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Gaucher Disease: A $G^{+1} \rightarrow A^{+1}$ IVS2 Splice Donor Site Mutation Causing Exon 2 Skipping in the Acid β-Glucosidase mRNA

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Summary

Gaucher disease is the most frequent lysosomal storage disease and the most prevalent Jewish genetic disease. About 30 identified missense mutations are causal to the defective activity of acid β-glucosidase in this disease. cDNAs were characterized from a moderately affected 9-year-old Ashkenazi Jewish Gaucher disease type 1 patient whose 80-year-old, enzyme-deficient, 1226G ($Asn^{370} \rightarrow Ser$ [N370S]) homozygous grandfather was nearly asymptomatic. Sequence analyses revealed four populations of cDNAs with either the 1226G mutation, an exact exon 2 (Δ EX2) deletion, a deletion of exon 2 and the first 115 bp of exon 3 (Δ EX2-3), or a completely normal sequence. About 50% of the cDNAs were the Δ EX2, the Δ EX2-3, and the normal cDNAs, in a ratio of 6:3:1. Specific amplification and characterization of exon 2 and 5′ and 3′ intronic flanking sequences from the structural gene demonstrated clones with either the normal sequence or with a $G^{+1} \rightarrow A^{+1}$ transition at the exon 2/intron 2 boundary. This mutation destroyed the splice donor consensus site (U1 binding site) for mRNA processing. This transition also was present at the corresponding exon/intron boundary of the highly homologous pseudogene. This new mutation, termed "IVS2 $G^{+1} \rightarrow A^{+1}$," is the first splicing mutation described in Gaucher disease and accounted for about 3.4% of the Gaucher disease alleles in the Ashkenazi Jewish population. The occurrence of this "pseudogene"-type mutation in the structural gene indicates the role of acid β-glucosidase pseudogene and structural gene rearrangements in the pathogenesis of this disease.

Introduction

Gaucher disease is the most frequent lysosomal storage disease and the most prevalent Jewish genetic disease (Frederickson and Sloan 1978; Desnick et al. 1982; Zimran et al. 1991). This autosomal recessive disease results from the defective activity of the enzyme acid β-glucosidase and the resultant accumulation of glucosyl ceramide primarily within cells of monocyte/macrophage lineage (Frederickson and Sloan 1978). Three major clinical types of Gaucher disease have been delineated on the basis of presence or absence of primary involvement of the central nervous system (Frederickson and Sloan 1978; Desnick et al. 1982). Gaucher disease types 2 and 3 have been termed "acute neuronopathic" and "subacute neuronopathic," respectively, and lead to mental retardation and visceral manifestations at differing rates (Dreborg et al. 1980; Desnick et al. 1982). Type 1 Gaucher disease is characterized by the lack of primary central nervous system disease and marked variability of the visceral signs, including hepatosplenomegaly, hypersplenism, and bony destruction. Although all types of Gaucher disease are panethnic (Frederickson and Sloan 1978; Desnick et al. 1982; Zimran et al. 1991), types 1 and 3 have clear ethnic predilection. Type 3 Gaucher disease has its highest frequency in the Swedish population which descended from founders in the Norrbottnian region. Type 1 Gaucher disease has its highest frequency ($q \sim .047$) in individuals of Ashkenazi Jewish extraction (Zimran et al. 1991).

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The acid β-glucosidase structural gene has been localized to chromosome 1 (Devine et al. 1982; Barneveld et al. 1983), and numerous point mutations (about 30) have been described in cDNAs derived from Gaucher disease patients (Tsuji et al. 1987, 1988; Latham et al. 1990, 1991; Zimran et al. 1990; for review, see Grabowski et al. 1990). The causality of many of these missense mutations has been demonstrated by the similar properties of the natural residual enzyme and that obtained after expression of mutagenized cDNAs in heterologous and autologous systems (Grace et al. 1990, 1991; Ohashi et al. 1991). The mutations encoding Asn^{370}→Ser (N370S [i.e., 1226G]) and Leu^{444}→Pro (L444P [i.e., 1448C]) substitutions account for about 50% and 10%, respectively, of alleles in Gaucher disease patients (Tsuji et al. 1987, 1988; Zimran et al. 1990). The 1226G mutation has been invariably associated, even in the heteroallelic state, with the nonneuronopathic (type 1) phenotype (Tsuji et al. 1988; Theophilus et al. 1989; Latham et al. 1991). In comparison, the 1448C mutation has been found in all types of Gaucher disease. The Norrbottnian and other type 3, subacute neuronopathic variants, result, in many cases, from homozygosity for the 1448C allele (Theophilus et al. 1989; Latham et al. 1991). Several alleles had multiple authentic point mutations (Hong et al. 1990; Zimran et al. 1990; Latham et al. 1991) which were also present in the corresponding exons of the highly homologous acid β-glucosidase pseudogene (Grabowski et al. 1990). These findings suggested that gene conversion or recombination between these two tightly linked loci could play an important role in the pathogenesis of Gaucher disease (Grabowski et al. 1990; Zimran et al. 1990). Indeed, a fusion of the pseudogene and the structural gene has been demonstrated in one family (Zimran et al. 1990).

In the present communication, we demonstrate the first splice-junction mutation which is causal to Gaucher disease. This relatively common point mutation results in the aberrant splicing of exon 2, with the production transcripts which are functionally null. This G^{+1}→A^{+1} substitution was also present at the corresponding exon 2/intron 2 boundary of the acid β-glucosidase pseudogene.

