Polymorphisms in Glucosylceramide (Glucocerebroside) Synthase and the Gaucher Disease Phenotype

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Abstract

Background: Gaucher disease results from the accumulation of glucosylceramide (glucocerebroside) in tissues of affected persons. Patients sharing the same genotype present with widely varying degrees of lipid storage and of clinical manifestations.

Objectives: To determine whether variation in the glucosylceramide synthase (UDPGlucose ceramide glucosyltransferase) gene, which encodes the enzyme that regulates the synthesis of glucocerebroside, could account for the variability and clinical manifestations.

Methods: Patients homozygous for the 1226G (N370S) mutation, the most common in the Ashkenazi Jewish population, were investigated. The exons and flanking sequences of the gene were sequenced using DNA derived from five very mild Gaucher disease patients and four patients with relatively severe Gaucher disease.

Results: One polymorphism was found in the coding region, but this did not change any amino acids. Seven other polymorphisms were found in introns and in the 5' untranslated region. Some of these were single nucleotide polymorphisms; others were insertions. The mutations appear to be in linkage equilibrium and none were found with a significantly higher frequency in either severe or mildly affected individuals.

Conclusions: Mutations in the glucosylceramide synthase gene do not appear to account for the variability in expression of the common Jewish Gaucher disease mutation.

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Marked variation of the clinical manifestations of Gaucher disease among patients with the same genotype, so-called variable penetrance, is commonly observed [1–5]. Efforts to explain this variability of disease phenotype have been unsuccessful.

Gaucher disease results from a deficiency in glucocerebrosidase, the enzyme that normally catabolizes the glycolipid glucocerebroside. The accumulation of glycolipid in Gaucher disease is a result of the balance reached between the synthesis of glucocerebroside on the one hand, and its catabolism by glucocerebrosidase on the other. The presumption that the activity of the glucocerebroside synthase plays a limiting role in the accumulation of glucocerebroside has gained support from the fact that inhibiting with butyldeoxynojirimycin (OGT 918), the ceramide-specific glucosyltransferase that initiates the glycosphingolipid biosynthetic pathway and catalyzes the formation of glucocerebroside, has resulted in clinical improvement in patients with Gaucher disease [6].

We have now examined the gene encoding glucocerebroside synthase to determine whether single nucleotide substitutions within the gene may be factors that determines the clinical severity of the disease.

Materials and Methods

The glucosylceramide synthase (UDP-glucose ceramide glucosyltransferase) genomic sequence was obtained from GenBank accession AL442066 and the mRNA sequence was obtained from GenBank accession number NM 03358.

Jewish Gaucher patients who were 1226G (N370S) homozygotes and who had low severity index scores (2 or less) or high scores (11 or more) were selected for sequencing of glucosylceramide synthase [7]. DNA samples from a Caucasian non-Jewish and a Jewish control were sequenced at the same time. All Jewish subjects were of Ashkenazi ancestry.

DNA was extracted from blood using standard techniques. DNA was amplified for sequencing using the polymerase chain reaction and the oligonucleotide primers listed in Table 1. PCR reactions contained 33.5 mM TrisCl pH 8.8, 8.3 mM $(NH_4)_2SO_4$, 3.35 mM $MgCl_2$, 85 μg/ml BSA, either 5% or no DMSO, 200 μM dNTPs, 250 ng of each oligonucleotide primer, 1.5 U Taq polymerase and 200 ng of DNA in 100 μl. After denaturing at 98°C for 4 minutes, 31 cycles of PCR were carried out at 94°C, 30 seconds, annealing temperature [Table 1], 30 seconds, and extension at 72°C, 30 seconds. Amplified fragments were purified for sequencing using a QIAquick PCR purification kit (Qiagen, Inc., Valencia, CA, USA). Sequencing was carried out by a fluorescent-tagged dideoxy chain termination method using an ABI Model 377 automated sequencer (Foster City, CA).

Results

In the nine exons of *UGCG* only one polymorphism was found; this was a c.861 A→G transition changing the normal CA**C** codon 287 to CA**G**. Both codons code for threonine. A number of other polymorphisms was identified: 292 basepairs upstream from the start ATG a 10 nt insert (CCCGCAGCCG) was present in some normal and Gaucher disease *UGCG* genes. The 68th nucleotide of intron one was usually a T, but 3 of 22 alleles examined were an A. A

