Sequencing (WES) were performed for variant identification. Candidate variants were checked for co-segregation with the phenotype using Sanger sequencing. Public databases including ExAC, GnomAD, dbSNP and the 1,000 Genome Project were searched to determine frequencies of the alleles. Conservation of the missense variants was ensured by aligning orthologous protein sequences from diverse vertebrate species.

Results We report on 3 children presented with Tay-Sachs Disease. The β hexosaminidase A enzyme activity was reduced in the Pakistani patient in one of the pedigrees. Genetic testing revealed 2 novel homozygous variants (p.Asp386Alafs*13 and p.Trp266Gly) in the gene HEXA in Pakistani and Moroccan patients respectively. The third family of Pakistani origin revealed a previously reported variant (p.Tyr427Ilefs * 5) in HEXA. p. Tyr427Ilefs*5 is the most commonly occurring pathogenic variation in Ashkenazi but was not reported in Pakistani population.

Conclusion Our study further expands the ethnic and mutational spectrum of Tay-Sachs disease emphasizing the usefulness of WES as a powerful diagnostic tool where enzymatic activity is not performed for Tay-Sachs disease. The study recommends targeted screening for these mutations (p. Tyr427Ilefs5) for cost effective testing of TSD patients. Further, the study would assist in carrier testing and prenatal diagnosis of the affected families.

Introduction

Tay-Sachs Disease (TSD) is a rare autosomal recessive genetic disorder associated with neurodegeneration. It represents the first well characterized lysosomal storage disease and is considered the prototype of GM2 gangliosidoses, which are caused by deficiency of either of 2 isoforms of β hexosaminidase namely β hexosaminidase A and B. TSD is caused by pathogenic variants in the HEXA gene leading to deficiency of β hexosaminidase A (HEXA) isoform activity. The human HEXA gene is located on chromosome 15 (15q23-q24) with 35.56 kb spans and contains 14 exons [1]. The encodedHEXA is an important lysosomal hydrolytic enzyme. Pathogenic variants in the HEXA-encoding gene leads to impaired activity of the enzyme, which loses its ability to cleave the terminal N-acetyl hexosamine residues of fatty acid derivatives known as GM2 gangliosides. As a result, N-acetyl hexosamine residues are massively accumulated in neuronal cells instead of further metabolizing into GM3 gangliosides [2]. Clinical features of the disease include seizures, hypotonia, weakness, regression of motor milestones, maculopathy with ‘cherry-red spot’, and cognitive deficits. Based on the onset of symptoms, TSD can be classified into 3 subtypes; infantile, juvenile and adult-onset [3].The infantile form of TSD has the most severe phenotype, typically characterized by regression of motor milestones and progressive weakness and hypotonia, and resulting often to early childhood death, while individuals with juvenile- or adult-onset disease encounter a delayed clinical course. The activity of HEXA isoform levels in TSD symptomatic individuals is deficient while that of β hexosaminidase B (HEXB) isoform, is either normal or elevated. Therefore, an assessment of β hexosaminidase enzyme activity in peripheral leukocytes, cultured fibroblasts or lymphoblasts is the test widely used internationally to diagnose the disease [4]. Recently, Next Generation Sequencing (NGS) has been proposed as an alternative screening method for TSD specially in populations with highly diverse ethnic origin.

Herein, we report on 2 novel mutations and one previously reported mutation in the HEXA gene in total 3 consanguineous families from Pakistan and Morocco presenting with infantile form of TSD. Novel homozygous variants (c.1157del; p.Asp386Alafs * 13 and c.796T >G; p.Trp266Gly) in HEXA were identified in a Pakistani (A) and a Moroccan (B) family respectively. In family C, a previously reported 4bp variant (c.1274_1277dupTATC; p.Tyr427Ilefs * 5) most commonly found in Ashkenazi Jews was identified [5]. Pakistani family A in our study is the first family to be genetically confirmed with a novel variant causing an infantile TSD in Pakistan expanding the mutational spectrum of HEXA in TSD.

