Comparison of Enzyme and DNA Analysis in a Tay-Sachs Disease Carrier Screening Program

Han-Wook Yoo, M.D.*, Kenneth H. Astrin, Ph. D.***, Robert J. Desnick, M.D., Ph.D.***

*Department of Pediatrics, School of Medicine, Gyeong-Sang National University Chinju, Korea
Division of Medical and Molecular Genetics, Department of Pediatrics, Mount Sinai School of
Medicine, New York, New York, U.S.A.*

Tay-Sachs disease (GM2 gangliosidosis, type 1; TSD) is an autosomal recessive GM2 gangliosidosis resulting from the deficient activity of the lysosomal hydrolase β -hexosaminidase A (Hex A). With a carrier frequency estimated at 1 in 25, it is a common lysosomal disorder in the Ashkenazi Jewish population. Tay-Sachs disease has provided the prototype for the prevention of severe recessive genetic diseases. Molecular analysis of the Hex A gene (HEXA) of Ashkenazi Jewish individuals affected with Tay-Sachs disease revealed that three common mutations cause the infantile and adult onset forms of the disease; a four base insertion in exon 11, a splice junction mutation in intron 12 and a point mutation in exon 7 (G269S). A study was undertaken to determine whether mutation analysis would be useful in TSD screening programs in identifying carriers and clarifying the status of individuals whose enzyme assays are inconclusive. Ashkenazi Jewish individuals who had been diagnosed as carriers, inconclusives by enzyme assay and non-carriers with low normal enzyme levels in the Mount Sinai Tay-Sachs Disease Prevention Program were examined for the presence of the three mutations using polymerase chain reaction (PCR) and allele specific oligonucleotide (ASO) hybridization. The insertion mutation was present in 29 of 34 carriers and 2 of 36 inconclusive individuals, the splice junction mutation was found in 4 of 34 carriers and the G269S mutation was found in 1 of 34 carriers. Of the 313 non-carrier individuals with normal enzyme activity in the lower normal range, one was positive for the splice junction mutation. DNA from all individuals screened in the Mount Sinai Tay-Sachs Disease Prevention Program was then examined in order to test the feasibility of using DNA analysis in routine screening. Blood samples from three or four individuals were pooled, the DNA amplified by PCR and subjected to mutation analysis. Of 400 individuals studied, 19 individuals (1 in 21) identified by enzyme assay as carriers had either the insertion (13) or splice (3) mutation except three who were negative for all three common mutations as were 10 of 12 individuals who were inconclusive by enzyme assay. DNA studies in conjunction with enzyme assay can be valuable in obtaining an accurate diagnosis of TSD carriers in the Ashkenazi Jewish population.

Key Words: *Tay-Sachs Disease (TSD), Carrier, DNA Analysis, Enzyme Assay*

Address for correspondence: Han-Wook Yoo, Department of Pediatrics, Gyeong-Sang National University, 92 Chilam-Dong, Chinju 660-280, Korea. (Tel) 0591-55-0111 ext. 8156, (Fax) 0591-52-9339.

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INTRODUCTION

Tay-Sachs disease (GM2 gangliosidosis, type 1, TSD) has provided the prototype for the prevention of genetic diseases (Kaback and Zeiger, 1972; Kaback et al,

1977). TSD is caused by a deficiency of the lysosomal enzyme β -hexosaminidase A (β -N-acetyl-D-hexosaminidase; Hex A; EC 3.2.1.52) (Okada and O'Brien, 1969; Sandhoff, 1969; Hultberg, 1969; Srivastava and Beutler, 1973; Sandhoff et al., 1989) which results in the progressive accumulation of GM2 gangliosides in neurons. Onset of the disease occurs at 4 to 6 months of life and is characterized by regression of acquired developmental milestones. The psychomotor retardation is progressive and affected children usually expire by five years of age. There are also juvenile and adult onset GM2 gangliosidoses which have a later onset and a more protracted clinical expression. Since no treatment for TSD is available, efforts have been directed towards prevention by prospective identification and counseling of heterozygous carriers of the TSD gene.

The rationale for prospective screening was based on the following: 1) the gene for TSD has a high frequency (=1:27) in the Ashkenazi Jewish population (Kaback et al., 1977; Peterson et al., 1983), 2) carriers of the gene can be detected by determining the β -hexosaminidase A activity in sources such as plasma, tears and peripheral leukocytes, and 3) prenatal diagnosis can accurately identify affected fetuses.

