Improving Accuracy of Tay Sachs Carrier Screening of the Non-Jewish Population: Analysis of 34 Carriers and Six Late-Onset Patients With HEXA Enzyme and DNA Sequence Analysis

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ABSTRACT: The purpose of this study was to determine whether combining different testing modalities namely β -hexosaminidase A (HEXA) enzyme analysis, HEXA DNA common mutation assay, and HEXA gene sequencing could improve the sensitivity for carrier detection in non-Ashkenazi (AJ) individuals. We performed a HEXA gene sequencing assay, a HEXA DNA common mutation assay, and a HEXA enzyme assay on 34 self-reported Tay-Sachs disease (TSD) carriers, six late-onset patients with TSD, and one pseudodeficiency allele carrier. Sensitivity of TSD carrier detection was 91% for gene sequencing compared with 91% for the enzyme assay and 52% for the DNA mutation assay. Gene sequencing combined with enzyme testing had the highest sensitivity (100%) for carrier detection. Gene sequencing detected four novel mutations, three of which are predicted to be disease causing [118.delT, 965A→T (D322V), and 775A→G (T259A)]. Gene sequencing is useful in identifying rare mutations in patients with TSD and their families, in evaluating spouses of known carriers for TSD who have indeterminate enzyme analysis and negative for common mutation analysis, and in resolving ambiguous enzyme testing results. (*Pediatr Res* 67: 217–220, 2010)

Tay-Sachs disease (TSD) is an autosomal recessive, neurodegenerative disorder caused by insufficient β -hexosaminidase A (HEXA) activity, leading to excess storage of G_{M2} ganglioside. Patients with the classical infant form manifest after the first few months of life. Symptoms include a cherry red spot on the fovea centralis of the macula, increased startle response, hypotonia, progressive weakness and loss of motor skills, seizures, and dementia. Death typically occurs between the ages of 2 and 5 y. Delayed onset (late infant, juvenile, or adult) forms occur less frequently; the later the onset, the less severe the disease. High-risk populations include Ashkenazi Jews (carrier frequency $\sim 1/30$), French Canadians, Cajuns, and Pennsylvania Dutch. Carrier frequency in other populations is $1/300$.

In the United States and Canada, carrier screening in the Ashkenazi Jewish population, instituted in the early 1970s, has resulted in a 90% decrease in incidence (three to five cases annually *versus* ~ 60) (1). Owing to this success, almost all newly diagnosed cases now have at least one parent of non-Ashkenazi Jewish descent. Thus, the American College of Obstetricians and

Gynecologists recommends carrier screening for couples of Ashkenazi Jewish, French-Canadian, and Cajun descent as well as for couples in whom only one member is at high risk based on ethnicity (2). Such screening is currently based on biochemical analysis of HEXA activity supplemented with molecular analysis for detection of the five most common HEXA gene mutations and two pseudodeficiency alleles associated with false-positive enzyme test results.

The biochemical assay often uses an uncharged, artificial substrate, 4-methylumbelliferyl- β -N-acetyl glucosaminide (MUG), to measure total hexosaminidase activity in serum (3). Total hexosaminidase is comprised of HEXA and hexosaminidase B (HEXB). HEXA is a heterodimer of alpha and beta chains, whereas HEXB is a homodimer of beta chains. In the biochemical assay, HEXB activity is measured after heat inactivation of the HEXA in a second serum aliquot. HEXA activity is then calculated as the difference between the total hexosaminidase and HEXB activities. Results are expressed as the percentage of HEXA activity relative to the total hexosaminidase activity.

Although the biochemical assay is reasonably accurate and cost effective, it does have several limitations. First, there is an indeterminate (gray-zone) range in which results from carriers and noncarriers overlap. As many as 10% of samples can have results in this range (4). Second, false-positive results occur in the serum of pregnant women and those taking oral contraceptives owing to the presence of another form of hexosaminidase P (*i.e.*, HEXP) (5,6). HEXP, which is not heat labile, reduces the calculated percentage of HEXA, and the woman may be incorrectly classified as a carrier. To circumvent this problem, the assay can be performed using white blood cells, which lack HEXP. Third, false-positive results (2% in Ashkenazi Jewish, 35% in other populations) occur in individuals who carry a pseudodeficiency allele (7,8). There are two such alleles: 739C>T, also termed ARG247TRP (R247W), and 745C>T, also designated as ARG249TRP (R249W). These alleles code for HEXA enzymes with reduced activity against the artificial MUG substrate but not against the cellular G_{M2} ganglioside. Thus, biochemical assay

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Abbreviations: HEXA, β -hexosaminidase A; HEXB, hexosaminidase B; HEXP, hexosaminidase P; MUG, 4-methylumbelliferyl- β -N-acetyl glucosaminide; TSD, Tay-Sachs Disease

results are likely to be consistent with a TSD carrier, though the phenotype is benign. Fourth, a B1 variant phenotype can result in a false-negative test (individual classified as normal rather than as a carrier) (9,10). Five *HEXA* mutations have been identified that cause this phenotype. Their protein product has reduced activity against cellular G_{M2} ganglioside, but normal activity against the artificial MUG substrate. Fifth, a rare thermolabile HEXB can result in an apparent increase in *HEXA* activity, potentially masking a TSD carrier. Finally, the biochemical assay cannot differentiate carriers of the milder juvenile/adult mutations from carriers of the more severe, infantile mutations.

