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Unusual expression of Gaucher's disease: cardiovascular calcifications in three sibs homozygous for the D409H mutation

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Abstract

Three sisters suffering from an unusual form of Gaucher's disease are described. These patients had cardiovascular abnormalities consisting of calcification of the ascending aorta and of the aortic and mitral valves. Neurological findings included ophthalmoplegia and saccadic eye movements in two patients, and tonic-clonic seizures in the third. The three patients died, two of them after having undergone aortic valve replacement. Tissue was obtained from one of the sibs and fibroblast and liver β-glucocerebrosidase activity was reduced to 4% and 11% of mean normal values. Genotype analysis indicated that the patient was homozygous for the D409H mutation. It is tempting to relate the phenotype of severe cardiac involvement to the D409H/D409H genotype, although further cases will be needed before this association can be confirmed.

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Gaucher's disease is characterised by glucocerebroside accumulation owing to deficiency of lysosomal β-glucocerebrosidase. Three types of the disease have been distinguished on the basis of the neurological involvement. Patients with type 1 are free of nervous system disease while neurological signs are present in patients with types 2 and 3. The disorder mainly affects the liver, spleen, bone marrow, skeleton and, in types 2 and 3, the brain. Cardiac complications are extremely rare in Gaucher's disease and mostly restricted to constrictive pericarditis in non-neuronopathic adult cases, or type 1.[1]

This autosomal recessive disease is caused almost exclusively by mutations in the gene encoding the lysosomal enzyme β-glucocerebrosidase (EC 3.2.1.45). The disease is particularly prevalent among the Ashkenazi Jewish population, in which only two mutations (N370S and 84GG) account for 80 to 90% of the cases. In non-Jewish patients, the two most frequent mutations are N370S and L444P, accounting for 50 to 70%.[2–4]

In the Spanish Gaucher's disease population, a third frequent mutation is D409H.[5] Here we report three sibs, homozygous for this mutation, suffering from an unusual form of Gaucher's disease. The three girls, the entire offspring of a healthy, non-consanguineous couple, were found to be affected with cardiovascular abnormalities consisting of calcification of the ascending aorta and of the aortic and mitral valves, in addition to ophthalmoplegia and the systemic findings characteristic of Gaucher's disease.

Material and methods

PATIENTS

Patients 1 and 2, the first and second girls born to a non-consanguineous couple, were admitted to hospital when 17 and 16 years old. Patient 1 was patient III·2 in Cormand *et al.*[5] The first symptoms were recurrent epistaxis and dyspnoea on exercise. Both had splenomegaly and, in patient 1, hepatomegaly was also present. Neurological manifestations started at 16 and 15 years of age and consisted of left ophthalmoplegia, saccadic eye movements, and hyporeflexia. Strabismus in the left eye and corneal opacities were also noted. Both patients had pes cavus. Laboratory tests showed pancytopenia, increased serum acid phosphatase activity, and normal serum calcium and phosphate. A bone marrow aspirate was consistent with the diagnosis of Gaucher's disease. Echocardiography and fluoroscopy disclosed substantial calcification of the mitral and aortic valves and of the ascending aorta. Doppler examination and cardiac catheterisation showed severe aortic stenosis with mild aortic regurgitation and moderate mitral stenosis. The patients died when 19 and 17 years old, one of them after receiving corrective surgery for aortic valve replacement. Necropsy was denied. Patient 3 was the youngest of the three sisters but the first to develop the disease. Hepatosplenomegaly was detected at 18 months of age. She underwent splenectomy at the age of 6 years to correct severe pancytopenia and examination of tissue disclosed the characteristic Gaucher cells. At 10 years of age she presented with generalised tonic-clonic seizures. Cardiovascular involvement was similar to her sisters. The patient died at 13 years during the postoperative course after surgical correction.

