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Glucocerebrosidase Gene Mutations in Patients With Type 2 Gaucher Disease

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Gaucher disease, the most common lysosomal storage disorder, results from the inherited deficiency of the enzyme glucocerebrosidase. Three clinical types are recognized: type 1, non-neuronopathic; type 2, acute neuronopathic; and type 3, subacute neuronopathic. Type 2 Gaucher disease, the rarest type, is progressive and fatal. We have performed molecular analyses of a cohort of 31 patients with type 2 Gaucher disease. The cases studied included fetuses presenting prenatally with hydrops fetalis, infants with the collodion baby phenotype, and infants diagnosed after several months of life. All 62 mutant glucocerebrosidase (GBA) alleles were identified. Thirty-three different mutant alleles were found, including point mutations, splice junction mutations, deletions, fusion alleles and recombinant alleles. Eleven novel mutations were identified in these patients: R131L, H255Q, R285H, S196P, H311R, c.330delA, V398F, F259L, c.533delC, Y304C and A190E. Mutation L444P was found on 25 patient alleles. Southern blots and direct sequencing demonstrated that mutation L444P occurred alone on 9 alleles, with E326K on one allele and as part of a recombinant allele on 15 alleles. There were no homozygotes for point mutation L444P. The recombinant alleles that included L444P resulted from either reciprocal recombination or gene conversion with the nearby glucocerebrosidase pseudogene, and seven different sites of recombination were identified. Homozygosity for a recombinant allele was associated with early lethality. We have also summarized the literature describing mutations associated with type 2 disease, and list 50 different mutations. This report constitutes the most comprehensive molecular study to date of type 2 Gaucher disease, and it demonstrates that there is significant phenotypic and genotypic heterogeneity among patients with type 2 Gaucher disease. Hum Mutat 15:181–188, 2000. Published 2000 Wiley-Liss, Inc.†

KEY WORDS: **Gaucher disease; acute neuronopathic; genotype–phenotype correlation; glucocerebrosidase; GBA; hydrops fetalis; congenital ichthyosis**

INTRODUCTION

Gaucher disease (MIM# 230800) results from the inherited deficiency of the lysosomal enzyme glucocerebrosidase (EC 3.2.1.45). Clinically, the disorder is divided into three types based upon the presence and rate of progression of neurologic manifestations. Type 2 or acute neuronopathic Gaucher disease (MIM# 230900) is the most severe form and is universally progressive and fatal, with death generally occurring before patients reach two years of age.

Classically, type 2 Gaucher disease was considered a disease of late infancy. Patients were described as normal at birth but later displayed increasing hepatosplenomegaly, the regression of developmental milestones, and an arrest of growth. The neurologic deterioration progressed rapidly with cranial nerve and extrapyramidal tract involvement. The age at death ranged from one month to two years, with an average of nine months [Frederickson and Sloan, 1972].

In recent years, the availability of a mouse model of type 2 Gaucher disease [Tybulewicz et al., 1992] led to the appreciation of a perinatal lethal vari-

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ant of Gaucher disease [Sidransky et al., 1992; Sidransky, 1997]. Affected individuals may be detected prenatally, often with hydrops fetalis [Sidransky et al., 1992, 1996b; Tayebi et al., 1997, 1998; Reissner et al., 1998; Strasburg et al., 1994; Stone et al., 1999b; Rowlands and Murray, 1997], or in the newborn period when they may present with a "collodion baby" phenotype [Lui et al., 1988; Sidransky et al., 1992, 1996a; Lipson et al., 1991; Stone et al., 1999a].

The gene for human glucocerebrosidase is located on chromosome 1q21. Over 100 mutations in the glucocerebrosidase gene have been described in the DNA of patients [Beutler and Gelbart, 1998; Grabowski and Horowitz, 1997]. A pseudogene for glucocerebrosidase, sharing 97% exonic sequence homology, is located 16kb downstream from the functional gene [Horowitz et al., 1989; Winfield et al., 1997]. The presence of this homologous sequence complicates mutation detection strategies, since many mutant alleles are point mutations which derive from the pseudogene sequence. PCR amplification of genomic DNA for sequencing must selectively amplify the functional gene.

