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Distribution of Three α-Chain β-Hexosaminidase A Mutations Among Tay-Sachs Carriers

Eugene E. Grebner and Jerzy Tomczak

Division of Medical Genetics, Jefferson Medical College, Thomas Jefferson University, Philadelphia

Summary

DNA from 176 carriers of the Tay-Sachs gene was tested for the presence of the three mutations most commonly found among Ashkenazi Jews: the so-called insertion, splice junction, and adult mutations. Among 148 Ashkenazi Jews tested, 108 had the insertion mutation, 26 had the splice junction mutation, five had the adult mutation, and nine had none of the three. Among 28 non-Jewish carriers tested, most of whom were obligate carriers, four had the insertion mutation, one had the adult mutation, and the remaining 23 had none of the three.

Introduction

The GM2 gangliosidoses are a group of neurodegenerative genetic diseases caused by a deficiency of hexosaminidase A that leads to a pathological accumulation of GM2 ganglioside (Sandhoff et al. 1989). Clinically, the diseases present in varying degrees of severity related to the extent of the hexosaminidase A deficiency. Tay-Sachs disease, the prototype of this group, has been diagnosed in a variety of ethnic groups but is most prevalent among Ashkenazi Jews. Three different mutations, all in the α-subunit gene, account for most Tay-Sachs disease in this group. They are a G-to-A inversion at the splice junction of intron 12 (Arpaia et al. 1988; Myerowitz 1988; Ohno and Suzuki 1988), a four-base insertion within exon 11 (Myerowitz and Costigan 1988) and a G-to-A inversion in exon 7 (Navon and Proia 1989; Paw et al. 1989). The first two cause the classical infantile form, and the latter, the adult form.

We have tested 176 carriers for the presence of these three mutations to help determine their distribution and to see how the DNA tests compare with the results of enzyme-based carrier tests.

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Address for correspondence and reprints: Eugene E. Grebner, Ph.D., 414 Medical Office Building, Thomas Jefferson University, 1100 Walnut Street, Philadelphia, PA 19107.

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Material and Methods

Genomic DNA was extracted from frozen leukocyte pellets essentially as described by Bell et al. (1981). The portion of genomic DNA containing the mutation of interest was amplified by PCR using the GeneAmp kit and a DNA Thermal Cycler, both from Perkin-Elmer Cetus. PCR was run for 30 cycles, each cycle consisting of 1 min at 94°C (denaturing), 55°C (annealing) and 72°C (extension). The amplified fragments were analyzed for the presence of each mutation as described below and in figure 1.

Insertion Mutation

A 159-bp fragment that flanks the mutation in exon 11 was amplified using primers described elsewhere (Myerowitz and Costigan 1988). The presence of the insertion mutation was detected by electrophoresing 10 μl of the PCR reaction mixture on a 4% agarose minigel or a 15% polyacrylamide gel.

Splice Junction Mutation

A 135-bp fragment that flanks the mutation in intron 12 was amplified using primers described elsewhere (Myerowitz 1988). The splice junction mutation was detected by electrophoresis of the amplified product after *Dde*I digestion (Myerowitz 1988). The reaction mixture for *Dde*I digestion consisted of 10 μl of the PCR reaction mixture and 2 μl of the appropriate 10 × buffer containing 1 unit of *Dde*I and was

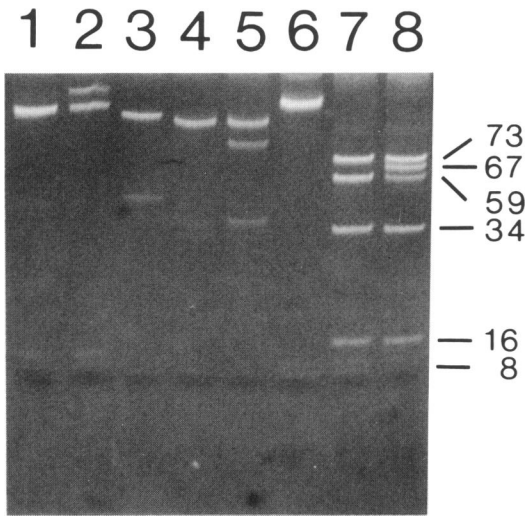

Figure 1 Identification of the three mutations by electrophoresis in 15% polyacrylamide gels. For the insertion mutation, the amplified fragments from individuals with two normal alleles appear as a single band (lane 1), while those from heterozygotes carrying the insertion mutation produce a second, slower-moving band (lane 2), which is presumably a hybrid duplex consisting of one normal fragment and one insertion-containing fragment (Shore and Myerowitz 1990; Triggs-Raine et al. 1990). For the splice junction mutation, the untreated amplified fragments migrate as a single 135-bp band (lane 3); digestion of normal fragments with *Dde*I trims short oligomers from each end to produce a 120-bp band (lane 4); *Dde*I digestion of fragments from heterozygotes for the slice junction mutation produces an additional 85- and 35-bp band resulting from a new *Dde*I site created by the mutation (lane 5) (Myerowitz 1988). For the adult mutation, the untreated amplified fragments migrate as a single 190-bp band (lane 6); digestion of normal fragments with *Scr*FI yields five smaller fragments of 73, 59, 34, 16, and 8 bp (lane 7); the mutation abolishes one of the *Scr*FI sites to produce a new 67-bp fragment in heterozygotes (lane 8).

incubated overnight at 37°C. The entire reaction mixture was electrophoresed on a 15% polyacrylamide gel.