Material, Patients, and Methods

Material

The following were from commercial sources: restriction endonucleases, polynucleotide kinase, and *Taq* polymerase (ProMega, Madison, WI); radiolabeled nucleotides (Amersham, Arlington Heights, IL); cDNA synthesis kit (BRL, Gaithersburg, MD); oligo(dT)-cellulose type 3 (Collaborative Research, Palo Alto, CA); Zetaprobe nylon filters (BioRad, Richmond, CA); X-ray film (Eastman Kodak, Rochester); TA cloning Kit (Invitrogen, San Diego); Sequenase (US Biochemicals, Cleveland); 4-methylumbel-liferyl-β-D-glucopyranoside (4MU-Glc; Genzyme, Cambridge, MA); 12[N-methyl-N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)]dodecanoic acid (C_{12}-NBD) (Molecular Probes, Junction City, OR); and sodium taurocholate (Calbiochem, San Diego). All other reagents were of the highest grade available. Oligonucleotides were synthesized (Applied Biosystems 380B DNA synthesizer) to contain the desired nucleotide sequences. N3 is a genomic clone containing the entire acid β-glucosidase structural gene locus, as well as 5′ and 3′ flanking regions (Theophilus et al. 1989).

C_{12}-NBD-glucosylceramide (NBD-GC) was prepared and purified according to a method described elsewhere (Grabowski et al. 1985). Natural glucosylceramide was purified from Gaucher disease splenic extracts, and glucosylsphingosine was prepared according to a method described elsewhere (Grabowski et al. 1985). Peripheral blood lymphocytes or cultured skin fibroblasts and long-term lymphoid lines were isolated or established, respectively, by routine methods, after informed consent was obtained according to institutional and NIH guidelines.

Patients

The pedigree of family L is shown in figure 1. The propositus, III-1, was a 9-year-old female with hepatosplenomegaly, anemia, and thrombocytopenia, all of which had been present since age 4 years. Extensive evaluation revealed growth retardation, massive splenomegaly, and bone marrow Gaucher cells but no significant bony disease. She is currently receiving Ceredase™ enzyme augmentation for her disease manifestations. Her younger brother (III-1) was 5 years of age and was diagnosed, at age 2 years, with moderate splenomegaly and anemia. Her paternal grandfather (I-1) is 80 years old and, during an evaluation for a platelet count of about 100,000/mm^3 at age 71 years, was discovered to have Gaucher cells in a bone marrow aspirate. Computed tomography showed his spleen to be slightly enlarged. He has no other symptoms. His brother (I-3) and sister (I-2) both had symptomatic Gaucher disease with moderate hepatosplenomegaly and hypersplenism. At age 65 years,

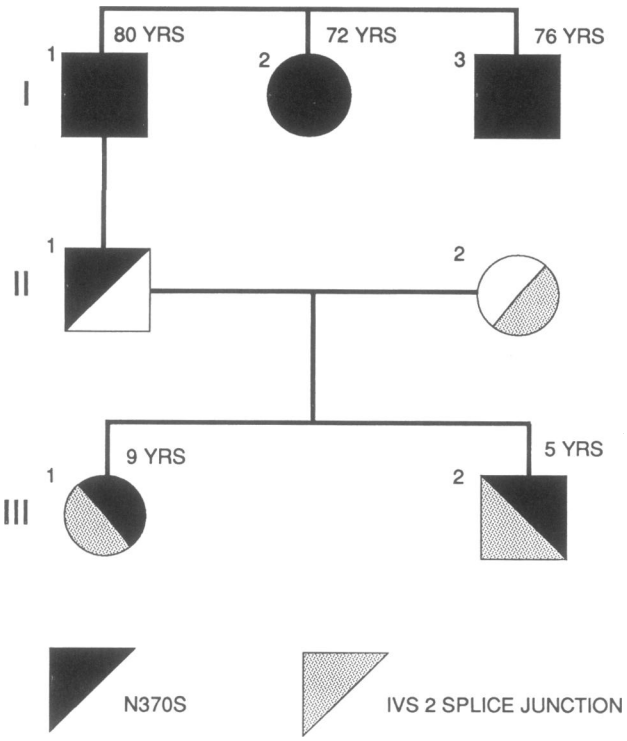

Figure 1 Pedigree of family L. Genotypes were determined by using SSO hybridization to PCR-amplified genomic regions. III-1 is the propositus.

I-2 had a splenectomy, for thrombocytopenia. Acid β-glucosidase assay of peripheral blood lymphocytes in I-1, I-2, I-3, III-1, and III-2, by natural and artificial substrates, indicated that these individuals were deficient (about 10% of mean normal) in enzyme activity. In comparable samples, II-1 and II-2 had about 65% of mean normal activity, which is similar to other carriers in our population (Grabowski et al. 1982). Comparable degrees of enzyme deficiency were demonstrated in cultured skin fibroblasts from I-1 and III-1. Long-term lymphoid lines and/or cultured skin fibroblasts were established on other patients with demonstrated acid β-glucosidase deficiency, by natural and artificial substrates (Grabowski et al. 1982).

RNA Isolation, cDNA Synthesis/PCR Amplification, and cDNA Cloning

Total RNA was isolated from the MS962 lymphoid cell line, and poly(A)+ RNA was selected on oligo(dT) cellulose according to the manufacturer's instructions (5 Prime-3 Prime; Westchester, PA). First-strand cDNAs were synthesized from about 2 μg poly(A)+ RNA by reverse transcription using oligo(dT) as a primer. One-tenth of the total cDNA product then was subjected to amplification using the PCR (50 μl) as follows: 5 μl of 10 × PCR buffer, 1 μl 25 mM of each dNTP, and 50 pmol of each 5′ and 3′ primer specific for untranslated sequences of the acid β-glucosidase cDNA (see below). The cDNA was denatured by incubating the above mixture at 94°C for 7 min. *Taq* polymerase (4 units) was added, and PCR amplification was conducted with 35 cycles of denaturation, annealing, and extension as follows: 30 s at 94°C, 4 min at 66°C, and 2 min at 72°C. An additional 7 min at 74°C was used in the final cycle, and then the mixture either was allowed to cool to room temperature or was cooled to 4°C. The products were analyzed on 1% agarose gels, with ethidium bromide staining. The region corresponding to a band of the expected size was extracted (Sambrook et al. 1988) and cloned. The primers were as follows: 5′-CTTCATCTAATGACCCTGAGG-3′ and 3′-CGTCCCGGTCACACTCGAAGT-5′. These correspond to cDNA bases −23 to −3, relative to the first ATG, and to 92 to 112, after the TGA (Stop), respectively.

