Tay-Sachs Disease—Carrier Screening, Prenatal Diagnosis, and the Molecular Era

An International Perspective, 1970 to 1993

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Objectives.—To provide an update of the international experience with carrier screening and prenatal diagnosis for Tay-Sachs disease (TSD), to assess the impact of these efforts, and to review the recent developments in DNA technology with application to TSD carrier detection and screening.

Design.—Through the International TSD Testing, Quality Control, and Data Collection Center, all testing centers in the world were surveyed annually to assess overall experience with carrier testing and prenatal diagnosis. Quality control and laboratory surveillance of testing centers were performed through an annual assessment, using samples provided by the center.

Setting.—Tay-Sachs disease testing centers around the world.

Participants.—Nearly 1 million young adults from both Jewish and non-Jewish populations.

Intervention.—Gene product screening (enzyme testing) and DNA-based mutation analysis (in some populations).

Main Outcome Measure.—Impact of screening program on disease incidence.

Results.—Data from all centers in the international TSD network on experience with TSD carrier testing and prenatal diagnosis since 1974 indicated that more than 36 000 heterozygotes were identified and 1056 couples found to be at risk for TSD in their offspring. A total of 2416 pregnancies at increased risk for TSD were monitored by amniocentesis or chorionic villus sampling. A dramatic decrease in the incidence of TSD in the Jewish populations was demonstrated. With both serum and leukocyte proficiency testing, there have been only 16 instances (of 845 cumulative laboratory evaluations) of one or more errors reported by a laboratory since 1983 resulting in nonaccreditation.

Conclusions.—This analysis represents a prototypic effort in coordinating adult education, carrier testing, and genetic counseling directed toward prospective prevention of a uniformly fatal childhood disease and demonstrates that such an effort can dramatically affect disease incidence.

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TAY-SACHS disease (TSD) is the prototype of the lysosomal sphingolipid storage disorders.[1] The hereditary nature of this uniformly fatal neurogenerative disease (autosomal recessive inheritance),[2,3] its ethnic predilection (infants of Central or Eastern European Jewish ancestry),[4-6] the characteristic neuropathological findings ("ballooning" of neurons with massive intralysosomal accumulation of lipophilic membranous bodies),[4,7] and the nature and structure of the stored intraneuronal material (GM_2 ganglioside)[8,9] are all well established. In 1969, the underlying biochemical defect in TSD was identified—a profound deficiency of activity of the lysosomal hydrolase β-hexosaminidase A (HEX A or GM_2 gangliosidase).[10-12] This isoenzyme is specifically required for the cleavage of the terminal β-linked *N*-acetylgalactosamine from GM_2 ganglioside. Failure to hydrolyze the terminal hexosamine leads to the continuous accumulation of GM_2 ganglioside in neuronal lysosomes. This results in progressive neuronal dysfunction (manifesting as hypotonia, blindness, dementia, and seizures), cell death, and subsequently the patient's demise, usually by 3 to 5 years of age.

The development and use of synthetic colorometric or fluorogenic substrates to quantify the activities of hexosaminidase isoenzymes[13] enabled development of relatively easy and accurate methods for diagnosis of TSD. This was accomplished in tissues and body fluids from infants affected with TSD as well as with cultured, second-trimester amniotic fluid cells derived from pregnancies in which fetuses had the disease.[14-16] Electrophoretic, thermal, and pH stability differences between the hexosaminidase isoenzymes provided methods to precisely quantify the HEX A component, thus allowing for accurate identification of the heterozygous carrier state (in which a significant reduction in HEX A activity is evident in tissues and body fluids from such individuals).[17-19]

Serum testing is used for carrier screening in men and nonpregnant women. Leukocyte HEX A testing must be used in pregnant women or in apparent serum-defined carriers who are taking oral contraceptives, are on unusual medications, or are suffering with a tissue-destructive disease (as determined by a personalized questionnaire that is completed by each individual at the time of testing).[17,18] In the program described herein, the laboratory charges for these tests are approximately \$20 to \$75, depending on whether one or both tests are used.

These advances provided the basis for

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A complete list of the International TSD Data Collection Network appears at the end of this article.

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community-based TSD education, carrier screening, and genetic counseling programs directed to the prospective prevention of this fatal disorder.[20] Because the disease occurs predominantly in a defined population and because of the availability of a simple, highly accurate, and relatively inexpensive carrier detection test (serum and/or leukocyte HEX A profiles), screening individuals of childbearing age in the high-risk Jewish population was proposed to identify healthy carriers of the disease gene and, most critically, those couples in which both partners are heterozygotes and therefore at risk for TSD in their offspring (prior to the birth of any affected children). Once identified, such couples could be provided comprehensive genetic counseling, including the option of prenatal monitoring of all pregnancies. In this way, at-risk couples could, if they chose, bring to term only those pregnancies in which the fetus was shown to be unaffected by this uniformly fatal disease. When prenatal testing indicated the fetus was afflicted with TSD (25% risk with each conception), families could make the difficult choice whether or not to abort such pregnancies. Other options that families might choose include the following: (*a*) deferring from having children of their own and considering adoption; (*b*) conceiving through artificial insemination or by ovum donation (being sure to exclude TSD heterozygosity in all potential donors); (*c*) simply taking their chances (recognizing they have a 75% chancewith each pregnancy of having a child unaffected by TSD); or (*d*) if single and found to be a carrier, to use such information in marriage or mating decisions to avoid marriage and/or reproduction with another carrier (as recommended by certain ultraorthodox Jewish groups).[21]

The first multiphasic public education, voluntary, carrier screening and genetic counseling effort for the prevention of TSD was initiated in a pilot program in Jewish communities of Baltimore, Md, and Washington, DC, in 1970.[20] Comparable initiatives in other cities of the United States, Canada, Israel, South Africa, South America, Europe, and Australia followed shortly thereafter.[22] Organizational, educational, and psychosocial aspects of these programs have been reviewed.[23-27] The purpose of this article is to update the international experience with carrier screening for and prenatal diagnosis of TSD and to assess the impact of these efforts on this prototypic disorder. In addition, the impact of recent developments in recombinant DNA technology and their applications to TSD carrier detection and screening programs are addressed.