Three mutations account for 92–98% of carriers among Ashkenazi Jewish individuals (11,12). The most common mutation is a TATC insertion at exon 11 (1278insTATC). A G to C transversion at the 5' splice site in intron 12 (1421 + 1G→C, also designated + 1 IVS12 G→C) is the second most common mutation, and a glycine to serine mutation in exon 7 (805G→A, also known as G269S) is the third. The 1278insTATC and the G269S mutations are also commonly found in non-Ashkenazi Jewish populations, along with an intron 9 splice site mutation (1073 + 1G>A, also termed + 1 IVS9 G>A) and a 7.6 kb deletion (7.6-KB DEL, EX1). These five mutations, along with the two pseudodeficiency mutations discussed earlier, make up our standard *HEXA* DNA common mutation test used for carrier screening and diagnosis. Although such DNA testing is preferable to the enzyme assay in the Ashkenazi Jewish population (4), it would only detect ~50% of carriers in other populations. The other 50% are attributed to nearly 100 disease-causing mutations present across the entire *HEXA* gene. Therefore, enzyme analysis has remained the method of choice in the non-Ashkenazi Jewish population. An unanswered question is whether extensive DNA sequencing, encompassing all coding regions and splice junction sites, would be an appropriate alternative to either the enzyme analysis or the standard DNA mutation analysis in these populations. This is an important question as the annual TSD death rate has remained at ~10 for the last several decades, an increase of three to four over that in the Ashkenazi Jewish population in the United States and Canada. In this work, we examine the potential of *HEXA* gene sequencing for carrier screening and diagnosis in a non-Ashkenazi Jewish population of purported TSD carriers.

MATERIALS AND METHODS

Patient information. Samples were obtained at the 2006 annual parents meeting of the National Tay-Sachs and Allied Diseases Association. Individuals were solicited who were either affected with TSD (all adult onset patients), self-reported parents of children or adults affected with TSD, or relatives of these index cases who have been told they were carriers of TSD. Obligate carrier status was known in only nine of the 33 (27%) self-reported, unaffected carriers. Informed consent was obtained and samples were col-

lected for both serum and blood/white blood cell preparations. This study was approved by the Western Institutional Review Board.

Study protocol. We performed the *HEXA* gene sequencing, *HEXA* DNA mutation test, and the *HEXA* enzyme assay on 34 self-reported TSD carriers, six late-onset patients with TSD, and one pseudodeficiency allele carrier. None of the carriers claimed to be of Ashkenazi Jewish descent, and none were pregnant at time of sample collection.

DNA extraction. DNA from most of the samples were extracted using Gentra Autopure LS (Minneapolis, MN) exactly as described by the manufacturer, and some samples were extracted using Qiagen Biorobot 9604 platform using MegAttract DNA blood M96 kit (Valencia, CA). Whole blood was used for DNA extraction in most cases, and in some cases, leukocytes were used. We observed no differences in sequencing quality between different extraction platforms and different starting materials.

HEXA gene sequencing. DNA sequencing and PCR were performed essentially as described by Huang *et al.* (13) except that DNA purification post-PCR was done using exonuclease (USB Corporation, Cleveland, OH)/calf intestinal alkaline phosphatase (Promega, Madison, WI) digestion, and postdye terminator reaction DNA was purified by ethanol precipitation and resuspended in Hi-Di formamide (ABI, Foster City, CA). Fourteen amplicons, each amplifying one of 14 exons, were generated through individual PCR reactions. Amplification efficiency and correctness of sizes were verified by agarose gel electrophoresis. Sequencing covered exons 1 through 14 of chromosome 15 and at least 20-bp flanking sequences. Samples were resolved on an ABI 3730 automated DNA analyzer and data were analyzed using SeqScape software (both from Applied Biosystems).

HEXA DNA common mutation test. Mutation analysis was performed for five common mutations and two pseudoalleles: 1278insTATC; 1421 + 1G→C (+1 IVS12 G→C); 805G→A [GLY269SER (G269S)]; 1073 + 1G→A (+1 IVS9 G→A); 7.6-KB DEL, EX1; 739C→T [ARG247TRP (R247W)]; and 745C→T [ARG249TRP (R249W)]. We used allele-specific primer extension to detect these mutations as per the method described by Strom *et al.* (14).

