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The first family with Tay-Sachs disease in Cyprus: Genetic analysis reveals a nonsense (c.78G>A) and a silent (c.1305C>T) mutation and allows preimplantation genetic diagnosis

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ABSTRACT

Tay-Sachs disease (TSD) is a recessively inherited neurodegenerative disorder caused by mutations in the *HEXA* gene resulting in β-hexosaminidase A (HEX A) deficiency and neuronal accumulation of GM2 ganglioside. We describe the first patient with Tay-Sachs disease in the Cypriot population, a juvenile case which presented with developmental regression at the age of five. The diagnosis was confirmed by measurement of HEXA activity in plasma, peripheral leucocytes and fibroblasts. Sequencing the *HEXA* gene resulted in the identification of two previously described mutations: the nonsense mutation c.78G>A (p.Trp26X) and the silent mutation c.1305C>T (p.=). The silent mutation was reported once before in a juvenile TSD patient of West Indian origin with an unusually mild phenotype. The presence of this mutation in another juvenile TSD patient provides further evidence that it is a disease-causing mutation. Successful preimplantation genetic diagnosis (PGD) and prenatal follow-up were provided to the couple.

Abbreviations: TSD, Tay-Sachs disease; PGD, Preimplantation Genetic Diagnosis; EEG, Electroencephalogram; MRI, Magnetic Resonance Imaging; MLPA, Multiplex Ligation Dependent Probe Amplification; STR, Short Tandem Repeat; PCR, Polymerase Chain Reaction; CVS, Chorionic Villus Sampling; ADO, Allele Dropout.

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Introduction

Tay-Sachs disease (TSD) or GM2 gangliosidosis variant B (MIM 272800) is an autosomal recessive neurodegenerative disorder due to β-N-acetyl-hexosaminidase A (Hex A) deficiency (Gravel et al., 2001). Hex A is a heterodimer of α and β subunits encoded by the *HEXA* and *HEXB* genes respectively. TSD is due to mutations in the *HEXA* gene localized on chromosome 15 (Myerowitz et al., 1985; Triggs-Raine et al., 1991). Mutations in the *HEXB* gene on chromosome 5 are responsible for Sandhoff disease. More than 130 mutations have been identified so far in the *HEXA* gene (http://www.hgmd.cf.ac.uk).

There are three different forms of TSD: classic infantile, juvenile and adult late-onset. The classic infantile form is the most common and is characterized by onset at 4–8 months and progressive neurological deterioration with macular cherry-red spots, blindness, intractable seizures and paralysis. Affected children rarely survive beyond five years of age. Juvenile and adult forms are very rare with a later onset and slower course. Juvenile or subacute GM2 gangliosidosis is characterized by gait disturbances, incoordination, speech problems and intellectual impairment. Behavior or psychiatric disturbances, proximal and distal weakness and muscle wasting tend to appear later in the disease course (Maegawa et al., 2006).

The overall prevalence of TSD is estimated to be about 1 in 200,000 live births in the general population (Gravel et al., 2001). However, a high prevalence is found in the Ashkenazi Jewish population, about 1 in 4000 live births, where three mutations are responsible for the majority of cases (Myerowitz and Costigan, 1988; Scott et al., 2010).

Although patients with Sandhoff disease (GM2 gangliosidosis variant O) have been diagnosed in Cyprus and a high frequency of carriers has been found in one community (Drousiotou et al., 2000; Furihata et al., 1999), no case of Tay-Sachs was diagnosed until now. In the present study we report the clinical, biochemical and molecular characterization of the first case of juvenile TSD in the Cypriot population. A successful preimplantation genetic diagnosis (PGD) was performed for the family.

Case report

The patient is a Greek Cypriot boy who presented at the age of 5 years old with developmental regression. He is the second child of healthy non-consanguineous parents. The first child, a girl, is reported healthy. Prenatal and perinatal history is reported normal. Initial concerns arose at four years due to poor fine motor skills and “night terrors”. Thereafter, he appeared gradually clumsier, developing an unsteady gait and slurred speech. Furthermore, he appeared to be hyperactive and with a limited attention span which affected his learning. An electroencephalogram (EEG) study showed right posterior-temporal spike wave discharges that were significantly potentiated by sleep. A brain Magnetic Resonance Imaging (MRI) showed mild, diffuse cerebellar atrophy. Evoked potentials, visual and auditory, were normal at the time of diagnosis. Ophthalmological examination did not reveal retinal cherry red spots.

On examination at nine years of age, the patient's occipitofrontal circumference, height and weight were around average. There were no dysmorphic features. He was drooling excessively and had feeding difficulties. He could only make a few steps unaided and his gait was unsteady. His deep tendon reflexes were increased at the knees and he had right-sided clonus. He was excessively restless and became easily agitated, upset and sometimes aggressive. Cardiovascular and the remainder of his examination were unremarkable. In general, he enjoys good health with no extensive hospitalizations, although he seems to be troubled by frequent upper respiratory tract infections. His sleep remains interrupted. He is on sodium valproate and risperidone but with very little effect. He cannot speak and communication is very limited. He attends a special educational needs school.

