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Molecular basis of adult-onset and chronic G_{M2} gangliosidoses in patients of Ashkenazi Jewish origin: Substitution of serine for glycine at position 269 of the α-subunit of β-hexosaminidase

(Tay–Sachs disease/subunit association/polymerase chain reaction)

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ABSTRACT **Chronic and adult-onset G_{M2} gangliosidoses are neurological disorders caused by marked deficiency of the A isoenzyme of β-hexosaminidase; they occur in the Ashkenazi Jewish population, though less frequently than classic (infantile) Tay–Sachs disease. Earlier biosynthetic studies had identified a defective α-subunit that failed to associate with the β-subunit. We have now found a guanosine to adenosine transition at the 3′ end of exon 7, which causes substitution of serine for glycine at position 269 of the α-subunit [designated 269 (Gly → Ser) substitution]. An RNase protection assay was used to localize the mutation to a segment of mRNA from fibroblasts of a patient with the adult-onset disorder. That segment of mRNA (after reverse transcription) and a corresponding segment of genomic DNA were amplified by the polymerase chain reaction and sequenced by the dideoxy method. The sequence analysis, together with an assay based on the loss of a *Scr*FI restriction site, showed that the patient was a compound heterozygote who had inherited the 269 (Gly → Ser) mutation from his father and an allelic null mutation from his mother. The 269 (Gly → Ser) mutation, in compound heterozygosity with a presumed null allele, was also found in fetal fibroblasts with an association-defective phenotype and in cells from five patients with chronic G_{M2} gangliosidosis. It was not found in β-hexosaminidase A-deficient cells obtained from patients with infantile Tay–Sachs disease nor in cells from individuals who do not have β-hexosaminidase A deficiency. However, there must be additional mutations with similar consequences, since the 269 (Gly → Ser) substitution was not present in fibroblasts from two patients with juvenile G_{M2} gangliosidosis even though these had an association-defective α-subunit.**

The G_{M2} gangliosidoses are autosomal recessive disorders caused by mutations in any of the three genes that encode the α- and β-subunits of β-hexosaminidase[§] and the activator protein. The resulting lack of functional β-hexosaminidase A isoenzyme (an αβ heterodimer) leads to lysosomal accumulation of undegraded *N*-acetylgalactosaminyl-β1→4-(*N*-acetylneuraminyl-α2→3)-galactosyl-β1→4-glucosyl-β1→1-ceramide (G_{M2} ganglioside) in neurons and other cells. The disorders display a spectrum of clinical severity, ranging from the most severe infantile forms of Tay–Sachs and Sandhoff disease, to juvenile, chronic, and adult-onset variants, which differ in age of onset and clinical course. The severity of the disease is inversely correlated with the residual level of β-hexosaminidase A activity towards G_{M2} ganglioside. In addition to this clinical heterogeneity, there is molecular heterogeneity within G_{M2} gangliosidoses of similar clinical presentation. The clinical, pathological, biochemical, and genetic aspects of the G_{M2} gangliosidoses have been recently reviewed (1).

Availability of cDNA (2, 3) and genomic (4) clones and sequence data has already resulted in precise identification of several mutations of the α-subunit gene that lead to infantile (classic) Tay–Sachs disease. These include deletion (5), loss of RNA splice site (6–8), amino acid substitution (9, 10), and premature termination (11). The Ashkenazi Jewish population, known to have a high (3%) frequency of heterozygotes, has been shown to harbor a splice-site mutation at intron 12 and at least one other mutation for infantile Tay–Sachs disease (6, 7).

The variant G_{M2} gangliosidoses are less well characterized, clinically or biochemically, than infantile Tay–Sachs disease. Patients present with signs of cerebellar and motor neuron involvement and, on occasion, with psychosis but with normal sensory function; they may carry diagnoses such as amyotrophic lateral sclerosis, atypical Friedreich ataxia, atypical spinocerebellar degeneration, or spinal muscular atrophy (1, 12–16). Cognition and intelligence deteriorate markedly in the juvenile but minimally in the chronic or adult-onset forms. Because they have been diagnosed mainly in the Ashkenazi population, sometimes in families with classic Tay–Sachs disease (17–21), it has been suggested that the variant G_{M2} gangliosidoses are due to compound heterozygosity of a rare mutation with a more common Tay–Sachs disease allele (1, 14, 16–22).

