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Mutations in the Lysosomal β-Galactosidase Gene That Cause the Adult Form of GM1 Gangliosidosis

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Summary

Three adult patients with acid β-galactosidase deficiency/GM1 gangliosidosis who were from two unrelated families of Scandinavian descent were found to share a common point mutation in the coding region of the corresponding gene. The patients share common clinical features, including early dysarthria, mild ataxia, and bone abnormalities. When cDNA from the two patients in family 1 was PCR amplified and sequenced, most (39/41) of the clones showed a C-to-T transition (C→T) at nucleotide 245 (counting from the initiation codon). This mutation changes the codon for Thr(ACG) to Met(ATG). Mutant and normal sequences were also found in that position in genomic DNA, indicating the presence of another mutant allele. Genomic DNA from the patient in family 2 revealed the same point mutation in one allele. It was determined that in each family only the father carried the C→T mutation. Expression studies showed that this mutation produced 3%–4% of β-galactosidase activity, confirming its deleterious effects. The cDNA clones from the patients in family 1 that did not contain the C→T revealed a 20-bp insertion of intronic sequence between nucleotides 75 and 76, the location of the first intron. Further analysis showed the insertion of a T near the 5′ splice donor site which led to the use of a cryptic splice site. It appears that the C→T mutation results in enough functional enzyme to produce a mild adult form of the disease, even in the presence of a second mutation that likely produces nonfunctional enzyme.

Introduction

A deficiency of lysosomal (acid) β-galactosidase (E.C.3.2.1.23) is the primary defect in the three clinical forms (infantile, juvenile, and adult) of the autosomal recessive disorder GM1 gangliosidosis and in Morquio B syndrome (O'Brien 1989). Although low levels of this enzyme are found in some tissues from patients with galactosialidosis and mucolipidoses II (I-cell disease) and III, the primary defect is not in the acid β-galactosidase gene (Reitman et al. 1981). In the case of galactosialidosis, a defect in the protector protein (Galjart et al. 1988) that stabilizes a large β-galactosidase/neuraminidase complex, is responsible for the disease (d'Azzo et al. 1982). Lysosomal β-galactosidase has a broad substrate specificity, including ganglioside GM1, asialo-GM1, glycoproteins, and keratan sulfate. Defects in this enzyme could be expected to have pleiotropic effects stemming from the storage of undigested substrates in various tissues.

The infantile form of GM1 gangliosidosis is the most severe, with onset of psychomotor retardation, hepatosplenomegaly, and bone abnormalities within the first year of life. GM1 gangliosides accumulate in the gray matter of the brain in a global manner and are thought to disrupt normal neural growth and function. Death usually occurs in early childhood. Patients with the juvenile form present with a progressive psychomotor loss, but with less severe somatic changes. Patients with the so-called adult form, who may manifest symptoms within the 1st decade of life, usually have dysarthria, mild somatic pathology, and a preserved intellect (O'Brien 1989). In the few adult brains that have come to autopsy (Goldman et al. 1981), storage of substrates was found to be confined mainly to the basal ganglia.

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Patients with Morquio B syndrome show primarily severe bone and connective-tissue pathology without the neurological impairment seen in the gangliosidoses.

In 1988, Oshima and others reported the cloning and sequencing of the cDNA encoding human lysosomal β-galactosidase (Oshima et al. 1988). The first reports of mutations in Japanese patients with the adult form of GM1 gangliosidosis were described in 1991 (Nishimoto et al. 1991; Yoshida et al. 1991). More recently, the cloning of the ~66-kb gene coding for this enzyme has been reported (Morreau et al. 1991). In the present paper we report the finding of two novel mutations in three Caucasian patients with the adult form of GM1 gangliosidosis. We show the inheritance of these mutations within the patients' families and demonstrate that one of the mutant alleles can express a small but significant amount of residual activity. This residual activity, even in the presence of a severe second mutation, is felt to be responsible for the relative mildness and slow progression of symptoms seen in these patients. A preliminary report on a portion of this research was presented as an abstract at the 8th International Congress of Human Genetics (Chakraborty et al. 1991).

Patients, Material, and Methods

The patients with GM1 gangliosidosis were identified on the basis of clinical histories (see below), deficient acid β-galactosidase activity, and normal sialidase activity (Wenger et al. 1974) as measured in sonicated leukocytes and cultured skin fibroblasts.

