Evaluation of the Risk for Tay-Sachs Disease in Individuals of French Canadian Ancestry Living in New England

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Background: The assessment of risk for Tay-Sachs disease (TSD) in individuals of French Canadian background living in New England is an important health issue. In preliminary studies of the enzyme-defined carrier frequency for TSD among Franco-Americans in New England, we found frequencies (1:53) higher than predicted from the incidence of infantile TSD in this region. We have now further evaluated the risk for TSD in the Franco-American population of New England.

Methods: Using a fluorescence-based assay for β-hexosaminidase activity, we determined the carrier frequencies for TSD in 2783 Franco-Americans. DNA analysis was used to identify mutations causing enzyme deficiency in TSD carriers.

Results: We determined the enzyme-defined carrier frequency for TSD as 1:65 (95% confidence interval 1:49 to 1:90). DNA-based analysis of 24 of the enzyme-defined carriers revealed 21 with sequence changes: 9 disease-causing, 4 benign, and 8 of unknown significance. Six of the unknowns were identified as c.748G>A p.G250S, a mutation we show by expression analysis to behave similarly to the previously described c.805G>A p.G269S adult-onset TSD mutation. This putative adult-onset TSD c.748G>A p.G250S mutation has a population frequency similar to the common 7.6 kb deletion mutation that occurs in persons of French Canadian ancestry.

Conclusions: We estimate the frequency of deleterious TSD alleles in Franco-Americans to be 1:73 (95% confidence interval 1:55 to 1:107). These data provide a more complete data base from which to formulate policy recommendations regarding TSD heterozygosity screening in individuals of French Canadian background.

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Tay-Sachs Disease (TSD)6 is an autosomal recessive lysosomal lipid storage disorder that is due to deficiency of the lysosomal enzyme β-hexosaminidase A (HEXA) [reviewed in (1)]. The HEXA isoenzyme is comprised of 1 α-subunit encoded by HEXA7 and 1 β-subunit encoded by HEXB. Two β-subunits can combine to form a 2nd isoenzyme with different but overlapping substrate specificities, β-hexosaminidase B (HEXB). Mutations in both alleles of HEXA result in a deficiency of HEXA, and mutations in both alleles of HEXB result in deficiencies of both HEXA and HEXB. Mutations at both alleles of either gene locus lead to a deficiency of HEXA activity and an accumulation of GM2 ganglioside, leading to similar lysosomal storage disorders, TSD and Sandhoff disease. There are also mutations in these genes that result in a partial deficiency of HEXA and lead to less severe forms of disease. Heterozygotes for deleterious mutations in HEXA and HEXB can be identified with an enzyme-based activity assay.

To identify TSD carriers by use of an enzymatic assay, the combined activities of HEXA and HEXB, as well as the activity of HEXB after heat-inactivation of HEXA, are determined. Heterozygotes for TSD have reduced HEXA

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6 Nonstandard abbreviations: TSD, Tay-Sachs Disease; HEXA, β-hexosaminidase A; HEXB, β-hexosaminidase B; CI, confidence interval; HEXS, β-hexosaminidase S; SSCP, single-strand conformational polymorphism.

7 Human genes: HEXA, alpha polypeptide gene; HEXB, beta polypeptide gene.
activity (observed as a reduced percentage of HEXA relative to the total β-hexosaminidase activities). This enzyme-based assay is routinely used by clinical laboratories that screen for heterozygotes for TSD (2).

Enzyme-based screening programs for HEXA deficiency were initiated in the 1970s for the Ashkenazi Jewish and, later, French Canadian populations that were each recognized to have an increased incidence of TSD (3, 4). Subsequently, an increased prevalence of TSD was discovered for individuals of Moroccan Jewish, Pennsylvania Dutch, and Southern Louisiana Cajun backgrounds (5–7). These assays also led to the recognition of increased TSD carrier frequencies among Franco-Americans living in New England (8, 9), Iraqi Jews (10), and Irish Americans (11).