We had previously shown that fibroblasts from several patients with adult-onset, chronic, and juvenile G_{M2} gangliosidoses synthesized a defective α-subunit that failed to associate with the β-subunit (23). The α-subunit was not converted to the mature lysosomal form, presumably because it could not be transported to lysosomes. However, it could be secreted from the cell, a point that distinguishes this defect from those in which the α-subunit is retained in the endoplasmic reticulum (24, 25).

In the present study, we have identified a mutation that gives rise to the association-defective α-subunit. Our index case was a California patient of Ashkenazi Jewish origin with adult-onset G_{M2} gangliosidosis (12, 26), whose fibroblasts had been shown to synthesize the defective α-subunit (23). The strategy was first to map the mutation to a small region of the α-subunit mRNA; this was done by hybridizing the mRNA to a series of antisense RNA probes and localizing the RNase-sensitive mismatch that permits cleavage of an otherwise

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Abbreviations: G_{M2} ganglioside, *N*-acetylgalactosaminyl-β1→4-(*N*-acetylneuraminyl-α2→3)-galactosyl-β1→4-glucosyl-β1→1-ceramide; PCR, polymerase chain reaction.

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§Listed as both β-*N*-acetylhexosaminidase (EC 3.2.1.52) and *N*-acetyl-β-glucosaminidase (EC 3.2.1.30).

protected RNA duplex (27, 28). Then the candidate region was amplified by the polymerase chain reaction (PCR) (29), and the mutation was identified by direct sequence analysis. A simple assay, based on the loss of a *Scr*FI restriction site, allowed the rapid identification of other affected individuals and carriers.

MATERIALS AND METHODS

Materials. Restriction and modifying enzymes were purchased from New England Biolabs and Stratagene. Deoxynucleotides and RNases A and T_1 were purchased from Pharmacia LKB; SP6 and T7 RNA polymerases and the Riboprobe transcription kit were purchased from Promega Biotec. Thermostable *Thermus aquaticus* (*Taq*) DNA polymerase was obtained from Perkin–Elmer/Cetus. Avian myeloblastosis virus reverse transcriptase was from Seikagaku. Modified T7 DNA polymerase (Sequenase) sequencing kit was from United States Biochemical. [α-^{32}P]CTP (400 Ci/mmol; 1 Ci = 37 GBq) was from Amersham, and [γ-^{32}P]ATP (7000 Ci/mmol) was from ICN. Oligonucleotide primers were synthesized by D. Glitz on a Dupont/Vega Coder 300 oligonucleotide synthesizer. Fetal bovine serum was from Irvine Scientific, and other cell culture reagents were from GIBCO.

Cell Strains and Culture Conditions. Normal human diploid fibroblasts (IMR90), fibroblasts from infantile Tay–Sachs patients (GM515 and GM2968), and lymphoblasts from chronic gangliosidosis patients (GM3441, GM3461, GM3770, and GM3575) were obtained from the Human Genetic Mutant Cell Repository (Camden, NJ). Fibroblast strains from California patients of Ashkenazi origin with adult-onset, chronic, or juvenile G_{M2} gangliosidosis (23) and from their parents were derived from skin biopsies obtained by M.M.K.; fibroblasts from an Assyrian juvenile G_{M2} gangliosidosis patient were provided by R. Gatti. Fetal fibroblasts deficient in β-hexosaminidase A were from our laboratory (M.M.K.) or obtained from E. E. Grebner. The fetal strains were classified as infantile Tay–Sachs if biosynthetic studies showed no α-subunit polypeptide and Northern blot analysis showed no α-subunit mRNA; they were classified as association-defective if biosynthetic studies showed no $\alpha\beta$ association and no maturation of the α-subunit but did show its secretion in the presence of NH_4Cl. Fibroblasts were cultured in modified Eagle's minimal medium with 15% (vol/vol) fetal bovine serum; lymphoblasts were cultured in RPMI 1640 with 10% fetal bovine serum.

