ORIGINAL PAPER

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Population-based genetic screening for reproductive counseling: the Tay-Sachs disease model

Abstract Since 1970, more than 1.4 million individuals worldwide have been screened voluntarily to determine if they are carriers of the mutant gene for Tay-Sachs Disease (TSD). Employing both enzymatic and molecular methods (for optimal sensitivity and specificity) more than 1400 couples have been identified to be at-risk for TSD in their offspring, i.e., both parents heterozygotes. Through prenatal testing of more than 3200 pregnancies, births of over 600 infants with this uniformly fatal neurodegenerative disease have been prevented. In the United States and Canada, the incidence of TSD in the Jewish population has been reduced by more than 90%. More that 100 mutations in the hexosaminidase A gene (the TSD locus) have been identified to date. Some are associated with later onset or more chronic forms of neuronal storage disease. Two mutations cause a carrier-like "pseudo-deficiency" when enzymatic testing is used (false positives). A number of practical, social, and ethical complexities have been identified in this prototypic population-based effort. Educational and counseling components must be provided both before and after screening. Issues of privacy and confidentiality of test results must be addressed. In certain cultures insurability and employment may be involved. The public perception of the biomedical community as advocates for wide-scale testing and screening may be interpreted, in some systems, as conflicts of interest on the part of entrepreneurial scientists, clinicians, and institutions.

Conclusion Many new opportunities for population-based screening will be evident in this era of genome-related discovery. Accordingly, some of the experiences with Tay-Sachs disease prevention may be instructive.

Key words Carrier testing · Mutation · Reproductive counseling · Screening · Tay-Sachs disease

Abbreviations *HEX A* β-hexosaminidase A · *TSD* Tay-Sachs disease

Introduction

Genome-related discoveries are occurring at an explosive pace and have dramatic implications both for the practice of medicine and for applications to public health. Genetic screening programs directed to specific populations (e.g., newborns, pregnant women, members of specific ethnic, racial or aged-related groups) already have provided, and will continue to provide, important opportunities for the application of newly acquired knowledge to the prevention and/or treatment of an ever-increasing number of human ailments. Population-based genetic screening could be employed for the

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pre-symptomatic detection of treatable disease (e.g. hemachromatosis in adults or MCAD deficiency in neonates), for the identification of predisposition to heritable disease (e.g. increased risk for breast/ovarian cancer in individuals of certain families or populations), or for the purpose of reproductive counseling where an at-risk status for affected offspring can be determined in healthy individuals prior to the birth of any afflicted children (e.g., heterozygote screening of reproductive-aged individuals in defined populations). In this last instance, through genetic counseling, and with the options of prenatal diagnosis and selective abortion, serious untreatable disease can be "avoided" and unaffected offspring attained selectively, even by identified at-risk families. Such a preventative approach has been considered for a wide range of disorders, from Tay-Sachs disease (TSD) and Canavan disease in Ashkenazi Jewish populations, to sickle cell anemia in American blacks, to β-thalassemia in mediterraneans and asians, to cystic fibrosis in caucasians, to aspartylglucosaminuria in Finnish couples, as well as others [1]. It is this last type of screening, i.e. for reproductive counseling, which is the focus of this report. In particular, the experience with TSD prevention, the prototype of this genetic screening approach will be examined.

Methods

The program for community-based education, carrier screening, and genetic counseling in the prevention of TSD was the first such effort of this type [4]. Initiated in 1970, shortly after the underlying defect in TSD, deficient activity of the lysosomal isoenzyme β-hexosaminidase A (HEX A), was defined [13, 16], wide-scale voluntary carrier screening directed to reproductive-aged individuals of European Jewish ancestry was initiated in the United States and Canada and subsequently developed in many countries throughout the world [6, 7]. The details of planning, organization, and delivery of this program have been reported previously [5, 6]. Through a voluntary data collection network (and a laboratory proficiency – quality control program as well), an annual review of this worldwide experience is achieved and testing accuracy maintained. Currently, a total of 75 testing sites and 43 laboratories where HEX A determinations or TSD mutation analysis is carried out, participate in this effort. The most recent survey provides data through June 30, 1999, and provides the basis of this report.

