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Ashkenazi-Jewish and Non-Jewish Adult G_{M2} Gangliosidosis Patients Share a Common Genetic Defect

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Summary

The adult form of Tay-Sachs disease, adult G_{M2} gangliosidosis, is an autosomal recessive neurological disorder caused by a partial deficiency of β-hexosaminidase A. We had previously identified, in Ashkenazi-Jewish adult G_{M2} gangliosidosis patients, a $Gly^{269}\rightarrow Ser$ mutation in the β-hexosaminidase α-subunit. All of the Ashkenazi patients were found to be compound heterozygotes with an allele containing the $Gly^{269}\rightarrow Ser$ mutation together with one of the Ashkenazi infantile Tay-Sachs alleles. We have now found the same $Gly^{269}\rightarrow Ser$ mutation in six adult G_{M2} gangliosidosis patients from four different non-Jewish families. Genomic DNA from three of the patients, two of whom were brothers, exhibited a hybridization pattern consistent with homozygosity for the $Gly^{269}\rightarrow Ser$ mutation. The remaining non-Jewish patients were compound heterozygotes of the $Gly^{269}\rightarrow Ser$ mutation together with an unidentified α-subunit mutation. The results demonstrate that individuals homozygous for the $Gly^{269}\rightarrow Ser$ change can be clinically affected. The same $Gly^{269}\rightarrow Ser$ mutation in both the Ashkenazi and non-Jewish patients may be the result of a common ancestor, given that the ancestry of these non-Jewish patients, like the Ashkenazim, can be traced to eastern Europe.

Introduction

The G_{M2} gangliosidoses are a group of autosomal recessive neurological disorders caused by a marked deficiency of the lysosomal enzyme β-hexosaminidase A (reviewed by Sandhoff et al. 1989). The enzyme deficit results in an accumulation of the natural substrate of β-hexosaminidase A, G_{M2} ganglioside, which in turn leads to neuronal malfunction. Deficiency of β-hexosaminidase A activity can be caused by mutations in either of the genes that encode the subunits, α or β, of the enzyme or in a third gene encoding the G_{M2} activator protein which is essential for the hydrolysis of G_{M2} ganglioside.

A total loss of enzyme activity as a result of mutations in the α-subunit gene causes the infantile form of Tay-Sachs disease, a lethal neurodegenerative disorder. The adult form of Tay-Sachs disease, termed adult G_{M2} gangliosidosis, is caused by defects in the α-subunit gene that result in a narrow range of residual enzyme activity. In the adult disease (reviewed by Navon et al. 1986), onset usually occurs in the second or third decade of life, with lower-motor-neuron, pyramidal tract, and cerebellar deterioration. In some patients psychosis precedes the other neurological symptoms. A notable feature of the adult disorder is its great variability even among affected siblings.

Recently progress has been made in the identification of the mutations in the β-hexosaminidase α-subunit that cause the G_{M2} gangliosidoses (reviewed by Neufeld 1989). The Ashkenazi-Jewish population has been of special interest because of an elevated incidence of both infantile Tay-Sachs disease and adult G_{M2} gangliosidosis. Two mutations have been identified in the α-subunit gene which underlie infantile Tay-Sachs disease among these patients; these two mutations are (1)

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a 4-bp insertion in exon 11, accounting for ~70% of the mutant alleles (Myerowitz and Costigan 1988), and (2) a splice-site mutation at the 5′ end of intron 12 in the remaining 30% (Arpaia et al. 1988; Myerowitz 1988; Ohno and Suzuki 1988). Both of these mutations result in a complete absence of β-hexosaminidase A activity. In Ashkenazi-Jewish patients with adult G_{M2} gangliosidosis, we (Navon and Proia 1989) and others (Paw et al. 1989) have identified a single base change at the 3′ end of exon 7 of the α-subunit gene that results in the substitution of Ser for Gly at position 269 in the α-polypeptide. All of the Ashkenazi patients were found to be compound heterozygotes for the $Gly^{269}\rightarrow Ser$ change together with one of the allelic infantile Tay-Sachs mutations. The heterozygosity in the Ashkenazi patients is likely a consequence of the high frequency of the infantile Tay-Sachs alleles together with a relatively rare $Gly^{269}\rightarrow Ser$ mutation in the Ashkenazi population. It is also possible that, because the $Gly^{269}\rightarrow Ser$ mutation gives rise to some residual β-hexosaminidase A activity (Conzelmann et al. 1983), individuals homozygous for this mutation might (*a*) express enzyme activity above a critical threshold, leading to a mildly affected or even asymptomatic state, and (*b*), as result, escape detection.

