*Am. J. Hum. Genet. 53:1198–1205, 1993*

# A Second Mutation Associated with Apparent ß-Hexosaminidase A Pseudodeficiency: Identification and Frequency Estimation

Z. Cao,* M. R. Natowicz,† M. M. Kaback,‡ J. S. T. Lim-Steele,‡ E. M. Prence,† D. Brown,‡ T. Chabot,* and B. L. Triggs-Raine*

*Department of Biochemistry and Molecular Biology, University of Manitoba, Winnipeg; †Shriver Center for Mental Retardation, Waltham, MA, and Harvard Medical School, Boston; and ‡Children's Hospital of San Diego and Department of Pediatrics, School of Medicine, University of California, San Diego

## Summary

**Deficient activity of ß-hexosaminidase A (Hex A), resulting from mutations in the *HEXA* gene, typically causes Tay-Sachs disease. However, healthy individuals lacking Hex A activity against synthetic substrates (i.e., individuals who are pseudodeficient) have been described. Recently, an apparently benign $C_{739}$-to-T (Arg247Trp) mutation was found among individuals with Hex A levels indistinguishable from those of carriers of Tay-Sachs disease. This allele, when in compound heterozygosity with a second "disease-causing" allele, results in Hex A pseudodeficiency. We examined the *HEXA* gene of a healthy 42-year-old who was Hex A deficient but did not have the $C_{739}$-to-T mutation. The *HEXA* exons were PCR amplified, and the products were analyzed for mutations by using restriction-enzyme digestion or single-strand gel electrophoresis. A $G_{805}$-to-A (Gly269Ser) mutation associated with adult-onset $G_{M2}$ gangliosidosis was found on one chromosome. A new mutation, $C_{745}$-to-T (Arg249Trp), was identified on the second chromosome. This mutation was detected in an additional 4/63 (6%) non-Jewish and 0/218 Ashkenazi Jewish enzyme-defined carriers. Although the Arg249Trp change may result in a late-onset form of $G_{M2}$ gangliosidosis, any phenotype must be very mild. This new mutation and the benign $C_{739}$-to-T mutation together account for approximately 38% of non-Jewish enzyme-defined carriers. Because carriers of the $C_{739}$-to-T and $C_{745}$-to-T mutations cannot be differentiated from carriers of disease-causing alleles by using the classical biochemical screening approaches, DNA-based analyses for these mutations should be offered for non-Jewish enzyme-defined heterozygotes, before definitive counseling is provided.**

## Introduction

The two isoenzyme forms of ß-hexosaminidase, Hex A and Hex B, differ in their subunit composition and substrate specificity (reviewed in Neufeld 1989; Neote et al. 1991). Hex A is composed of one α-subunit, encoded by the *HEXA* gene, and one ß-subunit, encoded by the *HEXB* gene; Hex B is composed of two ß-subunits. Only Hex A, together with a heat-stable protein called the "$G_{M2}$ activator," is able to hydrolyze the primary natural substrate, $G_{M2}$ ganglioside. Mutations in the *HEXA* gene that prevent normal Hex A function, causing the accumulation of $G_{M2}$ ganglioside, result in Tay-Sachs disease ($G_{M2}$ gangliosidosis variant B), an autosomal recessive lysosomal storage disorder (reviewed in Sandhoff et al. 1989). Tay-Sachs disease is typically fatal before the age of 5 years, but less severe juvenile and adult-onset forms of $G_{M2}$ gangliosidosis result when residual Hex A activity is present.

Successful heterozygote screening programs designed for Tay-Sachs disease prevention have been established for high-risk populations (Kaback et al. 1977*a*). Enzyme screening is routinely performed with a synthetic fluorogenic substrate, 4-methylumbelliferyl-ß-D-glucosaminide (4-MUG), which is hydrolyzed by both Hex A and Hex B. The increased heat stability of

Received June 23, 1993; revision received August 2, 1993.

Address for correspondence and reprints: Dr. B. Triggs-Raine, Department of Biochemistry and Molecular Biology, University of Manitoba, Winnipeg, Canada R3E OW3.



0002-9297/93/5306-0006$02.00

Hex B relative to Hex A allows the activities of the two isoenzymes to be differentiated. With this approach, Tay-Sachs disease carriers, noncarriers, and affected individuals can be reliably detected (Kaback 1972). However, several individuals who are healthy yet lack Hex A activity against the synthetic substrate (i.e., are pseudodeficient or Hex A–minus normal) have been identified through Tay-Sachs disease heterozygote detection programs (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). The individuals with Hex A pseudodeficiency who have been characterized to date are compound heterozygotes. They have a common disease-causing mutation in the *HEXA* gene on one allele and a benign $C_{739}$-to-T (Arg247Trp) mutation in exon 7 of *HEXA* on the second allele (Triggs-Raine et al. 1992). The $C_{739}$-to-T mutation does not impair $G_{M2}$ ganglioside hydrolysis (Kelly et al. 1976; Thomas et al. 1982; Grebner et al. 1986; Triggs-Raine et al. 1992), although it does prevent the hydrolysis of the synthetic substrate 4-MUG (Vidgoff et al. 1973; Kelly et al. 1976; Thomas et al. 1982; Grebner et al. 1986; Navon et al. 1986) and its sulfated derivative, 4-MUGS (Triggs-Raine et al. 1992). One of the pseudodeficiency subjects previously examined did not have the $C_{739}$-to-T mutation, suggesting that other benign mutations exist (Triggs-Raine et al. 1992).

