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A Pseudodeficiency Allele Common in Non-Jewish Tay-Sachs Carriers: Implications for Carrier Screening

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Summary

Deficiency of β-hexosaminidase A (Hex A) activity typically results in Tay-Sachs disease. However, healthy subjects found to be deficient in Hex A activity (i.e., pseudodeficient) by means of in vitro biochemical tests have been described. We analyzed the *HEXA* gene of one pseudodeficient subject and identified both a C_{739}-to-T substitution that changes Arg_{247}→Trp on one allele and a previously identified Tay-Sachs disease mutation on the second allele. Six additional pseudodeficient subjects were found to have the C_{739}-to-T mutation. This allele accounted for 32% (20/62) of non-Jewish enzyme-defined Tay-Sachs disease carriers but for none of 36 Jewish enzyme-defined carriers who did not have one of three known mutations common to this group. The C_{739}-to-T allele, together with a "true" Tay-Sachs disease allele, causes Hex A pseudodeficiency. Given both the large proportion of non-Jewish carriers with this allele and that standard biochemical screening cannot differentiate between heterozygotes for the C_{739}-to-T mutations and Tay-Sachs disease carriers, DNA testing for this mutation in at-risk couples is essential. This could prevent unnecessary or incorrect prenatal diagnoses.

Introduction

Tay-Sachs disease (G_{M2} gangliosidosis type I, reviewed in Sandhoff et al. 1989) is a neurodegenerative disorder resulting from deficiency or defective function of the α-subunit of β-hexosaminidase A (Hex A). There are two major forms of β-hexosaminidase, Hex A and Hex B (Robinson and Stirling 1968). Hex A comprises one α-subunit and one β subunit, while Hex B comprises two β subunits (Geiger and Arnon 1976; Srivastava et al. 1976; Mahuran and Lowden 1980). The α and β subunits are encoded by the *HEXA* and *HEXB* genes, respectively (Proia and Soravia 1987; Neote et al. 1988; Proia 1988). The assay of β-hexosaminidase with a synthetic substrate, 4-methylumbelliferyl-β-N-acetyl-glucosaminide (4-MUG), in combination with a heat denaturation step, can be used to differentiate the heat-stable Hex B from Hex A (Kaback 1972), allowing Tay-Sachs disease heterozygotes, homozygotes, and noncarriers to be reliably differentiated.

Healthy subjects found to have deficiency of Hex A activity when tested with standard synthetic substrates have been described elsewhere (Vidgoff et al. 1973; Kelly et al. 1976; O'Brien et al. 1978; Thomas et al. 1982; Grebner et al. 1986; Navon et al. 1986); they have been called "pseudodeficient" or "Hex A minus" normal. Most pseudodeficient subjects have been identified through enzyme screening or during prenatal diagnosis. One such subject was also the mother

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of a classical Tay-Sachs disease child (Vidgoff et al. 1973), suggesting that some of these subjects are compound heterozygotes of a Tay-Sachs disease allele and a pseudodeficiency allele. This indicated that, despite the biochemical defect associated with the pseudodeficiency allele, it must encode a protein with activity toward the natural substrate, G_{M2} ganglioside, compatible with normal life. Unfortunately, carriers of the pseudodeficiency alleles cannot be separated from carriers of Tay-Sachs disease alleles by using routine enzymatic methods (Vidgoff et al. 1973; O'Brien et al. 1978; Thomas et al. 1982; Grebner et al. 1986).

In North American non-Jews, the Tay-Sachs disease carrier frequency of 1/300, estimated by Hardy-Weinberg analysis, is almost half the frequency, 1/167, determined by enzyme screening (Kaback et al. 1978). This contrasts with the data for Ashkenazi Jews, in whom the carrier frequency estimated from the disease incidence, 1/30 (Myrianthopoulos and Aronson 1966), is consistent with the 1/31 frequency found by enzyme screening (Petersen et al. 1983). Greenberg and Kaback (1982) proposed that inconsistencies between the disease incidence and the enzyme-based carrier frequency in non-Jews could be accounted for by α-subunit mutations that make individuals appear as Tay-Sachs disease heterozygotes on the basis of standard biochemical tests, while they are either (*a*) not at risk for having offspring with infantile Tay-Sachs disease or (*b*) carriers of a Hex A minus allele.