Methods

Measurement of β-hexosaminidase activity

β-Hexosaminidase activity was measured in plasma and leucocytes with the use of the synthetic substrate 4-methylumbelliferyl-2-acetamido-2-deoxy-β-D-gluco-pyranoside (MUG) and the % Hex A was determined using heat denaturation, as described previously (Drousiotou et al., 2000). β-Hexosaminidase

activity in fibroblasts was measured using 4-methylumbelliferyl-β-D-N-acetylglucosamine-6 sulfate (MUGS), a synthetic substrate specific for Hex A.

Molecular analysis of the HEXA gene

Standard Sanger sequencing of the 14 exons and intron–exon boundaries of the *HEXA* gene was carried out using an ABI 3730 DNA analyzer (Applied Biosystems). For Multiplex Ligation Dependent Probe Amplification (MLPA) analysis the SALSA P199 *HEXA* from MRC Holland (www.mrc-holland.com) was used, following the instructions of the manufacturer.

Preimplantation genetic diagnosis (PGD)

The couple was extensively counseled before deciding to proceed with PGD. Blood samples from the proband, the parents and the grandparents were used for genotype and haplotype analysis. From each sample, both genomic DNA and single lymphocytes were isolated for the standardization of procedures. For the purpose of PGD, four Short Tandem Repeat (STR) markers closely linked to the *HEXA* gene were used as already described elsewhere (Altarescu et al., 2007). These were carefully selected following an initial workup with a number of STR markers, so that they would be informative for the family and flanking the *HEXA* gene (in order, D15S204 and TS-AAAT are 5′ and D15S215 and D15S169 are 3′ to the gene). Initial multiplex Polymerase Chain Reaction (PCR) amplification of all four STR's was followed by hemi-nested PCR. The reactions of 50 μl were set up for each step using Multiplex HotStart Taq (Qiagen, Germany) according to the manufacturer's instructions. In the hemi-nested reactions, the internal forward primers were labeled with fluorescent dyes and products were analyzed on an ABI 3130XL genetic analyzer.

After establishing haplotypes for the family, multiplex PCR reactions were optimized on single lymphocytes isolated from family members as well as from consenting donors. This was done with careful manipulation of individual primer concentrations and optimization of the PCR annealing temperature, until PCR efficiency and Allele Dropout (ADO) levels were at satisfactory levels (94% and 7% respectively). The optimized protocol was used in the PGD case.

Results and discussion

The patient described is the first case of TSD in the Cypriot population. The clinical features of the patient are similar to those reported for other juvenile TSD cases (Maegawa et al., 2006). The diagnosis was established with the measurement of Hex A activity. A low percentage of Hex A activity was found in plasma (5%, normal 52–78%), and leucocytes (12%, normal 49–70%). The deficiency of hexosaminidase A was confirmed in cultured fibroblasts using the MUGS substrate (total hexosaminidase and β-galactosidase were

Table 1
Biochemical and molecular data of Cypriot family with Tay-Sachs disease.

	β-Hexosaminidase activity						Genotype
	WBC nmol/h/mg protein		Plasma nmol/h/ml		Fibroblasts nmol/h/mg protein		
	Total NR: 700–3600	%HexA NR: 49–70	Total NR: 500–3000	%HexA NR: 52–78	Total[a]	% HexA[a]	
Proband	1673	12	352	5	116	1	c.78G>A;c.1305C>T
Mother	2110	61	679	45	–	–	c.1307C>T
Father	1565	48	412	51	–	–	c.78G>A

DNA mutation numbering is based on GeneBank accession number M16411 for *HEXA* considering nucleotide = 1 as the A of the ATG translation initiation codon.
WBC: white blood cells.
NR: normal range.
[a] Average value of controls run simultaneously: Total: 3588 nmol/h/mg protein, % HexA: 12.4%.

