

## ABSTRACT

Tay-Sachs Disease is an autosomal recessive disorder caused by mutations in the *HEXA* gene. Enzyme deficiency causes build-up of glycolipid GM2 in the central nervous system, leading to loss of motor control, blindness, severe mental retardation, macrocephaly, and death usually before age 5. Mutation-panel DNA testing is a commonly-used complement to biochemical testing that helps resolve pseudodeficiencies in most cases and clarifies carrier and diagnostic status in some cases.

Five mutations comprise the majority of mutations in the Ashkenazi Jewish population. However, over 120 mutations and 1 gross deletion in *HEXA* have been described, and very few of these have ethnic associations. Due to effective targeted enzymatic screening programs, the disease incidence has decreased greatly over the past 3 decades and there are now more Tay-Sachs births outside the Ashkenazi Jewish population than within. Availability of prenatal biochemical testing is limited. These factors suggest the need for a DNA-based Tay-Sachs test applicable to all populations.

In order to provide a method to identify rare mutations in unresolved diagnostic cases and improve on existing carrier screens for high-risk and non-Ashkenazi couples, we developed a sequencing assay covering all 14 exons of *HEXA* plus a PCR test to identify the 7.6 kb "French-Canadian" gross deletion. This test, the Ambry Test<sup>®</sup> Tay-Sachs Plus, has a 99% detection rate in all ethnicities. As part of the test validation procedure, nine samples from affected Jewish and non-Jewish patients were obtained from the Coriell Cell Repositories. Patients included 1 with ethnicity "black" and 3 with late-onset Tay-Sachs. Mutations were detected in all 18 alleles analyzed, including 5 that would not have been identified by a standard 5-mutation screening panel. Full gene sequence analysis for *HEXA* is useful to identify rare mutations in Tay-Sachs diagnostic cases and to provide comprehensive carrier screening for high-risk or mixed-ethnicity couples.

## INTRODUCTION

Tay-Sachs Disease is an autosomal recessive condition caused by mutations in the *HEXA* gene that lead to deficiency of the  $\beta$ -hexosaminidase A enzyme. Glycolipid GM2 accumulation in neuronal lysosomes leads to loss of motor control, blindness, severe mental retardation, macrocephaly, and death usually before age 5. Juvenile and adult-onset disease variants exist. The disease occurs in many ethnic groups but carrier frequencies in North America are highest, approximately 1/30, in those of Ashkenazi Jewish (AJ), Cajun, or French-Canadian descent.

Screening programs targeted to high-risk groups began in 1970 and have been effective in reducing disease incidence by more than 90% in the Jewish population.<sup>1</sup> Carrier screening should be offered to AJ individuals before conception or during early pregnancy, according to the American College of Obstetricians and Gynecologists' committee opinion.<sup>2</sup>

Biochemical carrier screening measures *HEXA* activity in serum and/or leukocytes to detect ~98% of carriers.<sup>2</sup> Approximately 1-2% of results are indeterminate<sup>3</sup>, falling between carrier and non-carrier ranges. Also, pseudodeficiency alleles account for approximately 35% of the positive carrier screens in the non-AJ population and 2% of those in the AJ population.<sup>4</sup> Pseudodeficiency is detected by DNA testing, requiring a second test on enzymatically-positive patients.

Standard mutation panels generally test for three to six disease-causing mutations and two pseudodeficiency mutations, and provide a carrier detection rate of ~95% in the AJ population and 25%-60% in the non-Jewish population<sup>1</sup>. The detection rate in the non-Jewish group can be further enhanced by the addition of select mutations to the basic panel, but it still remains significantly lower than the ~98% detection rate of enzymatic screening. This results from the variety of *HEXA* mutations described, currently numbering more than 1205 and including a 7.6 kb deletion found primarily in French-Canadians.<sup>5</sup>

Tay-Sachs carrier screening through biochemical and molecular methods offers the benefit of sensitive result confirmation in patients from select ethnic groups when results are negative. However, the existence of dual testing methods with potentially inconsistent results presents counseling dilemmas. Examples are indeterminate or positive biochemical screens with no common mutation detected. Molecular detection rates drop for couples of non-Ashkenazi descent, and prenatal cases arise in which one parent is unavailable for complete testing. There are few providers of biochemical testing on chorionic villus or amniotic fluid samples. For these reasons we developed a full gene sequence analysis of *HEXA* for clinical diagnostic and carrier testing use. We present here our experience in validating this test with banked research samples from affected patients.