Benign mutations have significant implications in heterozygote screening programs. Through biochemical screening of hexosaminidase enzyme activity, the Tay-Sachs disease carrier frequency in the non-Jewish population was shown to be 1/167 (Kaback et al. 1978), almost twice that predicted (1/300) on the basis of incidence of the disease (Myrianthopoulos and Aronson 1966). It was proposed that this discrepancy might be the result of benign mutations (Greenberg and Kaback 1982). Indeed, the benign $C_{739}$-to-T allele was detected in 32%–42% of non-Jewish enzyme-defined Tay-Sachs disease carriers and in as many as 3% of Jewish enzyme-defined carriers (Triggs-Raine et al. 1992; Kaback 1993; Tomczak et al. 1993). If the enzyme-defined carrier frequency in the non-Jewish population is calculated after heterozygotes for the $C_{739}$-to-T allele are removed, the frequency becomes 1/250, closer to the 1/300 estimated on the basis of the disease incidence. The remaining difference between the enzyme- and disease-based frequencies in the non-Jewish population likely reflects the existence of non-Jewish carriers of noninfantile forms of $G_{M2}$ gangliosidosis and possibly some additional low-frequency benign mutations.

We describe here a new, apparently benign $C_{745}$-to-T (Arg249Trp) mutation in *HEXA* in a healthy Hex A–deficient (i.e., pseudodeficient) proband. The $C_{745}$-to-T mutation was also found in an additional 4/63 non-Jewish and 0/218 Jewish enzyme-defined Tay-Sachs disease carriers. This mutation, together with the previously described benign mutation, $C_{739}$-to-T, accounts for approximately 38% of non-Jewish enzyme-defined carriers.

## Subjects and Methods

### *Subjects*

Leukocyte pellets from the proband (16819), her mother (16858), and her father (16859) were from the Boston Tay-Sachs Disease Prevention Program. Additional leukocyte pellets, leukocyte sonicates, or DNA samples from enzyme-defined carriers and noncarriers of Ashkenazi Jewish (descended from Jews from central or eastern Europe) and non-Jewish ancestry were identified by biochemical screening through the California and Philadelphia Tay-Sachs Disease Prevention programs. DNA for analysis was prepared from leukocyte pellets or sonicated leukocyte pellets as described elsewhere (Hoar et al. 1984).

### *ß-Hexosaminidase Assay*

The ß-hexosaminidase activities were determined using 4-MUG as a substrate (Kaback et al. 1977*b*). Hex A levels were specifically assayed using 4-MUGS as a substrate (Prence et al., in press).

### *Mutation Identification*

Previously described common *HEXA* mutations were tested for by PCR amplification of genomic DNA of the exon containing the mutation of interest. The presence or absence of a mutation was tested for by the destruction or creation of a restriction-enzyme site. Three disease-causing mutations common in the Ashkenazi Jewish population, a G-to-C substitution at the intron 12+1 junction (Arpaia et al. 1988; Myerowitz 1988), a 4-bp insertion at position 1278 of exon 11 (Myerowitz and Costigan 1988), and a G-to-A substitution at position 805 of exon 7 (Navon and Proia 1989; Paw et al. 1989) were screened for in PCR products as described elsewhere (Triggs-Raine et al. 1990). A disease-causing mutation common in the non-Jewish population, G-to-A at position +1 of intron 9 (Akli et al. 1991), was screened for by using a procedure described elsewhere (Akerman et al. 1992), as was the common benign mutation, $C_{739}$-to-T (Triggs-Raine et al. 1992).

The 14 *HEXA* exons were screened for uncommon

and novel mutations by using single-strand conformational polymorphism (SSCP) analysis (Orita et al. 1989). Primer pairs bounding each exon and the surrounding intron sequence were used for the preparation of the PCR product for SSCP analysis. This was performed as described elsewhere (Triggs-Raine et al. 1991), except that (*a*) only the room-temperature electrophoresis conditions were used for some exons and (*b*) the samples were not restriction enzyme digested prior to the analysis. PCR products that had an abnormal electrophoretic mobility were directly sequenced (Winship 1989).

### *Detection of $C_{745}$-to-T Mutation*

The exon 7 region was amplified from genomic DNA according to a PCR-based protocol described elsewhere (Triggs-Raine et al. 1991). A 20-μl aliquot of the PCR product was incubated for 2 h at 37°C with either 5 units of *Dde*I or 6 units of *Hpa*II (New England Biolabs, Beverly, MA). The restriction enzyme–digested samples were then separated on either a 10% polyacrylamide gel or a 2.5% agarose gel, for analysis.

