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Complex Alleles of the Acid β-Glucosidase Gene in Gaucher Disease

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Summary

Gaucher disease is inherited in an autosomal recessive manner and is the most prevalent lysosomal storage disease. Gaucher disease has marked phenotypic variation and molecular heterogeneity, and seven point mutations in the acid β-glucosidase (β-Glc) gene have been identified. By means of sequence-specific oligonucleotides (SSO), mutation 6433C has been detected homozygously in neuronopathic type 2 (acute) and type 3 (subacute) patients, as well as in children with severe visceral involvement who are apparently free of neuronopathic disease. To investigate the molecular basis for this puzzling finding, amplified β-Glc cDNAs from 6433C homozygous type 2 and type 3 Gaucher disease patients were cloned and sequenced. The Swedish type 3 Gaucher disease patient was truly homozygous for alleles only containing the 6433C mutation. In comparison, the type 2 patient contained a singly mutated 6433C allele and a "complex" allele with multiple discrete point mutations (6433C, 6468C, and 6482C). Each of the mutations in the complex allele also was present in the β-Glc pseudogene. SSO hybridization of 6433C homozygotes revealed that both type 2 patients contained additional mutations in one allele, whereas the 6433C alone was detected in both type 3 and in young severe type 1 Gaucher disease patients. These results suggest that the presence of the complex allele influences the severity of neuronopathic disease in 6433C homozygotes and reveal the central role played by the pseudogene in the formation of mutant alleles of the β-Glc gene. Analysis of additional cDNA clones also identified two new alleles in a type 3 patient, emphasizing the molecular heterogeneity of neuronopathic Gaucher disease.

Introduction

Gaucher disease results from inherited defects of the lysosomal hydrolase acid β-glucosidase (β-Glc; E.C.3.2.1.45). The resultant cellular accumulation of this enzyme's major substrate, glucosylceramide, leads to the diverse clinical manifestations of Gaucher disease, including hepatosplenomegaly, hypersplenism, and bony deterioration (Desnick et al. 1982). Three major types of this disease have been delineated, based on the absence (type 1 [nonneuronopathic]) or on presence and severity of neuronopathic manifestations (type 2 [acute neuronopathic] and type 3 [subacute neuronopathic]) (Frederickson and Sloan 1972). These three types are panethnic (Theophilus et al. 1989*a*), but types 1 and 3 have increased frequencies in the Ashkenazi Jewish (Fried 1973) and Swedish (Dreborg et al. 1980) populations, respectively. Although type 2 disease has a predictable, relentlessly progressive neurodegenerative course, type 3 and, particularly, type 1 disease have markedly variable clinical phenotypes even within ethnic groups. Thus, the development of diagnostic tests which correlate with the Gaucher disease types and and progression have important implications for the care of affected families as well as for assessment of the efficacy of therapeutic strategies.

The extensive heterogeneity of the kinetic and processing properties of the residual β-Glc from affected patients within and among the types (Ginns et al. 1982; Grabowski et al. 1985*a*, 1985*b*; Beutler and Kuhl 1986; Fabbro et al. 1987; Bergmann and Grabowski 1989),

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as well as the presence of normal amounts and sizes of β-Glc mRNA in Gaucher disease sources (Graves et al. 1986), suggested that several missense mutations could be causal to the disease. To date, four exonic point mutations have been identified in types 1, 2, and 3 Gaucher disease (fig. 1) by cloning and sequencing of either β-Glc genomic DNA (Tsuji et al. 1987, 1988) or cDNAs (Graves et al. 1988; Wigderson et al. 1989) from affected patients. An additional three missense mutations were identified by Theophilus et al. (1989*b*) during a comparative study of the usefulness of both RNase A and chemical cleavage, as well as of GC-clamped denaturing gel electrophoresis for the detection of single base mutations. From population studies of over 100 Gaucher disease patients (Tsuji et al. 1987, 1988; Theophilus et al. 1989*a*, 1989*b*; Zimran et al. 1989) two mutations, 6433C and 5841G, have been found in high frequency among affected individuals. These mutations predicted Leu^{444}→Pro and Asn^{370}→Ser substitutions, respectively. It is important to note that 5841G in the heteroallelic or homoallelic states was found only in patients with the nonneuronopathic (type 1) variants of Gaucher disease (Tsuji et al. 1988; Theophilus et al. 1989*a*; Zimran et al. 1989) and that milder type 1 phenotypes were associated with the 5841G homoallelic state. In comparison, alleles containing 6433C were found heterozygously in all types of Gaucher disease. It is curious that homoallelism for 6433C was associated with neuronopathic types (types 2 and 3) of Gaucher disease (Tsuji et al. 1987; Theophilus et al. 1989*a*) or with very severe visceral manifestations in young patients apparently free of neuronopathic involvement (Theophilus et al. 1989*a*). These observations indicated either a lack of correlation of the neuronopathic severity with the genotype at the β-Glc locus or that some alleles contained additional mutations which were not apparent by restriction endonuclease (i.e., *Nci*I) (Tsuji et al. 1987) or by sequence-specific oligonucleotide (SSO) probing of amplified genomic DNA (Theophilus et al. 1989*a*).