## METHODS

### SPECIMENS:

Specimens included in this study were from patients affected with Tay-Sachs disease whose DNA or cells had been deposited with Coriell Cell Repositories (<http://ccr.coriell.org>). For five of the specimens at least one mutation had been identified previously (NA00515, GM00527, GM01110, NA03461, and NA03575). Four specimens had unknown genotypes (GM00077, GM00221, GM03586 and GM13204). See Table 1 for patient details.

### DNA ISOLATION:

Genomic DNA (gDNA) was provided by Coriell for samples NA00515, NA03461 and NA03575. For the remaining samples, gDNA was isolated from cultured fibroblasts or lymphocytes using GFX isolation columns according to standard procedures (GE Healthcare). All gDNA samples were assessed for quality and quantity by spectrophotometry and agarose gel electrophoresis.

### GENE ANALYSIS:

All of the samples in this study were analyzed using the Ambry Test<sup>®</sup> Tay-Sachs Plus, which provides full sequence analysis of the *HEXA* gene (OMIM 272800) by direct dye-terminator sequencing and the analysis of the 7.6 kb "French-Canadian" gross deletion. Briefly, PCR primers were designed to cover the translated 14 exons plus at least 20 bp intronic flanking sequence. PCR amplification was performed using HotStarTaq Master Mix (Qiagen) with 100-150 ng input gDNA per reaction. Typical PCR conditions were: 1 cycle 95°C/15 min, 35 cycles of 94°C/30 seconds, 62°C/30 seconds, 72°C/30 seconds, followed by an extension at 72°C for 10 minutes and a terminal hold at 8°C. Annealing temperatures varied among primer pairs. PCR products were analyzed via agarose gel electrophoresis, followed by treatment with Exo-Sap-It (USB) according to manufacturer recommendations. Standard dye terminator cycle sequencing DTCS (Beckman-Coulter) was conducted followed by analysis on a CEQ8000 capillary sequencer. Exons were sequenced in both sense and antisense directions. All reported variations follow the nomenclature based on Genbank entry NC\_000015 complement (70422700..7045525).

In addition, a multiplex bridge PCR assay was designed in order to identify the 7.6 kb deletion. In the presence of a homozygous deletion, a PCR fragment amplifies at 438 bp. In the absence of the deletion, a 654 bp fragment is amplified. For heterozygous specimens both PCR fragments are amplified.

## RESULTS

TABLE 1: Summary of Patient Information and Results.

Mutations not tested by common 3-6 mutation panels are shown in bold blue type.

SPECIMEN ID	ETHNICITY	AGE	GENDER	RESULT	MUTATION NAME (ALTERNATE NAME)	COMPARED TO INCOMING GENOTYPE	TYPE OF MUTATION
NA00515	Jewish	1 yr	female	mutation 1 mutation 2	p.W392X (c.1176 G>A) c.1278insTATC	confirmed confirmed	stop codon mutation frameshift
GM00527	Caucasian	1 yr	male	mutation 1 mutation 2	p.W420C (c.1260 G>C) IVS 11+1 G>C (c.1330+1 G>C)	confirmed detected by Ambry	amino acid substitution splice site mutation
GM01110	Pennsylvania Dutch (consanguinity noted)	10 months	male	mutation 1 mutation 2	p.E482K (c.1444 G>A) p.E482K (c.1444 G>A)	confirmed confirmed	amino acid substitution amino acid substitution
NA03461	Ashkenazi	24 yrs late onset	male	mutation 1 mutation 2	p.G269S (c.805 G>A) IVS 12+1 G>C (c.1421 G>C)	confirmed confirmed	amino acid substitution splice site mutation
NA03575	Ashkenazi	31 yrs late onset	female	mutation 1 mutation 2	p.G269S (c.805 G>A) c.1278insTATC	confirmed confirmed	amino acid substitution frameshift
GM00077	Black	1 yr	male	mutation 1 mutation 2	c.436delG c.436delG	detected by Ambry detected by Ambry	frameshift frameshift
GM00221	Caucasian	3 yrs	male	mutation 1 mutation 2	c.1278insTATC c.1278insTATC	detected by Ambry detected by Ambry	frameshift frameshift
GM03586	Caucasian	3 yrs	female	mutation 1 mutation 2	c.1278insTATC c.1278insTATC	detected by Ambry detected by Ambry	frameshift frameshift
GM13204	Not Specified	45 yrs late onset	female	mutation 1 mutation 2	p.G269S (c.805 G>A) c.1278insTATC	detected by Ambry detected by Ambry	amino acid substitution frameshift