Family 1

Patient II-1.—This 38-year-old female had normal early childhood development. Speech problems were noted at age 4 years, and a neurological workup at age 19 years revealed a defect in articulation and impairment of both upper and lower extremity coordination. At present, she exhibits a severe and progressive stutter, hyperactive deep-tendon reflexes (especially in the lower extremities), and pes cavus. Her intellect, cranial nerve function, and fundoscopic exams are all normal. Although she has fewer vertebral changes than her brother, bilateral total hip replacement was required 5 years ago.

Patient II-2.—This 32-year-old white male was delivered normally from an uncomplicated pregnancy and developed normally through infancy. Like his sister, he presented with retarded speech development at age 4 years and was found to have dysarthria. Both a mild disturbance of upper-extremity movement and clumsy gait were noted on neurological exam. At age 10 years, a pneumoencephalogram showed ventricular dilatation, especially on the left, which was considered to be indicative of cerebral atrophy. Since that time, this patient's neurological picture has included progressive dysarthria, moderate ataxia, and intention tremor, but cranial nerve and fundoscopic examinations have been normal. Deterioration of short-term memory has also been noted. Bone changes include flattening of the vertebral bodies, progressive kyphosis, and subluxation of the right hip. Both siblings in family 1 have been described elsewhere (Wenger et al. 1980). Both sides of this family originate from the same small town in western Denmark.

Family 2

Patient II-1.—This patient is a 21-year-old white female, also of Scandinavian heritage, who resulted from a normal pregnancy and delivery. She presented at age 3 years with speech difficulties, which now remain as a severe stutter. On neurological examination, a spastic quadriparesis, especially in the lower extremities, was noted, along with a "scissoring" gait. The patient also has a history of urinary incontinence. Cranial nerve and fundoscopic examinations, as well as intellect, were found to be normal. Evaluation by magnetic resonance imaging revealed mild ventricular enlargement. The patient is also described as having short stature and scoliosis.

Cells

Human fibroblasts, started from forearm skin biopsies, were cultured in modified Eagle's medium alpha (αMEM), supplemented with 10% fetal bovine serum (FBS), 200 mM L-glutamine, and 2 mM gentamicin. Human lymphoblasts were isolated from human whole blood (Overhauser et al. 1990), washed twice in PBS, and resuspended in Iscove's medium (containing 20% heat-inactivated FBS, 5 mM L-glutamine, 80 μM gentamicin, 1 μg of phytohemagglutinin/ml, and 600 ng of cyclosporin A [Sandoz])/ml. Cells were then transformed with Epstein-Barr virus (Anderson and Gusella 1984) that had been isolated from cultures of B95-8 cells (provided by R. Nussbaum). Transformed cells were then maintained in OptiMEM (supplemented with 2% FBS, 200 mM L-glutamine, and 2 mM gentamicin).

Chemicals and Reagents

All chemicals and reagents were purchased from the following, unless otherwise noted: Bio-Rad Laborato-

Table 1

Oligonucleotides Used as PCR Primers and ASO Hybridization Probes

PCR Primer[a]	Location[b]	Sequence
1(+)	−20	5′ AGAGCCGGGAGGCTGGT 3′
2(−)	850	5′ CCACTGCTTCGGTCTTGAT 3′
3(+)	809	5′ ATACTGGCTGGCTAGATCAC 3′
4(−)	2249	5′ CCAGCCCTGCAGATATGTA 3′
5(+)	345	5′ ACTCGTGGTTATCCTGAGG 3′
5 (−)	345	5′ CCTCAGGATAACCAGCAGT 3′
6(−)	1894	5′ AACGTCACAGCACATAGTTC 3′
7(+)	1043	5′ TTGCTCTGCGAAACATCATC 3′
8(−)	1598	5′ CAGGCTTCATCATGGTGGC 3′
9(+)	78	5′ TGCCACCCAGAGGATGTTT 3′
10(−)	2072	5′ GGAGGATCTGTGAGGTATG 3′
A(−)	Intron 1	5′ CCTAGCAATGCCTCCCCG 3′
D(−)	Intron 2	5′ AGAACATCACACTGGACC 3′
Oligonucleotide Probe		
Insertion 75:[c]		
Normal		5′ GCTTGCGCGTAAGTCTGCG 3′
Mutant		5′ GCTTGCGCGTTAAGTCTGCG 3′
Transition 245:[d]		
Normal		5′ CCATCCAGACGTAAGTAGG 3′
Mutant		5′ CCATCCAGATGTAAGTAGG 3′

[a] + and − refer to the orientation of the sequence; the ASO probes are all in the + orientation.
[b] Refers to the most 5′ nucleotide with respect to the (+) orientation.
[c] Insertion of a T at the third position of intron 1, located between 75 and 76 in the cDNA.
[d] C→T transition at position 245 in the cDNA.

ries, Fisher Scientific, and Thomas Scientific. Tissue culture reagents were purchased from GIBCO. Radiochemicals were obtained from Dupont/NEN.