In an attempt to determine whether individuals homozygous for the $Gly^{269}\rightarrow Ser$ mutation express clinical manifestations, we screened non-Jewish adult G_{M2} gangliosidosis patients for this mutation. Our reasoning was that, if the $Gly^{269}\rightarrow Ser$ mutation was present in these patients, then it might be more likely to exist in a homozygous state because of the much lower frequency of infantile Tay-Sachs alleles in the non-Jewish population.

Methods

DNA Sources

The diagnosis of the adult G_{M2} gangliosidosis patients was based on a deficiency of β-hexosaminidase A activity together with a clinical presentation consistent with the disorder (Navon et al. 1986).

Family A (fig. 1), which has two siblings, 34 and 36 years old, who have adult G_{M2} gangliosidosis, has been described elsewhere (Mitsumoto et al. 1985). The non-Jewish paternal grandparents of the patients were from the Ukraine. Their non-Jewish maternal ancestors migrated from Yugoslavia.

A peripheral blood sample was obtained from a 36-year-old non-Jewish female patient (L-89-23) with adult G_{M2} gangliosidosis. Her maternal ancestors were from Poland, Ireland, and England, while the paternal side of the family was from "one of the Slavic countries."

Fibroblast cultures were derived from non-Jewish brothers, My.K. and Mi.K., 53 and 61 years old, respectively, who have adult G_{M2} gangliosidosis. One of their parents was from Yugoslavia, and the other was from Czechoslovakia.

D.W. is a 38-year-old male with adult G_{M2} gangliosidosis. His family (family B) is non-Jewish and of Polish descent. The mother's family was from the Rosanka region of Austria-Poland, and the father's family was from the Russian-Polish border.

A fibroblast culture was derived from an Ashkenazi-Jewish G_{M2} gangliosidosis patient, S.R., who has been described elsewhere (Navon and Proia 1989).

Genomic DNA was isolated from fibroblast cultures (in patients My.K., Mi.K., and S.R.) and blood samples (in family A, family B, L-89-23, and normal controls) according to methods described elsewhere (Maniatis et al. 1982).

Amplification and Detection of Specific DNA Sequences

The $Gly^{269}\rightarrow Ser$ mutation was detected by hybridization with allele-specific oligonucleotide probes. This method has been demonstrated to be reliable for the detection of this mutation (Navon and Proia 1989). Genomic DNA was amplified using *Taq* DNA polymerase (Perkin-Elmer Cetus) (Saiki et al. 1988) and oligonucleotides primers, 5′GGGTCCTACAACCCTGTCACCCAC 3′ and 5′AAGCTTCACTCTGAGCATAACAAG 3′, specific for exon 7 and intron 7, respectively, of the α-subunit gene (Proia and Soravia 1987). The DNA was subjected to amplification on a Perkin-Elmer Cetus thermocycler for 39 cycles. Each cycle was 2 min at 94°C for denaturation, 2.5 min at 60°C for annealing, and 3 min at 72°C for synthesis. The regions surrounding the Ashkenazi infantile Tay-Sachs mutations were amplified according to a method described elsewhere (Myerowitz 1988; Myerowitz and Costigan 1988).

The amplified DNA was blotted on GeneScreen Plus filters (New England Nuclear), denatured, neutralized, and dried. Prehybridization was for 1 h at 37°C in a solution containing 1 M NaCl, 0.005 M EDTA, 1% SDS, 100 μg denatured salmon-sperm DNA/ml. Hybridization was performed overnight at 37°C after addition of the appropriate [^{32}P]-labeled allele-specific oligonucleotide probe (~2.5 ng/ml). The sequence of the oligonucleotide probe specific for the $Gly^{269}\rightarrow Ser$ mutation was 5′TGGGGACCAAGTAAGAATG 3′. The

sequence of the corresponding normal probe was 5′TGGGGACCAGGTAAGAATG 3′. The sequences of the probes for detection of the Ashkenazi infantile mutations were as described elsewhere (Myerowitz 1988; Myerowitz and Costigan 1988).

