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Analysis and Classification of 304 Mutant Alleles in Patients with Type 1 and Type 3 Gaucher Disease

Vuk Koprivica,[1] Deborah L Stone,[1] Joseph K. Park,[1] Megan Callahan,[1] Amos Frisch,[2] Ian J. Cohen,[3] Nahid Tayebi,[1] and Ellen Sidransky[1]

[1]Clinical Neuroscience Branch, National Institute of Mental Health, National Institutes of Health, Bethesda; [2]Felsenstein Medical Research Center, Rabin Medical Center and Sackler Faculty of Medicine, and [3]Schneider Children's Medical Center of Israel, Rabin Medical Center and Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv

Gaucher disease results from the inherited deficiency of the enzyme glucocerebrosidase (EC 3.2.1.45). Although >100 mutations in the gene for human glucocerebrosidase have been described, most genotype-phenotype studies have focused upon screening for a few common mutations. In this study, we used several approaches—including direct sequencing, Southern blotting, long-template PCR, restriction digestions, and the amplification refraction mutation system (ARMS)—to genotype 128 patients with type 1 Gaucher disease (64 of Ashkenazi Jewish ancestry and 64 of non-Jewish extraction) and 24 patients with type 3 Gaucher disease. More than 97% of the mutant alleles were identified. Fourteen novel mutations (A90T, N117D, T134I, Y135X, R170C, W184R, A190T, Y304X, A341T, D399Y, c.153-154insTACAGC, c.203-204insC, c.222-224delTAC, and c.1122-1123insTG) and many rare mutations were detected. Recombinant alleles were found in 19% of the patients. Although 93% of the mutant alleles in our Ashkenazi Jewish type 1 patients were N370S, c.84-85insG, IVS2+1G→A or L444P, these four mutations accounted for only 49% of mutant alleles in the non-Jewish type 1 patients. Genotype-phenotype correlations were attempted. Homozygosity or heterozygosity for N370S resulted in type 1 Gaucher disease, whereas homozygosity for L444P was associated with type 3. Genotype L444P/recombinant allele resulted in type 2 Gaucher disease, and homozygosity for a recombinant allele was associated with perinatal lethal disease. The phenotypic consequences of other mutations, particularly R463C, were more inconsistent. Our results demonstrate a high rate of mutation detection, a large number of novel and rare mutations, and an accurate assessment of the prevalence of recombinant alleles. Although some genotype-phenotype correlations do exist, other genetic and environmental factors must also contribute to the phenotypes encountered, and we caution against relying solely upon genotype for prognostic or therapeutic judgements.

Introduction

Gaucher disease (MIM 230800), the most common glycolipid storage disorder, results from the inherited deficiency of the lysosomal enzyme glucocerebrosidase (E.C. 3.2.1.45). Clinically, Gaucher disease can be divided into three types, on the basis of the presence and rate of progression of neurologic manifestations. Type 1 Gaucher disease, which is the most common form, has no associated neurologic manifestations. However, patients with type 1 disease may have a wide spectrum of clinical presentations and may be asymptomatic or have a variable age of symptom onset. The most common findings in patients with type 1 Gaucher disease are organomegaly, cytopenia, and skeletal involvement. Type 2 disease, the rarest form, manifests with severe and progressive neurologic deterioration and is usually fatal in utero or within the first 2 years of life. Type 3, or chronic neuronopathic Gaucher disease, generally has an onset in childhood and, by definition, includes all patients with any form of neurologic involvement who have survived the first few years of life.

The gene for human glucocerebrosidase is located on chromosome 1q21, and there is a highly homologous pseudogene sequence located 16 kb downstream (Horowitz et al. 1989; Winfield et al. 1997). In the glucocerebrosidase gene, >100 different mutations have been identified (Grabowski and Horowitz 1997; Beutler and Gelbart 1998). Although there is a wealth of literature describing the screening of specific groups of patients with Gaucher disease for the presence or absence of certain common mutations, our understanding of genotype-phenotype correlations remains incomplete. There is significant phenotypic variation, not only among patients with the same disease type but also among patients with identical genotypes (Sidransky et

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Address for correspondence and reprints: Dr. Ellen Sidransky, Clinical Neuroscience Branch, National Institute of Mental Health, Building 49, Room B1EE16, 49 Convent Drive, MSC4405, Bethesda, MD, 20892-4405. E-mail: sidranse@irp.nimh.nih.gov

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al. 1994; Grabowski 1997). This is most notable among homozygotes for the relatively common N370S mutation, since these individuals may have a lifetime without symptoms (Azuri et al. 1998) or may develop massive organomegaly or incapacitating skeletal involvement. Even among patients from a geographic isolate—such as the Norrbottnian region of Sweden, where type 3 Gaucher disease is seen with increased frequency—a wide spectrum of presentations are encountered, although all of the patients are homozygous for the point mutation L444P (Erikson 1986; Dahl et al. 1990). It is usually even more difficult to predict the phenotype of compound heterozygotes.