PCR

Both fibroblasts and transformed lymphoblasts were harvested and used for preparation of RNA (Chomzynski and Sacchi 1987). First-strand complementary DNA was synthesized from 10–20 μg of total RNA, by using MMLV reverse transcriptase, primer, and reagents from BRL, and then were used directly for PCR. Genomic DNA for PCR reactions was isolated from cultured fibroblasts, transformed lymphoblasts, and heparinized human whole blood (Bell et al. 1981). Genomic DNA also was isolated from sonicated leukocyte pellets used initially for enzyme studies (Louie et al. 1991). Primers used for amplifying fragments from cDNA are listed in table 1 and were designed on the basis of published sequences (Oshima et al. 1988; Morreau et al. 1989; Yamamoto et al. 1990). It should be noted that nucleotides are numbered with the A of the initiation codon as "1." Most primers also contained either *Xba*I (sense primers) or *Sph*I (antisense primers) linkers for subcloning. PCR (Saiki et al. 1988) was performed on both Coy Labs and Perkin Elmer Cetus thermal cyclers, using buffers and *Taq* DNA polymerase purchased from either Perkin Elmer Cetus or Promega. Primers 1 and 2 were used to amplify the first half of the cDNA, while the 3′ half was amplified using primers 5(+) and 4. These products overlap by 500 bp. Several smaller overlapping PCR products were also generated and cloned into M13 for sequencing.

Subcloning and Sequencing

Restriction endonucleases and modifying enzymes were purchased from either Boehringer-Mannheim Biochemicals or New England BioLabs. All protocols and formulations used for cloning and sequencing, other than those specifically cited, were either based on standard methods (Sambrook et al. 1989) or in accordance with the manufacturer's instructions.

PCR products with incorporated *Xba*I or *Sph*I linkers were digested with these enzymes and directionally cloned into the polylinker of bacteriophage

M13 (mp18 and mp19). Single-stranded phage DNA was purified from insert-containing plaque supernatants, dissolved in sterile water, and sequenced in both directions by the dideoxy method (Sanger et al. 1977) using materials in the Sequenase kit (US Biochemicals). The PCR primers described above were also used as specific internal primers for sequencing. In some cases, 7-deaza-dGTP was used to sequence through compressions.

Allele-specific Oligonucleotide (ASO) Hybridizations

Oligodeoxynucleotides composed of 19 bp overlapping the region where each mutation occurred were designed in pairs, with one containing normal sequence and one containing mutated sequence (Ikuta et al. 1987). The probes used to detect each mutation are given in table 1. Note that the probe for the insertion mutation is composed of 20 bp. Genomic DNA containing the region of interest was PCR amplified for patients, their families, and control subjects. PCR primers 1 and A (table 1) were used to amplify the area surrounding the insertion at 75, while primers 9 and D were used to amplify the region near the point mutation at 245. The PCR products were loaded in duplicate slots of a Schleicher & Schuell slot-blot apparatus and were transferred to Zetabind membranes (CUNO). Blots were hybridized to the 5′ end–labeled oligonucleotide probes. The blots were then washed (after initial low-stringency washes) for 3–5 min in a high-stringency wash (2 × SSC and 0.1% SDS), on the basis of the calculated T_m of the oligonucleotide probe used, and were exposed to X-ray film (Lemna et al. 1990).

Northern Analysis

Twenty micrograms of formamide-formaldehyde–treated total RNA from patients in family 1 was electrophoresed on 1% agarose gels and was transferred to Hybond N (Amersham) nylon membranes by standard methods. Blots were probed with subcloned and sequenced PCR-amplified products from control subjects, as well as with part of the cloned acid β-galactosidase cDNA. Additionally, cloned DNA from the glyceraldehyde-3-phosphate dehydrogenase gene was used as a second probe to determine the relative amounts of RNA present on the blot.