After hybridization, the blots were washed twice for 30 min at room temperature in 2 × SSC containing 0.5% SDS and then for 5 min at 55°C with 2 × SSC.

Results

Two non-Jewish siblings with adult G_{M2} gangliosidosis have been described by Mitsumoto et al. (1985). The genomic DNA from these patients (II-1 and II-2), an unaffected sibling (II-3), and their parents was tested for the presence of the $Gly^{269}\rightarrow Ser$ mutation (fig. 1). Both affected siblings and their father were positive for the $Gly^{269}\rightarrow Ser$ mutation. Like the Ashkenazi patient, compound heterozygosity was indicated in the two non-Jewish patients because their DNA was also positive when tested with an allele-specific probe carrying the normal sequence from the region of this mutation. However, unlike the Ashkenazi patients, the non-Jewish patients did not carry either of the Ashkenazi infantile Tay-Sachs mutations, i.e., the intron 12 slice-junction mutation and the 4-bp insertion in exon 11 (not shown). Both non-Jewish patients are, therefore, compound heterozygotes of an allele bearing the $Gly^{269}\rightarrow Ser$ mutation inherited from their father, together with an unidentified mutant allele from their mother, who demonstrates levels of β-hexosaminidase A activity in the carrier range. The unaffected sibling shows normal levels of enzyme activity and is presumed not to carry a mutation.

The genomic DNA from four additional non-Jewish patients, two of whom (My.K. and Mi.K.) are siblings, was also found to be positive for the $Gly^{269}\rightarrow Ser$ mutation (fig. 2). Patient L-89-23 DNA, like the previously described non-Jewish patients, was positive when assayed with the allele-specific probe carrying the normal sequence and is presumed to be a compound heterozygote. This patient also tested negative for both Ashkenazi infantile mutations.

In contrast, the DNA from patients D.W., My.K., and Mi.K. was negative when assayed with the allele-specific probe carrying the normal sequence. This pattern of hybridization with these two allele-specific probes is indicative of homozygosity for the $Gly^{269}\rightarrow Ser$ mutation. However, if the allele carrying the $Gly^{269}\rightarrow Ser$ mutation was in compound heterozygosity with an allele harboring either a deletion or some other sequence change in the region of the $Gly^{269}\rightarrow Ser$ change, the same hybridization pattern would be observed. Homozygosity for the $Gly^{269}\rightarrow Ser$ mutation could be proved if maternal and paternal inheritance of the mutation could be demonstrated. As shown in figure 3, the mother of patient D.W. is positive for the $Gly^{269}\rightarrow Ser$ mutation. Although the father is deceased, paternal in-

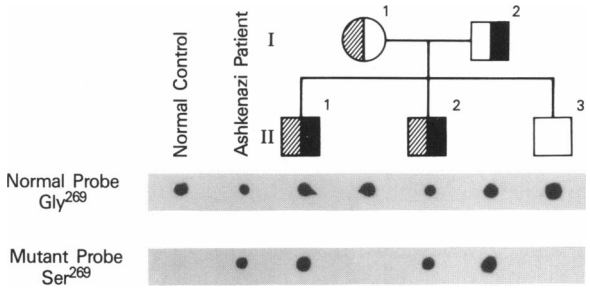

Figure 1 Analysis of a non-Jewish family with two affected siblings with adult G_{M2} gangliosidosis for the $Gly^{269}\rightarrow Ser$ mutation. Polymerase chain reaction (PCR)–amplified genomic DNA from a clinically normal individual, an Ashkenazi G_{M2} gangliosidosis patient, and a non-Jewish family, family A, with two affected siblings (II-1 and II-2) was assayed for the presence of the $Gly^{269}\rightarrow Ser$ mutation. The DNAs were hybridized with an allele-specific oligonucleotide probe specific for the $Gly^{269}\rightarrow Ser$ mutation (Mutant Probe) and with an oligonucleotide specific for the corresponding normal region (Normal Probe). A solid symbol indicates the presence of the $Gly^{269}\rightarrow Ser$ mutation. A hatched symbol indicates the presence of an unidentified α-subunit mutation. An open symbol indicates a normal α-subunit allele.