Implicit in the program for prevention of Tay-Sachs disease is the ability to accurately and reliably diagnose carriers. Since the discovery of the enzyme defect in Tay-Sachs disease in 1969, carrier detection has been accomplished by determination of β -hexosaminidase A activity in leukocytes, plasma and other sources using 4-methylumbelliferyl- β -D-N-acetylglucosaminide as a substrate (Dance et al., 1970; Kaback, 1972). Pregnant females or women on oral contraceptive medications may have a falsely low percentage of heat labile β -hexosaminidase A activity due to increased levels of a heat stable β -hexosaminidase B isoenzyme. In addition, there are individuals whose β -hexosaminidase A activity falls within an inconclusive range between carrier and non-carrier, making it difficult to reliably determine their carrier status. Thus, efforts have focused on the development of more reliable methods for detection of carriers and clarification of the carrier status of individuals with inconclusive enzyme values of β -hexosaminidase A.

The cloning and sequencing of the cDNA for the β -hexosaminidase A gene (Myerowitz and Proia, 1984; Myerowitz et al., 1985; Korneluk et al., 1986; Proia and Soravia, 1987) has provided the means for determining the molecular defects which cause Tay-Sachs disease among Ashkenazi Jewish TSD patients with either the infantile or adult forms of the disease. Three com-

mon mutations have been elucidated. The most common mutation in the infantile form is a four base insertion in exon 11 (Myerowitz and Costigan, 1988) while a second mutation, a splice junction transversion at the 5' end of intron 12, is less common (Arpaia et al., 1988; Myerowitz et al., 1988; Ohno and Suzuki et al., 1988). A third mutation, a guanine to adenine nucleotide substitution in exon 7 which leads to the substitution of a serine for glycine at amino acid position 269, is found in heterozygous form in individuals with adult onset TSD (Navon and Proia, 1989; Paw et al., 1989; Navon et al., 1990). The other Hex A allele in adult-onset TSD patients may contain either the insertion, splice junction or an unidentified mutation. A private mutation, has also been found in a single patient of Ashkenazi Jewish descent with infantile TSD involving a single base substitution of guanine for adenine (Myerowitz, personal communication). An additional point mutation has been found in exon 7 at position 739 (cytosine-thymine), mainly in individuals of non-Jewish descent (Triggs-Raine and Gravel, personal communication). Other mutations have also been described in other ethnic groups (Neufeld, 1989; Sandhoff et al., 1989; Paw et al., 1990).

Recent studies of obligate carriers of TSD have shown that 97% of carriers of Ashkenazi Jewish descent have one of these three common mutations. Of 90 TSD chromosomes studied, only 3 (or 3.3%) did not have one of the 3 common mutations (Triggs-Raine et al., 1990; Grebner and Tornczak, 1991). Therefore DNA studies of individuals of Ashkenazi Jewish descent should identify around 97% of carriers of TSD. The feasibility of using DNA studies in conjunction with enzyme analysis in a Tay-Sachs disease carrier detection program was investigated at the Mount Sinai Tay-Sachs Disease Prevention Program, New York.

MATERIALS AND METHODS

Specimens

Blood samples were obtained from individuals participating in the Mount Sinai Tay-Sachs Disease Prevention Program and from obligate Tay-Sachs disease carriers with informed consent. White blood cell pellets were isolated by sedimentation in 6% dextran in saline (Kaback et al., 1977). DNA for PCR amplification was isolated from whole blood or white blood cell pellets by standard techniques (Higuchi, 1989; Aldridge et al., 1984). Control fibroblast cell lines from individuals with infantile or adult Tay-Sachs disease containing one or more of the common Ashkenazi Jewish mutations were obtained from Coriell Institute, Camden, NJ.

β -Hexosaminidase A (Hex A) assay

White blood cell pellets were sonicated on ice in the Branson cup sonicator, followed by centrifuge at 600 rpm for 10 mins. The supernatant was used for enzyme assays according to standard protocol (Dance et al., 1970; Kaback, 1972). Diluted white blood cell extract in WBC buffer of 0.03 M citrate/0.04 M sodium phosphate buffer, pH 4.4 was duplicated for heat inactivation and non-heat inactivation of heat labile hexosaminidase A activity. After incubation with synthetic substrate (3m M4-methylumbelliferyl-2-deoxy-B-D-glucopyranoside) at 37°C for 15 mins, enzyme activities were assayed in heat inactivated and non-heat inactivated samples using a Turner Model #10 fluorometer. Consequently, by measuring total β -hexosaminidase activity and the remaining activity, the amount of β -hexosaminidase A was estimated by subtracting the "B" activity from the total activity. The criteria for the categorization as carrier, inconclusive, and lower normal Hex A activity group based on enzyme assay were as follows; carrier between 39% and 56%, inconclusive range between 57% and 60%, and lower normal range between 61% and 65% of Hex A activity.