Expression Studies of the Point Mutation at 245

Plasmid pTZ18R containing the full-length (2.4 kb) human β-galactosidase cDNA (Morreau et al. 1989) was the contribution of A. d'Azzo. The insert was subcloned into the *Xba*I and *Hin*dIII sites of the pBluescript SK+ polylinker. In order to generate a clone containing the C→T change, ~620 bp of the 5′ end of the cDNA was cleaved using *Hae*II and was replaced with a cloned, sequenced *Hae*II-digested PCR fragment containing the point mutation found in the patients. The new mutant construct was then cloned into the *Not*I and *Xba*I (this site was subsequently blunt-ended by filling-in with Klenow fragment) polylinker sites of the expression vector pRC-CMV (Invitrogen).

The normal construct was prepared by partial *Eco*RI digestion of the full-length cDNA, followed by *Hin*dIII digestion, and was inserted into the *Eco*RI-*Hin*dIII sites of Bluescript. It was removed from the polylinker by using *Not*I and *Xho*I (site blunt-ended). Finally, the entire fragment was cloned into pRC-CMV. The first 1,000 bp of both the normal and mutant constructs were sequenced and found to agree (except for the single C→T change in the mutant construct) with that published previously (Yamamoto et al. 1990). DNA of each of the constructs was purified from 500-ml plasmid preparations by using the Qiagen maxi-prep kit. A control construct comprising the expression vector without any inserted cDNA was also prepared. Twenty micrograms of construct DNA was transiently transfected into cultured skin fibroblasts from a patient with the infantile form of GM1 gangliosidosis by using the Transfinity calcium phosphate–based transfection kit (BRL). These cells contain essentially no intrinsic β-galactosidase activity. The 100-mm culture dishes of cells were also cotransfected with purified pBLCAT2 (Luckow and Schutz 1987) DNA (supplied by B. Saitta), harvested, and assayed. Acid β-galactosidase and total β-hexosaminidase activities were measured in transfected fibroblasts (Wenger et al. 1975) by utilizing fluorogenic substrates. Chloramphenicol acetyl transferase (CAT) was assayed using thin-layer chromatography (Sambrook et al. 1989). Protein concentrations were determined by the method of Lowry et al. (1951).

Results

It is evident from the enzymatic activities presented in figure 1 that all three patients have a deficiency of acid β-galactosidase. The parents of these patients have values that are roughly half of normal, thus confirming the autosomal recessive inheritance of this disease. While the paternal uncle of the patient in family 2 (family 2, I-3) is not a carrier of β-galactosidase deficiency, one of his children (family 2, II-3) is a carrier and inherited a mutation from his mother (who is also the mother of the patient).

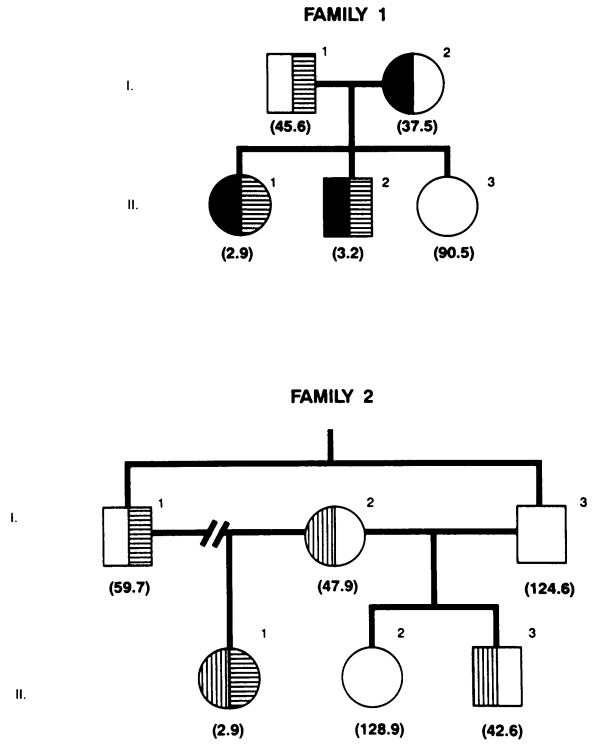

Figure 1 Pedigrees of the two families discussed in the present study. Family 1 contains two affected siblings, labeled "II-1" and "II-2." Family 2 contains one affected individual, labeled "II-1." The numbers in parentheses below each family member give the lysosomal (acid) β-galactosidase activity tested in leukocytes from each person. The enzyme activities are given as nanomoles of substrate cleaved per hour per milligram of protein. The mean ± SD in controls is 98 ± 20 in leukocytes. Dark shading indicates the presence of the splicing mutation at nucleotide 75. Horizontal bars depict the presence of the point mutation at 245. Vertical bars represent the unidentified mutation transmitted in family 2.