## Results

### *Enzyme Analysis*

In 1989, a pregnant female (16819) of non-Jewish origin was screened for Tay-Sachs disease carrier status at the same time as was her Ashkenazi Jewish spouse. Surprisingly, when her sample was tested with the synthetic substrate, 4-MUG, Hex A levels similar to those of individuals with Tay-Sachs disease were observed in both serum and leukocytes. At that time the proband was 38 years of age and showed no evidence of neurological impairment; she was believed to have at least one (perhaps two) benign mutation(s). The proband's parents (16858 and 16859) were also tested for Tay-Sachs disease carrier status, and both were found to have Hex A levels within the carrier range when they were tested with the synthetic substrate 4-MUG. Hex A activity in their samples was then determined using the sulfated substrate, 4-MUGS, which is specific for the Hex A isoenzyme (Kytzia and Sandhoff 1985). With this substrate, significant levels of Hex A were detected in the serum and leukocyte samples from 16819, greater than the concentrations seen in patients with Tay-Sachs disease. The results of the enzyme analyses are given in table 1.

### *Identification of the Benign Mutation*

DNA from the parents of the proband was tested for previously described mutations common in the Ashkenazi Jewish and non-Jewish population by using PCR-based methods. The mother, 16858, was found to carry the $G_{805}$-to-A (Gly269Ser) mutation that is associated with adult-onset $G_{M2}$ gangliosidosis (data not shown). This mutation was also determined to be present in the DNA of the proband, 16819 (data not shown).

Once the common known Tay-Sachs disease mutations were excluded (see Material and Methods), the DNA from 16859 was analyzed for uncommon or novel mutations, by SSCP analysis of the 14 *HEXA* exons. The exon 7 PCR product showed a band distinct from the normal pattern (fig. 1). Direct sequencing of the exon 7 PCR product revealed a single base change, a $C_{745}$-to-T transition corresponding to an Arg249Trp substitution in the α-subunit of β-hexosaminidase (fig. 2). No additional SSCP shifts were detected in the other exons (data not shown). The $C_{745}$-to-T change destroyed a *Hpa*II site in exon 7; this site was absent in approximately 50% of the exon 7 PCR product of 16859 (data not shown), consistent with its presence in the heterozygous state.

### *Confirmation of $C_{745}$-to-T Mutation*

Because the destruction of a restriction enzyme site cannot differentiate mutations affecting the same restriction-enzyme recognition site, we designed a strategy that would specifically detect the $C_{745}$-to-T mutation (fig. 3). Site-directed mutagenesis of the exon 7 region was performed by placing single nucleotide changes in each of the oligonucleotide primers used for PCR amplification of the exon 7 region. The mutation in primer B creates a *Dde*I restriction site in the exon 7 PCR product only in the presence of the $C_{745}$-to-T mutation. A single nucleotide substitution in primer A introduces a control *Dde*I site that will be present in all of the PCR products, even if they do not contain the $C_{745}$-to-T mutation. The $C_{745}$-to-T mutation was confirmed to be present in 16859 and in the proband, 16819 (fig. 3).

### *Screening for the $C_{745}$-to-T Mutation*

Enzyme-defined carriers, obligate carriers, and noncarriers of Tay-Sachs disease of both Ashkenazi Jewish and non-Jewish origin were tested for the presence of the $C_{745}$-to-T mutation, as outlined in figure 3; the results are summarized in table 2. The $C_{745}$-to-T mutation was identified in 4/63 non-Jewish enzyme-defined carriers but not in any of the other sample groups. The four DNA samples with the $C_{745}$-to-T mutation were also tested for the previously identified benign muta-

**Table 1**

**Hexosaminidase Levels in Serum and Leukocytes of Proband 16819 and Family**

| | Serum | | | Leukocyte | | |
|---|---|---|---|---|---|---|
| Sample | 4-MUG (nmol substrate cleaved/ml serum/h) | Hex A (%) | 4-MUGS[a] (nmol substrate cleaved/ml serum/h) | 4-MUG (nmol substrate cleaved/mg protein/h) | Hex A (%) | 4-MUGS (nmol substrate cleaved/mg protein/h) |
| 16819[b] ..... | 2,572 | 13.4 | 54 | 1,368 | 16.3 | 27 |
| 16858 ..... | 1,290 | 35.1 | 76 | 1,492 | 41.9 | 146 |
| 16859 ..... | 1,742 | 45.1 | 127 | 1,614 | 52.0 | 140 |
| Tay-Sachs disease ..... | 1,030 ± 280 | 7.5 ± 4.4 | 5 ± 1 | 2,092 ± 753 | 9.2 ± 3.9 | 5 ± 2 |
| Carrier ..... | 964 ± 262 | 43.2 ± 5.9 | ND | 2,035 ± 528 | 52.9 ± 2.7 | 130 ± 27 |
| Noncarrier ..... | 1,043 ± 236 | 66.0 ± 3.2 | 136 ± 31 | 2,049 ± 415 | 66.2 ± 3.2 | 246 ± 50 |