Elucidation of the molecular basis of TSD has made apparent an alternative approach for heterozygosity screening in some populations. In persons of Ashkenazi Jewish background, DNA-based carrier screening is possible because 3 mutations account for ~95%–98% of obligate TSD carriers (12, 13). In contrast, the molecular basis of TSD in most non-Jewish populations is highly heterogeneous, prohibiting DNA-based screening. Enzyme-based screening in the non-Jewish population is, however, often supplemented by molecular analysis for specific “pseudodeficiency” or benign mutations. These benign mutations cause partial enzyme deficiency, but the level of residual activity is adequate to prevent the pathological accumulation of G\textsubscript{M\textsubscript{2}} ganglioside (14).

In non-Jewish populations benign mutations account for differences between the carrier frequencies determined by enzyme screening (1:167) compared with those estimated on the basis of disease incidence (1:300) (13, 15, 16). Through molecular analysis of enzyme-defined carriers, the apparent risk for TSD within a population can be modified by adjusting for the population frequency of benign mutations. In the case of Irish Americans, such analyses lowered the carrier frequency from 1:25 to a maximum of 1:41 (11). In preliminary studies of Franco-Americans living in Massachusetts (MA), accounting for benign mutations reduced the risk from 1:57 to 1:66 (8).

Using a much larger study population and including analyses for additional mutations, we have expanded our studies of TSD carrier frequencies among persons of French Canadian background living in New England. We also characterize biochemical and structural aspects of a mutation found to have an unexpectedly high frequency in this population. Based on our analyses, we estimate the maximal TSD carrier frequency to be 1.73 [95% confidence interval (CI), 1.55 to 1.107].

**Materials and Methods**

**SAMPLES**

Samples were obtained from individuals referred to the E.K. Shriver Center in Boston, MA for TSD carrier screening; all specimens were obtained after donors gave written informed consents. Individuals who self-identified as being Franco-American or French Canadian, or who reported one or more grandparents of French Canadian background, were eligible for the study except as noted below. Individuals who were pregnant, had both Ashkenazi Jewish and French Canadian heritage, or indicated that they had a relative who was a carrier for or had TSD, were excluded from the study. The questionnaire used to identify appropriate study participants was previously published (8).

**ENZYME ANALYSIS**

Heterozygosity for TSD was determined with the standard heat-inactivation assay (2, 17). The percentages of serum HEXA activity used to identify heterozygotes and non-carriers for TSD were as reported previously (8). The diagnostic criteria for serum HEXA used to establish a diagnosis of heterozygosity and non-carrier status for TSD were: carrier, >55% HEXA and non-carrier, ≥61% HEXA. The diagnostic criteria for leukocyte HEXA were: carrier, <55% HEXA and non-carrier, ≥62% HEXA. Each case was individually evaluated on the basis of the serum HEXA %, leukocyte HEXA %, and total HEX activity. Samples with a percentage HEXA activity close to the cutoff for the carrier range and without a mutation detected by single-strand conformational polymorphism (SSCP) in our original study (9) were reassayed by a 2nd reference laboratory. Four samples were identified as non-carriers by the 2nd reference laboratory, and they are included as non-carriers in this analysis.

**DNA ISOLATION AND DETECTION OF MUTATIONS**

From the 43 individuals that tested as TSD carriers, leukocyte pellets or sonicates were available for 25; DNA was successfully isolated from 24 samples by use of previously described procedures (18). Nine enzyme-based assay positive samples that were not part of our previous analysis were tested for 4 DNA sequence variations, the 7.6 kb deletion (19), c.739C>T (15), c.805G>A (20, 21), and c.748G>A (9), according to established protocols (9). For samples with enough remaining DNA (22 samples) and no initially identified mutation, SSCP analysis of each exon was performed as described previously (9).

**EXPRESSION OF HEXA MUTATIONS**

We used methods described previously (14, 22) to assess the effect on HEXA activity of 2 novel amino acid substitutions, c.748G>A p.G250S and c.587A>G p. N196S. Mutations were introduced into the α-subunit cDNA with oligonucleotide primers 5’ ACG GCT CCG GAG TAT CCG TGT GC 3’ for c.748G>A, and 5’CAT GGC GTA CAG TAA ATT GAA CG 3’ for c.587A>G. The presence of only the desired change in the cDNA was confirmed by sequencing. Mutated cDNAs were subcloned into pSVL vectors to create pSVLG748A and pSVLA587G, again following the same approach previously used in our laboratory (14). Vectors with mutations associated with.
normal (αpSVL), TSD (αpSVLC508T), adult-onset TSD (αpSVLG805A), and pseudodeficiency (αpSVLC739T) phenotypes were used as described in previous studies (14, 22). These vectors were transfected into COS-7 cells, alone or in combination with the β-subunit cDNA (pCD43), and the HEXA (αβ dimer) and β-hexosaminidase S (HEXS) (αα dimer) activities, and α-subunit protein levels were monitored according to previously described procedures (14).