METHODS

Heterozygote Testing, Prenatal Diagnosis, and Disease Incidence

Beginning in the mid 1970s, the International TSD Testing, Quality Control, and Data Collection Center was established in California by a grant from the National Tay-Sachs Disease and Allied Disorders Association (NTSDAD), a nonprofit lay health organization in the United States. The purpose of the International Center is to survey all testing centers in the world annually to assess the overall experience in TSD carrier testing and prenatal diagnosis. In addition, through similar mail and telephone communications with all diagnostic centers in the United States and Canada, the number of newly diagnosed patients with TSD and other GM_2 ganglioside storage disorders are monitored each year.

This network is based on collegial participation. Although it is possible that a few centers may be screening or conducting diagnostic testing without our knowledge (particularly outside the United States and Canada), almost certainly these instances are rare and only small numbers of individuals are tested. To date, no center that we have ever contacted has refused to provide the data requested.

Each year, a questionnaire is communicated to all worldwide centers known or likely to be testing for lysosomal diseases or where patients with such disorders would likely be seen. Information about carrier testing experience, prenatal diagnosis, and newly diagnosed patients with TSD and related disorders is requested. Specific efforts are made to avoid duplication of prenatal or diagnostic cases by inclusion of the mother's initials on each reported prenatal case and, in the case of patient diagnosis, the initials and the date of birth of the patient. Particular efforts are made to avoid duplicate reporting of prenatal testing (eg, where chorionic villus sampling or amniocentesis is performed in one center and the sample is sent to a different center for laboratory studies).

Quality Control and Laboratory Surveillance

An additional commitment of the International Center is to conduct an annual assessment of all known carrier testing laboratories. This is achieved through an overnight mail shipment of 20 to 30 frozen serum and/or leukocyte samples. All samples are prepared and tested in the reference center laboratory and then coded, packaged, and shipped. All participating laboratories are contacted prior to shipment and are given a defined time within which results must be communicated back to the center. All samples are identified only by code number. The laboratory analyzes each sample, records its actual data and results, and is required to make a diagnosis on each. Both carrier and noncarrier samples (sera and leukocytes) are included. Parents of TSD children come to the reference laboratory annually and voluntarily provide samples for this survey. Noncarrier samples are prepared from local volunteers (including staff of the center) whose hexosaminidase profiles in both sera and leukocytes indicate noncarrier status and who have no known Jewish ancestry. Each laboratory must also report its detailed protocol and methodology for carrier testing. This is done so that if any diagnostic errors are made, the reference laboratory can assess methodological variables and make recommendations that, hopefully, will aid the testing center to correct the problem.

Because identical methodologies are not required, evaluation is primarily based on diagnosis of samples rather than on absolute assay values obtained. If one error or more occurs in either carrier or noncarrier samples, the laboratory is contacted by mail and/or telephone, possible problems are identified, and then a second set of coded samples is sent (phase II). Should multiple errors occur, critical interaction with the reference laboratory is suggested until methodology problems are resolved. Again, a defined time limit (2 to 3 weeks) for reporting phase II results is established. Any error with phase II samples or failure to report within the time allotted results in nonaccreditation.

This program is carried out under the auspices of the Quality Control Subcommittee of the Scientific Advisory Committee of the NTSDAD. To offset program costs, a fee is requested of each participating laboratory by the NTSDAD. This fee can be waived by the NTSDAD, because maximum participation is desired. Laboratory accreditation is granted only to laboratories that make no errors in both carrier and noncarrier sample identification (including phase II participants). The NTSDAD annually publishes a list of international centers that have been accredited in the proficiency testing program. This publication is circulated widely to synagogues, community centers, and other Jewish organizations with the recommendation that individuals or groups use only accredited laboratories for carrier screening or prenatal testing.

RESULTS

Carrier Screening

All centers in the international TSD network have provided data on their

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TSD carrier testing and prenatal diagnosis experiences since 1974. No program has refused to comply with these requests. Earlier summary experiences have been reported.[22,28]

The last annual survey conducted includes data from all programs through June 30, 1992. To that date, a cumulative total of nearly 1 million young adults throughout the world had been tested voluntarily to determine whether or not they were carriers of the TSD gene. These cumulative results are provided, by country or region, in Table 1. More than 36 000 heterozygotes have been identified and, most critically, 1056 couples have been found to be at risk for TSD in their offspring. None of these couples had a previous child with TSD (or any known GM_2 gangliosidosis). In some regions, testing has been almost exclusively among individuals (and their spouses) in families where TSD was known to have occurred in a close relative. Assuredly, this contributes greatly to the relatively high frequencies of at-risk couples identified in these areas. The possibility that some identified carriers represent false positives or are the result of laboratory error must also be considered.

After the first few years of the 1970s, when program start-ups were occurring worldwide, annual rates of worldwide testing have remained relatively constant at approximately 62 000 people per year. About 35 000 of these individuals are tested each year in the United States. This number also has remained relatively unchanged.