Protocol for DNA studies

The DNA studies were performed using two different protocols. The initial study examined DNA only from individuals who had been identified previously by enzyme assay as carriers, non-carriers with low normal enzyme levels or inconclusive individuals. In this study, the three common mutations were examined by PCR and allele-specific hybridization (Myerowitz and Costigan 1988; Navon and Proia, 1989). In the second part of the study, DNA from all individuals screened at the Mount Sinai Tay-Sachs Disease Prevention Program was examined, usually in pools of 3 to 5 samples. These studies were carried out using PCR and direct mutation analysis by change in electrophoretic mobility due to heteroduplex formation or alteration of restriction enzyme recognition site (Triggs-Raine et al., 1990). If a mutation was found in a pooled sample, the blood in the pooled sample was examined individually. In addition, if an individual was classified by enzyme analysis as a carrier or inconclusive but no mutation had been identified in their pooled sample, the patient's DNA was examined separately.

Table. 1. Oligonucleotide Primers for PCR and Dot Blot Hybridization

A. Oligonucleotide Primers for Multiplex PCR		
Genomic DNA Covered	Oligonucleotides	Expected Size
Exon 11-Intron 12		
Sense Primer ^a	5'GTGTGGCGAGAGGATATTCCAGT3'	ca 550 bp
Antisense Primer ^b	5'TCCTGCTCTCAGGCCCAACCCTC3'	
Exon 7-Intron 7		
Sense Primer	5'TCACCCACATCTACACAGCACAG3'	174 bp
Antisense Primer ^c	5'AAGCTTCACTCTGAGCATAACAAG3'	
B. Allele Specific Oligonucleotides		
Mutations Covered	Allele specific Oligonucleotides	
Exon 11 ^a		
Normal	5'GAACCGTATATCCTATGGC3'	
Mutant	5'GAACCGTATATCTATCCTA3'	
Intron 12 ^b		
Normal	5'CAGGCTCTGGTAAGGGTTT3'	
Mutant	5'CAGGCTCTGCTAAGGGTTT3'	
Exon 7 ^c		
Normal	5'TGGGGACCAAGTAAGAATG3'	
Mutant	5'TGGGGACCAAGTAAGAATG3'	

^aMyerowitz and Costigan, 1988

^bMyerowitz, 1988

^cNavon and Proia, 1989

Multiplex polymerase chain reaction

Polymerase chain reaction (Saiki et al., 1985) was used to amplify specific regions of hexosaminidase A genomic DNA. For allele-specific oligonucleotide (ASO) hybridization, the published PCR methods for amplifying the mutations in Tay-Sachs disease were modified to permit amplification of all three mutations simultaneously in a multiplex reaction. Two sets of primers, (Table 1) were utilized: one set of primers amplified the region of the Hex A gene which spanned the 4 base insertion (exon 11) and splice junction (intron 12) mutations and produced a PCR product of about 550 bp (Myerowitz and Costigan, 1988; Myerowitz, 1988). The other set of primers amplified the region of the Hex A gene which spanned the adult G269S mutation site in exon 7 and resulted in a PCR product of 174 bp.

Oligonucleotides were synthesized on an Applied Biosystems 380B DNA Synthesizer (Applied Biosystems, Foster City, CA). The PCR reaction mixture had a final volume of 100 μ l and consisted of 1X *Taq* polymerase buffer (50 mM KCl, 12mM Tris-HCl pH 9.0, 1.5mM MgCl₂, 0.01% gelatin (W/V) and 1% Triton-X-100; Promega Corporation, Madison, WI), 200 μ M of each deoxynucleotide, 1.0 μ M of each primer and 2.5 units of DNA *Taq* polymerase (Promega). 25 μ l of the whole blood or white cell lysates prepared by the rapid isolation method (Higuchi, 1989) or 1-2 μ g of genomic DNA prepared by the standard isolation method (Aldridge et al., 1984) was used as template DNA. The PCR cycle consisted of one minute at 94°C for denaturation, one minute at 66°C for annealing, and 2 minutes at 70°C for extension, for 30 cycles. Amplification of the DNA was confirmed by electrophoresis in 1.5% agarose.