[a] ND = not determined.
[b] Pregnant at the time of testing.

tion (Triggs-Raine et al. 1992), the G-to-A mutation at intron 9+1, common in non-Jewish populations (Akli et al. 1991; Akerman et al. 1992; Landels et al. 1992), and the three mutations common in the Ashkenazi Jewish population (Paw et al. 1990; Triggs-Raine et al. 1990; Grebner and Tomczak 1991; Landels et al. 1991). None of these mutations were detected in the $C_{745}$-to-T mutation–positive samples (data not shown). The ethnic/geographic origins of the five individuals carrying the $C_{745}$-to-T mutation were Polish/Irish, Philippine/Spanish, Hawaiian, Chinese, and French.

## Discussion

Healthy adults who appear Hex A deficient on standard biochemical tests and who are neurologically normal have been called "pseudodeficient" or "Hex A–minus normal." These individuals are compound heterozygotes, typically harboring a common disease-causing mutation on one allele and a benign mutation on the second allele. The benign mutations in the α-subunit of Hex A allow normal or adequate levels of hydrolysis of the natural substrate $G_{M2}$ ganglioside, resulting in a normal clinical phenotype. Because the standard synthetic substrate assay with 4-MUG cannot differentiate carriers of these mutations and carriers of disease-causing mutations (Triggs-Raine et al. 1992; present study), the characterization of benign mutations at the DNA level is important for Tay-Sachs disease prevention programs.

We identified a benign $C_{745}$-to-T mutation in exon 7 of the *HEXA* gene, a mutation that results in the substitution of Arg by Trp at position 249 of the α-subunit of β-hexosaminidase. The pseudodeficient subject in whom this mutation was identified (16819) has on the other allele the previously described $G_{805}$-to-A (Gly269Ser) mutation that is associated with adult-on-

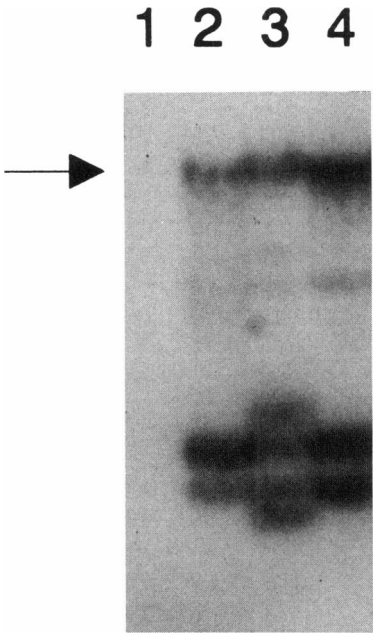


**Figure 1** SSCP analysis of exon 7 of *HEXA*. The $\alpha^{32}$P-dATP-labeled PCR products of 16858 (lane 2), 16859 (lane 3), and a normal control (lane 4) were denatured, and the single strands were separated on a nondenaturing polyacrylamide gel containing 10% glycerol. The arrow indicates the nondenatured band. Lane 1 is a water blank. The two extra bands in lane 3 result from a mutation that alters the conformation and, therefore, the mobility of the strands from one allele.

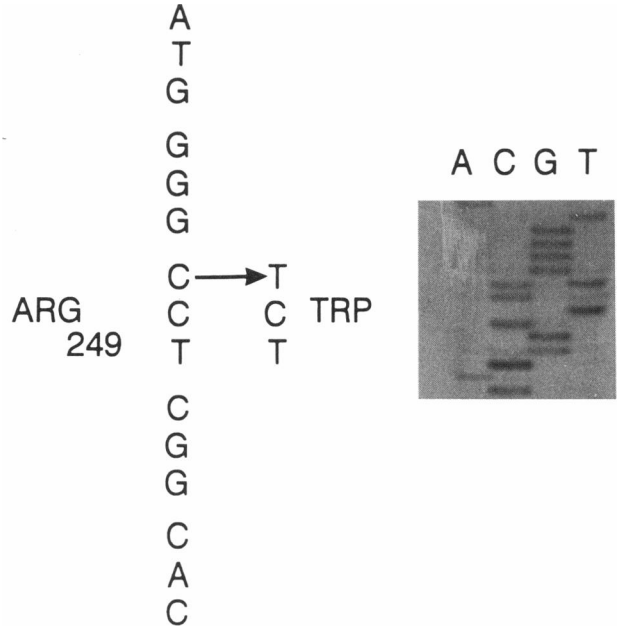


**Figure 2** Sequence of 16859 DNA. The region of exon 7 containing the $C_{745}$-to-T mutation is shown.

set $G_{M2}$ gangliosidosis. Presently, all of the pseudodeficient subjects who have been fully characterized have a benign mutation in *HEXA* on one allele and a common disease-causing mutation in *HEXA* on the other allele (Mules et al. 1992; Triggs-Raine et al. 1992; present study).