The Hex A from most individuals who are compound heterozygotes for a pseudodeficiency allele and a Tay-Sachs allele have a characteristic biochemical phenotype. The serum Hex A activity has been reported as 0%–15%, similar to that of a Tay-Sachs disease child or a person with a variant form of Tay-Sachs disease, while the leukocyte activity is 13%–24%, just above the level in some adult-onset patients but below the carrier range (Vidgoff et al. 1973; Kelly et al. 1976; Thomas et al. 1982; Grebner et al. 1986; Navon et al. 1986). Fibroblast levels vary 8%–26% (Kelly et al. 1976; O'Brien et al. 1978; Thomas et al. 1982; Grebner et al. 1986; Navon et al. 1986) between Tay-Sachs disease heterozygotes and individuals with variant forms of the disease. In contrast, however, in vivo and in vitro assays with the natural substrate of Hex A, i.e., G_{M2} ganglioside, give enzyme activities in the low normal range (Kelly et al. 1976; O'Brien et al. 1978; Thomas et al. 1982; Grebner et al. 1986).

A large number of mutations responsible for Tay-Sachs disease and its variants have been identified. Three *HEXA* mutations account for most Tay-Sachs disease and its variants in Ashkenazi Jews (Paw et al. 1990; Triggs-Raine et al. 1990; Grebner and Tomczak 1991; Landels et al. 1991), and more than 20 disease mutations have been identified in non-Jews (Tanaka et al. 1990; reviewed in Akli et al. 1991; Gravel et al. 1991; Paw et al. 1991; Triggs-Raine et al. 1991). In the present study, a mutation associated with Hex A pseudodeficiency in Ashkenazi Jewish and non-Jewish individuals is described. We show that this mutation accounts for a large proportion of non-Jewish enzyme-defined carriers but that it does not account for a significant proportion of Jewish enzyme-defined carriers.

Subjects and Methods

DNA Sources

Subject A (HSC 1107 [Hospital for Sick Children, Toronto]), subject B (GM04863 [Human Genetic Mutant Cell Repository, Camden, NJ], designated as patient 1 by Grebner et al. [1986]), subject C (designated as patient 2 by Grebner et al. [1986]), subject D (Bayleran et al. 1986), subject E (Navon et al. 1986), subject F (designated as II-2 by Kelly et al. [1976] and as patient 2 by Thomas et al. [1982]), subject G (The Kennedy Institute, Baltimore), and subject H (designated as patient 3 by Grebner et al. [1986]) were all healthy individuals identified to be Hex A deficient by means of the synthetic substrate, 4-MUG. DNA for analysis was prepared (Hoar et al. 1984) from fibroblasts or leukocytes.

Leukocyte pellets or sonicates from enzyme-defined carriers and noncarriers and from obligate carriers of Tay-Sachs disease were from the California, Boston, and Toronto Tay-Sachs Disease Prevention Programs. Most of the carrier samples from Ashkenazi Jews that were supplied by the Toronto and Boston progams had formed the basis of a previous study on the distribution of Tay-Sachs disease alleles in Ashkenazi Jews (Triggs-Raine et al. 1990). A sample was excluded from the biochemically defined carriers if it was from a person who was a biological relative of either another enzyme-defined carrier or an obligate carrier. Obligate carriers were defined as a biological parent of a fetus or child with any form of Tay-Sachs disease. DNA from two sibs with amyotrophic lateral sclerosis was provided by the Health Sciences Centre, Winnipeg.

Mutation Identification

The three mutations common in the Ashkenazi Jewish population were analyzed according to a method described elsewhere (Triggs-Raine et al. 1990). The DNA was then screened for novel mutations after PCR amplification of sequences flanking the 14 *HEXA* exons (Triggs-Raine et al. 1991). Single-strand conformational polymorphism (SSCP) analysis (Orita et al. 1989) was done by means of α^{32}P-dATP-labeled PCR products, with separation of the single- stranded DNA by electrophoresis on a 6% nondenaturing polyacrylamide gel containing 10% glycerol. The PCR product exhibiting a change in electrophoretic mobility was phosphorylated and cloned into *Eco*RV-cut pBS + (Stratagene, San Diego) by following standard procedures (Maniatis et al. 1982). Double-stranded DNA was sequenced with Sequenase (United States Biochemicals, Cleveland).