STATISTICAL ANALYSIS
We used a binomial distribution to calculate 95% CIs for the carrier frequencies for a proportion (NCSS Software).

Results
DETERMINATION OF THE TSD ENZYME-DEFINED CARRIER FREQUENCIES
Our prospective analysis of heterozygosity for TSD in individuals of French Canadian background living in New England included samples collected between 1989 and 2001, from 2783 nonpregnant donors. Samples were obtained from individuals from 6 New England states; the majority of the samples were from Massachusetts and New Hampshire residents (Table 1). In total, 43 TSD carriers were identified, giving an enzyme-based TSD carrier frequency in this population of 1 in 65 (95% CI, 1:49 to 1:90).

DETECTION OF KNOWN COMMON TSD MUTATIONS
For 24 of the 43 TSD carriers (above), DNA was available for molecular analysis of the HEXA gene. We first used direct analysis to test for common mutations in the samples; if the result was negative, we then used SSCP to screen for other mutations in the 14 HEXA exons. All variations were confirmed by sequencing. The results of the molecular analyses are reported in Table 2. In total, mutations were identified in 21 of 24 enzyme-defined carriers.

We categorized the identified variations on the basis of their associated phenotype—disease-causing, benign, or unknown (Table 2). In total, 9 of 21 samples had known disease-causing mutations and 4 of 21 samples had known benign mutations (c.739C>T or c.745C>T). Three variations of unknown significance, c.748G>A p.G250S, c.587A>G p.N196S, and c.1146 + 18A>G, accounted for the remaining 8 alleles. Six persons had the c.748G>A p.G250S variation.

FUNCTIONAL ASSESSMENT OF NOVEL AMINO ACID SUBSTITUTIONS
To assess the functional significance of the 2 novel amino acid changes, c.587A>G p.N196S and c.748G>A p.G250S, these changes were introduced into the α-subunit cDNA and transfected into COS-7 cells, as were several control vectors. Transfection of the α-subunit vectors alone to generate HEXS (αα) showed that the N196S variant had only a slight impact on α-subunit function, reducing the HEXS activity to 87% of the wild-type activity (Fig. 1A). This reduction was significantly less than the well-characterized benign mutation c.739C>T p.R247W, which reduced the HEXS activity to 37%. However, c.748G>A p.G250S reduced the activity of HEXS to only 4% of the activity of the wild-type α-subunit cDNA after the background is subtracted, an activity level similar to that of the c.805G>A p.G269S mutation that results in a late- or adult-onset form of TSD. These findings suggest that the c.748G>A encoded G250S substitution severely impacts the function of the α-subunit, whereas the N196S substitution encoded by c.587A>G has little effect.

To further investigate the functional effects of these changes, the normal and mutant α-subunit vectors (αpSVL, αpSVLC508T, αpSVLG805A, αpSVLC739T,
apSVLG748A, and apSVLA587G) were cotransfected with the β-subunit cDNA to generate both the HEXS (αα) and HEXA (αβ) β-hexosaminidase isoenzymes in COS-7 cells. This approach has been used previously and is known to amplify residual activity associated with the mutant α-subunit, allowing the differentiation of mutations associated with complete and partial HEXA deficiencies (14, 23, 24). Using this approach, we noted similar findings to that for the transfection of the α-subunit cDNAs alone (Fig. 1, Panel B and Fig. 2). The levels of HEXA and HEXS activities as well as mature α-subunit protein associated with the c.587A→G variation are clearly greater than that associated with the benign mutation (c.739C>T). In contrast, the c.748G>A mutation reduced the activities of HEXA and HEXS to levels comparable to the c.805G>A adult-onset mutation, although above that of the c.508C>T mutation associated with an infantile form of TSD. The levels of HEXA protein were also reduced by the c.748G>A mutation to a level similar to that of the adult-onset TSD mutation c.805G>A (Fig. 2).