Several overlapping PCR fragments originating from the cDNA of patients in family 1 were cloned and sequenced. A point mutation, changing the C at nucleotide 245 to one for T, was found (cDNA data not shown). This missense mutation changed the codon for amino acid 82 from ACG (threonine) to ATG (methionine). For the patients in family 1, a total of 41 clones from the 5′ half of the cDNA were examined. Thirty-nine of these clones carried the C→T mutation. Using total mRNA extracted from the fibroblasts of patient II-2 in family 1, northern analysis (not shown) revealed, as expected, mRNA of normal size (2.4 kb) but decreased amount. Only a sample of genomic DNA was available from the patient in family 2.

Nucleotide 245 is part of a split codon that straddles the intron between exon 2 and exon 3. When PCR-amplified genomic DNA products were sequenced, approximately half of the clones from each of the three patients were found to contain the C→T mutation. Figure 2 shows this mutation in the patient in family 2. The rest of the clones showed normal sequence at that nucleotide, confirming the presence of the point mutation in the heterozygous state. In order to further confirm this inheritance, family members were studied. In family 1, the father carried the 245 mutation in half of the clones examined, while neither the mother nor the noncarrier sister had this change in any clones sequenced (data not shown). The PCR products used for sequencing were then subjected to allele-specific oligonucleotide hybridization (ASO) as shown in figure 3. In family 1, both patients and their father showed hybridization with the mutant and normal probes, while the DNA of the mother and unaffected sister reacted only with the normal probe, consistent with results obtained by sequencing. In family 2, the patient and her father were both found to have one copy of the C→T allele. The rest of the family was not found to carry this mutation. These results are also presented in figure 1.

Seventeen unrelated unaffected individuals (34 al-

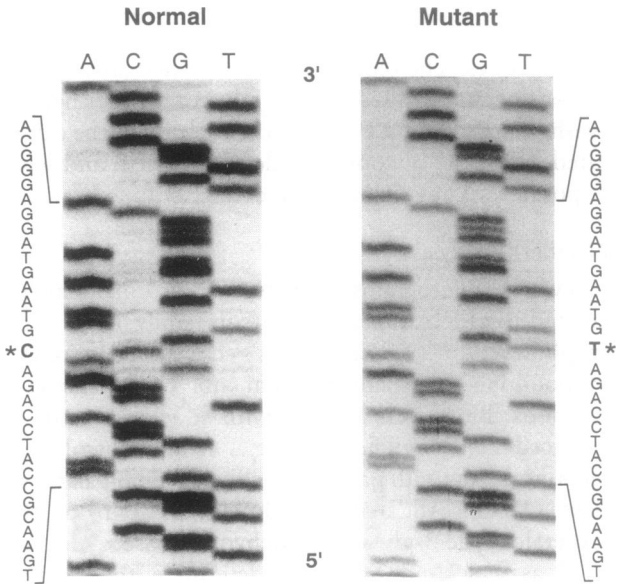

Figure 2 Autoradiograph of a sequencing gel, showing normal and mutant sequence from amplified genomic DNA of patient II-1 in family 2. The sequence, which is read 5′ to 3′, from the bottom, demonstrates the substitution of a C found in the normal sequence (*asterisk* [*]) for a T (*asterisk* [*]) in the mutated sequence. This mutation occurs at nucleotide 245 of the coding region and changes the codon ACG, for threonine, to ATG, for methionine.

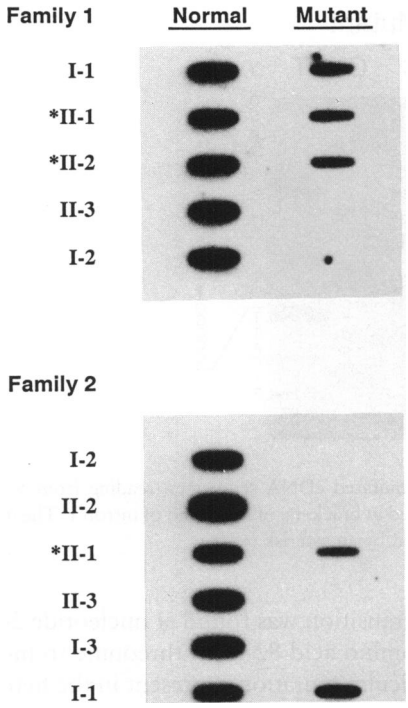

Figure 3 ASO hybridization of amplified genomic DNA from the region surrounding the C→T point mutation at nucleotide 245. The PCR products from members of families 1 and 2 were generated with primers 9 and D and hybridized to the probes listed in table 1. The numbers used to designate family members correspond to those in fig. 1. Patients are indicated by an asterisk (*).

leles), including several of probable Scandinavian descent, were screened by ASO, and none were found to carry the C→T substitution. Seven patients and several obligate carriers of the juvenile and infantile forms of GM1 gangliosidosis, as well as two siblings with Morquio B syndrome, were also screened, and none carried the point mutation; neither did an unrelated adult patient (data not shown). These data suggest that the change at 245 is not a common polymorphism.