The resultant PCR product was cloned directly into the plasmid by the TA cloning system, according to the manufacturer's instructions. The ligation with the TA cloning vector was conducted with a 1:1.7 mol:mol ratio of vector DNA:PCR fragment in 10 μl. After incubation at 12°C overnight, 1 μl of the ligation reaction was used for transformation. White colonies were selected for sequence analysis after plasmid amplification and purification according to a method described elsewhere (Theophilus et al. 1989). The cDNA inserts were sequenced using dideoxy chain-termination method adapted for dsDNA (Chen and Seeburg 1985). For determination of the complete cDNA sequence, the following primers were used (the numbers in parentheses refer to the nucleotide number in the normal cDNA sequence) (Sorge et al. 1985): 5′-CAATACGACTCACTATAGGG-3′ (from the T7 vector), 5′-GTTTTCCCAGTCACGAC-3′ (−40), 5′-CCATCCAGGCTAATCAC-3′ (281), 5′-CCTCCCAGAGGAAGATA-3′ (561), 5′-AGTGGATACCCCTTCCAGT-3′ (841), 5′-CAAAGCCACCCTACCC-3′ (1085), and 5′-TGCGTAACTTTGTCGAC-3′ (1306). Consistent with our previous results, the error rate for PCR or reverse transcriptase was about 1/500 bp in the final cloned product, as demonstrated by complete sequencing of several cDNA clones representing a single allele. Authenticity of a putative mutation was demonstrated by the consistency of the sequence in cloned cDNAs, sequence-specific hybridization to

PCR-amplified genomic DNA from the patient, and Mendelian inheritance in the involved family.

Amplification, Cloning, and Characterization of Specific Genomic DNA Fragments

Genomic DNA from either lymphoid lines or cultured skin fibroblasts was subjected to PCR amplification with primers spanning exon 2 and intronic flanking regions. On the basis of published sequences, primers were chosen to selectively hybridize to complementary sequences in either the structural gene or the pseudogene (fig. 2). The S23′ primer was specifically chosen to hybridize only to intronic sequences in the structural gene, since these sequences were deleted in the published sequence of the pseudogene. After amplification of either structural gene, the PCR products from the propositus and the mother were cloned and subjected to sequence analysis. The PCR products from the pseudogene of III-1 also were subjected to sequence-specific oligonucleotide (SSO) hybridization using the probes INT25′NG and INT25′MA (see below).

For dot blot analyses, one-tenth of the amplified products were denatured with 0.4 M NaOH and 25 mM EDTA in 200 μl. The samples were applied onto Zetaprobe nylon filters which had been presoaked in 10 × SSPE. The filters were baked for 2 h at 80°C, rinsed in 6 × SSPE, and prehybridized for 1 h at 55°C in 5 × SSPE, 5 × Denhardt's solution, and 0.5% SDS and then hybridized, for 5 h or overnight, at 55°C in the same solution containing ^{32}P-end labeled 19 mer SSO (1 × 10^6 cpm/ml) for the normal and mutant sequences: INT25′NG 5′-GGGCATCAGGTGAGT-GAGT-3′ and INT25′MA 5′-GGGCATCAGATG-AGTGAGT-3′, respectively. Similarly, these allele-specific oligonucleotides also were used to probe specifically amplified pseudogene sequences from II-1, II-2, and III-1. Genomic DNA was isolated from peripheral blood leukocytes according to a method described elsewhere (Theophilus et al. 1989). All samples to be tested were from unrelated individuals, unless otherwise specified. Dot blot analyses for the 1226G mutation were conducted according to a method described elsewhere (Grabowski et al. 1985).

