

# Population-Based Tay-Sachs Screening Among Ashkenazi Jewish Young Adults in the 21st Century: Hexosaminidase A Enzyme Assay Is Essential for Accurate Testing

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Received 3 March 2009; Accepted 29 July 2009

Tay-Sachs disease (TSD) carrier screening, initiated in the 1970s, has reduced the birth-rate of Ashkenazi Jews with TSD worldwide by 90%. Recently, several nationwide programs have been established that provide carrier screening for the updated panel of Jewish genetic diseases on college campuses and in Jewish community settings. The goals of this study were to determine the performance characteristics of clinical TSD testing in college- and community-based screening programs and to determine if molecular testing alone is adequate in those settings. Clinical data for TSD testing were retrospectively anonymized and subsequently analyzed for 1,036 individuals who participated in these programs. The performance characteristics of the serum and the platelet Hexosaminidase assays were compared, and also correlated with the results of targeted DNA analysis. The serum assay identified 29 carriers and the platelet assay identified 35 carriers for carrier rates of 1/36 and 1/29, respectively. One hundred sixty-nine samples (16.3%) were inconclusive by serum assay in marked contrast to four inconclusive samples (0.4%) by the platelet assay. Molecular analysis alone would have missed four of the 35 carriers detected by the platelet assay, yielding a false negative rate of 11.4% with a sensitivity of 88.6%. Based on the results of this study, platelet assay was superior to serum with a minimal inconclusive rate. Due to changing demographics of the Ashkenazi Jewish population, molecular testing alone in the setting of broad-based population screening programs is not sufficient, and biochemical analysis should be the assay of choice.

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## How to Cite this Article:

Schneider A, Nakagawa S, Keep R, Dorsainville D, Charrow J, Aleck K, Hoffman J, Minkoff S, Finegold D, Sun W, Spencer A, Lebow J, Zhan J, Apfelroth S, Schreiber-Agus N, Gross S. 2009. Population-based Tay-Sachs screening among Ashkenazi Jewish young adults in the 21st Century: Hexosaminidase A enzyme assay is essential for accurate testing.

Am J Med Genet Part A 149A:2444–2447.

Grant sponsors: Jonas Ehrlich Charitable Foundation; Lois B. Victor Foundation.

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Published online 26 October 2009 in Wiley InterScience (www.interscience.wiley.com)

DOI 10.1002/ajmg.a.33085

**Key words:** Tay-Sachs disease; population screening; platelet Hexosaminidase A; demographics of Jewish population; Ashkenazi Jewish; genetic testing

## INTRODUCTION

Tay-Sachs disease (TSD) carrier screening in the Ashkenazi Jewish (AJ) population, initiated in the 1970s with educational outreach and Hexosaminidase A (HexA) serum enzyme screening, has reduced the birth-rate of infants with TSD in the AJ community worldwide by 90% [Kaback, 2000]. Recently, several nationwide programs have been established to provide carrier screenings for the updated panel of Jewish genetic diseases on college campuses and in Jewish community settings, in keeping with the original model of population-based screening and education [Kaback et al., 1993]. However, there have been considerable demographic changes since the original screening programs [D'Souza et al., 2000, 2000–2001 NJPS report <http://www.ujc.org/page.aspx?id=46253>]. Specifically, unlike 30 years ago, college-aged students who identify themselves as Ashkenazi Jewish are less likely to be offspring of endogamous relationships. The past several years have seen a substantial increase in children from mixed Jewish and non-Jewish ethnic and racial backgrounds. In addition, many Jews do not know the countries of origin of their ancestors but they nonetheless self-identify as Ashkenazi Jews in a community setting. There have been reports in the literature that molecular screening can be highly sensitive in the AJ population, particularly in more homogenous populations [Fernandes et al., 1992; Bach et al., 2001]. However, biochemical testing has been available for decades and has the capability of detecting almost all carriers, irrespective of ethnic background [O'Brien et al., 1970; Suzuki et al., 1971; Navon and Padeh, 1972; Carmody et al., 1973; Singer et al., 1973; Saifer et al., 1976; Nakagawa et al., 1978a,b]. The goals of this study were to determine the performance characteristics of clinical TSD testing in current large scale, community-based screening programs and to determine if molecular testing alone is adequate in light of increasing genetic diversity within the Jewish community [D'Souza et al., 2000; 2000–2001 NJPS report <http://www.ujc.org/page.aspx?id=46253>; Vallance et al., 2006].