Prenatal Diagnosis of TSD

From 1969 when the prenatal detection of TSD was first accomplished[16] through June 1992, a total of 2416 pregnancies at increased risk for TSD in the fetus have been monitored either by amniocentesis or, more recently, by chorionic villus sampling. This overall experience is provided in Table 2. It is gratifying to note that as of this time more pregnancies have been monitored in couples without prior affected offspring (identified through screening) than in families who previously had children with TSD.

Several other points worthy of mention are in Table 2. While almost exactly one fourth of the monitored pregnancies in couples who had affected offspring revealed fetuses positively diagnosed with TSD (as expected), the fraction is considerably lower in the pregnancies identified through carrier screening. This results from the fact that a considerable number of at-risk pregnancies have been monitored (and included in this data) in which the actual risk was much less than 25%. Some pregnancies have been tested in which either partner or both partners have had inconclusive carrier testing results. Others have been tested, at the (carrier) woman's request, where the father of the fetus was unavailable or not identified. Further, the consideration of false-positive carrier identification may contribute to this finding. Also, 18 fetal diagnoses of TSD were made in which elective abortion was not carried out and the pregnancies were carried to term. Most of these cases occurred in the early 1970s, and the diagnoses were made in the third trimester of pregnancy, obviating the option of legal pregnancy termination. Several of these diagnoses were made in pregnancies in Hasidic (ultraorthodox) Jewish families with prior affected offspring. Rabbinical approval for termination was refused on religious grounds. All 18 of these pregnancies resulted in children who were, as predicted, afflicted with infantile TSD.

In essentially every instance where aborted fetal tissue was available for study, confirmation of the prenatal diagnosis was made by quantitative lipid analysis of central nervous system tissue, ultrastructural assessment by electron microscopy of the fetal brain and/or anterior motor horn cells of the spinal cord, and/or profound HEX A deficiency in various fetal tissues.

Also evident in Table 2 is that three fetal diagnoses of TSD were missed. All were due to sample mix-ups, laboratory error, or inadequate experience (and absence of appropriate controls) in the testing laboratories.

Perhaps the most important item in Table 2 refers to the 1881 infants born without TSD in this experience. As indicated by previous studies in families with children afflicted with TSD (before the advent of prenatal diagnosis), many of those children might never have been conceived or brought to term if this option had not been available.[29] While it is evident that this experience accounts for the prevention of nearly 500 births of children fatally afflicted with TSD, it should be emphasized that the TSD carrier screening experience is mostly about the birth of nearly 2000 healthy infants.

Impact on Disease Incidence

Prior to 1970, estimates of the incidence of TSD in both Jewish and non-Jewish populations were available only for the United States.[3,30] Since 1980, the International Data Collection Center in California has monitored the number of newly diagnosed cases of TSD and other GM_2 storage disorders on an annual or biannual basis. Approximately 60 new cases of TSD were identified each year in the United States in the pre-1970 era. These estimates were based on death-record–

Table 1.—Tay-Sachs Disease Heterozygote Screening, 1971 Through 1992

Country	No. Tested	Carriers	At-Risk Couples
United States	712 818	27 150	657
Israel	159 544	4229	263
Canada	55 922	2922	57
South Africa	11 638	1286	36
Europe	10 927	725	21
Brazil, Mexico	1682	96	20
Australia	473	10	2
Total	**953 004**	**36 418**	**1056**

Table 2.—Worldwide Prenatal Diagnosis of Tay-Sachs Disease, 1969 Through 1992

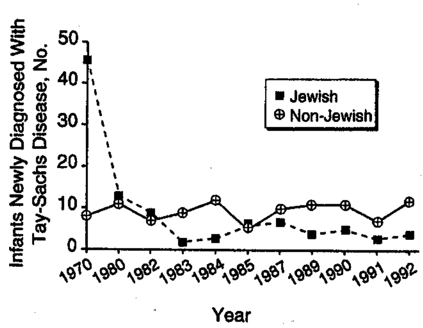
	Couples Identified At Risk		
Diagnoses	By Prior Offspring	By Carrier Screening	Total
Pregnancies monitored	1118	1298	**2416**
Affected fetuses	268	201	**469**
Elective abortions	250	201	**451***
Tay-Sachs disease fetuses missed	2	1	**3**
Unaffected offspring born	827	1054	**1881**

*Eighteen infants affected with infantile Tay-Sachs disease as predicted.

derived data and predicted an annual birth incidence of TSD among Ashkenazi Jews of about one in 4000 births. Among non-Jews, the birth incidence was predicted to be 100 times less frequent, indicating a carrier rate of about one in 300 in non-Jews as compared with one in 30 to one in 40 among European Jews living in the United States and Canada.[29]

With the development of population-based carrier screening and prenatal diagnosis programs in the 1970s throughout the major cities of the United States and Canada, a measurable impact already was evident by 1980 when only 13 newly diagnosed cases of TSD were reported.[28] The Figure illustrates the annual numbers of newly diagnosed cases of infantile TSD in both the Jewish and non-Jewish populations of the United States and Canada since 1970. A dramatic decrease in the incidence of TSD in the Jewish populations of these countries is readily apparent. Since 1983, when only two new cases were diagnosed, the annual number of newly diagnosed infants with TSD in the Jewish population has remained at around three to five cases per year. This represents a greater than 90% reduction in the incidence of TSD in the Jewish populations of these two countries over this interval (1970 to 1993). Unquestionably, the advent of prenatal diagnosis for TSD and the concomitant development of carrier detection and genetic counseling programs have contributed greatly to this dramatic decline.

