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SHORT COMMUNICATION

Identification of two *HEXA* mutations causing infantile-onset Tay–Sachs disease in the Persian population

Alireza Haghighi[1], Jamileh Rezazadeh[2], Azam Ahmadi Shadmehri[2], Amirreza Haghighi[3], Ruth Kornreich[4] and Robert J Desnick[4]

The β-hexosaminidase A (*HEXA)* mutations in the first reported cases of infantile Tay–Sachs disease in the Persian population were identified in two unrelated consanguineous families. The clinical diagnoses of the affected infants were confirmed by their markedly deficient levels of HEXA activity in plasma or peripheral leukocytes. The specific causative mutation in each family was determined by sequencing the *HEXA* alleles in both sets of related parents. Two mutations were identified: c.1A>G (p.MIV), which obliterated the initiating methionine in codon 1, and c.1177C>T (p.R393X), which predicted a termination codon or nonsense mutation.
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INTRODUCTION

Infantile Tay–Sachs disease (TSD) is a progressive neurodegenerative disorder due to the deficient activity of the lysosomal enzyme β-hexosaminidase A (HEXA), which results in the neuronal accumulation of GM2 ganglioside.[1,2] This autosomal recessive disease is characterized by onset at 4–8 months of life of neurological involvement, progressive psychomotor retardation, followed by paralysis QJ;and blindness. The cherry-red spot, due to lipid-laden ganglion cells, is the typical ophthalmologic feature. Affected children rarely survive beyond 5 years of age. In addition, juvenile- and adult-onset phenotypes have been described, which have a more protracted clinical course.[3]

The prevalence of TSD in general population is about 1 in 200 000 births, compared to 1 in 2500–3900 among Ashkenazi Jews.[4–6] As there is no treatment for TSD, efforts have been focused on prevention by prenatal carrier identification and genetic counseling for carrier couples.[5,6] TSD screening programs, designed to identify disease carriers in Ashkenazi Jewish populations, have successfully reduced the occurrence of the disease in this population.[5] We report the first cases of TSD in the Persian (Iranian) population and identify the disease-causing *HEXA* mutations in two unrelated families.

CASE PRESENTATIONS

Case 1

The female proband presented at 16 months of age with weakness and motor retardation. Her parents were first cousins once removed. She was normal until 14 months when her parents noted motor problems and she lost the ability to walk by 16 months. At 24 months, she could not sit unaided, and at 26 months she had her first convulsion.

Electromyography showed mild upper motor neuron involvement, but no evidence of a peripheral neuropathy or involvement of lower motor neurons. The electroencephalography was abnormal. Ophthalmological examination revealed the macular cherry-red spot and bilateral optic nerve atrophy. Abdominal ultrasonography was normal. She did not have any hearing problems.

On the basis of these findings, the diagnosis of infantile GM2 gangliosidosis or TSD was suggested and confirmed by demonstrating markedly deficient activity of leukocyte HEXA ($1.3\,\mathrm{nmol\,h^{-1}\,mg^{-1}}$; normal range: $90–260\,\mathrm{nmol\,h^{-1}\,mg^{-1}}$).

Cases 2 and 3

The probands were brothers from an Iranian family. The parents were third-degree relatives and their first child was a healthy girl. The patients were born after uneventful and full-term pregnancies. Case 2 was normal until 10 months of age when he experienced his first seizure. He steadily deteriorated and lost his vision, hearing and motor ability by 20 months. In the second year of life, myoclonic seizures became medically uncontrollable and he died at 32 months. Case 3 had some similar features but did not experience convulsions. He presented at 12 months of age with hypotonia and neurodevelopmental retardation. He lost his vision at the age of 18 months.

[1]Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, UK; [2]Milad Genetics Clinic, Behzisti Organization, South Khorasan, Iran; [3]The Hospital for Sick Children, University of Toronto, Toronto, Ontario, Canada and [4]Department of Genetics and Genomic Sciences, Mount Sinai School of Medicine of New York University, New York, NY, USA

Correspondence: Dr RJ Desnick, Department of Genetics and Genomic Sciences, Mount Sinai School of Medicine of New York University, Fifth Avenue, 100th Street, Box 1498, New York, NY 10029, USA.
E-mail: robert.desnick@mssm.edu
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Table 1 Primer pairs used to amplify the *HEXA* coding sequence, with sizes of PCR products