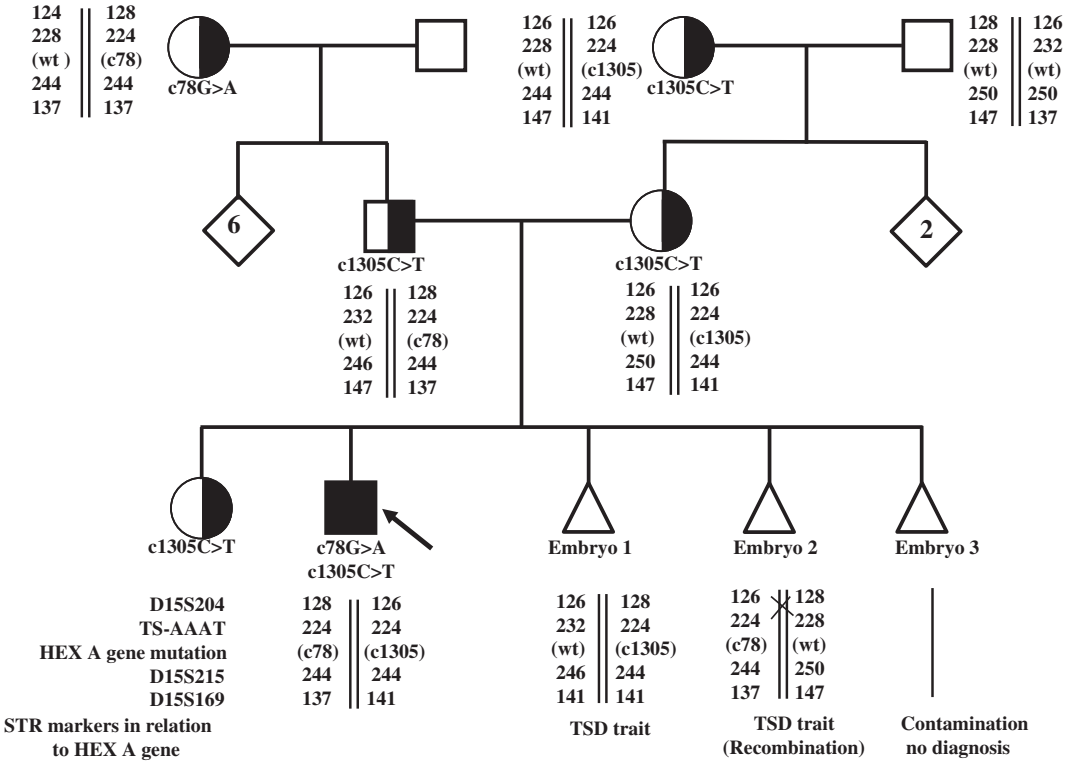

Fig. 1. Family pedigree showing genotypes and haplotypes used in PGD.

assayed as control enzymes): Hex A activity was found to be very low (116 nmol/h/mg protein with three controls run simultaneously giving an average value of 3588 nmol/h/mg protein). The father's Hex A activity was 51% in plasma and 48% in leucocytes while the mother's Hex A activity was 45% in plasma and 61% in leucocytes (Table 1).

Two previously described mutations have been identified in the proband and confirmed in the parents (Table 1). The first mutation, carried by the father, is the nonsense mutation c.78G>A (p.Trp26X) in exon 1. This mutation was initially identified in combination with the missense mutation p.Arg178Leu in a patient with infantile TSD of unspecified descent (Triggs-Raine et al., 1991). It was also described in homozygosity in two Arab patients (Drucker and Navon, 1993; Haghighi et al., 2011). This mutation does not result in any functional protein and therefore would not be expected to contribute to the residual Hex A activity found in our patient.

The second mutation, carried by the mother, is the silent mutation c.1305C>T (p.=) in exon 11. This mutation was at first considered to be neutral, because there is no change in the amino acid, and further attempts were made to identify the second mutation by sequencing the promoter area of the *HEXA* gene and using the MLPA method to identify any exon deletions or duplications. No other alteration was detected. We then came across a publication (Levit et al., 2010) describing a juvenile TSD patient of West Indian origin with an unusually mild phenotype (alive at 26) that carried this mutation in combination with the c.814G>A (p.Gly272Arg) in exon 8. The authors showed that the silent mutation c.1305C>T(p.=) results in a premature termination codon and that the aberrant transcripts are subjected to a relatively mild degradation by the non-sense-mediated mRNA decay surveillance mechanism. The identification of this silent mutation in a second patient with juvenile TSD with demonstrated segregation (Fig. 1) provides further evidence that this mutation is indeed disease-causing. Silent mutations have been previously shown to be disease-causing both in TSD (Akli et al., 1990; Wicklow et al., 2004) as well as in other genetic disorders (Cartegni et al., 2002).

Molecular and biochemical carrier screening in our family identified three more carriers, two with the c.1305C>T and one with c.78G>A (Fig. 1). Carriers with the c.1305C>T mutation had reduced β-hexosaminidase A levels in the plasma or leucocytes or both, thus supporting the pathogenic role of this mutation.

PGD for TSD is performed at a number of centers, but this is the first time the test has been performed in Cyprus. All stages of assisted reproduction and embryo biopsy were carried out as previously described (Palmer et al., 2002). Four cleavage-stage embryos (day 3) were biopsied and the single blastomere biopsies were analyzed for the four STR markers. One embryo failed to proliferate after the biopsy and was omitted from the analysis. Two embryos were diagnosed as TSD carriers. In the third embryo, contamination of the sample was detected and was rejected. Only embryo 1, which was diagnosed as a carrier of the silent mutation c.1305C>T(p.=), successfully reached the expanded blastocyst stage and was transferred on day 5.

Pregnancy test (hCG levels) after two weeks was positive. Prenatal diagnosis was performed on the 12th week of gestation on a Chorionic Villus Sampling (CVS) sample obtained trans-abdominally. Sequencing of the *HEXA* gene as well as biochemical determination of Hexosaminidase A on the sample, confirmed the genotype predicted from the PGD. Despite the difficulties of low ova numbers and the viability of embryos, probably due to advanced maternal age, the procedure was successful resulting in an unaffected baby.

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This paper is dedicated to the memory of Dr Stavros Hadjiloizou.

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