PCR = polymerase chain reaction
UGCG = UDP-glucose ceramide glucosyltransferase

Table 1. Oligonucleotide primers for UGCG PCR amplification and sequencing

Exon	Name	Amplification primer, 5′ to 3′	Annealing temp	DMSO	Fragment size (nt)	Sequencing primer
1	CGT1F	GGAGCCCGGCGGCGAGACG	69°C	5%	548	1FN
	CGT1R	GCCGGTCCGAGGGGCGAG				ACGGAGAGGCGGCGGAGG
2	CGT2F	TCAAAGCCATGCTTACCTGG	61°C	0	416	2FN
	CGT2R	AATACCTTCACCTTATACAGTG				GATTTATTGATAGCTTGTCAGTG
3	CGT3T	ATGGCAAGACTGCAGTTGTG	58°C	0	502	3T
	CGT3B	AGGACGTAAACACCTCTCAG				
4	CGT4F	GTTTTCCTTTTGAGAATGGACAG	57°C	0	378	4F
	CGT4R	AAAGAACTCATGAATGTATCTGAG				
5	CGT5F	TTGTATTCTCCTCAGAAAGGTGG	58°C	0	428	5F
	CGT5R	GGGCTTCTAGAACACAGCAGG				
6	CGT6F	AAAAACAGTTCGTGAACACCATG	58°C	5%	434	6FN
	CGT6R	AGGTGACAGAGCAAGACTCTG				CAGTTCGTGAACACCATGCTTT
7	CGT7T	GAATACCGGCAGTTGCCTGG	60°C	0	510	7T
	CGT7B	TTTGTATGGCCTACGCTCTGATG				
8	CGT8F	GAGAATGGTCTTTCTATCACAGG	58°C	0	409	8F
	CGT8R	GGGAAGCTGAAGAATGGCTCAG				
9	CGT9F	TCTTGAACGGTATAACATGGCAG	58°C	0	584	9F
	CGT9R	ATGAACGAACCCAGGTTCCTAG				

Table 2. Genotypes examined

		Polymorphisms							
	SI	292 Ins*	IVS1+68 T/A	IVS2+25 Repeat**	IVS3–123–126 Ins***	IVS4–69 T/C	IVS5+31 A/G	IVS7+122 G/T	Ex 8 861 A/G****
Gaucher disease 1226G homozygotes									
#1	2	+/-	T/T	3/4	+/–	T/C	A/A	G/G	A/G
#2	2	+/-	T/T	3/4	+/–	T/C	A/A	G/G	A/G
#3	2	+/-	T/T	3/3	–/–	T/T	A/A	G/G	A/G
#4	2	+/-	T/T	3/4	+/–	T/T	A/A	G/G	A/G
#5	1	+/-	T/T	3/3	+/–	T/T	A/A	G/G	A/G
#6	13	-/-	T/T	4/4	+/+	T/T	A/G	G/G	A/A
#7	16		T/T	3/3	+/+	T/T	A/A	G/G	A/A
#8	11	+/-	T/A	3/4	+/–	T/T	A/A	G/T	A/G
#9	13	+/-	T/T	3/4	+/–	T/T	A/A	G/G	A/G
Normal controls									
NJ-C		+/-	T/T	3/4	+/–	T/T	A/A	G/G	A/G
J-C		-/-	A/A		+/+	T/T	A/G	G/G	A/A
NJ-C				3/3					
J-C				3/4					

SI = Severity Index, NJ-C = non-Jewish control, J-C = Jewish control
* 10 nt insert (CCCGCAGCCG): + insert present; - insert absent
** GTTTT repeat
*** 4 nt insert (AAAT): + insert present, -insert absent
**** T287T

GTTTT sequence was repeated three times in some alleles and four times in others. The 69 nucleotide of intron 4, the 31st in intron 5 and the 122nd in intron 7 were all found to be polymorphic. The genotypes of the subjects examined are summarized in Table 2.

It is apparent, first of all, that none of the polymorphisms detected appeared to be significantly more common in either the severely or mildly affected group of Gaucher disease patients. Secondly, there seems to be no relationship between the inheritance of one or another of these polymorphisms in any given patient. Rather, they appear to be in linkage equilibrium, implying that they are relatively ancient.

Discussion

Many investigators have observed the marked variability in the penetrance of Gaucher disease [1,2,4,5,7–14]. It has been suggested that experience with viral infections might be one of the modifying

factors determining the clinical outcome of the disease [15–17], but Pines et al. [13] failed to demonstrate any correlation between prior Epstein-Barr virus or cytomegalovirus infection and the clinical course of Gaucher disease in patients homozygous for the common Jewish mutation. Their only positive finding was a higher level of anti-EBNA antibodies among patients with moderate/severe disease.

A polymorphism in the gene that encodes the enzyme required for the synthesis of glucocerebroside from ceramide seemed to be a logical candidate for a genetic factor that might explain differences between the clinical expression of the disease in persons with the same genotype. We found no coding polymorphisms in the gene, but the possibility that non-coding polymorphisms might either play a regulatory role or be linked to polymorphisms in upstream regulatory regions warranted investigating their possible relationship to disease severity. Comparing the pattern in the most severely affected homozygotes with those most mildly affected showed no differences between these two groups.

Gaucher disease is not alone among the so-called single gene disorders that have marked variations in penetrance. In addition to genetically linked, unlinked, and environmental causes that have been considered to include all of the possibilities [5], "epigenetic" modification must also be entertained [18]. In the mouse, retrotransposons that may regulate adjacent genes are activated stochastically, producing different phenotypes in genetically identical mice. It has been speculated that such a phenomenon may be important in the expression of some human disease states. Gaucher disease could be one, but evidence for such a phenomenon will be difficult to develop.

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Capsule

Clues into chloroquine resistance

Global resistance has made chloroquine, the traditional malaria drug, almost useless. Sidhu et al. investigated the correlation between chloroquine resistance among parasites and the occurrence of multiple point mutations in a gene called *pfcrt*, which encodes a membrane-spanning protein. Using allelic exchange, they transferred alleles of *pfcrt* from chloroquine-resistant parasites into a chloroquine-sensitive strain of *Plasmodium falciparum*. Clones of the engineered parasites displayed similar chloroquine resistance to those of the original without having had any prior exposure to the drug. The point mutations appear to confer quite specific structural recognition to chloroquine, so amodiaquine, a new antimalarial with a similar structure to chloroquine, is still effective against chloroquine-resistant parasites.

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