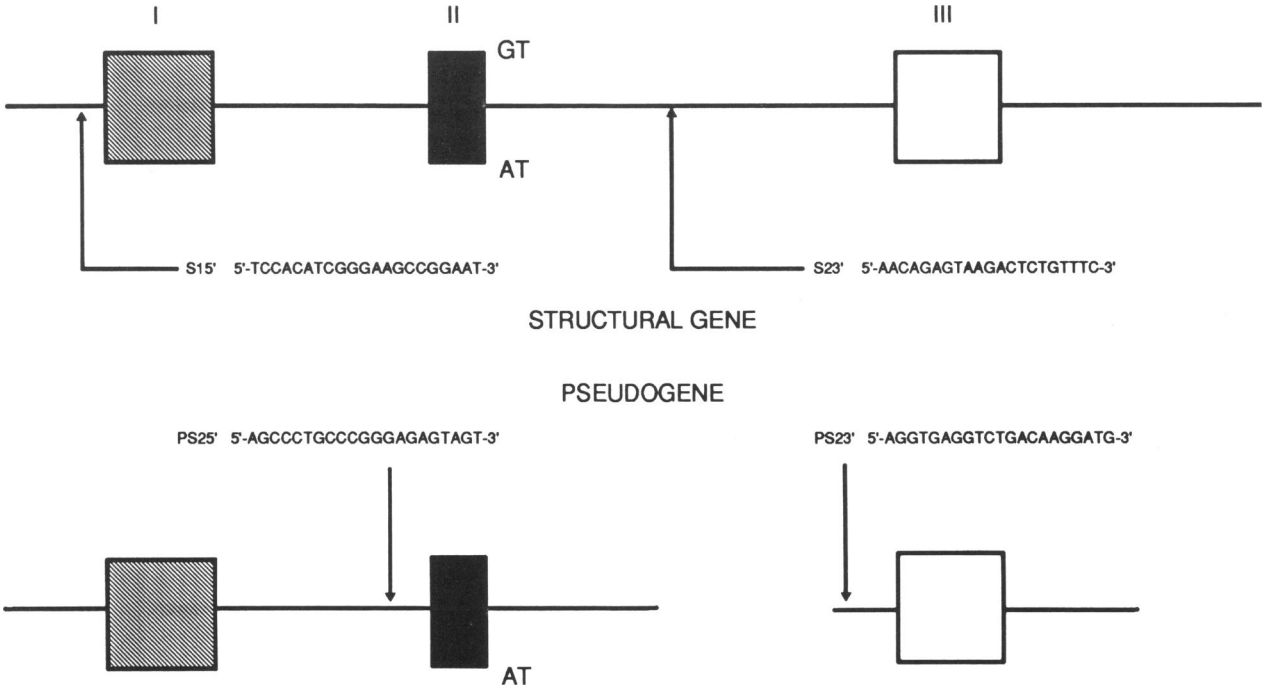

Figure 2 Strategy for the specific amplification of the acid β-glucosidase structural gene and pseudogene sequences. Sequences of exons I–III are shown as boxes and are shaded to correspond to those in fig. 3. The horizontal connecting lines indicate the intron sequences. The respective 5′ sense and 3′ antisense oligonucleotide primers for PCR amplification of the structural gene (S15′ and S23′) and pseudogene (PS25′ and PS23′) is shown. The gap in the pseudogene intron in the lower figure indicates a deletion compared to the corresponding intronic sequence of the structural gene.

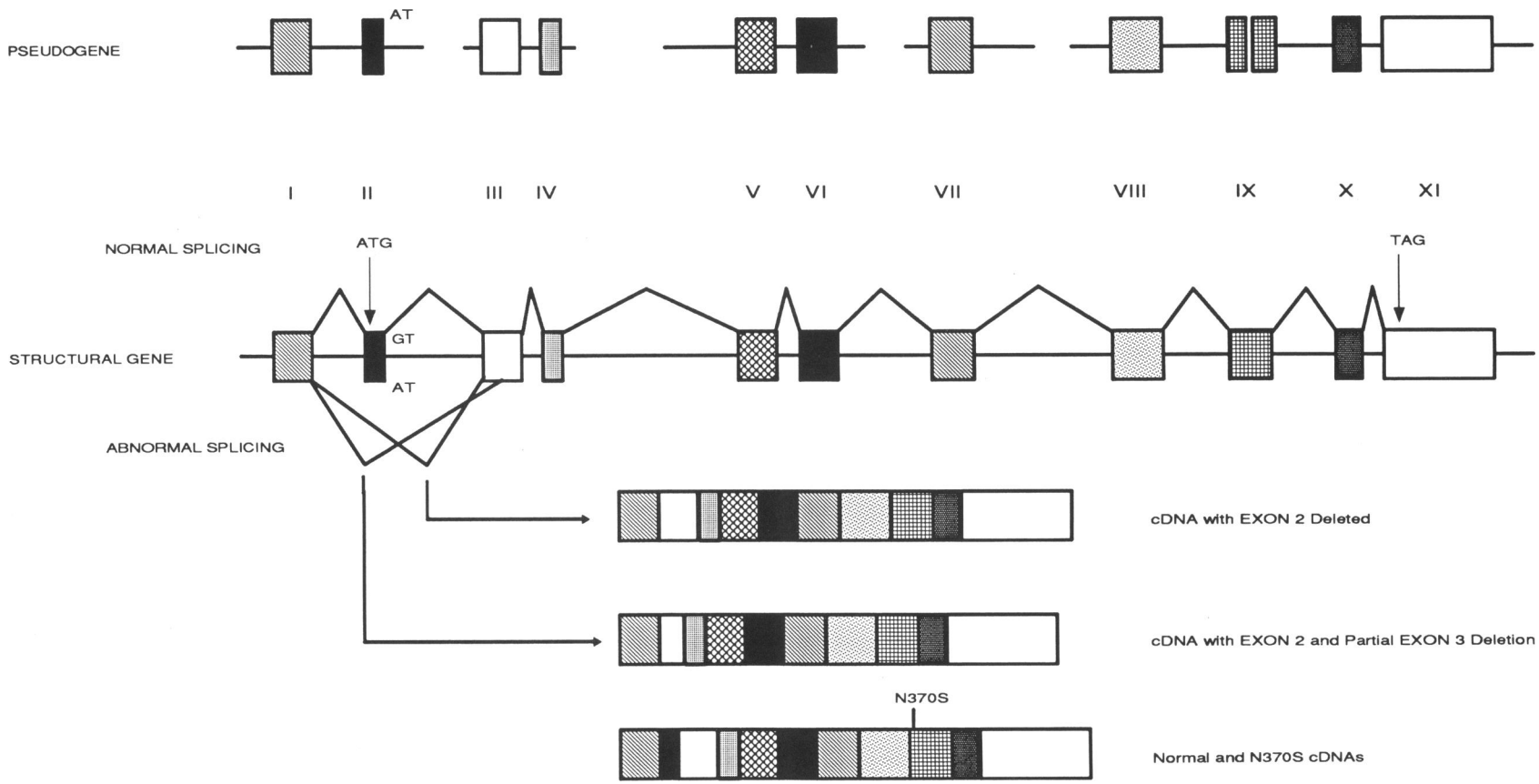

Figure 3 Schematic diagram of intron/exon organization of the acid β-glucosidase pseudogene and structural gene. The corresponding exons and introns from the pseudogene (*top*) and structural gene (*bottom*) are indicated by shaded boxes and horizontal straight lines. The initiating ATG and stop codon (TAG) are shown in exons II and XI, respectively. The gaps in the intronic sequences in the pseudogene indicate deletion relative to the structural gene. Splicing patterns are shown by the lines connecting the exon boxes. The exon 2/intron 2 boundary sequences in the normal (GT) and mutant (AT) genes are indicated, as is the AT sequence at the corresponding exon/intron boundary of the pseudogene. The resultant abnormally and normally spliced mRNAs (as cDNAs) are depicted in the lower part of the figure. Abnormal splicing results in cDNAs with an exact exon 2 deletion (i.e., Δ EX2) or the deletion of exon 2 and 115 bp of exon 3 (i.e., Δ EX2-3), because of the activation of a cryptic splice site. About 50% of the cDNAs contained the 1226G (N370S), whereas only rarely was a normal cDNA obtained, possibly because of allele skipping during PCR.