Reverse Transcription of Total RNA, and cDNA Amplification

Total RNA was isolated (Chirgwin et al. 1979), and 25 μg, dissolved in 20 μl of water, was denatured at 65°C for 5 min. Reverse transcription was done by immediately placing the denatured sample on ice and adding 80 units of RNasin (Promega Corporation, Madison), 1 μl of oligonucleotide primer (0.5 μg of dT_{15}), 25 μl of 2 × buffer (200 mM Tris-hydrochloride pH 8.0; 280 mM potassium chloride; 20 mM magnesium chloride; 2 mM of dATP, dCTP, dGTP, and dTTP; and 10 mM DTT), and 1 μl (3–5 units) of AMV reverse transcriptase (Bethesda Research Laboratories, Gaithersburg, MD). The mixture was incubated at 42°C for 1 h. The *HEXA* coding sequence was amplified from the resulting cDNA in three overlapping fragments. To generate each fragment, a 5-μl aliquot of the synthesized cDNA was amplified, in a standard PCR reaction mixture, with one of the following primer pairs (Triggs-Raine et al. 1990): exons 1–7, sense primer 5′CTCACCTGACCAGGGTCTC ACGT-3′ placed 5′ of exon 1 and antisense primer 5′-AAGTGTGGCCAGGAGTGTCA-3′ in exon 7; exons 6–11, sense primer 5′-TGTCATGGCGTACA-ATAAAT-3′ in exon 6 and antisense primer 5′-AGG-GGTTCCACTACGTAGAA-3′ in exon 11; and exons 7–14, sense primer 5′-TACAACCCTGTCAC-CCACAT-3′ in exon 7 and antisense primer 5′-CCTT-TCTCTCCAAGCACAGG-3′, 3′ of exon 14. The products were phosphorylated and subcloned into *Eco*RV-cut pBS + for sequencing as described above.

Detection of C_{739}-to-T Mutation

DNA (0.1–0.5 μg) prepared from fibroblasts or leukocytes was amplified according to a method described elsewhere (Triggs-Raine et al. 1990), except that the reaction cycle consisted of 32 cycles each of which comprised 30 s at 94°C, 30 s at 60°C, and 90 s at 72°C. A 10–15-μl aliquot was taken from the PCR product, and 1 μl (2 units) of the restriction enzyme *Nla*III (New England Biolabs, Beverly, MA) was added. After a 2-h incubation at 37°C the samples were analyzed on 2.5% agarose gels.

Hexosaminidase Enzyme Assay

All assays using 4-MUG as the substrate were performed according to the manual heat-inactivation methods described elsewhere (Lowden et al. 1976). Assays using 4-MUGS (4-methyl-umbelliferyl-6-sulfo-2-acetamido-2-deoxy-β-D-glucopyranoside; Toronto Research Chemicals) were performed by the same methods but without the heat-inactivation step.

Results

Identification of the Pseudodeficiency Mutation

During routine enzyme screening, subject A was found to be deficient in Hex A activity. We tested his DNA for the three known mutations common in Ashkenazi Jews. The exon 12 splice-junction mutation (Arpaia et al. 1988; Myerowitz 1988; Ohno and Suzuki 1988), an allele associated with the lethal infantile form of Tay-Sachs disease, was identified on one chromosome (data not shown). The second allele was detected by SSCP analysis. The exon 7 PCR product showed a band pattern distinct from that of the normal sequence (fig. 1). It was cloned and sequenced and revealed a single change, a C_{739}-to-T transition corresponding to an Arg_{247}-to-Trp substitution (fig. 2). The remainder of the *HEXA* coding region of subject A was examined by reverse transcription of total RNA and amplification of overlapping portions of the cDNA. This was simplified because the other mutant allele in subject A's DNA (exon 12 splice junction) produces a reduced quantity of mRNA (Ohno and Suzuki 1988). The amplified products were subcloned and sequenced. The C_{739}-to-T mutation was identified in all three clones, amd no other changes were detected.