Because community education and heterozygote screening has been targeted primarily to the higher-risk



Annual numbers of newly diagnosed cases of infantile Tay-Sachs disease in the United States and Canada.

Jewish community (although not excluding non-Jewish individuals who wish to be tested), the impact on disease incidence is predominantly in the target population. Whereas there were 10 to 15 cases of TSD estimated to occur in non-Jewish infants each year prior to 1970, that frequency has not shown much of a decline (Figure). In fact, whereas Jewish infants with TSD accounted for about 85% of the total annual cases before 1970, at present there are three to four times more cases of TSD being identified each year in non-Jewish infants than in Jewish children. This relative increase in cases in the non-Jewish population speaks to the effectiveness of the prevention effort in the target population, not to any real increase among non-Jews. Moreover, with increasing patterns of intermarriage, conversion, and decreasing information about one's ancestry and origins, the issue of "who is Jewish?" becomes increasingly problematic. In fact, the American College of Obstetricians and Gynecologists now recommends offering TSD carrier testing to couples where one member is of Jewish heritage, in addition to all Jewish couples.[31]

Quality Control

With the exception of 1979, a blinded proficiency testing exercise has been carried out each year with precoded serum and/or leukocyte samples in laboratories throughout the world known to be involved with TSD carrier testing. This currently includes a total of 57 international facilities in both academic and private settings. Of these, 43 laboratories are in the United States, six are in Canada, two in Europe, two in Australia, and one each in Israel, South Africa, Mexico, and Brazil. Error-free performance is required from each laboratory in order to receive accreditation from the NTSDAD.

After identifying and resolving a number of technical, preparative, shipping, and customs-related problems in the early phases of the program, the performance of testing laboratories with coded serum and/or leukocyte samples has improved greatly. Results from the initial few years of the program have been reported.[32]

Since 1983, with 45 to 53 laboratories participating yearly in the serum testing program, only on five occasions (out of 484 cumulative laboratory evaluations over this period) were one or more errors reported by a laboratory. This experience represents 7260 serum samples analyzed (of which about 3000 were obligate carrier samples) in which a total of only eight erroneous diagnoses were made, six in which carrier samples were missed and two in which noncarrier samples were incorrectly identified as carriers. However, this is after phase II testing was carried out in those two to three laboratories (out of more than 50) that made phase I errors.

The leukocyte effort, being considerably more complex, required early programmatic modifications. Difficulties related to the quality of the samples provided were encountered in a significant number of participating laboratories. Providing frozen leukocyte pellets (from sufficient quantities of blood from obligate heterozygotes) to accommodate each laboratory's customary methodology was difficult. In some instances, pellets stored for as long as 10 years were used. Beginning in 1986, this was changed so that only larger pellets (freshly prepared in the Reference Laboratory and one or two other major centers) were included. This resulted in clearly improved performance with leukocyte samples. With 36 to 47 laboratories participating annually in leukocyte proficiency testing since 1986, there have been 11 nonaccreditations out of 361 individual laboratory evaluations (after phase II).

Those centers not accredited are encouraged to interact with the Reference Laboratory to work out problems and to desist from patient testing until the next proficiency testing round. Over the years, a number of laboratories have elected to stop testing in their own facilities and decided rather to ship their local samples to an approved laboratory for analysis.

It should be clear that the implications of the predictive nature of the carrier detection test and the considerable time that might have to transpire before errors might become evident (defined by reproductive outcome) demand a zero tolerance for error. Critical reproductive decisions, the possible need for risk-associated obstetrical procedures (albeit minimal risk), and the potential for tragic misdiagnosis (of either kind) underscore the necessity to strive for essentially perfect laboratory performance in carrier and noncarrier identification. Since serum testing is invalidated by pregnancy (essentially 100% of pregnant women will appear to be carriers), it is particularly important that leukocyte testing be of the highest quality.

COMMENT

The Model and Its Application to Other Disorders

This experience represents the first population-based effort mounted for the prospective control of a lethal genetic disease. That multiphasic education, voluntary testing, and comprehensive genetic counseling can be coordinated in an effective disease prevention effort appears evident from this 22-year experience. Several key principles emerge from a critical analysis of this endeavor. From the beginning of the planning phase of the program, community leaders, medical practitioners, and religious advisers were brought into the planning process to develop their understanding, to gain their advice, and, if desired by them, to ensure their participation. The manpower needs for effective community education also were critically addressed well in advance of initiation of the program. Clearly, rigorous and highly accurate laboratory methods with exquisite sensitivity and specificity must be in hand and capable of flawlessly dealing with high volumes of samples. Timely delivery of results to all participants, with absolute control of the confidentiality of test results, is also essential. Professional genetic counseling (or its equivalent) is critical for those found to carry such a trait and must be readily available to clarify any misinformation, explain the realities and options, and allay unnecessary fears. Last, it should be underscored that all of these issues represent relatively unexplored areas of technology delivery, and simply recognizing the concerns does not guarantee that they have been dealt with effectively. For this reason, such efforts should be seen as experiments in technology transfer and should be initiated accordingly with built-in systems of evaluation.