For the 84GG mutation (Beutler 1991; Beutler et al. 1991*a*), amplified genomic DNA was obtained by using S15′ and S23′ primers (fig. 2) and the following normal and mutant probes: 84NG (normal) dACAG-GTTGCTTCTACTT and 84MG (mutant) dACAG-GATTGGCTTCTACTT.

Results

By means of mRNA isolated from a lymphoid line from III-1, first-strand cDNA was synthesized, and the entire coding region was amplified by PCR with primers complementary to sequences located 5′ and 3′ to the coding regions for the mature enzyme. In this way, only cDNAs of full length for the coding regions would be amplified. In addition, the primers were designed to be strictly complementary to exonic sequences only of the structural gene and contained mismatched bases for "exonic sequences" in the comparable 5′ and 3′ regions of the pseudogene. Four different subpopulations of cDNAs were identified and completely characterized by sequence analysis. As expected from the results of dot blot analysis of genomic DNA from III-1, half (50%) of these "full-length" cDNAs contained only the 1226G mutation. The remaining populations of cDNAs were mixtures of those with completely normal sequence, those with a complete and exact deletion of exon 2 (Δ EX2), and those with a deletion of both exon 2 and the most 5′ 115 bp of exon 3 (Δ EX2–EX3). These cDNAs are shown schematically at the bottom of figure 3. The relevant regions of the cDNAs for each of these are shown in figure 4*A* (normal), figure 4*B* (Δ EX2), and figure 4*C* (Δ EX2–EX3). The nucleotide sequence of several representatives of each mutant cDNA were completely determined, and, except for the aforementioned regions, the remainder of the sequences were completely normal. In a population of 10 cDNAs, the ratios of the normal, Δ EX2, and Δ EX2–EX3 cDNAs were about 1:6:3, respectively. The Δ EX2 and Δ EX2–EX3 cDNAs alter the

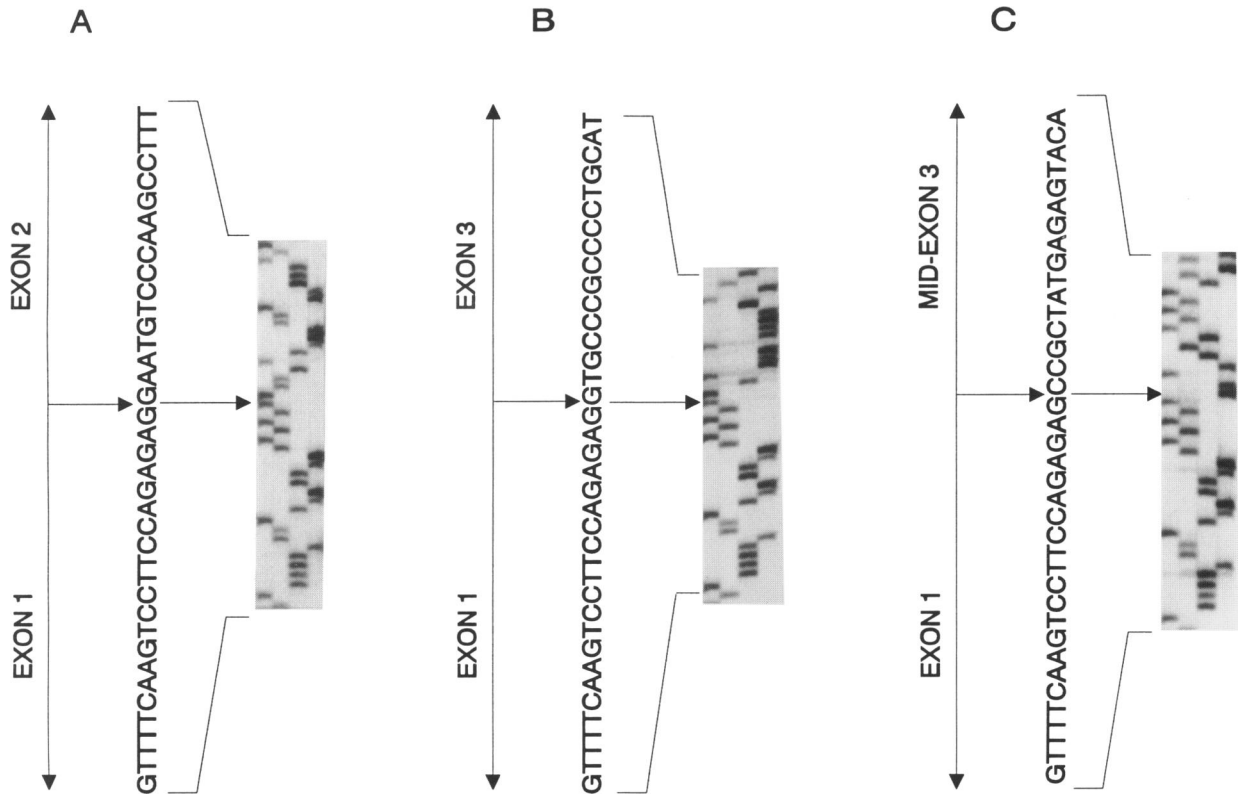

Figure 4 Sequence analyses of different cDNAs cloned from III-1. The cDNAs correspond to the schematic diagrams in the bottom of fig. 3. The panels show only representative sequences from the regions of interest, with the remainder of the nucleotide sequence corresponding exactly to that for the normal cDNA. The location of the exon1/exon2 (*A;* normal), exon1/exon3 (*B;* Δ EX2), and exon 1/mid-exon 3 (*C;* Δ EX2-3) are shown for the abnormally spliced mRNAs (as cDNAs) cloned from III-1. Δ EX2-3 has a complete deletion of both exon 2 and the 5′ 115 bp of exon 3.