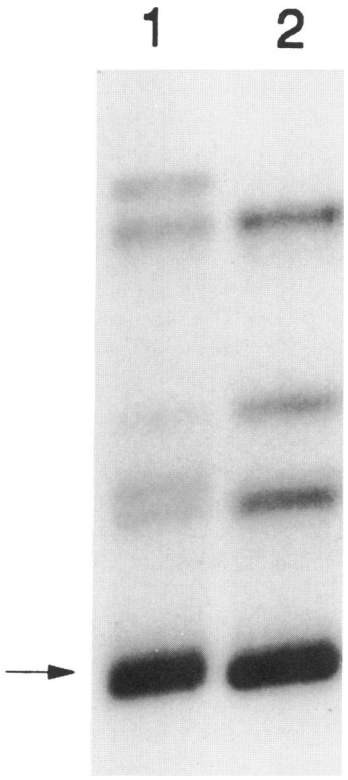

Figure 1 SSCP analysis of exon 7 of *HEXA*. The α^{32}P-dATP-labeled PCR products of subject A (lane 1) and of a normal control (lane 2) were denatured, and the single strands were separated on a nondenaturing polyacrylamide gel containing 10% glycerol. The arrow indicates the nondenatured band. The two extra bands in lane 1 result from a mutation that alters the conformation and, therefore, the mobility of the DNA strands from one allele.

Detection of the Mutation

The C_{739}-to-T mutation creates an *Nla*III site, which allowed for a strategy for the detection of this mutation, as outlined in figure 3. A region of exon 7 through intron 8, including the region containing the mutation, was amplified and tested for the presence or absence of the *Nla*III site. In addition, the forward primer was synthesized with a single base mismatch to create an independent *Nla*III site (5′-CCTGTCACCCACA-TGTACACAGCA-3′) to act as a control *NlaIII* site to assure that the restriction enzyme was active even if the C_{739}-to-T mutation was absent.

Testing for the C_{739}-to-T Mutation in Other Subjects with Pseudodeficiency

DNA samples from the Jewish subjects A–E, all of whom were healthy Hex A–deficient individuals, were each found to carry a single copy of the C_{739}-to-T

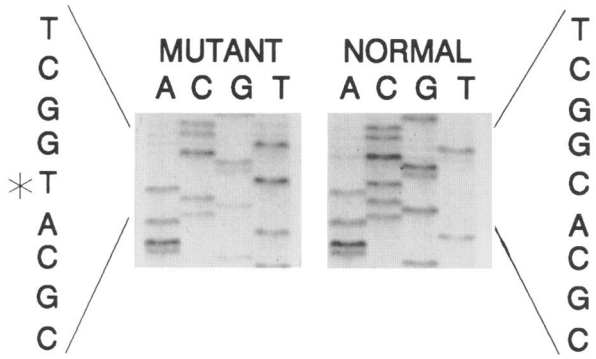

Figure 2 Sequence of normal and mutant DNA. The region of exon 7 containing the C_{739}-to-T mutation is shown. The asterisk indicates the altered base in the mutant sequence.

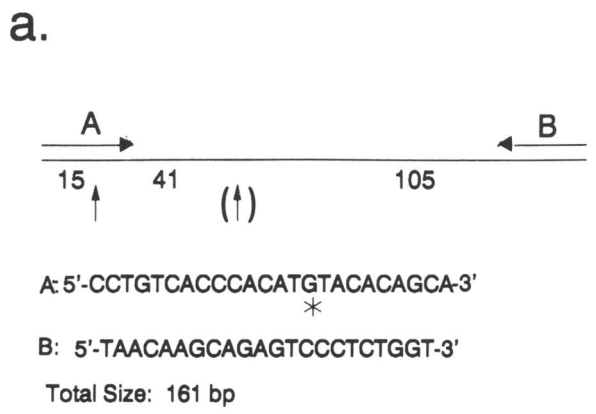

Figure 3 Strategy for the detection of the C_{739}-to-T mutation. *a*, *Nla*III sites, denoted by arrows, in the sequence. The parentheses indicate the site created by the presence of the C_{739}-to-T mutation. The asterisk under the A primer indicates the single base change introduced to create a control *Nla*III site. *b*, Agarose gel electrophoresis of the products of the strategy used with the A primer. Lane 1, Normal uncut. Lane 2, Normal, cut by *Nla*III. Lane 3, Mutant uncut. Lane 4, Mutant, cut by *Nla*III.