Allele specific oligonucleotide (ASO) hybridization

ASO hybridization was performed according to the method of Navon and Proia (1989) with minor modifications. One third of the PCR product was denatured by the addition of 300 μ l of 10 mM Tris-HCl pH 7.0 and 0.3 N NaOH, incubated at 65°C for 30 minutes, and neutralized with 300 μ l of 2 M ammonium acetate (Theophilus et al., 1989). The DNA was then applied to nylon membranes (Gene Screen, El duPont de Nemours and Co, Boston, MA) according to the manufacturer's protocols using a minifold I apparatus (Schleicher & Schuell, Keene, NH). The sequences of allele-specific oligonucleotides were as published for each mutation (Myerowitz, 1988; Myerowitz and Costigan, 1988; Navon and Proia, 1989; Table 1). The

oligonucleotides were end-labeled with γ ³²P-ATP (Amersham, Arlington Heights, IL) using polynucleotide kinase (Sambrook et al., 1989). Hybridization was carried out at 37°C for 4 hours in 1 M NaCl, 0.005 M EDTA, 1% sodium dodecylsulfate (SDS) and 100 μ g/ml denatured salmon sperm DNA. Washing solutions were as described in Navon and Proia (1989) except that the final washing temperature for the insertion and splice junction mutations was 52°C and for the G269S mutation, the final washing temperature was 54°C (Fig. 1).

Fig. 1. Tay-Sachs disease carrier screening by dot-blot hybridization using three sets of allele specific normal or mutant oligonucleotide probes in PCR-amplified genomic DNAs. Screenees 1, 2, 4, 5 turned out to be carriers of 4 bp insertion mutation in exon 11 and screenee 2, a carrier of the intron 12 splice junction mutation. Ex7+Ex11: control PCR-amplified genomic DNA for genetic compound of exon 7 G269S point mutation and exon 11 insertion mutation. Ex7+In12: control PCR-amplified genomic DNA for genetic compound of exon 7 G269S point mutation and intron 12 splice junction mutation.

Heteroduplex and restriction analysis

The second method for studying the three Ashkenazi Jewish mutations was heteroduplex or restriction analysis. The conditions and primers for PCR amplification of the pooled samples were similar to those reported (Triggs-Raine et al., 1990) with the following modifications: the concentration of the splice junction mutation primers was increased to 5 μ M (final concentration) and all three PCR reactions were amplified at an annealing temperature of 55°C. A portion of the exon 11 PCR reaction was analyzed directly on 8% acrylamide gel to check for the presence of 47 bp heteroduplex band characteristic of the heterozygous state for the 4-bp insertion mutation (Triggs-Raine and Gravel, 1990; Shore and Myerowitz, 1990) (Fig. 2). The presence of the insertion mutation could be confirmed by incubating the PCR product with *Hae* III (Promega) followed by analysis on an 8% acrylamide gel. Aliquots from the intron 12 and exon 7 reactions were incubated with either *Dde* I (Promega) for detection of the splice junction mutation or with *Eco*R II (Bethesda Research Laboratories, Gaithersburg, MD) for detection of the G269S mutation. The sam-

Fig. 2. Tay-Sachs disease carrier screening by restriction analysis or heteroduplex analysis. PCR-amplified genomic DNAs digested by *Dde*I were electrophoresed on 8% polyacrylamide gel in the first four lanes (H, N, 1, 2). Presence of intron 12 splice junction mutation created *Dde*I restriction site, yielding 85, 35 bp fragments by *Dde*I digestion as shown in heterozygous control (H) and screenee 2. A portion of exon 11 PCR product was analyzed directly on the same gel in the last four lanes (H, N, 1, 2) for the detection of 47 bp heteroduplex band resulted from heterozygous state for 4 bp insertion mutation in exon 11, which were present in heterozygous control (H) and screenee 1. M: marker DNA, ϕ χ 174 *Hae*III digested. N: normal control.

ples were then analyzed on 8% acrylamide gels for the presence of an 85 bp band characteristic of the splice junction mutation (Fig. 2) or a 52 bp band characteristic of the G269S mutation.

To decrease the number of PCR reactions required, three or four blood samples were pooled together, the DNA isolated (Higuchi, 1989) and amplified. 180 μ l of whole blood from three samples or 240 μ l of whole blood from four samples was mixed together and the DNA isolated. If a mutation was found in a pooled sample, the DNAs in the sample were analyzed individually. Also if an individual was determined to be a carrier or had inconclusive enzyme values by enzyme assay but no mutation was seen in the pooled blood sample, the DNA was also examined individually.