## MATERIALS AND METHODS

One thousand thirty-six individuals participated in screening programs run by The Victor Centers for Jewish Genetic Diseases at Albert Einstein Medical Center, Philadelphia and at Tufts Medical Center, Boston, by the Chicago Center for Jewish Genetic Disorders, and by the Jewish Genetic Diseases Center of Greater Phoenix. These programs offer education, genetic counseling, and screening in the community setting to individuals who self-identify as Ashkenazi Jewish and who are between 18 and 44 years old. Screening is provided at a reduced cost or no cost to these individuals who are not insured or whose insurance will not cover the cost of premarital/preconception testing.

All clinical samples were tested in the Human Genetics Laboratory at the Jacobi Medical Center by the serum and platelet HexA

enzyme assays as well as by targeted DNA mutation analysis. Serum enzyme assay was done using heat inactivation methodology while platelet assay was performed using charge separation with DEAE-cellulose columns; these have been described previously [O'Brien et al., 1970; Nakagawa et al., 1977, 1978b]. Genomic DNA was tested for seven common mutations in the *HEXA* gene using the Tag-It™ Ashkenazi Jewish Panel (Luminex Molecular Diagnostics, Toronto, Canada). The clinical data were retrospectively retrieved, anonymized, and analyzed.

## RESULTS

The performance characteristics of the serum and the platelet Hexosaminidase assays were compared, and also correlated with the results of targeted DNA analysis (Tables IA and IB).

### Carrier Detection by Enzyme Assays

For 1,036 individuals tested, the serum assay identified 29 carriers and the platelet assay identified 35 carriers (including all 29 identified by the serum assay) for carrier rates of 1/36 and 1/29, respectively.

### Inconclusive Rates

One hundred sixty-nine inconclusive samples (16.3%) were noted using serum assay. Of these 169 inconclusive samples, 83 were from females on oral contraception, 39 were from males, and the remaining 47 were from females who either were not taking oral contraceptives or did not comment in that field of the requisition form. If the 83 women who were on oral contraceptives are removed from this group, as they should not have been offered serum

**TABLE IA. Comparison of Performance Characteristics of Serum and Platelet Hexosaminidase Assays**

Assay	Non-carrier (%)	Carrier (%)	Inconclusive (%)
Serum	838 [80.9%]	29 [2.8%]	169 [16.3%]
Platelet	997 [96.2%]	35 [3.4%]	4 [0.4%]

**TABLE IB. Distribution of *HEXA* Mutations Observed**

Mutation	Number of times observed
Δ7.6 kb	0
R247W [pseudodeficient]	5 <sup>a</sup>
R249W [pseudodeficient]	0
G269S	2
IVS9[+1]G > A	0
1278insTATC	22 <sup>b</sup>
IVS12[+1]G > C	4

<sup>a</sup>One of these samples was inconclusive by the platelet assay [%HexA of 53].

<sup>b</sup>One of these samples was inconclusive by the platelet assay [%HexA of 50].

screening alone, the inconclusive rate is 86/1,036 (8.3%). In marked contrast to the serum assay, the platelet assay yielded only four inconclusive samples (0.4%); all four also were found to be inconclusive by serum assay.

### Correlation to Targeted DNA Analysis

Thirty-one of the 35 individuals classified as carriers by the platelet assay had detectable *HEXA* mutations with the breakdown shown in Table IB. Notably, four individuals who were classified as carriers by the platelet assay did not have any of the seven common mutations (Table II). Each of these platelet positive/DNA negative individuals had at least one non-Ashkenazi Jewish parent. Using the serum assay, one of these four samples was identified as a carrier and three were inconclusive (Table II). Two individuals whose samples were inconclusive by platelet and serum assays had detectable DNA mutations (Table IB), bringing the total number of individuals with *HEXA* detectable mutations up to 33. Finally, none of the individuals identified as non-carriers by enzyme assays had any of the seven common mutations.

### DISCUSSION

Our data suggest that the platelet HexA enzyme assay is a very effective method for TSD large scale carrier screening, associated with a very high detection rate (1 in 29; 3.4%) and a very low inconclusive rate (0.4%). This inconclusive rate is ~40-fold lower than that seen with the serum assay. These findings likely relate to the fact that platelets are a homogeneous source of Hexosaminidase enzymes, with clear separation between the non-carrier and carrier groups. Moreover, isoenzyme activity patterns, as assessed in platelets via charge separation, do not appear to be altered by pregnancy, oral contraceptive use, or certain diseases [Nakagawa et al., 1978a; Nitowsky et al., 1979]. Other advantages of this method include the small volume of blood needed, the ease and rapidity of platelet isolation, and the ability to freeze platelets with no apparent loss of activity ([Nakagawa et al., 1977, 1978a], and references therein). While serum enzyme analysis is widely used and may be appropriate in certain situations, the higher rate of inconclusive results compared to the platelet assay makes it less practical in large-scale screenings where up to hundreds of individuals are tested within a short time frame [Prence et al., 1993; Casal et al., 2005; Vallance et al., 2006]. However, since the cell separation and the column chromatography steps of the platelet assay can be labor intensive (and hence costly) when large numbers of samples are involved, one option is to perform serum screening as the first line test and reflexing to the platelet assay for inconclusive and carrier

samples. Further study would be required to determine whether the platelet assay could be the biochemical assay of choice when applied in an individualized setting such as in a general obstetrical practice. Finally, the leukocyte-based approach [Suzuki et al., 1971] is known to be highly effective in overcoming serum inconclusive results. Further comparative analysis would be necessary to ascertain the effectiveness of the platelet vis-à-vis leukocyte assay.