The first mutation described in an infant with type 2 Gaucher disease, L444P [Tsuji et al., 1987], continues to be the mutation most often encountered in this patient group. However, this mutation is present in the glucocerebrosidase pseudogene and can occur alone or as part of several different recombinant or fusion alleles [Tayebi et al., 1998]. Laboratories relying on PCR based genotyping techniques may not be able to discriminate between a recombinant allele and an allele with the L444P point mutation only.

In this paper we present the mutant alleles identified in a series of 31 patients with type 2 Gaucher disease. Fourteen of these cases were included in a previous review [Tayebi et al., 1998]. We have also summarized other published mutations encountered in patients with type 2 Gaucher disease.

MATERIALS AND METHODS

Mutation Analysis

High molecular weight DNA was isolated from blood or cultured cell lines from affected individuals and/or their parents. In most cases, patient DNA was first screened for the common N370S, L444P, R463C, 84insG, IVS2+1 G→A, and 55 bp deletion (c.1263-1317del) mutations, as previously described [Tayebi et al., 1996b; Tayebi et al., 1998]. Long template PCR amplification of the entire glucocerebrosidase gene was performed to look for large deletions or insertions [Tayebi et al., 1996a]. All exonic sequences and most intronic sequences were selectively amplified in three fragments ranging from 1.7 kb to 3 kb in length. A fragment encompassing exons 1–5 was amplified using 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 using the forward primer 5′-GACCTCAAATGATATACCTG-3′ and the reverse primer 5′-AGTTTGGGAGCCAGTCATTT-3′ (annealing temperature 58.5°C, extension time 2 min). Finally, a fragment extending across exons 8–11 was amplified using the forward primer 5′-TGTGTGCAAGGTCCAGGATCAG-3′ and the reverse primer 5′-ACCACCTAGAGGGGAAAGTG-3′ (annealing temperature 61°C, extension time 90 sec). These amplified segments were purified before sequencing or restriction digests were performed, using either a GeneClean II kit (Bio 101, LaJolla, CA) or a QIAquick PCR Purification Kit (Qiagen, Santa Clarita, CA). Cycle sequencing was accomplished using the Dye Terminator Cycle Sequencing kit (Applied Biosystems, Porter City, IA), and all mutations were confirmed by sequencing using both forward and reverse primers (Table 1). Novel mutations were confirmed by restriction digestion of an amplified PCR fragment when the mutation created or obliterated a restriction site.

Southern Blot Analyses

Genomic DNA was digested with the restriction enzymes *Sst*II or *Ssp*I, electrophoresed on a 0.6% I.D.$_{NA}^{TM}$ agarose gel, transferred to nitrocellulose and hybridized to a ^{32}P-labeled glucocerebrosidase cDNA probe as previously described [Tayebi et al., 1998].

Western Blot Analyses

Total protein was extracted from frozen fibroblast cell pellets from patients 2, 3, 5, 7, 9, 10, 11, 17–22, 26, and 30. The pellet was resuspended in 60mM potassium phosphate, pH 5.9 containing 0.1% Triton X-100, sonicated several times at 4°C using a 50W Cell Disrupter Model 225R (Heat Systems Ultrasonics Inc., Farmingdale, NY), and centrifuged at 12,000g for 5 min at 4°C. Western blots with rabbit polyclonal antibody were performed using 15–30μg of the protein supernatant, which was electrophoresed on either 8% or 12% SDS PAGE-Tris-glycine gel as described previously [Tayebi et al., 1998].