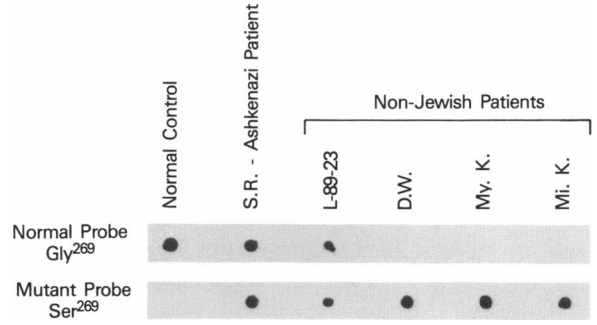

Figure 2 Identification of the $Gly^{269}\rightarrow Ser$ mutation in four non-Jewish adult G_{M2} gangliosidosis patients. PCR-amplified genomic DNA from a clinically normal individual, an Ashkenazi-Jewish adult G_{M2} gangliosidosis patient, and four non-Jewish adult G_{M2} gangliosidosis patients was assayed for the presence of the $Gly^{269}\rightarrow Ser$ mutation. The DNA were hybridized with an allele-specific oligonucleotide probe specific for the $Gly^{2696}\rightarrow Ser$ mutation (Mutant Probe) and with a oligonucleotide specific for the corresponding normal region (Normal Probe).

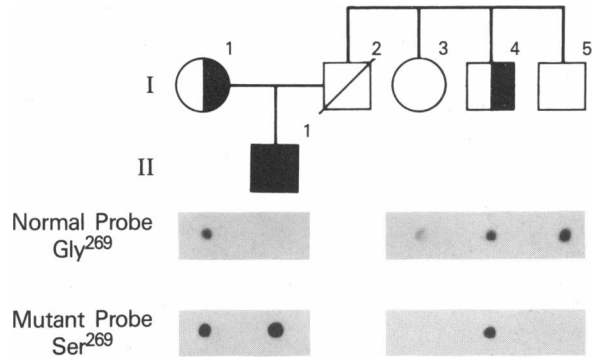

Figure 3 Demonstration of homozygosity of the Gly^{269}→Ser mutation in a non-Jewish adult G_{M2} gangliosidosis patient. PCR-amplified genomic DNA from a non-Jewish patient, D.W., and his family, family B, was assayed for the presence of the Gly^{269}→Ser mutation. The DNA was hybridized with an allele-specific oligonucleotide probe specific for the Gly^{269}→Ser mutation (Mutant Probe) and with an oligonucleotide specific for the corresponding normal region (Normal Probe). A solid symbol indicates the presence of the Gly^{269}→Ser mutation. An open symbol indicates a normal α-subunit allele.

heritance of the mutation is indicated by the presence of the Gly^{269}→Ser change in the DNA of one of the father's siblings (I-4). This individual displays heterozygote levels of β-hexosaminidase activity that are consistent with the presence of the Gly^{269}→Ser mutation.

Discussion

Very small variations in residual enzyme activities are believed to greatly influence the clinical course of patients with late-onset lysosomal storage diseases (Conzelmann and Sandhoff 1984). Since the Gly^{269}→Ser mutation gives rise to some residual activity, it is conceivable that homozygous individuals might be asymptomatic or less severely affected than the patients who have the Gly^{269}→Ser mutation in allelic combination with one of the null, Tay-Sachs infantile mutations. The results presented here demonstrate that individuals homozygous for the Gly^{269}→Ser mutation can be clinically affected. Although the homozygous patients appear to be at the mild end of the clinical spectrum of adult G_{M2} gangliosidosis (Navon et al. 1986), the effect of homozygosity of the Gly^{269}→Ser mutation on clinical severity cannot be determined at this time, because of the small number of patients. In any case, this genotype would be a factor in very few patients, because the majority are Ashkenazi Jews, who, thus far, have proved to be compound heterozygotes. Other components, both genetic and environmental, may play a role in the extreme variability of clinical severity in this disorder in most of patients.