Recombinant Plasmids. The plasmid containing the entire β-hexosaminidase α-subunit cDNA, pβHα5 (2), was provided by R. Myerowitz. The insert was digested with *Stu* I, *Sac* I, *Pvu* II, and *Hinc*II restriction endonucleases to generate fragments corresponding to exons 1, 1–3, 3–6, 6–9, 9–13, and 13–14; the restriction map and exon placements are found in refs. 2 and 30, respectively. The fragments were subcloned into pGEM3Z to serve as template of antisense RNA synthesis.

RNase Protection Assay. Total RNA was isolated by a published procedure (31). Poly(A)$^+$ RNA was purified by oligo(dT) chromatography. RNase protection assay was performed essentially as described (32). Radiolabeled antisense RNA probes were synthesized from linearized plasmids (described above) with T7 or SP6 RNA polymerase and [α-^{32}P]CTP as recommended by the manufacturer. Full-length probes were recovered from urea/polyacrylamide gels and hybridized (5×10^5 cpm per assay) with 3 μg of poly(A)$^+$ RNA at 65°C for 16 hr. The resulting RNA hybrids were digested with RNase A (40 μg/ml) and RNase T_1 (500 units/ml) at 30°C for 1 hr, followed by serial purification and analysis as described (32).

Reverse Transcription and PCR Amplification of cDNA. About 40 μg of total RNA was reverse transcribed with 80 units of reverse transcriptase, 6 μg of antisense primer hybridizing to exon 10 (5′ CCATAAGAAGAGACGATGTCCAGC 3′), and 6 μg of sense primer hybridizing to exon 7 (5′ GAAAGGGGTCCTACAACCCTG 3′).

PCR amplification (29) of the resulting cDNA was performed in a Perkin–Elmer/Cetus DNA thermal cycler by using the following profile: 2-min denaturation at 94°C, 3-min annealing at 43°C, 5-min extension at 72°C for 35 cycles, under conditions adapted from a protocol provided by New England Biolabs.

PCR Amplification of Genomic DNA. High molecular weight genomic DNA was isolated as described from fibroblasts and lymphoblasts (33) and from leukocyte pellets (34). Genomic DNA (1 μg) was annealed to 4 μg each of sense primer complementary to exon 7 (above) and antisense primer complementary to intron 7 (5′ ACATTCTTCTAAGGACCAAGGCT 3′). Amplification was carried out for 35 cycles as follows: 2-min denaturation at 94°C, 2-min annealing at 45°C, and 3-min extension at 72°C.

Direct Sequencing of PCR Products. PCR products were purified by gel filtration over a 1-ml bed volume of Sephadex G-50, two extractions with phenol/chloroform, and two precipitations with ethanol. An internal sequencing primer complementary to exon 7 (5′ CCCTGTCACCCACATCTACA 3′) was 5′-radiolabeled and annealed to the PCR product. Dideoxy sequencing (35) was performed with Sequenase as recommended by the manufacturer. A primer complementary to exon 8 (5′ GATAAAAATCTGGGAAGACAGAG 3′) was used to sequence the opposite strand.

***Scr*FI Restriction Analysis.** About 100 ng of PCR-amplified fragments of genomic DNA, purified as described above, were incubated with 5 units of *Scr*FI at 37°C for 1 hr; restriction fragments were analyzed by electrophoresis on a 15% nondenaturing polyacrylamide gel.

Assay for Intron 12 Splice-Site Mutation. Genomic DNA was annealed to an exon 12 sense primer (5′ CTGAGCAGAAGGCTCTGG 3′) and intron 12 antisense primer (5′ GGACAACTCCTGCTCTCAG 3′) and subjected to amplification. The allele-specific oligonucleotide hybridization was performed by a minor modification of a published procedure (36); the oligonucleotide probes for the intron 12 mutation were as described (6).

RESULTS

Localization of the Adult-Onset G_{M2} Gangliosidosis Mutation by RNase Protection. The mRNA encoding the α-subunit, isolated from fibroblasts of a patient with adult-onset G_{M2} gangliosidosis (12, 23, 26), was found by Northern blot analysis to be of normal size but only 15% of the normal level (data not shown). It was analyzed by the RNase protection assay by using a set of antisense RNA probes that jointly spanned the entire length of the α-chain mRNA. The only probe to show a specific mismatch with the patient's mRNA was the one generated from a 390-base-pair (bp) *Sac* I–*Pvu* II cDNA fragment, corresponding to exons 6–9. In addition to the protected band of 390 bp, two cleavage products of 245 and 145 bp resulted from RNase digestion of the probe hybridized to the patient's mRNA, indicating a mismatch located 40% of the way from one of the termini (Fig. 1). The two smaller products were not seen when normal mRNA was used. Moreover, they were not seen when the mRNA was derived from fibroblasts of a patient with infantile Tay–Sachs disease; although these fibroblasts appear to have essentially no α-subunit mRNA in Northern blot analysis, the small amount that is present (8, 37, 38) is detected in the more sensitive RNase protection assay.