Adult Mutation

A 190-bp fragment that flanks the mutation in exon 7 was amplified using primers described elsewhere (Navon and Proia 1989). The adult mutation was detected by electrophoresis after *Scr*FI digestion of the amplified product (Paw et al. 1989). The reaction mixture for *Scr*FI digestion consisted of 10 μl of the PCR reaction mixture and 2 μl of the appropriate 10 × buffer containing 2 units of *Scr*FI and was incubated for 6 h at 37°C. The entire reaction mixture was electrophoresed on a 15% polyacrylamide gel.

Results and Discussion

We have analyzed DNA from 176 putative carriers of the Tay-Sachs gene for the insertion, splice junction, and adult mutations. The carriers, all unrelated, were originally identified as such through our carrier-detection screening program, or because they had a child with Tay-Sachs disease. Of the 176 carriers, 148 were Jewish and 28 were non-Jewish.

Of the 148 Jewish carriers, 139 had one of the three mutations, and nine had none of the three. The insertion mutation was the most common (78%), followed by the splice junction mutation (19%) and the adult mutation (3%) (table 1). The distribution was essentially the same between 24 obligate carriers and 115 Jewish carriers identified by enzyme assay.

Of the nine Jewish carriers that had none of the three mutations, seven had been identified by enzyme assay and two were obligate carriers. Obviously the two obligates carried either a new mutation or one of the other known mutations for which we have not tested. For the seven identified by enzyme assay, there exists the additional possibility that they were misclassified and are actually not carriers. While we cannot rule out this possibility at present, all seven had test

Table 1

Distribution of Three Different α-Chain Hexosaminidase A Mutations among Tay-Sachs Carriers

Mutation	Jewish Carriers		Non-Jewish Carriers	
	Screened[a]	Obligate	Screened[a]	Obligate
Insertion	89	19	0	4
Splice Junction	21	5	0	0
Adult	5	. . .[b]	1	. . .[b]
None of the above	7	2	6	17
Totals	122	26	7	21

[a] Carriers that were originally identified as such by enzyme assay based on hexosaminidase A levels in serum and leukocytes.
[b] Parents of patients with adult Tay-Sachs disease were not included.

values unequivocally in the mid–carrier range for both serum and leukocytes as determined by the standard heat inactivation assay (Kaback et al. 1977) and by an assay using the sulfated substrate.

The distribution of the three mutations among Ashkenazi Jews reported here is virtually identical to that recently reported in a combined study from screening centers in Toronto and Boston, which was composed of 278 Jewish carriers (Triggs-Raine et al. 1990). However, the results differed slightly from ours in the number of obligate carriers that had none of the three mutations. The Toronto-Boston study showed that only 1 of 62 obligate carriers had none of the three mutations, while we found 2 of 27 who did not, but the total numbers are too small to attribute any significance to the difference. What is apparent is that most obligate carriers have one of the three mutations.

By contrast, the Toronto-Boston study showed that 39 (18%) of 216 carriers identified by enzyme assay did not have any of the three mutations, which led the researchers to conclude that most were probably false positives. In our study, only seven (5.7%) of 122 had none of the three mutations. The most likely explanation for some of the difference between the two studies is the delineation of the carrier range, which determines whether individuals with borderline values will be classified as carriers or inconclusive. However, even if one excludes the borderline cases, there are still individuals from both studies who have unambiguous carrier values by enzyme assay, yet have none of the three mutations. It remains to be determined how many of these result from other biological factors influencing hexosaminidase A activity, inconsistencies in the enzyme assay, or the presence of additional mutations.

Among the non-Jewish carriers, four had the insertion mutation and one had the adult mutation, which is most easily explained by presuming Jewish ancestry of which they were unaware. The remaining carriers, most of whom were obligate carriers, had none of the three mutations (table 1).

For screening purposes, we regard DNA testing as a useful adjunct to the enzyme assay. It is reassuring to be able to confirm the diagnosis of most carriers identified by enzyme assay. It has increased our confidence in the assignment of our carrier range by demonstrating that most patients whose values fell in the inconclusive range were probably not carriers. (Of 15 individuals diagnosed by us as inconclusive with both serum and leukocytes over the past 2 years, 13 had none of the three mutations.) But probably the most important benefit is the ability to identify carriers of the adult mutation who are indistinguishable, by enzyme assay alone, from carriers of the infantile form, a distinction which could have significance in prenatal diagnosis.

But, in practice, DNA testing has not yet made a significant impact on our routine carrier screening because we would still be reluctant to label as false positive any carrier, identified by enzyme assay, who had none of the three mutations. At this stage, the absence of the three mutations does not exclude the presence of others.

Note added in proof: Since the submission of the manuscript of the present paper, another report has appeared on the distribution of these mutations (Paw et al. 1990).

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