Initial efforts should be construed as pilot studies with critical psychosocial analytic components included, such as assessing educational effectiveness; compliance and noncompliance factors; possible individual, family, or community stigmatization effects; and ongoing impact of the program. It is of interest that many of these principles were not addressed in the early 1970s when carrier

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screening programs for sickle cell trait were initiated in many parts of the United States. These and other sociopolitical reasons may account in large part for the less-than-optimal response these programs received in black communities throughout America.[33]

On the other hand, organizational and planning strategies similar to those used in the TSD program were employed in the highly successful programs directed at "control" of β-thalassemia in Sardinia, Italy, and subsequently in other areas of the Mediterranean basin.[34-36] These efforts have led to a major reduction in the incidence of homozygous β-thalassemia in these regions, and thousands of at-risk couples have been identified and counseled to enable them to have children unaffected by this serious disease.[37]

As technologies have advanced, the model developed with TSD also has been considered for carrier detection and prenatal diagnosis of cystic fibrosis.[38] Wide differences in the clinical spectrum of this disease, its more panethnic distribution, and the limitations of different sensitivities of current heterozygote detection methods in various ethnic groups make population-based carrier screening for cystic fibrosis more difficult. In addition, rapid advances in effective treatment of this gastrointestinal/pulmonary disorder also underscore the need to proceed cautiously with population-wide heterozygote detection screening efforts.[39,40]

More recently, a population-based carrier screening program (again, targeted to Ashkenazi Jews) for detection of heterozygotes of type I Gaucher disease has been suggested.[41] This is another lysosomal storage disease, but (like cystic fibrosis) a disease that does not involve the nervous system and is highly variable in its manifestations (many patients are symptom-free well into adult life). Moreover, recent experiences with enzyme replacement therapy, albeit quite expensive at this time, indicate considerable somatic benefits in symptomatic patients.[42] If therapeutic intervention is effective and available, then (as in treatment of phenylketonuria) perhaps the screening should be directed to Jewish newborns rather than employing reproduction-oriented carrier testing (performed prior to pregnancy or in already-pregnant couples). Clearly, the nature of the disease and the availability of treatment must be major considerations in deciding whether or not the prospective prevention approach, as in TSD, is applicable.[43] The availability of an accurate and sensitive carrier detection test is necessary if such a program is to be initiated, but it alone is not sufficient to embark on such an effort.

The TSD program, as initially construed, was targeted to individuals and couples prior to pregnancy. This would provide the broadest range of reproductive options to individuals or couples discovered to be at risk for the disease in their offspring. This underscores the rationale for community-based testing efforts. However, the nature of the US health care system tends to focus health care behaviors to times of perceived need, rather than to a more preventive (prospective) philosophy. Accordingly, in most centers throughout this country and others, TSD carrier testing is perceived as a reproduction-related test and is generally not thought of or pursued until pregnancy. Pregnancy is also one of the few times young adults access their health care system, making it a far simpler and less expensive time for health professionals to provide testing, compared with mounting a community-based education and screening program.

An alternative approach is that developed among the ultraorthodox Jews in the United States, Israel, and several other countries. This is the so-called Dor Yeshurim program.[21] Because there is a religious proscription against abortion and because marriages are arranged and require prior approval of the rabbinate in this group of Jews, this community has organized its screening effort with the goal of avoiding carrier-carrier matings. Anonymous testing (coded samples) is carried out and a centralized record of test results is kept, available only to the rabbi. When a proposed marriage is to be considered, the rabbi can decide whether a different match should be made. This is done without disclosure of the carrier test information, in order to avoid stigmatization of the individual or family. Within the belief system of this religious subgroup, this seems a reasonable use of modern technology. Any generalization to other individuals or groups, however, would likely be highly questionable. No new cases of TSD have been reported in the Israeli Dor Yeshurim program in recent years.[44]

Screening and the Delineation of Variant GM_2 Storage Disorders

With the advent of wide-scale population screening and readily available diagnostic testing, a number of HEX A–deficient conditions other than classical infantile TSD have been delineated.[1] These include GM_2 gangliosidoses with symptom onset later in childhood than infantile TSD (B_1 variant[45] and juvenile[46]), in addition to even later onset forms (also HEX A deficient) known as chronic[47] or adult-onset GM_2 gangliosidosis.[48] It is apparent that the age of onset of symptoms in these conditions is directly correlated with the amount of residual GM_2 ganglioosidase activity present in cellular lysosomes (ie, "leaky mutants").[49]

Additionally, a few infants with a classic TSD phenotype have been described whose tissue HEX A activities (with synthetic substrates) are within the normal range, but who nonetheless have massive GM_2 ganglioside accumulation in their neurons.[50] These infants have been shown to lack GM_2 activator, a distinct lysosomal glycoprotein required in vivo for the catabolism of GM_2 ganglioside (GM_2 activator serves a detergent-like function, facilitating the interaction of the hydrophobic glycolipid, GM_2 ganglioside, with its catalytic hydrolase, HEX A or GM_2 gangliosidase). This is the activator-deficient form of TSD, or the "AB variant."[50]

On rare occasion, healthy adult individuals have been identified with near-total deficiencies of HEX A activity (measured with synthetic substrates) but are entirely asymptomatic. Such individuals, identified through screening programs or extended family testing, have been followed up for as many as 15 years and remain entirely well, some into their sixth decade. These persons have been called "HEX A minus, normal" and are now recognized to be compound heterozygotes (with different mutations in each of their two HEX A genes). One mutation is disease-related, while the other is considered a "pseudodeficient mutation" that results in a protein that has reduced capacity to hydrolyze synthetic substrates, but no loss of ability to catalyze the breakdown of the natural substrate, GM_2 ganglioside.[51] Therefore, the individual with both mutations will appear to have little or no activity of HEX A with the usual fluorogenic substrate, but will appear to be a carrier (with 50% of the activity of a noncarrier) by natural substrate assay.[52]

Obligate heterozygotes (parents) of all of the variant mutations associated with GM_2 storage disorders have reduced HEX A levels like TSD carriers and cannot be distinguished one from another by enzymatic testing. This includes carriers of the pseudodeficient mutation(s) as well. The exceptions to this are carriers of the B_1 variant mutation, a binding site affinity alteration, in whom HEX A levels are in the low noncarrier range. Fortunately, these exceptions are extremely rare, particularly in the Jewish population where screening is targeted. Carriers of the activator mutations also would not be evident by the usual HEX A heterozygote detection test.