In the present paper we report the cloning and sequencing of cDNAs encoding the complete β-Glc poly-

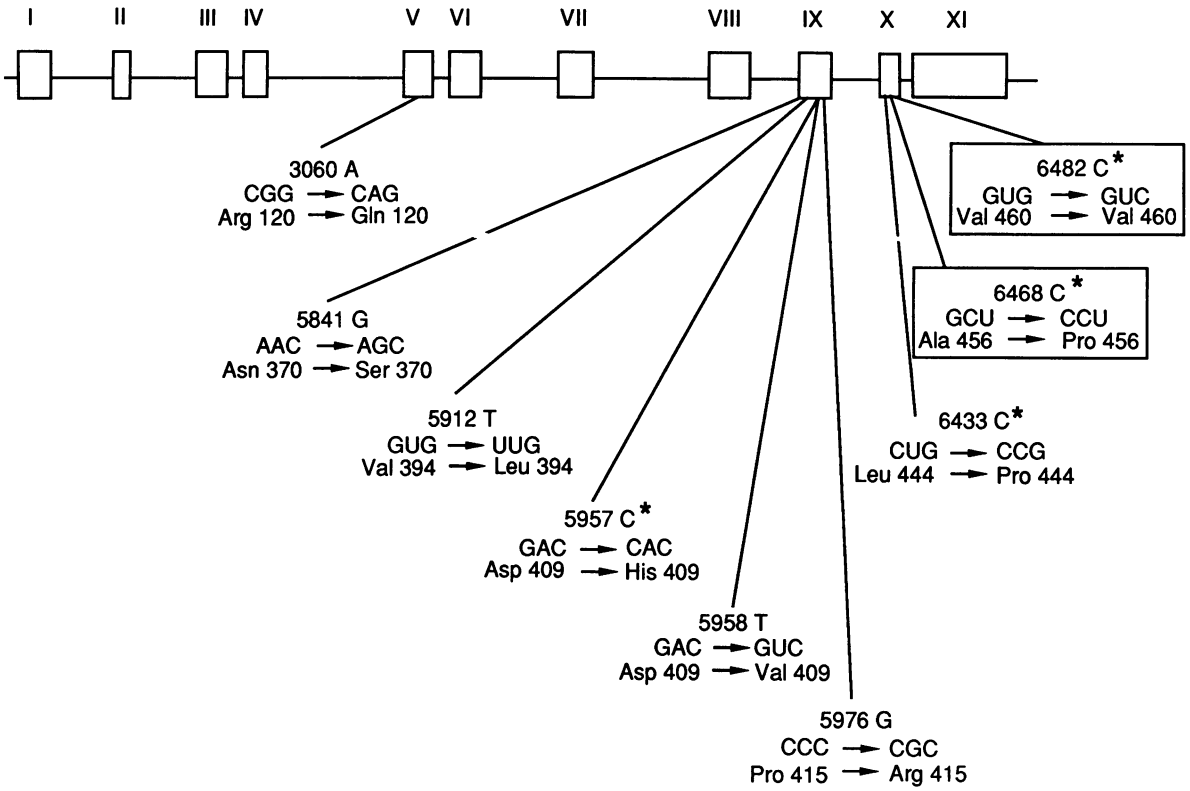

Figure 1 Schematic diagram depicting positions of the nine point mutations identified in the β-Glc genes of Gaucher disease patients. Exons are represented as open boxes and are numbered with roman numerals. Mutations marked with an asterisk are also present in the pseudogene (Horowitz et al. 1989). Mutations characterized in the present report are boxed.

peptide sequence from each allele of selected Gaucher disease patients. These studies (1) show that some alleles bearing the 6433C mutation indeed do contain additional base substitutions and (2) suggest that the presence of such "complex" alleles may influence the severity of neuronopathic involvement in Gaucher disease. Analysis of additional cDNA clones also identified two new alleles in a type 3 patient, emphasizing the molecular heterogeneity of neuronopathic Gaucher disease.