The frequency of specific mutated alleles varies in different populations. It has been postulated that founder effects may account for such population differences (Diaz et al. 1998; Diaz et al. 1999; Lau et al. 1999). For example, one group has shown that mutations N370S, L444P, c.84-85insG, and IVS2+1G→A account for >96% of the mutated alleles in Ashkenazi Jewish patients, although they constitute <75% of the mutated alleles in non-Jews (Beutler et al. 1992; Beutler and Gelbart 1993). Among Japanese patients with Gaucher disease, neither mutation N370S nor c.84-85insG are seen, but L444P and F213I are relatively common, lending further support to the founder-effect theory (Kawame et al. 1993; Eto and Ida 1999). In the Portuguese Gaucher population, the N370S mutation accounts for 63% of the mutated alleles, and two other rare mutations, G377S and N396T, are commonly encountered (Amaral et al. 1993; Amaral et al. 1999). The evaluation of individuals homozygous for the mutations that are common in different ethnic groups provides a clearer picture of genotype-phenotype relationships in Gaucher disease. Recently, more comprehensive mutation profile studies have been undertaken in two smaller patient populations in Europe (Germain et al. 1998; Hodanova et al. 1999).

A limitation of most genotype-phenotype studies published in the literature is that they relied solely on PCR-based mutation-detection techniques to screen for the presence or absence of specific mutations. This approach has inherent problems, because a complex allele with more than one mutation, a deleted allele, or a mutation located at a primer site would go undetected. In addition, recombinant alleles resulting from crossover or gene-conversion events between the glucocerebrosidase gene and its pseudogene—including fusions, duplications, or deletions—would not be identified. An allele carrying a portion of the pseudogene sequence will not be amplified by primers designed to be specific for the glucocerebrosidase gene, and the patient may mistakenly be designated as a homozygote for the second allele (Tayebi et al. 1996*b*). Other strategies are necessary to more accurately genotype patients with Gaucher disease.

In this study, we performed genotypic analyses on samples from 152 patients with Gaucher disease, (128 with type 1 disease and 24 with type 3 disease) using complete-gene sequencing (Tayebi et al. 1998; Stone et al. 2000), long-template PCR (Tayebi et al. 1996*b*), restriction-site mutation detection (Sidransky et al. 1994), and the amplification refraction mutation system (ARMS) (Mistry et al. 1992). The allelic frequencies of specific mutations in these patients were compared with respect to ethnic background. The 128 patients with type 1 Gaucher disease included individuals of Ashkenazi ($n = 64$) and non-Ashkenazi ($n = 64$) origin. We also used the same strategy to establish the genotypes of 24 non-Jewish patients with type 3 Gaucher disease. Many rare or novel mutations and recombinant alleles were detected. Since >97% of the total mutant alleles were identified, this work represents a comprehensive analysis of the mutations encountered in our patient population. However, the study also demonstrates that even when the majority of DNA mutations are known, phenotypic differences among patients cannot be fully accounted for by genotype alone, and that modifier genes or environmental factors must also be considered.

Material and Methods

High-molecular-weight DNA was isolated from white blood cells or from cultured fibroblast or lymphoblast cell lines from affected individuals. A single proband was evaluated in each family. A majority of the patients were seen under an NIMH Institute Review Board–approved protocol at the Clinical Center at the National Institutes of Health, and informed consent was obtained. Sixty of these patients were included in a previous study screening for the presence of five specific mutations (Sidransky et al. 1994). Ten probands with type 1 disease were evaluated at the Rabin Medical Center in Israel and were referred because they were known to have at least one rare Gaucher allele, on the basis of preliminary mutation screening in Israel.