Transient-transfection experiments were initiated to show that the single base change at 245 caused decreased β-galactosidase activity. Constructs were made as described above and contained either the normal β-galactosidase cDNA, the mutant—i.e., that with the single C→T substitution—or the expression vector without any insert. It was felt that any cell type chosen for transfection should have negligible endogenous β-galactosidase activity in order to detect low-level expression of a mutant allele above the background. Cultured fibroblasts of a patient with the infantile form of GM1 gangliosidosis were selected for transfection and were used within a few passages from primary culture, in order to increase the transfection efficiency. The plasmid pBLCAT2, which carries the bacterial CAT gene (Luckow and Schutz 1987), was cotransfected along with the β-galactosidase constructs, as well as with the insertless vector, and served as an internal control for transfection efficiency.

Figure 4 shows the enzymatic activity of the constructs after transfection. Three sets of transfections in duplicate were performed, and the data are summarized in this figure. Analysis of variance calculations (F-test) found the difference in expression between normal and mutant constructs to be highly statistically significant at $P < .01$. The mutant activity, when compared with background activity, was also highly significant, at $P < .05$. Values for total β-hexosaminidase activity were similar for all transfected treatment groups studied. The transient-transfection experiments demonstrate that expression of the mutant construct results in β-galactosidase activity levels that are 3%–4% of that found for the normal construct.

Genomic sequencing and ASO experiments indicated

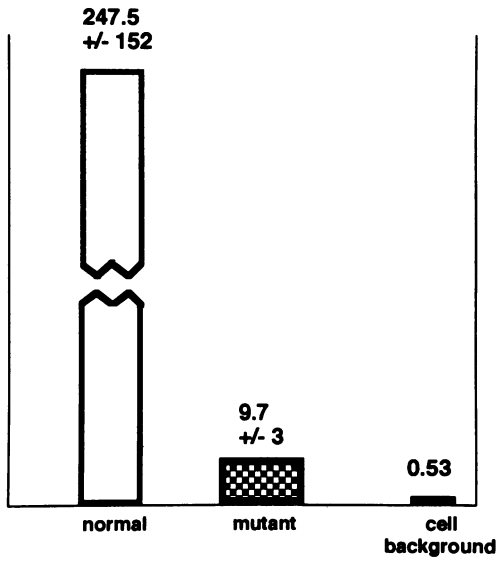

Figure 4 β-Galactosidase activity of cells transiently transfected either with constructs containing the full-length normal human β-galactosidase cDNA or the mutant cDNA or with the expression vector containing no insert (cell background). The numbers above each bar (not drawn to scale) show the average enzyme activity of transfected cell extracts, in nanomoles of substrate converted per hour per milligram of protein. The averages and SDs represent values of three sets of experiments done in duplicate. These values were adjusted on the basis of the CAT activity (data not shown) of the extracts, which served as an internal control of transfection efficiency.

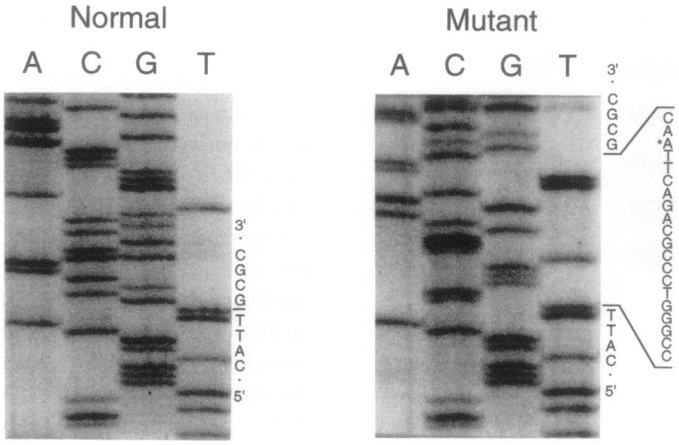

Figure 5 Autoradiograph of a sequencing gel containing both normal and mutated cDNA sequences (reading from 5′ to 3′ in the antisense strand) from patient II-1 (family 1). The mutant cDNA retains 20 bp (indicated in brackets) of the 5′ end of intron 1. The insertion of a T (shown as an A in the antisense strand), in the retained 20-bp fragment, is indicated by an asterisk (*).