Sequence Analysis of the Adult-Onset G_{M2} Gangliosidosis Mutation. A 450-bp fragment of cDNA spanning all of exons 7–9, obtained by reverse transcription of RNA followed by

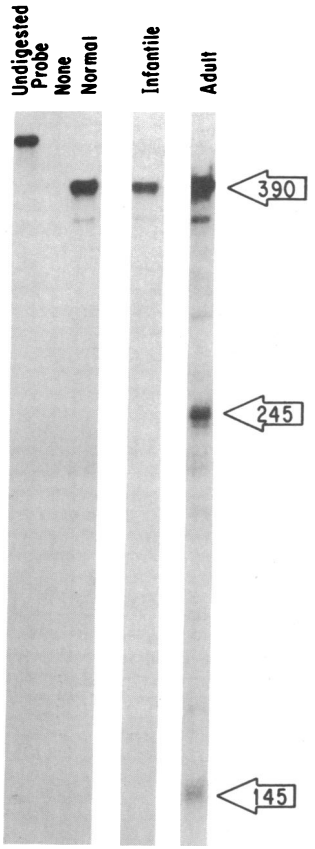

FIG. 1. Localization of the adult-onset G_{M2} gangliosidosis mutation by RNase protection. A labeled antisense RNA probe corresponding to exons 6–9 was annealed to poly(A)$^+$ RNA derived from normal, infantile Tay–Sachs disease, and adult-onset G_{M2} gangliosidosis fibroblasts. The RNA duplexes were digested with RNases A and T_1 to generate the protected fragments indicated by the arrows. The size difference between the probe (438 bp) and the normal protected fragment (390 bp) corresponds to the pGEM3Z polylinker sequence present in the probe. In the lane designated "none," the probe had been annealed to 20 μg of RNA depleted of polyadenylylated mRNA as a control for complete digestion of unprotected RNA. The figure is a composite of several radioautograms; the exposure time was varied to provide approximately equal intensity of the 390-bp band. The residual 390-bp band in the adult-onset sample is attributed to incomplete digestion.

PCR amplification, was sequenced directly by using an internal primer. A transition of guanosine to adenosine was found at position 805 (in exon 7) of the α-subunit mRNA (Fig. 2 *Upper*); the mutation would result in a substitution of serine (AGT) for glycine (GGT) at position 269 of the α-polypeptide [designated the 269 (Gly → Ser) substitution]. The nucleotide change was confirmed by sequencing the opposite strand (data not shown). The RNase cleavage pattern expected from this nucleotide change in the α-subunit mRNA corresponds exactly to that observed in Fig. 1.

Direct sequencing of the corresponding fragment of genomic DNA, amplified by PCR, showed the presence of both

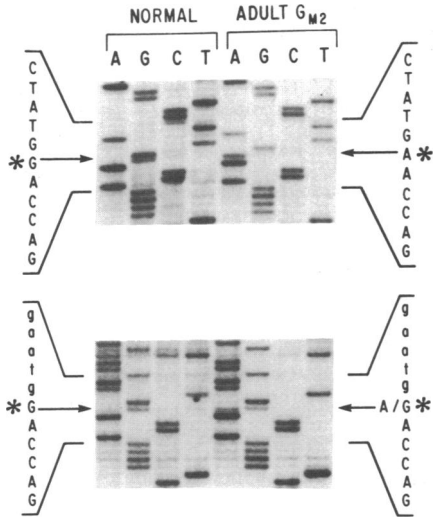

FIG. 2. Sequence analysis of the adult-onset G_{M2} gangliosidosis mutation. The sequences of the critical sections of cDNA (*Upper*) and genomic DNA (*Lower*) near the 3′ boundary of exon 7 are shown. Arrows point to the mutation.

the mutant adenosine and the normal guanosine at the last nucleotide position of exon 7 (Fig. 2 *Lower*). Thus the patient is a compound heterozygote, with only one allele having the guanosine to adenosine mutation in exon 7. Since the product of this allele was the only one seen in the α-subunit mRNA sequence, the other allele though normal in the exon 7 region, must not produce stable mRNA.