The proband in this study is now 42 years of age and exhibits no symptoms of $G_{M2}$ gangliosidosis. Patients with adult-onset $G_{M2}$ gangliosidosis, including persons homozygous for the Gly269Ser mutation (Navon et al. 1986; Sandhoff et al. 1989), typically exhibit symptoms before 40 years of age (Navon et al. 1990; M. R. Natowicz, unpublished data). Although a detailed neurological assessment could not be obtained from the proband in this study, discussions with her revealed no symptoms of neurodegenerative disease. Final confirmation that this individual is pseudodeficient would require that a tissue sample be tested for its capacity to hydrolyze $G_{M2}$ ganglioside, but fibroblasts were not available. However, the absence of clinical symptoms in the fifth decade indicates that, if the $C_{745}$-to-T mutation causes disease, the clinical phenotype must be very mild.

The first benign mutation identified, $C_{739}$-to-T (Arg247Trp), is associated with a characteristic enzyme phenotype. When the standard synthetic substrate, 4-MUG, was used, compound heterozygotes for the $C_{739}$-to-T mutation and a disease-causing mutation exhibited low or absent serum Hex A activity but slightly higher levels in fibroblasts and/or leukocytes (Thomas et al. 1982; Grebner et al. 1986; Navon et al. 1986; Triggs-Raine et al. 1992). The mutant α-subunit also did not hydrolyze the sulfated derivative of 4-MUG, 4-MUGS (Triggs-Raine et al. 1992). However, in cases where samples were available for testing, $G_{M2}$ ganglioside was hydrolyzed either in cultured fibroblast cells or in $G_{M2}$ gangliosidase assays (Kelly et al. 1976; O'Brien et al. 1978; Thomas et al. 1982; Grebner et al. 1986; Triggs-Raine et al. 1992). Insofar as $G_{M2}$ ganglioside hydrolysis studies are technically difficult, DNA testing is the only simple way to differentiate between compound heterozygotes with the $C_{739}$-to-T mutation

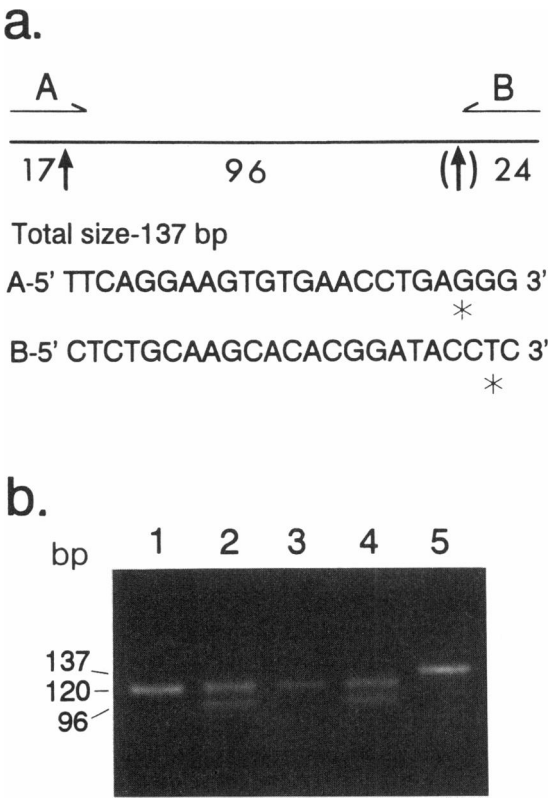


**Figure 3** Strategy for the detection of the $C_{745}$-to-T mutation. *a*, *Dde*I sites (*arrows*). The parentheses indicate the site created in the presence of the $C_{745}$-to-T mutation. The asterisks under primers A and B indicate the single base changes introduced to create a control *Dde*I site in primer A and to create a *Dde*I site in the presence of the $C_{745}$-to-T change when primer B is used in the PCR reaction. *b*, Agarose gel electrophoresis of the products of the strategy used with the A and B primers. Lane 1, Normal DNA cut by *Dde*I. Lane 2, 16819 cut by *Dde*I. Lane 3, 16858 cut by *Dde*I. Lane 4, 16859 cut by *Dde*I. Lane 5, Normal DNA uncut.

**Table 2**

**DNA Analysis of Tay-Sachs Disease Carriers and Noncarriers**

| Heritage and Status | Total No. Tested | No. (%) of $C_{745}$-to-T |
|---|---|---|
| Non-Jewish: | | |
| Carriers[a] | 63[b] | 4 (6%) |
| Noncarriers[a] | 10 | 0 |
| Obligate carriers | 6 | 0 |
| Ashkenazi Jewish: | | |
| Carriers[a] | 218[c] | 0 |
| Noncarriers[a] | 10 | 0 |

[a] Status of sample is based on an enzyme assay with the synthetic substrate, 4-MUG.

