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Preface
Genetic screening and counseling

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Guest Editors

The human genome has now been reliably and almost completely sequenced. In view of the huge investment required to reach this milestone, there are tremendous pressures to apply this information. Putting this knowledge to work in the field of medicine has the potential to immediately quell critics and show a direct benefit to mankind. With the unraveling of the human genome sequence, new challenges in genetic counseling have emerged for health care providers. For example, the burden to offer patients testing for genetic diseases can no longer be dismissed, interpretation of tests has become more complex, previously unrecognized ethical issues have emerged, and of course the cost of providing health care services has not diminished.

New demands placed on the obstetrician-gynecologist to provide genetic counseling caused us to devote the first two articles of this issue of *Obstetrics and Gynecology Clinics of North America* to genetic counseling. We hoped to offer some proof that genetic counseling is more than a casual conversation with a patient or couple. We start our discussion of genetic screening for specified diseases with a discussion of cost efficacy. Screening programs already in place for Tay-Sachs disease in the United States and β-thalassemia in Sardinia have proven effective; however, they target a very specific ethnic group. Canavan disease screening is relevant because it is straightforward and like Tay-Sachs and β-thalassemia targets a specific ethnic group. New standards for carrier screening of cystic fibrosis have recently been established through a combined effort of the American College of Obstetricians and Gynecologists, American College of Medical Genetics, and National Institutes of Health. Although directed at specific ethnic groups, these guidelines guarantee the obstetrician-gynecologist will need to have a minimal understanding of the clinical and genetic aspects of cystic

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fibrosis. Finally, we included discussions of breast cancer because of its immediate association with women's health care and tri-nucleotide repeat disorders (e.g., fragile X syndrome) because of their importance in the etiology of mental retardation and a spectrum of additional disorders affecting the nervous system.

We hope this issue of the *Obstetrics and Gynecology Clinics* will confer on all obstetrician-gynecologists the exciting promise that the "new genetics" offers for genetic screening and medicine in general.

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Obstet Gynecol Clin N Am
29 (2002) 255–263

OBSTETRICS AND GYNECOLOGY CLINICS of North America

Elements of a genetics counseling service

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The mission of a genetics counseling service is to provide education and facilitate health care of individuals who believe they are at risk for (or known to have) a genetic disease. These individuals might also believe (or know they have) an increased risk to pass on a genetic disease to their offspring. To provide this service properly the counseling team must address: 1) education related to specific disorders including natural history and treatment options; 2) availability of genetic testing when appropriate; 3) genetic risk assessment; 4) reproductive options; 5) psychosocial implications of information provided. This diversity creates the need for a team of healthcare professionals, many with special training in genetics. Primary personnel that compose the genetics service are the genetic counselors, physician clinical geneticists, genetics clinic nurses, and support staff (eg, receptionists, transcriptionists). With the explosion of discoveries in molecular biology and human genome sequencing, it has become common for genetics counseling services to focus on specialty areas that involve specialty trained healthcare professionals. For example, a prenatal genetics service might involve close collaborations among reproductive geneticists, maternal-fetal medicine specialists and sonographers. A cancer genetics service would interact more closely with oncologists, surgeons and internists. The specialty training of each team member allows that person to contribute in a unique way to the team's overall goal of providing comprehensive education and to facilitate patient care. Independent of the specific focus of a particular clinic, the primary personnel of the genetics counseling service remain the same.

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The genetics service

Genetic counselors

Physicians today are becoming increasingly dependent on the services provided by genetic counselors. Not all are aware of the training that these health care professionals undertake to achieve certification. The cornerstones of the genetics service, the genetic counselors are Master's degree trained health care professionals with specialized training in medical genetics and the psychosocial implications that the receipt of genetics information can impose on the patient. From 1982 through 1990 the American Board of Medical Genetics (ABMG) offered certifying examinations to genetic counselors. Since 1993 certification has been offered jointly by the American Board of Genetic Counseling (ABGC) and the ABMG. Recent data indicates that certification has been granted to 1,410 people [3]. Through a delineated education process, this Board requires genetic counselors to achieve specific competencies (Box 1). As physicians contemplate

Box 1. American Board of Genetic Counseling Required Competencies [1]

- Elicit and interpret individual, family, medical, developmental, and reproductive histories;
- Determine the mode of inheritance and risk of transmission of genetic conditions and birth defects;
- Discuss the inheritance, features, natural history, means of diagnosis, and management of these conditions;
- Identify, coordinate, interpret, and explain genetic laboratory tests and other diagnostic studies;
- Assess psychosocial factors, recognizing social, educational, and cultural issues;
- Evaluate the client's or family's responses to the condition or risk of recurrence and provide client-centered counseling and anticipatory guidance;
- Communicate information to their clients in an understandable manner;
- Facilitate informed decision making about testing, management, and reproductive alternatives;
- Identify and effectively utilize community resources that provide medical, educational, financial, and psychosocial support and advocacy; and
- Provide accurate written documentation of medical, genetic, and counseling information for families and health care professionals.

how to offer genetics counseling services within their own offices, it is important for them to understand the specific competencies of the genetic counselor. For board eligibility (now called Active Candidate Status) the counselor must have graduated from an ABGC-accredited Master's level genetic counseling training program. The oldest such program dates to 1971 when the first class of Master's degree genetic counselors graduated from Sarah Lawrence College in Bronxville, NY. There are currently 25 such programs in the United States and two in Canada. Certification requires passing a two-part examination that is held every three years. A genetic counselor unable to successfully pass both examinations within two consecutive examination cycles is no longer considered an Active Candidate. Certification is valid for a maximum of 10 years for genetic counselors who passed the board examination in 1996 or later. Those who successfully passed before 1996 have unlimited certification [1]. Licensure for genetic counselors is new and currently only required in California and Utah, although additional states are likely to mandate this in the near future.

The National Society of Genetic Counselors is the organization that represents genetic counselors. This society has a well defined vision: "to be the leading voice, authority and advocate for the genetic counseling profession" [2]. Furthermore their mission statement, "to promote the genetic counseling profession as a recognized and integral part of health care delivery, education, research and public policy" [2] makes it clear that genetic counselors must be the cornerstone of a genetics counseling service. This organization boasts about 1842 members and is an excellent resource for locating genetic counselors across the world [2].

Clinical geneticists (MD or DO)

Physicians with special training in clinical genetics represent another component of a genetics counseling service. The clinical geneticist can conduct physical examinations, make diagnoses of genetic disorders, manage the clinical care of these disorders, as well as contribute to the counseling and education of the patient and family. The clinical geneticist also serves as a resource person for other healthcare providers. They should be capable of providing more detailed basic science and medical knowledge on a case by case basis. Physicians interested in clinical genetics can attain certification by the ABMG. The graduate medical training requirements for certification in clinical genetics can follow any of three paths. The first requires a minimum of 24-months of training in an American College of Graduate Medical Education (ACGME) accredited genetics "residency program" after completing a minimum of 24 months of specialty training in one of several American Board of Medical Specialties recognized disciplines (eg, pediatrics, obstetrics and gynecology). The ABMG and another specialty (eg, American Board of Obstetrics and Gynecology [ABOG]) could subsequently certify one. A second option is the completion of 48 months of training in an ACGME accredited residency program dedicated to both genetics and other rotating clinical experiences. One would only be

certified by the ABMG. Third is the completion of a combined pediatrics and medical genetics residency over a 60 month period. The latter method of certification gives the trainee the opportunity to seek certification in pediatrics through the American Board of Pediatrics and clinical genetics (ABMG). Since the inception of board certification in the area of clinical genetics in 1982, there have been 1,006 certificates issued [3]. Of note, of all of those with ABMG certification (clinical or laboratory discipline) 112 have been certified by ABOG [4]. By contrast there are 33,026 active diplomats of the American Board of Obstetrics and Gynecology and 1,419 of these are certified in the subspecialty of maternal-fetal medicine [5].

After successful ABMG certification, physicians may seek membership as a Fellow of the American College of Medical Genetics (ACMG). This organization has a clear mission statement (Box 2) directed at its members and the public. The

Box 2. American College of Medical Genetics Mission Statement [6]

- Advance the art and science of medical genetics by maintaining high standards in education, practice and research.
- Increase access to medical genetic services and improve public health.
- Advocate for and represent geneticists and providers of clinical genetic services.
- Develop clinical practice guidelines.
- Develop laboratory services directories, databases, population screening guidelines and position papers.
- Establish uniform laboratory standards, quality assurance and proficiency testing.
- Promote effective and fair health policies and provide technical assistance to government agencies, professional organizations and other medical specialties.
- Sponsor educational programs for geneticists, other health care providers and the public, including the Annual Clinical Genetics Meeting.

ACMG provides education resources and a voice for the medical genetics profession. It also serves to make genetic services available to and improve the health of the public, through the implementation of methods to diagnose, treat and prevent genetic disease [6].

The training and focus of an ABMG certified clinical geneticist and an ABOG physician having special competency in maternal-fetal medicine overlap but are quite different. Both disciplines share a general interest in fetal development, the etiology of abnormalities of fetal development (eg, vascular, chromosomal, single

gene), and risks of recurrence. However, the geneticist who has seen a fetus with a specific birth defect has had the opportunity and responsibility to study the birth defect through childhood and into adulthood. By contrast, a thorough understanding of the consequences of birth defects beyond neonatal life is not part of the training of maternal fetal medicine sub-specialists. Maternal fetal medicine training in genetics is limited to prenatal genetics; the genetics of gynecologic cancer, reproductive endocrinology, and non-gynecologic organ systems (eg, musculoskeletal, nervous, and cardiac) receive far less attention if any. The clinical geneticist should know the gamut of human genetics including syndromes not ordinarily seen by obstetricians and gynecologists, population genetics, Bayesian analysis, metabolic diseases, cytogenetics, and polygenic inheritance (Box 3). Geneticists must possess an in-depth understanding of

Box 3. Areas Examined for ABMG Certification in Clinical Genetics* [3]

- **I. Basic Principles**
  - A. Genetic mechanisms
  - B. Pedigree analysis/risk assessment
  - C. Biochemical genetics
  - D. Cytogenetics
  - E. Molecular genetics
  - F. Screening
- **II. Clinical Diagnosis**
  - A. Metabolic disease
  - B. Dysmorphology/teratology
  - C. Cytogenetic disorders
  - D. Genetic disease recognition
  - E. Prenatal diagnosis
- **III. Patient Management**
  - A. Legal/ethical issues
  - B. Counseling
  - C. Anticipatory guidance
  - D. Treatment

* May not be all inclusive

molecular principles and terminology. They should understand recombination, tri-nucleotide repeats, genetic heterogeneity, compound heterozygosity, and genotype-phenotype correlation within the context of human disease. All of this added training allows the geneticist to offer a perspective to potential parents that is detailed and precise. The maternal-fetal medicine physician who is not a geneticist may have a greater appreciation of the physiologic perturbations that

underlie abnormal fetal development. Properly trained maternal-fetal medicine physicians are experts in the use of ultrasound as a tool to diagnose the fetus. The maternal-fetal medicine physician can provide a differential diagnosis that includes more common non-genetic entities and is quite excellent at relaying prognostic information and recurrence risk information to their patients.

Genetics nurses

Three programs offer graduate level genetics training for nurses; there is no accreditation process and nurses who have not completed a master's degree through an ABGC accredited program are not eligible for ABGC certification. That is, nurses seeking ABGC certification must now follow the same course as outlined for genetic counselors, irrespective of prior degrees. However, The International Society of Nurses in Genetics (ISONG) [7] is currently restructuring the credentialing process for nurses interested and qualified to care for persons with medical genetics needs in order for them to receive appropriate recognition of their training and knowledge of genetics. One would expect genetic nurses to be most useful in disease specific areas (eg cancer, muscular dystrophy, cystic fibrosis).

Evolving credentialing processes

When compared to other disciplines in medicine, clinical genetics is fairly young. Furthermore, that genetic counseling has a future in almost every medical discipline cannot be debated. Rapid advances in genetics and recognition of the future of genetic counseling have lead to at least one recent challenge to the very young certification processes currently in place. Initially, the Institute for Clinical Evaluation (ICE), a non-profit organization established by the American Board of Internal Medicine (ABIM), had proposed special competencies, credentialing and certification for persons offering family cancer risk assessment and management. Sufficient overlap between the purview of this Institute and the already established ABGC and ABMG resulted in the withdrawal of this Institutes proposal (November 2001 meeting of the ABIM) [8]. However, other established boards and credentialing entities might attempt similar proposals. This example serves to underline the need to remain current on the training requirements and certification required of the members of a genetic counseling service.

Support staff

The efficiency and organization of any genetics counseling service is dependent on the available support staff. The size of this personnel pool will vary depending on clinic volume and specific needs. The support staff's primary responsibilities may include the routine office needs such as scheduling patients, verifying insurance information, assembling, filing, and retrieving patient charts. However, the support staff can also assist the genetic counselors and medical

geneticist by requesting medical records, retrieving journal articles, as well as obtaining case specific information. The support staff needs to be cognizant that many patients are distraught and anxious when calling for appointments. The patient's first impression of the genetics counseling service is set by the professionalism of the support staff.

Tools of the genetics counseling service

A genetics counseling service has at its disposable a number of tools that assist in conveyance of up to date, accurate information. These include books, videotapes, pamphlets, and the World Wide Web. Books can be helpful in providing case specific general medical information. Video tapes or slide shows can be used to reach a large audience requiring the same or similar general information (eg, advanced maternal age counseling). They can also be used to supplement or reinforce information already presented. Genetic counseling visual aids are helpful during counseling sessions to explain complex genetic information such as modes of inheritance, linkage analysis and other molecular testing methods in a diagrammatic fashion. Many genetics counseling services offer an assortment of easy to read pamphlets, which allow the patient to review information presented during the counseling session. Organizations like the National Organization for Rare Diseases (NORD) and the March of Dimes offer pamphlets that are easy to read, readily available, and have been proven effective. However, one of the most useful tools to a successful genetics counseling service is access to the World Wide Web and e-mail. The internet allows geneticists access to genetic laboratory databases, parent support groups, and educational resources (Table 1).

Laboratory services

A genetics counseling service is accustomed to working closely with multiple genetic testing laboratories, both research and clinical. Although there may be some laboratories with which the service works with more than others, there is not a single laboratory capable of performing all necessary genetic testing. In addition, due to constraints dictated by insurance carriers, it is unrealistic for a genetics counseling service to work exclusively with one or two laboratories. Geneticists and genetics laboratories therefore work to provide accurate and precise information to families. It is not uncommon for laboratories to contact the genetics counseling service when additional samples from the patient or other family members are needed in order to complete or more accurately interpret results.

When to refer to a genetics counseling service

Molecular information has already proven invaluable to many areas of healthcare. For example, we now know some of the genes that can impact a

Table 1
World Wide Web medical genetics resources[a]

Site	Web Address	Target Audience	Content
GeneTests	www.geneclinics.org	Health care providers	Directory (International):
GeneClinics	www.genetests.org	Consumers	Genetics laboratories
GeneReviews			Genetic clinics
			Prenatal diagnosis clinics
			Full text review articles
Online Mendelian Inheritance in Man (OMIM)	www3.ncbi.nlm.nih.gov/omim/	Health care providers	Catalog: Genetic diseases
European Directory of DNA Laboratories	www.eddnal.com	Health care providers	Directory (EU, Switzerland, Norway):
		Consumers	Genetics laboratories
Center for Disease Control Services	www.cdc.gov/genetics	Health care providers	Resource:
		Consumers	Internet links
Kansas University Medical Center	www.kumc.edu/gec/geneinfo.html	Health care providers	Resource:
		Consumers	Internet links (e.g. support groups)
March of Dimes	www.modimes.org	Health care providers	Resource:
		Consumers	Education
Genetic Alliance	www.geneticalliance.org	Consumers	Resource: Education
Reprotox	www.reprotox.org	Health care providers	Resource: Teratogen exposure
MUMS-National parent to parent network	www.netnet.net/mums	Consumers	Resource: Support groups

[a] Table is not an all inclusive listing of informative sites. Overlap of information among some sites does occur.

person's cholesterol level, some that predispose to Alzheimer disease, as well as some that cause cancer. In the future, the discovery of other genes will make it possible for physicians to predict which patients are at increased risk for common health problems and which patients are presymptomatic. Knowledge of a patient's genetic information has implications not only for the patient, but also for the patient's family members who may have similar genetic information. When is it appropriate for a healthcare provider to refer a patient to a Genetics Counseling Service? The answer to this question is not always straightforward. Any healthcare provider performing genetics counseling must be appropriately educated in order to provide accurate information and address the larger issues of how this will impact the patient, the patient's family, and the patient's reproductive history. Further, they must be prepared to address concerns related to

insurability and medical coverage. Thus some indications for referral to a prenatal genetics counseling service include patients with known or suspected ultrasound diagnoses, medical or family history that requires further investigation and patients with known or suspected genetic diagnoses. Under the umbrella of genetic counseling are the specific tasks of educating, providing the psychosocial needs of the patient, confirming patient diagnoses through further clinical examinations or laboratory testing, and facilitating health care administration for the patient. These enormous responsibilities are made more manageable by utilizing an appropriately staffed genetics counseling service.

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[8] Institute for Clinical Evaluation Web Site. http://www.icemed.org/. Accessed March 10, 2002.

Obstet Gynecol Clin N Am
29 (2002) 265–276

OBSTETRICS AND GYNECOLOGY CLINICS of North America

A general approach to genetic counseling

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Genetic counseling is the process whereby an individual or family is provided information about a real or possible genetic problem. In educating and counseling about genetics, the counselor must provide information that is understandable. This information should include basic information concerning the disease, its course, and severity. Importantly, this discussion should include disease diagnosis as well as the nature of available treatment or management options. Counseling sessions must cover the concepts of inheritance patterns, the tools available for refining risk, and their limitations.

Generally speaking, patients who seek genetic counseling are operating under one of two premises (Fig. 1): (1) a family member or fetus was diagnosed with a disorder for which they believe they are at increased risk or (2) their age, race, gender, or ethnicity places them at increased risk for disease. In the first circumstance, it is absolutely required that the counselor establish that a correct and accurate diagnosis was made in the proband (index family member with the disease). This involves taking a medical and family history, performing clinical examinations, and obtaining relevant medical records and laboratory test results. Once the correct diagnosis has been confirmed (which is not always possible), additional clinical information is provided to assist the patient in understanding the medical facts of the disease. We view the medical facts as the common platform from which the remainder of the counseling session proceeds. Often, patients have some understanding of the disease for which they have sought counseling and the medical facts are not disputed. Finally, the clinical nature of the disease is often easier for patients to understand than the genetic principles that follow. The counselor can use this phase to better understand the patient's general knowledge level and to realize how best to gear the remainder of the discussions. Once the counseling session moves beyond this phase it is rarely

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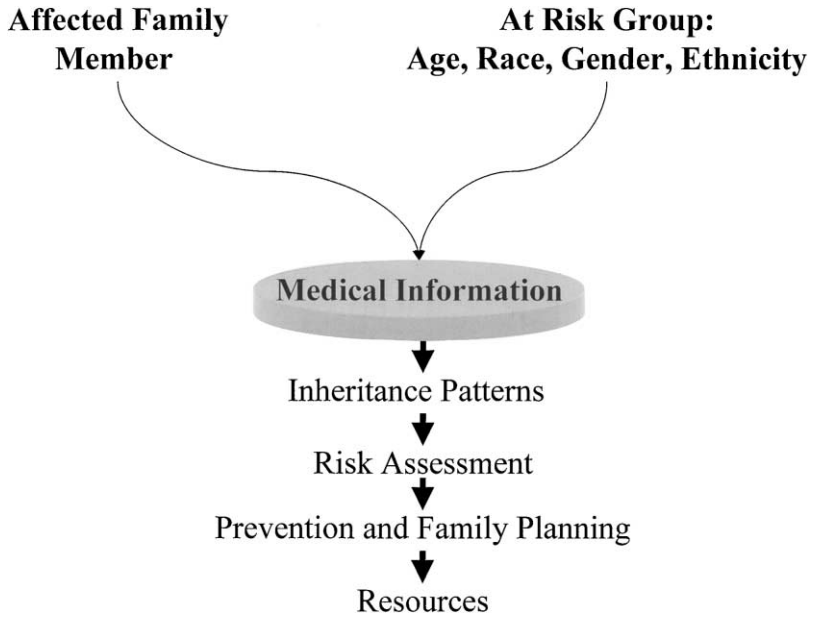

Fig. 1. Antecedents of a genetics consultation and the common platform from which counseling proceeds.

necessary to return. The patient has often learned a great deal by this time, and the counselor has a better understanding of the patient. When patients present after operating under the second proposed premise, there is no need to confirm a known or suspected diagnosis. Instead, the clinical condition discussed with the patient is determined by the patient's age, race, gender, ethnicity, or desire.

From this common platform forward, the general principles of counseling are independent of the specific condition being considered. The counselor must be able to provide information regarding the mode of inheritance, risks of recurrence, genetic and medical implications of the disorder, prevention, family planning, and additional information resources available. Because genetic counseling is directly concerned with human behavior, it must be based on an understanding of the psychological meanings of health and illness, procreation, and parenthood. Genetic information can bring bad news; thus, the counselor must be a resource for individuals and families dealing with sadness, loss, anger, guilt, or anxiety. The counselor has a responsibility to help individuals and families adjust psychologically and socially to their genetic condition.

Communication

At the heart of genetic counseling lies the necessity to educate patients about the genetic facts and issues relevant to their circumstances. One would assume

that the higher an individual's educational level, the better he/she should be able to understand complicated and unfamiliar biologic and medical concepts. For common genetic counseling situations (e.g., advanced maternal age), prepared literature packets can be provided prior to the counseling session so that the patient can become familiar with the information to be discussed during the counseling session. Such printed materials not only initiate the education process but also serve as a resource to which the patient may wish to refer at a later time. They also have the advantage of emphasizing that a patient's problem is not unique, further standardizing the informational content so that the counseling process is consistent. Patients may wish to share these materials with members of their family.

Nondirective counseling

Carl Rogers, a clinical psychologist, is credited with being the first to describe the psychotherapeutic approach of not advising, interpreting, or guiding clients as "nondirectiveness" [1]. In 1969, the World Health Organization Expert Committee on Genetic Counseling endorsed the nondirective approach to genetic counseling, an attitudinal strategy that has become universally accepted by virtually all professionals providing genetic counseling services. The counselor makes clear from the onset that the process is educational and that no decisions will be made for the patient. The counselor tries to remain impartial and objective in providing information that will allow patients to make their own rational decisions commensurate with their private concerns and desires. A variation on this theme is that some counselors will not comment on the decisions families suggest, whereas others will support any decisions families make. This is in contrast to the directive approach physicians typically take when dealing with patients with medical problems. For example, a physician who detects a breast lump in a patient will usually impart a strong recommendation as to what steps should or should not be taken.

Completely nondirective counseling is probably unrealistic. The tendency for counselors to interject their own biases by either verbal or nonverbal messages always exists. Indeed, to provide counseling implies that it is necessary. Despite conscious efforts to provide nondirective counseling, counselors may unwittingly give directive signals. For example, a simple gesture (nonverbal body language) such as shifting in one's chair or raising eyebrows may be interpreted as approval or disapproval of a decision. Repetition of certain points or presenting them in a louder voice may influence the way an individual weighs the information. Silence at certain crucial moments in a discussion may give the loudest message of all. These biases may reflect the attitudes of the genetic counselor about the nature and meaning of health and disease, the seriousness of the genetic condition in question, perception of quality of life, and the appropriateness of decisions related to genetic counseling and testing, acceptance or rejection of advice, and other important issues.

A second aspect of nondirective counseling is called "procedures aimed at promoting the autonomy and self directedness of the client." In contrast to not giving any advice, some believe genetic counselors can give advice and still remain nondirective. The trick lies in how the advice is given. By preceding advice with comments that make the client aware that the genetic counselor is just giving suggestions that may or may not be taken, the goal of nondirectiveness is still achieved [2].

Counseling providers

Genetic counseling cannot and should not be exclusively provided by a single specialist, but rather should be an interdisciplinary activity. There is also a growing recognition of the need to ensure that those providing genetic counseling are competent. Increasingly, primary care physicians, who may not be thoroughly trained in medical genetics, are providing genetic services. Although this has been viewed with skepticism by some, in straightforward situations the primary care physician may be the most appropriate person to provide the counseling because he or she knows the family, their personal attitudes, and socioeconomic background better than a consultant. In more complex situations, the primary care physician may lack the specific knowledge, time commitment, knowledge of necessary diagnostic tests, or skills needed to provide proper genetic counseling.

Anyone who holds themself out to the public as engaging in genetic counseling services must possess sufficient knowledge, training, and skill to provide these services in a reasonable manner. Practitioners must respect the limits of their individual competence, and avoid acting beyond the scope of their ability. A genetic team approach has been endorsed as consistent with the notions of competence and appropriately shared responsibilities.

Medical information

The medical information gathered for purposes of genetic counseling may be historical or come out of a recently performed physical exam or laboratory procedure. The mainstay of any counseling session is the ability to obtain accurate family information, which derives from collection of a thorough and accurate pedigree.

Medical history

Some obstetricians consider it useful to obtain genetic information through questionnaires or checklists, often constructed to require action only to positive responses. When similar forms were implemented in the past, they revealed that 21.4% of couples in a prenatal clinic showed at least one positive response, with 7.8% of the original sample requiring formal genetic counseling. Advanced maternal age was the most common indication [3]. For all pregnancies, it is stand-

ard practice to determine whether a couple or anyone in either of their families has a disorder that might prove heritable. The same holds true for gamete donors [4].

One should construct a pedigree through the inquiry of the health of first-degree relatives (siblings, parents, offspring), second-degree relatives (uncles, aunts, nephews, nieces, and grandparents), and third-degree relatives (first cousins). Record all abnormal reproductive outcomes such as repetitive spontaneous abortions, stillbirths, and anomalous live-born infants. Couples who have had two or more first trimester miscarriages have perhaps a 4–5% chance that one of them will be a carrier for a balanced chromosome translocation [5]. Chromosome analysis of the blood of both partners should be offered. If a history exists of a previous stillbirth or abnormal live-born child, it is imperative to obtain autopsy reports and chromosome analyses if available. Without a reliable diagnosis, giving a risk of recurrence is not possible.

Subsequent genetic counseling may be sufficiently complex to warrant referral to a geneticist, or it may prove sufficiently facile for the informed clinician to handle. If a birth defect is detected in a second- or third-degree relative, the likelihood of that anomaly occurring in a pregnancy rarely proves significantly increased. Identification of a second- or third-degree relative with an autosomal recessive trait will ordinarily place the couple at little increased risk for an affected offspring, the exception being if the couple is consanguineous or the condition is common, such as cystic fibrosis or sickle cell anemia. Nonetheless, one should inquire about the status of relatives as distant as first cousins (of the fetus) because identification of certain disorders in such relatives may be the only clue that the couple may be at increased risk for autosomal dominant disorders characterized by decreased penetrance or for X-linked recessive disorders.

In addition to identifying relatives with genetic disorders, one should record drug exposure to the woman and her partner. When discussing drug exposure, it is important to determine at what stage in the pregnancy the exposure occurred. If the exposure occurs in the first 2 weeks after conception, it is unlikely that the exposure will have an effect on the growing fetus. The explanation for this paradox is that organogenesis does not begin until approximately 2 weeks after conception or 4 weeks after the last menstrual period (4 gestational weeks). If the drug exposure occurs after the first 2 weeks of embryogenesis, there is a higher level of concern for the fetus. When searching for information on drug use during pregnancy, an excellent resource is the online drug database Reprotox. Subscribers are able to obtain summaries of published information on the use of specific drugs during pregnancy in minutes. Many states also have a toll-free teratogen hotline manned by health professionals including genetic counselors who are available to answer questions concerning exposure to medication during pregnancy.

Parental age is an important aspect to prenatal counseling and should be documented during the counseling session. It is well known that the most common indication for prenatal diagnosis is advanced maternal age, defined in the United States as 35 years or older at the time of delivery. Advanced maternal age warrants discussion independent of a patient's difficulties in achieving pregnancy or a physician's personal convictions regarding pregnancy termination.

Although offspring of fathers in their fifth or sixth decade have an increased risk of new dominant mutations, risk estimates on an individual basis are not possible.