TABLE 1. Primers Used for Sequencing

Exon	Forward primer	Reverse primer
2	5′-GAGAGTAGTTGAGGGGTGGA-3′	5′-CAAAGGACTATGAGGCAGAA-3′
3	5′-ATGTGTCCATTCTCCATGTC-3′	5′-GGTGATCACTGACACCATTT-3′
4	5′-GGTGTCAGTGATCACCATGG-3′	5′-ACGAAAAGTTTCAATGGCTCT-3′
5	5′-GCAAGTGATAAGCAGAGTCC-3′	5′-AGCAGACCTACCCTACAGTTT-3′
6	5′-CTCTGGGTGCTTCTCTCTTC-3′	5′-ACAGATCAGCATGGCTAAAT-3′
7	5′-TTGGCCGGATCATTCATGAC-3′	5′-AGTTTGGGAGCCAGTCATTT-3′
8	5′-TGTGTGCAAGGTCCAGGATCAG-3′	5′-TTTGCAGGAAGGGAGACTGG-3′
9	5′-CACAGGGCTGACCTACCCAC-3′	5′-GCTCCCTCGTGGTGTAGAGT-3′
10	5′-CAGGAGTTATGGGGTGGGTC-3′	5′-GAGGCACATCCTTAGAGGAG-3′
11	5′-GTGGGCTGAAGACAGCGTTGG-3′	5′-ACCACCTAGAGGGGAAAGTG-3′

RESULTS

The mutant alleles identified in the DNA of the 31 patients studied are summarized in Table 2. All of the sixty-two mutant alleles were characterized. Thirty-three different mutant alleles were identified. These included point mutations, splice junction mutations, deletions, fusion alleles, and recombinant alleles. Among the base changes found, 11 were mutations which are newly described by our group: H311R and V398F [Stone et al., 1999b], c.533delC [Tayebi et al., 1997], R131L, H255Q, S196P, c.330delA, F259L, R285H, Y304C and A190E. These new mutations were confirmed by restriction digestion whenever the

TABLE 2. Type 2 Gaucher Disease

Ethnic background	Genotype[+]	Clinical presentation	Clinical description
Patients with manifestations in utero			
1) Ashkenazi	rec A/IVS 10+2 T>G	Hydrops	Reissner et al., 1998
2) Afghan*	rec D/rec D	Hydrops	Sidransky et al., 1996b
3) Turkish*	c.533delC/c.533delC	Hydrops	Tayebi et al., 1997
4) Cape Verdean*	H311R/H311R	Hydrops	Stone et al., 1999b
5) North American	rec B/rec B	Hydrops	Tayebi et al., 1998
6) North American	rec D/rec D	Hydrops	
7) Lebanese*	rec D/rec D	Ichthyosis/Hydrops	Rowlands & Murray, 1997
Patients diagnosed at birth			
Ethnic background	Genotype[+]	Clinical presentation	Clinical description
8) Dutch/Surinamese	R359X/V398F	Ichthyosis	Stone et al., 1999b
9) Australian	c.1263-1317del/R257Q	Ichthyosis	Lipson et al., 1991
10) Greek	rec C/H255Q	Hepatosplenomegaly	Sidransky et al., 1992
11) Lebanese	R120W/S196P	Ichthyosis	Liu et al., 1988
12) Lebanese*	S196P/S196P	Respiratory difficulty	
13) North American	F259L/N188K	Opisthotonus	
14) North American	c.1263-1317del/R285H	NA	
Patients identified after several months			
Ethnic background	Genotype	Age at presentation	Clinical description
15) Black American	rec E/E41K	8 mo	Sinclair et al., 1998
16) North American	L444P/G202R	8 mo	Tayebi et al., 1998
17) North American	L444P/R163	7 mo	Tayebi et al., 1998
18) North American	L444P/rec F	5 mo	Tayebi et al., 1998
19) North American	L444P/rec F	NA	Cell line GM 877
20) Mexican	R131L/R131L	5 mo	Fujimoto et al., 1995
21) North American	L444P/P415R	NA	Cell line GM 1260
22) Mexican	L444P+E326K/rec G+L444P	NA	Cell line GM 8760
23) Swedish	L444P/c.330delA	3 mo	
24) Belgian*	G202R/G202R	6 mo	
25) North American	rec G+L444P/R257Q	6 mo	
26) North American	L444P/Y304C	12 mo	
27) North American	A190E/G325R	9 mo	
28) North American	L444P/IVS2+1G>A	4 mo	
29) North American*	G202R/G202R	5 mo	
30) Kenyan/Filipino	L444P/IVS2+1G>A	4 mo	
31) North American	G325R/C342G	NA	Cell line GM 2627

*Known consanguinity
NA, information is not available.
[+]The designation rec A-G refer to a recombinant allele with the site of crossover as shown in Figure 1.

mutation created or obliterated a restriction site. The specific enzymes used are listed in Table 3. Many other rare mutations were also found, including IVS10+2T>G, E41K, G325R, C342G, R359X, R257Q, R120W, G202R, R163X, N188K, and P415R. One mutant allele identified had the amino acid change E326K in addition to mutation L444P. An E326K + L444P allele has previously been described in another infant with type 2 Gaucher disease who carried mutation E233X on the second allele [Grace et al., 1999].