The Molecular Decade and TSD Testing

Previous studies demonstrated that human HEX A is a heterodimeric gly-

Table 3.—α-Subunit Gene of Human Hexosaminidase A: Classes of Mutations as of March 1993

Class of Mutation	No. Reported
Missense	28
Nonsense	5
Splice site 5′ and 3′	10
Single codon deletions	2
Large deletions	1
Frameshifts (small deletions and insertions)	7
Total	**53***

*Twenty-one of 53 mutations occur at CpG dinucleotide sites.

Table 4.—Mutations Associated With Later-Onset Forms of Hexosaminidase-A–Deficient GM_2 Gangliosidoses*

Form of Disease	Mutation	Ethnic Group
"B_1 Variant" (late infantile or juvenile onset)	Arg178 → His	Portuguese, European
	Arg178 → Cys	Czech
	5 other mutations reported	Diverse other
Juvenile GM_2	Arg499 → His	Scotch, Irish
	Arg504 → His	Assyrian, Armenian
	Gly250 → Asp	Lebanese
Chronic or adult GM_2	Gly269 → Ser	Ashkenazi Jewish, diverse other
	Lys197 → Thr	Dutch

*Identified in both homozygous and compound heterozygous states. Arg indicates arginine; His, histidine; Cys, cystine; Gly, glycine; Asp, aspartic acid; Ser, serine; Lys, lysine; and Thr, threonine.

coprotein comprising a single α subunit (54 kd) and one β subunit (55 kd).[53,54] The genes directing the synthesis of α and β subunits have been localized to chromosomes 15q23q24 and 5q13, respectively.[55,56] The previously mentioned activator protein, also required for in vivo GM_2 hydrolysis, is produced from a different gene, regionally localized to chromosome 5q.[57] Defects in any of these three gene products can result in lysosomal GM_2 accumulation and subsequent disease. Deficient or defective α subunits result in HEX A deficiencies associated with TSD and its variants. Abnormalities in β subunit production result in both HEX A and HEX B deficiencies (HEX B is composed of two β subunits) since the β subunit is common to both enzymes. Disorders resulting from β-subunit gene mutations include Sandhoff disease (the prototype β-subunit disorder) and its variants. These are discussed elsewhere.[1]

Knowledge of the subunit structure and partial amino acid sequence of these enzymes made immunologic and molecular methods applicable first to the isolation and cloning of the α-subunit cDNA,[58] and subsequently to the characterization of the entire genomic α-subunit nucleotide sequence.[59] This gene spans approximately 35 000 base pairs (35 kilobases), contains 14 exons, and has both 5′ regulatory elements (TATA) and 3′ untranslated regions. There is extensive homology (>60%) with the β-subunit gene on chromosome 5, strongly suggesting that both are derived from a common ancestral gene.[60]

Southern blotting and polymerase chain reaction–linked techniques (single strand confirmational polymorphisms,[61] denaturing gradient gel electrophoresis,[62] and chemical mismatch cleavage[63]) have been used to identify 53 different mutations in the α-subunit gene to date, spanning all 14 exons and including all classes of mutations (Table 3). Included in this compilation, but not listed in Tables 4 or 5, are two mutations, *Arg247Trp*[51] and *Arg249Trp*,[64] that have been identified in association with the pseudodeficient state.

Specific mutations associated with later clinical onset forms of GM_2 gangliosidosis, including the B_1, juvenile, and adult variants, have been defined, usually in compound heterozygosity with one of the more common infantile alleles (Table 4). The great majority of these mutations have been detected in single families (private mutations) or in only a small number of patients where consanguinity or a high level of inbreeding is evident in the parents.

Similarly, among infants with the classical TSD phenotype (infantile onset), a wide variety of individual mutations have been delineated (Table 5). Nearly 40 different mutations, occurring in patients of widely diverse races and ethnic origins, are associated with infantile TSD.[65] Again, as with the later-onset forms of the disease, most of these alterations have been identified in single individuals or in very few cases. There are, however, several important and noteworthy exceptions when mutations are quantified on a subpopulation basis.

Mutation Analysis Among Jews and Non-Jews

In the Ashkenazi Jewish population of North America, three specific mutations (two associated with infantile disease and one with the adult onset form) account for between 92% and 98% of all mutant alleles.[66,67] These are the first three mutations listed in Table 6. Among enzymatically defined unrelated Jewish heterozygotes identified by screening, 3% are carriers for the adult-onset mutation, *Gly269Ser*, and 2% are carriers for a pseudodeficient mutation, *Arg247Trp*. Two other rare disease-related mutations have been found in these Jewish carriers, and about 5% carry mutations not identified by this six-mutation assessment.

When the same six mutations are evaluated in non-Jewish individuals identified as carriers by the enzymatic screening test, an entirely different distribution of mutations is found (Table 6). Here, only about 60% of the mutations carried by these individuals are identified. As in the non-Jewish parents (obligates) of infants with TSD, a large number of other mutations (different from the Jewish obligate carriers) are involved (as listed in Table 5). Only a small fraction (8%) of non-Jewish heterozygotes carry the mutation (*+TATC 1278*) most commonly found in Jewish carriers. The second most frequent Jewish allele (*+1 IVS 12*), also associated with infantile disease, has not been found as yet in any of more than 200 non-Jewish carriers examined. The adult-onset mutation, *Gly269Ser*, is found in 5% of non-Jewish carriers, slightly more common than the frequency among Jewish carriers.