Patients and Methods

Patient Description

Fibroblast cultures GM877 and GM878 were obtained from the NIGMS Human Genetic Mutant Cell Repository (Camden, NJ). All patient samples were obtained by informed consent or assent of minors, according to institutional and NIH guidelines. The Swedish type 3 patients had visceral involvement and seizures and/or mental retardation at ages 25 (MSM80) and 10 (MSM79) years. The Ashkenazi Jewish/Irish type 3 patient developed psychomotor retardation and myoclonic seizures by 5 years and died at 6 years. Genomic DNA from normal individuals and from Gaucher disease patients was extracted from fibroblast or lymphoblastoid cell lines and was characterized according to the presence or absence of β-Glc mutations by using SSO according to a method described elsewhere (Theophilus et al. 1989*a*, 1989*b*). All patients had deficient β-Glc activity (4%–12% of normal) in cultured skin fibroblasts and/or in peripheral blood leukocytes (Grabowski et al. 1985*b*).

Mutation Notation

Numbering of nucleotides throughout the present paper is according to the β-Glc genomic sequence of Horowitz et al. (1989) (EMBL/Genbank Data Libraries accession no. J03059). Mutations were designated according to the altered genomic nucleotide; for example, 3060A refers to the substitution of an A at genomic nucleotide 3060 (fig. 1).

Preparation of Probes and Primers

Oligonucleotides were synthesized on an Applied Biosystems 380B DNA synthesizer. Probes were labeled with ^{32}[P]-ATP by using polynucleotide kinase (Maniatis et al. 1982), and unincorporated nucleotides were removed by centrifugation through Quick Spin G-25 Sephadex columns (Boehringer Mannheim Biochemicals, Indianapolis).

Polymerase Chain Reaction (PCR) Amplification of β-Glc-specific cDNA

RNA was isolated (Wilkinson 1988) from the characterized cell lines described above, and poly(A)$^+$ RNA was selected on oligo(dT) cellulose columns according to manufacturer's instructions (5 Prime–3 Prime, Westchester, PA). cDNAs were made from approximately 2 μg poly(A)$^+$ RNA by reverse transcription using oligo-dT as primer (cDNA synthesis kit; Bethesda Research Laboratories/Life Technologies, Gaithersburg, MD). One-tenth of the cDNA product was then added to a solution containing 67 mM Tris (pH 8.8), 16.6 mM ammonium sulfate, 1.5 mM magnesium chloride, 10 mM β-mercaptoethanol, 170 μg BSA/ml, 10% (v/v) dimethylsulfoxide, and 33 μM each of dATP, dTTP, dCTP, and dGTP, as well as 5 pmol each of oligonucleotide primers specific for 5′ and 3′ untranslated regions of the β-Glc cDNA sequence; ECOR546, a 32-mer containing and *Eco*RI site and 24 bases corresponding to β-Glc bases 546–568, and ECOR6764, a 31-mer containing an *Eco*RI site and 23 bases complementary to β-Glc bases 6742–6764. The cDNA then was denatured by incubating the above mixture at 94°C for 7 min, 2.5 units of *Thermus aquaticus* polymerase (*Taq;* Perkin Elmer–Cetus, Norwalk, CT) were added, and the mixture was overlaid with mineral oil (Sigma, St. Louis). PCR amplification (Saiki et al. 1985) was conducted with a DNA thermal cycler (Perkin Elmer–Cetus). Each amplification cycle was 30 s at 94°C followed by 5 min at 66°C. After 15 cycles, the reaction was interrupted to allow the addition of 2.5 units of *Taq* polymerase and 50 pmol each of a second set of β-Glc primers that were located just inside the first primer set: i.e., ECOR557, a 31-mer containing an *Eco*RI site and 26 bases corresponding to β-Glc bases 557–582, and ECOR6722, a 28-mer containing an *Eco*RI site and 20 bases complementary to β-Glc bases 6703–6722. An additional 30 cycles of amplification then were conducted. The products were extracted with an equal volume of phenol:chloroform (50:50 [v/v]), ethanol precipitated, resuspended, and digested with the restriction enzyme *Eco*RI (New England Biolabs, Beverly, MA). Analysis on an ethidium bromide–stained 1% agarose gel revealed a band of the expected size (approximately 1.7 kb), which was cut out and purified using a Gene-Clean kit (Bio 101, La Jolla).