Restriction Enzyme Assay for the Mutation. The mutation can be detected by a simple assay, based on the loss of a *Scr*FI restriction site (5′ CCNGG 3′) where the inner guanosine (underlined) is replaced by adenosine. The principle of the assay is shown schematically in Fig. 3. The exon–intron 7 boundary region is amplified by PCR, and the resulting 250-bp product is digested with *Scr*FI. The cleavage, which occurs at sites denoted by arrows in Fig. 3, yields a 114-bp or a 122-bp fragment from the allele that is normal or mutant, respectively, in this region. The restriction fragments are separated by electrophoresis in a polyacrylamide gel and visualized with ethidium bromide. The enzyme also produces fragments of 78, 34, and 14 bp from both alleles, but only the larger of these invariant fragments remains in the gel.

As predicted, the restriction enzyme assay showed only the 114-bp fragment in DNA derived from normal fibroblasts and both the 114- and 122-bp fragments¶ in DNA derived from fibroblasts of the adult-onset G_{M2} gangliosidosis patient (Fig. 3). This is in accordance with the results of DNA sequencing, which had shown this patient to be a compound heterozygote. Amplified DNA from his father also had the two fragments, whereas that from his mother had only the shorter one (Fig. 3). Thus, consistent with Mendelian segregation, the father must be the carrier of the exon 7 adult-onset G_{M2} gangliosidosis mutation, whereas the mother must be the carrier of the functionally null allele.

Loss of the *Scr*FI site in the allele was observed in DNA derived from the following cells: fibroblasts from the California patient with chronic G_{M2} gangliosidosis, previously described as having an association-defective α-subunit (23), and from this patient's mother; lymphoblast lines GM3441, GM3461, GM3770, and GM3575, all derived from patients with chronic G_{M2} gangliosidosis (13); and fibroblasts from two fetuses diagnosed as β-hexosaminidase A-deficient and later shown by biosynthetic studies to have an association-defective α-subunit (E.F.N., unpublished results). The electrophoretic separation of the two restriction fragments is shown in Fig. 3 for the DNA sample derived from lymphoblast line GM3461; identical patterns were produced by the others (data not shown). To rule out the possibility that a different mutation had obliterated the *Scr*FI site, the presence of the guanosine to adenosine transition was confirmed by direct sequencing of the PCR-amplified genomic DNA.

There was no loss of the *Scr*FI restriction site in 37 control samples of DNA tested. These negative samples were derived from fibroblasts of nine infantile Tay–Sachs disease patients of Ashkenazi origin (GM515, shown in Fig. 3; GM2968; and seven fetal strains), one of French-Canadian origin (WG884), and one of different ethnic background; from leukocytes or fibroblasts of 21 Ashkenazi and three non-Ashkenazi individuals, who are not carriers of β-hexosaminidase A deficiency; and from fibroblasts of two patients with other disorders. These results show that the exon 7 mutation

¶The higher yield of the 122-bp fragment than of the 114-bp fragment when the two were present in the same sample (Fig. 3) results from the manner in which the PCR procedure was performed. After amplification is carried out for 35 cycles, oligonucleotides may be depleted; the amplified fragments would anneal to each other in the last cycle in a theoretical ratio of 1:1:2 for normal homoduplexes, mutant homoduplexes, and heteroduplexes, respectively. Since the heteroduplexes, like the mutant homoduplexes, would not be cleaved by *Scr*FI, the ratio of 122- to 114-bp fragments would be 3:1.