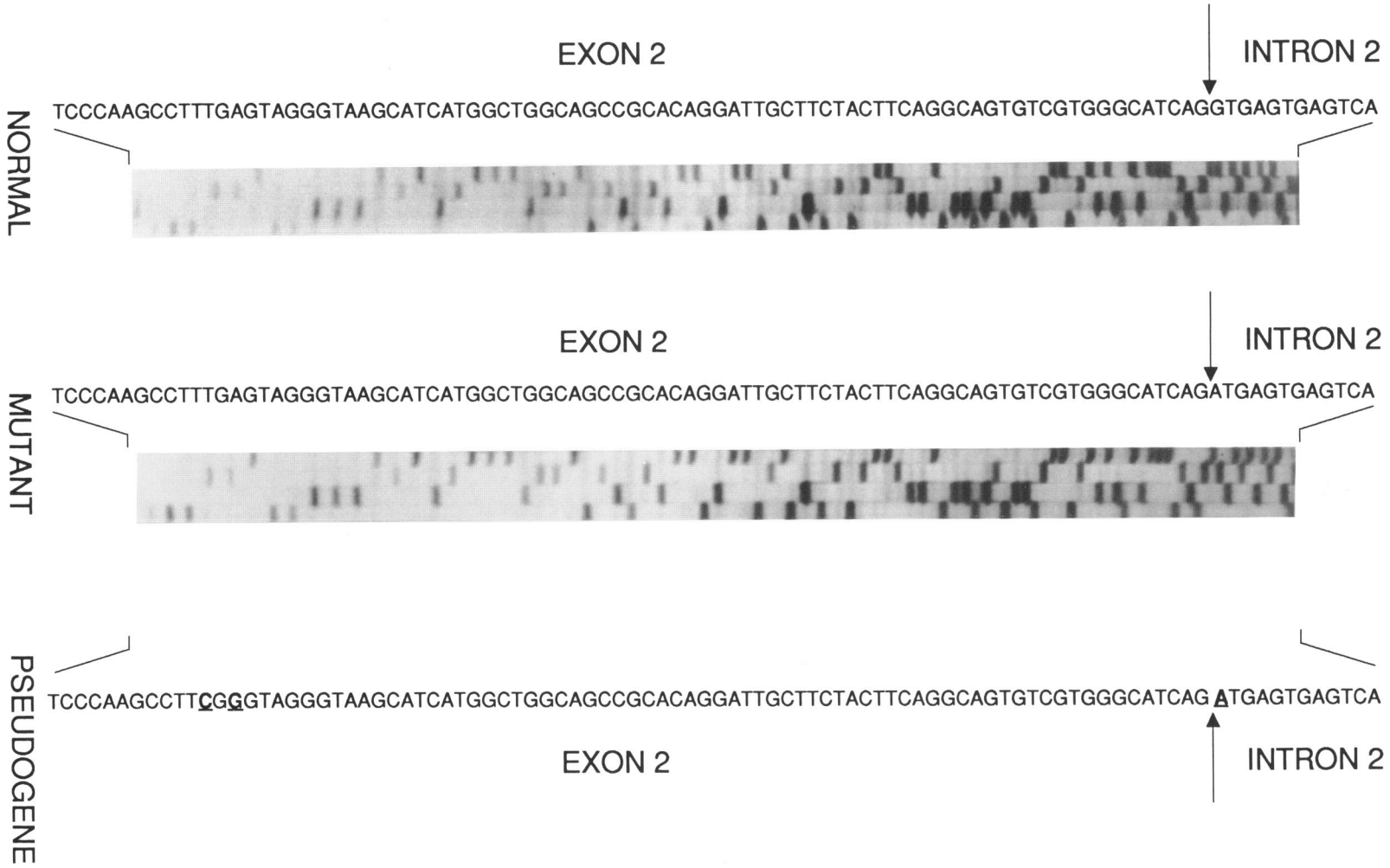

Figure 5 Sequence analyses of PCR-amplified genomic DNA from III-1. The normal and mutant sequences across all of exon 2 and into intron 2 are shown. The $G^{+1} \rightarrow A^{+1}$ transition was found at the exon 2/intron 2 boundary. This transition was the only difference in this region of the acid β-glucosidase alleles from III-1. In comparison, the pseudogene sequence contained the $G^{+1} \rightarrow A^{+1}$ transition, as well as two additional substitutions (*boldface and underlined*) near the 5′ end of pseudogene exon 2.

reading frame of acid β-glucosidase, which leads to stop codons within exon 3. If proteins were made from the Δ EX2 or Δ EX2–EX3 mRNAs, they would be highly truncated and mislocalized, since they would be missing the entire hydrophobic leader sequence.

The finding of cDNAs with skipped exons suggested the presence of a genomic mutation leading to alternative splicing. As shown in figure 2, PCR primers were synthesized and used to selectively amplify the structural gene from III-1. To avoid amplification of the highly homologous pseudogene, the 3′ antisense primer sequence for the structural gene was chosen to correspond to a deletion in intron 2 of the pseudogene (fig. 2). After PCR amplification and cloning of genomic DNA from III-1, 20 independent clones were isolated and subjected to sequence analysis. Two equal populations of sequences were present (fig. 5). One population had the normal genomic sequence at the 5′ exon 2/intron 2 boundary with G^{+1}, and the other population had a point mutation with A^{+1} present at the splice donor site. No other differences from either the published genomic sequence (Horowitz et al. 1989) or that determined from N3, a clone containing the normal structural gene (G. A. Grabowski, unpublished observation), were found in the region amplified. The published pseudogene sequence contains this same $G^{+1}\rightarrow A^{+1}$ at the corresponding exon 2/intron 2 boundary (fig. 3, *top*). To exclude the possibility that the pseudogene sequence had been amplified, PCR amplification, cloning, and sequence analysis were conducted using the primers shown in figure 2 for the pseudogene gene. Representative pseudogene sequences from III-1 contained the $G^{+1}\rightarrow A^{+1}$ transition, as well as two additional differences (Horowitz et al. 1989) from the structural gene (boldface, underlined letters in fig. 5). This was also demonstrated by dot blot analyses of amplified genomic DNA from III-1, II-1, and II-2, by use of the appropriate oligonucleotides (see Material and Methods) (figs. 6 and 7). These results clearly demonstrate that the $G^{+1}\rightarrow A^{+1}$ mutation was present in the structural gene from III-1 and that the converse, $A^{+1}\rightarrow G^{+1}$, was not present in the pseudogene.