HEXA enzyme assay. The serum *HEXA* enzyme assay has been described previously (3). For this study, we set the normal *HEXA* activity level at 57–80% and the carrier/affected level at 0–51%.

RESULTS

There was a single patient (male) who was determined to be a non-TSD carrier by all three testing modalities: Hex A enzyme analysis on serum, *HEXA* DNA common mutation assay, and *HEXA* gene sequencing. Therefore, this patient is unlikely to be a true carrier and will be eliminated from further discussion.

The *HEXA* gene sequencing assay detected two mutant alleles in six of the six adult-onset TSD-affected individuals. Thus, gene sequencing correctly classified 100% of TSD cases. Gene sequencing correctly classified 30 of 33 (91%) TSD unaffected carriers. No mutation was detected in two of the remaining cases, and a novel mutation (775A→G), predicted to be silent, was detected in the final case. Gene sequencing also successfully detected a pseudodeficiency mutation [739C→T (R247W)] in the TSD pseudodeficiency carrier. Table 1 details the test results obtained from all three screening methods on the cases that were misclassified by gene sequencing. The average enzyme levels for late-onset TSD and TSD carriers were 10 and 43%, respectively.

Table 1. Sequencing assay discrepant results

Sample	Phenotype	Sequencing assay	% HEXA activity	DNA mutation assay*
TS50	Carrier	No mutation	48 (A/C)	No mutation
TS12	Carrier	775A→G†	51 (A/C)	No mutation
TS53	Carrier	No mutation	51 (A/C)	No mutation

* DNA mutation assay includes detection of 7.6-KB DEL, EX1; 739C→T; 745C→T; 805G→A; 1073 + 1G→A; 1278insTATC; and 1421 + 1G→C.

† Novel mutation; no change in phenotype expected.

A/C, in affected/carrier range; N, in normal range.

Table 2. Sensitivity of the enzyme, DNA mutation, and gene sequencing assays

Phenotype	Patients no.	Enzyme assay (%)	DNA mutation assay* (%)	Enzyme + DNA mutation (%)	Sequencing assay (%)	Enzyme + sequencing (%)
TS disease	6	6/6 (100)	6/6 (100)	6/6 (100)	6/6 (100)	6/6 (100)
TS carrier	33	30/33 (91)	17/33 (52)	32/33 (97)	30/33 (91)	33/33 (100)
TS disease/carrier	39	36/39 (92)	23/39 (59)	38/39 (97)	36/39 (92)	39/39 (100)
Pseudo	1	Indeterminate	1/1 (100)	1/1 (100)	1/1 (100)	1/1 (100)

* DNA mutation assay includes detection of 7.6-KB DEL, EX1; 739C→T; 745C→T; 805G→A; 1073 + 1G→A; 1278insTATC; and 1421 + 1G→C.

Table 3. Enzyme assay discrepant results

Sample	Phenotype	% HEXA activity	DNA mutation assay*	Sequencing assay
TS36	Pseudodeficient	53 (I)	739C→T	739C→T
TS38	Carrier	55 (I)	805G→A	805G→A
TS16	Carrier	82 (A)	No mutation	533G→A

* DNA mutation assay includes detection of 7.6-KB DEL, EX1; 739C→T; 745C→T; 805G→A; 1073 + 1G→A; 1278insTATC; and 1421 + 1G→C. I, in indeterminate range; N, in normal range; A, above normal range.

Table 4. Novel mutations detected by HEXA gene sequencing

Sample	Phenotype	Novel mutation	Predicted protein damage*	Known mutation	% HEXA activity
TS21	Carrier	759G→A V253V	No	TATCins1278	35 (A/C)
TS33	Carrier†	965A→T D322V	Yes	None	44 (A/C)
TS32	Carrier†	965A→T D322V	Yes	None	48 (A/C)
TS23	Carrier	118Tdel	Yes	None	49 (A/C)
TS12	Carrier	775A→G T259A	No	None	51 (A/C)

* PolyPen and SHIFT web-tools were used for protein folding prediction.

† Blood relatives (mother/daughter).

A/C, in affected/carrier range.

Sensitivity of the gene sequencing assay was substantially higher than that of the DNA common mutation test: 91% of TSD carriers and affected were correctly classified by gene sequencing *versus* only 52% correctly classified by the DNA mutation test (Table 2). The enzyme assay sensitivity and gene sequencing had similar sensitivities (91%). Of the two cases with indeterminate enzyme results, gene sequencing correctly classified one as a pseudodeficiency carrier and the other as a TSD carrier. In addition, gene sequencing detected a TSD-associated mutation (533G→A) in a TSD carrier who had an enzyme assay result above the normal range. The 533G→A mutation is associated with the rare B1 variant phenotype known to overestimate HEXA enzyme activity when a neutral substrate such as MUG is used (15,16). Thus, gene sequencing helped clarify enzyme test results that did not correlate with the phenotype (Table 3). We also tested six Sandhoff carriers for *HEXA* DNA common mutation and *HEXA* gene sequencing assays and found no mutation in the *HEXA* gene. Combined use of the enzyme assay and gene sequencing had the greatest sensitivity (100%).