Ethnicity is a significant aspect of any counseling session (Table 1). This information has the potential to alter the threshold for concern for diseases with higher prevalence in specific ethnic groups. For example, Ashkenazi Jews are at increased risk for offspring with Tay-Sachs disease, Gaucher disease, cystic fibrosis, inherited breast cancer, Canavan disease, and others. Ethnic specific disease often results from a high carrier frequency of recessive traits among groups closed to inter-ethnic marriage. Screening for Tay-Sachs disease and Canavan disease is recommended for all Ashkenazi Jewish couples, as well as couples in which only one partner is Jewish. Increasing availability of prenatal diagnostic techniques also makes advisable routine heterozygote screening for β-thalassemia in Mediterranean people and Chinese, sickle cell anemia in blacks, and α-thalassemia in Southeast Asians, Chinese, and Filipinos [6]. Screening for cystic fibrosis should be offered to Caucasians and Ashkenazi Jews and should be made available to individuals of other ethnic groups (Asians, Hispanics, and African-Americans) [7]. In addition to Tay-Sachs disease, Canavan disease, and cystic fibrosis, Ashkenazi Jews may wish to be screened for additional disorders: Familial dysautonomia, Gaucher disease (type 1), Niemann-Pick disease (type A), Fanconi anemia (type C), and Bloom syndrome. This reasoning is based on efficiency screening in that ethnic group, given that only a limited number of mutations are responsible for most mutations in a given disorder. Overall, 1 in 7 Ashkenazi is heterozygous for one of the above disorders. Many laboratories group testing for these diseases together, making it easier to obtain carrier screening for more than one condition.

Confirming the diagnosis

Good genetic advice requires certainty of diagnosis; even the best counseling cannot compensate for an inaccurate diagnosis. In addition to taking a detailed family history, the practitioner should carefully examine the proband, as well as

Table 1
Ethnic group and at-risk disease

Ethnicity	At-risk disease
Ashkenazi Jews	Tay-Sachs[a], Gaucher (type 1), cystic fibrosis[a], Canavan[a], familial dysautonomia, inherited breast cancer, Niemann-Pick (type A), Fanconi anemia (type C), Bloom syndrome
Northern European Caucasians	Cystic fibrosis[a]
African-Americans	Sickle cell[a]
Mediterraneans	β-Thalassemia[a]
Chinese	β-Thalassemia, α-Thalassemia[a]
Southeast Asians	α-Thalassemia[a]
Filipinos	α-Thalassemia[a]

[a] American College of Obstetricians and Gynecologists recommends routine screening.

other family members at risk. If the proband is no longer living, the appropriate medical records should be sought and reviewed. The possibility of nonpaternity must also be considered.

Laboratory studies needed to establish the diagnosis may include chromosome analysis, DNA studies, or biochemical tests of blood, urine, or cultured cells. Improved techniques now permit DNA analysis of archival specimens (e.g., paraffin-embedded tissue blocks) in some cases. Nongenetic factors can mimic genetic factors in the production of disease (phenocopies); a good history and various clinical and laboratory studies may help resolve questionable cases.

Despite the most intensive efforts, a precise diagnosis sometimes cannot be established. For some families, the counselors' answer "We do not know" leads to frustration and dissatisfaction with the counseling experience. In contrast, other families receive a measure of satisfaction and relief from the knowledge that all reasonable steps have been taken to answer their questions.

Laboratory testing

As laboratory genetic testing is made available for an increasing number of diseases, locating a laboratory to perform the test is becoming more complicated. An invaluable resource is *GeneTests*, a free online directory. *GeneTests* is supported by grants from the National Library of Medicine of the National Institutes of Health and the Maternal and Child Health Bureau of the Health Resources and Services Administration. *GeneTests* can be accessed by health care professionals via the Internet at www.genetests.org. One-time registration is required, and within 24 hours a user ID and password will be sent via e-mail.

The Medical Genetics Laboratory Directory at the *GeneTests* web site has a variety of search options, with the most commonly used being disease name. Other options include searching by laboratory director, laboratory, and gene name. If searching by disease name, one will be given a list of laboratories testing for the particular condition. Whether the test is done on a research or clinical basis will be noted along with the laboratory director, main contact phone number, e-mail, CLIA number (see below), and methodology. The cost of the test is not included in the *GeneTests* directory, making it necessary to call the individual laboratories to compare.

When choosing a laboratory, considerations include laboratory reputation, cost of testing, billing practices, and turnaround time. All laboratories in the United States that perform testing on human samples for the purpose of diagnosis, prevention, or treatment of disease are subject to the Clinical Laboratories Improvement Amendment of 1988 (CLIA). Commercial DNA laboratories must complete a certification process and receive a CLIA number. Laboratories outside the United States and those performing only research tests are not required to have CLIA certification [8]. Many laboratories have information packets and web sites containing test descriptions, price lists, billing information, and turnaround time

that are helpful in comparing different laboratories. Another important question practitioners may wish to ask is who will be available to interpret test results. Does the laboratory have a geneticist or genetic counselor available for queries?

For uncommon conditions, finding a laboratory to do a DNA test may be especially difficult. The first question is whether or not the gene for the particular condition has been discovered. If not, DNA analysis will naturally not be available, although sometimes knowledge of just the chromsomal region may permit linkage analysis. If the gene has been discovered but there is no listing in *GeneTests*, testing may or may not be available on a clinical basis. The discovery of the gene is only the first step in a long process and commercial testing may be delayed for several years. DNA tests continue to evolve in the initial years. After the initial discovery of a gene, researchers continue to elucidate such issues as detection rate (sensitivity), the number of different mutations causing the disease, affected versus unaffected range of certain mutations, and differences among ethnic groups. Research laboratories will often accept samples during this period and not charge for the analysis; however, their turnaround time is often highly variable, even to months. One way to proceed is to contact the researchers who discovered the gene directly and inquire whether clinical testing is available. If not, are they nonetheless willing to accept samples on a research basis? If it is agreed to send a sample, one should determine whether the ''research'' results could be reported to the referring physician and family. Sometimes, the results are not expected to be used in clinical situations and may not be reported. In any event, samples that are sent on a research basis require informed consent from patients or their family members. Remember to ask for a copy of consent forms before obtaining samples.

Risk estimates

Mendelian inheritance patterns, multifactorial disease estimates, gonadal mosaicism, and Bayesian analysis are available methods that allow counselors to refine disease or recurrence risk estimates. Implicit in each of these is a general knowledge of the inheritance pattern of the disease or disorder in question.

Mendelian inheritance

Concepts of Mendelian inheritance are applicable for single gene disorders. Applications of these simple methods for risk estimation are most often used when a couple has had a previously affected child and wishes to know their recurrence risk for a second child with the same disorder. For dominant conditions the risk is 50%, for recessive conditions the risk is 25% for any given offspring.

Multifactorial disease estimates

The mode of inheritance for traits that show continuous variation is termed polygenic or multifactorial. Examples of these traits include height, weight, and

blood pressure; however, the same mode of inheritance, polygenic/multifactorial, is appropriate to explain traits that demonstrate discontinuous variation. To conclude this mode of inheritance requires that the trait in question is not associated with anomalies in more than one organ system. Examples include most isolated cardiac defects, diaphragmatic hernia, congenital hip dislocation, omphalocoele, and posterior urethral valves. Population-based information allows for the calculation of genetic risk for diseases or birth defects (Table 2) that are not single gene in origin. For these polygenic entities, empiric risk estimates are used, usually 2–5% recurrence following the birth of one affected child to clinically normal parents.

Bayesian analysis

Bayesian analysis is most commonly applied to risk calculation for X-linked conditions (e.g., Duchenne muscular dystrophy). This analysis takes into consideration the reproductive history of the mother in establishing the risk to her future offspring. This consideration has led to the phrase "risk modified by reproductive history," which is synonymous with Bayesian analysis [9]. To illustrate, suppose a 30-year-old woman is referred for genetic counseling because of a previous child with Duchenne muscular dystrophy (DMD). She is 8 weeks pregnant, has two normal sons, and there is no other family history of DMD. What is her risk of being a carrier for DMD and of having another affected son? Approximately one third of isolated cases of DMD are caused by new gene mutations, whereas in the other two thirds the mother is heterozygous. The two normal sons must also be taken into account when determining risk using a Bayesian calculation (Table 3). The patient's risk of being a carrier for DMD is $\sim$33%; thus, her chance of having another son affected with DMD is $\sim$16.5% (33% $\times$ $^1/_2$ likelihood of transmitting the X chromosome with the mutation). When the patient desires prenatal testing in such circumstances, we recommend a search in *GeneTests* to find laboratories that offer DNA testing for

Table 2
Examples of multifactorial diseases and recurrence risks

Disease	Recurrence risk (%)[a]
Congenital heart disease	2–5%
Unilateral cleft lip	4%
Bilateral cleft lip	6.7%
Unilateral cleft lip and cleft palate	4.9%
Bilateral cleft lip and cleft palate	8%
Gastroschisis	<1%
Spina bifida	3% (without folic acid supplementation) 1% (with folic acid supplementation, first 8 weeks of gestation)
Club Foot	2–5%

[a] Assumes one affected sibling and nonsyndromic and nonchromosomal etiology of the index case.

Table 3
Example of calculations made in performing Bayesian analysis

	Patient is a carrier	Patient is not a carrier
Prior probability	2/3	1/3
Conditional probability	1/4[a]	1
Joint probability	2/12[b]	1/3
Posterior probability = 2/12 ÷ (2/12 + 1/3) = 1/3 or ~ 33%[c]		

[a] The conditional probability was determined by multiplying the 1/2 chance of having an unaffected boy if the patient is a carrier for each child that is unaffected.

[b] The joint probability was determined by multiplying the a priori and conditional probability.

[c] The posterior probability was determined by dividing the joint probability by the sum of the joint probabilities.

this condition. A phone call to the laboratory of choice might reveal that about 60–65% of men with DMD have a detectable deletion or duplication by current testing standards. Blood is thus drawn on the affected child and sent for analysis. Because of time restraints, the mother's blood is usually drawn at the same time. Suppose DNA mutation analysis reveals a deletion of exons 45–50 in the DMD gene of the affected son. Analysis of the mother's DMD genes might indicate that she does not have this deletion in her blood cells; however, the laboratory cautions that about 15% of women with no mutation in their blood cells will have germline mosaicism for the DMD mutation and, hence, still be at risk for another affected son. After reviewing the results of the DNA tests, your patient decides to pursue chorionic villus sampling. This reveals that she is having a female, which ordinarily cannot be affected. The mother declines further DNA studies, leaving for her daughter the decision of carrier testing when she is sufficiently old. Bayesian analysis can also be used to calculate recurrence risk for autosomally inherited diseases and those with variable penetrance and variable expressivity.

Patient perceptions and risk

The counselor must establish precisely what information the individual, couple, or family wants to learn about their genetic situation. People often have less interest in the label of a disorder and its mechanism of action than how to predict a disorder, its effect on physical and mental functioning, and how intrusive, difficult, or effective existing treatment or alternatives might be. One should always be mindful that patients generally remember the level of risk they were assigned during a counseling session. Their memory may be categorical rather than numerical (i.e., they may recall only whether they fall in a high- or low-risk group.) Different people perceive their genetic risks differently. The counselor should strive to help the family understand the consequences of the genetic problem. Factual information must be conveyed concerning its significance and natural history. Such explanations should be offered in small, discreet steps, with frequent pauses enabling patients to ask questions. This may require

several counseling sessions, given that individuals may not absorb all the requisite information at the initial meeting.

Perception of risk is also highly dependent on the individual's subjective experiences and expectations and is related to the manner in which they receive the information and their experiential, emotional, religious, and situational concerns. For example, a cleft lip may be perceived as a major tragedy by some parents, whereas other couples may readily accept a child with Down syndrome.

The manner in which risk figures are presented has been an important influence in how they are interpreted. Telling a 35-year-old woman that her risk of having a child with Down syndrome is one in 385 might be interpreted differently than telling her she has a four-fold higher risk as compared with a woman age 20. Regardless of the actual risk, patients often perceive risks as being "all or nothing," i.e., it will either happen or not happen. Although most counselors claim to use a nondirective approach, few deny that an element of counselor bias is always present. Risk figures might usefully be presented in several alternative ways; however, beyond near comprehension of numerical risks, genetic counseling must assist individuals in determining their own acceptable risk. Having said this, the counselor must always bear in mind that for the individual couple or family for which the feared event actually occurs, the risk is now 100%; the gamble was lost.

All the above implies that the more accurate information the couple has, the more likely they are to make a final decision that is consistent with their own values. As a matter of policy, this conclusion underlies the doctrine of informed consent in genetic counseling and its purpose: the promotion of self determination and rational decision making in situations that critically affect one's own life.

Summary

Genetic counseling can best be performed if a systematic approach is taken. The geneticist or genetic counselor has the difficult task of conveying complex information to patients in an understandable form. At the same time, this must be done in a nondirective manner, affording the patient the right to make his or her own decisions. Obtaining accurate family and medical history information is crucial to the genetic counseling process. Given the nature of genetic information, multiple sessions may be necessary to ensure that the patient understands the risks involved in his or her particular situation.

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OBSTETRICS AND GYNECOLOGY CLINICS of North America

Variables that underlie cost efficacy of prenatal screening

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Genetic screening is defined as a search in a population for persons possessing genotypes that put themselves or their offspring at risk for genetic disease [1]. Although, in the past genetic screening occurred following the diagnosis of a family member, diseases of large public health concern would not benefit from this form of screening alone [2]. Significant advances in genetic research and technology have permitted population-based carrier screening for a large number of recessive diseases. The benefits of the information generated by screening include reassurance, opportunity to prepare psychologically and medically, and the ability to pursue other reproductive options. However, there are significant costs that should also be taken into consideration when applying current genetic technology to clinical practice. These include the cost of pretest education and consent, genetic counseling of at-risk couples, the cost of sample collection and performing and interpreting the test, and prenatal diagnosis. In the following pages, we discuss variables that contribute to the cost-effectiveness of prenatal genetic screening.

Carrier frequency/test sensitivity

When implementing a genetic screening program, one must first define the population that will be offered screening. This is usually determined by the frequency and severity of the disease within a particular ethnic group. Such

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examples include Tay-Sachs disease screening in the Ashkenazi Jewish population, sickle cell screening among African-Americans, and cystic fibrosis screening within the northern European population. Knowledge about the carrier frequency of the diseased allele in a specified population is extremely important when assessing the usefulness of screening, as it is tightly associated with the sensitivity of the screening test. This can be demonstrated using Tay-Sachs disease (TSD) and cystic fibrosis (CF) as examples.

Without a positive family history, approximately 1 in 25 Ashkenazi Jews is a carrier of Tay-Sachs disease, whereas only 1 in 250 individuals in the general population is a carrier. Greater than 70 mutations in the hexosaminidase A gene (the enzyme responsible for Tay-Sachs disease) have been identified to date, with some mutations being more prevalent than others [3]. Three mutations account for >90% of heterozygotes in the Jewish population [4]. As a result, the detection rate among Ashkenazi Jews is approximately 98%, although it is only 60% in the non-Jewish population due to the increased prevalence of less common mutations [5]. A second example is cystic fibrosis. The risk of being a carrier for one of the cystic fibrosis mutations varies by ethnicity, with risks ranging from 1 in 25 for European-Americans, 1 in 46 for Hispanic-Americans, 1 in 60 for African-Americans, and 1 in 150 for Asian-Americans [6]. Greater than 900 mutations in the gene responsible for cystic fibrosis have been identified to date. The most common mutation among patients of northern European ancestry (ΔF508) accounts for 70% of mutant alleles in this population, but for only 48% in African-Americans, and 30% in Asian Americans. Therefore, since the allelic frequency varies by ethnic group, screening for a combination of mutations can improve the sensitivity to 90–95% in several populations [7], but the test sensitivity will remain low if used in populations with a low disease frequency and/or rare allele mutations.

Since there can be hundreds of distinct mutations for a single recessive disorder, it is not cost-effective to screen for all of them. The number of mutations included in a testing panel affect not only the detection rate, but also the cost of the screening. Therefore, an appropriate balance between increased sensitivity/specificity and decreased costs must be considered. It has been suggested that in order for the mutation to be included in a screening panel, it should have a frequency of at least 0.1% in the targeted population. For Ashkenazi Jewish and other Caucasians, 20 to 25 cystic fibrosis mutations would satisfy this criteria [8]. However, most panels for CF screen for only 5 to 10 of the most common mutations [9], resulting in a carrier detection rate of 85–90% for Caucasians [10]. Sensitivity is considerably less when applied to individuals of other ethnic backgrounds.

Pretest education

Once the population has been defined, it is crucial to know how accepting these individuals will be to genetic screening. Pretest education and voluntary

consent are imperative for the implementation of effective carrier screening programs. Unintentional coercion for genetic screening may result [11], since the level of knowledge amongst medical professionals surpasses that of their patients. This exemplifies the importance of community education to a successful screening program. Genetic counseling is an important component of screening programs, by increasing the understanding of screening and acceptance of information, as well as promoting informed choice. Most groups have found that patients could be adequately educated prior to screening through a combination of brochures, videotapes, and personal contact with ancillary personnel (either group or individual) [8,12,13]. The majority of women (70 to 85%) questioned by Mennie et al believed that they received enough prescreen information about CF carrier testing. Over 85% of patients in other studies reported an understanding of CF screening after reading an information leaflet and receiving a face-to-face explanation in the office, and felt that they had enough time and information to make a decision regarding testing. The majority of women (65 to 80%) retain this knowledge, even up to a year from testing [14,15].

In addition to patient education and understanding, participation in carrier screening has been affected by factors related to convenience, cost, views regarding pregnancy termination, concerns about the low sensitivity of the test, and concerns about confidentiality and insurability. Prenatal CF screening acceptance rates have ranged from 50 to 85% [7,16]. Several authors have reported the demographics and reasons women may choose not to be tested. Mennie et al found that women who declined carrier testing were more likely to be multiparous and were also more likely to decline AFP screening. A woman's opinion on pregnancy termination was the reason reported by most (54%) for declining screening. Less common reasons included a fear that the test would generate unacceptable levels of anxiety (15%), reluctance of the partner to participate (10%), perceived risk of having an affected child as low (7%) and the inability of the test to detect all carriers (6%) [16]. Similar reasons have been reported by others [13,15,17,18]. Major factors that led to a decision to be tested have included a desire to know one's carrier status, ease of testing, hope of reassurance, and the value of prenatal diagnosis [13]. Cuckle et al reported that 42% of women accepted screening to find out "if anything was wrong with the baby," [17] and both Levenkron et al and Loader et al determined that the single major reason for acceptance was a desire for reassurance that the risk of having a child with CF was low [15,19]. In contrast to women who declined testing, women who accept testing are more often nulliparous, well-educated, and more informed about the targeted disease. They also regard having an affected child as more serious, believe themselves to be more susceptible to having such a child, and are more likely to terminate a pregnancy if the fetus is affected [15,19,20].

An important issue is the concern regarding the potential psychological consequences of carrier screening. Although approximately half of women are reassured by screening, a quarter will experience anxiety related to the process. The majority of screened women, however, do not report increased levels of

psychological distress compared to the general population, nor do carriers display more pessimism toward their pregnancies [12–14,21], although some studies have indicated that carriers of genetic disease, although healthy, may consider their future health more negatively than prior to screening. As Marteau et al stated, "learning carrier status may have subtle, unintended effects on self-perception [22]."

An additional concern is whether disclosure of one's carrier status might affect one's family relationships, employment, or insurability. With regards to family, the majority of women identified as carriers do not report any change in family relations because of the genetic testing, and, of those that do, most acknowledge a beneficial effect for the family. Conflict only resulted in the presence of pre-existing poor family support [23]. Although most women have rejected the notion that they would feel stigmatized [13], one-third reported their concern that personal, genetic information may be learned by the "wrong people," in turn resulting in stigmatism and discrimination. A clear example of this was the great misuse of genetic information during population-based screening for sickle cell anemia in the 1970s, which led to widespread discrimination and stigmatization [24]. Screening programs today must take into account the social and racial differences that accompany the acquisition of genetic information [11].

Despite the decision to accept or decline testing, the majority of women have expressed satisfaction with their decision [13], and most believe that carrier screening should be routinely offered [17,25]. Even after 1 year, 83% to 97% of women believed that they had benefited from testing [14,15].

Performing the test

Two methods of screening have been proposed, sequential screening and couple screening. Sequential testing involves testing one member of the couple first (the woman) and testing the partner only if the first test is positive. Couple screening involves collecting specimens from both partners and issuing a single result, either positive or negative, for the couple [12]. Another method of couple screening would be to issue individual results simultaneously. There are several advantages and disadvantages that accompany each method. For instance, sequential screening is more time consuming and requires more staff time, including an increased need for genetic counseling for positive women [8,18]. One way to bypass this would be to provide genetic counseling only after the partner has been tested. Either method would result in increased knowledge of the disorder and its inheritance [21,26]. Couple screening, on the other hand, requires more laboratory time and organization than does sequential testing, and fails to provide individuals with the opportunity to inform family members of their carrier status when given as a single result. It may also lead to false reassurance about a negative result, as well as the false belief that retesting would not be required in future pregnancies with new partners [18,21]. Thus, couple screening with issuing individual test results would be preferable.

For CF, laboratory testing appears to be the largest contributor to cost, estimated at almost half, while patient education is responsible for approximately 20% of the overall costs [8]. The cost of the test is dependent on the number of mutations included in the panel [6,7,27]. This does have the potential to decrease, though, if assays were manufactured in larger quantities.

Reproductive options

Certainly, access to genetic testing enhances the ability of couples that are carriers of autosomal recessive diseases to make reproductive choices, including avoidance of mating or taking the 75% chance of having a healthy child. It allows them prenatally to consider options of gamete donation, preimplantation diagnosis, or adoption. Once a pregnancy is established, prenatal diagnosis offers the benefit of obtaining pertinent information regarding the status of the fetus at the cost of incurring the risk of an invasive procedure-related fetal loss. The risk of fetal loss that women are prepared to accept increases consistently as the risk to the fetus increases. Maternal age, parity, and education are other factors that also contribute to acceptance. Older women, women without children, and less educated women are more willing to risk fetal loss from invasive procedures than their counterparts [28]. The majority of high-risk couples (66%–93%), however, do opt for prenatal diagnosis [8,15,23,25,29,30]. It was assumed prior to implementation of screening programs that carrier couples would pursue prenatal diagnosis with subsequent pregnancy termination of an affected fetus [20]. These assumptions, however, do not always hold true, as 90% of participants in screening for TSD indicated consideration of prenatal diagnosis but only 60% of those participants would consider terminating an affected fetus [31]. The percentage of individuals who would consider termination of pregnancy if the fetus was found to be affected with CF has ranged in the literature from 32% to 88% [8,13,21,25,30], although this may not accurately represent the decisions made by couples faced with this very scenario. In fact, all couples with an affected fetus chose termination of pregnancy in a report by Brock [29]. Thus, some raised the concern that carrier screening and prenatal diagnosis would lead to unacceptable increases in the number of abortions and losses of unaffected fetuses. On the contrary, the use of prenatal diagnosis leads to an increase in the number of couples choosing further offspring, an increase in the births of healthy children and a decrease in the number of affected offspring [32]. In a setting without the availability of prenatal diagnosis, only 30% of parents of CF children choose further offspring. This number increases to 60% to 85% when DNA diagnosis is available [23,32].

Cost-effectiveness

Economic models have been derived to evaluate the costs and benefits of genetic screening programs, but, as noted previously, there are many variables

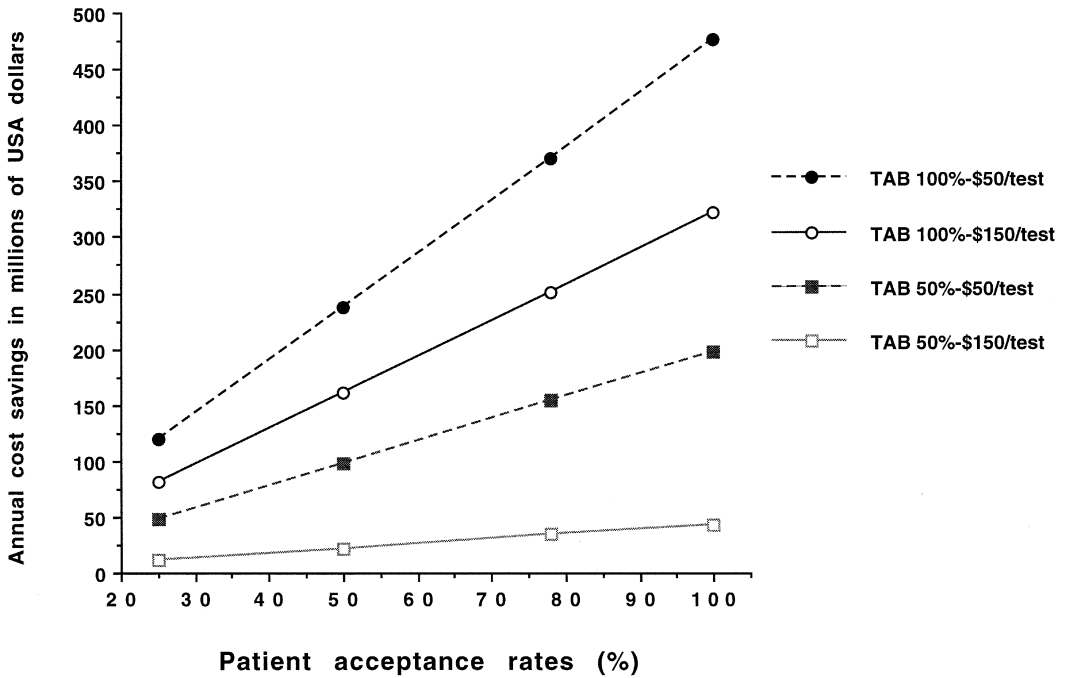

Fig. 1. Sensitivity analyses for white pregnant women using different costs per screening test and therapeutic abortion (TAB) rates. (*From* Vintzileos et al: A cost-effectiveness analysis of prenatal carrier screening for cystic fibrosis. Obstet Gynecol 91:529–34, copyright 1998; with permission.)

that need to be taken into consideration. Cost-effectiveness analysis has been utilized routinely in healthcare to determine the most effective use of public funding by assessing the value of different public health programs [33]. Unfortunately, it has been nearly impossible to agree upon the quantification of health care benefits. Cost-effectiveness analysis makes the assumption that a program may have value without defining monetary worth of benefits. For example, screening for TSD has allowed for informed reproductive choice and a net increase in the health of carrier couples by preventing an affected infant and subsequently preventing the psychological costs [34]. It has been estimated that the benefits of carrier screening for TSD are worth approximately $3.20 to $6.40 for each $1.00 spent, assuming a 100% termination rate of affected fetuses [33]. However, as variables (such as carrier frequency, test sensitivity, laboratory costs, and termination acceptance rates) are altered, the cost savings balance may change. In a strategy proposed by Wildhagen et al, couple screening for CF was found to be more costly than sequential screening. For sequential screening, testing for the most common mutation in the female and an expanded panel in her partner, costs would exceed savings if the carrier frequency is lower than 1:36 [35]. As the carrier frequency decreases, the cost savings balance worsens. Vintzileos et al. generated a cost benefit formula to determine the maximum allowable cost per CF sequential screening test for a program to be cost-effective [36]. The formula was used to estimate the net cost savings per prenatally diagnosed case of CF and assumed termination rates ranging from 50% to 100%. Other variables included in the model were the cost of screening, the number of women required to be screened to identify one case of fetal CF, the fertility rate (because subsequent pregnancies would not require retesting), the carrier frequency, the sensitivity of the mutational analysis, and the cost of the prenatal diagnosis package (either amniocentesis or CVS). For cost-effectiveness, they found that the cost (in 1997 US dollars) of each screening test should not exceed $189 for Caucasians or $135 for African Americans. In Asians and Hispanics for whom the test has a much lower sensitivity, it should not cost more than $22 and $72, respectively, in order for the screening program to be cost-effective. Higher screening costs can be absorbed once programs are well established [36]. The actual net benefit derived will depend not only on the ethnic/racial make-up of the population but also on patient acceptance rates of testing, prenatal diagnosis, and pregnancy termination rates (Fig. 1). In the model derived by Vintzileos et al, screening is associated with net losses for African-Americans, Asians, and Hispanics, but the net benefits per prenatally diagnosed cases among Caucasians are so large they would compensate for these losses. This would lead to overall net savings in the United States approaching $250 million annually. For couples planning more than one pregnancy, the cost-effectiveness of carrier screening improves, as the costs are distributed over more pregnancies per couple. Asch et al used a decision-analytic model to define economic outcomes from several CF screening strategies [9]. The lowest cost per CF birth avoided from society, patient, and health care payer perspectives, was achieved with sequential screening using an expanded panel of mutations for the partner and interruption of the

pregnancy if amniocentesis reveals an affected fetus. This cost is extremely sensitive to the proportion of couples that decide to terminate, and increases if the chance of non-paternity is high. For couples planning two pregnancies, the cost-effectiveness ratios are approximately half those of single pregnancy cases. In contrast, Lieu et al evaluated the cost effectiveness of routinely offering CF prenatal screening to women of European descent with no family history and found that testing did not result in net savings from the perspective of the health care payer because they erroneously assumed that CF testing is repeated with each subsequent pregnancy [27]. Assumptions made in their model were that 78% of women would choose to have the test and 80% of couples testing positive would proceed to prenatal diagnosis. Costs were found to increase as test sensitivity decreases, laboratory costs increase, test acceptance increases, and termination acceptance rates decrease. In addition, one-half of high-risk pregnancies would be missed because of late prenatal care, women who decline screening, and a test sensitivity that is not 100%, and only 12% of all potential CF births would be avoided. Non-medical costs and intangible benefits, however, were not taken into consideration; only direct medical costs were included. Cuckle et al, assuming all women have two pregnancies and that all carrier couples accept prenatal diagnosis for CF, found that the costs of screening are highly sensitive to the cost of the DNA test and the proportion of carriers it can detect [17].