that another mutated allele was present in these families. Two of forty-one cDNA clones examined in patients in family 1 did not contain the point mutation described. These two clones were found to contain an insertion of 20 bp between nucleotides 75 and 76, as presented in figure 5. The CGC shown immediately above the brackets is normally the last codon in exon 1, with the C in the third position located at nucleotide 75. This is precisely the junction where intron 1 occurs (Morreau et al. 1991). The 20-bp insert includes the normal 5′ splice donor sequence of intron 1, except for the insertion of a T at the third position in the intron. The extra T, shown in figure 5, in the donor splice sequence causes a splicing anomaly where a cryptic splice site distal to the normal donor is now utilized (fig. 6). The result is a retention of 20 bp of intron within the mRNA produced from this allele. A frameshift occurs, causing premature termination. No other mutations were found in the cDNA of these patients.

ASO hybridization studies (data not shown) confirmed that patients in family 1 and their mother are heterozygous at the genomic DNA level, for the insertion of the extra T that causes this splicing mutation. Neither the father nor the unaffected sister shows evidence of this change. The patient in family 2 does not have this mutation. Other patients with different clinical forms of GM1 gangliosidosis were screened, and none had this mutation.

Discussion

We have demonstrated the presence of a new point mutation in the coding region of acid β-galactosidase. A C→T transition was found at nucleotide 245, which changes amino acid 82 from threonine to methionine. This particular mutation is present in the heterozygous state in two siblings and in one unrelated patient with similar clinical features of the milder, adult form of

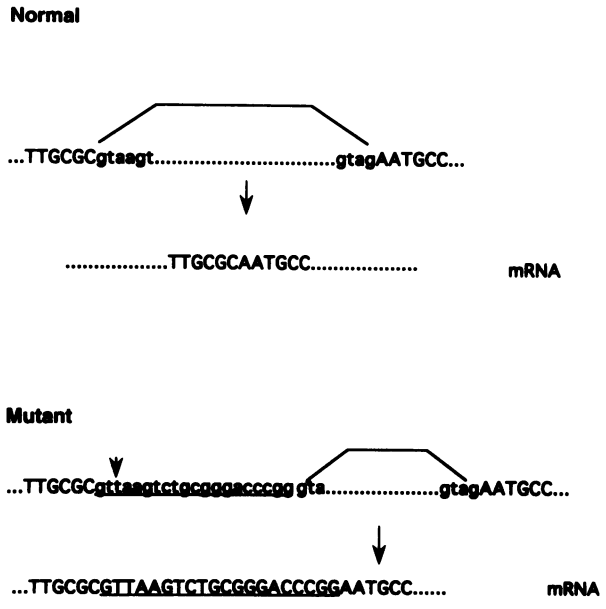

Figure 6 Diagram of the splicing mutation that occurs in family 1. In the normal human β-galactosidase gene, intron 1 (which is >12 kb) is completely spliced out (*top*). The insertion of an extra t (*bottom, arrow*) in the normal 5′ splice donor region activates a new downstream splice donor site, which results in the retention of 20 bp of intron 1 (*underlined*).

	-3	-2	-1	/	+1	+2	+3	+4	+5	+6
consensus	C/A	A	G		g	t	a/g	a	g	t
normal	C	G	C		g	t	a	a	g	t
mutated	C	G	C		g	t	**t**	a	a	g
distal site	C	G	G		g	t	a	c	g	g

Figure 7 Comparison of exon 1/intron 1 splice junctions. The 9-bp consensus 5′ donor splice junction, which contains sequence from the exon (*negative nos., uppercase letters*) and intron (*positive nos., lowercase letters*) involved in splicing, is shown. The exon 1/intron 1 splice junction found in the normal human β-galactosidase gene is also shown, along with the mutated junction, which contains an extra t (boldface) as found in family 1. Finally, the distal splice junction actually used in these patients is displayed. A slash (/) separates the exon 1/intron 1 boundary, while nucleotides that do not match those found in the consensus sequence are underlined.

GM1 gangliosidosis. All three of these patients are Americans of Scandinavian descent, and in both families the mutation was found in the genomic DNA of the patients' fathers. In addition, DNA from a panel of unrelated noncarriers, as well as from several patients and carriers of other clinical forms of β-galactosidase deficiency, was screened and found not to contain the mutation.