Cloning and Sequencing of β-Glc cDNA

The gel-purified PCR products were ligated into lambda ZAPII that had been digested with *Eco*RI, packaged (GigapackII Gold packaging extract), and plated

out on the recA–*Escherichia coli* host strain XL1-blue according to the manufacturer's instructions (Stratagene, La Jolla). Plaques were screened for the presence of all suspected mutations by SSO hybridization (Theophilus et al. 1989*a*, 1989*b*). At least two plaques of each expected genotype were plaque purified, and the plasmids containing the inserts were excised from the lambda ZAPII vector according to a method described by the manufacturer (Stratagene). Each plasmid DNA was then amplified and purified according to the method of Birnboim and Doly (1979). The cDNA inserts were sequenced using the dideoxy chain-termination method, adapted for dsDNA by Chen and Seeburg (1985), by using Sequenase™ kits (United States Biochemical, Cleveland) and oligonucleotide primers according to a method described elsewhere (Graves et al. 1988). Each cDNA encoded both the complete mature β-Glc and the leader-peptide amino acid sequences.

PCR Amplification of β-Glc Genomic DNA and Dot Blot Analyses

Selective PCR amplification of structural gene-derived sequences and dot blot analyses were performed according to a method described elsewhere by Theophilus et al. (1989*a*). The SSOs and washing conditions used to detect the mutations at positions 3060, 5841, 5976, and 6433 have been described by Theophilus et al. (1989*a*), and those for mutations at positions 5912, 5957, and 5958 have been described by Theophilus et al. (1989*b*). The SSOs used to detect the mutation at position 6468 were 5′-TGGCTCTGCTGTTGT-3′ (normal) and 5′-TGGCTCTCCTGTTGT-3′ (mutant); washing temperature was 44°C in 4 × SSC. The SSOs used to detect the mutation at position 6482 were 5′-TGGTCGTGCTAAACC-3′ (normal) and 5′-TGGTCGTCCTAAACC-3′ (mutant); washing temperature was 45°C in 4 × SSC.

Results

Identification of the Norrbottnian Type 3 Mutation and Complex Alleles

Table 1 summarizes the results of genotype analyses by SSO screening of PCR-amplified genomic DNA of the nine Gaucher disease patients relevant to the present study. The genotype analyses by SSO for mutations 3060A, 5958T, and 5976G are not shown, since the corresponding normal sequences were detected in all listed patients. Two Swedish type 3 (MSM79 and MSM80), two type 2 (GM877 and MSM78), and two young patients tentatively classified as type 1 (MSM45 and MSM51) were found to be homozygous for alleles

Table 1

SSO Analyses of Gaucher Disease Patients

	Genotype[a]						
	5841	5912	5957[b]	6433[b]	6468[b]	6482[b]	Ethnicity
Type 3:							
MSM79[c]				−/−			Swedish
MSM8O				−/−			Swedish
MSM74[c]		+/−	+/−	+/−	+/−	+/−	Ashkenazi Jewish/Irish
Type 2:							
GM877[c]				−/−	+/−	+/−	Caucasian
MSM78				−/−	+/−	+/−	German/English
Type 1:							
MSM45[d]				−/−			Hispanic
MSM51[d]				−/−			Italian
MSM43	+/−			+/−	+/−	+/−	Italian/Irish
MSM52[c]	+/−	+/−					Ashkenazi Jewish

[a] All patients were screened for the presence of all known mutations; when only the normal sequence was detected the column is blank; +/− = heterozygous for a mutation at the indicated position; −/− = homozygous for a mutation at the indicated position.

[b] The mutant sequence at this position is present in the β-Glc pseudogene (Horowitz et al. 1989).

[c] cDNAs representing both mutant β-Glc alleles from these patients were sequenced.

[d] The classification of these young patients with severe visceral manifestations as type 1 is tentative, owing to their age (5 years) and to the possibility that neuronopathic manifestations could develop (Theophilus et al. 1989*b*).

containing the 6433C mutation. cDNAs from one Swedish type 3 (MSM79) and from one type 2 (GM877) patient were amplified by PCR, cloned, and sequenced. A single consistent missense mutation, 6433C, was found in all four cDNA clones analyzed from MSM79.

Sequencing of several cDNA clones from the type 2 patient, GM877, revealed two populations of alleles. One population contained only the 6433C mutation, while the other population contained the 6433C mutation and two additional base substitutions, 6468C and 6482C. The 6468C mutation encoded a missense substitution, Ala^{456}→Pro, whereas the 6482C was a conservative mutation (fig. 1). Figure 2 shows examples of these sequences, compared with that for the normal cDNA. To demonstrate the authenticity of these point mutations, SSO probing of PCR-amplified genomic DNA from both the patient (GM877) and his mother (GM878) was conducted with individual oligonucleotides representing each substitution. As shown in figure 3, GM878 carried the allele containing the three base substitutions 6433C, 6468C, and 6482C.