RESULTS

The frequency of the three common TSD mutations was determined in obligate carriers of non-Jewish and Ashkenazi Jewish descent using PCR and ASO hybridization. As shown in Table 2, 15 carriers of Ashkenazi Jewish descent and five adult onset Tay-Sachs disease patients were studied, for a total of 25 TSD alleles. Eleven (73%) of the obligate carriers had the insertion mutation and 4 (27%) the splice junction mutation while the adult Tay-Sachs disease patients all had the G269S mutation and either the insertion mutation (4 of 5) or splice junction mutation (1 of 5). Of the 14 obligate carriers of non-Ashkenazi Jewish descent studied, 6 (42%) had the 4-bp insertion mutation, 3 (21%) had the 5' splice junction mutation and 5 (36%) had none of the three common Ashkenazi Jewish Tay-Sachs mutations.

Individuals in the Mount Sinai Tay-Sachs Disease Screening Program who were identified as carriers, inconclusives or non-carriers with low normal levels by enzyme analysis were examined for the presence of three common mutations. Individuals classified as carriers had between 39 and 56% Hex A in their white blood cells, inconclusives 57 to 60% Hex A and low non-carriers 61 to 65% Hex A. From the 1300 people screened, 37 individuals were classified as carriers, 36 individuals had Hex A enzyme values in the inconclusive range and 313 individuals were classified as non-carriers with Hex A levels below 65%.

As shown in Table 3, 29 of 37 enzyme positive carriers (78%) had the insertion mutation while 4 (11%) had the splice junction mutation. One individual had the adult Tay-Sachs G269S mutation while 3 (8.3%) individuals did not have any of the three common mu-

tations. All the individuals who did not have any of the common Jewish mutations were of Ashkenazi Jewish descent as determined by intake history. By enzyme assay, thirty six individuals were classified as inconclusive. However, only two of these individuals had the insertion mutation, while other 34 were negative for all three common mutations.

DNA from 313 individuals with white blood cell Hex A enzyme activities between 61 and 65% Hex A, the low non-carrier range, were also examined (Table 3) one individual, whose Hex A enzyme values in WBC was 60.17%, had the splice junction mutation.

These pilot DNA studies conducted on individuals whose carrier status had already been determined by enzyme assay suggested that DNA testing was clearly valuable in identifying or clarifying carrier status. Routine DNA screening of individuals studied in the Mount Sinai Tay-Sachs Disease Prevention Program was therefore undertaken with the samples analyzed in a pool of three or four samples. Preliminary studies, with blood samples from individuals with known mutations, showed that a mutation present in only one out of the three or four blood samples in the pool is detectable after PCR amplification and gel electrophoresis. Samples analyzed individually and in pools were also found to give exactly the same results, thus eliminating the

possibility of unequal amplification of samples. In the study of 400 pooled samples, 16 of 19 (84%) individuals identified as carriers by enzyme assay had either the insertion (13 individuals) or the splice mutation (3 individuals). Two of 12 individuals classified as inconclusive also had the insertion mutation.

DISCUSSION

The use of DNA testing as an adjunct to enzyme analysis to improve carrier diagnosis in a Tay-Sachs disease carrier screening program has been investigated. For DNA diagnosis to be efficacious, the mutations studied must be present in a high proportion of obligate carriers and affected individuals. In our preliminary study of Ashkenazi Jewish obligate carriers and adult-onset TSD patients, all the individuals were found to have one of the three common Ashkenazi Jewish Tay-Sachs disease mutations. Sixty percent had the four base insertion mutation, 20% the splice junction mutation, and 20% the G269S point mutation. Similar results were observed in other studies where only three out of 87 (3.4%) alleles from Ashkenazi Jewish carriers and patients did not have one of the common Ashkenazi Jewish mutations (Triggs-Raine et al., 1990; Grebner and Tomczak, 1991). Thus,

Table 2. Identification of Mutations in Tay-Sachs Disease Obligate Carriers/Patients

Ethnic Background	No. of Subjects	No. of TSD Alleles	No. of Mutant Alleles Identified			
			Insertion	Splice	G269S	Other
Ashkenazi Jewish	15 carriers + 5 adult TSD patients	25	15	5	5	0
Non-Jewish	14	14	6	3	0	5