With the same primers (fig. 2) and PCR-amplified genomic DNA from a normal individual and from I-1, II-1, II-2, III-1, and N3, dot blot analyses were conducted using sequence-specific probes for the normal and mutant sequence in the structural gene. As shown in figure 8, I-1, a 1226G homozygote, and II-1, a 1226G obligate heterozygote, hybridized, as expected, only with the G^{+1} (normal) probe. In comparison, II-2, a heterozygote by acid β-glucosidase assay and negative

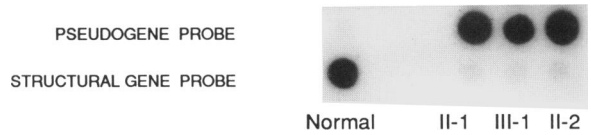

Figure 6 SSO analyses of PCR-amplified pseudogene sequences from members of family L. Primers were used to specifically amplify the pseudogene (fig. 2). The probes (see Material and Methods) for the pseudogene (i.e., Int25′MA) and structural gene (i.e., Int25′NG) differed only by the $G^{+1}\rightarrow A^{+1}$ transition at the exon 2/intron 2 boundary in the respective genomic sequences. The dot labeled "Normal" was the amplified structural gene from genomic DNA of a normal individual and was the result of using both the INT25′ NG probe and the primers shown in fig. 2.

for the 1226G mutation, and III-1, the affected propositus by clinical criteria and by acid β-glucosidase assays and a heterozygote for the 1226G mutation, hybridized with both the normal and mutant (A^{+1}) probes. For specifically amplified pseudogene sequences, the corresponding G^{+1} and A^{+1} oligonucleotide probes were used to demonstrate that only the sequence containing A^{+1} was present in the pseudogene in members of this family (fig. 6). As shown in figure 6 the normal amplified structural gene DNA was hybridized with the G^{+1} probe as a control, whereas this probe did not hybridize to the pseudogene sequences amplified from II-1, II-2, or III-1.

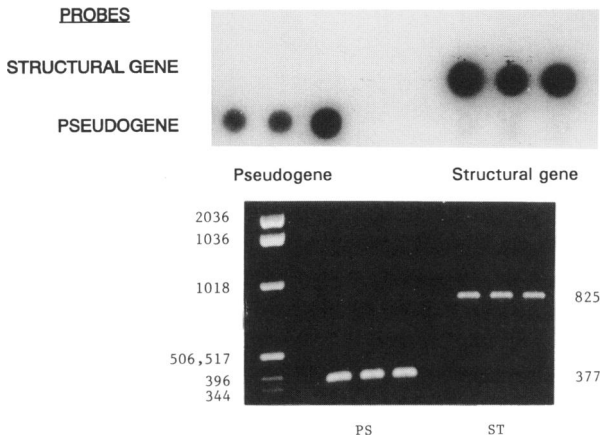

Figure 7 Dot blot analyses in representative Ashkenazi Jews. The specificity of PCR amplification for the structural gene (ST) and pseudogene (PS) are demonstrated by the correct sizes of the respective PCR products (*bottom panel*) in agarose gels stained with ethidium bromide. Selective hybridization to sequence-specific probes (*top panel*) for the structural gene and pseudogene were shown by 5′-CAAGCCTTTGAGTAGGGT-3′ and 5′-CAAGCCTTCGGGTAGGGT-3′, respectively (see fig. 5). The expected sizes of the pseudogene and structural gene PCR products were 377 bp and 825 bp, respectively.

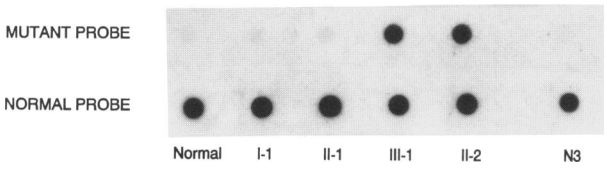

Figure 8 SSO analyses of PCR-amplified genomic DNA from members of family L. Primers were used to specifically amplify the structural gene (fig. 2). The dot labeled "N3" is a cosmid clone containing the entire chromosomal locus for the acid β-glucosidase structural gene. I-1 was homozygous for 1226G. II-1 was an obligate heterozygote for 1226G. II-1 and II-2 were carriers for Gaucher disease, on the basis of acid β-glucosidase activity determinations. III-1 was heteroallelic for 1226G and the IVS2 $G^{+1}\rightarrow A^{+1}$ mutation.

To determine the frequency of the $G^{+1}\rightarrow A^{+1}$ mutation in the Gaucher disease population, DNA from peripheral blood leukocytes was obtained, PCR amplified, and subjected to sequence-specific dot blot analyses with the normal and mutant probes. In figure 7 the specificity of the PCR amplifications is shown in dot blot analyses and ethidium bromide–stained PCR structural and pseudogene products from representative patients. For the screening study, unrelated Ashkenazi Jewish Gaucher disease type 1 patients were screened for the 1226G, the 1448C, and 20 other rare alleles (Grabowski et al. 1990), by means of sequence-specific probes and PCR-amplified genomic DNAs (Theophilus et al. 1989*a*, 1989*b*; Latham et al. 1990, 1991). As shown in table 1, the $G^{+1}\rightarrow A^{+1}$ mutation accounted for about 3.4% of the total Gaucher disease alleles in the Ashkenazi population. The mutation was always present in the heterozygous state. In addition, these populations were screened for the presence of the 84GG mutation (Beutler 1991; Beutler et al. 1991*a*). This mutation accounted for about 10% of the alleles in this Ashkenazi Jewish Gaucher disease type 1 population. The IVS2 and 84GG mutations were not found in non-Jewish type 1 patients, in type 2 patients, or in type 3 patients.