SSO screening was conducted with amplified genomic DNA from 46 unrelated Gaucher disease patients (most of whom have been described by Theophilus et al. 1989*a*) to determine the frequency of the two new mutations in the structural gene for β-Glc. A summary of the nine relevant patient genotypes is shown in table 1; the other 37 patients had the genotypes as described by Theophilus et al. (1989*a*, 1989*b*). Of the six Gaucher disease patients who were homozygous for the 6433C mutation, the additional 6468C and 6482C mutations were found only in the two type 2 patients (GM877 and MSM78). SSO probing of PCR-amplified genomic DNA from MSM78 and her family revealed that the mother carried the allele containing the three base substitutions 6433C, 6468C, and 6482C, whereas the father carried the singly mutated, 6433C, allele (fig. 3). From the population study, alleles containing the two new mutations, 6468C and 6482C, also were found heterozygously in one type 3 patient who was of Ashkenazi Jewish/Irish extraction and in one type 1 patient who was of Italian/Irish background. In all instances segregation of the mutations 6468C and 6482C were concordant with the 6433C mutation. Furthermore, no patient was found to be homozygous for a complex allele.

Characterization of Another Complex β-Glc Allele and an "Ashkenazi" Mutation

As shown in table 1, the structural genes from the Ashkenazi Jewish/Irish type 3 patient (MSM74) were found to have five base substitutions by SSO hybridiza-

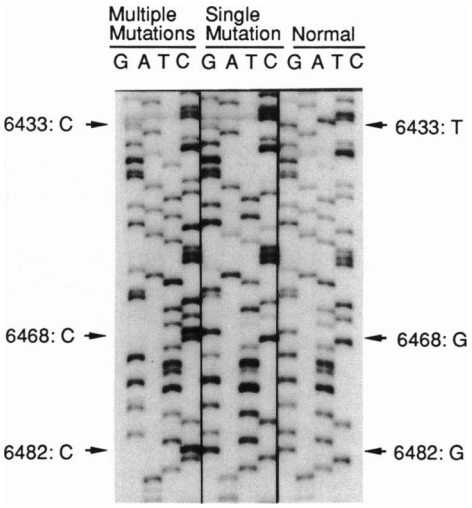

Figure 2 Sequencing of PCR-amplified β-Glc cDNA clones from GM877. The relevant portions of the sequencing gels are shown for the multiply mutated and for the singly mutated alleles, as well as for a normal control. The nucleotides indicated on the left or right refer to the mutant or normal genomic base, respectively.

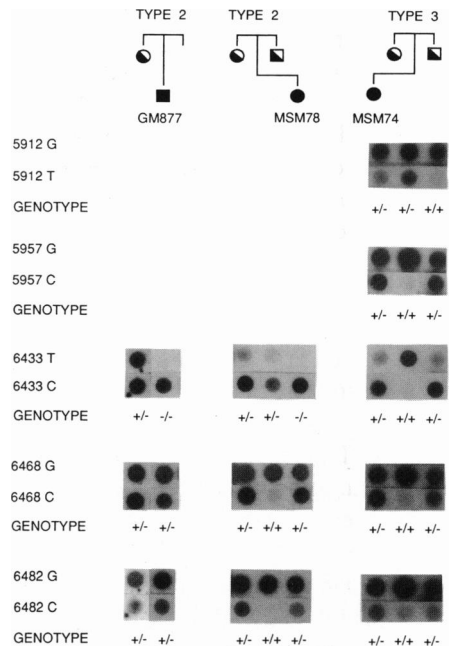

Figure 3 SSO hybridization to PCR-amplified genomic DNA from Gaucher disease families who have complex alleles. In the left column the upper and lower genomic base designations refer to the normal or mutant sequence, respectively. The genotype designation refers to the presence of the normal (+) or mutant (-) sequence. For patient MSM74, the mother and father were of Ashkenazi Jewish and Irish extraction, respectively.

tion. To assign these base substitutions to alleles, cDNAs from this patient's fibroblast mRNA were amplified by PCR, cloned, and sequenced. No additional authentic mutations were found in several complete cDNA sequences. Four mutations—5957C, 6433C, 6468C, and 6482C—were present on one allele, and the other allele contained only the 5912T mutation. The 5957C and 5912T missense mutations predicted Asp^{409}→His and Val^{394}→Leu substitutions in β-Glc, respectively. SSO analyses of amplified genomic DNA from MSM74's parents demonstrated that this complex allele was inherited from the Irish father and that the allele containing the missense mutation 5912T was from the Ashkenazi Jewish mother (fig. 3).