The most frequent disease-related identifiable mutation among non-Jews (predominantly in California) is the splice site alteration *+1 IVS 9*. This accounts for about 10% of the mutations in this group and is a mutation that has been particularly associated with individuals of Celtic and French origins.[68,69] It is also one of the alleles that seems to have been introduced to both Pennsylvania Dutch and Cajun communities in the past, as studies of infants with TSD in these population isolates have revealed.[70,71]

Of particular relevance to screening, the single most common mutation (32%) discovered among non-Jewish carriers (nonobligates) is a pseudodeficiency mutation, *Arg247Trp*. A second recently described pseudodeficient alteration, *Arg249Trp*,[64] was also found in about 4% of these individuals. Thus, while only about 2% of screening-identified TSD Jewish carriers are found to have a benign pseudodeficient mutation, these alterations account for approximately 36% of non-Jewish carriers. The implications of these findings are critical for genetic counseling and for possible prenatal diagnostic testing and interpretation.

Clearly, DNA-based mutation analysis is indicated in all at-risk couples (Jewish and non-Jewish) identified by the HEX A screening test. While it would be impractical (and prohibitively expensive) to conduct population screening by

Table 5.—Mutations Associated With Tay-Sachs Disease (Infantile Hexosaminidase-A–Deficient GM_2 Gangliosidosis)*

Mutation	Ethnic Group
7.6-kilobase deletion, exon 1	French Canadian
+1 IVS 12 (G → C)	Ashkenazi Jewish
+ TATC 1278†	Ashkenazi Jewish, Cajun, many others
+1 IVS 9 (G → A)†	Celtic, French, Cajun, Pennsylvania Dutch
+1 IVS 7 (G → A or C)	French Canadian
ΔF 304 or *305*†	Moroccan Jewish
−1 IVS 4 (G → T)	American black

*These and 30 additional independent mutations reported in diverse worldwide ethnic groups as of March 1993.

†Identified in homozygous and in compound heterozygous states.

Table 6.—Distribution of Hexosaminidase-A α-Subunit Mutations in Jewish and Non-Jewish Unrelated Heterozygotes*

	Jewish		Non-Jewish	
Mutation	Obligate, % (n=47)	Screening, % (n=239)	Obligate, % (n=22)	Screening, % (n=187)
+ TATC 1278	81	80	32	8
+1 IVS 12	15	9	0	0
Gly269Ser	2	3	0	5
Arg247Trp	0	2	0	32
Arg249Trp	0	0	0	4
+1 IVS 9	0	0	14	10
Other (various)	0	1	4	2
None identified	2	5	50	39
Total	**100**	**100**	**100**	**100**

*Enzymatically defined, California Tay-Sachs Disease Prevention Program.

mutation analysis for all 53 currently known mutations, it is essential that once identified as a carrier by gene-product testing (ie, reduced HEX A levels), individuals should undergo follow-up ethnic-specific DNA testing and pseudodeficiency mutations should be ruled out. Certainly, this is indicated in all non-Jewish enzyme-defined carriers before genetic counseling is provided and, at minimum, in both members of all Jewish couples where both partners appear to be carriers. These studies should be conducted on carriers before prenatal testing is performed, in order to delineate the nature of the disorder for which they might be at risk and to obviate any fetal testing if either parent carries a pseudodeficient mutation (and therefore is not at risk for any neurologically significant disorder in their offspring). Currently, in its initial stages, laboratory charges for DNA mutation analysis range from approximately \$150 to \$200 per person tested. However, with anticipated developments in technology and automation, it is likely that these costs can be reduced.

Further mutations in the HEX A gene (as well as additional pseudodeficiency alterations) are expected to be discovered in the future. For this and the aforementioned reasons, initial screening should be at the gene product level (ie, enzyme, protein, mRNA, etc). This approach should identify the vast majority of all mutations with few false-negatives and avoid the problems of the exquisite specificity and reduced sensitivity associated with DNA-based mutation-specific analyses. Optimally, both methods employed in sequence as described, result in the most efficient and comprehensive use of the technology.

Population Frequencies and Implications

Based on testing data derived from the experience of the California TSD Prevention Program since 1973, it is possible to calculate the relative carrier frequencies and predicted TSD incidence in both Jewish and non-Jewish populations (at least as represented by those in California). Since this program is one of the few in the world where major activities in outreach, community-based testings are still maintained, there is a sizable experience with non-Jewish persons who elect to be tested. Primarily, this is accomplished through annual testings on approximately 20 college and university campuses throughout the state. Testing is available to anyone who wishes this information, and although directed primarily toward Jewish students, many non-Jews choose to be screened. In addition, through completed self-administered questionnaires on each person tested, all individuals with family members known to have TSD or with blood relatives who have been found to be carriers can be excluded from such calculations. If included, such individuals would skew the carrier rates and disease incidence figures significantly.