Mutation analysis using a panel of founder mutations and pseudo-deficiency alleles identifies from 92% to 99% of carriers in a homogeneous AJ population [Yoo et al., 1993; Bach et al., 2001]. Based on the results of our study, in broad-based population screening programs where ethnicity is based on self-identification, the ideal test for Tay-Sachs carrier status cannot be analysis of founder AJ mutations alone. Using targeted mutation analysis alone, 4 out of 35 carriers identified by enzyme screening would have been missed, yielding an 11.4% false negative rate with 88.6% sensitivity to detect carriers. This false negative rate, also reflected in the literature by Kaback [2000], likely reflects demographic changes in the AJ population with higher intermarriage rates, adoption, and individuals uncertain about their ancestral origins [National Jewish Population Survey (NJPS) [2003] 2000–2001, <http://www.ujc.org/page.aspx?id=46253>]. Using the criteria proposed by ACMG [Gross et al., 2008], testing should have a sensitivity of greater than 90%. Certainly in a more homogeneous population, DNA testing may reach above this level. However, this study demonstrates that in the case of a mixed, broad-based young adult population, the use of mutation detection alone for TSD falls below that level and thus is not an acceptable method of testing self-identified Ashkenazi Jews. Notwithstanding, mutation analysis still has an integral role and should be employed for confirmatory diagnosis, to clarify borderline/inconclusive results, and to identify the causal mutation in affected/carriers and their families [Yoo et al., 1993; Natowicz and Prence, 1996]. With respect to the latter, mutation analysis also allows one to identify patients with pseudodeficient alleles (who are not at risk for infants with Tay-Sachs) or alleles for later-onset forms of TSD, for whom the genetic counseling is different from the infantile form. Finally, *HEXA* sequencing analysis should be employed for the enzyme positive/targeted DNA mutation negative cases and may also be considered in the platelet enzyme inconclusive/DNA negative cases. Indeed, our sequencing analysis has shown that a platelet positive/targeted DNA mutation negative sample described here carries an *HEXA* mutation that is not one of the seven common mutations tested for in the targeted mutation assay (manuscript in preparation).

As a final point, our finding of individuals who have at least one non-Ashkenazi Jewish parent and are Tay-Sachs carriers (Table II) underscores the importance of having an accurate and sensitive

TABLE II. Demographics of Platelet Assay Positive/DNA Negative Individuals

Patient gender	Serum HexA	Platelet HexA	DNA: AJ mutation panel	Ancestry
F	Inconclusive	Carrier	No mutation	Romania/Spain/Belarus
M	Inconclusive	Carrier	No mutation	Unknown/adopted
M	Carrier	Carrier	No mutation	Russia AJ/Irish/UK/Nova Scotia (not AJ)
F	Inconclusive	Carrier	No mutation	Serbia/Hungary/Ukraine

method to screen individuals who are not 100% Ashkenazi Jewish. The carrier rate for Tay-Sachs disease varies, but there are other populations in which there is a higher carrier rate than the general population such as the Irish, Cajun, and French Canadian [Kaplan, 1998]. As the gene pool of those who identify as Ashkenazi Jewish is diversifying, more individuals will be tested who have some non-Jewish heritage. HexA enzyme analysis is a well-tested example of an accurate and reliable screening method that transcends ethnicity and can be used in any population group [Casal et al., 2005; Vallance et al., 2006]. Since more individuals of partial non-Jewish heritage may undergo testing through these large screening programs, future research should explore whether enzyme analysis may allow for expanded screening programs in non-Jewish populations.

## ACKNOWLEDGMENTS

We are grateful to Lois B. Victor, the founder of the Victor Centers for Jewish Genetic Diseases for her support of the work of AS and JH. We thank the Jonas Ehrlich Charitable Foundation for their generous support to the Carrier Testing Program at the Jacobi Human Genetics Laboratory.