Material and Methods

Ethical approval and blood collection

The present study was approved by the institutional review boards of Arid Agriculture University Rawalpindi, Pakistan, and UCL Institute of Neurology, Queen Square, London. After obtaining informed consent, pedigrees were drawn using information given by the wellinformed elders of the families (Fig. 1a, d & g). Blood samples were collected from probands, their parents and unaffected siblings. Whole genomic DNA was extracted from available blood samples using standard procedures. A dried blood spot(DBS) specimen from the proband of family A (IV-1) was collected by applying a few drops of blood, drawn by lancet from the heel, onto specially manufactured absorbent filter paper. The blood was allowed to thoroughly saturate the paper and air dried for several hours. The specimen was stored in low gas-permeability plastic bags with desiccant added to reduce humidity at 4–8 °C. The (DBS) specimen was then shipped away to Synlab MVZ Leinfelden-Echterdingen, Germany for further testing, where the clinical analyte β hexosaminidase A was removed from the filter paper by placing the disc in a solvent or buffer and quantified.

Next generation sequencing

To investigate the genetic cause of the disease, Whole Exome Sequencing (WES) was performed in one of the affected probands in each family. For WES, Nextera Rapid Capture Enrichment kit (Illumina) was used according to the manufacturer instructions. Libraries were sequenced in an Illumina HiSeq3000 using a 100-bp paired-end reads protocol. Sequence alignment to the human reference genome (UCSC hg19), variants calling, and annotation were performed as described elsewhere [6]. After removing all synonymous variants, single nucleotide variants (SNVs) and indels in exonic and donor/acceptor splicing variants were selected for further analysis. In accordance with the pedigree and phenotype, priority was given to rare variants [$<1\%$ in public databases, including 1000 Genomes project, NHLBI Exome Variant Server, Complete Genomics 69, and Exome Aggregation Consortium (ExAC v0.2)] that were fitting a recessive or a de novo model.

In silico analysis

Prediction of functional effects of non-synonymous single nucleotide substitutions (nsSNPs) was done using software SIFT (Sorting Intolerant From Tolerant) (available at <http://sift.jcvi.org/>), Polyphen2 (Polymorphism Phenotyping v2) (available at <http://genetics.bwh.harvard.edu/pph2/>) and MutationT@ster (available at <http://www.mutationtaster.org/>). Prediction of protein stability changes

upon single point mutation was done using I-Mutant2.0 (<http://folding.biolfold.org/i-mutant/i-mutant2.0.html>). Evolutionary conservation of the amino acid residues of Hex A was analyzed using ClustalW program available online at (<http://www.uniprot.org/help/sequence-alignments>).

Results

Clinical Findings

The proband of family A (IV-1) is a 2.5-years old female, born to consanguineous parents from Pakistan, presented with regression of milestones and hypotonia with an onset at the age of 7 months (Fig. 1a). She is the third child of the family. 2 older female siblings had previously presented with similar phenotype and died at the age of approximately 3 years old. They both had a history of generalized hypotonia, delayed motor milestones and speech, and febrile seizures with an onset of around 6 months. AIV-1 was additionally diagnosed with generalized epileptic disorder, with an EEG showing a generalized pattern of bursts of sharp-and-slow-wave complexes. On examination, the proband had significant degree of trunk and limb hypotonia and weakness, being unable to sit on her own and hold her head up. She was awake and alert, but not able to follow commands or make visual contact. The proband had no history of epileptic seizures and EEG was reported normal. Brain MRI showed no signs of cerebral or cerebellar atrophy, but interestingly revealed some diffuse T1/T2-hyperintensities of subcortical white matter and hypointense appearance of thalamus in T2 and FLAIR images. Subsequent DBS testing for the enzymatic activity of β hexosaminidase was reported to be diagnostic for Tay-Sachs. (Proband: 0.03 nmol/spot, Reference range: 0.6–2.4 nmol/spot).

The proband of family (VI-2) was a 17-months old female, born to second-degree consanguineous parents from Morocco (Fig. 1d). She was born at term gestation with a normal weight and no adverse perinatal events were reported. She had normal early development and presented with psychomotor regression and hypotonia since the age of 9 months. She also had a history of generalized myoclonic epileptic seizures. She is the only child of the family and has a first cousin with a similar history. On examination, she had truncal hypotonia and was not able to make visual contact. Fundoscopy revealed bilateral maculopathy with the presence of ‘cherry-red spot’.