Previously, Theophilus et al. (1989*b*) had detected the 5912T mutation in exon 9 of the structural β-Glc gene from an Ashkenazi Jewish Gaucher disease type 1 patient (MSM52) by screening PCR-amplified exons for point mutations. To determine whether this allele was identical to that in the type 3 patient described above, β-Glc cDNA from this type 1 patient was amplified by PCR, cloned, and sequenced. Two populations of alleles were obtained: one contained only a common Ashkenazi Jewish Gaucher disease mutation, 5841G, and the other singly substituted allele was identical to that from the Ashkenazi Jewish/Irish type 3 patient (MSM74) and contained only the 5912T mutation. It should be noted that all the above-mentioned mutations were detected both by sequencing multiple cDNA clones and by SSO analysis of PCR-amplified genomic DNA, to effectively exclude the possibility that the mutations were PCR or cloning artifacts.

Discussion

Elucidation of the relationships between the genetic defects in β-Glc and the phenotypes in the Gaucher disease variants has major clinical and basic importance. Recent studies (Tsuji et al. 1987, 1988; Graves et al. 1988; Theophilus et al. 1989*b*; Wigderson et al. 1989) have identified seven missense mutations in the structural β-Glc genes from Gaucher disease patients, indicating the extensive molecular heterogeneity in this disease. It is important to note that the presence of at least one allele having the 5841G mutation was associated with the absence of neuronopathic involvement (Tsuji et al. 1988; Theophilus et al. 1989*a*; Zimran et al. 1989). However, the apparent homozygosity for the 6433C mutation in both acute (type 2) and subacute (type 3) neuronopathic variants of Gaucher disease, as well as in young patients with severe visceral manifestations, was puzzling. Because of the rarity of neuronopathic Gaucher disease, the number of patients analyzed was small. However, the results of the current studies demonstrate that the presence of an additional missense mutation on at least one of the alleles bearing the 6433C mutation was correlated with the most severe Gaucher disease variant, type 2 acute neuronopathic disease. True homoallelism for the 6433C mutation appears to be associated either with a less severe variant, type 3 subacute neuronopathic disease, or with rapidly progressive, massive, visceral, and, in both cases, lethal manifestations of Gaucher disease in children with apparent absence of neuronopathic involvement. This finding has major implications for the diagnosis and management of patients and families at risk for severe variants of Gaucher disease.

The two type 3 Gaucher disease patients who were homozygous for the 6433C mutation (table 1) were of lineage derived from the northern (Norrbottnian) region of Sweden. Previously, Dahl et al. (1988) found an *MspI* polymorphism which was tightly linked to the Norrbottnian type 3 allele. Indeed, all 11 patients with this variant of Gaucher disease who were analyzed were homozygous for this polymorphism. The 6433C mutation results in an *MspI* polymorphism, consistent with the results of Dahl et al. (1988; data not shown), and was the only alteration present in the cDNAs of the Swedish type 3 patient sequenced in the present study. Although other mutations within the coding region of the β-Glc gene may be present in the Norrbottnian population, genealogical studies indicate that all studied patients in this region derive from one (Svennerholm et al. 1982) or at most two (Iselius et al. 1989) families. Consequently, homozygosity for the 6433C mutation will likely account for a majority of affected Norrbottnian type 3 patients, and therefore a simple mutation-specific test for detection of carriers could be implemented.

Sequencing of both alleles from the Ashkenazi Jewish/Irish patient with Gaucher disease type 3 demonstrates the molecular diversity in this phenotype. One allele from this patient contained a Val^{394}→Leu substitution (mutation 5912T) which also was present as the only mutation on an allele from an Ashkenazi Jewish type 1 patient. The other allele in the heterozygous type 3 patient contained three missense mutations, 5957C in exon 9 and 6433C and 6468C in exon 10, as well as a conservative base substitution, 6482C, in exon 10 (table 1). The complex allele from the heterozygous type 3 patient encodes a β-Glc with three amino acid substitutions, Asp^{409}→His, Leu^{444}→Pro, and

Ala^{456}→Pro. This is a critical region of β-Glc, since it contains the nucleophile for catalysis, Asp^{443} (Dinur et al. 1986). Furthermore, the presence of Leu^{444}→Pro (6433C) mutation alone leads to the expression of a highly unstable, but catalytically active, β-Glc present in fibroblasts (Bergmann and Grabowski 1989) or expressed in insect cells from the specifically mutagenized cDNA (Grace et al. 1989). Although expression studies of complex alleles will be necessary, it is very likely that an allele containing additional "pseudogene" mutations would express a severely compromised protein. The fact that the singly substituted allele bearing the apparently undramatic Val^{394}→Leu mutation (5912T) was insufficient to prevent the development of neuronopathic manifestations provides further evidence for the physiological importance of this region of the protein. Furthermore, the lack of neuronopathic disease in the Ashkenazi Jewish type 1 patient (46 years old), whose genotype was 5841G/5912T, provides further support for the "protective" effect of the 5841G allele in Gaucher disease patients (Theophilus et al. 1989*a*).