The Ashkenazi patients studied to date carry the Gly^{269}→Ser mutation in compound heterozygosity with one of the two Ashkenazi infantile Tay-Sachs mutations. In contrast, the non-Jewish patients were either homozygous for the Gly^{269}→Ser mutation or were heterozygotes for this mutation with an unidentified mutation. The apparent increased homozygosity for the Gly^{269}→Ser mutation among non-Jewish patients compared with Ashkenazi patients can be attributed to the different frequencies of the infantile Tay-sachs disease alleles among the Ashkenazi (1/31) and the non-Jewish (1/167) populations, together with a relatively rare Gly^{269}→Ser mutation in both groups (Greenberg and Kaback 1982; Navon and Adam 1985; Sandhoff et al. 1989). The absence of either of the Ashkenazi infantile mutations—i.e., the 4-bp insertion in exon 11 and the splice-site mutation in intron 12—in the non-Jewish compound heterozygotes is consistent with a much lower occurrence of these mutations in the non-Jewish population (E. E. Grebner and R. Myerowitz, personal communication).

Thus far, all adult G_{M2} gangliosidosis patients tested, Jewish and non-Jewish, have been found to carry the Gly^{269}→Ser mutation. It is interesting that the ancestry of the non-Jewish patients can be traced to eastern Europe, the region of the historical origins of Ashkenazi Jews. This suggests that the mutation in both groups of patients may have originated in a common ancestor.

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References

Arpaia E, Dumbrille-Ross A, Maler T, Neote K, Tropak M, Troxel C, Stirling JL, et al (1988) Identification of an altered splice site in Ashkenazi Tay-Sachs disease. Nature 333:85–86

Conzelmann E, Kytzia HJ, Navon R, Sandhoff K (1983) Ganglioside G_{M2} *N*-acetyl-β-D-galactosaminidase activity in cultured fibroblasts of late-infantile and adult G_{M2} gan-

gliosidosis patients and of healthy probands with low hexosaminidase level. Am J Hum Genet 35:900–913

Conzelmann E, Sandhoff K (1984) Partial enzyme deficiencies: residual activities and the development of neurological disorders. Dev Neurosci 6:58–71

Greenberg DA, Kaback MM (1982) Estimation of the frequency of hexosaminidase variant alleles in the American Jewish population. Am J Hum Genet 34:444–451

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

Mitsumoto H, Sliman RJ, Schafer IA, Sternick CS, Kaufman B, Wilbourn A, Horwitz SJ (1985) Motor neuron disease and adult hexosaminidase A deficiency in two families; evidence for multisystem degeneration. Ann Neurol 17: 378–385

Myerowitz R (1988) Splice junction mutation in some Ashkenazi Jews with Tay-Sachs disease: evidence against a single defect within this ethnic group. Proc Natl Acad Sci USA 85:3955–3959

Myerowitz R, Costigan FC (1988) The major defect in Ashkenazi Jews with Tay-Sachs disease is an insertion in the gene for the α-chain of β-hexosaminidase. J Biol Chem 263:18587–18589

Navon R, Adam A (1985) Frequency of hexosaminidase A variant alleles among Ashkenazi Jews and prenatal diagnosis of G_{M2} gangliosidosis. Am J Hum Genet 37: 1031–1033

Navon R, Argov Z, Frisch A (1986) Hexosaminidase A deficiency in adults. Am J Med Genet 24:179–196

Navon R, Proia RL (1989) The mutations in Ashkenazi Jews with adult G_{M2} gangliosidosis, the adult form of Tay-Sachs disease. Science 243:1471–1474

Neufeld EF, (1989) Natural history and inherited disorders of a lysosomal enzyme, β-hexosaminidase. J Biol Chem 264:10927–10930

Ohno K, Suzuki K (1988) A splicing defect due to an exon-intron junctional mutation results in abnormal β-hexosaminidase α-chain mRNA. Biochem Biophys Res Commun 153:463–469

Paw B, Kaback MM, Neufeld EF (1989) 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. Proc Natl Acad Sci USA 86:2413–2417

Proia RL, Soravia E (1987) Organization of the gene encoding the human β-hexosaminidase α-chain. J Biol Chem 262:5677–5681

Saiki RK, Gelfand DH, Stoffel S, Scharf SJ, Higuchi R, Horn GT, Mullis KB, et al (1988) Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science 239:487–491

Sandhoff K, Conzelmann E, Neufeld EF, Kaback MM, Suzuki K (1989) The G_{M2} gangliosidoses. In: Scriver CR, Beaudet AL, Sly WS, Valle D (eds) The metabolic basis of inherited disease, 6th ed. McGraw-Hill, New York, pp 1807–1842