Although studies such as these can give some insight as to whether carrier screening for a particular genetic disorder would be beneficial from economic and health care perspectives, a simple comparison between the costs of a screening program and that of medical care sought for these disorders is not entirely possible, as intangible costs and benefits would not be appropriately taken into account. These would include the emotional costs of abortion and the possibility of wrongful diagnosis with the subsequent termination of an otherwise healthy fetus [37]. Quantification of such intangibles has proven to be extremely difficult [38]. It is also difficult to quantify the value of the health and social benefits of screening programs. In order to determine the efficacy of large-scale screening for genetic disease, the total benefits must exceed the total costs of implementing such a program. Therefore, in order to determine if a screening program would be effective, both measurable and intangible costs and benefits must be considered.

Conclusion

In conclusion, population-based screening programs should incorporate appropriate education, counseling, and supportive services for individuals seeking such delicate genetic information [39]. The cost-efficacy of such programs must incorporate both the measurable and intangible costs and benefits associated with each individual screening program. As the era of genetic research and technology continues, the issues presented in this paper must be considered as we are entering the beginning of a great expansion in population-based screening for genetic disease in our country.

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Tay-Sachs disease
Screening and counseling families at risk for metabolic disease

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Goals

This article will discuss carrier screening and prenatal testing for Tay-Sachs disease. At the completion of the article the reader should be able to discern:

- Who should be offered carrier testing
- What testing methods to use
- How to interpret lab results
- How to counsel couples at risk

Overview

Disease

Tay-Sachs disease is an autosomal recessive, neurodegenerative disorder that results from excess storage of the cell membrane glycolipid, G_{M2} ganglioside, within the lysosomes of cells. Tay-Sachs disease is characterized by normal motor development in the first few months of life, followed by progressive weakness and loss of motor skills beginning around 2 to 6 months of age. This is followed by decreased social interaction and an increased startle response. Physical examination reveals hypotonia, hyperreflexia, and a cherry red spot on the fovea centralis of the macula. Individuals experience progressive neurodegeneration with macrocephaly (due to accumulation of storage material within the brain) and typically develop seizures, blindness, spasticity, and complete disability. Death from pneumonia usually occurs between 2 and 5 years of age.

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Tay-Sachs disease is uniformly fatal and, at present, there is no effective treatment. Prior to carrier screening programs, the disease incidence was $\sim$1/3600 in Ashkenazi Jews (carrier frequency of 1/30) and $\sim$1/360,000 in other populations (carrier frequency of 1/300).

History

Warren Tay was a British ophthalmologist who first described what is now known as Tay-Sachs disease. In 1881, Dr. Tay described a 1-year-old child with a cherry-red spot of the macula and named the disease "infantile amurotic idiocy [1]." In 1896 the American neurologist Bernard Sachs described the distended cytoplasm of neurons that is characteristic of Tay-Sachs disease. He noted that the disease was familial and also recognized the increased prevalence in the Ashkenazi (Central and Eastern European) Jewish population [2,3]. In the 1930s the German biochemist Ernst Klenk identified that the distention of the cytoplasm of neurons was due to accumulation of acidic glycosphingolipids that he named gangliosides [4]. In 1968, Robinson and Stirling extracted Hexosaminidase from human spleen and distinguished two isoforms by electrophoresis: Hex A (the acidic, heat-labile form) and Hex B (the basic, heat-stable form) [5]. In 1969, O'Brien demonstrated a deficiency of Hex A activity in Jewish individuals affected with Tay-Sachs disease [6]. In the early 1970s, large-scale enzyme-based screening for Tay-Sachs disease began in Jewish communities across North America. Similar programs were subsequently established in Israel and other countries with high numbers of Ashkenazi Jews [7]. The cloning of the HEXA gene in 1987 [8] led to identification of Tay-Sachs disease causing mutations in Ashkenazi Jews and other populations. Other groups with a high incidence of Tay-Sachs disease include the Pennsylvania Dutch [9], Cajun [10], and French Canadian populations [11]. Currently, carrier testing uses both enzyme assay and DNA mutation analysis.

Biochemical defect

Tay-Sachs disease results from a deficiency of the isoenzyme β-Hexosaminidase A (Hex A). The hexosaminidases are produced in the endoplasmic reticulum and are then transported to the lysosomes where they hydrolyze G_{M2} ganglioside, as well as other glycoproteins, glycosaminoglycans, and glycolipids, which are derived from cell membranes. There are three isoenzymes of β-Hexosaminidase called β-Hexosaminidase A (Hex A), β-Hexosaminidase B (Hex B), and β-Hexosaminidase S (Hex S). These are made from combining the proteins from two different genes, HEXA and HEXB. HEXA codes for the protein called the α-subunit, while HEXB codes for the protein called the β-subunit. These three isoenzymes of β-Hexosaminidase (Hex A, Hex B, and Hex S) are made by combining the α and β subunits. The isoenzyme Hex A is composed of one α and one β subunit (αβ). Hex B is made of two β subunits

(ββ), and Hex S is made of two α subunits (αα). In Tay-Sachs disease there is a deficiency of Hex A activity with normal or increased activity of Hex B. Hex B deficiency results in another G_{M2} gangliosidosis, Sandhoff disease. Rare cases of deficiency of a Hex A cofactor, known as activator protein, have been reported to result in a Tay-Sachs phenotype with normal Hex A and Hex B activities. Measurement of Hex A and Hex B activity in serum or leukocytes can be utilized to diagnose Tay-Sachs disease and identify carriers for the disease.

Genetic basis (DNA mutations)

The deficiency of Hex A (αβ) that results in Tay-Sachs disease is due to mutations in the HEXA gene that codes for the α subunit of the β-Hexosaminidases. Mutations in HEXA were first described in 1986 [11]; to date, 100 different mutations have been identified in the HEXA gene (http://data.mch.mcgill.ca/). In populations where the carrier frequency is high (Ashkenazi Jews, Pennsylvania Dutch, Cajuns in Southern Louisiana, and French Canadians in Eastern Quebec), there are a small number of common mutations. This results from an initial mutation in an individual that is subsequently passed to offspring (carriers). These carriers pass the mutation on to their children, and thus the mutant gene is spread through the population. For Tay-Sachs disease in the Ashkenazi Jewish population, it has been estimated that the initial mutation occurred after the second Diaspora of the Jews from Palestine (between 70 AD and 1100 AD) in central/eastern Europe [12]. It has been hypothesized that the increased incidence of Tay-Sachs carriers (and other lysosomal storage disorders such as Gaucher disease and Niemann-Pick) resulted from carriers being less susceptible to tuberculosis and other infections [13]. There are two relatively common mutations (polymorphisms) that decrease the activity of Hex A against the artificial substrate used in the enzyme assay, which do not affect the Hex A activity against G_{M2} ganglioside (and thus do not cause disease). The importance of this will be discussed in sections on enzyme assay and interpretation of testing data.

Ashkenazi Jews

There are two common mutations in HEXA associated with Tay-Sachs disease and one mutation associated with a late onset form of the disease. A four base-pair insertion into exon 11 of the HEXA gene (1278insTATC) accounts for 75% to 80% of all mutations in this population. A splice site mutation in intron 12 ($1421+1G \rightarrow C$) accounts for 15% of mutations. Three percent of carriers have a late onset allele (G269S) and 2% have a pseudodeficiency polymorphism (R247W). After screening those who are carriers (based on biochemical testing) for the six most common HEXA mutations (including the two pseudodeficiency alleles) 2% will have unidentified mutations. All types of mutations (deletions, insertions, point mutations) have been reported in all 14 exons and most introns of the HEXA gene [7,14]. Table 1 summarizes common mutations.

Table 1
Summary of the frequency of the six mutations commonly screened for by DNA mutation analysis [6].

	Disease causing alleles[a]				Pseudodeficiency alleles[b]	
Population	1278insTATC	1421 + 1G → C	G269S	1073 + 1G → A	R247W	R249W
Ashkenazi Jews	80%	15%	2%	–	2%	
Non-Jews	20%	–	5%	15%	32%	4%

[a] Incidence of mutation in carriers for Tay-Sachs disease (not including those who are pseudodeficient).

[b] Incidence of pseudodeficiency alleles in individuals whose Hex A enzyme analysis results are in the carrier range.

French Canadians, Eastern Quebec

Common mutations include a large (7.6 kb) deletion at the 5′ end of the gene, a splice site mutation in intron 7 (805 + 1G → A) and the common 4 base-pair insertion in exon 11 seen in Ashkenazi Jews (1278insTATC) [15,16].

Cajuns of Southern Louisiana

The intron 9 splice site mutation (1073 + 1G → A) and the 4 base-pair insertion in exon 11 (1278insTATC) are the most common alleles [10].

Pennsylvania Dutch

Most of the disease causing alleles are the intron 9 splice site mutation (1073 + 1G → A). The Pseudodeficiency allele (R247W) is also frequently seen [9].

Others

Of non-Jews with carrier range enzyme results, 36% will be found to have one of the two pseudodeficiency alleles (R247W, R249W). Of carriers (not including pseudodeficient individuals), 20% have the same insertional mutation in exon 11 (1278insTATC) seen in most Jewish carriers in carriers. A splice site mutation in intron 9 (1073 + 1G → A) accounts for 15% of mutations. This splice site mutation has not been reported in Jews and the common Jewish intron 12 splice site mutation (1421 + 1G → C) has not been found in non-Jews. About 40% to 50% of non-Jewish carriers will not have one of the six common mutations (including the two pseudodeficiency alleles) identified on routine DNA analysis [7,14]. Common mutations in other groups are listed in Table 1.

Screening programs

Screening programs to detect carriers of Tay-Sachs disease were begun in Ashkenazi Jews in North America in 1970. Beginning in the mid-1970s, the international Tay-Sachs Disease Testing, Quality Control and Data Collection Center was established in California through funding from the National Tay-Sachs Disease and Allied Disorders Association. The center monitors and certifies labs performing both enzyme and DNA testing for Tay-Sachs disease

and carrier status. Information is collected yearly from most labs around the world involved in testing [7]. As of 1998, over 900,000 individuals in the US have been screened and 35,000 carriers identified. Of those, there were 795 US couples at-risk to have a child with Tay-Sachs Disease [13]. Since 1983, when only two new cases of Tay-Sachs disease were diagnosed in North America, the annual number of newly diagnosed infants with Tay-Sachs disease in the Jewish population has remained at 3 to 5 cases per year. This reflects a 90% reduction in the incidence of Tay-Sachs disease in the Jewish population in the U.S. and Canada [7]. The carrier frequency of Tay-Sachs disease in the Jewish population has remained constant. This is due to the fact that screening programs do not select against carrier pregnancies but only target pregnancies affected with Tay-Sachs disease. Most Tay-Sachs carriers come from families where only one parent is a carrier (1/30 Ashkenazi Jewish families) rather than from families where both parents are carriers (1/900 Ashkenazi Jewish families). Therefore, even if carrier-carrier unions are discouraged (as in the Dor Yeshurim program) [7], there will not be any decrease in carrier frequency in the population.

Prior to population carrier testing, Jewish infants with Tay-Sachs disease accounted for 85% of newly diagnosed cases. At present, the incidence of Tay-Sachs disease is 3 to 4 times higher in the non-Jewish population, compared with North American Ashkenazi Jews [7]. The success of this program has led to recommendations from the American College of Medical Genetics and the American College of Obstetrics and Gynecology to recommend screening for all couples where one or both individual(s) are of Jewish descent [17].

Carrier screening is approached differently in ultraorthodox (Hassidic) Jewish groups, where there is a strict religious prohibition against pregnancy termination. In this screening program (Dor Yeshurim) anonymous testing is performed and the coded results are available only to the rabbis of the community, who must approve of all marriages. When a marriage is proposed the rabbi can decide whether a match should be made, using the carrier testing to prevent carrier matings [7]. This prevents carrier matings without stigmatizing a family [14].

Who to test

In accordance with guidelines from the American College of Obstetrics and Gynecology and the American College of Medical Genetics, carrier testing for Tay-Sachs disease should be offered to individuals whose ancestry is fully or partially Jewish [17]. As well, other groups with a high frequency of Tay-Sachs carriers should be offered testing, including Pennsylvania Dutch, Cajuns of Southern Louisiana, and French Canadians of Eastern Quebec. Because Tay-Sachs is an autosomal recessive disease, there do not have to be prior affected family members for an individual to be a carrier or for a couple to be at risk. Therefore, individuals should not be reassured by the absence of genetic disease in their family.

Ideally, testing should be performed when an individual or couple is considering starting a family and before they become pregnant. Testing before

becoming pregnant is for reasons of adequate counseling, ease of testing and for planning options for intervention in the event of an at-risk pregnancy. Counseling should be provided by an obstetrician, genetic counselor, or geneticist who has an understanding of methods of testing, can interpret test results, and convey the options for couples at-risk. Individuals offered testing should understand the following points:

- Individuals of Jewish ancestry have a 1/30 chance of being a carrier; if both partners are of Jewish ancestry their risk of both being carriers is 1/900 (1/30 × 1/30), even if there are no prior affected individuals in either family
- Testing using DNA analysis offers >99% certainty of carrier status in Ashkenazi Jews
- If both prospective parents are carriers, there is a 25% risk of having an affected child with each pregnancy.
- Options for intervention include testing of a pregnancy by chorionic villus sampling or amniocentesis, egg or sperm donor assisted reproduction, preimplantation diagnosis, and adoption

Carrier testing should be done prior to becoming pregnant, or as soon as an expectant couple comes to medical attention. This is to ensure that individuals can give full consideration to their options for testing and intervention. Testing of pregnant women using enzyme assay is also more complicated because pregnancy can alter serum levels of Hex A and therefore enzyme analysis of pregnant women must be done on leukocytes.

If only one member of a couple is of Ashkenazi Jewish descent, that person should be tested first. This is because the enzyme assay and DNA test are more accurate in Ashkenazi Jews. They have a much lower false positive carrier status secondary to pseudodeficiency alleles (2% in Ashkenazim versus 35% in non-Jews) and much higher DNA mutation detection rate (98% in Ashkenazim versus 40% in non-Jews) [7]. If the person of Jewish ancestry is established to be a carrier, the partner should be tested. If the Jewish partner is not a carrier there is no need to test the non-Jewish partner.

Carrier test results are typically available within 2 weeks of testing; testing of a pregnancy by amniocentesis or chorionic villus sampling typically takes 5 to 6 weeks for completion of studies.

Diagnosis and carrier testing

Enzyme assay

Enzyme assay for diagnosis of Tay-Sachs disease and carrier status is a fluorimetric study that measures activity of both Hex A and Hex B in either serum or leukocytes. Carriers of Tay-Sachs disease have decreased activity of

Hex A with normal or increased activity of Hex B. The reaction uses an artificial substrate (typically a 4-methylumbelliferyl glucosamine) that permits fluorimetric detection of the hydrolyzed product. The advantage of the Hex A and Hex B assay in serum is that the assay is simple and cheap. However, there are three limitations to the serum assay: (1) There is an indeterminate range where carriers and non-carriers may overlap, (2) The assay is unreliable in pregnant women and in women taking oral contraceptives, and (3) carriers of pseudodeficiency alleles cannot be distinguished from carriers of disease-causing mutations.

In published studies of Ashkenazi Jewish testing programs, it is estimated that the false positive rate of serum enzyme assay is 1.1% (excluding pseudodeficiency cases), giving a specificity of 98.9% [14]. Of those tested by enzyme assay, 10% were in the inconclusive range and further testing (either by enzyme assay on leukocyte/platelet or by DNA mutation analysis) was required. Of those in the inconclusive range 2% were found to be carriers by DNA mutation analysis; 98% had no pathogenic mutation detected [18]. Because of these limitations, abnormal or inconclusive results of enzyme assay should be clarified with enzyme assay on leukocytes and/or DNA mutation analysis.

Women who are pregnant or taking oral contraceptives may have elevated serum Hex A enzyme levels, that will result in an inability to detect that they are carriers (false negative results). If the individual to be tested is taking oral contraceptives, or is pregnant, the enzyme assay should be done on leukocytes, not on serum. If pregnant, the DNA mutation analysis should be sent at the same time as the enzyme assay, since expeditious identification of carrier status is important.

The use of an artificial substrate for assay of Hex A and Hex B enzyme activity confers simplicity and low cost. However, the artificial substrate causes false positive results in 2% of Ashkenazi Jews and 35% of non-Jews [9]. This is because of two common variations (polymorphisms) in the HEXA gene (R247W and R249W) that impair Hex A activity against the artificial substrate used in the enzyme assay but do not impair the ability of Hex A to hydrolyze the natural substrate, G_{M2} ganglioside. Thus, individuals whose enzyme assay indicates carrier status should have DNA mutation analysis for the two common pseudodeficiency alleles (R247W and R249W) [7,9]. Rarely, individuals will carry both a pseudodeficiency allele and a disease causing mutation. The enzyme assay results will be in the range of individuals affected with Tay-Sachs disease, though the individual will not have a Tay-Sachs disease phenotype. DNA mutation analysis for common mutations and pseudodeficiency alleles can clarify the enzyme results in these cases.

All individuals who are in the carrier or indeterminate range on enzyme analysis should have DNA testing to verify the results and in anticipation of prenatal testing. While enzyme analysis can be done on cultured amniocytes or chorionic villus cells, DNA mutation analysis is less prone to error and is the preferred method of prenatal diagnosis when one of the common mutations is identified. Enzyme analysis for prenatal diagnosis should be reserved for cases where the mutation is unidentified.

DNA diagnosis

DNA diagnosis for Tay-Sachs disease and carrier status involves testing for common mutations only. Clinical labs do not search for all (~ 100) mutations that have been reported to date. DNA is extracted from blood or cultured amniocytes/chorionic villus cells and the DNA is amplified by polymerase chain reaction (PCR). The entire HEXA gene is not amplified; rather the particular areas of the HEXA gene where common mutations occur are amplified and then used in testing. Most labs use some reliable and sensitive methodology, such as allele specific oligonucleotide hybridization (ASO, aka dot-blot) testing. These amplified DNA fragments are hybridized with oligonucleotides (short sequences of single stranded DNA synthesized to specifically match either the normal or mutant DNA sequence). The process of hybridization is where the labeled single stranded oligonucleotide joins with the complementary PCR product to form a double stranded piece of DNA. A radioactive detection method is typically used. Although more rapid methods may soon come into wide use, it is unlikely that there will be expansion of the number of mutations tested.

It is important to know which mutations the laboratory is testing for. Some labs test for only the three common disease-causing Ashkenazi Jewish mutations. These labs, while appropriate for testing Ashkenazim, are not appropriate for testing non-Jews. The clinician ordering the test should check with the lab to be sure that they test for the common mutations seen in a particular population. For example, if a non-Jew is being tested, the laboratory selected should test for the $1073+1G \rightarrow A$ mutation, which accounts for 15% of mutations in the non-Jewish population.

Sensitivity of testing for those of Ashkenazi Jewish descent is >98% when testing for the three most common alleles (1277insTATC, $1421+1G \rightarrow C$, and G269S) [14]. The carrier frequency in Ashkenazi Jews is 1/30; the DNA test false negative rate is $<2\%$ (2/100). Therefore, in Tay-Sachs carrier testing of Ashkenazim, one out of every 1500 ($1/30 \times 2/100$) carriers will be missed by DNA testing. Put the other way around, 1499/1500 Ashkenazi carriers will be correctly identified. Thus, 99.9% of Ashkenazi Jewish carriers of Tay-Sachs disease are identified by DNA mutation analysis for the three most common alleles.

Interpreting results

Testing of both Ashkenazi Jewish individuals and non-Jews for Tay-Sachs carrier status may utilize enzyme analysis, DNA diagnostics, or both studies in combination. Two percent of Ashkenazi Jews and 35% of non-Jews who have enzyme analysis results in the carrier range will carry pseudodeficiency alleles and not disease causing mutations. As well, DNA testing can confirm carrier status, and identification of the disease causing mutation can permit accurate prenatal diagnosis. Thus, all individuals found to be carriers or in the indetermi-

nate range by enzyme assay should have DNA mutation analysis. Those who have enzyme activity in the carrier range and have a pseudodeficiency allele (R247W or R249W) are not carriers for Tay-Sachs disease. Therefore, prenatal diagnosis is not indicated. Those found to have a disease causing mutation (1278insTATC, $1421+1G\rightarrow C$, G269S, $1073+1G\rightarrow A$) are carriers for Tay-Sachs disease. They should be counseled that they are carriers and offered prenatal testing by DNA analysis of cultured amniocytes/chorionic villus cells if the partner is also a carrier of a known mutation. Those who have carrier range serum enzyme results and no disease causing or pseudodeficiency mutation detected should have a leukocyte enzyme assay. If this is also indicative of carrier status, the individual should be counseled that he/she is a carrier and offered prenatal testing (by enzyme assay of cultured amniocytes/chorionic villus cells) if the other partner is also a carrier.

Summary

Carrier testing for Tay-Sachs disease should be offered to couples when at least one individual is of Ashkenazi Jewish (carrier frequency 1/30), Pennsylvania Dutch, Southern Louisiana Cajun, or Eastern Quebec French Canadian descent. Ideally, testing is done prior to conception. For Ashkenazi Jews, in whom DNA testing identifies 99.9% of carriers, DNA testing is the preferred method to ascertain carriers [14]. For non-Jewish individuals seeking carrier testing, enzyme assay should be done initially and positive or indeterminate results should be confirmed by DNA mutation analysis. If only one partner is descended from a high-risk group, that person should be tested first; only if he/she is a carrier should the other partner be tested. If the couple is pregnant at the time carrier testing is requested, both partners should have enzyme testing (leukocyte assay for the pregnant woman and serum assay for the father) and DNA testing sent concomitantly to expedite counseling and action. Carriers are individuals with a disease causing DNA mutation or carrier range enzyme analysis results on both serum and leukocytes with no detectable mutation and no pseudodeficiency alleles. Noncarriers are individuals with normal enzyme results or carrier range enzyme results and a pseudodeficiency allele on DNA mutation analysis. If both partners are found to be carriers they should be counseled of a 25% risk of having an affected child with each pregnancy. Options to modify this risk include prenatal diagnosis by amniocentesis or chorionic villus sampling, egg or sperm donation, preimplantation diagnosis or adoption.

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Obstet Gynecol Clin N Am
29 (2002) 297–304

OBSTETRICS AND GYNECOLOGY CLINICS of North America

Canavan disease
Prenatal diagnosis and genetic counseling

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Canavan disease, also known as spongy degeneration of the brain, is a severe neurodegenerative disease that leads to early death. The disease is caused by a deficiency of the enzyme aspartoacylase (ASPA) [1]. This leads to increased levels of N-acetylaspartic acid (NAA) in brain and the excretion of NAA in urine. Recent developments in carrier identification have led to two major findings: (1) that Canavan is not a rare disease among Ashkenazi Jews [2], and (2) molecular diagnosis of carriers among Ashkenazi Jews can be determined in about 98% of the cases. These two aspects of Canavan disease make prenatal diagnosis and the requisite genetics counseling an important consideration for obstetricians serving at risk populations.

Historical background

Clinical and pathologic features of Canavan disease were described (e.g., enlarged head and spongy degeneration of the brain) in 1931 [3]. In a 1928 report, a patient with Canavan disease was described, however, the diagnosis of Schilder's disease was made [4]. Disease criteria were better defined and an at risk population identified (ie, Ashkenazi Jewish) in 1949 [5]. That report for the first time recognized Canavan disease as a distinct genetic entity. The term *Canavan disease* has remained as the term for this condition in the American

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literature. Since the 1949 decription numerous cases have been reported, with the highest prevalence among Ashkenazi Jewish individuals [6].

Enzyme abnormality

Canavan disease results from a deficiency of a biochemical enzyme specific to the central nervous system, aspartoacylase [7]. Keeping in mind that under normal circumstances, the concentration of NAA in brain is second only to glutamate in the free amino acid pool [8]. This deficiency leads to accumulation of NAA in brain, and NAA in CSF, plasma, and urine. Oddly, the role of NAA in mammalian brain is unclear. There are suggestions that it provides acetate for myelin synthesis component or as an osmolite [9,10]. It may be that NAA is important in the maintenance of intact white matter. Thus, the pathogenesis of the hallmark pathologic feature, severe spongy degeneration of the brain, remains and enigma.

Molecular basis

Canavan disease is panethnic; however, it is most prevalent among Jews of Eastern European ancestry. Of 200 Canavan disease patients referred, two thirds were of Jewish extraction. Screening of healthy Ashkenazi Jewish individuals revealed that 1/37 to 1/58 was a carrier for Canavan disease [11–13]. This range is validated by the observed incidence for Canavan disease in this population, approximately 1 in 6000 to 13,000. This high incidence, and the accuracy of carrier testing using mutation analysis, make genetic screening desirable in this population.

Among non-Jewish patients the mutations are more diverse. This would diminish the utility of screening for these patients. Although faced with a suspicious family history, one should keep in mind that Canavan disease has been reported among Europeans, Middle-Easterners, Turks, Gypsys, African-Americans, and Japanese.

The gene for Canavan disease (ASPA) was cloned in 1993. It is has been mapped to chromosome 17 p-ter [14]. The gene is 20 kb and the cDNAs 6 exons codes for a 313 amino acid protein with a molecular mass of 36 kDa. The aspartoacylase gene is conserved among species. The coding sequence of the bovine and mouse cDNA show 92% and 86% identity with the human cDNA [15].

Mutations in ASPA can be determined for affected individuals and carriers. The most common mutations of Canavan disease are shown in Table 1. There are only two are common among Ashkenazi Jews [14]. These include a missense mutation in exon 6; 285Glu → Ala. This represents 86% of the diseased alleles among this population. The other is a nonsense mutation in exon 5; 231 Try → X (termination codon) found in 13.6% of the alleles tested. Thus, these mutations account for approximately 98% of all Jewish patients with Canavan disease [14].

A common mutation found in non-Jewish Canavan disease patients of European ancestry is Ala 305 → Glu, in exon 6. The incidence of this mutation ranges from 40% to 48% of non-Jewish European individuals. Thus far there have been more than 30 mutations among non-Jewish individuals identified [14,16–21].

Table 1
Aspartoacylase common mutations

Nucleotide change	Amino acid change	Residual enzyme activity (%)
Jewish		
854A → C	285 Glu → Ala	2.5
693 C → A	231 Tyr → X	0.0
Non-Jewish		
914 C → A	305 Ala → Glu	0.0

The diversity of mutations, and the lower carrier frequency of mutations in ASPA among non-Jewish populations, prohibits the establishment of effective screening programs in these groups.