In most of the cases, patient DNA was first screened for the common N370S, L444P, R463C, c.84-85insG, IVS2+1G→A, and c.1263-1317del mutations, as described elsewhere (Tayebi et al. 1996*a;* Tayebi et al. 1998). The presence of these mutations was then confirmed by sequencing. If mutation L444P was detected, or if no common mutant alleles were identified, Southern blot analyses using the restriction enzymes *Ssp*I and *Sst*II and long-template PCR amplification of the entire glucocerebrosidase gene were performed to look for large deletions, recombinations, or duplications (Tayebi et al. 1996*b;* Tayebi et al. 1998). Then, the exonic se-

quences and most intronic sequences were amplified in three fragments of 1.7–3 kb in length, by use of primers designed to amplify selectively the glucocerebrosidase gene and not the pseudogene sequence. A fragment encompassing exons 1–5 was amplified by means of the forward primer 5′-CCTAAAGTTGTCACCCATAC-3′ and the reverse primer 5′-AGCAGACCTACCCTACAGTTT-3′ (annealing temperature 57°C, extension time 3 min). A second fragment covering exons 5–7 was amplified by use of the forward primer 5′-GACCTCAAATGATATACCTG-3′ and the reverse primer 5′-AGTTTGGGAGCCAGTCATTT-3′ (annealing temperature 58.5°C, extension time 2 min). Lastly, a fragment extending across exons 8–11 was amplified by means of the forward primer 5′-TGTGTGCAAGGTCCAGGATCAG-3′ and the reverse primer 5′-ACCACCTAGAGGGGAAAGTG-3′ (annealing temperature 61°C, extension time 1 min 30 s). These amplified segments were purified by use of either a GeneClean II kit (Bio 101) or a QIAquick PCR Purification Kit (Qiagen) before sequencing or restriction digests was performed. Cycle sequencing was accomplished by use of the Dye Terminator Cycle Sequencing kit (Applied Biosystems), and all identified mutations were confirmed by sequencing with both forward and reverse primers, as described elsewhere (Stone et al. 2000).

Results

The patient DNA was initially screened for the presence or absence of four commonly encountered mutations, IVS2+1 G→A, c.84-85insG, N370S, and L444P. Figure 1 summarizes the results of this screening and shows the allelic distribution among the patients with type 1 Gaucher disease in our series. The 10 patients from Israel were excluded because of the sampling bias. The distribution of the four mutant alleles differed between the Ashkenazi and non-Jewish populations. Among the Ashkenazi patients studied, screening for these four mutations successfully identified 93% of the mutant alleles. However, when screening for the four common mutations in the non-Jewish patients, only mutations N370S and L444P were detected, accounting for 80 (62%) of 128 mutant alleles. PCR screening detected L444P in 36 of the non-Ashkenazi patients. Subsequent sequencing and Southern blotting demonstrated that 17 patients actually had L444P as part of a recombinant allele carrying additional pseudogene sequence alterations (fig. 2) and that 19 patients had mutation L444P as a single point mutation. Thus PCR screening for individual mutations correctly identified only 63 (49%) of 128 mutant alleles in the non-Jewish patients.

The specific genotypes identified in patients with type 1 Gaucher disease are listed in tables 1 and 2. Most

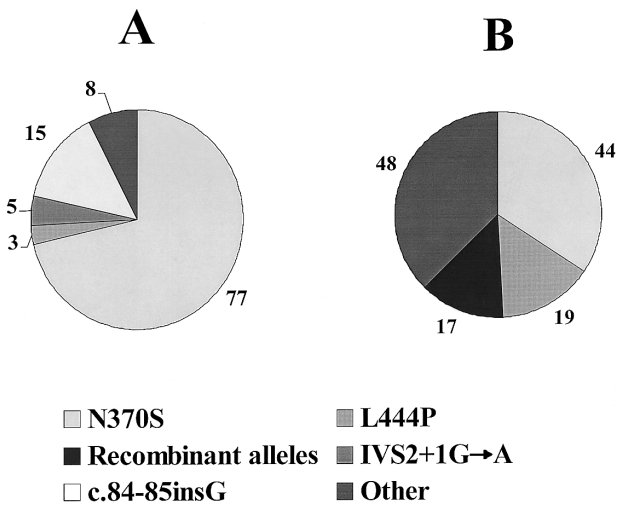

Figure 1 The distribution of five mutant alleles among patients with type 1 Gaucher disease. *A*, Ashkenazi Jewish patients (108 alleles). *B*, Non-Jewish patients (128 alleles).

(97%) of the mutant alleles were identified. In some of the cases where one allele remained unknown, complete sequencing could not be performed, because of limitations in the quantity of available DNA. There were consistent differences between the Ashkenazi Jewish and non-Jewish patients. All of the N370S homozygotes identified were Ashkenazi Jews. Recombinant alleles, which were not found in any of the Ashkenazi patients, were found in 30% of the non-Jewish patients studied. Among the 19 patients identified with recombinant alleles, five different sites of crossover between the glucocerebrosidase gene and pseudogene were found (fig. 2) (Tayebi et al. 1999*b*). All five were located between intron 8 and exon 10, a region in which the gene and the pseudogene share high sequence homology (Horowitz et al. 1989). Mutation R463C was identified in 9 (14%) of the non-Jewish patients, but in only 1 Ashkenazi Jewish patient. Genotype R463C/G202R was encountered in three unrelated, non-Jewish probands.