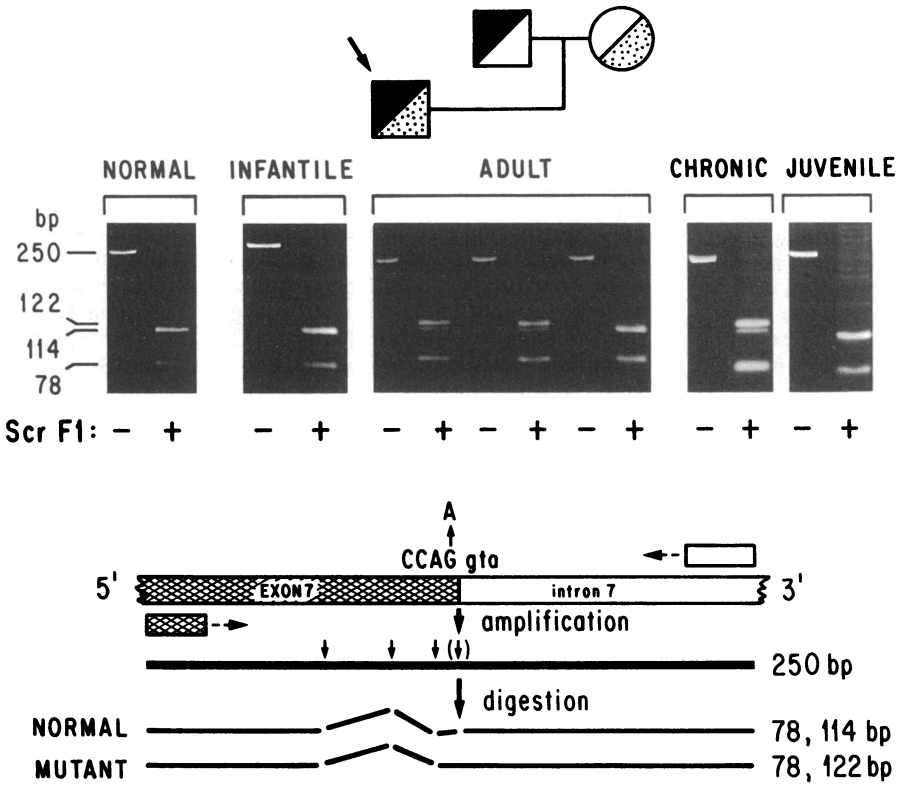

FIG. 3. *Scr*FI restriction assay for the exon 7 mutation. Ethidium bromide-stained polyacrylamide gels, showing electrophoretic separation of fragments obtained after incubation of PCR-amplified genomic DNA segment with (+) or without (−) *Scr*FI restriction endonuclease are depicted. The origin of the fragments is explained schematically below the gels. The DNA samples were derived (from left to right) from normal fibroblasts (IMR90) and fibroblasts from patients with infantile Tay–Sachs disease (GM515); adult-onset G_{M2} gangliosidosis, and the patient's father and mother; chronic G_{M2} gangliosidosis (GM3461); and juvenile G_{M2} gangliosidosis. Loss of the 250-bp fragment following treatment with *Scr*FI shows that cleavage by the endonuclease was complete.

is not found in individuals who either have a normal level of β-hexosaminidase or have two alleles that give rise to infantile Tay–Sachs disease.

In addition, the *Scr*FI site was present in the DNA from fibroblasts of two patients with juvenile G_{M2} gangliosidosis (Fig. 3) even though these have an association-defective α-subunit (ref. 23; unpublished results). One of the patients is of half-Jewish origin (39); the other is Assyrian. These cases demonstrate that the association-defective phenotype can result from mutations other than 269 (Gly → Ser).

Identification of the Mutation in the Second Allele. The DNA of the eight compound heterozygotes identified by *Scr*FI restriction analysis was tested by hybridization with specific oligonucleotides for the intron 12 guanosine to cytidine splice-site mutation that has been observed in some patients with infantile Tay–Sachs disease (6–8). Three samples derived from GM3441, GM3461, and the California chronic G_{M2} gangliosidosis patient hybridized to the mutant oligonucleotide (data not shown). The other samples, two of which had been shown to carry a functionally null allele by comparison of cDNA and genomic sequences, presumably carry a different null mutation for infantile Tay–Sachs disease.