Clinical features

Like most inherited enzymopathies, Canavan disease is autosomal recessive. Newborns appear normal at birth and for the first few months of life. After the first few months of life, infants with Canavan disease fail to achieve developmental milestones. Hypotonia is a characteristic of Canavan disease in the early months of life. Head control remains poor and is a constant feature of the disease. The head size gets progressively larger after the age of 6 months. The head lag, hypotonia, and large head with diffuse white matter in the MRI of the brain should suggest Canavan disease. Children with Canavan disease can acquire some developmental milestones during the first year of life; however, overall delays are profound and ultimately they are unable to sit, walk or talk. A 3-year-old child with Canavan disease is seen in Fig. 1. As patients get older they become progressively spastic, even opisthotonic. Sleep disturbances, optic atrophy, and feeding difficulties with gastroesophageal reflux may develop. The latter may result in the need for nasogastric feeding or permanent feeding gastrostomy. With improved nursing and general medical care, some patients with Canavan disease can survive into their second decade. Other than supportive care, there are no effective therapies for Canavan disease. Experiments using gene therapy have been on-going in a very limited number of patients [22].

Differential diagnosis

When considering the differential diagnosis of Canavan disease, the feature of macrocephaly associated with MRI confirmed white matter disease is pivotal. Neurogenerative diseases that deserve some consideration include Alexander's disease, benign megalencephaly with leukodystrophy, and Krabbe disease. One must remember that Canavan disease is not necessarily a rapidly progressive disease such that children may be considered to have static encephalopathy. The child remains stable for a while with developmental delay

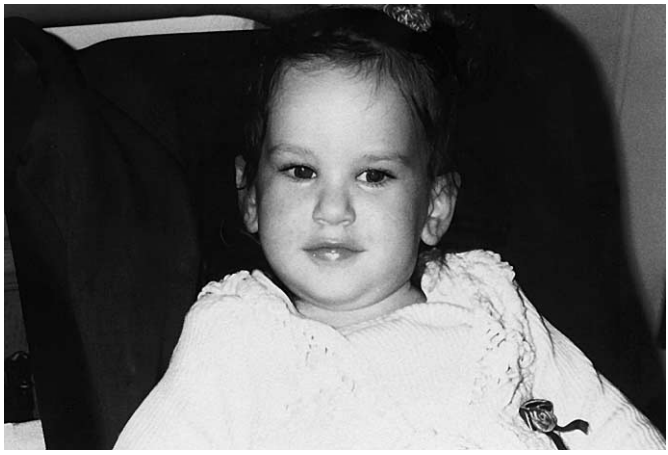

Fig. 1. A 3-year-old Jewish girl with Canavan disease is shown. She has a macrocephaly and requires head support. She is homozygous for mutation 285 Glu → Ala.

but no deterioration. Such infants may be misdiagnosed as suffering from perinatal birth asphyxia. This can result in a considerable delay in diagnosis and subsequent preventive counseling. Figs. 2 and 3 show the brain of a 9-month-old diagnosed with perinatal asphyxia. The white matter changes were ascribed to perinatal birth asphyxia. Subsequently, the diagnosis of Canavan disease was made. Indeed many of these children in the past were labeled with cerebral palsy. It is important to note that with Canavan disease, the head circumference increases and remains large while in most cases of perinatal asphyxia there is microcephaly.

Diagnosis

Histopathology

Histopathology of the brain was once used as the primary diagnostic tool for Canavan disease before the discovery of the enzyme defect. Microscopy reveals spongy degeneration throughout the white matter, including the subcortical regions. The astrocytes are swollen, and electron microscopy studies show elongated and distorted mitochondrion. The grey matter, which is spared in the early stages of the disease, becomes atrophied and spongy. The observation of spongy changes in the brain raised the question of NAA involvement in regulating water content of the brain cells [23–25].

Enzyme assay

Cultured skin fibroblasts are the most suitable tissue for determining aspartoacylase activity. Enzyme activity in carriers is frequently one half normal values

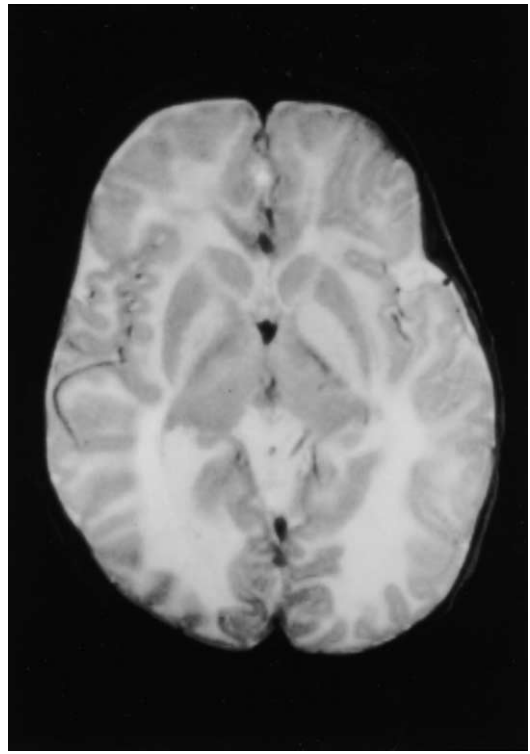

Fig. 2. MRI study of the brain of a 9-month-old child with Canavan disease, showing diffuse white matter disease involving the sub cortical regions, the posterior fossae, and the internal and external capsules are more severely involved.

and is virtually unmeasurable in affected patients. One should keep in mind that enzyme assays can be cumbersome, and reliability of these results is a function of laboratory expertise with this assay. Enzyme assay cannot be performed using a blood specimen, chorionic villi, or amniocytes. Aspartoacylase activity in cultured amniocytes or CVS is very low in a normal pregnancy. Therefore, the activity cannot be relied upon for prenatal diagnosis [2].

Analyte assay

The diagnosis of Canavan disease today relies on increased levels of NAA in the urine and other body fluids using gas chromatography-mass spectroscopy. During an established at risk pregnancy, when mutation analysis is either of no assistance or cannot be performed in a timely manner, amniotic fluid assays of NAA may assist in establishing an affected fetus [1,24]. Of note, the elevation of NAA in amniotic fluid is less than that found in urine. Stable isotope dilution increases the accuracy for measuring NAA in amniotic fluid.

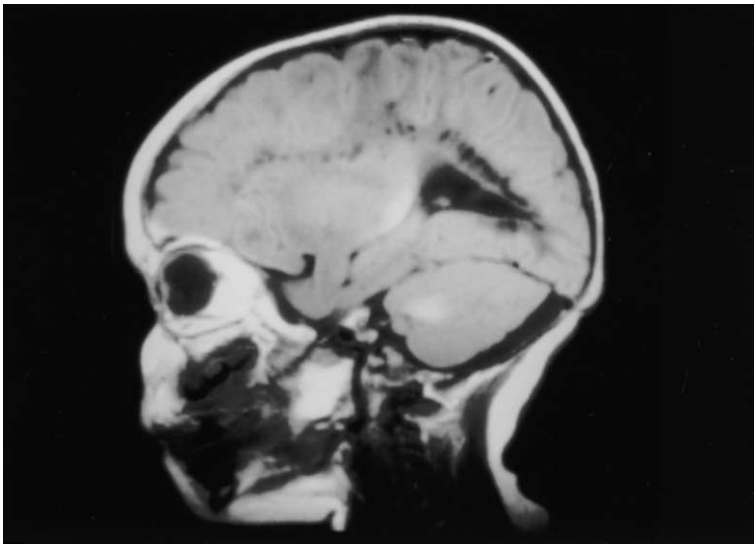

Fig. 3. MRI of the sagittal region of the same child shows cystic periventricular leukomalacia. These changes are frequently caused by perinatal anoxia. The coronal views show similar periventricular involvement.

This requires specialized laboratories with experience in performing such a test [26,27].

Laboratories that offer testing for Canavan and other genetic disorders are listed at www.genetests.org.

Mutation analysis

Mutation analysis plays a defined role in Canavan disease. Identification of specific mutations as a result of testing an affected proband should be used to provide the most accurate genetic counseling services. This information may be important in establishing the carrier status of other members in the pedigree, and identification of an affected conceptus (using cells obtained from amniocentesis or chorionic villous sampling) [28–30].

Mutation analysis is also a major component of the implementation of carrier testing of at risk populations (i.e, Ashkenazi-Jewish). Prevention programs through carrier testing are being offered for Jewish individuals and pregnancies for couples of Jewish ancestry. These programs together with the prenatal diagnosis have considerably reduced the incidence of Canavan disease among Jewish people. A Committee of the American College of Obstetrics and Gynecology has recommended that molecular carrier screening be offered to all Jewish couples [31]. This view echoes one that had previously been put forth by the American College of Medical Genetics [32].

Mouse model

A knockout mouse for Canavan disease has been engineered [33]. The mouse has a phenotype of neurodegenerative disease. The mouse has spongy degeneration of the brain, increased NAA in urine, and asparoacylase deficiency. This mouse is being used for experimentation with gene therapy and for the understanding of the pathogenesis of Canavan disease. A rat with aspartoacylase deficiency, tremor rat, was discovered in Japan. The rat has spongy degeneration of the brain and increased NAA [34].

Summary

Canavan disease is a severe leukodystrophy more common among Ashkenazi Jews. The enzyme defect, apartoacylase, has been identified, and the gene cloned. Only two mutations account for over 98% of all Jewish alleles with Canavan disease. The carrier frequency among healthy Jews is 1:37–58. Carrier detection and prenatal diagnosis can be accurately carried out using molecular analysis. When mutations are unknown, analysis of amniotic fluid for NAA using stable isotope dilution technique can be used for prenatal diagnosis.

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OBSTETRICS AND GYNECOLOGY CLINICS of North America

Screening for thalassemia
A model of success

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The β-thalassemias are a markedly heterogeneous group of autosomal recessive disorders resulting from reduced (β^+) or absent (β^0) production of the β-globin chains, which together with the α-chains make up the hemoglobin tetramere (α_2 β_2) [1,2]. The shortage of β-chains results in an excess of unassembled α-chains, which precipitates damaging the membrane and determining premature apoptosis of the red blood cell precursors, thereby resulting in ineffective erythropoiesis.

Three different clinical and haematological conditions are recognized, ie, the β-thalassemia carrier state (heterozygous β-thalassemia), thalassemia intermedia, and thalassemia major (Table 1). The latter two result from homozygosity or compound heterozygosity for β-thalassemia alleles. Homozygosity refers to the presence of the same mutation in both copies of the gene. Compound heterozygosity refers to the concept that each copy of the gene is mutated, but the mutation is distinctly different.

The β-thalassemias have a remarkably high frequency in the Mediterranean area, the Middle East, the Far East, and East Asia. A relatively high incidence is also observed in people of African origin. However, due to population flow, the β-thalassemias are now widespread and also occur in Continental Europe, North

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Table 1
Clinical and hematological characteristics of β-thalassemias

	Clinical picture	Hematological picture
Silent carrier	Silent	Silent/borderline HbA2
Carrier	Silent	Reduced MCV and MCH and mild reduction of Hb Increased HbA2 α/β ratio > 1.2–2.0
Thalassemia intermedia	Pallor and jaundice (moderate/severe) Hepato-splenomegaly Thalassemia-like bone modifications No regular transfusion required	Microcytic hypochromic anemia NRBC and moderate morphological changes in peripheral blood HbF 5–99% α/β ratio > 2.0
Thalassemia major	Severe anemia Hepato-splenomegaly Severe bone deformities Growth retardation	Severe hypochromic anemia NRBC and severe morphological changes in peripheral blood HbF 70–99% α/β ratio > 3.0

and South America, and Australia. The best available estimate indicates that approximately 240 million people world-wide are heterozygous for β-thalassemia and at least 200,000 affected homozygotes are born every year [1–3].

In the past decades, the molecular pathology of the β-thalassemias and the mutation-phenotype relationships has largely been elucidated. This knowledge has been applied to carrier identification and prenatal diagnosis in a number of Mediterranean populations and has resulted in a dramatic reduction in the homozygous state in several populations at risk. In this article we review these advances.

Clinical features

The β-thalassemia carrier state is clinically asymptomatic. Haematologically it is characterized by microcytosis [low mean corpuscular hemoglobin volume (− MCV)], a reduced hemoglobin content per red blood cell [low mean corpuscular hemoglobin (− MCH)], and an increased percentage of hemoglobin A_2 (higher than 3.5%). Hemoglobin A_2 (α_2 δ_2) is a minor normal component of adult red blood cells. There may be an inconstant minor increase in fetal hemoglobin (2–5%). In vitro biosynthesis using radioactive globin chains shows an imbalance between α and β chain production. Ratios ranging from 1.2–2.5 have been reported [4].

The condition, thalassemia intermedia, is more broadly characterized than either β-thalassemia carrier state or thalassemia major. The vast clinical heterogeneity in this condition is a result of wide heterogeneity at the molecular level

[2,5–7]. This group of patients presents late in life and can sustain a hemoglobin level consistent with life without the need for transfusion therapy. However, the clinical phenotype of this condition includes anemia and usually some degree of splenomegaly. The haematologic indices can range between those of β-thalassemia carriers and patients with thalassemia major. Depending on disease severity, the management approaches in this condition may be minimal to nearly as intense as that used to treat thalassemia major.

Thalassemia major is characterized by severe microcytic anemia, spleen and liver enlargement, and characteristic skeletal abnormalities. These result from massive expansion of the bone marrow in an attempt to compensate for the premature destruction of red blood cell precursors. The skeletal abnormalities can be seen as dysmorphic facies, and marked osteoporosis. Haematologic indices are a function of β-globin chain production (β^0 or β^+) and any history of prior administration of blood transfusions. Hemoglobin electrophoresis demonstrates absent to minimal hemoglobin A (0–30%) and variable hemoglobin A_2 (2% to 5%). The percentage of hemoglobin F dominates (95% to 70%). After blood transfusion, haematologic indices cannot be relied upon for diagnosis due to the presence of transfused red blood cells in peripheral blood.

The present management of thalassemia major consists of a regular blood transfusion program and iron chelation with Desferrioxamine B to eliminate the iron overload secondary to multiple red blood cell transfusions, and to a lesser extent to increased iron absorption. Life expectancy with this management extends into the third decade. An alternative to traditional management is bone marrow transplantation from HLA identical siblings which, in patients at low risk (absence of iron overload and iron-mediated parenchymal damage), results in a disease-free survival of about 90% to 95% [8]. Further perspectives rely on finding effective agents that boost fetal Hb production, thus compensating for the shortage of β-chains, or on transfecting a normal β or γ globin gene by viral vectors into pluripotent stem cells (gene therapy) [9].

Molecular pathology

To date, β-thalassemia can be attributed to approximately 200 different molecular defects [10,11]. A repository of mutations causing β-thalassemia is regularly updated and published [11]. Despite the marked molecular heterogeneity, in each population at risk prevalent mutations are limited. In fact, 8 to 10 mutations usually account for the molecular lesions on chromosomes among Mediterranean and Asian people [12] (Table 2). An example of ethnic specific mutations can be seen among black people. The most common causes of β-thalassemia in this group are -88 C $\rightarrow$ T, -29 A $\rightarrow$ G, and codon 24 T $\rightarrow$ A mutations. The appreciation of these molecular characterizations obviously facilitates diagnosis and screening.

Most frequently β-thalassemia results from either a single nucleotide substitution or by single oligonucleotide addition or deletion that affects the coding region

Table 2
Population distribution of common β-thalassemia mutations

Population	Mutations
Mediterranean	− 87C → G, IVS1-1 G → A, IVS1-6 T → C, IVS1-110 G → A, cd 39 C → T, IVS2-745 C → G
Middle East	Cd8-AA, cd 8/9 + G, IVS1-5 G → T, cd 44-C, IVS2-1, 41/42 - TTCT
Indian	− 619 bp deletion, cd 8/9 + G, IVS1-1 G → T, IVS-5 G → C, 41/42
Chinese	−28A → G, 17A → T, 19 A → G, IVS1-5 G → G, 41/42 - TTCT, IVS2-654 C → T
African/American Black	− 88 C → T, −29A → G, IVS1-5 G → T, cd 24 T → A, IVS11-949 A → G, A → C

or sequences critical for β-globin gene function. In contrast to α thalassemia, β-thalassemias rarely result from the mechanism of gross gene deletion. However, due to the size of the β globin cluster (recall this cluster accounts for ε, γ, and δ globin chains), and nucleotide sequence similarity in this region, complex rearrangements can occur. These may result in a number of complex β-thalassemias (δβ-thalassemia and εγδβ-thalassemia), which result from the deletion of a variable extent of the β-globin gene cluster.

About half of the known mutations that cause β-thalassemia inactivate the β-globin gene completely, thus resulting in β^0-thalassemia. Mutations of this type include frameshift, nonsense, initiation codon, and RNA processing mutations (Fig. 1). The remaining group of mutations lead to a variable output of β-globin chains from the affected locus, thus producing β^+-thalassemia of varying severity. Mutations that cause β^+-thalassemia have been ascribed to the promoter, splice sites, or those that alter polyadenylation signal mutations. The net effect of each of these is reduced quantity, of otherwise normal β globin chains. Among the β^+-thalassemia alleles, one Hb variant worth mentioning is HbE [2]. This variant is the most common structural variant capable of impairing the normal splicing process. HbE shows a high frequency in India and South Asia.

Prediction of a mild phenotype

Informative and reliable genetic counseling requires an accurate description of the clinical phenotype. This is particularly important when evaluating carriers whose offspring are at risk for thalassemia intermedia [6,7,13]. Under the diagnosis of thalassemia intermedia, we comprehend a spectrum of disorders of varying severity that lies between that seen for the β-thalassemia carrier state and thalassemia major. The large majority of patients with thalassemia intermedia are homozygotes or compound heterozygotes for β-thalassemia. By identifying those capable of molecular compensation, clinical disease prediction can be predicted. Studies carried out in the past few years have delineated a number of molecular mechanisms capable of reducing the ratio of α: non α (β + γ) haemoglobin chains. Importantly, this ratio correlates with the clinical phenotype. An example is the inheritance, in homozygosity or

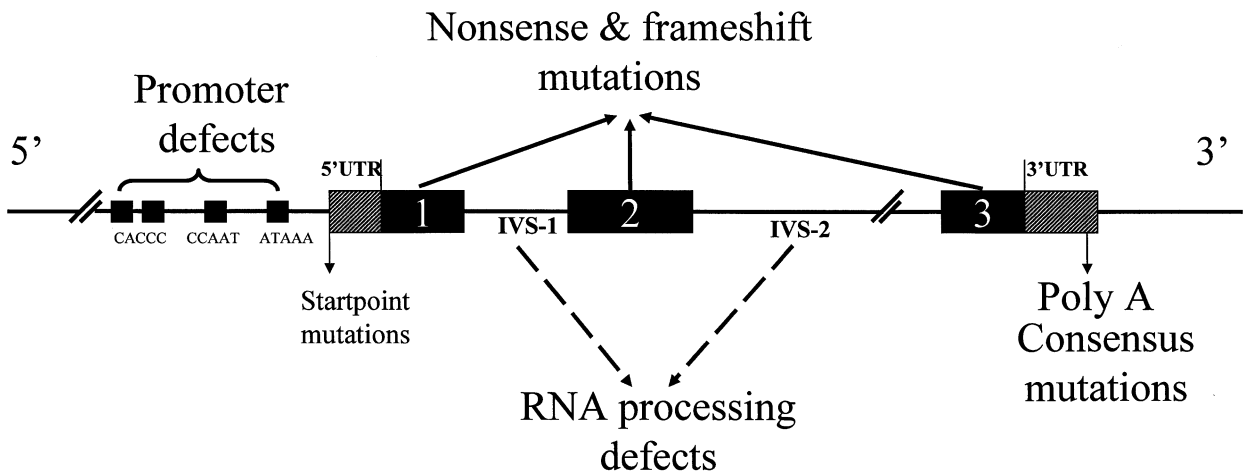

Fig. 1. Variability in point mutation in the β-globin gene.

compound heterozygosity, of a mild or silent β-thalassemia mutation. These are associated with a substantial residual output of β-thalassemia from the affected locus (Table 3). Another example is homozygosity or compound heterozygosity for typical β-thalassemia and δβ-thalassemia. This is associated with high γ-chain production. An alteration of the α:non-α ratio may occur as a result of co-inheritance of α-thalassemia [6,14,15]. This leads to a reduction in α-globin gene output and hence to a reduced α/non α inbalance. Co-inheritance of a genetic determinant capable of sustaining continuous production of γ-chains in adult life [deletion and non-deletion hereditary persistence of fetal Hb (HPFH)], may also cause altered ratios of the α:non-α globin chains. Finally, some cases of mild β-thalassemias result from the co-inheritance with homozygous β-thalassemia of heterocellular HPFH (alterations at loci other than the globin chain loci). This condition is genetically heterogeneous and may

Table 3
Mild and silent β-globin mutations causing β-thalassemia

	Silent	Mild β^+
Transcriptional mutants in the proximal CACC box	− 110 C → T	− 90 C → T
	− 92 C → T	− 88 C → T
		− 88 C → G
		− 87 C → T
		− 87 C → G
		− 87 C → A
		− 86 C → T
		− 86 C → G
TATA box		− 31 A → G
		− 30 T → A
		− 29 A → G
5′ UTR	+ 1′ A → C	+ 22 G → A
		+ 10 -T
		+ 33 C → G
Alternative splicing	Cd27 G → T (Hb Knossos)	Cd19 A → C (Hb Malay)
		Cs24 T → A
Consensus splicing	IVS I-5 G → A Plus 7.2 Kb deletion (Corfu δβ-thal.)	IVS I-6 T → C
IVS	IVS 2-844 C → G	
3′ UTR	+ 6 C → G	
Poly A site	AATAAG	AAGAAA
		AATGAA

Mild β° [a]	Frameshift	Splicing junction
	Cd6−A	IVS2 + 1 G → A
	Cd8−AA	

[a] Associated with increased HbF production.

be linked or unlinked to the β-globin gene cluster. To date, two loci have been mapped, one on the Xq 22.2-22.3 region, the other on chromosome 6 (6q22.3-23.1) [16–18]. Nevertheless, many others are likely to exist as well [19]. More rarely, thalassemia intermedia results from double heterozygosity for the triple α-globin gene arrangement, which is associated with high α-chain output, and typical β-thalassemia, or also from the presence of highly hyper unstable β-globin (dominant β-thalassemia) [20–23].

We may conclude that, besides β-globin gene analysis, α and γ globin gene analysis has the potential to better define the phenotype thus improving genetic counselling. However, it should be noted that only inheritance of homozygosity for mild/silent β-thalassemia is consistently associated with a mild phenotype.

Carrier identification of β-thalassemia

Several genetic factors may modify the haematologic phenotype of the β-thalassemia carrier state (low MCV and MCH, and an increase in the HbA_2 percentage). When present these may cause difficulties in identification of patients that are carriers (Table 4). Co-inheritance of heterozygous β-thalassemia and α-thalassemia may raise the MCV and the MCH high enough to determine normal values at least in some of these double heterozygotes. This may occur as a result of either a deletion of two α-globin structural genes or as a non-deletion lesion affecting the major α_2-globin gene (the two functional α-genes, denominated as α_1 and α_2, have a relative expression of 1:3). Fortunately, these carriers may be easily identified due to their high HbA_2 levels [24–25].

Elevation of HbA_2 is the most important feature in the identification of heterozygous β-thalassemia, but a substantial group of β-thalassemia heterozygotes may have normal HbA_2. The first mechanism to account for the abnormally low HbA_2 levels in a β-thalassemia carrier is the presence of a specific mild β-thalassemia mutation, such as the β^+ IVS-1 nt 6 mutation [26].

Table 4
Phenotype characteristic of atypical β-thalassemia carriers

Phenotype	Genotype
Normal MCV/MCH	α and β-thalassemia interaction
Normal HbA2 level	Coinheritance of δ and β-thalassemia Some mild β-gene mutations γδβ-thalassemia
Normal MCV/MCH and HbA2 level (silent)	Silent β-gene mutations α globin gene triplication
Severe heterozygous β-thalassemia	Hyperunstable hemoglobins Coinheritance of heterozygous β-thalassemia and triple α-globin gene

A second, common mechanism is co-inheritance of heterozygous β-thalassemia and δ-thalassemia. The decreased output of the δ-globin chains may result in normalisation of HbA_2 levels [27,28]. Also, γδβ and δβ-thalassemia have normal HbA_2. All these normal HbA_2 atypical heterozygotes, however, have low MCV-MCH. Because of this phenotype, normal HbA_2 β-thalassemia heterozygotes should be differentiated from α-thalassemia heterozygotes by globin chain synthesis analysis and/or by α, β, and δ-globin gene analysis. δβ-Thalassemia, in addition, may easily be defined by the variable but markedly increased HbF.

Another major problem in carrier screening is the identification of silent β-thalassemia or the triple α-globin gene arrangement, both of which may lead to the production of intermediate forms of β-thalassemia by interacting with typical heterozygous β-thalassemia [20–22,29]. Silent β-thalassemias are characterised by normal MCV and MCH values and normal HbA_2, and by the fact that they are defined only by the slight imbalance in the α-globin/non α-globin synthesis. Nevertheless, on examining the hematological features of these carriers, one may find borderline HbA_2 or MCV-MCH values that may alert for the presence of atypical β-thalassemia, thus requiring further studies (globin chain synthesis or gene analysis). The most common silent β-thalassemia is the $\beta^+ -101\ C \rightarrow T$ mutation; others are very rare [29]. The triple α-globin gene arrangement may show a slight imbalance of α/non α chain synthesis or, more commonly, may be completely silent [20–22].

Compound heterozygosity for silent and typical β-thalassemia, and double heterozygosity for typical β-thalassemia and the triple α-globin gene arrangement result in markedly attenuated forms of thalassemia (thalassemia intermedia) [20,29].

An extreme, though rare, instance of thalassemia gene combination, which may result in a carrier diagnosis pitfall, is the presence of α, δ, and β-thalassemia together, which may lead to a completely silent phenotype [30].

Awareness raising and population education

Since the late 1970s, population screening programs of adults at child-bearing age, genetic counselling, and prenatal diagnosis have been introduced among the populations at risk in the Mediterranean area, including Sardinians, continental Italians, Greeks, and Cypriots. Although details regarding these programs have been described elsewhere, the most relevant characteristics are discussed herein, focusing on those that have been operating over a long period, and about which a larger amount of data is available (Sardinia, Greece, Cyprus) [1,12,13,31,32]. These programs were directed either to couples with previous affected children (retrospective diagnosis) or to childless couples (prospective diagnosis). Though very useful for individual families, retrospective diagnosis has a limited effect on the control of homozygous β-thalassemia at the population level. All these programs have been characterised by intensive education campaigns with the population. Education has been conducted mostly through mass media, including

local newspapers, radio, television, and magazines (Fig. 2). In some programs, especially in Sardinia at the beginning, lectures have been organized for the general public at large in factories, large stores, and shops. Family physicians, obstetricians, pediatricians, midwives, and nurses have all been trained in this new field of preventive genetics. Posters and information pamphlets have been made available at marriage registry offices, general practitioners and obstetricians offices, as well as family clinics. The pamphlets provide the following information: (a) that the β-thalassemia carrier state can be easily identified with the appropriate methodology; (b) who should get the test, where, and how carrier tests are available; (c) that heterozygotes are not at a disadvantage; (d) the natural history of the homozygous condition is described and it is stressed that this is a severe disease for which a continuous transfusion program combined with iron chelation therapy is needed for survival, and that cure may be achieved through bone marrow transplantation in the limited proportion of homozygotes that have an HLA identical donor sibling; and (e) that the homozygous state can be safely prevented by that fact that couples identified as being at risk, in the case of both members being carriers, have a number of options, including fetal diagnosis, to avoid giving birth to affected children. Every year at a meeting about thalassemia in Cyprus, magazines and booklets are distributed. Seminars with parents' associations are held periodically with the purpose of planning proper means of information to educate the population. In addition, community-based parents'

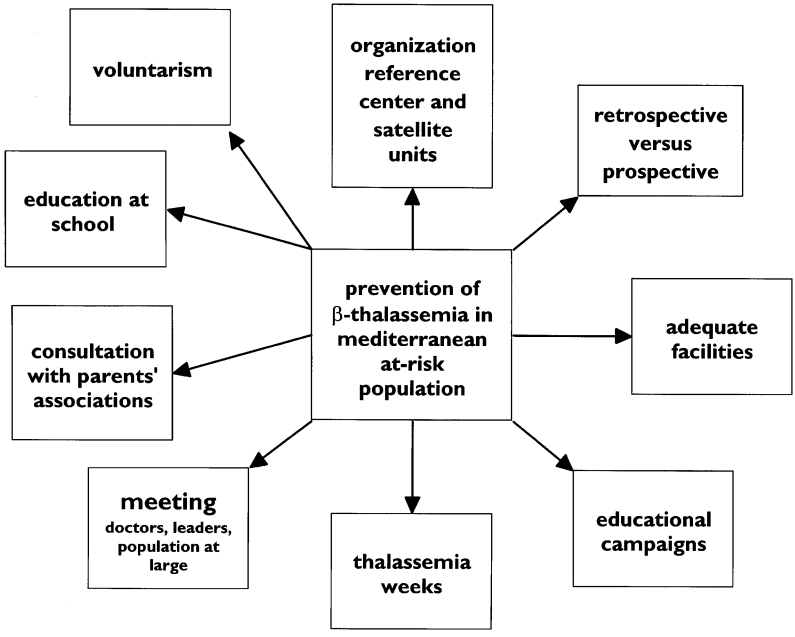

Fig. 2. Prevention of β-thalassemia in Mediterranean at-risk population.

associations have played an invaluable role as an influential group by increasing the financial support for public education and providing psychological assistance to patients and families. In Cyprus, the Greek Orthodox Church has given a substantial contribution by requiring marriage candidates to produce a certificate stating that β-thalassemia carrier testing has been carried out. Educational videotapes have also been introduced in secondary schools to teach students about inherited anemias and especially β-thalassemias. In Sardinia, for the past 3 years this educational session has been followed by carrier testing at the end of secondary school (see later). This procedure has indirectly led to raise awareness of β-thalassemia among the tested children's parents. Since 1980 the educational campaign has not been held at the population level in Sardinia. The only educational activity carried out at present is the teaching of β-thalassemia in secondary schools and the introduction of β-thalassemia as a topic in courses for doctors, nurses, and obstetricians.