Eleven different genotypes were detected in our 24 patients with type 3 Gaucher disease (table 3). Of the commonly screened mutations, mutation N370S was not detected in this group. Two patients had a single allele with mutation c.84-85insG, and two carried IVS2+1G→A. Genotype L444P/L444P was identified in ten patients, but there were no L444P heterozygotes. Six patients were heterozygous for a recombinant allele, and three different sites of crossovers were noted (fig. 2), all in the same region as those identified in patients with type 1 Gaucher disease. No patients were homozygous for a recombinant allele. Mutation R463C was

also prevalent among patients with type 3 Gaucher disease and was found in ten patients.

Discussion

By using restriction-site mutation detection, ARMS, long-template PCR, Southern blotting, and complete gene sequencing, we have identified 97% of the 304 mutant alleles present in the 152 patients in this study. The identified mutations included point mutations, frameshift mutations, recombinant alleles, deletions, splice mutations, and insertions. In the 64 Ashkenazi patients with type 1 Gaucher disease, 16 different mutant alleles were identified, and 32 were identified in the 64 non-Jewish type 1 patients. In the 24 patients with type 3 Gaucher disease, 13 distinct mutant alleles were detected.

Among the patients with type 1 Gaucher disease, our results roughly support the previously described allele distributions for Ashkenazi and non-Ashkenazi patient populations (Grabowski 1997). Screening for four common Gaucher mutations by PCR-based techniques would identify 93% of the alleles of the American Ashkenazi Jewish subjects. In the non-Jewish population, only 49% of the alleles would have been identified correctly, and, by this screening method, 17 individuals with recombinant alleles would have been misclassified as having only point mutation L444P. The patients with type 1 Gaucher disease that we studied had a higher percentage of rare mutations than those in previously published American studies (Grabowski 1997; Beutler and Gelbart 1998). This may be attributed to the sampling bias inherent in studying samples at a tertiary referral center, since we often receive referrals of patients with mutations that were not detected by standard clinical laboratory testing. This was true of the samples from Israel, which were sent to us because the patients were known to have at least one rare allele (table 1).

We continue to caution that there are basic limitations

Table 1

Genotypes Encountered in American and Israeli Ashkenazi Jewish Patients with Type 1 Gaucher Disease

Genotype	No. of Patients
American patients:	
N370S/N370S	26
N370S/L444P	2
N370S/IVS2+1G→A	4
N370S/c.84-85insG	15
N370S/V394L	1
N370S/F213I	1
N370S/R463C	1
N370S/Y135X	1
L444P/V394L	1
IVS2+1G→A/R496H	1
R48W/R120W	1
Israeli patients:	
N370S/V394L	4
N370S/R120W	1
N370S/c.1122-1123insTG	1
N370S/R120Q	1
N370S/W184R	1
N370S/R285C	1
N370S/?	1

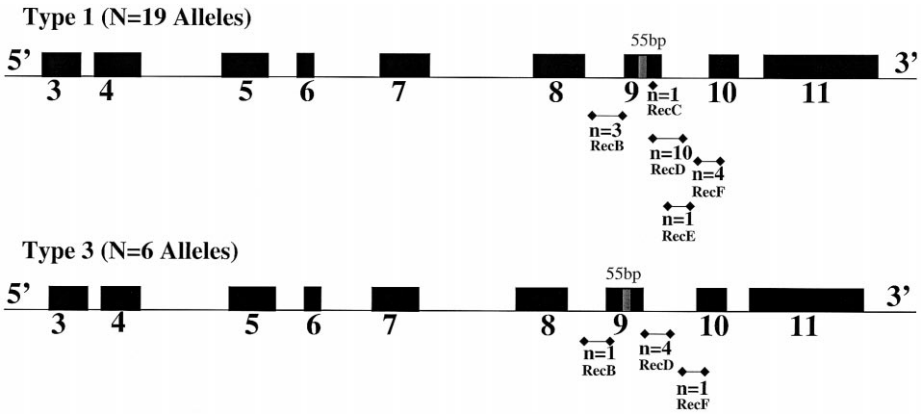

Figure 2 Distribution of the identified sites of crossover between the glucocerebrosidase gene and pseudogene in patients with type 1 and type 3 Gaucher disease. A total of 19 recombinant alleles were detected in patients with type 1 Gaucher disease and 6 in patients with type 3 Gaucher disease. The horizontal lines connecting the diamond symbols represent the general regions where the crossover must have occurred, n= the number of recombinant alleles identified within the region shown. The approximate sites are as follows: RecA, (not shown - identified in a patient with type 2 Gaucher disease (Stone et al. 2000) intron 3; RecB, intron 8 or the beginning of exon 9 (before the 55 bp deletion c.1263-1317 del); RecC, exon 9; RecD, end of exon 9 or beginning of intron 9; RecE, intron 9; RecF, end of intron 9 or beginning of exon 10.