[b] Of these 63, 19 were examined in a previous study (Tomczak et al. 1993); 14 of these 19 were shown to have a previously described mutation and thus were not tested for $C_{745}$-to-T; the remaining 44 samples were tested only for the $C_{745}$-to-T mutation, unless they were $C_{745}$-to-T positive.

[c] Of these 218, 209 were examined in a previous study (Tomczak et al. 1993); 197 of these 209 were shown to have a previously described mutation and thus were not tested for $C_{745}$-to-T; the remaining 9 samples were tested only for the $C_{745}$-to-T mutation.

and those with "true" Tay-Sachs disease mutations (Triggs-Raine et al. 1992).

The Arg249Trp mutation described here is associated with a different biochemical phenotype than is the Arg247Trp mutation. The Arg247Trp mutation results in different levels of Hex A activity in serum and leukocyte samples, whereas the pseudodeficient subject with this mutation in combination with the Gly269Ser mutation on the other allele had low levels of Hex A in both serum and leukocytes when tested with the 4-MUG and 4-MUGS substrate. The levels of Hex A were found to be significantly higher than those in Tay-Sachs disease patients when determined with 4-MUGS.

The two benign mutations, Arg247Trp (Triggs-Raine et al. 1992) and Arg249Trp (present study), fall within a highly conserved region of the α-subunit of β-hexosaminidase (fig. 4). Specifically, a stretch of 10 amino acids in this region is entirely conserved in several mammalian ß-hexosaminidases (Myerowitz et al. 1985; O'Dowd et al. 1985; Korneluk et al. 1986; Bapat et al. 1988; Beccari et al. 1992). Much of this region is also conserved in the more distantly related ß-hexosaminidase of *Dictyostelium discoideum* (Graham et al. 1988). Although this region of the *Vibrio parahemolyticus* chitobiase showed little homology in its original computer alignment (Soto-Gil and Zyskind 1989), realignment demonstrated considerable similarity between amino acids 431–440 of chitobiase and amino acids 244–253 of the α-subunit of β-hexosaminidase. Little is known about the structure of the ß-hexosaminidase proteins. The Arg249Trp mutation described here could affect the heat stability and/or substrate recognition/binding of the α-subunit.

The previously described benign $C_{739}$-to-T mutation accounts for 32%–42% of non-Jewish enzyme-defined carriers (Triggs-Raine et al. 1992; Kaback 1993; Tomczak et al. 1993). We found that the $C_{745}$-to-T and $C_{739}$-to-T mutations together account for approximately 38% of enzyme-defined non-Jewish carriers. Only the $C_{739}$-to-T mutation, however, has been detected in Jewish enzyme-defined carriers, albeit in a small proportion (<3%) (Kaback 1993; Tomczak et al. 1993). If both of these mutations are taken into account in the calculation of the enzyme-defined carrier frequency in the non-Jewish population, the frequency becomes approximately 1/280, similar to the 1/300 expected on the basis of the disease incidence in non-Jews. These data, therefore, support the hypothesis that benign mutations account for the difference in the enzyme-defined and disease-based carrier frequencies.

DNA testing for benign mutations should be available through comprehensive Tay-Sachs disease prevention programs. Testing for the $C_{739}$-to-T and $C_{745}$-to-T mutations could rule out the risk for Tay-Sachs disease in approximately 38% of non-Jewish and 3% of Ashkenazi Jewish enzyme-defined carriers (Kaback 1993; Tomczak et al. 1993). DNA testing for benign mutations in non-Jewish enzyme-defined Tay-Sachs disease

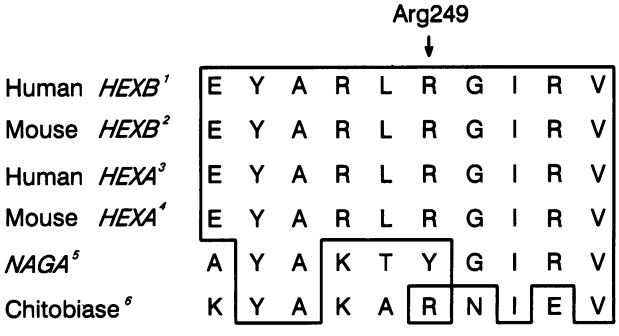


**Figure 4** Homology between exon 7 region of *HEXA* and other ß-hexosaminidases. Superscript nos. refer to references, as follows: 1 = O'Dowd et al. (1985); 2 = Bapat et al. (1988) and B. L. Triggs-Raine and R. Gravel (unpublished data); 3 = Myerowitz et al. (1985) and Korneluk et al. (1986); 4 = Beccari et al. (1992); 5 = Graham et al. (1988); and 6 = Soto-Gil and Zyskind (1989).

carriers should, in most instances, precede final counseling for carrier status. At a minimum, before prenatal diagnosis, testing for benign mutations should be offered for both Jewish and non-Jewish enzyme-defined carriers in "at-risk" couples. If unidentified, both the $C_{739}$-to-T and $C_{745}$-to-T mutations can lead to unnecessary and confusing prenatal diagnoses (Triggs-Raine et al. 1992).