A critical evaluation of the information channel through which the population at large has been informed has been carried out twice in Sardinia; once at the beginning of the program and again more recently [12]. At the start of the program most spouses were informed through the mass media (44%), general practitioners (31%), and obstetricians (23%). This trend has been modified recently, since the information is now given by physicians (family doctors, obstetricians, and genetic counsellors), and reaches more than 70% of the population.

Target population

The target population for screening has been couples at marriage, preconception, or early pregnancy. Nevertheless, even nowadays a limited number of couples request testing when already pregnant, which may lead to marked emotional stress. In Cyprus and Sardinia, the number of young unmarried people requesting screening is increasing steadily, this being a clear indication of improved awareness of the disease and related prevention methodology. As mentioned above, both in Cyprus and in Sardinia, screening of adolescents and school children has been introduced recently [1,12].

In these populations, heterozygote screening has been carried out on a voluntary basis. Though informed consent was not requested in these programs, every effort has been made to inform the patient about the meaning of the carrier state and the potential adverse effect associated with its detection.

Efficacy of carrier screening

At present, in Cyprus and Sardinia at least, the large majority of couples both before and after marriage present voluntarily at the screening centers for testing and counseling. In both countries adequate facilities for screening have been provided; in Sardinia, for instance, we have 13 centers spread all over the country (Fig. 3). Screening of relatives, informed about the risks by the counselled carrier,

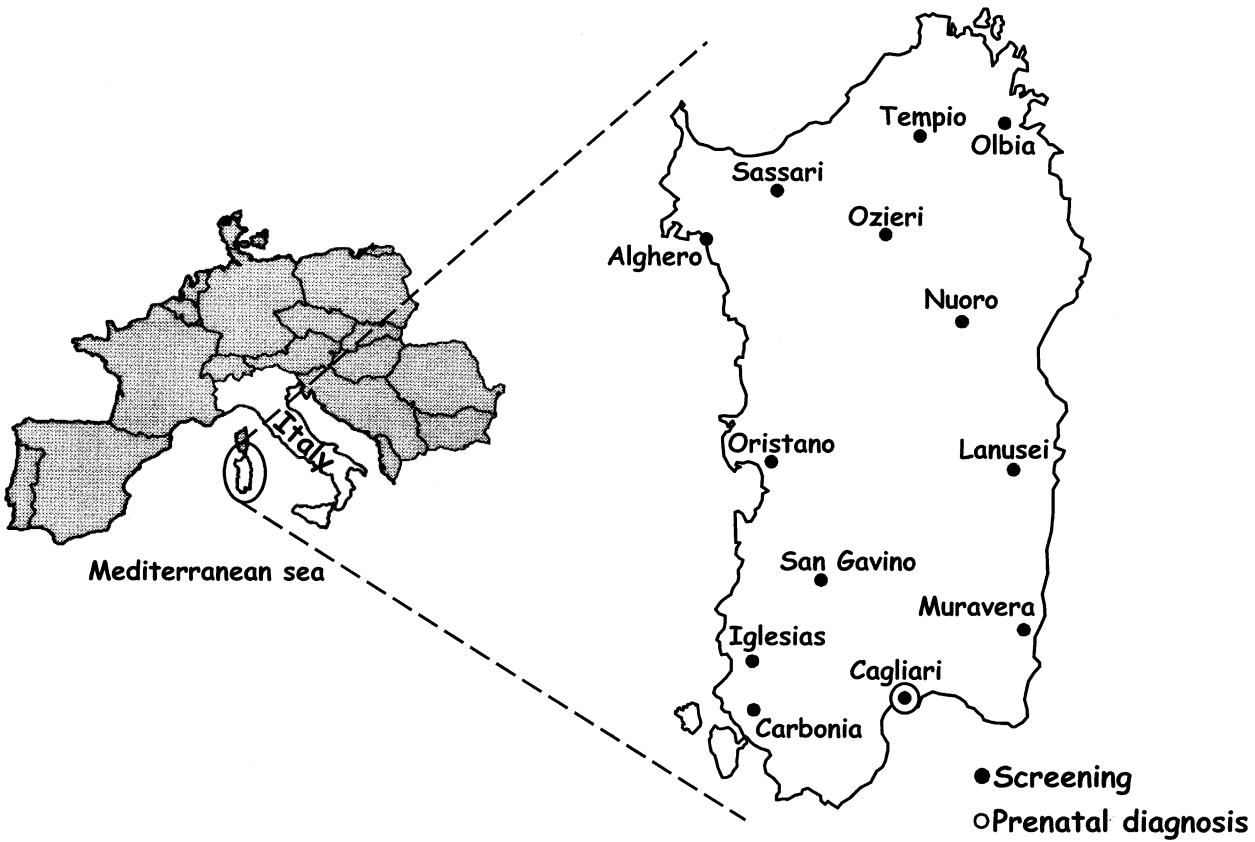

Fig. 3. Centers for screening for β-thalassemia in Sardinia.

has strengthened the efficacy of the screening process. In Sardinia, for instance, we managed to detect most of the couples at risk, ie, approximately 90% of those predicted on the basis of the carrier rate, by screening only a small proportion ($\sim$11%) of the population at child-bearing age [12].

Counseling

Counseling has been non-directive and generally based on a private interview with the individual or couple [1,12]. The provided information is aimed at giving an informed basis on which to make a reproductive choice, ie, birth control, mate selection, adoption, fetal testing, or artificial selection by a donor. Particular emphasis has been given to details of fetal analysis, ie, sampling procedure, risk for the fetus, failure, and misdiagnosis. An explanatory booklet is usually provided. The predicted natural history of the disease based on the genotype at the α, β, and γ loci is usually discussed. In Sardinian families with previous normal or heterozygous children, we propose and eventually carry out HLA typing on fetal DNA to assess whether a sibling is HLA-identical, and thus a suitable bone marrow donor. This information allows the parents to have further alternatives in the event of an affected fetus, ie, bone marrow transplantation. Prenatal diagnosis uptake has been very high since first trimester diagnosis became available (see later) [33]. Finally, in the counseling session we inform the carriers about the risks to their relatives, and recommend that they should inform them so that they can choose to take the test as well. In Sardinia most relatives opted to be tested.

Carrier detection procedure

Several procedures have been proposed for β-thalassemia carrier screening [12]. The cheapest and simplest is based on MCV and MCH determination followed by HbA_2 quantitation for subjects showing microcytosis (low MCV) and reduced Hb content per red blood cell (low MCH). However, since with this procedure a considerable proportion of double heterozygotes for β and α thalassemia may be missed (these are found in many populations, such as Sardinians, where both disorders are common), it can only be used in populations with a low frequency of α-thalassemia [24,25]. At our center, in the first set of examinations we include MCV and MCH determination and hemoglobin chromatography by HPLC, which can quantitate HbA_2 and HbF and can detect the most common hemoglobin variants (HbS, HbC, and HbE) that may result in a hemoglobin disorder by interacting with β-thalassemia (Fig. 4). It should be stated that HPLC is also capable of detecting Hb *Knossos*, a mild β-thalassemia allele, which is not identified by using common procedures for hemoglobin analysis. In the presence of low MCV and MCH and elevated HbA_2 levels, a diagnosis of heterozygous β-thalassemia is made.

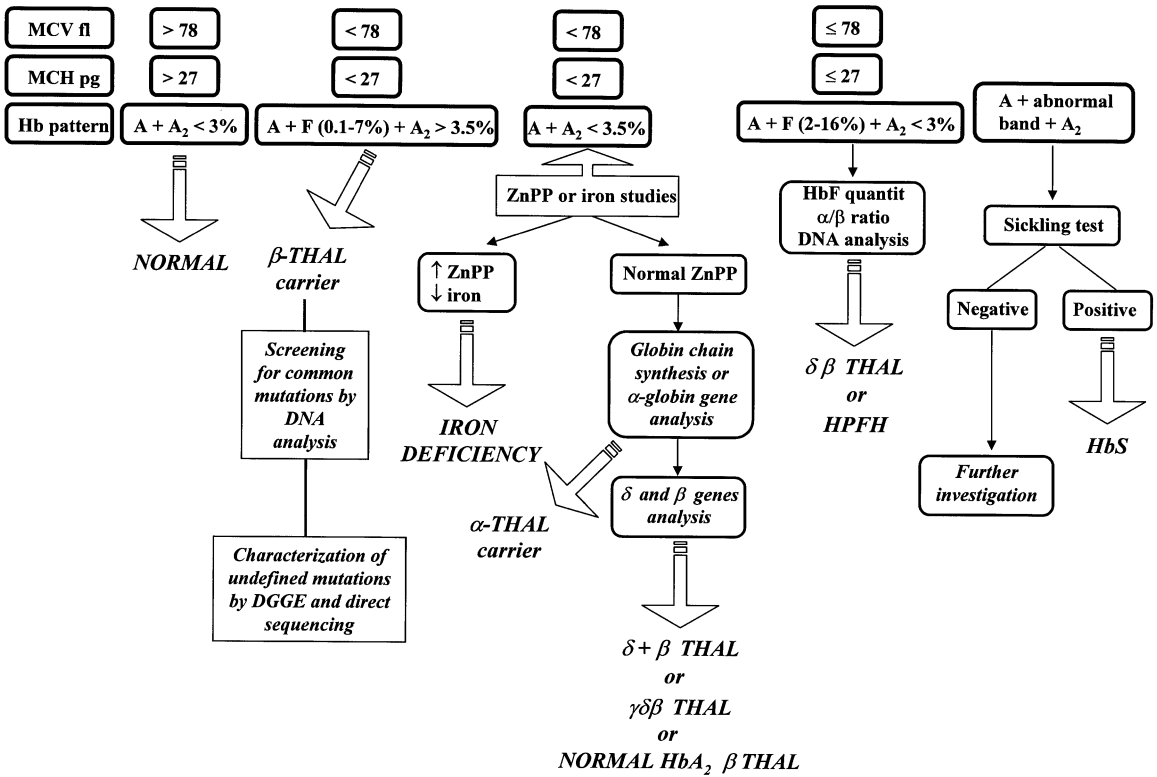

Fig. 4. Flow chart used in the carrier detection program at the Ospedale Regionale per le Microcitemie in Cagliari.

A phenotype characterised by microcytosis, hypochromia, normal-borderline HbA_2, and normal HbF may result from iron deficiency, α-thalassemia, γδβ-thalassemia, β + δ thalassemia, or mild β-thalassemia. After excluding iron deficiency through appropriate studies (zinc protoporphyrin determination, transferrin saturation), the different thalassemia determinants leading to this phenotype are discriminated by globin chain synthesis analysis and eventually by α, β, and δ globin gene analysis [12]. In the presence of normal MCV and borderline HbA_2 levels, we are inclined to suspect the presence of a silent mutation or the triple α-globin gene arrangement and therefore proceed directly to α- and β-globin gene analysis, since the α/β globin chain synthesis ratio could also be normal [34]. Definition of the type of thalassemias in these carriers is solely recommended when they mate with a carrier of a typically high HbA_2 β-thalassemia or an undetermined type of thalassemia. In those rare cases showing normal or low MCV-MCH, normal or reduced HbA_2 levels, and high HbF, we suspect the presence of δβ-thalassemia, which should be differentiated from HPFH. This distinction is performed by globin chain synthesis analysis (normal in HPFH and unbalanced in δβ-thalassemia), and/or β-cluster gene analysis.

Molecular diagnosis

In couples at risk identified by the above described carrier detection procedure, the specific β-thalassemia mutation is defined by one of the several available PCR-based methods [35]. The most widely used procedures are primer-specific amplification (ARMS) [36] and reverse dot blot analysis (RDB) [37] with a series of primers or probes complementary to the most common mutations in the specific population [12]. As mentioned above, in each population at risk, β-thalassemia results from a limited number (4–20) of common mutations and a variable number of rare mutations. Alternatively, the specific mutation may be defined by denaturing gradient gel electrophoresis (DGGE), which has the additional advantage of detecting also the unknown mutations [38–40] (Fig. 5). Following localization by DDGE, the mutation is defined through direct sequencing of the DNA contained in the abnormal migrating fragment. If the mutation is not detected by DDGE, we search for the presence of small deletions through polyacrylamide gel electrophoresis of the PCR-amplified products prepared for ARMS or RDB analysis, which may lead to the detection of small deletions of the β-globin gene, whose presence may be suspected by finding very high HbA_2 levels. Larger deletions of the cluster may be identified with restriction fragment length polymorphism analysis carried out with PCR-based procedures. In a very limited number of cases (0.1% in our experience), direct sequencing from position −600 bp to 60 bp downstream from the β-globin gene failed to detect a disease-causing mutation that may lie elsewhere in the genome (locus control region or genes coding for transcription factors). Counseling and decision-making can be quite difficult in these cases

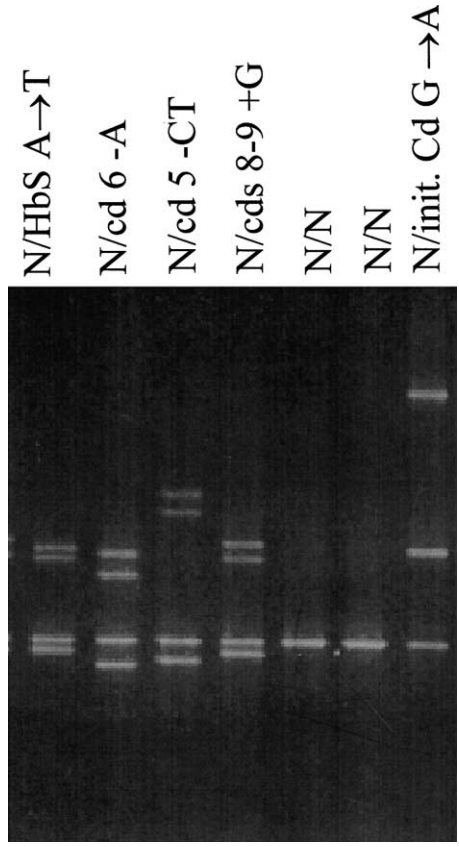

Fig. 5. Denaturating gradient (42% → 72%) gel electrophoresis in heterozygotes for β-thalassemia. The β-globin genotype of each subject is indicated on top.

(see later) [41]. Finally, mutation detection by either DNA high pressure liquid chromatography (DHPLC) (Fig. 6) or oligonucleotide microchip array are very appealing new approaches [42,43].

As previously mentioned, δ-globin gene analysis may be necessary to define double heterozygotes for δ and β-thalassemia that may be mistaken for α-thalassemia heterozygotes. The suspicion of interacting δ-thalassemia may arise when borderline HbA_2 levels are found or when family studies show segregating δ-thalassemia (characterised by normal MCV-MCV and low HbA_2) and β-thalassemia. Identification of δ and β double heterozygotes, however, may be accomplished by globin chain synthesis analysis and/or α, β, and δ-globin gene analysis.

Definition of the δ-thalassemia mutation may be carried out using one of the previously mentioned PCR-based methods. As in β-thalassemia, also in δ-thalassemia, each population at risk has its own spectrum of common δ-thalassemia mutations that may be defined through a limited number of spe-

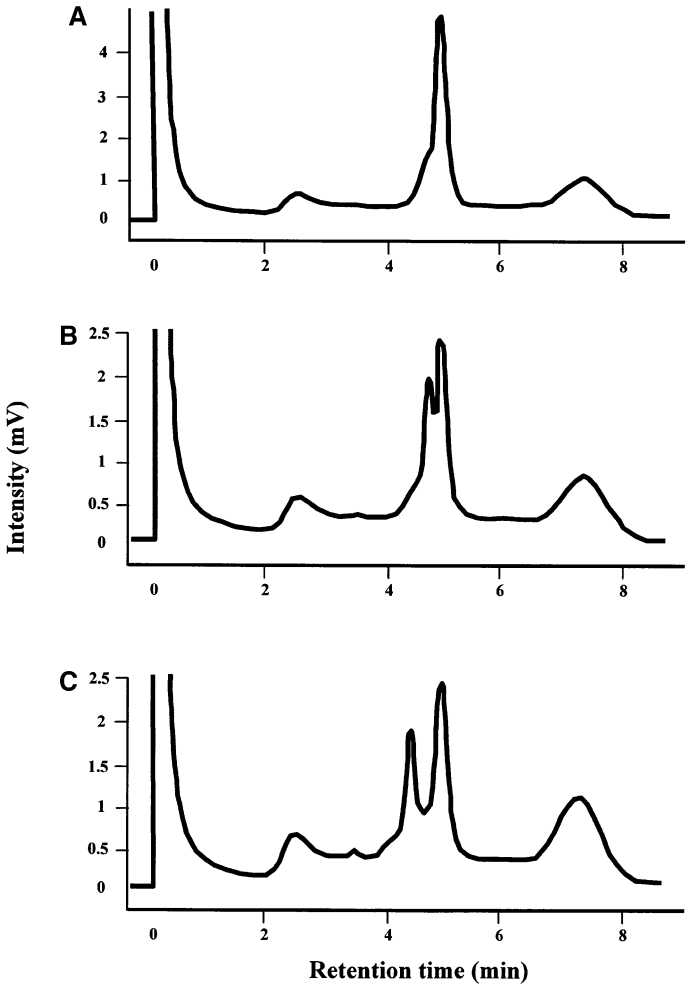

Fig. 6. β-globin gene analysis by denaturing high performance liquid chromatography (DHPLC), a recently developed methodology for detection of heteroduplexes formed in DNA samples containing mismatches between wild type and mutant strands. Chromatograms of exon 2 of the β-globin gene: (A) normal control, (B) heterozygotes for β° 39 mutation, (C) heterozygotes for the β° 76 mutation.

cific primers/probes. In Sardinians, for instance, only three δ-thalassemia mutations have been detected so far. The list of δ-thalassemia mutations is available at the repository of the human β and δ-globin gene mutation [11]. Though most of the δ-thalassemia determinants are in *trans* (on opposite chromosomes) to β-thalassemia, some have also been detected in *cis* (on the same chromosome) [27,28,44–47].

Definition of the α-globin gene arrangement may be carried out to discriminate between heterozygosity for α-thalassemia and double heterozygosity for δ and β-thalassemia or γδβ-thalassemia. This analysis could also be useful in

defining co-inherited α-thalassemia in homozygous β-thalassemia, which may lead to the prediction of a mild clinical condition. Deletion α° or α^{+}-thalassemias are detected by PCR using two primers flanking the deletion breakpoint, which amplify a DNA segment only in presence of specific deletions. As a control, DNA from a normal chromosome is simultaneously amplified using one of the primers flanking the breakpoint and a primer homologous to a DNA region deleted by the mutations. Non-deletion α-thalassemia may be detected by restriction endonuclease analysis or allelic oligonucleotide specific probes on selectively amplified α_1- and α_2-globin genes [10,48].

Definition of co-inherited HPFH determinants can be useful in predicting the development of the phenotype of an affected fetus. As mentioned above in fact, on increasing the γ-chain output, co-inherited HPFH with homozygous β-thalassemia may lead to a milder phenotype.

The presence of high HbF in the parents may lead to the suspicion of double heterozygosity for β-thalassemia and HPFH. At present, the molecular definition of HPFH is limited to two forms of non-deletion HPFH, namely −196 C → T A γ and −158 C → T Gγ, which have been proved to be capable of ameliorating the clinical phenotype of homozygous β-thalassemia. These HPFH determinants may easily be detected through restriction endonuclease or dot blot analysis with oligonucleotide-specific probes on PCR-amplified DNA. In the future, the identification of the HPFH determinants as linked or unlinked to the β-cluster may lead to improving the capability of predicting the phenotype.

Prenatal diagnosis

Prenatal diagnosis for the β-thalassemias was carried out successfully in the 1970s through the use of globin chain synthesis analysis of fetal blood [49]. Thanks to the molecular characterization of the β-thalassemias, the introduction of chorionic villous analysis, and the development of PCR-based methods for DNA analysis, it became possible to assess the fetal genotype within the first trimester of pregnancy by fetal trophoblast analysis [40,50,51].

Fetal sampling

In the 1970s, fetal blood for analysis was obtained by placentocentesis, later in the early 1980s by foetoscopy, and finally since 1984 by cordocentesis. With the introduction of the methodologies that allowed the direct detection of fetal DNA mutations, we used amniocentesis (1983–1984), transcervical chorionic villous sampling (TC-CVS) (1984–1986), and, since 1986, transabdominal chorionic villous sampling (TA-CVS) with the free hand technique [52–54]. Table 5 summarizes the overall results obtained at our center. In our experience TA-CVS appears to be the safest and most reliable procedure. Additional

Table 5
Invasive procedures in 5800 prenatal diagnosis of β-thalassemia

Technique	No.	Wks.	Failures	Losses (%)	Misdiagnosis
Placentacentesis	981	18–24	10	5.2	2
Fetoscopy	67	18–24	2	5.6	-
Cordocentesis	120	18–24	-	2.1	-
Cardiocentesis	6	18–24	-	-	-
Amniocentesis	203	16–18	6	2.6	-
Trascervical CVS[a]	572	9–13	1	4.2	1
Transabdominal CVS[b]	3851	6–24	-	1.3	-

[a] by biopsy forceps.
[b] by freehand technique and single spinal needle.

advantages are simplicity, speed, better patient acceptance [55], and lower risk of infection and bleeding. Moreover, TA-CVS can be performed at any gestation period, which is particularly important for couples who present late in gestation. The use of prenatal diagnosis increased dramatically (99.4% versus 93%) after the introduction of CVS (Table 6) [55].

Fetal DNA analysis

Nowadays fetal DNA is analyzed by one of the above mentioned PCR-based methods [12,39,40]. In order to reduce the occurrence of misdiagnoses, in our center we use two different PCR-based procedures for each case (eg, RDB hybridisation and primer-specific amplification). Fig. 7 shows the molecular analysis of a typical example of a fetus at risk for the compound heterozygous state for two different β-thalassemia mutations, and Table 7 reports the overall results obtained at our center. DNA analysis gave very accurate results. We observed misdiagnosis only by fetal blood analysis and by oligonucleotide hybridisation on electrophoretically separated non-amplified DNA fragments, but never by PCR-based methods. However, misdiagnosis may occur for several reasons, such as failure to amplify the target DNA fragment, false paternity, maternal contamination, and sample exchange. Misdiagnosis due to failure of DNA amplification may obviously be less frequent with the duplicate methodology used in our center. In order to limit the possibility of misdiagnosis due to false paternity or maternal contamination, besides mutation analysis, we also carry out studies of an appropriate polymorphic sequence. The effect of maternal contamination can also be limited by

Table 6
Uptake of prenatal diagnosis of β-thalassemia following introduction of CVS

	Couples counselled (No.)	Couples deciding for prenatal diagnosis	%
Before CVS[a]	1125	1047	93.1
After CVS	3045	3016	99.4

[a] by fetal blood sampling and amniocentesis.

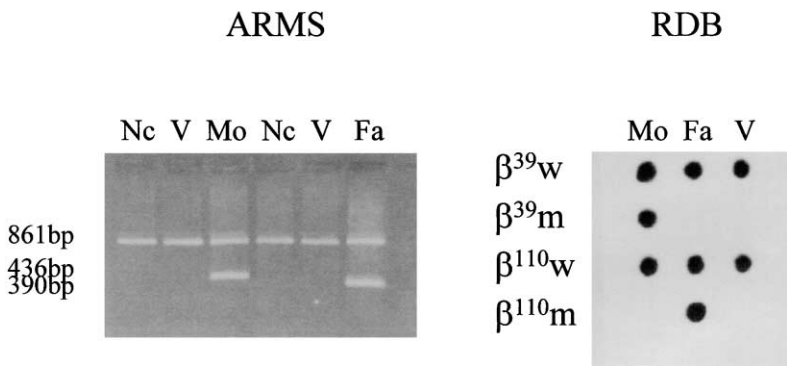

Fig. 7. Prenatal diagnosis by mutation analysis in a fetus at risk for β39/βI-110 mutations: Left: ARMS analysis for β39 (first 3 lines) and βI-110 (last 3 lines) mutation; Nc, normal control; V, villus DNA from fetus normal for both mutations; Mo, mother heterozygous for β39 mutation (436 bp fragment); Fa, father heterozygous for β IVSI-110 (390 bp fragment). Right: RDB analysis (w, wild type; m, mutant); V, villus DNA from fetus normal for both mutations.

careful dissection of the maternal decidua from the fetal blood trophoblast under the inverted microscope.

Efficacy of prevention programs

All prevention programs have been very successful, since the large majority of populations at risk improved their knowledge of β-thalassemia and practised its prevention without any substantial adverse effect [13]. Following non-directive counseling, the large majority of couples at risk opted in favor of prenatal diagnosis. Moreover, in all the populations in which education and counseling were introduced in the Mediterranean area, we observed a substantial decline in the birth rate of thalassemia major. For example, in Sardinia, the incidence of thalassemia major declined from 1:250 live births to 1:4000, with an effective prevention of the large majority of cases that would have probably been born had a prevention program not been in place (Fig. 8).

The most frequent reasons for the limited number of births that are affected with thalassemia major in our population today include the absence of informa-

Table 7
Overall results of prenatal diagnosis of β-thalassemia in Sardinia

	PCR-based analysis	Enzymatically restricted DNA with ASO probes	Fetal blood
Pregnancies monitored	3590	1194	1131
Homozygous fetuses	879	325	286
Failures	0	7 (0.6%)	10 (0.9%)
Misdiagnosis	0	1 (0.08%)	2 (0.2%)

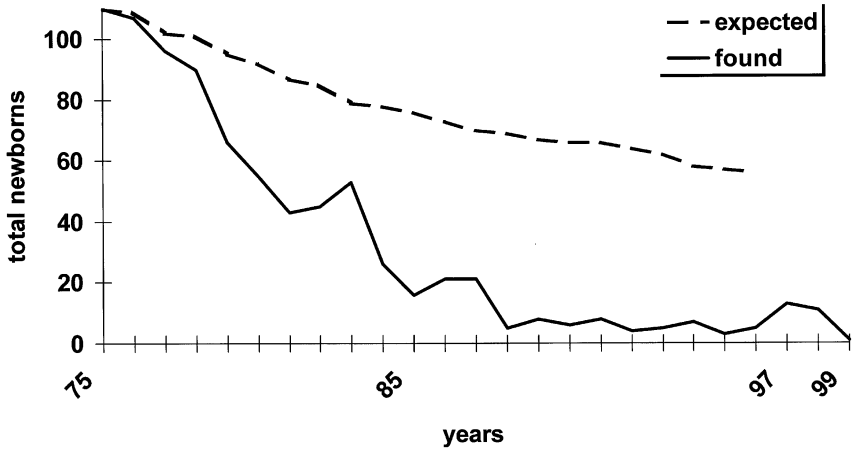

Fig. 8. Fall of the birth rate of babies with homozygous β-thalassaemia in Sardinia.

tion, misdiagnosis, false paternity, and adverse attitude towards prenatal diagnosis and/or unwillingness to terminate pregnancy. Very similar results have been obtained in all the other Mediterranean populations in which screening and counseling have been introduced [1,12,32].