The proband of Family C (IV-3) was a 2.5 years old female, born to consanguineous parents from Pakistan (Fig. 1g). She was well until about 6 months of age after which, a delay in the attainment of newer milestones was noticed by the parents. Justlike a proband of a Pakistani family A, this proband also had significant degree of trunk and limb hypotonia and weakness and was unable to sit on her own and hold her head up. She had a history offebrile seizure and later was presented speech problems along with intellectual impairment. Her older siblings were normal. Unfortunately, the probands of family B and C were deceased by the time of genetic confirmation of Tay-Sachs and therefore not available for enzymatic activity testing.

Genetic Findings

Whole-exome sequencing revealed a homozygous frameshift variant Asp386Alafs * 13 in HEXA in the affected individual of family A of Pakistani origin (Fig. 1b). Data analysis of family B of Moroccan origin revealed a homozygous missense variant Trp266Gly in HEXA gene (Fig. 1e). Affected individual in family C from Pakistan was found to carry a homozygous 4bp insertion variant leading to frameshift Tyr427Ilefs * 5 in HEXA gene (Fig. 1h).

The Asp386Alafs * 13variant was present within the most significant homozygous block (chr:15; 59619222–99132077), the Trp266Gly variant was present within block (chr:15; 69341532– 74317043), while Tyr427IlefsTer5 variant was present within block (chr:15; 50182703–84645608) identified in the homozygosity mapping analysis (performed using the WES data). Parents of the affected individuals were found heterozygous forthe variants identified in their offspring. Mutation prediction tools including SIFT, Polyphen2 and MutationT@ster predicted the identified variants as disease causing. By conservation analysis, tryptophan (W) at amino acid position 266 was found conserved among different species (Fig. 1f) and comparison of amino acids of HEXA in unaffected and affected individuals is shown in Figs. 1c & i. The free energy change value (DDG) of the protein stability decreased to 2.03 Kcal/mol upon single-site mutation Trp266Gly at exon 7 predicted by I-mutant2.0.

Discussion

In this study, 3 consanguineous families with different ethnic origin were clinically and genetically investigated for mutations in HEXA gene causing Tay-Sachs disease (TSD). All families presented an infantile form of TSD. We identified two novel pathogenic mutations (p.Asp386AlafsTer13; p.Trp266Gly) occurring at

the functionally active site of the alpha subunit of β hexosaminidase A expanding the mutational spectrum of HEXA in TSD. We also found a 4bp duplication (c.1274_1277dupTATC) in third family of Pakistani origin. This variant(c.1274_1277dupTATC) is considered the most common recurrent mutation in Ashkenazi Jewish population. We report this variant for the first time in Pakistani population. [7] reported that the c.1274_1277dupTATC variant is one of the 3 most common hot spot mutations for TSD patients from India. Due to same geographical background and origin, it can be suggested that the common variant p.(Tyr427Ilefs*5) identified in family C might be used for carrier screening of the Pakistani population.

As per Human Gene Mutation Database (HGMD), nearly 190 mutations have been reported so far in HEXA causing TSD; that include 136 single base substitutions, 29 small deletions, 9 small insertions, 3indels and 1 large deletion of 7.6 kb (<http://www.hgmd.cf.ac.uk/>). The highest incidence of TSD identified in Ashkenazi Jews is likely due to a shared genetic background with a 4bp insertion c.1274_1277dupTATC (p.Y427Ifs5) in exon 11 of HEXA gene being the most common mutation in this group. Almost 15% of them harbor a splicing mutation (c.1421+1G>C; IVS12+1G>C) and 2% bear a later-onset mutation (c.805G>A) [5]. TSD has also been reported in individuals of non-Ashkenazi Jewish origin including Arab, Iranian, Indian, Iraqi and Turkish populations in Middle East and South Asia [3, 5]. Recently a single case of juvenile TSD with normal anterior chamber and bilateral healthy fundi has been reported in Pakistani population [7]. It has been reported that mutations resulting in gross alterations in the hexosaminidase alpha subunit sequence are generally found in the severe infantile form. However, missense mutations causing amino acid substitutions have also been found in both the infantile and late onset phenotypes [8]. The 2 homozygous frameshift and missense mutations found in our study are associated with severe infantile form of TSD.

