

Five New Gaucher Disease Mutations

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ABSTRACT. DNA from 17 individuals with 20 unidentified alleles was subjected to single-stranded conformation polymorphism analysis and/or sequencing and 5 previously undescribed mutations have been identified: 245T, 259T, 635G, 914C del, and IVS10(+2). Two of these mutations, 914C del and IVS10(+2), are null, or "lethal" mutations. Because the other mutation each of these two patients carried was "mild", the phenotype was type I disease. In addition to the new mutations we describe, the second example of the rare 1448G mutation has been documented in one of the patients. This mutation is particularly interesting because in samples studied by restriction analysis with *NciI* it can readily be confused with the common 1448C mutation. Reexamination of 28 patients who had previously been diagnosed as carrying the 1448C mutation were confirmed to be 1448C.

INTRODUCTION

A deficiency of the enzyme glucocerebrosidase leads to accumulation of insoluble glucocerebroside in the tissues, resulting in the clinical manifestations of Gaucher disease. This disorder, the most common of the glycolipid storage diseases, is characterized by hepatosplenomegaly, skeletal lesions, and in the rare type II and type III forms of the disease, neurologic symptoms.

We recently summarized the 44 glucocerebrosidase mutations known to cause Gaucher disease (1). Since our previous report 4 other mutations have been published (2-4). We have found one of these new mutations (1448G) in a patient and now document this and an additional 5 previously undescribed mutations in patients with Gaucher disease.

MATERIALS AND METHODS

Patient Population

DNA was extracted from leukocytes using standard methods. To date, we have examined the DNA of 245 unrelated Gaucher disease patients (136 Jewish, 102 non-Jewish, and 7 half-Jewish). Each sample was examined by the methods indicated previously described (1) until the mutations on both alleles had been identified.

Examination of DNA from Patients with Unidentified Mutations

DNA from 17 individuals (15 non-Jewish, 2 Jewish) with 20 unidentified alleles were the subject of this study. Sixteen of these samples were subjected to single-stranded conformation polymorphism analysis (SSCP) (5). With the exception of two samples from which insufficient DNA was available to examine one exon in each

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case, all exons and genomic nt 201-396 (6) containing the putative promotor were examined. In two cases (patient #1 and #5) abnormal bands were encountered. In these the DNA from that region of the glucocerebrosidase gene was amplified by the polymerase chain reaction (PCR) and sequenced (7) using the Applied Biosystems (Foster City, CA) automatic sequencer to determine the exact site of the mutation. The promotor and entire coding region (with the exception of exon 1) of the glucocerebrosidase gene of five patients was sequenced. The DNA of four of these patients had shown no abnormal bands on SSCP and in the case of the fifth (patient #2) SSCP had not been performed. In each case in which a mutation was found it was confirmed by sequencing the opposite strand. The creation of a restriction endonuclease site by the mutation served to further validate each mutation.

Analysis of Messenger RNA

Total cellular RNA was obtained from patient #5, in whom a splice site abnormality was identified. Freshly drawn whole blood was mixed in a 3:1 ratio with 5% polyvinyl pyrrolidone in 3% sodium citrate and incubated at 37C for 30 minutes. The upper phase, containing the mixed leukocytes, was centrifuged to pellet the cells and

approximately 1×10^7 cells were mixed with Trizol Reagent (Life Technologies Inc., Gibco BRL, Gaithersburg, MD) according to manufacturer's instructions. 10 μ g of total RNA was obtained.

First strand synthesis was initiated with 400 units of SuperScript II RNase H Reverse Transcriptase (Life Technologies Inc., Gibco BRL, Gaithersburg, MD) using 2 μ g of total RNA as template. The reaction was primed with 50 ng of a specific oligonucleotide primer at cDNA number 1703-1722 located 111bp downstream from the glucocerebrosidase stop codon. Double stranded cDNA was amplified with the polymerase chain reaction for 35 cycles (92 C, 30 sec; 58 C 30 sec; 72 C, 45sec) using an upstream primer cDNA number 1251-1270 and a nested downstream primer cDNA 1621-1640.

RESULTS

Patient Characteristics

New mutations were found in 6 of the patients. The clinical characteristics of these patients are summarized in Table I.

Patient #1 was a 46 year old Israeli Ashkenazi Jewish woman who has been known for about 10 years to have Gaucher disease.