The reason(s) for success

If all these programs have been successful, it is first of all due to the educational campaign that fully informed the large majority of adults about thalassemia at, or before, marriage, thus offering them the opportunity of making an informed decision about reproduction. This population education program, at least in Sardinia, took advantage of the fact that most of the population was distributed in small villages of 2,000–3,000 inhabitants where it was easier to spread the knowledge of thalassemia. As mentioned above, the efficacy of the screening program was magnified by the fact that the relatives of carriers and patients were also screened. Lately, the introduction of teaching on thalassemia in secondary schools may also have played a role in further reinforcing the knowledge of thalassemia. Since screening, counseling, and prenatal diagnosis were introduced through the Social Health Service, they are completely free of charge. This obviously removed potential economic access barriers to health facilities. Another very important prerequisite for success was the implementation of adequate facilities to meet the demand for screening, counseling, and prenatal diagnosis before the educational campaign. Finally, we believe the program would not have been so successful without the collaboration of a very motivated staff, especially at critical times such as for counseling and prenatal diagnosis.

Further prospects

Technical speaking, the oligonucleotide microchip procedure [43] is very likely to be introduced. This may lead to direct mutation detection as a screening procedure and subsequent elimination of carrier detection based on haematological studies. Thanks to microchip analysis, moreover, other common disorders of our population, such as Wilson's disease and autoimmune polyendocrinopathy type I may be included in the carrier screening process.

Chorionic-villous DNA analysis could be simplified by using an automated procedure, such as DNA high pressure liquid chromatography analysis (DHPLC) [42]. We have already set up the procedure of pre-implantation diagnosis through the analysis of a single blastomere from an eight-cell embryo following in vitro fertilization [56]. The option for pre-implantation diagnosis will soon be discussed in the counseling session, especially with couples who have had several interrupted pregnancies due to affected fetuses, and are therefore against further pregnancy termination. It is worth mentioning that in a study carried out at our center on preimplantation genetic diagnosis, the large majority of women who underwent CVS with subsequent pregnancy interruption prefer preimplantation diagnosis in future pregnancies [57].

As at other centers, we too are carrying out studies to make prenatal diagnosis feasible in clinical practice through the analysis of fetal cells in maternal circulation [58,59]. Promising and encouraging results have been obtained by isolation of nucleated-red blood cells by micro dissection under light microscopy and non-radioactive PCR analysis following density gradient separation of mononuclear cells from maternal blood, enrichment of fetal cells by magnetically activated cell sorting using the anti-transferrin receptor antibody, and immunostaining of fetal cells by anti-fetal or embryonic Hb antibodies.

Nevertheless, the most important challenge for the future is the organization of this kind of program in populations where β-thalassemia is prevalent; such an enterprise is still not possible at the present state of development.

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Genetic screening for cystic fibrosis

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Cystic fibrosis is the most common autosomal recessive condition affecting Caucasian people of European or Ashkenazi Jewish descent. The carrier frequency is 1/29 and the incidence is 1/3300 [1]. In this article, we shall consider clinical manifestations, frequency in various ethnic groups, the spectrum of responsible mutations, and current guidelines for genetic screening.

Clinical manifestations

The name "cystic fibrosis" was derived from the histopathologic findings noted on inspection of the pancreas from affected individuals [2]. Later it was suggested that the primary physiologic perturbation, and thus the clinical manifestations noted below, rested in the inability to clear viscous mucous from pulmonary and gastrointestinal tissue [3]. Recognition of the recessive inheritance pattern of this disease dates to the 1940s [4], but the gene was not isolated until 1989 [5–7]. Important progress in understanding the molecular pathogenesis of cystic fibrosis has also been recent (1980s onward).

Chronic and progressive are important terms used to describe the clinical manifestations of cystic fibrosis. These clinical characteristics can be divided into typical and atypical. Typical features involve the respiratory and gastrointestinal system. Within the respiratory tract, these most commonly include cough, wheezing, and recurrent pneumonia. The cough is similar to that described for patients with chronic obstructive pulmonary disease. The cough is hacking in nature, later turning to a productive cough. The cough is worse in the morning and after activity [8]. Obstruction secondary to an inability to clear mucus produced in the bronchial tree combined with recurrent infection (*Staphylococcus aureus*, *Hemophilius influenzae*, and *Pseudomonas aeruginosa*) results in progressive deterioration of lung function [8]. Unlike either pure

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obstructive or restrictive pulmonary disease, lung function studies (Fig. 1) of patients with cystic fibrosis demonstrate progression from a pattern of airway obstruction (secondary to retained mucous secretions) to a more restrictive pattern (scarring and damage of fibroelastic tissue from recurrent infection) [8,9]. The hyperinflation often seen on early chest X-ray evaluation gives way to bronchiectasis, bronchial wall thickening, and enlarged hilum. Clinical progression of disease is also accompanied by complications commonly observed among patients with chronic lung diseases of either the obstructive or restrictive type (Fig. 1) [8].

The second major affected organ system is gastrointestinal. A gastrointestinal feature that may be manifest as early as the second trimester of in utero life is echogenic bowel. In one recent series, cystic fibrosis was confirmed in 5% of cases of echogenic bowel [10]. Estimates are that 10–20% of neonates with cystic fibrosis will develop meconium ileus in the neonatal period. Inability to pass meconium is believed to be caused by a change in stool consistency that results from abnormal pancreatic enzyme secretion and reduced water content of stools [8]. Recurrent bowel obstruction, intussusception, and rectal prolapse are gastrointestinal tract complications that result from cystic fibrosis. Pancreatic insufficiency results in protein and fat malabsorption and in most cases of cystic fibrosis is present from birth [8]. Along with the expected impact on growth, added complications attributable to malabsorption of fat include vitamin deficiencies. Skin and eye disease (vitamin A), bone demineralization (vitamin D), neuronal dystrophy (vitamin E), and predisposition to hemorrhage (vitamin K) are a few of the complications that can result from

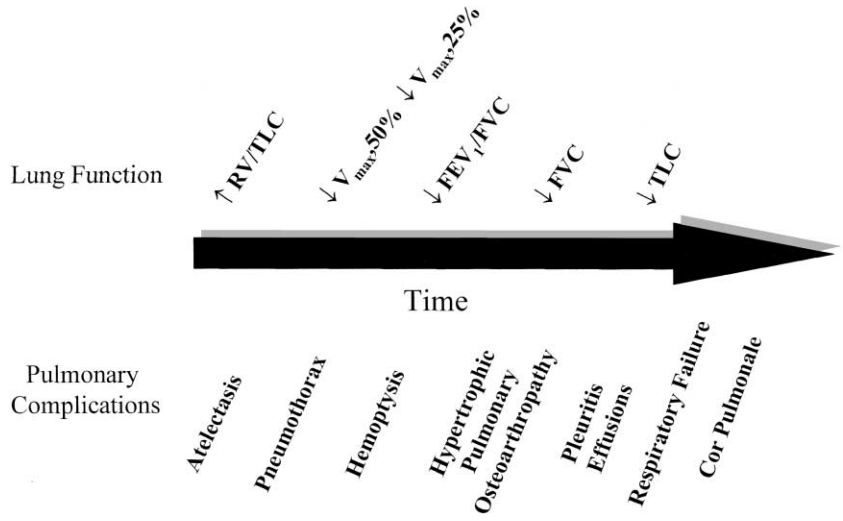

Fig. 1. Deterioration in pulmonary function and progression of clinical features among patients with cystic fibrosis.

these vitamin deficiencies. Diabetes mellitus may be observed, especially in older patients [11]. Inspissation of biliary secretions leads to focal biliary cirrhosis. Often this is asymptomatic but associated with a mild elevation in alkaline phosphatase [12]. Although no more than 5% of patients with this histologic feature become symptomatic, those who do may present with features similar to those with end-stage liver disease (i.e., hyperbilirubinemia, ascites, edema, and bleeding esophageal varices). Hepatosplenomegaly in a patient with cystic fibrosis establishes the presence of portal hypertension [8]. Table 1 represents a compilation of many of the secondary complications that can be seen among patients with cystic fibrosis. Recognition of these complications may be important to those taking a medical or family history from a patient at risk for cystic fibrosis or the carrier state. In some cases, the complications listed are the presenting feature or only clue of cystic fibrosis. Family members may only recognize or recall the cause of death as resulting from the secondary complication (e.g., massive hemorrhage from a bleeding esophageal variceal).

The clinical course of cystic fibrosis is variable. This variability may lead to confusion when discussing the merits of screening for this disease during pregnancy. As noted earlier, the disease is chronic and progressive. In about two thirds of cases, the diagnosis is made during the first year of life, and these cases are always severe [1]. Median survival of patients affected with cystic fibrosis ranges from 27–29 years [13]; however, patients representing both extremes in survival have been described. Progression of pulmonary disease is not a function of the specific genetic perturbation. Rather, climate, race, age at diagnosis, and gender play a combined role in establishing disease progression [8]. A hallmark more recently appreciated is that clinical severity within ethnic groups can vary as a result of the molecular principle known as genetic heterogeneity (mutations within a gene are varied, and this

Table 1
Pulmonary and gastroenterologic complications observed in cystic fibrosis

Organ system	Complications
Pulmonary	Atelectasis
	Pneumothorax
	Hemoptysis
	Aspergillus fumigatus
	Hypertrophic pulmonary osteoarthropathy
	Pleuritis/pleural effusion
	Respiratory failure
	Cor pulmonale
Gastroenterologic	Meconium ileus
	Bowel obstruction
	Intussusception
	Rectal prolapse
	Malabsorption of fat soluble vitamins
	Diabetes
	Non-alcoholic cirrhosis

variability accounts for some of the observed variation in the clinical phenotype). When clinical manifestations of disease can be predicted according to the mutation detected on the maternal and paternal copy of the gene, this is called genotype-phenotype correlation. With the exception of pancreatic insufficiency, genotype-phenotype correlation is not a typical feature of cystic fibrosis (Table 2).

Diagnosis

Along with the clinical features of the pulmonary (cough and recurrent pneumonia) and gastrointestinal (malabsorption and poor weight gain) tracts, the ''gold standard'' diagnostic test for cystic fibrosis is historically the sweat chloride test (>60 meq/L) [14]. Although the number and structure of eccrine sweat glands in skin is normal, the salt content is not. Sodium chloride and potassium are elevated; the primary pathogenesis of this is attributed to a failure to reabsorb chloride along the sweat gland. These baseline electrolyte losses establish a predisposition for hypochloremic acidosis in younger children. It is believed that chronic salt depletion underlies the observed lower systolic and diastolic blood pressure among patients with cystic fibrosis compared with appropriate controls [15]. Since elucidation of the gene in 1989, a molecular diagnosis has become possible. The gene CFTR encodes a chloride channel. Identification of two abnormal CFTR alleles is diagnostic of cystic fibrosis, but as will be pointed out, failure to identify mutations on each chromosome does not rule out cystic fibrosis [8]. Molecular diagnosis is particularly helpful in that abnormalities in sweat chloride testing can occur as a result of other clinical entities. Furthermore, intermediate sweat chloride values (40–60 meq/L) and even normal values (presence of splice site mutation 3849 + 10kbC → T) have been documented for some patients with cystic fibrosis [16].

Mutation analysis

The CFTR gene located on chromosome 7q31.2 consists of 27 coding exons, and the genomic sequence spans 230 Kb [14]. A classification scheme

Table 2
Genotype-phenotype relationship

Organ system	Correlation
Pulmonary	
Onset of lung disease	No
Severity of lung disease	No
Progression of lung disease	No
Gastrointestinal	
Pancreatic insufficiency	Yes
Cirrhosis	No

Table 3
CF carrier screening mutation panel

Mutation	(Ethnicity[a])/frequency[b] Caucasians	Mutation	Ethnicity/frequency Caucasians	Mutation	Ethnicity/frequency Caucasians
Δ F508	(Danish)/66	G542X	(Spanish;Ashkenazi Jewish)/2.4	A455E	
R553X	(German)/0.7	R117H	0.7	711 + 1G → T	(French-Canadian)
R1162X	(Italian)/0.3	R334W		3659delC	
2184delA		3849 + 10kbC → T		N1303K	(Italian)/1.3
3120 + 1G → A	(African-American)[c]	G551D	(English)/1.6	R560T	
Δ I 507		1717-1G → A	(Italian)/0.6	1898 + 1G → A	
621 + 1G → T	(French-Canadian)/0.7	R347P		I148T	(French-Canadian)
G85E		2789 + 5G → A	(Spanish)		
1078delT		W1282X	(Ashkenazi Jewish)[d]/1.2		

[a] Most common worldwide ethnic/racial groups listed.
[b] Expressed as % of US cystic fibrosis alleles in Caucasians.
[c] 11% frequency among African-American individuals.
[d] 60% frequency among Ashkenazic-Jewish individuals.

has been proposed that attempts to correlate the cellular dysfunction caused by the abnormal cystic fibrosis transmembrane conductance regulator protein and the type of mutation [8]. To date, nearly 1000 CFTR mutations have been identified. In an effort to standardize the laboratory approach to screening, the Subcommittee on Cystic Fibrosis Screening, Accreditation of Genetic Services Committee, American College of Medical Genetics (ACMG) recommended the use of a pan-ethnic panel that includes all mutations with an allele frequency of at least 0.1% in the general US population for use in screening (Table 3). Currently, 25 mutations make up this panel. In addition to those shown in Table 3, under certain circumstances laboratories have been mandated to perform reflex tests in an effort to decrease the chances of an incorrect diagnosis of ΔF508 homozygosity. The I506V, I507V, and F508C are not associated with classic cystic fibrosis. Laboratory testing may result in these alleles being interpreted as ΔF508 when the opposite chromosome is known to be ΔF508. Reflex testing for exon 8 thymidine tracts of five, seven, or eight nucleotides is performed when the R117H allele is identified (Fig. 2). The significance of this allele and the relationship of this allele to the thymidine sequence are shown. Furthermore, a recommendation was made for continued surveys of allele frequencies within ethnic groups and fluidity with respect to the list of mutations screened for [17]. The prevalence of specific mutations

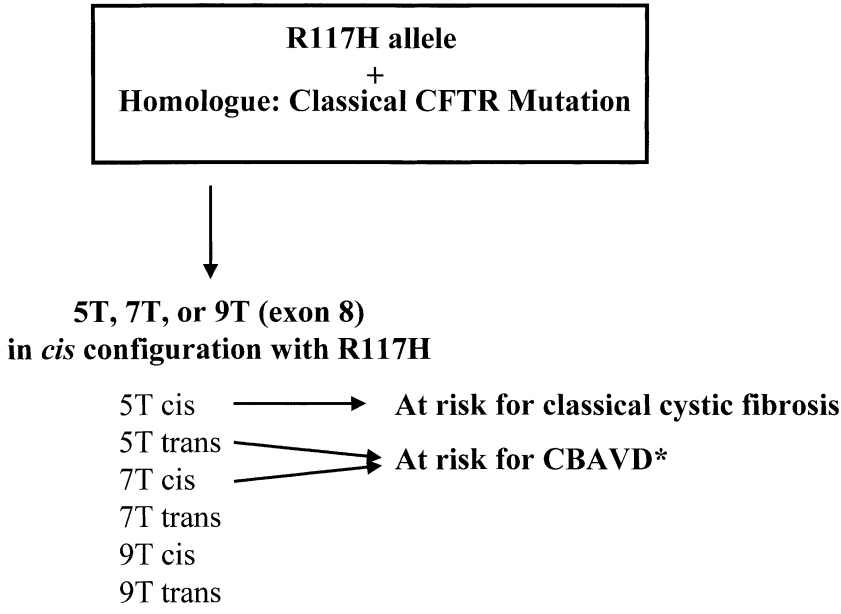

Fig. 2. Reflex testing performed and its significance when one allele identified is R117H.

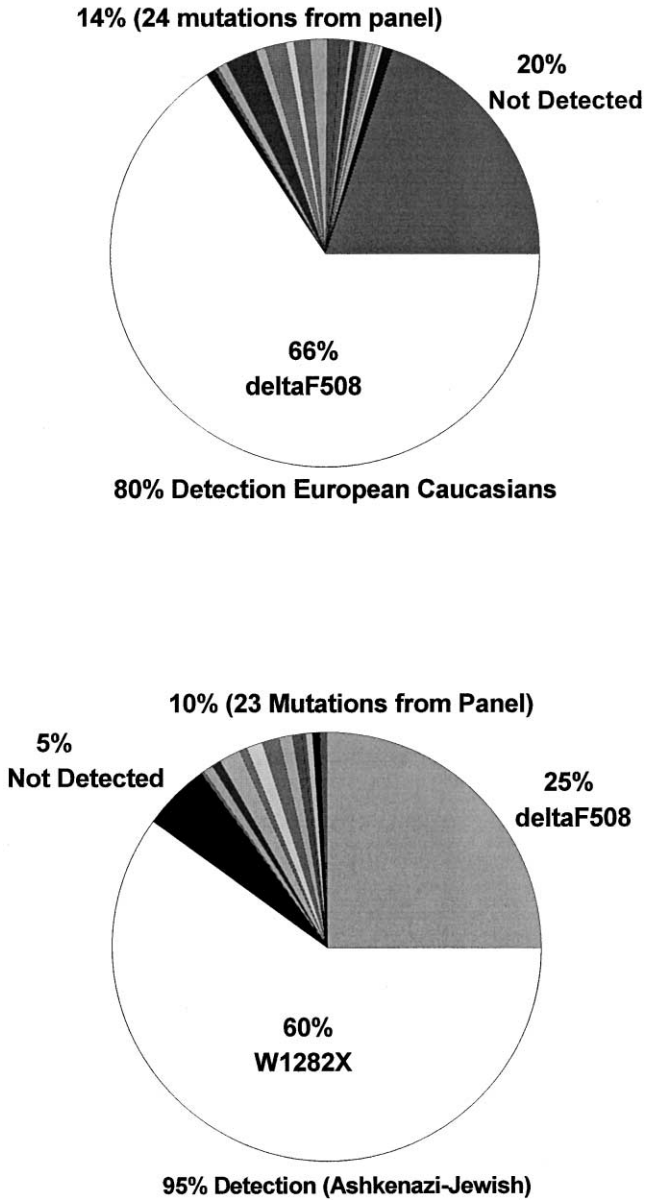

Fig. 3. European Caucasians compared with Ashkenazi Jewish population with respect to detection rate and most common mutations.

among ethnic groups varies widely. One can see that even among the ethnic groups for which screening should be offered, the prevalence of specific mutations varies considerably (Fig. 3).

Table 4
Risk estimates for high-risk population

Risk without screening	Individual risk	Risk to pregnancy without screening	
Ashkenazi Jew	1/29	1/29 × 1/29 × 1/4 = 1/3,364	
Caucasian	1/29	1/29 × 1/29 × 1/4 = 1/3,364	
Risk with screening (negative)	Individual risk screening (negative)	Both negative[a]	One negative[a], one not tested
Ashkenazi Jew	1/930	1/930 × 1/930 × 1/4 = 1/3,459,600	1/930 × 1/29 × 1/4 = 1/107,880
Caucasian	1/140	1/140 × 1/140 × 1/4 = 1/78,400	1/140 × 1/29 × 1/4 = 1/16,240
Risk with screening (positive)	One positive, one negative	One positive, one not tested	Both positive
Ashkenazi Jew	1 × 1/930 × 1/4 = 1/3,720	1 × 1/29 × 1/4 = 1/116	1 × 1 × 1/4 = 1/4
Caucasian	1 × 1/140 × 1/4 = 1/560	1 × 1/29 × 1/4 = 1/116	1 × 1 × 1/4 = 1/4

Residual risk values are a function of ethnicity and the frequency of specific mutations tested in screening panel.

[a] Refers to one or both members that make up the couple being tested.

Table 5
Risk estimation among low-risk populations

Individual risk assessment		Detection rate	Carrier frequency		Risk carrier negative test
Hispanic-American		57%	1/46		~1/105
African-American		69%	1/65		~1/207
Asian-American		Unknown	1/90		Unknown
Risk of offspring with cystic fibrosis	No testing	One[a] negative one not tested	One positive one negative	One positive one not tested	Both negative
Hispanic-American	1/8,464	1/19,320	1/420	1/184	1/44,100
African-American	1/16,900	1/53,820	1/828	1/260	1/171,396
Asian-American	1/32,400	Unknown	Unknown	1/360	Unknown

[a] Refers to one or both members that make up the couple being tested.

Screening

Whom to screen and when to offer screening have been addressed by the combined efforts of The American College of Obstetricians Gynecologists (ACOG), the National Institutes of Health (NIH), and the ACMG. Perhaps the most subtle aspect of the recommendations made recently is the distinction of the terms *offering screening* to couples in which at least one member is of a high risk ethnic group versus *making screening available* to persons of "other" ethnic groups. Offering screening requires the direct participation by the physician or support staff in educating and discussing medical aspects of cystic fibrosis, the testing procedure, and the interpretation of test results. Making screening available requires the dissemination of information to the couple through printed materials. One should keep in mind that screening must always be voluntary and always requires informed consent. The manner by which a couple expresses they would use the information gained from screening should not be a factor in the health-care provider's decision to offer or make screening available. The most recent recommendations were as follows [1]: Screening should be *offered* to (1) adults with a family history, (2) reproductive partners of individuals with cystic fibrosis, (3) couples in whom one or both are Caucasian (including Ashkenazi Jewish) and are planning a pregnancy, and (4) couples in whom one or both are Caucasian (including Ashkenazi Jewish) and are seeking prenatal care. Screening should also be *made available* to couples where one or both partners are not Caucasian or Ashkenazi-Jewish.

Approach to screening

Many factors enter into the equations used to determine efficacy of screening programs. One factor important in a prenatal screening program aimed at a recessive condition is the need to establish carrier status of both partners in order to determine risk to the fetus. The time at which patients present for prenatal care, limitations on the gestational age after which pregnancy cannot be terminated,

and duration of the expected pregnancy are important in this discussion, but as a rule they cannot be impacted by the obstetrician or are already predetermined. Screening couples generally follows one of two approaches: (1) sequential screening is performed when the gravida is screened first, with the man screened only if the woman tests positive, or (2) concurrent screening attempts to screen the female and male partners at the same time. Sequential screening attempts to exploit the fact that fewer than 1% of at-risk couples will be screen positive. Accessibility of the other partner or cases in which ethnic groups are not the same within a couple are commonly cited reasons for utilizing this approach. Concurrent screening is more sensitive to time constraints for decision-making and when more than one recessive condition is being screened for this approach maximizes time for decision making. Perhaps the most important reason for care providers and patients to adopt either method preferentially is that residual risk after testing is altered. Recently issued recommendations make it clear that either approach is acceptable.

Informing the patient

Patients need to be informed in order to decide if screening for cystic fibrosis is desired. Salient information includes: (1) the purpose of screening, (2) the voluntary nature of screening, (3) medical aspects of cystic fibrosis, (4) genetics of cystic fibrosis, (5) population-based estimates of carrier status, (6) how to interpret test results, and (7) individual and cultural values.

Interpreting screening test results

Independent of whether patients are offered screening or screening is merely made available, there will likely be the need to present to patients information regarding the risk of being carriers and what that risk means for their planned or ongoing pregnancy. Risk assessment requires an understanding of each person's individual risk of carrying a mutation on one copy of the gene. Additionally, by focusing on the same risk for the partner allows one to understand the risk to the pregnancy. Any individual has a risk of being a carrier for cystic fibrosis that is directly related to their ethnicity as already explained. Furthermore, the sensitivity of carrier testing is a function of the number of mutations searched for and the individual's ethnicity. Of note, even when screening is negative there remains some chance that an individual still carries a copy of a CFTR mutation. The remaining risk after testing is called the residual risk. Residual risk can be calculated for each permutation of results (Tables 4 and 5), but residual risk is never equal to zero. This is because the recommended laboratory panel of mutations has an ethnic-specific sensitivity that never reaches 100%. There is always some possibility of having an affected child. On the opposite end of the spectrum, when both partners of a couple have a positive test result, it is not

certain that a child will have cystic fibrosis; instead, this risk is 25%. The risk increases to a maximum of 50% when an affected parent establishes a pregnancy with a known carrier. Thus, the goal of screening is to inform patients of their individual residual risk and thus the residual risk of a conception affected with cystic fibrosis. Subsequent to this is the responsibility to inform patients of their reproductive options when the risk is sufficiently high. These include the option of chorionic villous sampling or of amniocentesis to establish the exact risk of an affected fetus. The exact risk for any pregnancy is determined by either of these invasive methods. As shown in Tables 4 and 5, a number of permutations of test results are possible when providing antenatal screening for cystic fibrosis; therefore, communicating results to patients requires correspondence or direct conversation that is capable of addressing any of these outcomes. Sample form letters for communicating results to patients have been prepared [1]. In addition, patients found to be carriers may wish to communicate the results of their testing to other family members because others may also be carriers. To facilitate this process, a sample form letter that the patient may find useful is available [1].

Screening pitfalls

Pitfalls are an unfortunate part of any screening program, and that proposed for cystic fibrosis is no exception. Most important of all is that screening does not detect all mutations. One must recognize that screening during pregnancy assumes properly identified paternity. Residual risk estimates (Tables 4 and 5) assume no family history. Risk can be more accurately deduced with information that includes the known mutations in the affected family member. Estimates of residual risk are specific to the couple tested. Testing may reveal a fetus at risk for male infertility, and not pulmonary dysfunction. Genotype-phenotype correlation cannot be assumed.

Summary

The importance of the recent recommendations that address cystic fibrosis carrier screening cannot be overemphasized. For the first time, a systematic approach to offering or making screening available to all pregnant women in the hopes of providing refined risk estimates for a genetic disease has been established. Caucasian of European or Ashkenazi-Jewish descent should be offered screening. Within the proposed guidelines are ethnic-specific carrier frequencies (1/29) used to establish who should be offered testing and to whom testing should be made available. Recent recommendations have made clear that in a pan-ethnic population a frequency of 1/1000 is required for inclusion into the cystic fibrosis mutation panel. A general framework for screening during pregnancy has been established (either concurrent or sequential). It will be interesting to watch as the fruits of the human genome project are inspected

and applied to everyday clinical practice. No doubt the cost of screening will be reduced through advances in technology. The combined efforts of NIH, ACOG, and ACMG have provided the first set of comprehensive standards for screening of recessive diseases. How time changes these guidelines deserves following.

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Counseling the at risk patient in the *BRCA1* and *BRCA2* Era

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There are a variety of factors that increase a woman's risk of developing either breast or ovarian cancer. One of the most significant risk factors for these cancers is genetic predisposition. With the identification of the *BRCA1* [1] and *BRCA2* [2] genes in 1994 and 1995 respectively, a new era of genetic risk assessment and genetic testing for the predisposition of breast and ovarian cancer began. The identification of these genes has given individuals the choice to find out whether they are at increased risk to develop cancer due to a genetic predisposition. The knowledge of one's genetic status has clinical significance with regard to prevention, screening and treatment as well as psychological impact on the individual and their family.

The discovery of these genes and the numerous articles on genetic predisposition to cancer in the lay press has resulted in a rise in demand for genetic testing by individuals with a strong family history as well as those with a more moderate family history. Women from low risk families have also shown interest in genetic testing [3] since some will overestimate their risk to develop cancer.

In this article we will focus on the genetic predisposition of breast and ovarian cancer due to mutations in the *BRCA1* and *BRCA2* genes. In addition, we will provide guidelines for genetic counseling for cancer predisposition and recommendations for follow-up of women who have undergone testing.