DISCUSSION

We have described a mutation of the α-subunit gene of β-hexosaminidase, a guanosine to adenosine substitution at the 3′ end of exon 7, present in one allele of patients with chronic or adult-onset G_{M2} gangliosidosis. The mutation in the other allele was identified as functionally null by the absence of the corresponding mRNA in two cases and by the presence of the intron 12 splice-site mutation (6–8) in three others. Therefore the exon 7 mutation, which causes a replacement of glycine by serine at position 269, must be responsible for the abnormal α-subunit and the low but finite level of β-hexosaminidase A activity seen in these patients. A finding of the same mutation in patients diagnosed as chronic and adult onset is not remarkable, since the clinical distinction between the disorders is somewhat arbitrary (1, 14). It is unlikely that the exon 7 mutation is a neutral polymorphism, as it was not present in the DNA from 37 individuals who did not have an association-defective phenotype. On the other hand, other mutations, as yet unidentified, may also result in the association-defect, as seen in two cases of juvenile G_{M2} gangliosidosis.

From data obtained in voluntary screening programs, it has been estimated that the frequency of alleles causing the variant G_{M2} gangliosidosis in the Ashkenazi population is 1 in 1200, or one-fortieth the frequency of alleles causing infantile Tay–Sachs disease (22). Since screening tests based on hexosaminidase A activity do not discriminate between carriers of the infantile and the variant disorders, 1 in 20 Ashkenazi heterozygous couples, advised that they were at risk to have a child with Tay–Sachs disease, might in fact be at risk for a child affected with a variant G_{M2} gangliosidosis. A simple DNA-based test, such as the *Scr*FI restriction assay or allele-specific oligonucleotide hybridization, should allow correct identification if applied to all enzymatically-diagnosed couples at risk. However, the information may not provide easy choices for the families, as the variant disorders are not necessarily benign. The chronic and adult-onset G_{M2} gangliosidoses are characterized by great variability of clinical manifestations, even between siblings (1, 14, 15). While the condition may be mild in some, it may present as severe psychosis or debilitating neurological disease in others. Such intrafamilial variability, which presents serious problems for genetic counseling, means that the formation and effectiveness of a marginal amount of β-hexosaminidase A can be influenced by other factors. These would include the many genetic and perhaps environmental factors that impinge on the synthesis of lysosomal enzymes, as well as on the synthesis and degradation of G_{M2} ganglioside.

At the protein level the 269 (Gly → Ser) substitution gives rise to a nonassociating α-subunit. This finding should not be construed as evidence for a direct effect of the substitution on subunit contact points or even on the association process itself. The amino acid change may affect conformation or perhaps some posttranslational modification that is required

for association to occur. Because the process of $\alpha\beta$ subunit association is poorly understood (40), it is not possible to choose among the possibilities. The 269 (Gly → Ser) mutation and other mutations, whether naturally occurring or generated by site-directed mutagenesis, may help elucidate the mechanism by which $\alpha\beta$ association is accomplished.

The guanosine to adenosine transition that causes the 269 (Gly → Ser) substitution occurs in the last nucleotide of exon 7. Similarly located mutations in other genes have been shown to result in splicing defects. The *spf*^{ash} mutation of murine ornithine transcarbamylase, a guanosine to adenosine transition in the last nucleotide of exon 4, activates a cryptic splice site that is used in preference to the normal one and produces an elongated mRNA (41). A transition of guanosine to adenosine in the last nucleotide of exon 6 of the human type 1 collagen gene causes skipping between exons 5 and 7 (42). The possibility that some missplicing also occurs in the mutation of adult-onset or chronic G_{M2} gangliosidosis cannot be ruled out. The amount of α-polypeptide synthesized by the adult-onset G_{M2} gangliosidosis patients' fibroblasts was found to be one-fourth of normal (23), and the amount of α-subunit mRNA observed by Northern blot analysis and RNase protection assay was about one-sixth. Given the compound heterozygosity with a null allele, approximately half the normal amount of steady-state mRNA and of newly synthesized polypeptide would be expected. To account for the deficit, we speculate that some missplicing might have occurred, giving rise to a nonfunctional, unstable mRNA that would not have been observed in sequence analysis.

Note. While this manuscript was in preparation, we learned that similar results had been obtained by Navon and Proia (43), who discovered the 269 (Gly → Ser) mutation in other patients with adult-onset G_{M2} gangliosidosis.

Note Added in Proof. After a 4-bp insertion in exon 11 was reported to be the major cause of infantile Tay–Sachs disease in the Ashkenazi population (44), we determined that the patients with a 269 (Gly → Ser) mutation on one allele had either an intron 12 splice-site transversion or an exon 11 insertion on the other allele.

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