Table I: Characteristics of 6 Patients Found to have New Gaucher Disease Mutations

Patient #	Current Age	Onset Age	Jewish	Ethnic Origin	Neurologic Signs	Severity Score [†]	Genotype (New mutations bold)	Disease Type
1	46	34	Yes	Ashkenazi	No	7	1226G/ 245T	I
2	21	20 mo	No	Bedouin	No	13	259T /1448G	I
3	56	35	No	Scotch/Welch	No	6	1226G/ 635G	I
4	33	3 mo	No	Scotch/Welch	No	14	1226G/ 914C del	I
5	6	5	Yes	Ashkenazi	No	6	1604A/ IVS10(+2)	I
6	7	7	No	Afro-American	No	4	259T /?	I

New mutations are shown in bold type.

[†]Calculated as in Zimran et al. (8).

She presented with progressive splenomegaly and hypersplenism, moderate hepatomegaly and intermittent low-back pain; there were no fractures or episodes of bone crisis. Partial splenectomy was performed at age 40 years, after the platelet count had fallen to 15,000. However, the re-growth of the splenic remnant with re-emergence of thrombocytopenia to 20,000 necessitated enzyme replacement therapy. Patient #2 was a 21 year old Bedouin Arab who had been diagnosed at age 20 months when he was assessed for "failure to thrive". He was splenectomized at age 15 because of massive splenomegaly and anemia. He subsequently developed aseptic necrosis of both femoral heads and in the left proximal tibia, and a moderate abnormality in pulmonary function was documented. Patient #3 was a 56 year old woman whose Gaucher disease had been discovered 21 years earlier as a result of the investigation of thrombocytopenia. A splenectomy had been carried out with normalization of the platelet count. The platelet count was subsequently relatively stable for many years, but in the past 5 years had declined from 106,000 to 56,000/ μ L. There were no skeletal symptoms or radiologic evidence of bone involvement. Patient #4 was diagnosed when only 3 mo of age. She

was splenectomized at age 8 and had fairly extensive bone disease. Patient #5 was a child of 6 who had relatively mild disease, diagnosed only a year earlier. There was a moderate degree of splenomegaly, occasional bone pains but no X-ray abnormalities of the bones, and mild thrombocytopenia. The mutation in patient #6 was discovered by screening the DNA from patients with unidentified mutations for the novel 259T mutation. She was a black child with massive splenomegaly and marked pancytopenia, but no bone or central nervous system involvement.

DNA Analysis

The location and characteristics of the new mutations are summarized in Table II. Of the 5 new mutations that were found, 4 are in the coding region, two missense (245T and 259T), 1 nonsense (635G), and one deletion (914C). The fifth mutation is a T to G transversion in the second position of the splice "donor" site of intron 10. In addition to these new mutations, a second example of the recently reported (3) 1448G (L444R) was encountered.

Table II: Characteristics of the New Mutations Detected in this Study

Patient	cDNA #	Genomic #	Exon	nt Substitution	Amino Acid	AA Substitution	Detection†	SSCP
1	245	1749	3	C→T	43	Thr ⁴³ →Ile	+ <i>AccI</i>	abnormal
2	259	1763	3	C→T*	48	Arg ⁴⁸ →Trp	(+ <i>SlyI</i>)	normal
3	635	3429	6	C→G	173	Ser ¹⁷³ →Term	+ <i>HinfI</i>	normal
4	914	4263	7	C-del	N/A	Frameshift	+ <i>AccI</i>	normal
5	IVS10(+2)	6492	intron 10	t→g	N/A	Splice	+ <i>NciI</i>	abnormal

* The sequence change corresponds to the normal pseudogene sequence

† Rapid detection is achieved through digestion with the indicated restriction endonuclease; () indicates that a *SlyI* site is created artificially by the use of a mismatched primer. + indicates that the site is created.

mRNA Analysis

The cDNA amplified from messenger RNA of the patient #5 (splice mutation) showed two bands on polyacrylamide gel electrophoresis in this region of the glucocerebrosidase gene. One had the expected size of 389bp the other was approximately 100bp shorter in length. Sequencing of this region of the amplified cDNA revealed that exon 10 which is 117bp in length had been removed; no other consensus splice donor sites are present in exon 10 or intron 9 and consequently the next available splice site at the exon 9/intron 9 junction was utilized.