The common Gaucher mutations that were identified in our patients were IVS2+1 G>A, L444P, and the 55bp deletion (c.1263-1317del). Mutation IVS2+1 G>A was identified in the DNA from two patients, point mutation L444P was detected on 25 patient alleles (41%), and the 55bp deletion was seen in the DNA of two pa-

TABLE 3. **Mutations in Glucocerebrosidase Encountered in Patients With Type 2 Gaucher Disease**

Exon/ intron	Amino acid substitution	Site of nucleotide substitution	Restriction site change	First description in a type 2 patient
Intron 2	Splice site ψ	IVS 2+1 G→A	–*Hph*I	Horowitz et al., 1993
Exon 3	E41K ψ	c.238G→A	–*Bsr*DI	Sinclair et al., 1998
Intron 2	Recombinant (rec A)[a]			Reissner et al., 1998
Exon 4	Frameshift at aa71	c.330delA		Present study
Exon 4	K74X (Null)	c.337A→T		Grace et al., 1997
Exon 4	S107L	c.437C→T	+*Tsp*509I	Demina & Beutler, 1998
Exon 5	R120W ψ	c.475C→T	–*Nci*I	Tayebi et al., 1998
Exon 5	R131C	c.508C→T	–*Aci*I	Sinclair et al., 1998
Exon 5	R131L	c.509G→T	–*Aci*I	Present study
Exon 5	Frameshift at aa139	c.533delC	+*Msl*I	Tayebi et al., 1997
Exon 5	K157Q	c.586A→C	+*Scr*FI	Latham et al., 1991
Exon 6	R163X (Null)	c.604C→T	–*Hph*I/+*Dde*I	Present study
Exon 6	P178S	c.649C→T	–*Bsa*JI	Choy and Wei, 1995
Exon 6	N188K ψ	c.681T→G	+*Sty*I	Present study
Exon 6	A190E	c.686C→A	+*Mnl*I/–*Bsr*BI	Present study
Exon 6	G195E	c.701G→A	+*Hin*fI	Cormand et al., 1998
Exon 6	S196P ψ	c.703T→C	–*Tsp*45I	Present study
Exon 6	G202R ψ	c.721G→A	–*Nci*I	Beutler et al., 1994
Exon 6	F213I ψ	c.754T→A		Kawame & Eto, 1991
Exon 7	E233X (Null)	c.814G→T	+*Mse*I/–*Pvu*II	Grace et al., 1999
Exon 7	H255Q	c.882T→G	+*Fnu*4HI	Present study
Exon 7	R257Q	c.887G→A	–*Bsm*AI	Present study
Exon 7	F259L	c.894C→A	+*Mse*I	Present study
Exon 7	R285H	c.971G→A	–*Cac*8I	Present study
Exon 8	Y304C	c.1028A→G		Present study
Exon 8	H311R	c.1049A→G	+*Mae*II	Stone et al., 1999b
Exon 8	G325R ψ	c.1090G→A	+*Bsu*36I	Eyal et al., 1990
Exon 8	C342G	c.1141T→G	–*Stu*I	Eyal et al., 1990
Exon 8	R359X (Null)	c.1192C→T	–*Sau*3AI	Beutler & Gelbart, 1994
Intron 8	Recombinant (rec B)[a]			Hatton et al., 1997
Exon 9	D380A	c.1256A→C	+*Scr*FI	Walley & Harris, 1993
Exon 9	383-400del ψ	c.1263-1317 (55bp)del		Walley & Harris, 1993
Exon 9	G389E	c.1283G→A	–*Mnl*I/+*Mbo*II	Cormand et al., 1998
Exon 9	N392I	c.1292A→T	–*Mfe*I/+*Msl*I	Cormand et al., 1998
Exon 9	V398F	c.1309G→T	–*Sal*I	Stone et al., 1999b
Exon 9	D399N	c.1312G→A	–*Taq*I	Beutler & Gelbart, 1994
Exon 9	D409H ψ	c.1342G→C	–*Sty*I	Horowitz et al., 1993
Exon 9	P415R	c.1361C→G	+*Hha*I	Wigderson et al., 1989
Exon 9	Recombinant (RecTL)(rec C)[a]			Tayebi et al., 1998
Intron 9	Recombinant (rec D)[a]			Tayebi et al., 1998
Intron 9	Recombinant (rec E)[a]			Sinclair et al., 1998
Exon 10	Recombinant (RecNciI)(rec F)[a]			Hong et al., 1990
Exon 10	445-450del	c.1447-1466del,insTG		Uchiyama et al., 1994
Exon 10	L444P ψ	c.1448T→C	+*Nci*I	Tsuji et al., 1987
Exon 10	L444R	c.1448T→G	+*Nci*I	Uchiyama et al., 1994
Exon 10	N462K	c.1503C→G	–*Bsr*BI/+*Hae*II	Hatton et al., 1997
Exon 10	Splice site	c.IVS 10-1 G→A	–*Msp*I	Seri et al., 1995
Intron 10	Splice site	c.IVS 10+2 T→G	–*Age*I/+*Nci*I	Reissner et al., 1998
Exon 11	D474Y	c.1537G→T	–*Bam*HI	Choy et al., 1998
3′UTR	Recombinant (rec G)[a]			Present study