mutation in combination with a previously identified mutation associated with infantile Tay-Sachs disease (table 1). Two additional DNA samples were from subjects F and G, non-Jewish Pennsylvania Dutch in-

Table 1

DNA Analysis of Pseudodeficient Subjects

Subject (age in years)	Presentation	Allele 1	Allele 2	Heritage	Reference
A (47)	Screening[a]	C_{739}→T	Exon 12[b]	Ashkenazi-Jewish	Present study
B (12)	Prenatal[c]	C_{739}→T	Exon 11[d]	Ashkenazi-Jewish	Grebner et al. 1986
C (14)	Prenatal[c]	C_{739}→T	Exon 11[d]	Ashkenazi-Jewish	Grebner et al. 1986
D (30s)	Screening[a]	C_{739}→T	Exon 11[d]	Ashkenazi-Jewish	Bayleran et al. 1986
E (39)	Screening[a]	C_{739}→T	Exon 11[d]	Ashkenazi-Jewish	Navon et al. 1986
F (56)	Screening[a]	C_{739}→T	Unknown	Pennsylvania Dutch and ?[e]	Thomas et al. 1982
G (13)	Prenatal[c]	C_{739}→T	Unknown	Pennsylvania Dutch	Present study
H (36)	Screening[a]	Unknown	Unknown	Non-Jewish	Grebner et al. 1986

[a] Identified as hexosaminidase A deficient during Tay-Sachs disease screening.
[b] Exon 12 splice junction mutation (Arpaia et al. 1988; Myerowitz 1988; Ohno and Suzuki 1988).
[c] Identified by prenatal diagnosis because both parents were found to be carriers during standard Tay-Sachs screening.
[d] 4-bp insertion in exon 11 (Myerowitz and Costigan 1988).
[e] Heritage of the subject's mother is unknown.

dividuals who are part of a kindred with an elevated incidence of Tay-Sachs disease (Kelly et al. 1975). In these cases, the C_{739}-to-T mutation was independently identified by direct sequencing of the exon 7 PCR amplification product. The second allele in subjects F and G remains unidentified, although it is presumed to be an infantile Tay-Sachs disease allele that is segregating in this population. Subject H, a pseudodeficient subject whose hexosaminidase had biochemical properties different from those of the hexosaminidase of other subjects (Grebner et al. 1986), did not have the C_{739}-to-T allele.

Enzyme Assay of a Pseudodeficient Subject

Serum and leukocyte samples from subject B and from subject B's carrier mother and sib were assayed with both the standard substrate 4-MUG and the α-subunit-specific substrate 4-MUGS (tables 2 and 3). The mother, a carrier of the C_{739}-to-T mutation, had Hex A levels just outside the carrier range, i.e., in the "inconclusive" region. The sib of subject B, a carrier of the exon 11 +TATC mutation, had Hex A levels in the carrier range. Subject B, who has both of these mutant alleles, had Hex A levels well below the carrier range, similar to or slightly higher than those associ-

Table 2

Hexosaminidase Levels in Leukocytes of Subject B and Family

	4-MUG		4-MUGS	
Source	Specific Activity (nmol substrate cleaved/mg protein/h)	% Hex A	Specific Activity (nmol substrate cleaved/mg protein/h)	MUGS/MUG (×100)
Subject B	1,300	17.2	16.1	1.2
Sib of B[a]	1,330	51.4	53.9	4.0
Mother of B[b]	1,230	58.5	61.1	5.0
Carriers:				
Average	1,080	50.6	44.4	4.1
Range (n = 12)	752–1,350	41.7–54.5	27.3–62.1	3.6–4.6
Normals:				
Average	1,160	69.7	73.0	6.3
Range (n = 12)	910–1,540	65.4–72.2	56.4–95	5.8–7.3

[a] Carrier of 4-bp insertion in exon 11, which is associated with infantile Tay-Sachs disease.
[b] Carrier of C_{739}-to-T mutation in exon 7, which is associated with Hex A pseudodeficiency.