## REFERENCES

- Bach G, Tomczak J, Risch N, Ekstein J. 2001. Tay-Sachs screening in the Jewish Ashkenazi population: DNA testing is the preferred procedure. *Am J Med Genet* 99:70–75.
- Carmody PJ, Rattazzi MC, Davidson RG. 1973. Tay-Sachs disease—the use of tears for the detection of heterozygotes. *N Engl J Med* 289:1072–1074.
- Casal JA, Cano E, Tutor JC. 2005. Beta-hexosaminidase isoenzyme profiles in serum, plasma, platelets and mononuclear, polymorphonuclear and unfractionated total leukocytes. *Clin Biochem* 38:938–942.
- D'Souza GA, McCann CL, Hedrick J, Fairley C, Nagel HL, Kushner JD, Kessel R. 2000. Tay-Sachs disease carrier screening: A 21-year experience. *Genet Test* 4:257–263.
- Fernandes MJ, Kaplan F, Clow CL, Hechtman P, Scriver CR. 1992. Specificity and sensitivity of hexosaminidase assays and DNA analysis for the detection of Tay-Sachs disease gene carriers among Ashkenazic Jews. *Genet Epidemiol* 9:169–175.
- Gross SJ, Pletcher BA, Monaghan KG, for the Professional Practice and Guidelines Committee. Carrier screening in individuals of Ashkenazic Jewish descent. 2008. *Genet Med* 10:54–56.
- Kaback M. 2000. Population-based genetic screening for reproductive counseling: The Tay-Sachs disease model. *Eur J Pediatr* 159:S192–S195.
- Kaback M, Lim-Steele J, Dabholkar D, Brown D, Levy N, Ziegler K. for the International TSD Data Collections Network. 1993. Tay-Sachs disease—carrier screening, prenatal diagnosis, and the molecular era. An international perspective, 1970 to 1993. *JAMA* 270:2307–2315.
- Kaplan F. 1998. Tay-Sachs disease carrier screening: A model for prevention of genetic disease. *Genet Test* 2:271–292.
- Nakagawa S, Kumin S, Nitowsky HM. 1977. Human hexosaminidase isozymes: Chromatographic separation as an aid to heterozygote identification. *Clin Chim Acta* 75:181–191.
- Nakagawa S, Kumin S, Chandra P, Nitowsky HM. 1978a. Human hexosaminidase isoenzymes: Assay of platelet activity for heterozygote identification during pregnancy. *Clin Chim Acta* 88:249–256.
- Nakagawa S, Kumin S, Fox D, Nitowsky HM. 1978b. Human hexosaminidase isozymes. III. Distribution and activity of isozymes in peripheral blood leukocytes and platelets. *J Lab Clin Med* 91:922–928.
- National Jewish Population Survey (NJPS). 2003. 2000-01: Strength, Challenge and Diversity in the American Jewish Population. NJPS: Rates of Inter-marriage <http://www.ujc.org/page.aspx?id=46253>.
- Natowicz MR, Prence EM. 1996. Heterozygote screening for Tay-Sachs disease: Past successes and future challenges. *Curr Opin Pediatr* 8:625–629.
- Navon R, Padeh B. 1972. Urinary test for identification of Tay-Sachs genotypes. *J Pediatr* 80:1026–1030.
- Nitowsky HM, Davis J, Nakagawa S, Fox D. 1979. Human hexosaminidase isozymes. IV. Effects of oral contraceptive steroids on serum hexosaminidase activity. *Am J Obstet Gynecol* 134:642–647.
- O'Brien JS, Okada S, Chen A, Fillerup DL. 1970. Tay-Sachs disease. Detection of heterozygotes and homozygotes by serum hexosaminidase assay. *N Engl J Med* 283:15–20.
- Prence EM, Natowicz MR, Zalewski I. 1993. Unusual thermolability properties of leukocyte beta-hexosaminidase: Implications in screening for carriers of Tay-Sachs disease. *Clin Chem* 39:1811–1814.
- Saifer A, Amoroso J, Perle G. 1976. Identification of Tay-Sachs by hexosaminidase analysis of urine and tear samples. *Adv Exp Med Biol* 68:339–366.
- Singer JD, Cotlier E, Krimmer R. 1973. Hexosaminidase A in tears and saliva for rapid identification of Tay-Sachs disease and its carriers. *Lancet* 2:116–119.
- Suzuki Y, Berman PH, Suzuki K. 1971. Detection of Tay-Sachs disease heterozygotes by assay of hexosaminidase A in serum and leukocytes. *J Pediatr* 78:643–647.
- Vallance H, Morris TJ, Coulter-Mackie M, Lim-Steele J, Kaback M. 2006. Common HEXB polymorphisms reduce serum HexA and HexB enzymatic activities, potentially masking Tay-Sachs disease carrier identification. *Mol Genet Metab* 87:122–127. Epub 2005 Dec 13.
- Yoo HW, Astrin KH, Desnick RJ. 1993. Comparison of enzyme and DNA analysis in a Tay-Sachs disease carrier screening program. *J Korean Med Sci* 8:84–91.