Table 2

Genotypes Encountered in Non-Jewish Patients with Type 1 Gaucher Disease

Genotype[a]	Frequency
N370S/L444P	11
N370S/RecB	1
N370S/RecC	1
N370S/RecD	6
N370S/RecE	1
N370S/RecF	3
L444P/D409H	1
L444P/A90T	1
R463C/R285C	1
N370S/T369M	1
R463C/R257Q	1
N370S/R463C	2
L444P/R170C	1
R463C/G202R	3
RecD/R463C	1
R170P/R170P	1
L444P/R48W	1
N370S/S196P	1
K79N/?	1
N370S/G377S	1
N370S/R131L	1
R257Q/F216Y	1
N370S/D399Y	1
RecD/c.222-224delTAC	1
N370S/R257Q	1
N370S/c.1263-1317del(RecB)	2
RecD/V352L	1
RecF/N117D	1
N370S/c.203delC	2
L444P/A341T	1
N370S/R120W	1
RecD/c.153-154insTACAGC	1
N370S/E326K+c.203-204insC	1
N370S/R120Q	1
L444P/R463C	1
N370S/T134I	1
L444P/L444P	1
N370S/?	5

[a] RecB–RecF refer to recombinant alleles with the site of crossover as indicated in figure 2.

in our ability to predict the severity of a point mutation on the basis of the associated patient phenotype. It is clear from many inherited disorders that monogenic traits are not necessarily simple (Scriver and Waters 1999). Modifier genes, contiguous genes, transporter proteins, activator proteins, and environmental factors may contribute significantly to phenotype, as has been demonstrated in other genetic disorders. For example, in cystic fibrosis, the expression at a modifier locus helps to define whether a patient with cystic fibrosis will develop the pancreatic insufficient form of the disease (Estivill 1996). Patients with Hunter syndrome with atypical phenotypes have been shown to have deletions encompassing contiguous genes (Timms et al. 1997). In phenylketonuria, both *cis* alleles and *trans*-acting factors contribute to phenotype (Scriver and Waters 1999). In α-1-antitrypsin deficiency, both environmental factors—such as smoke—and molecular factors—such as chaperones and proteases—play a role (Perlmutter 1998). Nonetheless, our extensive genotyping data was evaluated to determine whether certain genotype-phenotype generalizations were possible, especially regarding the newly described mutant alleles.

In our study, 14 novel mutations were detected, 12 of which were identified in patients with type 1 Gaucher disease (table 4). Three of these, Y135X, W184R, and c.1122-1123insTG, were found in Ashkenazi Jewish patients. In all three, the second allele was N370S. Since the N370S mutant allele appears to express enough enzyme to "protect" against neuronopathic Gaucher disease, our study did not enable speculation about the severity of these mutations.

Nine novel mutations were identified among the non-Jewish patients with type 1 Gaucher disease (table 4). Five of the mutations—A90T, N117D, R170C, c.222-224delTAC (which deletes a downstream threonine) and c.153-154insTACAGC (which results in a duplication of the amino acids tyrosine and serine)—were detected in black patients, and the clinical cases will be discussed elsewhere (unpublished data). All five of these were encountered with more-severe mutations, either a recombinant allele or mutation L444P, on the second allele, and thus we suspect that they are relatively mild mutations. The other four new mutant alleles were each accompanied by an allele with N370S. Two novel point mutations, A341T and D399Y, were encountered in patients with Swedish ancestry, and a third mutation, T134I, was identified in an American patient. A fourth patient carried a novel insertion mutation, c.203-204insC (introducing a stop codon at a position 34 nucleotides downstream in exon 3), together with a pos-

Table 3

Genotypes Encountered in 24 Patients with Type 3 Gaucher Disease

Genotype[a]	No. of Patients
L444P/L444P	10
RecD/R463C	3
c.84-85insG/R463C	2
IVS2+1G→A/R463C	2
RecD/D409H	1
A190T/G377S	1
Y304X/R463C	1
RecF/N188S	1
D399N/R463C	1
c.1263-1317del(RecB)/?	1
R463C/A176D	1

[a] RecB–F refer to recombinant alleles with the site of crossover as shown in figure 2.