The clinical features of infantile TSD seen in our patients were consistent with the defined phenotype including progressive loss of neurological function, hyperirritability, and progressive weakness. However, none of the cases had hepatosplenomegaly. Assaying for the activity of β hexosaminidase A in the serum via DBS testing revealed a deficiency of this enzyme in proband of family A. DBS testing has been the standard of care for neonatal screening for a variety of diseases. Moreover, this technology holds promising diagnostic services. With the emergence of advanced analytical technique, the use of DBS in clinical chemistry has been

broadened and many clinical analytes, including nucleic acids, small molecules and lipids, have been successfully measured [9]. DBS tests offer as timely and valid diagnosis of lysosomal storage disorders (LSDs) by measuring lysosomal enzymes. As a positive result will always be confirmed or denied by further specific tests, in this regard DBS testing may further supplement a valid molecular diagnosis via WES. Researchers worldwide have combined newborn screening (NBS) programs with NGS and explored potential advantages of the long-term collection of DBS samples [10]. Hence it is recommended to the clinicians to use the DBS testing as a primary diagnostic test for timely diagnosis of TSD. The MRI brain was available in one case (AIV-2) and revealed hypointense appearance of thalami in T2 and FLAIR images along with some diffuse T1/T2-hyperintensities of subcortical white matter. However, no signs of cerebral or cerebellar atrophy were found. Unfortunately, none of the probands underwent detailed electrophysiology testing to evaluate brainstem responses, including blink reflex and the presence or absence of exaggerated startle reflex. No clinical signs of hyperekplexia were reported in any of the 3 probands whatsoever, although probands of families of B and C were deceased at the time of genetic diagnosis and therefore not available for follow-up examination.

The 2 homozygous frameshift mutations (p.Asp386Alafs * 13; p.Tyr427Ilefs * 5) found in Pakistani families A and C respectively are predicted to cause protein degradation either through nonsense mediated decay or by the production of truncated protein lacking the C-terminal amino acids resulting in a completely nonfunctional version of β -hexosaminidase A leading to severe infantile form of TSD. The novel homozygous mutation (p.Asp386Alafs * 13) found in family A was not found in ExAC, 1000Genome, GnomAD, dbSNP and was predicted to be disease causing by MutationTaster(www.mutationtaster.org), Polyphen-2 (<http://genetics.bwh.harvard.edu/pph2/>) and CADD (<https://cadd.gs.washington.edu/>).

The missense variant (p.(Trp266Gly)) identified in Moroccan family (B) locates in Glycosyl hydrolase family 20 catalytic domain (amino acids 167–488). This domain contributes an essential function in the GM2-hydrolyzing activity of β -hexosaminidase A [7]. The wild-type residue tryptophan at amino acid position 266 is more hydrophobic than the mutant residue glycine which is very flexible and can disturb the required rigidity of the protein at this position. The variant was conserved in the respective orthologues (Fig. 1f). The mutation p.(W266G) most likely causes loss of hydrophobic interactions in the core of the protein leading to 2.03 Kcal/mol

decrease in free energy value of HEXA protein identified using the software I-Mutant2.0. Thus, the single-site mutation (Trp266Gly) destabilizes the HEXA protein and is associated with severe infantile phenotype. This is in accordance with previous observations that missense mutations responsible for infantile TSD were generally located in a functionally important region [11]. We believe that the deceased siblings of indexed cases harbored the same disease with same mutation.

Our study contributes to improving carrier testing in previously underexplored populations and prenatal diagnosis in the affected families. Screening for the most common variants will decrease the cost burden on population.

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Contributor's Statement

F. Bibi and S. Efthymiou drafted the manuscript. T. Bourinakis, Y. Kriouile, T. Sultan, S. Haider provided clinical information and revised the manuscript. V. Salpietro and A. Ullah analysed the data. H. Houlden and G. K. Raja supervised the study.

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Footnote

Fig. 1 Pedigrees of families showing autosomal recessive inheritance of Tay-Sachs disease a, d, g. Sequencing of the HEXA showing mutations (c.1157del, c.796T>g, and c.1274_1277dup) identified in family a, d and g respectively. Point of mutation is represented by arrows b, e, h. Comparison of amino acids of HEXA in unaffected

and affected individuals c, i. Tryptophan w amino acid (p.W266) represented in red
is conserved across different species f