Results

Over the past 30 years, more than 1.4 million individuals have been screened voluntarily for TSD carrier status throughout the world (Table 1). The vast majority of these persons have been Jewish individuals of child-bearing age (> 18 years), although anyone of age volunteering for the screening test is offered it, regardless of religious background. In some programs, screening is offered to premarital aged persons (with parental permission) [2, 10]. About 51,000 carriers have been identified (either by reduced HEX A activity in serum and/or leukocytes or by direct mutation analysis), and most critically, nearly 1400 couples have been identified to be

Table 1 TSD heterozygote screening 1971–1999

Country	Tested (n)	Carriers (n)	At-risk couples (n)
United States	960,815	36,750	803
Israel	334,500	7,803	400
Canada	68,188	3,431	63
South Africa	15,651	1,603	52
Europe	18,440	1,173	37
South America	1,766	103	20
Australia	4,187	123	4
Total	1,403,547	50,986	1,379

at-risk for TSD in their children, i.e., both partners heterozygotes. None of these couples have had prior affected offspring. As illustrated in Table 2, over 3200 pregnancies have been monitored by amniocentesis or chorionic villus sampling in at-risk families, 628 fetuses with TSD diagnosed, and all but 19 aborted electively. Most importantly, more than 2550 children unaffected by TSD have been born to these families, in striking contrast to reproductive outcomes in years prior to carrier screening and prenatal diagnosis programs [12]. Since 1970, the incidence of TSD in the Jewish populations of the United States and Canada has been reduced by more than 90% [7]. In some ultra-orthodox Jewish communities, where abortion is proscribed, screening is conducted in late childhood and rabbinical approval of (by chance) proposed carrier/carrier marriages is withheld [2, 9]. In this way, disease is avoided through testing-based "mate selection". Although effective in these autocratic communities, extension of this approach to other social structures would seem highly problematic.

Mutation analysis in Tay-Sachs disease

With the positional cloning of the HEX A α-subunit gene in 1985, molecular characterization of mutations at this chromosome 15q.23 locus became possible [11]. Not only have numerous and diverse mutations been characterized associated with infantile TSD, but gene alterations resulting in dramatically reduced but not totally deficient enzymatic activity, and thereby later onset of disease, also have been identified [14, 15]. More than 100

Table 2 Prenatal diagnosis of TSD: worldwide experience 1969–1999

	Couples identified at-risk by:	
	Prior offspring (n)	Carrier screening (n)
Pregnancies monitored	1,447	1,814
Affected fetuses	350	278
Elective abortions	332[a]	277[b]
TSD fetuses missed	2	1
Unaffected offspring born	1,071	1,484

[a] 18 infants affected with infantile TSD as predicted
[b] 1 infant affected with adult onset TSD as predicted

mutations now have been characterized in the α-subunit gene of HEX A [8]. As shown in Table 3, in the Jewish population, three mutations account for 93% of all enzymatically identified carriers: 1278_1279insTATC (relative frequency 77%) and IVS12 + 1g > c (12%) are both associated with infantile TSD, whilst c.805G > A (G269S; 4%) is associated with adult onset TSD. Among non-Jewish enzymatic carriers, 13% have a IVS9 + 1g > A mutation, 9% the 1278insTATC insertion, and 4% the same late onset (adult) alteration noted above in Jews.

Most importantly, 43% of enzymatically-defined carriers amongst non-Jews and about 3% of Jewish enzymatic carriers have a "pseudo-deficiency mutation". Such an alteration negates enzymatic activity with synthetic substrates (routinely used for TSD carrier identification) but does not interfere with cleavage of the natural substrate GM_2 ganglioside, thereby making the mutation of no biologic consequence. Two such mutations have been characterized: c.739C > T (R247W), and a neighboring c.745C > T (R249W) [3, 18]. Pseudo-deficiency states have been reported with numerous enzymatic systems where synthetic substrates are employed to assay catalytic activities [17]. Similar examples of benign mutations (polymorphisms) should be anticipated as other systems are evaluated in the future. Clearly, the capability to have both mutation detection data and functional assessment of the gene product provide the most optimal information for accurate interpretations to be made.

Using molecular methods, which detect the six most common mutations (including the two known pseudo-deficiency alterations), about 35% of non-Jewish enzyme-defined carriers and 4% of Jewish carriers (by enzyme) reveal no identifiable mutation. This decrease in sensitivity with DNA testing and the false-positives with enzymatic analyses (pseudo-deficiencies), underscore the rationale for the recommended policy of using both methods in sequence, enzymatic screening initially, followed by DNA-based mutation testing in all those found to have carrier range HEX A activities. This greatly improves genetic counseling (i.e. particularly for couples found to be at-risk for late-onset disease) and avoids unnecessary procedures and anxieties in couples who enzymatically appear to be, but who are not, at-risk for neurologically significant disease in their children (i.e. one or both parents carrier of pseudo-deficient mutations).