Table 4

Rare Mutations Encountered in Patients With Type 1 Gaucher Disease

Mutation Type	Mutations in	
	Ashkenazi Patients	Non-Jewish Patients
Novel	Y135X	A90T
	W184R	N117D
	c.1122-1123insTG	T134I
		R170C
		A341T
		D399Y
		c.222-224delTAC
		c.203-204insC
		c.153-154insTACAGC
Also reported in type 2 patients	R120W	R120W
	F213I	R131L
		S196P
		G202R
		R257Q
		D409H
		c.1263-1317del
Known as "mild"	R48W	R48W
	R496H	K79N
		F216Y
		V352L
		G377S
Previously published (severity unknown)	R120Q	R120Q
	R285C	R285C
	R463C	T369M
	V394L	R463C
		c.203delC

sible polymorphism, E326K (Grace et al. 1999), on the same allele.

Of the 21 other rare alleles encountered in our patients with type 1 Gaucher disease (table 4), eight (R120W, R131L, F213I, S196P, G202R, R257Q, D409H, and c.1263-1317del) have been previously reported in patients with type 2 Gaucher disease (Stone et al. 2000) and thus are more likely to be severe. Six others (R48W, K79N, F216Y, V352L, G377S, and R496H) have been previously reported as mild (Beutler and Gelbart 1998). In our patients, mutations R48W, V352L, and R496H were encountered in patients who also had the more severe mutations L444P, R120W, R257Q, or IVS2+1 G→A, all four of which are described in patients with type 2 Gaucher disease, thus confirming that the former three mutations are likely to be mild. The severity of the five other previously described rare alleles—R120Q, R285C, T369M, V394L, and R463C—cannot be established from our data. The frameshift mutation c.203delC (introducing a stop codon 58 bp downstream in exon 3), presumably a null mutation, was encountered in two unrelated probands together with N370S.

A total of 19 patients with type 1 Gaucher disease were found to have recombinant alleles. Five different sites of crossover were identified, as illustrated in figure 2. In two patients, the recombinant allele was exclusively a 55-bp deletion in exon 9, c.1263-1317del(RecB), presumably resulting from gene conversion, whereas, in the other 17 patients, larger regions of pseudogene sequence that included point mutation L444P were incorporated, as detected by Southern blot analyses and sequencing. The terms RecA→F (tables 2 and 3) (fig. 2) refer to the approximate site of crossover, but it should be noted that alleles incorporating different-sized segments of pseudogene sequence can share the same crossover site. For example, a crossover at site RecB resulted in mutation c.1263-1317del in two patients, whereas, in a third patient, a crossover at this site resulted in a recombinant allele that included c.1263-1317del, together with mutations D409H and L444P and other pseudogene sequence. Several of the recombinant alleles carried a fusion between the gene and pseudogene, and others resulted from gene conversion. Since homozygosity for a recombinant allele results in early lethality (Tayebi et al. 1999*a;* Stone et al. 2000), the second allele encountered in each of the 19 individuals with recombinant alleles is, presumably, less severe.

Our results have direct implications for laboratories screening for mutations in patients with type 1 Gaucher disease. For example, mutation V394L was found in

9% of the Ashkenazi Jewish patients studied. When screening this patient population, adding mutation V394L to the panel could improve the sensitivity of the screening. For non-Jewish patients, the strategy used to identify mutant alleles could be altered to detect mutations more commonly seen in this population. Specifically, we did not detect mutation IVS2+1G→A or c.84-85insG in our series of non-Jewish type 1 patients, although we have identified these mutations in non-Jewish patients with type 2 and type 3 Gaucher disease (Stone et al. 2000). Mutations R463C, G202R, and recombinant alleles were found more frequently in non-Jewish subjects as compared to Ashkenazi patients. We strongly advocate that DNA from all patients identified with mutation L444P be further evaluated by direct sequencing and Southern blotting to establish the presence or absence of a recombinant allele or a duplicated or fusion allele.