Table 3. Comparison of Enzyme and DNA-Based Screening of Ashkenazi Jewish Individuals for Tay-Sachs Disease

Enzyme Diagnosis ^a	No. of Subjects Analyzed	DNA Diagnosis (No. of Mutant Alleles Identified)			
		Insertion	Splice	G269S	Other
		MUTATIONS STUDIED BY ASO/DOT BLOT HYBRIDIZATION			
Carrier	37	29	4	1	3
Inconclusive	36	2	0	0	34
Lower Normal	313	0	1	0	312
MUTATIONS STUDIED BY RFLP ANALYSIS ^b					
Carrier	19	13	3	0	3
Inconclusive	12	2	0	0	10

^a% Hexosaminidase A activity in white blood cells

^b Total number of samples examined either in pools or individually = 400

out of 116 Ashkenazi Jewish TSD alleles studied in three laboratories, only 3 (2.6%) did not have one of the three common mutations. In the Ashkenazi Jewish population, the four base insertion mutation in exon 11, the splice junction mutation in intron 12 and the point mutation G269S in exon 7 are present in 97% of the TSD carriers and would be expected to be present in over 90% of the carriers detected by enzyme assay.

Among the carriers of non-Jewish descent, our study found a higher frequency of the common Ashkenazi mutations (64%) in the small non-Jewish population studied compared to other reports (Triggs-Raine et al., 1990; Paw et al., 1990; Grebner and Tomczak, 1991). Analysis of individuals classified as carriers by enzyme assay in the Mount Sinai Tay-Sachs Disease Prevention Program determined that 50 out of 56 (89.3%) had one of the three common Ashkenazi Jewish Tay-Sachs mutations. The five individuals who were negative for the three common mutations had Hex A values as measured in white blood cells, tears and serum clearly in carrier range. These individuals also appear to be of Ashkenazi Jewish descent as determined by intake history. The percentage of carriers lacking any of the common TSD mutations in our study (10.7%) is higher than that in two other studies (Paw et al., 1990; Grebner and Tomczak, 1991) which found that 6-8% of their enzymatically diagnosed Jewish carriers did not have any of the three common Jewish mutations but lower than a third study (Triggs-Raine et al., 1990) which found 18% of the carriers without the known common mutations. These apparent false positives could be the result of unidentified or untested mutations, other biological factors affecting Hex A activity besides mutations in the Hex A gene or inconsistencies in the enzyme assay. However, individuals with low % Hex A activity but no identified DNA mutations must still be considered Tay-Sachs disease carriers.

The clarification of the carrier status of individuals classified as inconclusive by enzyme assay was possible by the DNA analysis. Since only four of the 48 inconclusive individuals had one of the common Jewish mutations, it is probable that most of the inconclusive individuals are actually non-carriers. Although it is not possible to rule out absolutely that individuals with inconclusive enzyme levels but without any of the three common Ashkenazi Jewish mutations are not carriers, it clearly decreases the probability of their being carriers.

Only one out of 313 individuals classified as non-carriers with low normal enzyme activity had one of the common Ashkenazi Jewish mutations. This in-

dividual had a Hex A enzyme level of 60.1% in white blood cells and is the only known false negative detected for a rate of 0.3%. The DNA studies thus indicate that the enzyme assay is a reliable test for carrier detection and that very few carriers would be missed.

DNA analysis of individuals being screened for carrier status of Tay-Sachs disease is useful in clarifying individuals whose status is unclear from enzymatic studies. DNA analysis identified five individuals as carriers who were classified as either non-carriers or inconclusive. The study also identified six individuals classified as carriers and 44 individuals with inconclusive enzyme values who have a decreased risk of being carriers since they lack the common Jewish Ashkenazi TSD mutations. It is necessary, however, to use both enzymatic assays and DNA analysis to determine the carrier status most accurately, since additional mutations occurring at very rare frequencies probably exist within the Ashkenazi Jewish population. DNA analysis also allows one to distinguish between carriers of the adult mutation, G269S which causes the adult form of Tay-Sachs disease and those individuals who carry the insertion and splice junction mutations.

The examination of the DNA mutations in pooled samples of blood permits rapid and efficient identification of mutations. Mutations are reliably observed in the pooled samples of three to five individuals and can be confirmed by amplifying the DNA from the individual blood sample. The development of multiplex PCR reactions along with pooling of blood samples also make use of DNA analysis more economical in TSD carrier screening programs.

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