BIOLOGICAL MATERIAL

Skin fibroblast cultures were established according to routine procedures in Eagle's minimum essential medium. Aqueous homogenate of liver was centrifuged at 100 000 *g*, and the pellet was resuspended in water and used as

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Residual β-glucocerebrosidase activity in tissues of patient 1, in types 1 and 3 Gaucher's disease patients, and controls

	Stearoyl-β-glucocerebrosidase (nmol/h/mg protein)		*MU-β-glucosidase (nmol/h/mg protein) fibroblasts†*		
	*Liver**	*Fibroblasts**	*T+TX*	*PS+SAP*	*PS+SAP ratio T+TX*
Control					
Mean (SD)	15·0 (7·6) (n=5)	177 (46) (n=20)	198 (80) (n=55)	343 (122) (n=30)	1·3 (0·2)
Patient 1	2·1	19	7·6	9·5	1·2
Type 1					
Mean (SD)		20·1 (5·8) (n=7)	18·2 (9·6) (n=18)	77 (41) (n=18)	4·2 (1·6)
Type 3					
No 1	ND		4·5	7·1	1·6
No 2			2·2	4·4	2·0

* Assayed in the presence of taurocholate (T) and Triton X-100 (TX).
† Assayed in the presence of T and TX, or with phosphatidylserine (PS) and a sphingolipid activator protein preparation (SAP).
ND = not detectable.

enzyme source. Sphingolipid activator protein preparation was partially purified from human brain.[6]

ENZYME ASSAY

Glucocerebrosidase activity was measured with N-stearoyldihydroglucosylceramide (1 mmol/l) and 4-methylumbelliferyl-β-glucopyranoside (MU-βGlc, 4·5 mmol/l) in the presence of sodium taurocholate (T, 1·5% w/v) and Triton X-100 (TX, 0·2% v/v). β-glucocerebrosidase activity in patient's fibroblasts was also measured in the presence of the physiological activators phosphatidylserine (PS, 5 μg) and an activator protein preparation (SAP, 150 μg) using MU-βGlc as substrate.

DETECTION OF THE D409H MUTATION

Genomic DNA amplification and allele specific oligonucleotide (ASO) hybridisation were carried out as previously described.[5] For SSCP analysis, a different fragment of 174 bp, corresponding to the 3′ end of exon 9, was amplified using the following primers: 5′-ACTGGAACCTTGCCCTGAAC-3′ and 5′-ATAGGCCTGGTATGGAATGG-3′. SSCP conditions were those described in Bayés *et al.*[7] For direct sequencing, PCR products were amplified by Wizard™ PCR Preps (Promega) and sequenced using the Sequenase Version 2·0 DNA Sequencing Kit (USB) according to the manufacturer's conditions.

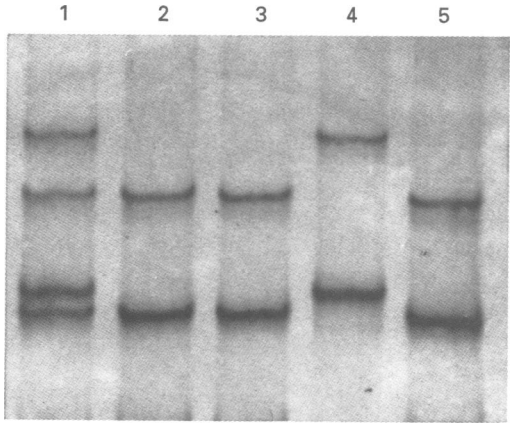

Figure 1 SSCP analysis of part of exon 9 of the β-glucocerebrosidase gene. Lane 5 corresponds to a healthy subject. The sample from patient 1 (homozygous for the D409H mutation) is shown in lane 4. The patient in lane 1 is heterozygous for D409H, while the two other patients (lanes 2 and 3) do not bear mutations in this region of the gene.

Results

Enzymatic and mutation genotype analysis were carried out in patient 1.

ENZYMATIC ANALYSIS

In the patient's cultured fibroblasts β-glucocerebrosidase was reduced to 4% (substrate MU-β Glc) and to 11% (substrate stearoylglucocerebroside) of normal levels. With the natural substrate, liver enzyme activity was 14% of mean controls. Measurement of residual enzyme activity was also carried out in the presence of the natural activators, since a selective reconstitution of glucocerebrosidase activity in type 1 patients, but not in types 2 or 3, by PS[8] and SAP[9] has been pointed out. In the presence of SAP and PS, β-glucosidase displayed low levels of activity (9·5 nmol/h/mg protein) so that the mutant enzyme expressed 3% of control mean activity. This value as well as the ratio PS + SAP/T + TX is similar to those found in two cases with type 3 Gaucher's disease (table).