The genotyping results of the patients with type 3 Gaucher disease also have relevance for genetic counseling. In this group, it was particularly striking that clinically similar patients had many different genotypes. None of the patients were found to have mutation N370S, confirming that the presence of N370S is predictive of nonneuronopathic disease. A significant proportion of the patients (41%) were L444P homozygotes. However, all five of the patients who appeared heterozygous for L444P actually had a recombinant allele, and, among these five patients, two different sites of crossover between the gene and pseudogene (RecD and RecF) were identified. An additional patient carried the 55-bp deletion c.1263-1317del(RecB). Mutation R463C, which was present in 10 (41%) of the patients, was the second-most-common allele among patients with type 3 Gaucher disease. Two novel mutations, A190T (a point mutation) and Y304X (which results from a 1-bp deletion in exon 8), were encountered in our patients, each with mutation R463C on the second allele. Five rare alleles—A176D, G377S, N188S, D399N, and D409H—were also detected. Two of these, N188S and G377S, have previously been described in patients with type 1 Gaucher disease and were presumed to be mild (Laubscher et al. 1994; Kim et al. 1996; Choy et al. 1999).

We have recently completed the genotypic analysis of 31 patients with type 2 Gaucher disease, and all 62 mutant alleles were successfully identified (Tayebi et al. 1999*a;* Stone et al. 2000). Homozygosity for a recombinant allele results in perinatal lethal type 2 Gaucher disease, and it appears that most recombinant alleles are null alleles. Homozygosity for point mutations S196P, G202R, and H311R also result in type 2 Gaucher disease. Homozygosity for mutation L444P is usually associated with type 3 Gaucher disease, most often in patients with compromised horizontal saccadic eye movements. However, there are a few published exceptions to this general observation. In our series and that of others, there have been young patients with this genotype who have no apparent neurologic involvement (Kawame et al. 1993; Cox and Schofield 1997). The patient which we have included is only four years old, but her affected younger brother does appear to have early involvement of his extraocular movements. Long-term followup of these patients is necessary, as many of these children later developed neurologic symptoms (Ida et al. 1999). We did not find any true L444P homozygotes in our series of 31 patients with type 2 Gaucher disease (Stone et al., in press), and believe that many of the patients with type 2 disease described in the literature as having genotype L444P/L444P may actually have at least one recombinant allele.

Homozygosity for mutation N370S is always associated with type 1 Gaucher disease, and heterozygosity for N370S is also predictive of type 1 disease. By use of the generalizations made from patients with other homozygous genotypes, correlations can be attempted in those with heterozygous genotypes. All of our patients with one L444P allele and one recombinant allele have type 2 Gaucher disease. Likewise, genotypes L444P/IVS2+1G→A and L444P/G202R have only been encountered in type 2 patients. Thus, it appears that one L444P allele together with a null allele results in type 2 Gaucher disease, whereas L444P homozygotes usually have type 3 disease. A recombinant allele was encountered together with A90T, c.222-224delTAC, V352L, N117D, or c.153-154insTACAGC in patients with type 1 Gaucher disease, which would indicate that these five mutations are relatively mild. However, a recombinant allele—together with either mutation D409H, mutation N188S, or mutation R463C—resulted in type 3 Gaucher disease. Patients described in the literature with genotype D409H/D409H have a unique phenotype, with features including aortic involvement, oculomotor involvement, and hydrocephaly (Abrahamov et al. 1995; Uyama et al. 1997). Our patient with a recombinant allele and mutation D409H shared these unusual features (Stone et al., in press). However, mutation N188S, seen in a type 3 patient, had been previously described primarily in Asian patients with relatively mild disease and was even suggested to be protective from neuronopathic disease (Kim et al. 1996; Choy et al. 1999).

It is particularly difficult to understand the phenotypic consequence of mutation R463C. In the literature, genotype R463C/R463C was described in an adult patient with type 1 Gaucher disease (Hatton et al. 1997) and in a Turkish patient with type 3 disease (Gurakan et al. 1999). In our series, all patients with R463C/N370S have had type 1 disease. However, we have had patients with R463C/R257Q, R463C/R285C, R463C/

G202R, R463C/RecD, and R463C/L444P with type 1 Gaucher disease and patients with R463C/RecD, R463C/IVS2+1G→A, R463C/c.84-85insG and R463C/A176D with type 3 Gaucher disease. Since G202R, R257Q, IVS2+1G→A, c.84-85insG, and recombinant alleles are all severe or null mutations found in patients with type 2 Gaucher disease, these relationships are inconsistent.

Several of the mutations discussed here have been expressed previously in different in vitro–expression systems (Ohashi et al. 1991; Choy et al. 1996; Grace et al. 1997, 1999; Pasmanik-Chor et al. 1997). Although, in all these studies, the enzymatic activity of expressed enzyme with mutation N370S was higher than with mutation L444P, it is very difficult to compare the enzyme activity associated with mutant alleles among different studies, as these studies use different preparation and assay systems.