[a]See Figure 1.
ψ Denotes a mutation which is present in the pseudogene sequence.

tients. Southern blots and direct sequencing demonstrated that mutation L444P occurred alone on nine alleles, with E326K on one allele, and as part of a recombinant allele on 15 alleles.

A total of 17 recombinant alleles were identified in our patients, accounting for 27% of the mutant alleles. In two individuals the recombinant allele was a 55 base pair deletion originating from the pseudogene sequence [Tayebi et al., 1996b]. Among the 15 others, there were seven different sites of recombination identified, ranging from intron 3 to the 3′ untranslated region (Fig. 1). Southern blot results indicated that five of the recombinant alleles were fusion alleles in which the sequence located between the gene and the pseudogene and portions of the gene and pseudogene sequence were deleted [Tayebi et al., 1998].

Western blot analyses were performed on protein extracted from 15 fibroblast cell lines, 11 of which were shown in a previous publication [Tayebi et al., 1998]. Under our conditions, bands corresponding to human glucocerebrosidase isoforms were either very weak or absent in samples from patients 2, 3, 5, 7, 17–19, 21, 22, and 30. The 62.5 kDa isoform of the enzyme could be clearly seen in samples from patients 9, 10, 11, 20, and 26.

DISCUSSION

We are now aware of 50 different mutations encountered in patients with type 2 Gaucher disease (Table 3). These mutations occur in almost every exon and in several introns of the glucocerebrosidase gene. Among the 33 different mutations identified in our patients, there were 19 amino acid substitutions, 8 recombinant alleles, 2 nonsense mutations, 2 frameshift deletions, and 2 splice site mutations.

The patients with type 2 Gaucher disease described in this study had variable phenotypes, with life spans ranging from intrauterine death at 22 weeks of gestation to survival to the age of 30 months. Seven patients (cases 1–7) presented prenatally with hydrops fetalis. Recombinant alleles were particularly prevalent in this group, and were identified on nine of the fourteen alleles. Homozygosity for a recombinant allele was always associated with perinatal lethality.

A second group of seven patients (cases 8–14) was diagnosed and died in the newborn period. Several of these babies were noted to have congenital ichthyosis and many required ventilatory assistance. In this group, 11 different mutant alleles were identified.