	Primer sequence (5′–3′)		
Exon	*Forward*	*Reverse*	*Product length (bp)*
1	TGTAAAACGACGGCCAGTCCAGGCCGGAAGTGAAAG (70455525)	CAGGAAACAGCTATGACCCTCCTGATTGAACCGTAGTCCTA (70454924)	638
2	TGTAAAACGACGGCCAGTTAGGGTCTTGGTTTTGCCTG (70436081)	CAGGAAACAGCTATGACCAGGCCATCCAGAGTTACAGC (70435851)	267
3	TGTAAAACGACGGCCAGTGTCCAGTGATTTATATAGAATATCTGGTC (70435106)	CAGGAAACAGCTATGACCAACACCAACCTTCCCACATC (70434894)	249
4	TGTAAAACGACGGCCAGTTGCTCTGCTACATTGAGAACC (70433219)	CAGGAAACAGCTATGACCCAATATTGGGATCCAACCCC (70433026)	230
5	TGTAAAACGACGGCCAGTTTGTCTTCATCTCCCTGTGC (70432646)	CAGGAAACAGCTATGACCGGAACTTGGTCTGTCCGTTG (70432391)	292
6	TGTAAAACGACGGCCAGTCCAACATCGCAAGTTTGAGG (70430700)	CAGGAAACAGCTATGACCGCCACAGCCAGATTCAGAC (70430467)	268
7	TGTAAAACGACGGCCAGTTGTGGGCATTTTGAGTATCTTC (70430121)	CAGGAAACAGCTATGACCAGCCAGTGCCCTGAAGC (70429843)	315
8	TGTAAAACGACGGCCAGTTTACGTGTAGGACTGTGCGTG (70428723)	CAGGAAACAGCTATGACCCCTCGGGTGCTAACTTCTATTC (70428403)	357
9-10	TGTAAAACGACGGCCAGTTAATCCCCAGGCATTAGGC (70427594)	CAGGAAACAGCTATGACCTCTGTAGAGGCAGGGAGGAG (70427002)	629
11-12	TGTAAAACGACGGCCAGTGACATACTTTGCTGCTGGGG (70426230)	CAGGAAACAGCTATGACCCTTCAGAAGGCTCGTTGCAC (70425485)	782
13	TGTAAAACGACGGCCAGTGGTAGCAGCCTGTGGATGTC (70425021)	CAGGAAACAGCTATGACCCTCTCTAAGGGGTTCCCCAG (70424772)	286
14	TGTAAAACGACGGCCAGTGTGTGAAAAGTGTTGCTGGG (70423603)	CAGGAAACAGCTATGACCTGCCACATTACTCTTTATTGAATG (70423295)	345

Abbreviation: β-hexosaminidase A.
All the primers were tagged with M13 sequencing primers at the 5′ end. The number given in the primer table at the end of each primer reflected the location of the position in the primer at the 5′ end (without M13) as to the NCBI reference sequence NC_000015.8.

Fundoscopy revealed the typical macular ‘cherry-red spot’. The diagnosis was confirmed by demonstration of a severe deficiency of plasma HEXA activity (0.07 $mU\,ml^{-1}$; normal range: 0.47–2.60 $mU\,ml^{-1}$). The patient died at 18 months of age.

MATERIALS AND METHODS

Molecular analysis of the *HEXA* gene

Genomic DNA was extracted from peripheral leukocytes by standard procedures. All *HEXA* exons and at least 20 bp of flanking sequence were amplified in 12 PCR mixtures using the primers in Table 1. Amplification was performed for each fragment in a 25-μl final volume containing 50 ng of genomic DNA, 0.2 mM of each dNTP, 0.2 μM of each primer, 1.0 U of Platinum *Taq* polymerase (Invitrogen Corp, Carlsbad, CA, USA), 1.5 mM $MgCl_2$ and 2.5 μl of 10× buffer for 5 min at 95 °C, followed by 35 cycles of amplification consisting of 30 s at 95 °C, 30 s at 60 °C and 30 s at 72 °C, and a final elongation of 7 min at 72 °C in an ABI 9700 Thermal Cycler (Applied Biosystems, Foster City, CA, USA). PCR products were then purified and sequenced using ABI PRISM Big Dye Terminator Cycle Sequencing (Applied Biosystems) on an ABI 3730xl automated sequencer (Applied Biosystems). Data were analyzed using ‘Sequencher’ software (Gene Codes Corporation, Ann Arbor, MI, USA).

RESULTS

Molecular analysis of the *HEXA* gene in the parents from case 1 revealed that both were heterozygous for c.1177C>T (p.R393X), a nonsense mutation in codon 393. DNA from the proband was not available; homozygosity for this mutation predicts a premature termination of the enzyme protein.

In cases 2 and 3, sequencing of their genomic DNAs revealed homozygosity for a missense mutation c.1A>G, which altered the initiation methionine to a valine.

DISCUSSION

Here, we report the first cases of TSD in the Iranian population. The *HEXA* mutations in three probands from two consanguineous Persian families were identified. The c.1A>G mutation was first described in an American black TSD patient[7] and in a 2-year-old black child with juvenile-onset TSD disease whose other mutation Y37N (c.109T>A) had residual activity consistent with the juvenile-onset phenotype.[8] Our patient is the first non-black TSD patient.

In cases 2 and 3, the *HEXA* mutation c.1177C>T in exon 11 caused the nonsense mutation p.R393X, which occurred at CpG dinucleotide, a known hot spot for mutations.[9] This mutation was initially identified in a French infant with infantile TSD[10] and was also described in a Turkish infant.[11]

To date, more than 130 mutations in the *HEXA* gene have been characterized that cause TSD.[3,12] There are ethnic specific mutations responsible for TSD in certain populations (that is, Scott *et al*.[6]), but most mutations are sporadic and frequently found in diverse populations.[3] In the Ashkenazi Jewish population, three ‘founder’ mutations account for 98% of all the mutant alleles.[12,13] These include a four-base duplication c.1274_1277dupTATC (81%), the splicing mutation c.1421+1G>C (IVS12+1G>C; 15%) and a later-onset mutation c.805G>A (p.G269S; 2%).[6,12] In contrast, the major mutations in non-Jewish populations include the duplication c.1274_1277dupTATC and the missense mutation G269S that only occur at frequencies of 30 and 5%, respectively.[13]

As there is no effective treatment for TSD, current efforts are focused on screening populations to identify disease at-risk carrier couples,[14] and then offering prenatal diagnosis. Identification of mutations in the Persian population will have direct application to this effort.

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