With more than 91 000 Jewish and 81 000 non-Jewish persons tested, apparent carrier rates (based on serum and/or leukocyte HEX A levels) of one in 30 (0.033) among Jews and one in 167 (0.006) among non-Jews are observed (Table 7). Importantly, as described in the preceding section, molecular studies indicate that some of these individuals are heterozygotes for later-onset disorders, and even more critically, others carry pseudodeficient mutations. When corrections are made for the observed pseudodeficiency allele frequencies in each population, and when reasonable estimates are applied for mutations causing noninfantile diseases, substantial changes in the apparent frequencies result. While the adjusted carrier rate for Jewish individuals becomes one in 31, the frequency of disease-related carriers among non-Jews becomes one in 277. These adjusted carrier rates predict incidences of infantile TSD in these populations that are in close agreement with observed incidence data.

The impact of TSD carrier detection and the provision for selective reproduction in at-risk couples has led to questions as to the possible influence such efforts might have on the gene frequency for TSD mutations in the Jewish and general populations. Wouldn't the additional births from carrier-carrier couples of offspring unaffected by TSD (each of whom would have a two-thirds risk of being a carrier) dramatically increase the disease gene frequency? In fact, such a program does not increase the gene frequency at all. Since the vast majority of autosomal recessive traits are passed from one generation to the next through carrier-noncarrier matings, it is clear that the impact on overall gene frequency is minimal. Actually, the long-term effect of such a program is to reduce slightly the rate at which the gene would otherwise be expected to be lost from the population.[72]

Why Is TSD in the Jewish Population?

The relatively high frequencies of three different mutations associated with HEX A α-subunit deficiency in the Ashkenazi Jewish population and the persistence of the same and additional α-subunit alterations in other diverse non-Jewish populations raises the question as to the genetic rationale for the retention or maintenance of these deleterious or lethal alleles in these populations. Two major arguments have been proposed (which, in fact, may not be mutually exclusive). Some contend that founder effect (the chance occurrence of a carrier being one of a small ancestral group of a rapidly expanding population) and genetic drift (random fluctuations in gene frequencies in small populations) could account for this observation.[73,74] Alternatively, others argue that some selective environmental factor may confer (or has conferred) a biologic advantage (increased fitness) on heterozygotes carrying these mutations.[75] The suggestion has been made that relative

Table 7.—Carrier Frequencies of Hexosaminidase-A α-Subunit Mutations in Jewish and Non-Jewish Populations*

	Jewish	Non-Jewish
Total tested	91 217	81 846
Carrier rate		
Observed	0.033 (1/30)	0.006 (1/167)
Corrected†	0.032 (1/31)	0.0036 (1/277)
Predicted TSD birth incidence‡	0.0002 (1/4100)	0.000003 (1/340 000)

*California Tay-Sachs Disease (TSD) Prevention Program data only.
†Corrected for pseudodeficiency allele frequencies.
‡Noninfantile mutations: 5% in Jewish, 10% in non-Jewish (estimates).

resistance to certain pulmonary diseases, particularly tuberculosis, might have been the selective force, which could have been operative for many centuries in Eastern and Central Europe where the Ashkenazi Jews lived, often under extremely demanding environmental conditions.[6,76]

The observed increase in incidence of three other genetically distinct lysosomal lipid storage disorders, Gaucher disease, Niemann-Pick disease, and mucolipidosis type IV, in this same population (possibly resulting in similar benefits to heterozygotes for these autosomal recessive mutations) lends weight to this hypothesis.[77,78] It is intriguing to speculate that some subtle (as yet undetermined) alteration in cell membrane sphingolipid composition or distribution, the result of diminished lysosomal hydrolase activity in the heterozygote, could affect cellular responses and/or other host defense mechanisms that could in turn lead to reduced susceptibility to pathogens like mycobacterium tuberculosis. Further research is needed to explore this hypothesis.

CONCLUSIONS

This article reports the international experience with TSD heterozygote screening and prenatal diagnosis from 1970 to 1993. It represents a prototypic effort in the coordination of adult public education, voluntary carrier testing, and comprehensive genetic counseling directed to the prospective prevention of an untreatable and uniformly fatal childhood disease. Clearly, the data provided indicate that such an effort, carefully organized, can have a dramatic impact on disease incidence. The principles of organization and delivery of this program can serve (and already have done so) as a model for similar efforts directed to different subpopulations for the control of other serious recessively inherited disorders.

It should be emphasized, however, that programs such as this, which achieve reduction of disease incidence by the prevention of births of affected infants through alternative reproductive mechanisms or by elective abortion of affected fetuses, represent a less-than-ideal method of disease control. Assuredly, a simple, relatively inexpensive, and highly effective therapy (or even a "cure") would provide, at least ethically, a more appealing intervention. For this reason, prevention programs like the TSD effort should be seen as interim approaches and should not curtail continued research directed toward the effective therapy or cure of such disorders.

Having stated that, a note of realism is also necessary. Numerous approaches to the effective treatment of TSD and other GM_2 storage disorders, including purified enzyme replacement, cellular infusions, and even bone marrow transplantation, have proven uniformly unsuccessful in patients with these conditions.[1] As the disease process begins in early fetal life, it may be necessary to initiate therapies in utero if they are to be effective. The blood-brain barrier, once matured, seems a formidable blockade to both enzymatic and/or cellular replacement. Even if purified targeted enzyme or enzymatically normal cellular elements could be introduced in the central nervous system, are neurons (the primary target cells of the disease process) capable of receptor-mediated endocytosis? Will the affected individual's immune system "reject" the introduced or newly produced enzyme because it might be seen as a foreign antigen?

Clearly, major future breakthroughs will be necessary before realistic prospects for treatments or cures of these disorders are at hand. Accordingly, until such achievements are accomplished, carrier screening and genetic counseling (with the option of prenatal diagnosis) are likely to continue for the foreseeable future as mainstays in the preventive management of these disorders.

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