The remaining 17 infants (cases 15–31) presented after several months of age. Some were noted to have gross skin abnormalities, including ichthyosis, and others were noted to have skin abnormalities by ultrastructural analyses [Sidransky et al., 1996a]. Fourteen different genotypes were identified in this group. Although 10 of these patients were found to have point mutation L444P, none were homozygous for this mutation only. Three carried the L444P point mutation together with a recombinant allele.

Ten of the 31 patients appeared to be homoallelic for the mutant allele identified. In seven of these cases (2–4, 7, 12, 24, and 29), there was known consanguinity. To explore the possibility of an undetected total or partial gene deletion on a second allele, we examined parental DNA in the cases in which it was available. The sequence at known polymorphic sites in the gene was evaluated, since heterozygosity would confirm the presence of two alleles. Lastly, gene dosage was estimated by comparing band intensities of the gene and pseudogene on Southern blots of genomic DNA digested with the enzyme *Ssp*I. However, even using these strategies, the possibility of a large deletion on one allele of cases 5, 6, and 20, could not be eliminated conclusively.

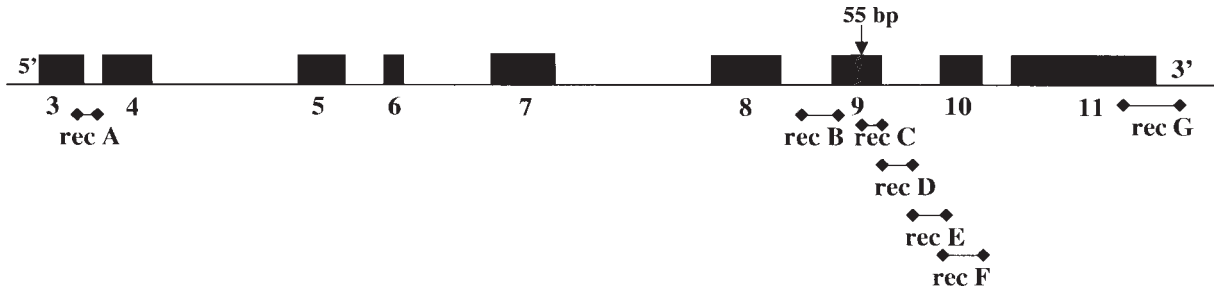

FIGURE 1. **Illustration of seven identified sites of crossover between the glucocerebrosidase gene and its pseudogene, encountered in patients with type 2 Gaucher disease. The approximate sites are as follows: rec A, intron 3; rec B, intron 8 or beginning of exon 9; rec C, exon 9; rec D, end of exon 9 or beginning of intron 9; rec E, intron 9; rec F, end of intron 9 or beginning of exon 10; rec G, 3′ untranslated region.**

Several of the rare mutant alleles identified were encountered in more than one individual with type 2 Gaucher disease (Table 2). Patients 12 and 13, who were first cousins, carried mutation S196P. Mutation IVS2+1 G>A was identified in two unrelated individuals and has been described previously in patients with type 2 Gaucher disease and the genotype L444P/IVS2+1G>A [Ida et al., 1996; Horowitz et al., 1993]. Mutation G202R was encountered in three unrelated probands, and has also been reported twice in type 2 patients homozygous for the mutation [Grace et al., 1997; Zimmer et al., 1999]. The 55bp deletion in exon 9 (c.1263-1317del) was identified in two unrelated probands. The rare mutations R257Q and G325R were found in two patients each in our series.

Although mutation L444P was found frequently among the patients with type 2 Gaucher disease, no patients were homozygous for this point mutation. The genotype L444P/L444P is often encountered in patients with type 3 Gaucher disease, and has also been seen in young Gaucher patients without any evidence of neurologic involvement. In a mouse model of Gaucher disease in which mutations were introduced by a single insertion mutagenesis procedure (SIMP), homozygous L444P mice had a neonatal lethal phenotype [Liu et al., 1998]. It is not clear why humans with two copies of L444P seem to be protected from type 2 Gaucher disease, while those who have L444P and a presumably more severe mutation develop type 2 Gaucher disease. All of the type 2 disease patients carrying the L444P point mutation presented several months after birth, rather than at birth or prenatally.