DISCUSSION

We have previously suggested that Gaucher disease-producing mutations can conveniently be divided into three categories (1). *Null* (or lethal) alleles are defined by a molecular defect that prevents the formation of any enzyme. Some of the more common mutations in this category are the 84GG frameshift mutation and the IVS2(+1) splicing mutation. No patient who has inherited two such alleles has ever been encountered; presumably the absence of any glucocerebrosidase activity is lethal before birth in man as it is in the mouse (9). *Severe* alleles are defined as those that can produce neuronopathic disease, either when inherited with another severe or with a null allele. The most common example of such a mutation are is 1448C (L444P). *Mild* mutations are defined as those which are not associated with neurologic disease, even when found together with a null or a severe mutation. The most common example of such a mutation is the common Jewish 1226G (N370S) mutation. The relationship between the category of mutation and the clinical phenotype is summarized in Table III.

It is often difficult to judge the severity of the Gaucher disease phenotype produced by a mutation found in a patient with type I disease. This is particularly true when the other allele is one that produces mild disease. This was the situation in both patient #1 and patient #5 (see below).

Only when a mutation is found in the context of a null mutation or a severe mutations (mutations associated with type II or type III Gaucher disease) can one be reasonably certain of its capability of causing neurologic disease. However, since patient #1 had only very indolent disease, having experienced her first disease manifestations at the age of 34 and having reached the age of 44 without severe symptoms of Gaucher disease, it is likely that the 245T mutation is a relatively mild one.

Table III: Interaction between different types of Gaucher disease mutations

		One Allele		
		Null	Severe	Mild
Other Allele	Null	Non-viable	Type II/III	Type I
	Severe	Type II/III	Type II/III	Type I
	Mild	Type I	Type I	Type I

Patient #2 is from a relatively inbred group, the Bedouin Arabs. Thus, he would have been expected to be homozygous for a rare mutation. Instead, he proved to have two different rare mutations, the 1448G mutation, which has been documented only once before (3) and the new 259T mutation. Nucleotide 1448 is same nucleotide at which a relatively common T-C transition is found. The new 1448G mutation creates an *NciI* restriction site as does the more common 1448C mutation. The majority of our patients were diagnosed by allele specific oligonucleotide hybridization (ASOH) but in some of our earlier studies we used the *NciI* restriction endonuclease to detect mutations at nt 1448. Twenty-eight patients in whom the diagnosis had been made by restriction endonuclease analysis were confirmed to have the 1448C mutation by ASOH or *BsiE* I digestion, which is specific for the 1448G mutation. Thus, none of our previously diagnosed 1448C patients had been misdiagnosed.

The 1448G mutation is clearly a severe one, since it was associated with type II disease in the patient in which it was originally reported in combination with a frame shift (lethal; null) mutation. Thus, the 259T mutation may be classified as mild; the patient was 21 years of age and had no neurologic manifestations. One other patient (#6) was also found to have the 259T mutation. She is a Black female diagnosed at age 7 with massive splenomegaly. Her other mutation has not yet been detected, so that at present she is classified as 259T/?.

The deduced result of the 635G mutation is to change ¹⁷³serine to a stop codon. The 914C deletion produces a frameshift and the IVS10(+2) mutation produces an alternatively spliced mRNA. As a result, the exon immediately upstream from the defective splice site is missing. Such exon skipping appears to be a regular occurrence when there are defects in the 5' splice site (10) and we have observed it in the glucocerebrosidase pseudogene (11), where some of the splice consensus sites have been lost. These mutations, found in patients #3, #4, and #5 respectively, are all therefore null. However, because each patient had also inherited either the relatively mild 1226G or 1604A mutation, all had type I disease, albeit with different degrees of severity.

The 1604A mutation, in particular, appears to produce mild enzyme deficiency. In the other four Gaucher disease patients with this mutation we have encountered, only one had inherited the relatively mild 1226G mutation, while the other three had the 84GG null mutation. Since the 84GG mutation is only one-tenth as common in the general Jewish population as is the 1226G mutation (12), the selective occurrence of the latter mutation with the 1604A mutation implies that patients with the 1226G/1604A genotype rarely come to medical attention. Recent unpublished data indicate that this mutation has a gene frequency of approximately 0.2% in the Jewish population, making it the third most frequent mutation after 1226G and 84GG.

Forty-eight mutations that cause Gaucher disease have been described previously. This

study expands this number to 53 mutations. Rare mutations such as the ones described here are of particular value in the families in which they are found. Heterozygote detection based on enzyme assay is notoriously unreliable, and when the mutation is known the ascertainment of heterozygotes by DNA analysis becomes a simple matter. Usually, as in the mutations we have described here, this may be accomplished quite easily by restriction endonuclease analysis.

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