When a cDNA construct containing this mutation was expressed in transient-transfection studies, the mutant expressed 3%–4% of the activity demonstrated by the normal construct. These results show that the mutation at 245 is not merely a polymorphism but that, in fact, it causes a functional impairment that would contribute to disease. Furthermore, the expression studies correlate with what we find by in vitro enzyme assay, in which the patients' cells show residual enzyme activity.

Further work with family 1 revealed that 2/41 cDNA clones contained an insertion of 20 bp of intronic sequence between nucleotides 75 and 76 of the coding sequence. The insertion of a T was found within the splice donor region of intron 1, activating a new, distal splice site. This inserted T was found in the genomic DNA of the patients and mother in family 1. It is important to note that the point mutation at 245 and the insertion at 75 are mutually exclusive, in that no clone sequenced was found to contain both. These mutations therefore exist as different alleles.

When the normal and mutated exon 1/intron 1 splice junctions are examined, it becomes obvious that the insertion of an extra T at position +3 (as shown in fig. 7) greatly reduces the favorability of this mutated site as a splice donor when both are compared with the 9-bp consensus (Mount 1982; Ohshima and Gotoh 1987). In the original normal splice junction, seven of the nine splice donor sequence positions match those of the consensus, while the addition of an extra T at the +3 position reduces the similarity to only four of nine matching. The distal donor sequence used in the patients has six of nine positions matching the consensus, including the highly conserved consensus positions of −1, +3, and +5, which makes it as favorable as the normal site. The frameshift caused by use of the cryptic splice site results in the creation of an early termination codon within 155 nt. Any protein product would be severely truncated and almost certainly nonfunctional. In fact, several lines of evidence suggest that even the mRNA resulting from this mutation is unstable and not likely to contribute significantly to any enzyme produced by the patients. While ASO screening of the patients shows roughly equal amounts of genomic DNA with the extra T and without it, only 2 of 41 cDNA clones sequenced from patients in family 1 contained the inserted T. All the others contained the C→T at nucleotide 245. Northern analysis data corroborate this, where patient II-2 shows a decreased amount of mRNA specific for acid β-galactosidase. Studies by d'Azzo's group, on a Finnish patient who is homozygous for the same splicing mutation as is described in the present paper, found him to have barely detectable (<2%) β-galactosidase mRNA levels and an infantile phenotype (Morrone et al. 1994). The adult patients in family 1, however, are heterozygous, and they also have an allele with the Thr82Met mutation, which, though itself straddling a splice site, produces mRNA that expresses a protein with some residual activity. This residual activity is felt to account for the late onset and relative mildness of the patients' clinical signs and symptoms.

It is tempting to speculate about the phenotype that individuals homozygous for the C→T mutation might display. Would they be asymptomatic or have an extremely late onset of disease? Previous histological studies of brains of patients with the adult form of GM1 gangliosidosis (Goldman et al. 1981) showed storage to be focal, confined mainly to basal ganglia. If patients homozygous for the Thr82Met allele were also to have focal storage, they might present in their 5th, 6th, or even 7th decade of life, with symptoms resembling those of diseases of basal ganglia, such as Parkinson disease. Investigation of families (especially those of Scandinavian descent) in which symptoms of late-onset basal ganglia dysfunction are prevalent may prove fruitful in this regard.

Two mutations in Japanese patients with adult GM1 gangliosidosis have been described. All except one pa-

tient were found to be homozygous for an allele that resulted in a Ile51Thr change (Nishimoto et al. 1991; Yoshida et al. 1991). These Japanese patients are reported to have intellectual impairment but otherwise share clinical features similar to those of the adults whom we have described here. An additional point mutation, Arg457Gln, which was reported in one Japanese adult patient, existed in the heterozygous state along with the Ile51Thr change.

Three mutations that change amino acids 273, 482, and 509 of acid β-galactosidase in patients with Morquio B syndrome have also been described (Oshima et al. 1991). These patients mainly display pathology of bone and connective tissue, which may reflect selective impairment of the degradation of hydrophilic substrates (e.g., keratan sulfate). The mutations found in the adult Morquio patients are located toward the carboxyl terminus of the enzyme. The prevalent mutation found in adult Japanese patients with GM1 gangliosidosis, as well as those found in our patients, tend to cluster near the amino terminus of the protein. Future studies such as three-dimensional analysis of the enzyme and its active site(s) may afford greater insight into the disparate phenotypic presentations that can arise from mutations within the same gene.

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