**Discussion**

The assessment of the risk for TSD among individuals of French Canadian background in the New England states is an important health issue because up to 10.3% of the residents of these states declared French Canadian heritage in the 2000 census and a much larger proportion reported French ancestry (25); the latter, in turn, almost certainly relates to the heavy migration of French Canadians to the New England states (26). The risk for TSD in this group, however, cannot be easily predicted by comparison to the TSD carrier frequency found in French Canadians living in Quebec because the latter varies between regions, from 1:13 to 1:300 (4). In our study of 2783 individuals, we found an enzyme-defined TSD carrier frequency of 1:65, before considering the molecular basis of HEXA deficiency in this group. This risk is lower than that of the Ashkenazi Jewish population (~ 1:25), but much higher than the enzyme-defined carrier frequency in the general non-Jewish population (1:167).

We sought to further assess the risk for TSD in this group through a mutational analysis of HEXA heterozygosity. Of the 24 enzyme-defined carriers for whom DNA was available, we identified 9 individuals with known disease-causing mutations, 4 with known benign mutations, and 8 with mutations of unknown significance. The 3 individuals for whom mutations were not identified may have had mutations that were not detectable by SSCP, because this method does not detect 100% of mutations. Furthermore, general strategies to detect large
duplications or insertions that could also account for the enzyme deficiency were not performed. In these 3 individuals, however, the relatively high percentages of HEXA activity in the serum (59.8%, 58%, 54.2%) and white blood cells (49.5%, 53.4%, 54.1%) suggest that the results may be false positives of the enzyme assay.

The 9 known disease-causing mutations that were identified in this study were largely representative of those that have previously been identified in individuals of French Canadian background. Most of these individuals (5 of 9) had the 7.6 kb deletion, a mutation unique to the French Canadian population that originated in the region surrounding the Bas-St.-Laurent-Gaspésie region of Quebec and the Madawaska Valley in the province of New Brunswick (27). A 2nd mutation unique to French Canadians, c.805 + 1G>A (28), was not identified in these samples. The c.805 + 1G>A mutation originated in the Saguenay-Lac St-Jean region of Quebec (28), and its absence from our samples suggests a different geographic origin for the Franco-Americans that we examined. Two of the other known disease-causing mutations that were identified, c.1287insTATC and c.508C>T, have previously been identified in French Canadians or Franco-Americans (29,30) and c.409C>T p.R137X has been described in a cohort of European subjects (31).

The known benign variants, c.739C>T p.R247W and c.745C>T p.R249W, accounted for 4 of the mutations in enzyme-defined TSD carriers in this study. These mutations have been well characterized and when taken into account reduce the estimated frequency of heterozygosity for deleterious HEXA alleles in the non-Jewish population from ~1:67 to ~1:167. When we accounted for these known benign mutations in individuals with enzyme-defined heterozygosity, the number of carriers was reduced from 43 to 39.

We further considered the 3 mutations of unknown significance in our population, c.748G>A p.G250S, c.587A>G p.N196S, and c.1146 + 18A>G, to try to determine their impact on the enzyme-defined TSD carrier frequency. We found these sequence changes to be absent both in 50 enzyme-defined non-carriers (9) and in an additional 100 controls (data not shown) and therefore consider them likely to be the cause of enzyme-deficiency in these individuals. In addition, the c.1146 + 18A>G change was not predicted to have a major impact on splicing (data not shown), no other variant was identified by denaturing HPLC analysis of the HEXA exons (data not shown). The c.587A>G p.N196S was identified only once among our enzyme-defined carriers, although we had identified it in an earlier study in a carrier who was pregnant (9). This substitution did not significantly reduce the HEXA and HEXS activity and may be a benign mutation that leads to apparent HEXA deficiency. If c.587A>G p.N196S is included as a benign sequence variant, the number of enzyme-defined carriers is reduced to 38, giving a maximal deleterious Tay-Sachs carrier frequency of 1:73 (95% CI 1:55 to 1:107).