## Acknowledgments

This work was supported by grants from the Manitoba Health Research Council (MHRC) and by Medical Research Council (MRC) of Canada grant MT-11708 (all to B.T.-R.), by from the Department of Mental Retardation of the Commonwealth of Massachusetts contract 100220023SC (to M.N.), and by both a contract from the Genetic Disease Branch, Maternal and Child Health, California Department of Health and a grant from the National Tay-Sachs Disease and Allied Disease Association (both to M.M.K.). The authors thank Dr. Roy Gravel for providing many of the oligonucleotide primers used in this study and thank Dr. Gene Grebner and Mr. Jerzy Tomczak for providing DNA samples previously tested for common mutations.

## References

Akerman BR, Zielenski J, Triggs-Raine BL, Prence EM, Natowicz M, Lim-Steele JST, Kaback MM, et al (1992) A mutation common in non-Jewish Tay-Sachs disease: frequency and RNA studies. Hum Mutat 1:303–309

Akli S, Chelly J, Lacorte J-M, Poenaru L, Kahn A (1991) Seven novel Tay-Sachs mutations detected by chemical mismatch cleavage of PCR-amplified cDNA fragments. Genomics 11:124–134

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

Bapat B, Ethier M, Neote K, Mahuran D, Gravel RA (1988) Cloning and sequence analysis of a cDNA encoding the β-subunit of mouse β-hexosaminidase. FEBS Lett 237:191-195

Beccari T, Hoade J, Orlacchio A, Stirling JL (1992) Cloning and sequence analysis of a cDNA encoding the α-subunit of mouse β-N-acetylhexosaminidase and comparison with the human enzyme. Biochem J 285:593–596

Graham TR, Zassenhaus HP, Kaplan A (1988) Molecular cloning of the cDNA which encodes β-N-acetylhexosaminidase A from *Dictyostelium discoideum*. J Biol Chem 263:16823–16829

Grebner EE, Mansfield DA, Raghavan SS, Kolodny EH, d'Azzo A, Neufeld EF, Jackson LG (1986) Two abnormalities of hexosaminidase A in clinically normal individuals. Am J Hum Genet 38:505–514

Grebner EE, Tomczak J (1991) Distribution of three α-chain β-hexosaminidase A mutations among Tay-Sachs carriers. Am J Hum Genet 48:604–607

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

Hoar DI, Haslam DB, Starozik DM (1984) Improved direct molecular diagnosis and rapid fetal sexing. Prenat Diagn 4:241–247

Kaback MM (1972) Thermal fractionation of serum hexosaminidases: applications to heterozygote detection and diagnosis of Tay-Sachs disease. Methods Enzymol 28:862–867

——— (1993) Tay-Sachs disease: molecular issues in carrier screening and prenatal disease. In: Molecular biology of lysosomal diseases, 2d ed. International Duodecim Symposium of the Finnish Medical Society, Helsinki, p 43

Kaback MM, Hirsh P, Roy C, Greewald S, Kirk M (1978) Gene frequencies for Tay-Sachs (TSD) and Sandhoff's disease (SD) in Jewish and non-Jewish populations. Pediatr Res 12:530A

Kaback M, Rimoin PL, O'Brien JLS (eds) (1977*a*) Tay-Sachs disease: screening and prevention. AR Liss, New York

Kaback MM, Shapiro LJ, Hirsch P (1977*b*) Tay-Sachs disease heterozygote detection: a quality control study. In: Kaback MM, Rimoin DL, O'Brien JLS (eds) Tay-Sachs disease: screening and prevention. AR Liss, New York, pp 267–279

Kelly TE, Reynolds LW, O'Brien JS (1976) Segregation within a family of two mutant alleles for hexosaminidase A. Clin Genet 9:540–543

Korneluk RG, Mahuran DJ, Neote K, Klavins MH, O'Dowd BF, Tropak M, Willard HF, et al (1986) Isolation of cDNA clones coding for the α-subunit of human β-hexosaminidase. J Biol Chem 261:8407–8413

Kytzia H-J, Sandhoff K (1985) Evidence for two different active sites on human ß-hexosaminidase A. J Biol Chem 260:7568–7572

Landels EC, Ellis IH, Fensom AH, Green PM, Bobrow M (1991) Frequency of the Tay-Sachs disease splice and insertion mutations in the UK Ashkenazi Jewish population. J Med Genet 28:177–180

Landels EC, Green PM, Ellis IH, Fensom A, Bobrow M (1992) β-Hexosaminidase splice site mutation has a high frequency among non-Jewish Tay-Sachs disease carriers from the British Isles. J Med Genet 29:563–567