BRCA1

The first major breast and ovarian cancer predisposition gene was located on chromosome 17q21 in 1989 [4] and subsequently cloned in 1994 [1]. It is a large gene containing 24 exons and encodes a protein of 1863 amino acids. The *BRCA1*

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gene functions primarily as a tumor suppressor gene with mutation of the remaining copy of the *BRCA1* gene in the tumor cells of women who carry a constitutional mutation [1]. Recent studies have demonstrated that the normal function of the BRCA1 protein is in recognition of DNA damage [5]. Thus, loss of *BRCA1* function predisposes somatic cells to an increase in mutations and subsequent tumorigenesis. Surprisingly, there is no evidence for somatic mutations in the *BRCA1* gene in breast cancers of women who do not inherit a *BRCA1* mutation [6] and only a few somatic *BRCA1* mutations have been reported in sporadic ovarian cancers [7]. There is, however, significant scientific controversy as to the regulation of the *BRCA1* gene and protein in sporadic breast cancers, which is beyond the scope of this article.

To date more than 800 constitutional mutations in the *BRCA1* gene have been reported to the Breast Cancer Information Core [8]. The majority of the mutations are frameshift or nonsense, which lead to premature protein termination. These mutations can be found throughout the entire gene. Most mutations are unique to one or a few families and are termed: ''private mutations.'' Only a handful are seen repeatedly and termed ''founder mutations.'' The best-studied founder mutations are two mutations that are prevalent in the Ashkenazi Jewish population. The first mutation is known as 185delAG, which has been found in 1% of the Ashkenazi Jewish population and in 21% of women of Ashkenazi Jewish descent with breast cancer [9]. The second founder *BRCA1* mutation is 5382insC, which is present in 0.1% of Ashkenazi women [10]. Other founder mutations are specific to other populations including the Dutch [11,12] and Icelandic [13]. Within the United States, the only founder mutations for which direct testing is routinely performed are the Ashkenazi Jewish mutations. For other individuals full sequencing of the gene is performed as described below.

In addition to clearly deleterious truncating mutations, several missense mutations and variants have been found in the *BRCA1* gene. For some of the missense variants it is not clear whether they are disease causing or benign polymorphisms and their clinical significance is unknown. Splice mutations have also been identified in the *BRCA1* gene, which are caused by base substitutions, insertions or deletions in the introns, which alter consensus splice sites and can lead to a loss of an exon [8]. Detailed information regarding deleterious mutations and variants in the *BRCA1* gene can be viewed on the BIC website at the National Institutes of Health [14].

BRCA2

In 1995, the second breast cancer susceptibility gene localized to chromosome 13q12 was cloned by Stratton and colleagues [2]. The *BRCA2* gene is even larger than *BRCA1* and contains 27 exons and encodes a protein of 3418 amino acids. Similar to *BRCA1*, many recent studies have demonstrated that the BRCA2 protein plays an important role in DNA repair [5]. Also, as seen for *BRCA1*, mutations in *BRCA2* follow the two hit hypothesis with tumor samples of women who carry a constitutional mutation demonstrating somatic mutation in the second

copy. Once again, there is little evidence for mutations in the *BRCA2* gene in truly sporadic breast or ovarian cancer [15,16].

More than 100 mutations have been reported and similar to *BRCA1* the majority cause premature chain termination. The prevalence of *BRCA* mutations for women with breast cancer under the age of 36 is estimated at 2.4% and between the ages of 36 to 45 the estimated prevalence is 2.4% [17]. An Ashkenazi Jewish founder mutation, 6174delT, has also bee found in the *BRCA2* gene. This specific mutation is found in about 1% of the Ashkenazi population [10,18]. Overall, 2.5% (one in forty) of the general Ashkenazi population carries one of the three founder mutations in *BRCA1* and *BRCA2.*

Prevalence of BRCA1 and BRCA2 mutation carriers among cancer patients

Early studies demonstrated that approximately half of all cases of familial breast cancer are due to mutations in the *BRCA1* gene and in 80% of families with 4 or more cases of early onset breast cancer (under age 50) in a family [19]. However, these studies were based on heavily affected early-onset families that were ascertained for linkage studies and probably represent overestimates. More recently, Ford et al [20] examined 237 high-risk families and found 52% of the families carried a *BRCA1* mutation, 32% carried a *BRCA2* mutation and 16% neither gene. In the same study, 76% of the families with both male and females affected with breast cancer, mutations in *BRCA2* were found [20]. This discrepancy between the predicted prevalence of a *BRCA1* mutation based on linkage studies and the number of mutations actually found could be partially due to the method (PCR-based direct sequencing) used today to detect mutations. PCR based methods are likely to miss large rearrangements or deletions of the *BRCA1* gene. Petrij-Bosch et al [12] studied breast cancer families in the Netherlands and found that approximately 36% of *BRCA1* mutations would not be detected by direct sequencing methods. Several genomic rearrangements are more frequent in specific populations, for example, an exon 13 duplication, which causes a frameshift mutation, has been found in 3 unrelated US families of European decent, one family of Portuguese descent [21] as well as in families of British descent. Unger et al [22] studied 42 US families with breast and ovarian cancer previously found to be negative for both *BRCA1* and *BRCA2* mutations by full sequencing. Five of 42 families had a rearrangement. These recent findings emphasize the need to incorporate detection of genomic rearrangements into clinical testing for high-risk families.

A large number of studies of women from less highly selected populations have focused on *BRCA1* and *BRCA2* mutation frequency in those with less significant family histories. In a population-based cohort of women with breast cancer, unselected for family history, 5.9% to 6.2% of the affected women under 35 years of age were found to carry a *BRCA1* mutation and 3.4% were carriers of a *BRCA2* germline mutation [23, 24]. In the same study of women under 45 years of age with breast cancer and a first-degree relative with breast cancer,

7.1% carried a germline mutation in *BRCA1* and 4.9% carried a *BRCA2* mutation. Also noted in these studies was the positive correlation between the number of affected family members, the earlier age of diagnosis and the mutation frequency. These results were also confirmed by Peto et al [17] who detected *BRCA1* mutations in 3.1% of women with breast cancer under the age of 36 and 1.9% in women with breast cancer between ages 36 to 45. Couch et al. [25] found that among women with breast cancer at any age and a moderate family history of breast cancer (but no ovarian cancer) only 16% of the women studied were found to carry a *BRCA1* mutation. This frequency increases to 30%–40% if the proband is of Ashkenazi Jewish descent or if there is ovarian cancer in the family. Although these studies all used different methods they highlight that the majority of women with very early onset of breast cancer do not carry mutations in *BRCA1* or *BRCA2.*

In ovarian cancer families, Narod [26] found that 92% of families with breast cancer and 2 or more cases of ovarian cancer showed linkage to mutations in *BRCA1.* A recent large population-based study [27] of 515 women with ovarian cancer found that 11.7% were carriers of *BRCA1* and *BRCA2* mutations. This increased to 18.4% of women diagnosed between ages 40 and 50. In addition, women who have had both breast and ovarian cancer are very likely (>80%) to carry a *BRCA1* mutation [28]. Thus, the likelihood of finding mutations is a function of age of onset, number of affected relatives and presence of ovarian cancer. As described below, most cancer genetic and high-risk clinics use computer models to estimate for the individual patient the likelihood of finding a mutation in these genes.

Many studies demonstrate that carriers of mutations in the *BRCA2* gene have an increased risk of early-onset breast cancer and ovarian cancer but the risks are somewhat lower than for women who are carriers of a *BRCA1* mutation [20, 29]. Krainer et al [29] found that *BRCA2* mutations are less frequent than *BRCA1* mutations among women with early-onset breast cancer in the general population, which may be due to the lower prevalence of these mutations in the population. Risch et al. found that the average age of diagnosis of ovarian cancer in *BRCA2* carriers to be 57.5 years compared to 51.2 years for *BRCA1* carriers [27]. Of women who carried a mutation those diagnosed with ovarian cancer over the age of 60 were more likely to be *BRCA2* carriers and for women diagnosed with ovarian cancer under 50 years of age, the majority were *BRCA1* carriers.

Cancer incidence in BRCA1 and BRCA2 mutation carriers

Since the initial identification of linkage to either *BRCA1* or *BRCA2,* significant effort has been made to determine the likelihood of developing a particular cancer in mutation carriers. Table 1 summarizes these risks and they are described in detail below. These studies have often obtained different results based on the design of the study and potential for biased ascertainment. In addition, the range and variation in cancer rates could reflect the presence of other modifying genetic factors. Studies are now underway that are examining the genetic and environ-

Table 1
Estimated lifetime risks associated with *BRCA1* and *BRCA2* mutations

Cancer	*BRCA1*	*BRCA2*
Breast (♀)	50–85% [27,31–33]	50–85% [14,27,33]
Breast (♂)		6–7.5% [14]
Ovarian	15–40% [27,31–33]	14–27% [14,33]
Colon	6%* [32]	
Prostate	8–16%* [32]	7–16% [33]
Pancreas		1.5–2.1 [14]

♀ Female; ♂ male
* The risks were not confirmed by other studies

mental modifiers of penetrance in *BRCA1* and *BRCA2* mutation carriers. Some candidates for modifier genes have been described including DNA repair genes, which contain polymorphisms, associated with an increased relative risk for breast cancer [30].

Breast cancer

From several studies of high-risk families it is estimated that *BRCA1* mutations confer a lifetime risk of 50% to 85% for developing breast cancer [27,31,32]. and on average, approximately 59% of women will develop it premenopausally. However, in population-based studies of Ashkenazi Jewish women carriers of one of the three founder mutation in *BRCA1* and *BRCA2* the risk for breast cancer was lower and estimated at 55% by age 70 [33]. Similar results have been seen by Fodor et al., which found a 36% lifetime risk for breast cancer for carriers of 185delAG in *BRCA1* and 6174delT in *BRCA2* in breast cancer patients of Ashkenazi Jewish decent in the New York area [34]. Despite the range in breast cancer risk estimates, the majority of studies find a significantly increased risk of breast cancer with a substantial shift towards development of the cancer at an early age.

Women with breast cancer who are mutation carriers are at increased risk for developing contralateral breast cancer [32,35,36]. Ford et al [32] studied 33 high-risk *BRCA1* families and found the diagnosis of a second breast cancer at 48% and 64% by age 50 and 70, respectively. Similarly, Verhoog et al studied 164 patients from *BRCA1* positive families and found 40% of the women diagnosed with the first breast cancer under the age of 50 and 12% of the women diagnosed over age 50 had developed contralateral breast cancer [36].

Male breast cancer has been found to be associated with *BRCA2* mutations and in some populations this association is stronger than in others. For example, in the Swedish population in 7/34 (20%) male patients with breast cancer were carriers of a *BRCA2* mutation even without a family history of breast and/or ovarian cancer [37]. In another population-based study of men in the US with breast cancer 30% had a family history of breast or ovarian cancer and of those only 4% were carriers of a *BRCA2* mutation [38]. This difference

may reflect the frequency of founder *BRCA2* mutations in the Swedish population compared with the general US population. Studies in the US have found lifetime estimates for breast cancers in male *BRCA2* mutation carriers of 2.8% to 6.3% [39].

Reproductive cancers

Early studies of families with *BRCA1* mutations demonstrated a 15% to 40% lifetime risk or developing ovarian cancer [27,31,32]. In women with breast cancer that carry a *BRCA1* mutation, there is an estimated risk for ovarian cancer of 29% and 40% by age 50 and 70, respectively [32]. Although early linkage studies emphasized that ovarian cancer was more associated with *BRCA1* mutations, it has become clear that women who carry *BRCA2* mutations have an increased risk as well [27]. Interestingly, it appears that the risks of breast and ovarian cancer in *BRCA2* mutation carriers are related to the specific site of the mutation in the gene. Gayther et al reported on families with *BRCA2* mutations with increased ovarian cancer and relatively less breast cancer [40]. These families were found to have mutations within exon 11 between nucleotides 3035 and 6629 and this region was called the "ovarian cancer cluster region (OCCR). In a recent study, Thompson et al confirmed this finding [41]. In this study of 164 *BRCA2* families, mutations in the OCCR conferred a cumulative risk of 32.6% of developing breast cancer by age 70 compared with a cumulative risk of 46.2% outside the OCCR region. The cumulative risk for ovarian cancer by age 70 was estimated to be 19.5% within the OCCR region compared to 10.9% outside this region. Of note, the Ashkenazi founder *BRCA2* mutation is found within the OCCR region.

The likelihood that women with the diagnosis of borderline ovarian tumors carry mutations in *BRCA1* and *BRCA2* has been reported by several groups. Gotlieb et al studied the rates of the Ashkenazi Jewish founder mutations in 46 Ashkenazi Jewish women diagnosed with borderline ovarian tumors and unselected for family history [42]. Only one woman (2.2%) was found to carry one of these mutations—which is comparable to the expected population frequency—compared to 32% of women affected with invasive ovarian cancer of the same population group. In a second population-based study [27] of women affected with ovarian cancer and unselected for family history no mutation in either *BRCA1* or *BRCA2* were found in 134 women with borderline ovarian tumors. Thus, the diagnosis of borderline ovarian cancer does not increase the likelihood that a woman carries a mutation in *BRCA1* or *BRCA2* and emphasizes the need to review pathology records of women in the family reported to have ovarian cancer.

Recently, some attention has been given to women diagnosed with fallopian tube carcinoma. Aziz et al studied the importance of genetics as the possible cause of this cancer [43]. They found an increase in the risk of ovarian cancer (relative risk 2.2) and of early onset breast cancer (relative risk 2.4) in first degree relatives of women with fallopian tube carcinoma. They also found that 11% of the women

harbored a *BRCA1* mutation, 5% harbored a *BRCA2* mutation, and 28% of the women diagnosed before age 55 harbored a mutation in either gene.

Other cancers

There remains some controversy whether other cancers are associated with mutations in the *BRCA1* gene including colon cancer and prostate cancer. In a large population-based study of Ashkenazi individuals an increase in the risk for colon cancer in relatives of *BRCA1* carriers was found [33]. Similarly, a study from the Breast Cancer Linkage Consortium reported an increased risk for prostate cancer in carriers of *BRCA2* mutations [14]. However, other investigators have not been able to confirm these findings. In studies of Ashkenazi Jewish men with prostate cancer in Israel and the United States the rate of the *BRCA1* and *BRCA2* founder mutations did not differ significantly from the rate in the general Ashkenazi population [44–46].

Risch et al examined cancer risk in first-degree relatives of *BRCA1* carriers [27]. They found the following increased relative risks of cancers; ninefold for ovarian cancer, fivefold for breast cancer, sixfold for stomach cancer and a threefold for leukemias and lymphomas. These investigators also report that colorectal cancers occurred in family members of *BRCA2* carriers when the mutations were within the OCCR (relative risk 3.4). Overall, there appears to be a higher incidence of other cancers (non breast and ovarian) in families that carry *BRCA2* mutations, including, prostate, pancreatic, gallbladder, bile duct, stomach and malignant melanoma [14,39]. In particular, an increased risk of pancreatic cancer was found with a 2.1% cumulative risk by the age 70 (BCLC) and the pancreatic cancers were earlier in onset [14].

Other syndromes that predispose to breast and ovarian cancer

There are a number of other inherited cancer syndromes that predispose to breast and/or ovarian cancer that are listed in Table 2 (see Nathanson et al for a recent review [47]). Li Fraumeni syndrome (LFS) is characterized by early-onset breast cancer, childhood and adult sarcomas, brain tumors and other cancers. Mutations in the *p53* gene have been found in about 80% of the families with LFS [48] and in some families, mutations in the *hCHK2* gene have been found [49].

Cowden syndrome (CS) is a dominantly inherited syndrome characterized by hamartomas in different tissues such as breast, skin, thyroid, gastrointestinal tract, endometrium and brain. Another common feature of this syndrome is skin findings such as trichilemmomas, oral papillomatoses, acral and palmoplantar keratoses. Women with CS have a 20% to 25% risk for developing breast cancer at an early age (<50) and an increased incidence of thyroid cancer [50]. Germline mutations in the *PTEN* gene were found to cause CS. Hereditary nonpolyposis colon cancer (HNPCC) is associated with early-onset colon cancer, endometrial

Table 2
Other genetic conditions that predispose women to breast and or ovarian cancer

Condition	Gene	Associated cancers
Li-Fraumeni syndrome	*TP53*	Childhood sarcomas, bone, breast, brain, leukemia and lung cancers, adrenocortical carcinoma
Ataxia telangiectasia heterozygotes	*ATM*	Breast cancer in carriers
Hereditary Non-Polyposis Colon Cancer	*hMSH2*, *hMLH1*	Colorectal, endometrial, ovarian, biliary tract and ureteral cancers
Cowden syndrome	*PTEN*	Breast, thyroid and colorectal cancers
Peutz-Jeghers syndrome	*STK11*	Gastrointestinal polyps, ovarian and breast cancers

cancer and other GI malignancies (reviewed by Lynch [51]). There is also an increased incidence of ovarian cancer in HNPCC kindreds. HNPCC can be caused by mutations in one of five different mismatch repair genes.

Inheritance pattern of mutations in BRCA1 and BRCA2 genes

Mutations in *BRCA1* and *BRCA2* are transmitted as dominant traits. An individual who inherits a mutation (constitutional mutation) from one parent and a normal copy of the gene from the other parent is at increased cancer risk. Thus,

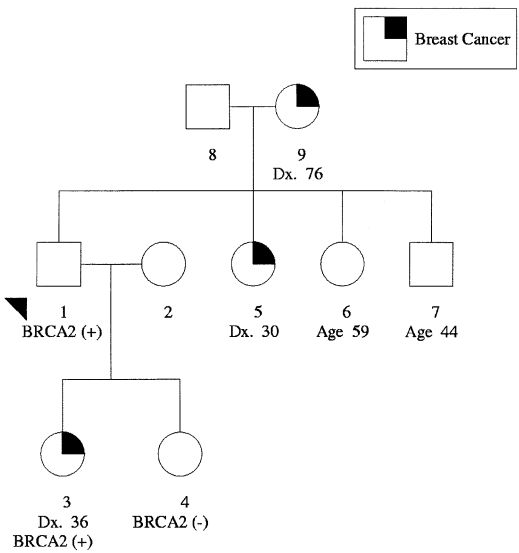

Fig. 1. Example of paternal inheritance of a *BRCA2* mutation. The proband (arrowhead) is an adult male with a sister and daughter with early onset breast cancer. DNA testing reveals that he and his affected relatives carry a truncating mutation in the *BRCA2* gene. Circles are females, squares are males. Dx.—age of cancer diagnosis.

with each pregnancy there is a 50% chance that the inherited susceptibility to cancer will be passed down to the next generation. This is true whether the abnormal gene is from the paternal lineage or the maternal. It is equally likely that a woman will inherit a *BRCA1* or *BRCA2* mutation from her mother or father. This fact may be overlooked when taking family histories. An example of a paternal inheritance of a *BRCA2* mutation may be seen in Fig. 1. Overall, for a person found to carry a mutation there is a 50% risk that their siblings and/or offspring have also inherited the mutation. Although at a genetic level these mutations do not "skip" generations, having a *BRCA1* or *BRCA2* mutation does not always lead to the development of cancer so a mutation carrier woman may be unaffected yet have a daughter with early onset cancer as seen in Fig. 2. Other factors such as penetrance of a specific mutation, eg mutations in the OCCR region, environmental factors, and or other modifying genes play a role and may determine whether a person will develop cancer.

Genetic counseling for breast and ovarian cancer predisposition

The complexities and the psychological impact of genetic testing for *BRCA1* and *BRCA2* mutations gave rise to specialized cancer genetics and high-risk clinics. The majority of these specialized clinics have adopted The National Society of Genetic Counselors guidelines for developing comprehensive genetic programs [52]. These genetic programs offer genetic testing in the context of pre-

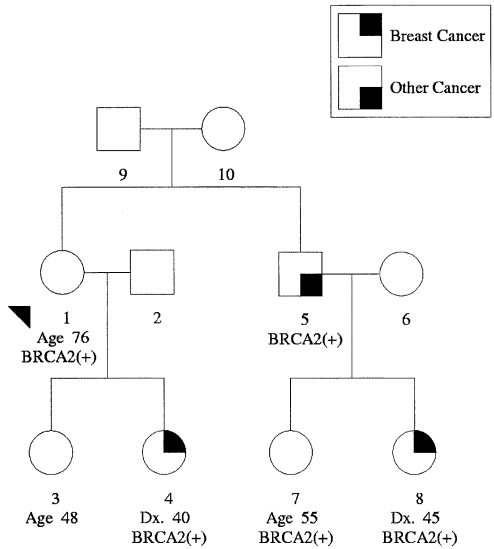

Fig. 2. Example of non-penetrance of a *BRCA2* mutation. The proband in this family is a 76 year old woman who is found to carry a *BRCA2* mutation. She has no history of cancer although her daughter who also carries the mutation was diagnosed with breast cancer at age 40.

testing and post-testing counseling by trained professionals with knowledge of the discussed issues [52,53]. Similar guidelines have also been offered by the American Society of Clinical Oncology [54], the American Society of Human Genetics [55,56] as well as the American College of Obstetricians and Gynecologists [57]. Based on the recommendations of these groups, a comprehensive cancer genetics program includes the following phases:

Phase I. Pre-test counseling

Individuals can be referred from many different sources such as a primary care physician, gynecologist, oncologist and general surgeon. Indications for referral for a genetic evaluation can be family history of cancer, an individual with early onset breast or ovarian cancer or multiple cancers.

Appropriate referrals for a cancer genetics evaluation:

- An individual with a personal or close family history of pre-menopausal breast cancer
- Families with multiple individuals with breast and/or ovarian cancer
- A young women with bilateral breast or breast and ovarian cancer
- A personal or family history of male breast cancer
- An individual with a personal or close family history of ovarian cancer
- Women of Ashkenazi Jewish descent even with less significant personal or family histories

Self-referrals are not uncommon and are usually prompted by personal concern or concern for their adult children. The decision to have a genetic evaluation and genetic testing differs between women and men. Women report their primary reason for seeking genetic counseling is for further clarification of their own risk of developing cancer [58] and men are mostly concerned about their children's risk [59,60]. Other reasons for seeking genetic counseling and genetic testing were for reassurance, increase in cancer screening, and to improve personal health.

Several studies have tried to evaluate whether genetic counseling and/or genetic testing have any impact on cancer screening behavior and relief of anxiety. Lerman et al. studied 200 women 35 years or older with a first-degree relative with breast cancer for the impact of genetic counseling on these individuals compared to individuals who received general health counseling [61]. Women who received an individualized breast cancer risk counseling were more likely to improve their understanding of their risk, however, in both the study and control group two thirds continued to overestimate their cancer risk. Black et al found that women between 40 to 50 years old overestimate their cancer risk [62]. Croyle et al recently reviewed the extensive literature on the psychological impact of cancer genetic testing [63].

At the Baylor College of Medicine Cancer Genetics Clinic the pretest counseling process involves two phases: the first phase is the initial contact with the person requesting counseling normally done over the phone. Questions asked

in this phase are listed in Table 3. Over-estimating one's cancer risk is a frequent finding and has found to influence the decision whether or not to participate in cancer screening programs and genetic testing [64]. Therefore, it's important to determine the individual's goal and address their emotional distress. A detailed family history is an important step in this pre-visit phase. The details should include all family members with cancer and their age at diagnosis on both paternal and maternal sides as well as healthy family members and their ages. Information about all cancer types is important since other genetic disorders may predispose to a number of cancer types (Table 2). Before embarking on *BRCA1* and *BRCA2* testing, these other genetic conditions should be considered.

Medical records are important to confirm diagnoses in the relatives in particular for ovarian cancer which is often confused with other reproductive cancers that may have much less impact on the likelihood of their being a *BRCA1* or *BRCA2* mutation. Once the family history is completed it is useful to classify the family by risk status. A high-risk family (for example, see Fig. 3) is one with a remarkable family history often including very early-onset breast cancer cases and is very likely to carry a mutation in one of the susceptibility genes. A moderate risk family (Fig. 4) has a less remarkable family history but will often still have several affected members but with an older age of breast cancer diagnosis and no ovarian cancer. A low risk family may have only one or a few family members affected with breast cancer or ovarian cancer (Fig. 5).

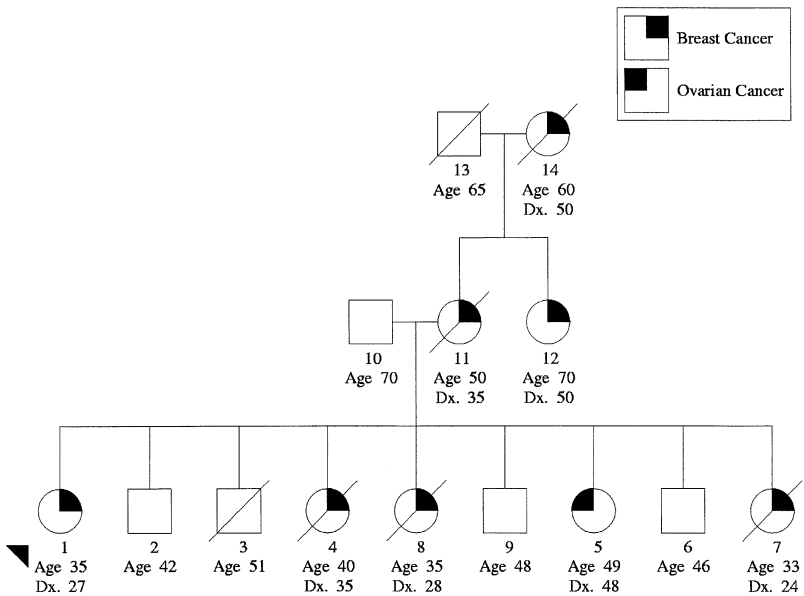

Fig. 3. Example of a high risk family. The proband was diagnosed with breast cancer at age 27. She has multiple sisters and maternal relatives with very early-onset breast cancer or ovarian cancer. She has a very high risk to carry a *BRCA1* or *BRCA2* mutation of 98% based on the BRCAPRO model.

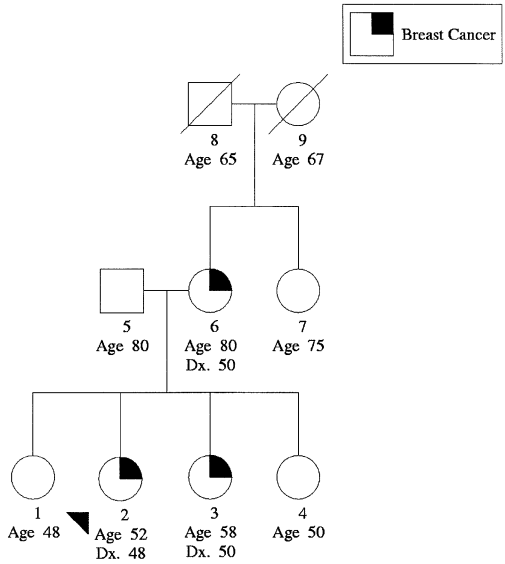

Fig. 4. Example of a moderate risk family. The proband was diagnosed with breast cancer at age 48. Her sister and mother were diagnosed at breast cancer at age 50. There are no female relatives with ovarian cancer. She has a moderate risk to carry a *BRCA1* or *BRCA2* mutation of 11.3% based on the BRCAPRO model.

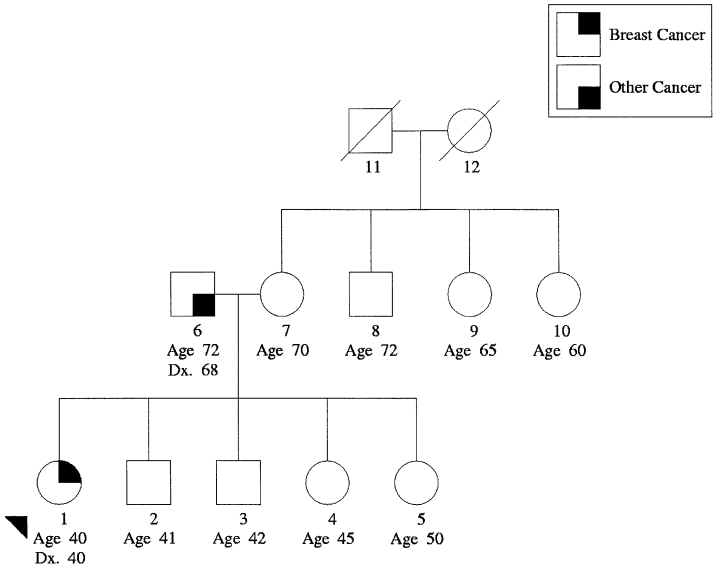

Fig. 5. Example of a low risk family. The proband was diagnosed with breast cancer at age 40. However, she has multiple female relatives who have not had cancer. Thus, her risk to carry a *BRCA1* or *BRCA2* mutation is low at 1.2% based on the BRCAPRO model.