MUTATION ANALYSIS

Mutation analysis on the DNA of patient 1 was carried out by SSCP analysis and ASO hybridisation (not shown), and confirmed by sequencing. Fig 1 shows the SSCP analysis carried out on amplified DNA from exon 9 of patient 1 (lane 4), of a healthy subject (lane 5), and of three other unrelated Gaucher's disease patients, one of them heterozygous for the 409 mutation (lane 1). The three different patterns, corresponding to homozygous for, heterozygous for, and non-carrier of the 409 mutation, are clearly distinguished.

The rest of the glucocerebrosidase gene was exhaustively analysed to rule out the presence of other mutations. Genomic DNA was amplified by PCR in 14 overlapping fragments which covered all the coding region of the gene. Four different SSCP conditions were tested for each fragment. No additional abnormal SSCP patterns were detected.

Sequencing of amplified exon 9 DNA shows the G to C transversion leading to the D409H substitution (fig 2).

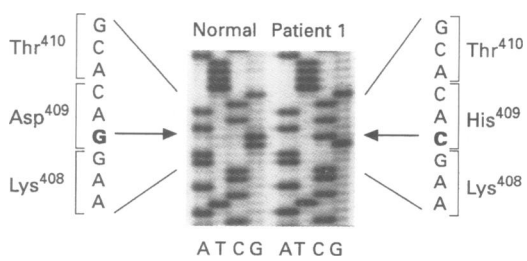

Figure 2 Sequence analysis of exon 9 of the β-glucocerebrosidase gene. PCR products from patient 1 and a normal subject were directly sequenced as described in Material and methods. Patient 1 is homozygous for a G to C transversion (arrow) at cDNA position 1342, resulting in an Asp[409] to His substitution.

Discussion

Cardiac involvement consisting of constrictive pericarditis[10–12] and diffuse infiltration of the myocardium by Gaucher cells[13] has been described in patients with type 1 Gaucher's disease. In these patients constriction and calcification of the pericardium were related to unrecognised haemorrhagic pericarditis.[12]

Heart disease has been considered a frequent finding in patients with type 3b Gaucher's disease.[14] However, complications of the cardiovascular system of a similar extent and severity to those present in our three patients have only been reported in a 15 year old boy with Gaucher's disease and cardiac abnormalities also consisting of calcification of the ascending aorta and aortic and mitral valves.[15] No neurological evaluation of this child was provided. Recently, two sibs with Gaucher's disease and mitral and aortic valve lesions have been reported.[16]

In our patients, the neurological manifestations were restricted to ophthalmoplegia and dysmetric saccadic eye movements (patients 1 and 2) and to myoclonic seizures (patient 3). Reconstitution of mutant glucocerebrosidase activity by PS and SAP confirmed the assignment of these patients to the subacute neuronopathic form of Gaucher's disease. The importance of oculomotor disturbances in the diagnosis of certain forms of type 3 has been pointed out by Stowens *et al*,[17] being in some patients the sole manifestation of the disease.[18] Interestingly, gaze paralysis was also reported in a type 3 Gaucher's disease case presenting with aortic involvement.[19]

Genotyping showed that patient 1 is homozygous for mutation D409H. This is the third most frequent mutation detected in Spanish Gaucher's disease patients, although it only accounts for 5·7% of mutated alleles.[5]

A single allele D409H does not predict development of neurological disease including ophthalmoplegia. In fact, from a series of 46 Spanish patients with the disease, this allele was present in the heterozygous state in one out of 36 patients with type 1 (1·38%), in one out of seven children with type 2 (7·14%), and in one out of three unrelated cases with type 3 Gaucher's disease (apart from patient 1 and her sisters, homozygous for the mutation). Nevertheless, none of the patients carrying a single copy of the D409H allele was suffering from either heart disease or ophthalmoplegia.

It is tempting to relate homozygosity for mutation D409H to severe cardiac involvement in Gaucher's disease although further cases are needed before this association can be claimed. In this respect, a group of Arab patients homozygous for the D409H mutation presenting heart disease with similar symptoms to those of our patients has been reported.[20]

Should this association be proven, it would provide the first clear genotype-phenotype correlation in neuronopathic Gaucher's disease.

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