Discussion

In addition to the organizational, technical, and practical considerations, which underlie this three decade experience, a number of fundamental issues have surfaced which have obvious implications for genetic screening programs of this kind. Clearly, the capacity of a genetic test result to accurately predict reproductive risk (or lack thereof) for a specific phenotype is paramount. The increasing diversity of mutations at any given locus and the necessity to appreciate polymorphisms (and/or "leaky" mutations) with their impact on alterations in phenotype, are critical issues with molecular testing. There are limitations with biochemical methods as well, as seen with the pseudo-deficiencies. Such concerns point to the critical need for "comprehensive science" upon which to build such programs. Variations, both in the nature and frequency of mutations between diverse sub-populations must be appreciated. This can result in considerable variation in sensitivities and predictive values where only mutation-based tests are employed. Further, the burgeoning problem of needs for retesting may surface, as future allelic mutations are identified (which were not examined at initial testing opportunities).

It is generally agreed that reproduction-related genetic screening of this type should be provided only on a voluntary basis, rather than be mandatory. Few would disagree that from an ethical perspective, mandatory carrier screening could not be justified. But there are attendant issues with voluntary programs which introduce their own complexities. The critical need for effective educational materials, appropriate and perhaps specific for targeted sub-populations, and the optimal

Table 3 Distribution of HEX A α-subunit mutations in Jewish and non-Jewish unrelated heterozygotes

Mutation	Phenotype	Jewish		Non-Jewish	
		Obligate (n = 87)	Screening[a] (n = 432)	Obligate (n = 54)	Screening[a] (n = 270)
+TATC 1278	Infantile	82% (71)	77% (331)	17% (9)	9% (24)
+1 IVS 12	Infantile	11% (10)	12% (53)	0	0
+1 IVS 9	Infantile	0	0	17% (9)	13% (35)
Gly 269 → Ser	Adult	6% (5)	4% (16)	0	4% (10)
Arg 247 → Trp	Benign	0	2% (11)	0	40% (108)
Arg 249 → Trp	Benign	0	<1% (1)	0	3% (9)
None of above	Infantile	1% (1)	4% (18)	20% (11)	25% (67)
Other (various)	Infantile	0	<1% (2)	46% (25)	6% (17)
Total		100%	100%	100%	100%

[a] Enzymatically defined California TSD Prevention Program

mechanisms for delivering such information must be defined. The "level" of understanding required for a reasonably informed decision to occur (consent) is also a complex matter. Further, all efforts of this type require a comprehensive counseling capacity in order to meet the varying needs of participant individuals, perhaps both before and after testing. Considerations regarding as yet unconceived or unborn children and any given individual's future reproduction are abstract notions. The ability to understand such abstractions and probabilistic information (as well as to integrate them into critical decision making) is not given, but rather raises complex implications, both for the providers of such genetic health services as well as the targeted consumers. Much needs to be learned in the future to facilitate these processes.

Widespread attention has been drawn to issues of privacy and confidentiality with regard to genetic testing [1]. Questions have been raised regarding the implications of carrier identification and at-risk status both for individual insurability and employment. Such concerns are particularly relevant in a free market economy with private health and life insurance systems, but may be relevant to other systems as well. Major legislative and judicial efforts may be required to ensure individual protections in this context. Further, the pervasive development of bioscience in the market place has added new concerns to such endeavors. An increasing number of laboratory and health professionals are entrepreneurially involved in the development and delivery of such technologies and thereby are perceived as having a certain "conflict of interest" as advocates of wide-scale technical application. In capitalistic systems where revenues are generated for the institution, the academician, as well as the clinician through laboratory and service related efforts, advocacy roles can present an apparent dilemma for the health care provider.

Undoubtedly, population-based genetic screening directed toward reproductive counseling raises a broad range of considerations relevant to social, medical and ethical concerns. The anticipated complete delineation of the human genome should dramatically increase the potential applicability of such knowledge to population-based screening efforts. As such, the prior experience with programs such as that described here should provide important foundations for comparison and reflection.

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