Based on genomic reference sequence NC\_000015 complement (70422700..7045525); cDNA NM\_000520.

- Previously detected mutations were confirmed on 9/9 alleles and previously undetected, known deleterious mutations were detected on all 9 of the remaining alleles.
- In 18 alleles, 5 mutations (W392X<sup>7</sup>, W420C<sup>8</sup>, IVS11+1 G>C<sup>5</sup>, E482K<sup>9</sup>, and c.436delG<sup>10</sup>, shown in bold blue font) of the 8 different mutations identified by the Ambry Test<sup>®</sup> Tay-Sachs Plus would not have been identified by standard 3-6 mutation panels.
- Five previously unknown alleles of the common c.1278insTATC mutation<sup>11</sup> were identified in these specimens.
- Three individuals manifested late-onset Tay-Sachs (NA03461, NA03575, and GM13204). All were compound heterozygous for one classic Tay-Sachs mutation and the known late-onset G269S mutation.<sup>12</sup>

## DISCUSSION

Patients represented in our sample group were delineated by age of onset into infantile and late-onset Tay-Sachs groups. At least one non-Caucasian patient was represented. Given the range of patient characteristics and the large number of published *HEXA* mutations, it is not surprising that we found 8 different mutations among the 18 alleles. Only 3 of these 8 mutations are tested by common mutation panels.

However, our samples were not collected at random and do not necessarily represent a cross-section of Tay-Sachs patients. Samples were supplied by Coriell Cell Repositories, to which investigators voluntarily contribute genetic material along with variable amounts of anonymized clinical and analytical data. Rather than describe a pattern of mutation occurrence, which was not our intention in this validation study, the results attest to the ability of the methodology to detect a variety of mutations.

TABLE 2: Estimates of Revised Carrier Risks after *HEXA* Gene Sequence Analysis.

These calculations assume a negative test result with no family history. Risk adjustments were not made to account for enzyme analysis results.

ETHNICITY	TAY-SACHS CARRIER RISK BEFORE TESTING	REVISED CARRIER RISK AFTER TAY-SACHS PLUS
Ashkenazi Jewish, <sup>13</sup> Cajun, French-Canadian <sup>14</sup>	1/30	1/2901
Other populations at general risk <sup>1,15</sup>	1/280	1/27,901
Irish-American <sup>15</sup>	1/44	1/4301

The late-onset presentation in patients NA03461, NA03575, and GM13204 is consistent with their compound heterozygosity for the G269S mutation and another mutation.

With two mutations detected in each of the 9 patient samples, full gene sequence analysis completed genotyping for 5 patient samples (GM00527, GM00077, GM00221, GM03586, GM13204) and confirmed previously detected genotypes in the other 4. Gene sequencing has a sensitivity of approximately 99% and our results of 100% mutation detection in a small series of known affected patient samples is consistent. Given potential complexities of carrier screening by biochemical methods and common mutation testing, *HEXA* gene sequence analysis is a useful tool for minimizing risk and detecting suspected but less common mutations. Table 2 provides revised risk information for carrier tests in various populations.

## CONCLUSIONS

In this validation of the Ambry Test<sup>®</sup> Tay-Sachs Plus assay, we demonstrated 100% mutation detection in a series of known affected patients of various ethnicities and ages of onset. In clinical practice this test is expected to have a detection rate of 99% and can be useful in the following situations common to prenatal genetic counseling:

- mutation identification for biochemically positive suspected carriers without a common mutation
- resolution of indeterminate biochemical carrier results
- prenatal testing when biochemical testing requirements cannot be met or parental testing cannot be completed

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