It is interesting that the identical base substitutions in the complex alleles so far described contain base substitutions that also occur in the corresponding highly homologous exons of the β-Glc pseudogene, which is tightly linked to the structural gene on chromosome 1 (Horowitz et al. 1989). Previously, Tsuji et al. (1987) found a single pseudogene mutation, 6433C, in Gaucher disease patients. Theophilus et al. (1989*b*) also demonstrated the presence of a different single pseudogene exon 9 mutation, 5957C, in a β-Glc structural gene allele from a Gaucher disease type 1 patient of Greek descent and also identified a patient of racially mixed South African descent who had both the 6433C and 5957C pseudogene mutations in his β-Glc structural genes. Recently, Zimran et al. (1990) have isolated a β-Glc cDNA that contained the 6433C, 6468C, and 6482C pseudogene mutations. In the present paper, as well as identifying a similar allele to that described by Zimran et al. (1990), we have identified an allele that carries four pseudogene mutations: 5957C, 6433C, 6468C, and 6482C. Thus, pseudogene mutations in the exon 9 and exon 10 regions of the β-Glc structural gene can occur singly or in a variety of combinations of missense mutations resulting in various complex alleles which are causal for Gaucher disease. The ethnic diversity of the patients having such pseudogene missense mutations in their structural β-Glc genes indicates that multiple independent genetic events, such as recombination or gene conversion, are important to the etiology of Gaucher disease; for example, Zimran et al. (1990) showed that unequal crossover between the functional β-Glc gene and the pseudogene was probably the molecular event responsible for the creation of the allele they described.

From these and previous studies, the molecular etiologies of Gaucher disease are emerging as an extremely heterogeneous group of singly and multiply (complex) substituted β-Glc alleles, many of which remain to be characterized. However, it is becoming apparent that the various combinations of "severe" and "mild" alleles at the β-Glc locus affect the clinical expression of the disease and that the pseudogene plays an important role in the genesis of Gaucher disease. Continued delineation of the genotype/phenotype relationships in the Gaucher disease types will have importance in the management of this inborn error of metabolism.

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References

Bergmann JE, Grabowski GA (1989) Posttranslational processing of human lysosomal acid β-glucosidase: a continuum of defects in Gaucher disease type 1 and type 2 fibroblasts. Am J Hum Genet 44:741–750

Beutler E, Kuhl W (1986) Glucocerebrosidase processing in normal fibroblasts and in fibroblasts from patients with type I, type II, and type III Gaucher disease. Proc Natl Acad Sci 83:7472–7474

Birnboim HC, Doly J (1979) A rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nucleic Acids Res 7:1513–1523

Chen EY, Seeburg PH (1985) Supercoil sequencing: a fast and simple method for sequencing plasmid DNA. DNA 4:165–170

Dahl N, Erikson A, Hammarstrom-Heeroma K, Pettersson U (1988) Tight linkage between Type III Gaucher's disease (Norrbottnian Type) and a *Msp*I polymorphism within the gene for human glucocerebrosidase. Genomics 3:296–298

Desnick RJ, Gatt S, Grabowski GA (eds) (1982) Gaucher disease: a century of delineation and research. Alan R Liss, New York

Dinur T, Osiecki KM, Legler GL, Gatt S, Desnick RJ, Grabowski GA (1986) Human acid β-glucosidase: isolation and

amino acid sequence of a peptide containing the catalytic site. Proc Natl Acad Sci USA 83:1660–1664

Dreborg S, Erikson A, Hagberg B (1980) Gaucher disease—Norrbottnian Type. Eur J Pediatr 133:107–118

Fabbro D, Desnick RJ, Grabowski GA (1987) Gaucher disease: genetic heterogeneity within and among the subtypes detected by immunoblotting. Am J Med Genet 40:15–31

Frederickson DS, Sloan HR (1972) Glucosyl ceramide lipidoses: Gaucher's disease. In: Stanbury JB, Wyngaarden JB, Frederickson DS (eds) The metabolic basis of inherited disease, 3d ed. McGraw-Hill, New York, pp 730–759