We were especially interested in analyzing the c.748G>A p.G250S mutation because it was identified in 6 of our enzyme-defined TSD carriers but, to our knowledge, has never been identified in a TSD patient. This suggested to us that it may be another pseudodeficiency mutation. Surprisingly, our expression analysis showed the G250S substitution decreased HEXA and HEXS activities as well as α-subunit protein to levels well below that of a known benign mutation and similar to that of an adult-onset TSD mutation. Based on our expression system and functional and immunochemical assays, the G250S substitution has the potential to cause a late-onset form of TSD.

Interestingly, 3 different mutations have been identified at the G250 position. The first, G250D, was found to cause a juvenile form of TSD in a Lebanese Marronite family (32). A G250V change was subsequently found among enzyme-defined TSD carriers of Iraqi Jewish origin (33). The G250S change was identified among Franco-American enzyme-defined TSD carriers (9) and in this study. The risk for TSD associated with the G250V and G250S changes is difficult to assess because neither has been detected in an individual with any form of TSD or pseudodeficiency state. Nonetheless, as described above, the behavior of the G250S-containing α-subunit is similar to that of the adult-onset G269S mutation and has a more severe impact on function than the pseudodeficiency mutation R247W. Thus, our expression analyses suggest the G250S mutation is more likely to be disease-causing than benign.

The x-ray crystal structure of a functionally mature glycosylated form of human lysosomal HEXA was recently determined (34). As shown in Fig. 3, the α- and β-subunits of HEXA are kidney-shaped, 2-domain proteins that form dimers. A number of disease-causing mutations in the α-subunit of HEXA have been mapped to the interface between domains I and II, including the G250S variation discussed here. Additional notable mutations occurring at this interface include G250D (35), R166G (36), and E482K (37). In each case, the interface is disrupted by either loss of a salt bridge or a hydrogen bond, as suggested for R166K and E482K, respectively, or overpacking and burying a polar residue, as suggested by G250D (34). An amino acid substitution of G250 cannot be accommodated, because the added side chain cannot pack efficiently against the α-helix of domain I (Fig. 3). It appears that the larger and more polar the substituted residue at this position, the more detrimental it is to the proper folding and catalytic function of HEXA. Interestingly, the benign mutations R247W and R249W appear to have a much less dramatic effect on folding and function because these residue positions occur on an α-helix of domain II such that a bulky substitution is accommodated by pointing the side chains toward the solvent; thus, they do not appear to seriously affect packing or electrostatic interactions (Fig. 3).
A retrospective analysis of the incidence of TSD in northern New England suggested the carrier frequency for TSD in this population is lower than that found in the Ashkenazi Jewish community (i.e., ~1:25), although a specific frequency statistic was not determined (38). Because that study ascertained cases of TSD by analysis of death records of pediatric individuals from the 3 most northern New England states, any pediatric individuals who were not correctly diagnosed or who did not receive a correct ICD disease code, as well as all individuals with late-onset phenotypes of TSD, would not have been identified. In addition, using an estimate of 1:73 for heterozygosity for deleterious alleles at the HEXA locus based on the data from this work, that retrospective analysis would likely not have had adequate statistical power to detect the predicted number of cases of TSD. Consequently, the data from this study augments that of the retrospective analysis.

Current policy recommendations regarding TSD heterozygosity testing in persons of French Canadian background specify that screening for carrier status should be offered to such individuals before pregnancy if one or both members of a couple are of French-Canadian or Cajun descent (39). Molecular genetic testing of the TSD gene locus affords the ability to detect alleles associated with pseudodeficiency, lethal infantile-onset, and juvenile- and adult-onset phenotypes. This information must be incorporated both in personal decision-making when heterozygosity screening or prenatal testing is done and in the formulation of policy recommendations. Overall, the data from the current work add to earlier studies and provide a considerably more robust database with which public health policy recommendations concerning TSD carrier screening can be formulated.

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References


Fig. 3. The HEXA crystal structure in complex with the mechanistic inhibitor NAG-thiazoline (NGT) (PDB 2GK1). The α-subunit is drawn as a ribbon diagram with domain I colored red and domain II colored blue. The β-subunit, which by way of dimerization is rotated ~120° toward the viewer relative to the α-subunit, is colored white. NAG-thiazoline bound into the active site of each subunit is colored by element and drawn as a space-filled molecule. The inset to the left of the HEXA structure highlights the G250S/D/V mutation hot spot and the 2 benign mutations R247W and R249W.