Our study demonstrates that by employing several different mutation-detection strategies, including direct sequencing, 97% of the mutant alleles could be identified in our large population of patients with Gaucher disease. Fourteen novel mutations are also described. We specifically searched for recombinant alleles, and we found them in 15% of the patients with type 1 and 25% of the patients with type 3 Gaucher disease. This study not only identifies a large number of novel and rare mutations in patients with types 1 and 3 Gaucher disease but accurately assesses the prevalence of recombinant alleles. These two features enhance our ability to attempt genotype-phenotype correlations. Although relevant generalizations can be made, our data suggest that additional factors must contribute to the range of phenotypes encountered in patients with Gaucher disease (fig. 3). Clearly, the identification of specific point mutations is useful, but whether these mutations are part of a complex or recombinant allele is important. Also, since the region on chromosome 1q21 encompassing human glucocerebrosidase is particularly gene rich, with seven genes and two pseudogenes within an ~75-kb region (Winfield et al. 1997), the possible contribution of genes contiguous to glucocerebrosidase in certain patients is particularly intriguing. Another factor which could contribute to phenotype is the involvement of an alternate substrate. Glucosylsphingosine, a lipid which is also degraded by the enzyme glucocerebrosidase, is elevated in tissues from patients with Gaucher disease, including the brains of patients with neuronopathic Gaucher disease (Nilsson and Svennerholm 1982; Orvisky et al. 1999). The rate of accumulation and degradation of glucosylsphingosine or the amount stored may be responsible for certain disease manifestations in patients.

As now seen in many Mendelian disorders, other genes may also contribute to the range of symptoms

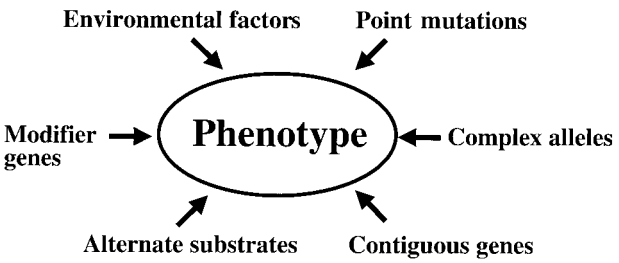

Figure 3 Factors which may contribute to phenotype in patients with Gaucher disease.

encountered. In patients with Gaucher disease, two related genes are those encoding for sphingolipid activator proteins (SAPs) and lysosome-associated membrane proteins (LAMPs). The SAPs are small nonenzymatic glycoproteins that are necessary for the degradation of lysosomal sphingolipids (Morimoto et al. 1990). SAP-C is essential for the degradation of glucocerebroside and patients with mutations in the gene encoding SAP-C demonstrate glucocerebroside storage with hepatosplenomegaly and Gaucher cells in the bone marrow, along with oculomotor and neurologic abnormalities (Pampols et al. 1999). It has recently been shown that the lysosome-associated membrane proteins, LAMP-1 and LAMP-2, may participate in the intracellular delivery of glucocerebrosidase from the endoplasmic reticulum to lysosomes, and that this transport may be defective in patients with Gaucher disease (Zimmer et al. 1999). It is likely that other modifier genes will also be identified.

Lastly, environmental factors clearly contribute to the heterogeneity observed in patients with Gaucher disease (fig. 3). It has been shown that, in Gaucher disease, siblings with identical genotypes may have different clinical manifestations, different disease complications, and even different responses to therapy (Cox and Schofield 1997; Grabowski 2000). Factors such as viral illnesses, surgery, pregnancy, and trauma may precipitate the development or progression of manifestations in certain patients.

Therefore, we caution against relying solely on genotypes to predict prognosis or the need for therapy in patients with Gaucher disease. A better understanding of the functional consequences of specific mutations at the protein level, the possible involvement of genes contiguous to human glucocerebrosidase (Winfield et al. 1997) and the role of activator proteins (Morimoto et al. 1990), transporter proteins (Zimmer et al. 1999) and other modifier genes may enhance genotype-phenotype studies. The generation of mouse models homozygous for specific Gaucher mutations may also better clarify

the phenotypic consequences of the observed mutant alleles.

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Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

GenBank, http://www.ncbi.nlm.nih.gov/Genbank (for the sequence surrounding human glucocerebrosidase [accession number AFO23263] and for human glucocerebrosidase [GBA] [accession number JO3059])

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for Gaucher disease [MIM 230800]; Gaucher disease, type 2 [MIM 230900]; and Gaucher disease, type 3 [MIM 231000])

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