Recombinant alleles were prevalent in our patient population, and multiple sites and mechanisms of recombination were identified. Seven different sites of recombination were discovered, and all except one occurred between exons 8 and 11 (Fig. 1). Southern blots demonstrated that in five mutant alleles, recombination had occurred as a result of reciprocal recombination between the gene and pseudogene, resulting in a fusion allele [Tayebi et al., 1998]. In others, results of sequencing and Southern blots support gene conversion as the most likely mechanism of recombination (Tayebi et al., unpublished results).

Nineteen of the mutations detected in this patient population resulted in single amino acid substitutions, which did not cluster about any particular region of the gene. Until the three-dimensional structure of the glucocerebrosidase protein is better established, the consequences of these specific amino acid changes cannot be fully appreciated. Of these 19 mutations, 18 resulted in substitutions of amino acids which are conserved between the mouse and human glucocerebrosidase proteins [O'Neill et al., 1989]. The sole exception was mutation A190E, because the murine protein has an arginine at this position.

Most of our patients had heterozygous genotypes, which contributed to the difficulty in interpreting the Western Blot results. However, the three cases (patients 2, 5, and 7) who were homozygous for recombinant alleles had no protein detectable by a polyclonal antibody to human glucocerebrosidase. Patient 3, who is homozygous for a frameshift mutation c.533delC and is the only patient described thus far with no RNA detected by Northern analysis [Tayebi et al., 1997; Tayebi et al., 1998], also had no detectable protein. These results support our previous observation that glucocerebrosidase could not be demonstrated in those patients who died in utero [Tayebi et al., 1998]. Western analyses also showed markedly reduced amounts of the 62.5 kDa isoform of glucocerebrosidase in protein samples from patients 18, 19, and 21, each of whom carried mutation L444P together with a recombinant allele. On the other hand, patient 20, who was homozygous for mutation R131L, clearly showed the 62.5 kDa isoform of the protein.

Several of the mutant alleles identified in our patients have been tested in different in vitro expression systems [Grace et al., 1997; Choy et al., 1996; Ohashi et al., 1991]. However, the published literature describing expression studies of specific mutations is confusing, and it is difficult to compare the activity of mutant proteins among studies [Grace et al., 1997; Pasmanik-Chor et al., 1999]. Also, the correlation between phenotype and the results of residual enzyme determinations and Western analyses are not always straightforward [Pasmanik-Chor et al., 1999; Tayebi et al., 1998; Zimmer et al., 1999]. Other factors, including sphingolipid activator proteins [Morimoto et al., 1990], contiguous genes [Winfield et al., 1997], or the defective intracellular transport of mutant glucocerebrosidase from the endoplasmic reticulum to lysosomes [Zimmer et al., 1999], could contribute to the clinical phenotype encountered.

Thus, making predictions of phenotype from genotype alone continues to be difficult. There is significant genotypic heterogeneity among clinically similar infants, including those with the most severe manifestations. Since many recombinant alleles and unique or rare mutations are encoun-

tered in this patient population, screening PCR amplified DNA for common point mutations is not adequate, and the diagnosis is still best established by enzymatic determinations. Ultrastructural studies of skin may also prove valuable in discriminating young patients with type 2 Gaucher disease from those with milder forms of the disorder [Sidransky et al., 1996a].

Even so, the results of this study permit several generalizations about phenotype based upon genotype. Homozygosity for a recombinant allele appears to result in prenatal lethality due to hydrops fetalis. From our series, it seems that L444P homozygotes do not develop type 2 disease. Therefore, all patients with type 2 Gaucher disease who are identified with the genotype L444P/ L444P should be studied further, using both direct sequencing and Southern blots, to rule out the presence of a recombinant allele. The combination of one allele with point mutation L444P and another with a null or very severe allele seems to be associated with classic type 2 Gaucher disease and survival for several months. We show that homozygosity for five other mutations c.533delC, S196P, R131L, G202R and H311R is also associated with type 2 Gaucher disease. Finally, no patients with type 2 Gaucher disease have been described with the common N370S mutation. An improved understanding of the structure of the glucocerebrosidase protein may result in better predictions of the functional consequences of the mutant alleles encountered and enable better genetic counseling based upon genotype.

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