Mules EH, Hayflick S, Dowling CE, Kelly TE, Akerman BR, Gravel RA, Thomas GH (1992) Molecular basis of hexosaminidase A deficiency and pseudodeficiency in the Berks County Pennsylvania Dutch. Hum Mutat 1:298–302

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

Myerowitz R, Piekarz R, Neufeld EF, Shows TB, Suzuki K (1985) Human β-hexosaminidase α chain: coding sequence and homology with the β chain. Proc Natl Acad Sci USA 82:7830–7834

Myrianthopoulos NC, Aronson SM (1966) Population dynamics of Tay-Sachs disease. I. Reproductive fitness and selection. Am J Hum Genet 18:313–327

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

Navon R, Kolodny EH, Mitsumoto H, Thomas GH, Proia RL (1990) Ashkenazi Jewish and non-Jewish adult $G_{M2}$ gangliosidosis patients share a common genetic defect. Am J Hum Genet 46:817–821

Navon R, Proia RL (1989) The mutations in Ashkenazi Jews with adult GM2 gangliosidosis, the adult form of Tay-Sachs disease. Science 243:1471–1474

Neote K, Mahuran DJ, Gravel RA (1991) Molecular genetics of β-hexosaminidase deficiencies. Adv Neurol 56:189–207

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

O'Brien JS, Tennant L, Veath ML, Scott CR, Bucknall WE (1978) Characterization of unusual hexosaminidase A (HEX A) deficient human mutants. Am J Hum Genet 30:602–608

O'Dowd BF, Quan F, Willard HF, Lamhonwah A-M, Korneluk RG, Lowden JA, Gravel R, et al (1985) Isolation of cDNA clones coding for the β subunit of human β-hexosaminidase. Proc Natl Acad Sci USA 82:1184–1188

Ohno K, Suzuki K (1988) A splicing defect due to an exon-intron junctional mutation results in abnormal β-hexosaminidase α chain mRNAs in Ashkenazi Jewish patients with Tay-Sachs disease. Biochem Biophys Res Commun 153:463–469

Orita M, Suzuki Y, Sekiya T, Hayashi K (1989) Rapid and sensitive detection of point mutations and DNA polymorphisms using the polymerase chain reaction. Genomics 5:874–879

Paw BH, Kaback MM, Neufeld EF (1989) Molecular basis of adult-onset and chronic GM2 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

Paw BH, Tieu PT, Kaback MM, Lim J, Neufeld EF (1990) Frequency of three *HEX A* mutant alleles among Jewish and non-Jewish carriers identified in a Tay-Sachs screening program. Am J Hum Genet 47:698–705

Prence EM, Natowicz MR, Zalewski I. Unusual thermolability properties of leukocyte beta-hexosaminidase: implications for Tay-Sachs disease carrier screening. Clin Chem (in press)

Sandhoff K, Conzelmann E, Neufeld EF, Kaback MM, Suzuki K (1989) The GM2 gangliosidoses. In: Scriver CR, Beaudet AL, Sly WS, Valle D (eds) The metabolic basis of inherited disease. McGraw-Hill, New York, pp 1807–1839

Soto-Gil RW, Zyskind JW (1989) N,N′-diacetylchitobiase of *Vibrio harveyi*. J Biol Chem 264:14778–14783

Thomas GH, Raghavan S, Kolodny EH, Frisch A, Neufeld EF, O'Brien JS, Reynolds LW, et al (1982) Nonuniform deficiency of hexosaminidase A in tissues and fluids of two unrelated individuals. Pediatr Res 16:232–237

Tomczak J, Boogen C, Grebner EE (1993) Distribution of a pseudodeficiency allele among Tay-Sachs carriers. Am J Hum Genet 53:537–539

Triggs-Raine BL, Akerman BR, Clarke JTR, Gravel RA (1991) Sequence of DNA flanking the exons of the *HEXA* gene, and identification of mutations in Tay-Sachs disease. Am J Hum Genet 49:1041–1054

Triggs-Raine BL, Feigenbaum ASJ, Natowicz M, Skomorowski M-A, Schuster SM, Clarke JTR, Mahuran DJ, et al (1990) Screening for carriers of Tay-Sachs disease among Ashkenazi Jews—a comparison of DNA-based and enzyme-based tests. N Engl J Med 323:6–12

Triggs-Raine BL, Mules EH, Kaback MM, Lim-Steele JST, Dowling CE, Akerman BR, Natowicz MR, et al (1992) A pseudodeficiency allele common in non-Jewish Tay-Sachs carriers: implications for carrier screening. Am J Hum Genet 51:793–801

Vidgoff J, Buist NRM, O'Brien JS (1973) Absence of β-N-acetyl-D-hexosaminidase A activity in a healthy woman. Am J Hum Genet 25:372–381

Winship PR (1989) An improved method for directly sequencing PCR amplified material using dimethyl sulphoxide. Nucleic Acids Res 17:1266