Fried K (1973) Population study of chronic Gaucher disease. Isr J Med Sci 9:1396–1398

Ginns EI, Brady RO, Pirrucello S, Moore C, Sorrell S, Furbish FS, Murray GJ, et al (1982) Mutations of glucocerebrosidase: discrimination of neurologic and non-neurologic phenotypes of Gaucher's disease. Proc Natl Acad Sci USA 79:5607–5610

Grabowski GA, Dinur T, Osicki KM, Kruse JR, Legler G, Gatt S (1985*a*) Gaucher disease Types 1, 2, and 3: differential mutations of the acid β-glucosidase active site identified with conduritol B epoxide derivatives and sphingosine. Am J Hum Genet 37:499–510

Grabowski GA, Goldblatt J, Dinur T, Kruse J, Svennerholm L, Gatt S, Desnick RJ (1985*b*) Genetic heterogeneity in Gaucher disease: physicokinetic and immunologic studies of the residual enzyme in cultured fibroblasts from non-neuronopathic and neuronopathic patients. Am J Med Genet 21:529–549

Grace ME, Goldberg L, Berg A, Grabowski GA (1989) Gaucher disease: dissection of the enzymatic pathology by site-directed mutagenesis and expression of acid β-glucosidase cDNAs. Am J Hum Genet 45:A190

Graves PN, Grabowski GA, Eisner R, Palese P, Smith FI (1988) Gaucher disease Type I: cloning and characterization of a cDNA encoding acid β-glucosidase from an Ashkenazi Jewish patient. DNA 7:521–528

Graves PN, Grabowski GA, Ludman MD, Palese P, Smith FI (1986) Human acid β-glucosidase: Northern blot and S1 nuclease analysis of mRNA from HeLa cells and normal and Gaucher disease fibroblasts. Am J Hum Genet 39:763–774

Horowitz M, Wilder S, Horowitz Z, Reiner O, Gelbart T, Beutler E (1989) The human glucocerebrosidase gene and pseudogene: structure and evolution. Genomics 4:87–96

Iselius L, Hillborg PO, Lindsten J (1989) The distribution of the gene for the juvenile type of Gaucher disease in Sweden. Acta Paediatr Scand 78:592–596

Maniatis T, Fritsch EF, Sambrook J (1982) Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY

Saiki RK, Sharf S, Faloona F, Mullis KB, Horn G, Ehrlich HA, Arnheim N (1985) Enzymatic amplification of β-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. Science 230:1350–1355

Svennerholm L, Dreborg S, Erikson A, Groth CG, Hillborg PO, Hakansson G, Nilsson O, et al (1982) Gaucher disease of the Norrbottnian Type (Type 3): phenotypic manifestations. In: Desnick RJ, Gatt S, Grabowski GA (eds) Gaucher disease: a century of delineation and research. Alan R Liss, New York, pp 67–94

Theophilus BDM, Latham T, Grabowski GA, Smith FI (1989*a*) Comparison of RNase A, a chemical cleavage and GC-clamped denaturing gradient gel electrophoresis for the detection of mutations in exon 9 of the human acid β-glucosidase gene. Nucleic Acids Res 17:7707–7722

——— (1989*b*) Gaucher disease: molecular heterogeneity and phenotype-genotype correlations. Am J Hum Genet 45: 212–225

Tsuji S, Choudary PV, Martin BM, Major JA, Barranger JA, Ginns EI (1987) A mutation in the human glucocerebrosidase gene in neuronopathic Gaucher's disease. N Engl J Med 316:570–575

Tsuji S, Martin BM, Barranger JA, Stubblefield BK, Lamarca ME, Ginns EI (1988) Genetic heterogeneity in type 1 Gaucher disease: multiple genotypes in Askenazic and non-Askenazic individuals. Proc Natl Acad Sci USA 85:2349–2352

Wigderson M, Firon N, Horowitz Z, Wilder S, Frishberg Y, Reiner O, Horowitz M (1989) Characterization of mutations in Gaucher disease patients by cDNA cloning. Am J Hum Genet 44:365–377

Wilkinson M (1988) RNA isolation: a miniprep method. Nucleic Acids Res 16:10933

Zimran A, Sorge J, Gross E, Kubitz M, West C, Beutler E (1989) Prediction of severity of Gaucher's disease by identification of mutations at DNA level. Lancet 2:349–352

——— (1990) A glucocerebrosidase fusion gene in Gaucher disease: implications for the molecular anatomy, pathogenesis, and diagnosis of this disorder. J Clin Invest 83: 219–222