Table 3

Serum Levels of Hexosaminidase in Subject B and Family

Source	4-MUG (nmol substrate cleaved/ml serum/h)	4-MUG % Hex A	4-MUGS Specific Activity (nmol substrate cleaved/ml serum/h)	MUGS/MUG (×100)
Subject B	784	9.6	9.5	1.2
Sib of B[a]	1,070	44.5	51.2	4.8
Mother of B[b]	790	53.4	51.0	6.5
Carriers:				
Average	950	41.5	49.1	5.2
Range ($n=12$)	679–1,190	38.0–46.8	35.6–61.2	4.5–6.3
Normals:				
Average	1,050	59.6	78.3	7.5
Range ($n=12$)	799–1,370	56.1–63.1	60.5–103	7.1–8.1

[a] Carrier of 4-bp insertion in exon 11, which is associated with infantile Tay-Sachs disease.
[b] Carrier of C_{739}-to-T mutation in exon 7, which is associated with Hex A pseudodeficiency.

ated with classical or variant forms of Tay-Sachs disease. In all cases, the extent of the forms of the deficiency was found to be similar by both synthetic substrates, i.e., 4-MUG and 4-MUGS.

Screening for the C_{739}-to-T Mutation in Tay-Sachs Disease Carriers and Noncarriers

The samples initially screened for this mutation were from Ashkenazi Jews who had been biochemically screened as part of the regular Tay-Sachs disease prevention programs in California, Boston, and Toronto. We thought that this mutation might account for some of the enzyme-defined carriers who did not have any of the other common mutations causing Tay-Sachs disease in this population. As shown in table 4, none of the 36 Ashkenazi Jewish enzyme-defined carriers previously shown not to have one of the three mutations common in that population had the C_{739}-to-T mutation.

Samples from non-Jews who had also been enzymatically screened as part of the California and Boston Tay-Sachs disease prevention programs, as well as samples from non-Jewish obligate carriers of Tay-Sachs disease, were analyzed for the C_{739}-to-T mutation. It is significant that the mutation was found in 20/62 non-Jewish enzyme-defined carriers. Fifty-five noncarriers and 33 obligate carriers of Tay-Sachs disease did not carry the mutation (table 4). Finally, the mutation was identified in two middle-aged non-Jewish sibs with familial amyotrophic lateral sclerosis (ALS) who had carrier (44% and 49%) Hex A activity. An additional middle-aged sib, who did not have ALS, also had carrier levels of enzyme activity. The non-Jewish carriers with this allele had Hex A levels scattered throughout the carrier range and could not be differentiated from true Tay-Sachs disease carriers by means of standard methods. Most of the non-Jewish individuals with the C_{739}-to-T allele had some ancestral origin in western Europe, suggesting that this allele may have originated in this region.

Table 4

DNA Analysis of Enzyme-defined Carriers, Enzyme-defined Noncarriers, and Obligate Carriers of Tay-Sachs Disease

Heritage and Status	Total No.	No. (%) with C_{739}-to-T Mutation
Non-Jewish:		
Carrier	62	20 (32)
Noncarrier	15	0 (0)
Jewish:		
Carrier	36[a]	0 (0)
Noncarrier	40	0 (0)
Non-Jewish obligate carrier[b]	33	0 (0)

[a] These 36 carriers did not have one of the three mutations common in the Ashkenazi Jewish population identified in a previous study (Triggs-Raine et al. 1990).
[b] Seventeen of the obligate carriers are actually represented by 10 Tay-Sachs disease–affected offspring (3/20 alleles in the affected offspring either were inherited from a parent of Ashkenazi Jewish origin or were already represented by an obligate carrier, leaving 17 non-Jewish obligate alleles).

Discussion

We found a C_{739}-to-T (Arg247-to-Trp) mutation in seven pseudodeficient subjects of either non-Jewish or Jewish origin. In the five Ashkenazi Jewish individuals, who come from a population where the mutations causing Tay-Sachs disease are largely known, the second allele was identified as one of the common Tay-Sachs disease–causing alleles. This confirms earlier hypotheses that pseudodeficient subjects would be compound heterozygotes for a Tay-Sachs disease allele and a "pseudodeficiency" allele.