Discussion

The present studies identify the first mutation in Gaucher disease that destroys an intronic 5′ donor site for mRNA splicing and leads to the deletion of an exon. This mutation is relatively common and accounts for about 3.4% of the Gaucher disease alleles in the Ashkenazi Jewish population. Except for the recently described single-base exonic insertion, 84GG, described by Beutler's group (Beutler 1991; Beutler et al. 1991*a*), all other Gaucher disease alleles are due to missense mutations which result in in-frame amino acid substitutions in acid β-glucosidase (Tsuji et al. 1987, 1988; Grabowski et al. 1990; Latham et al. 1990, 1991; Zimran et al. 1990). In comparison, the IVS2 $G^{+1}\rightarrow A^{+1}$ mutation described here results in the loss of exon 2 or of exon 2 and part of exon 3 and leads to mRNAs which could encode only nonfunctional, truncated fragments of acid β-glucosidase. These findings and the fact that the IVS2 $G^{+1}\rightarrow A^{+1}$ mutation also occurs in the pseudogene provide insight into the molecular pathogenesis of Gaucher disease.

To explain the variation in the clinical and cellular involvement, in Gaucher disease (Grace et al. 1991) in particular and in other lysosomal storage diseases (Conzelmann and Sandhoff 1983) in general, a threshold model has been suggested. This model proposes that the variation in the severity of these diseases has as its major determinant the level of residual acid β-glucosidase or other specific lysosomal hydrolases, within the lysosome (Conzelmann and Sandhoff 1983). Many of the missense mutations in Gaucher

Table 1

Frequency of IVS2 and 84 GG Mutations in the Gaucher Disease Population

Source[a]	No. of Patients with Genotype					
	1226G/1226G	1226G/1448C	1226G/84GG	1226G/IVS2	1226G/?[b]	IVS2/?[b]
Ashkenzai type 1 (n = 88).....	41	12	18	5	11	1
Non-Jewish:						
Type 1 (n = 12).....	0	4	0	0	8	0
Type 3 (n = 7)	0	0	0	0	0	0
Type 2 (n = 9)	0	0	0	0	0	0

Note.—All patients were screened for all known mutations.

[a] "n" is the total number of patients screened.

[b] "?" denotes unidentified or private alleles within a family.

disease produce functional, albeit defective, enzymes (Grace et al. 1990, 1991). In comparison, the IVS2 $G^{+1}\rightarrow A^{+1}$ allele leads to aberrantly spliced mRNAs which are missing all of exon 2 and have altered reading frames. Consequently, no functional enzyme can be derived from these mRNAs. The low (i.e., less than 5%) level of normal cDNAs cloned probably results from a PCR artifact and indicates the need for caution in using this technique for mutation identification. If the IVS2 $G^{+1}\rightarrow A^{+1}$ did occur in the homozygous state, the affected patient would be expected to have very severe, potentially rapidly fatal Gaucher disease. This was suggested by the fact that III-1 is affected with Gaucher disease and is much more severely involved than her 1226G homozygous paternal grandfather. As a result, in affected patients who are heterozygous for this mutation, the amounts of functional, residual enzyme within patient's cells or tissues arise only from the 1226G allele (Grace et al. 1990).

Since the first description of a mutation in the acid β-glucosidase causing Gaucher disease (Tsuji et al. 1987), it was suggested that the pseudogene may be important to the pathogenesis of this disease. Horowitz et al. (1989) determined the entire sequence of the acid β-glucosidase structural gene and its pseudogene and indicated that both were contained in a 32-kb fragment of genomic DNA from chromosome 1 (Zimran et al. 1990). The high degree of homology of the two sequences and the very close physical location suggested that genetic recombination or conversion events between these loci may be relatively frequent. Indeed, mutant cDNAs with multiple, authentic nonconservative substitutions have been identified (Hong et al. 1990; Latham et al. 1990, 1991) and result in catalytically inactive and/or highly unstable acid β-glucosidases (Reiner et al. 1987; Grace et al. 1990). In addition, several of the singly mutated alleles from Gaucher disease patients have single exonic substitutions which are present in the corresponding exons from the pseudogene (Latham et al. 1990, 1991). The present studies characterize the first such nonexonic mutation in Gaucher disease. The present studies also demonstrate that the $G^{+1}\rightarrow A^{+1}$ transition at the exon 2/intron 2 boundary was present in the pseudogenes from the propositus, as well as in those of her parents, thereby excluding the occurrence of a gene conversion or homologous reciprocal recombination in the mother. These results also exclude the possibility that III-1 had a different pseudogene form which, in the exon 2 region, contained structural gene sequences.

The carrier frequency for Gaucher disease in the Ashkenazi Jewish population has been estimated to be about 1/10 (Zimran et al. 1991). However, a significant percentage of the alleles in this population could not be identified (Tsuji et al. 1987, 1988; Grabowski et al. 1990; Latham et al. 1990, 1991; Zimran et al. 1990, 1991). In the present population of Ashkenazi Jewish Gaucher disease type 1 patients, the IVS2 $G^{+1}\rightarrow A^{+1}$ accounted for about 3% of the alleles in this population. Screening for the 84GG mutation recently described by Beutler (1991) and Beutler et al. (1991*a*) identified about 10% of the total Gaucher disease alleles in this population. Consequently, the 1226G (128/176 [72%]), 1448C (12/176 [6.8%]), 84GG (18/176 [10%]), and IVS2 $G^{+1}\rightarrow A^{+1}$ (6/176 [3.4%]) account for about 92% of the alleles in the Ashkenazi Jewish Gaucher disease type 1 population screened in these studies. Thus, a screening program based on these four mutant alleles would detect about 92% of the Gaucher disease alleles in heterozygotes for this disease in the Ashkenazi Jewish population. The highly variable clinical manifestations of Gaucher disease type 1 in this population, the lack of absolute correlation of genotype with type 1 disease severity (Tsuji et al. 1987, 1988; Theophilus et al. 1989; Zimran et al. 1990), and the availability of effective specific therapy for this disease (Barton et al. 1990, 1991; Beutler et al. 1991*b;* Fallet et al. 1992) raise several difficult ethical and counseling issues which require attention prior to the institution of such a program on a mass scale.

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