Gene sequencing detected four mutations (Table 4) we believe to be novel, because we found no report of them either in the Tay-Sachs mutation database at McGill University (<http://www.hexdb.mcgill.ca/?Topic=HEXAdb>) or in the PubMed database. A single nucleotide deletion, 118.delT, in exon 1 was found in one carrier. This leads to one nucleotide frame shift resulting in a truncated protein that ends at exon 2. Therefore, this is most likely a disease-causing mutation. A missense mutation, 965A→T (D322V), causes an amino acid change from a positively charged aspartic acid to a hydrophobic valine. This is likely to be disease-causing because one of

the two individuals with this mutation is an obligate carrier and has a HEXA activity result in the carrier range and no other detected mutation. In addition, PolyPen and SHIFT web-based tools suggest damage at the ligand-binding site that interferes with protein folding (17,18). Another missense mutation, 775A→G changes a hydrophilic threonine to a hydrophobic alanine at amino acid position 259 (T259A). It may be a disease-causing mutation because the enzyme activity was in the carrier level (51% HEXA) and no other TSD-associated mutation was detected. However, the predicted protein folding seems to be minimal according to PolyPen and SHIFT web-based tools. A silent mutation, 759G→A (V253V), in exon 7 was detected in a carrier who also has a 1278insTATC allele. Thus, 759G→A is most likely a benign polymorphism.

DISCUSSION

The data demonstrate that no single test is capable of identifying all carriers of TSD. The presence of a B1 variant in 33 obligate carriers in this study demonstrates that enzyme testing alone cannot correctly identify all TSD carriers. DNA sequencing alone similarly did not detect three obligate carriers. The combination of Hex A enzyme assay and *HEXA* gene sequencing detected all obligate carriers, whereas the combination of Hex enzyme assay and *HEXA* DNA common mutation test detected all but the one carrier with the B1 allele.

The most cost effective and accurate TSD carrier detection method would be a combination of Hex A enzyme assay with a DNA common mutation test that was expanded to include the B1 allele, because the allele leads to false-negative Hex A

enzyme analysis. The particular B1 variant (533G→A) we detected is present in wide geographic and ethnic distribution (19), and its frequency is especially high in Portuguese population, where its prevalence is estimated to be 0.84 per 100,000 live births (20).

DNA sequencing did not detect a Hex A mutation in three TSD carriers. The sequencing method, however, is limited in that it does not identify large deletions or insertions or mutations well into an intronic sequence. Such mutations seem to be relatively rare, though, and our sequencing assay covers all of the known intronic mutations. DNA sequencing is also limited in that the promoter region is not sequenced, but no mutations have been reported in this region thus far. Mutations in genes other than the *HEXA* gene are not identified, though to date none are known to be associated with the TSD phenotype. Nevertheless, sequencing is not 100% sensitive, and the cost is prohibitively high for use in population-based carrier screening.

When compared with the *HEXA* enzyme assay and the DNA common mutation assay, the primary benefit of gene sequencing in this study was to increase overall sensitivity, clarify discrepant results obtained by the other two assays, and identify novel mutations. In our study environment, we compared test results to a “known” phenotype/carrier status; however, this is not the environment in which these assays are used. Is there an appropriate role for *HEXA* gene sequencing in the clinical environment that seeks to identify carriers and affected individuals?

Multiple authors have recommended common DNA mutation testing for high-risk populations such as the Ashkenazi Jewish population (4). The ACOG Committee on Genetics does not specifically recommend a screening method for high-risk populations, but does recommend use of the enzyme test for individuals in low-risk populations (2). For the non-Ashkenazi Jewish population, our data show the *HEXA* enzyme assay will detect a greater percentage of TSD carriers than a DNA common mutation test. Because the enzyme assay indirectly quantitates *HEXA* activity, however, ambiguous results are often obtained. *HEXA* gene sequencing analysis is appropriate for obligate carriers or affected patients whose mutation has not been identified, for carrier detection in spouses of known carriers who have indeterminate enzyme analysis and negative for the common mutation and to resolve diagnostic dilemmas from ambiguous enzyme testing.

In conclusion, we have successfully detected various small mutations in the *HEXA* gene through gene sequencing and, by combining the *HEXA* enzyme assay and the *HEXA* gene sequencing assay, we were able to clarify TSD carrier status in 100% 34 obligate TSD carriers. No single testing modality

was able to identify all obligate TSD carriers. This has important implications if TSD population screening is expanded to non-AJ populations.

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