A characteristic enzyme phenotype is associated with compound heterozygosity for the C_{739}-to-T mutant allele and a Tay-Sachs disease allele. On the basis of synthetic substrates, these individuals exhibit low or absent serum Hex A activity, but the levels are somewhat higher in fibroblasts and/or leukocytes (Thomas et al. 1982; Grebner et al. 1986; Navon et al. 1986; present study). Substantial levels of G_{M2} ganglioside hydrolysis in cultured cells from subjects B, C, and F have been reported (Thomas et al. 1982; Grebner et al. 1986). M. M. Kaback and K. Sandhoff (unpublished data) found similar levels of enzyme activity in subject D by using an in vitro G_{M2} gangliosidase assay (489.7 pmol G_{M2} ganglioside degraded/h/μg protein; normal range 384–787; adult-onset-disease range 13–33). Therefore, the Arg247-to-Trp change in the α-subunit does not appear to affect the capacity of Hex A to hydrolyze the natural substrate. Further, the α-subunit containing the Arg247-to-Trp mutation appears to be made, processed, and secreted normally from fibroblasts (Thomas et al. 1982; Grebner et al. 1986). The reduced activity toward 4-MUGS compared with G_{M2} ganglioside was unexpected, since the two substrates have been shown to be specific to the α-subunit (Kytzia and Sandhoff 1985). Indeed, their different behavior toward Hex A in subjects with the pseudodeficiency allele suggests that the mutated region of the protein may be involved in substrate recognition.

The C_{739}-to-T mutation accounted for 32% of enzyme-defined carriers among non-Jews. The high prevalance of the allele in this population could explain the discrepancy between the enzyme-defined carrier frequency of 1/167 and the 1/300 frequency predicted by disease incidence. If the carriers of the C_{739}-to-T mutation are removed from the calculation, then the carrier frequency becomes 1/250, which is closer to that predicted. Since one pseudodeficient subject in our study did not have the C_{739}-to-T mutation, there may be additional alleles among non-Jewish carriers that do not cause disease.

The C_{739}-to-T allele was not identified in any of the Ashkenazi Jewish enzyme-defined carriers who were previously analyzed and shown not to have any of the three mutations common in this population (Triggs-Raine et al. 1990). A larger study will be required to establish the frequency of this allele within Ashkenazi Jewish carriers.

Assessment of the clinical phenotype associated with the C_{739}-to-T mutation in pseudodeficient subjects is of crucial importance in providing a prognosis for these individuals and others with the same genotype. Subject A was healthy when he was first tested and still appears healthy at age 47 years, although no formal neurological assessment has been done. Subject E had a neurological assessment 4 years ago, at the age of 35, and was found to be normal. Subject D was healthy at the time of testing in her mid-30s and remained so in the next decade, but no further update is available. Subject F, now 56 years of age, similarly reports no health problems. It is not yet possible to say that compound heterozygotes carrying a Tay-Sachs disease allele in combination with the pseudodeficiency allele will be disease free throughout their lives. However, until more detailed neurological assessments can be made, especially in older subjects, we would counsel that the C_{739}-to-T allele appears to pose little or no clinical risk.

The atypical Hex A activities associated with the C_{739}-to-T mutation also complicate prenatal diagnosis when there is another disease-related allele, as was the case for subjects B, C, and G. Prenatal diagnosis in two cases was uncertain. With Hex A levels between that of a Tay-Sachs disease homozygote and that of a Tay-Sachs disease heterozygote (for subject B, 3.5% in amniotic fluid and 25% in cultured amniotic fluid cells; for subject C, 2% in amniotic fluid and 8% in amniotic fluid cells; and, for subject G, 14%–21% in cultured amniotic fluid cells), subjects C and G were considered at risk for a variant form of Tay-Sachs disease. DNA testing for the C_{739}-to-T mutation should prove to be a useful adjunct to enzyme procedures in distinguishing the allele from those causing variant clinical forms of the disease.

We believe that testing for this allele should be available in programs for carrier testing for Tay-Sachs disease in both Ashkenazi Jews and non-Jews. In particular, the anxiety and expense associated with antenatal diagnosis, as well as the difficulty in interpreting the

unusual enzyme levels, make it important to identify the C_{739}-to-T mutation in couples presumed to be at risk. As we have shown in one instance, neither 4-MUG nor 4-MUGS could distinguish between a carrier of the C_{739}-to-T allele and a carrier of a known disease allele. This has been extended to a broader survey of carriers (M. M. Kaback, unpublished data). While the natural substrate assay would appear to be an appropriate alternative, the instability of the radiolabeled natural substrate, the requirement for the biological activator protein, and the complexity of the assay make it impractical. DNA testing is currently the only simple method for identifying this allele.

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