Phase II. Cancer genetic counseling

The second phase is the counseling visit. Some cancer genetic programs may use a first visit to obtain the family history information and then have the counseling session occur during a second visit. As shown in Table 3, the counseling visit includes a discussion on the genetics of breast and ovarian cancer susceptibility, the risk of cancer for the individual based on family history and other risk factors, and the risk for carrying a genetic mutation in one of the cancer susceptibility genes. A number of models have been developed to estimate a woman's individual risk for developing breast cancer and to estimate the risk of carrying a mutation in either *BRCA1* or *BRCA2*. Perhaps one of the greatest challenges during this visit is making sure the patient understands the difference between their risk of developing cancer and their likelihood of carrying a cancer susceptibility mutation.

Models used for cancer risk estimate

Several models have been developed to assist in estimating the risk a woman has to develop breast cancer during her lifetime. The Gail mode is a multivariate model that is used extensively by family practitioners and medical oncologist and is based on data from the Breast Cancer Detection and Demonstration Project [65].

Parameters used in the Gail Model Risk Assessment Tool:

- Race
- Age (valid for women $\geq$ 35)
- Age at first menarche
- Age at first live birth
- Number of first degree relatives with breast cancer (mother, sisters, daughters)
- Number of breast biopsies
- Was atypical hyperplasia found on breast biopsy?

Table 3
Phases of a comprehensive cancer genetics evaluation

Phase I	Phase II
Determine reason for referral and referral source	Review family history and medical records
Determine patient motivation for genetic evaluation	Calculate cancer risk and likelihood for carrying a mutation
Obtain a detailed family history and construct pedigree	Discussion with the patient about the genetics of breast and ovarian cancer susceptibility
Request medical records to confirm cancer	Discuss patient's risk for developing breast and/or ovarian cancer
	Discuss patient's risk for detecting a mutation in either *BRCA1* or *BRCA2*

It is used to estimate a woman's 5-year and lifetime risk to develop breast cancer. It takes into account reproductive factors (age at first menarche, age at first live born child), limited family history (first-degree relative only) with breast cancer, number of breast biopsies and findings of atypia. It also contains a modification for the woman's race. The Gail model is currently being used to determine eligibility for the STAR trial, which compares Tamoxifen and Raloxifene in reducing the incidence of breast cancer in high-risk women. However, the Gail model was developed before the era of *BRCA1* and *BRCA2* mutation testing. The limited family history information that is included in the Gail model results in an underestimate for women who have a more extensive family history of early-onset breast cancer, ovarian cancer, male breast cancer or paternal relatives with breast cancer. For this reason we typically do not use Gail model results in very high-risk families.

The Claus Model utilizes much more extensive family history data to calculate cancer risk and assumes Mendelian inheritance [66]. It takes into account first and second-degree relatives with breast cancer and their age of diagnosis. The model however, does not use paternal family history or ovarian cancer. This model is more appropriate for women with a family history of early-onset breast cancer. Both models are specific for breast cancer risk and do not provide information with regard to risk of other malignancies including ovarian cancer. At a practical level, during the counseling session we provide estimates based on the Gail and Claus models and briefly explain which factors these models use to determine a woman's risk.

Genetic testing

A risk estimate for carrying a mutation in either *BRCA1* or *BRCA2* genes can be calculated by using models that were developed for this purpose. Obtaining the family history information in advance allows these estimates to be made prior to the appointment and the discussion can thus be tailored appropriately. The Couch Model [25] is based on empiric data and estimates the risk of carrying a *BRCA1* mutation when accounting for the following factors: average age of breast cancer diagnosis, Ashkenazi Jewish descent and a family history of ovarian cancer. This model does not calculate the risk for carrying a *BRCA2* mutation and thus it is an underestimate of the likelihood of finding a mutation in both genes. The BRCAPRO model is a computer model that calculates the Bayesian probabilities from pedigree data [67]. It is based on mutation frequency and penetrance estimates for each gene and considers affected women as well as non-affected women in the family. Age of onset of breast and ovarian cancer are important factors. This model is being used by many clinics for both research studies and clinical decision-making. As an example of the range of values generated by the BRCAPRO model, we calculated the likelihood of finding a mutation in the three sample pedigrees shown in Figs. 3, 4 and 5 assuming that the probands were not of Ashkenazi descent. The high risk proband has a 98% risk, the moderate risk

proband has a 11% and the low risk proband has a 1.2% of being mutation positive. The results of this analysis would tailor how we counseled the patient during the session.

The discussion on genetic testing should be in a nondirective manner and should include its benefits and limitations, specificity and sensitivity and the clinical applications. The American Society of Clinical Oncology recommends clinical testing for women with a risk of 10% or greater of carrying a mutation [54]. However, women with lower likelihood of carrying a mutation may still want to proceed with testing, but they should understand the high probability of a negative result.

Genetic testing results are more informative if a family member affected with breast and/or ovarian cancer is the one to first have genetic testing. Although testing an affected relative may delay the process we strongly recommend it to our patients. This may not always be possible since some affected family members may be deceased or are not interested in having genetic testing. In addition, genetic testing provides information not only regarding the individual undergoing testing but also regarding the individual's parents, siblings and children whether or not they wish to learn this information. These implications should be discussed prior to genetic testing. After reviewing this information there should be a discussion of the different potential results of testing as described below.

Once estimates of cancer risk and mutation likelihood are disclosed there is normally a two-way discussion to explore the goals the individual wishes to achieve by genetic testing, their readiness for testing and coping abilities, confidentiality issues, and costs involved, as part of the informed consent process (see box).

Issues to be discussed for informed consent

- The purpose of genetic testing
- The possible results and the implications of the test
- Alternatives to genetic testing
- Inheritance pattern of the mutation
- Detection rate and the accuracy of the test
- The costs involved
- The psychological impact involved with the knowledge of genetic status
- The potential risks of insurance discrimination
- The options available for treatment, prevention and surveillance

At the conclusion of the pretest counseling a blood sample can be obtained or if the individual requires more time to decide, a blood sample can be obtained at a later date.

Insurance issues

Full sequence analysis of *BRCA1* and *BRCA2* usually takes 4 to 5 weeks to complete. The cost of full sequencing is approximately $2600. For Ashkenazi Jewish patients, testing for the three founder mutations is performed first at the cost of approximately $400. Insurance coverage is highly variable with some insurance companies paying for testing in full or 50% to 80% of the cost. Many companies have developed their own criteria for approving testing based on patient medical history, family history and potential impact on medical treatment.

Genetic testing for inherited predisposition to cancer is associated with concerns of insurance discrimination, denial of coverage, increase in premiums and problems with life insurance. This fear may be one of the reasons why a person chooses not to have genetic testing. Lynch et al. studied the various reactions to disclosure of genetic test results and found that approximately 25% of the participants feared disclosure of positive test results to their primary care physician because of the perceived fear of insurance discrimination [68]. However, this perception has not been substantiated for cancer testing although other cases of discrimination based on genetic testing have occurred. States including Colorado, Florida, Minnesota, North Carolina, Ohio and Texas have legislation in place, which protects certain individuals against discrimination, based on genetic testing. Concerns about health insurance discrimination may be less for individuals with a group policy, which is usually obtained through employment, and coverage is provided without regarding personal health [69].

Phase III. Post-test counseling

The goal of this session is to provide the genetic test results. Most cancer genetics and high-risk clinics give the results in person. The individual may be alone but we recommend they be accompanied by a friend or relative. During this time they will learn if they are at increased risk, if their risk is reduced or if testing was not informative. Emotional distress may be anticipated whether the results are positive or negative [63].

Interpretation of results

I. A positive result. A positive result is when a deleterious mutation has been found in either *BRCA1* or *BRCA2* and thus it is an informative result. The psychological response to a positive result is highly variable. The knowledge that one carries a mutation can be anxiety provoking, in particular for women who are unaffected with cancer. In a short-term follow-up of 60 women who were found to be carriers of *BRCA1* mutations, those unaffected with cancer showed a higher level of distress 1 to 2 weeks following the results than noncarriers and carriers affected with cancer [70]. In some cases, it can be a relief because carriers may be satisfied to no longer have the uncertainty of their risk status and in the case of women affected with cancer they may welcome an explanation as to why they

developed early-onset cancer. A positive result also may help clarify their medical decisions and plan their screening strategies. A number of investigators have carried out extensive studies on the psychological impact of *BRCA1* and *BRCA2* testing [63].

All other close family members of this patient should be offered testing for this specific mutation. Controversy surrounds the issue of who is responsible for notifying "at risk" family members. In general, genetic information should be protected by the legal and ethical principle of confidentiality however there are exceptions to this rule. As noted in the ASHG statement on professional disclosure of familial genetic information [71] a professional could disclose genetic information if attempts by the patient have failed, in a potentially harmful situation, if the relative is identifiable, if the condition is preventable and treatable or if early intervention will reduce the genetic risk. At the Baylor College of Medicine Cancer Genetics Clinic we provide a letter to the patient to distribute to family members. The letter does not include the patient's name but indicates that a family member has been found to carry a mutation (the specific mutation is indicated), recommends that the family member seek an evaluation of their cancer risk and provides a phone number for the family member to call to aid in identifying a cancer genetics clinic in their area.

II. A negative result: Understanding a negative result can be more confusing for both the patient and the physician. A negative test result means that no mutation was identified in either the *BRCA1* or *BRCA2* gene using current methodologies. The interpretation of a negative result is highly dependent on whether a disease causing mutation has already been identified in the family. We consider a "true negative" when an at-risk family member is negative for a known deleterious mutation found in a first or second-degree relative affected with cancer. A negative result in this setting means that the woman did not inherit the high-risk allele and thus is not at increased risk of cancer based on her family history. The at-risk woman would then have a lifetime risk of developing breast and/or ovarian cancer equal to a woman in the general population. This type of negative result often provides relief of anxiety and suggests the decreased need for cancer surveillance, chemoprevention or prophylactic surgery. However, the reaction towards a negative result may vary and can sometimes cause disbelief in the results. Lynch et al found several individuals, who were found to be negative for familial mutations, yet expressed their disbelief in the results and stated that a negative result would not change their screening habits [68]. Survivor guilt is another emotion that was expressed by several women in this study.

Other negative results may be uninformative for several reasons. The interpretation of a negative result is highly dependent on whether the person tested is affected with ovarian cancer or early onset breast cancer. If the woman affected with cancer is negative then the cancer is not due to a detectable mutation in *BRCA1* or *BRCA2*. The cancer in the family may be due to a mutation in *BRCA1* or *BRCA2* that is not detected by current methods. For example, as described previously, recent publications suggest that rearrangements may be found in some women who test negative for sequence-based tests. The use of

tests that detect these mutations will likely become available over time. The family may also have a mutation in another cancer susceptibility gene, which has not been tested for or has not yet been identified. There is active ongoing research to identify a *BRCA3* gene [72]. Overall, it is important for the patient, especially if from a high-risk family, to understand that a negative result does not mean that there is no increased risk of cancer, just that current testing has not identified the cause of cancer in her family. Studies of the psychological impact of genetic testing have found this scenario (rather than a positive test) to result in the greatest level of emotional distress [73].

In many cases a negative result may be found because the cancer is sporadic and not due to any hereditary cause. This is most likely in low-risk families with one or few affected women. Finally, the individual tested may have not been the ''ideal person'' to be offered testing. Genetic testing should be offered to an affected family member with the youngest age of diagnosis of breast cancer or a diagnosis of ovarian cancer and we strongly encourage our patients to investigate this possibility before testing an unaffected woman first. However, this is not always possible since many family members that were affected with cancer have died and other living members may be logistically far away and/or are not interested in being tested. In this case, a negative result is uninformative because we do not know if the women affected with cancer in the family carried a detectable mutation. In this situation, further screening recommendations for the unaffected individuals should be based on their family history almost as if the testing hadn't been performed. In summary, interpretation of a negative *BRCA1* and *BRCA2* test result is complex and is highly dependent on the exact circumstances of the person tested and her family history. Although many patients are relieved to hear ''the test is negative,'' it is important to spend time explaining the implications of a negative result in women who may remain at increased risk for breast and ovarian cancer.

III. A variant of unknown clinical significance: This finding is usually a missense mutation or a single base pair change in either *BRCA1* or *BRCA2* for which it's effect is unknown [8]. This variant may or may not increase the risk of breast and/or ovarian cancer. Additional information can be sought to try and determine the significance of the variant [74] including determining if the variant segregates with cancer in the family, whether there are any functional studies reported of this particular variant or whether this variant has been seen in women who also carry a clear deleterious mutation. In the latter case, it is unlikely that a patient would carry two deleterious mutations and suggests that the variant may be a polymorphism.

Recommendations and surveillance for BRCA1/2 carriers

The absence or presence of a *BRCA1* or *BRCA2* mutation has considerable medical as well as psychological impact. The main goal of genetic testing is to identify women at risk for developing breast and/or ovarian cancer and offer them screening measures that could detect cancer at an early stage. The knowledge of

one's genetic status may have an affect on screening behavior whether the results of genetic testing were positive or negative for a mutation in either *BRCA1* or *BRCA2* and long term follow-up studies of women who have been tested are underway. For example, one study of Ashkenazi women receiving a negative *BRCA1* result did not find any significant change in mammography behavior when comparing compliance prior to and post testing [75]. Lerman et al. found that one year after *BRCA1* and *BRCA2* testing a minority of women who tested positive opted to have prophylactic surgery [76]. Younger women who tested positive continued to have surveillance by mammography. The options available to women who are at increased risk for developing these cancers can be divided into three categories: screening and close surveillance, prophylactic surgery and chemoprevention (reviewed by Hartmann [77]).

I. Screening and surveillance. Mammography is the method used today for breast cancer screening. The American Cancer Society recommends mammography for every women aged 40 or older. However, the sensitivity of this tool is highest among women age 50 and older. Kerlikowske et al found that the sensitivity of mammography is lowest among women younger than 50 and especially low if the intervals between mammograms was two years and when women have a family history of breast cancer [78]. The decrease in sensitivity may be due to the density of the breast tissue and tumors in young women develop more rapidly. High risk women including *BRCA1* and *BRCA2* mutation carriers should have more frequent breast exams starting at a younger age than women of the general population should. The current screening recommendations proposed by an NIH panel include a clinical breast exam every 6 months and mammograms every 6 to 12 months beginning at age 25 [79].

The use of other imaging techniques such as MR imaging and ultrasound for the early detection of breast cancer are now being investigated. Ultrasound of the breast has been used for the last decade as an adjunct to mammography. When combined with mammography it is found to increase the sensitivity and the specificity of a breast exam [80]. Ultrasound has also been shown to be more accurate in detecting malignant lesions in women under the age of 50 [81]. MRI has been shown to be highly sensitive [82] but its specificity remains moderate [83]. Kuhl et al found MRI to have a high sensitivity and a high predictive value when compared to mammography and ultrasound [84]. Others claim MRI can be useful when used together with mammography in high-risk patients. Tilanus-Linthorst et al reported their experience with MRI in screening young women at increased risk for breast cancer (over 25% risk) and found MRI to increase the detection of breast cancers, which were not picked up by mammography or ultrasound in this group of women [85]. The limitations of MRI are the lack of feasibility, its high cost and the high false-positive rate [86]. Large-scale studies are needed to validate the use of this method, particularly in young high-risk women.

Given the high mortality of ovarian cancer, there has been growing interest in developing measures for early detection of ovarian cancer that will be effective in reducing the mortality rate from this cancer. For *BRCA1* and *BRCA2* mutation

carriers who have a much higher *a priori* risk of ovarian cancer an NIH consensus panel recommends annual transvaginal ultrasounds, CA-125 levels and biannual pelvic exams to detect ovarian cancer at an early stage [79]. However, this was based on expert opinion and there is no clear data that these measures reduce mortality from ovarian cancer in carriers of *BRCA1* and *BRCA2* mutations.

II. Prophylactic surgery. Removal of healthy tissue prophylactically is offered to women with inherited susceptibility to breast and ovarian cancer. There is significant controversy over the appropriateness of this approach [77] although there are now several studies that are beginning to provide data with regard to whether prophylactic surgery results in a reduction in cancer risk for *BRCA1* and *BRCA2* carriers.

Mastectomy with contralateral prophylactic mastectomy is also considered for women with a new diagnosis of breast cancer who are deciding upon treatment. The increased risk of a second primary breast cancer and the need for careful follow-up surveillance should be explained to the patient who decides upon breast conservation treatment. An accelerated schedule of testing (with an increase in cost) is offered by Myriad Genetics Laboratories (Salt Lake City, Utah) for women in this situation.

A cohort of healthy women who underwent prophylactic mastectomy due to a family history of breast cancer at the Mayo Clinic between 1963 to 1993 has been reported [87]. The women were divided into two groups: a high-risk group of women (more likely to be mutation carriers) and a moderate risk group based on their family history of breast cancer. The investigators found a 90% reduction in the incidence and mortality of breast cancer in both groups of women after prophylactic mastectomy. Follow-up psychological studies of these women have revealed that 70% were satisfied with the procedure and in particular expressed decreased concern about their risk of developing breast cancer, however, a minority of women reported negative effects of the procedure on a number of measures [88].

Bilateral prophylactic oophorectomy (BPO) is considered for women with an increased risk for breast and ovarian cancer. This option is often considered after childbearing has been completed. The removal of the ovaries in premenopausal women reduces hormone exposure, which reduces the risk of breast cancer. Recently, the impact of BPO in women who are carriers of a *BRCA1* or *BRCA2* mutation has been reported. Rebbeck et al studied a cohort of 43 unaffected women who were carriers of a *BRCA1* mutation, for the reduction in breast cancer following a bilateral prophylactic oophorectomy [89]. The study found a 50% reduction in the risk of breast cancer compared to a control group of 79 *BRCA1* carrier women who had not had prophylactic surgery. This breast cancer risk reduction increases 10 years following the BPO and was most prominent for women who had their BPO before age 50. Similar results were found by Narod and colleagues [88]. Analysis of the reduction in ovarian cancer diagnosis after BPO has been presented in abstract form and also demonstrated a significant reduction in ovarian cancer incidence [90]. Women at risk for ovarian cancer

based on their family history still have a 2% risk for primary peritoneal cancer after oopherectomy [91].

III. Chemoprevention. In 1998, the United States Food and Drug Administration (FDA) approved the use of tamoxifen for the reduction of breast cancer and contralateral breast cancer risk. The use of this agent was approved based on the results of the Breast Cancer Prevention Trial (BCPT) [92]. This trial included more than 13,000 women ages 35 to 59 with a calculated 5 year risk for breast cancer of at least 1.66% (based on the Gail model) by 60 years old. However, it is important to note that only a small percentage of these women are estimated to be mutation carriers. The women were given tamoxifen or a placebo. The data from this trial suggests that tamoxifen reduced the risk of breast cancer by 49%: 44% in women 49 years or younger, 51% in women 50 to 59 years and 55% in women 60 years or older.

Narod et al compared 209 women with bilateral breast cancer to 384 women with unilateral breast cancer [93]. All women were carriers of either a *BRCA1* or *BRCA2* mutation. The case control analysis found a 50% reduction in the risk of bilateral breast cancer in *BRCA1* and *BRCA2* carriers who were treated with tamoxifen for their first breast cancer. They also found that the protective effect of tamoxifen was greatest for use up to 4 years. Raloxifene has been approved by the FDA for preventing and treating osteoporosis in postmenopausal women. It has also been shown that raloxifene reduces the risk of breast cancer and inhibits the growth of existing breast cancer [94]. There is no data available on the impact of raloxifene in *BRCA1* and *BRCA2* carriers.

Conclusions

As reviewed in this chapter, significant progress has been made in identifying the genes responsible for inherited susceptibility to breast and ovarian cancer. There has now been more than five years experience in genetic testing for *BRCA1* and *BRCA2* mutations. This experience has resulted in recommendations for performing counseling and genetic testing in a meaningful way for patients. Although a relatively complex procedure, genetic evaluation and testing can result in a much better understanding about cancer risks for the patients who undertake the process. The objectives of ongoing research in the field are to (1) identify additional cancer predisposing genes, (2) define the genetic and environmental factors that influence cancer risk in those found to carry a mutation and (3) improve knowledge about surveillance, chemoprevention and surgery to reduce the early morbidity and mortality seen in *BRCA1* and *BRCA2* families.

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Fragile X and other trinucleotide repeat diseases

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Hereditary unstable DNA

According to the laws of Mendelian genetics, genes are passed unchanged from parent to progeny. New gene mutations can occur, but once they do, the mutations are also passed on unchanged. Although this concept still applies to many genes or traits, it is now recognized that certain genes are inherently unstable, and their size and function may be altered as they are transmitted from parent to child. These intergenerational genetic changes explain such puzzling genetic phenomena as anticipation and skipped generations, and are responsible for several important diseases; at least 20 diseases caused by hereditary unstable DNA have been identified.

Hereditary unstable DNA is composed of strings of trinucleotide repeats. Trinucleotide repeats are stretches of DNA in which three nucleotides are repeated over and over (i.e., CAGCAGCAGCAG). Triplet repeats composed of all combinations of nucleotides have been identified, but CGG and CAG are the most common [1]. These repeats are found in several sites within genes: in the noncoding region, in introns (gene segments that are translated into RNA but are then excised before the mRNA is translated into a protein), or in exons (gene segments that are translated into mRNA and are not excised). Triplet repeats found within exons may be in the untranslated region, or in the region that is translated into protein (Fig. 1) [2].

Depending on their location within the gene, the number of triplet repeats in a string can change as it is passed on to offspring. Although decreases in the number of repeats can occur, the number usually increases. Once the number of repeats reaches a critical size, it can have a variety of affects on gene function. The repeats may cause a loss of gene function, as in fragile X. However, in the majority of triplet diseases the result is the gain of a new, abnormal protein and thus a new function. For example, if the triplet repeat is composed of CAGs, (which encode glutamine), and is located in a coding region, the translated

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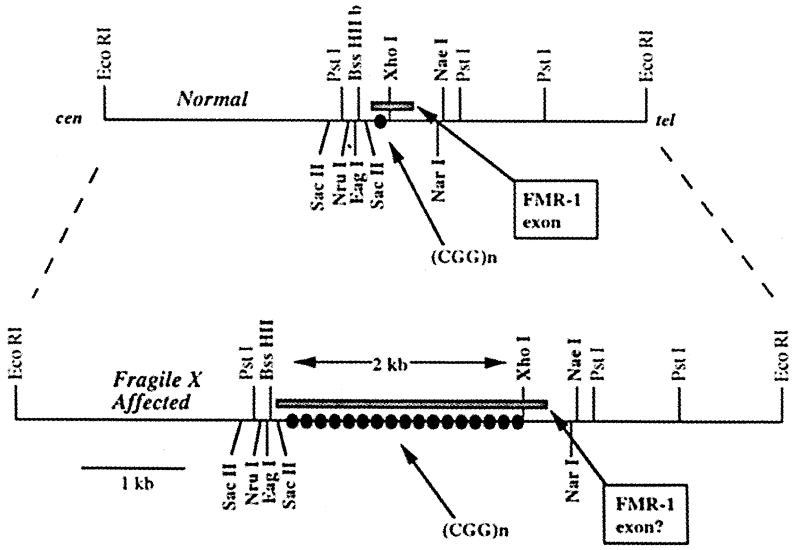

Fig. 1. Diagram of the presumed structure of the FMR 1 gene. Map of the 5.2-kb fragment in Xq27.3 produced by digestion with restriction enzyme *Eco*RI. The fragment contains the CGG repeats (●) mutated in fragile X syndrome in normal and fragile X-affected forms. Restriction sites for other enzymes and the exon of FMR-1 are indicated. Restriction sites in bold type are sensitive to methylated cytosine residues in CpG dinucleotides. Cen refers to the centromere and tel to the telomere portion of each chromosome. (*From* Nelson DL. Fragile X syndrome: Review and Current Status, Growth Genetics & Hormones 1–4, 1993; with permission.)

protein will include a string of glutamines. Polyglutamine regions have a high charge density, and thus may change the protein's function and seriously alter cellular operations. If the triplet repeat is outside the coding region in an untranslated region, the ultimate effect may be on mRNA function or gene processing. Table 1 lists several triplet repeat diseases, the identity of the triplets involved, the location of the triplets within the gene, and the theorized result (gain or loss of function) [3]. Although all these diseases are interesting and merit consideration, this discussion will focus primarily on fragile X syndrome, myotonic dystrophy, and Huntingtons disease.

Fragile X syndrome (Martin-Bell Syndrome)

Background

Fragile X syndrome is the second most common form of genetic mental retardation (after Down syndrome), and is the most common form of familial mental retardation. It accounts for 4% to 8% of all mental retardation in males and females, and is found in all ethnic and racial groups. Affected individuals have a variety of neurologic problems, including mild to severe mental retardation, autistic behavior, attention deficit-hyperactivity disorder, speech and lan-

Table 1
Comparison of the features of the most common trinucleotide repeat diseases

Disease	Chromosome	Locus	Location in Associated Gene	Repeat	Size in Normal	Size in Carrier	Size in Affected	Change in Gene Function
Kennedy disease (SBMA[a])	Xq11-12	AR	Exon 1	CAG (gln)	12–34	–	40–62	Gain
Huntington disease	4p16.3	HD	Exon 1	CAG (gln)	6–37	–	35–121	Gain
Spinocerebellar ataxia type 1	6p22-23	SCA1	Exon 8	CAG (gln)	6–39	–	41–81	Gain
Dentatorubral pallidolusyian atrophy	12p12-13	DRPLA	Exon 5	CAG (gln)	7–34	–	54–70	Gain
Machado-Joseph disease	14q32.1	MJD	Internal exon?	CAG (gln)	13–36	–	68–79	Gain
Fragile X syndrome	Xq27.3	FRAXA (FMR1)	5' untranslated	CGG	5–52	43–200	230–>2,000	Loss
Dystrophia myotonica	19q13.3	DM	3' untranslated	CTG (CAG)	5–37	44.46	50–>2,000	RNA stability?
Mental retardation?	Xq27.3	FRAXE	??	GGC (CGG)	6–25	116–133	200–>850	??
(None)	Xq28	FRAXF	??	GGC (CGG)	6–29	–	300–500	??
(None)	16p13.11	FRA16A	??	GGC (CGG)	16–49	–	1,000–2,000	??

From Nelson DL. Allelic expansion underlies many genetic diseases. Growth Genetics & Hormones 1996;12:1–4 with permission.

[a] Spinal and bulbar muscular atrpohy

guage problems, and occasionally seizures [4]. The physical phenotype includes a narrow face with large jaw, long prominent ears, and macro-orchidism in postpubertal males.

Familial mental retardation affecting only males has been recognized for many years, and in the past was generally classified as *X-Linked mental retardation*. However, the term X-linked is nonspecific, and this generic designation likely included a variety of different X-linked clinical entities. Then in 1969, Lubs described a subgroup of mentally retarded males who had a fragile site in their X chromosome [5]. A fragile site is a specific, non-random point on a chromosome that appears as a nonstaining gap after exposure to certain chemical agents or specific culture conditions [6]. In this case, it was a fragile site at Xq27.3 which became apparent after culturing the cells in folate deficient medium. When this fragile site was also found in the mentally retarded males of a family originally described by Martin and Bell, the Martin-Bell syndrome of X-linked mental retardation became synonymous with fragile X syndrome. Since that original report, four more fragile sites in this area have been discovered. By convention, the original site is called FRAXA and the others are designated FRAXB through E. Only FRAXA and E are associated with (different) mental retardation syndromes.

One of the earliest observations about fragile X syndrome was that it has an unusual inheritance pattern. Although males are primarily affected, a proportion of females are affected as well, and can exhibit a wide range of phenotypic features from very mild to severe. In addition, in contrast to typical X-linked disorders in which only one or a few individuals in every generation is affected, the number of family members with fragile X syndrome typically increases with each generation. This observation came to be called the *Sherman Paradox* after the investigator who first noted that the probability of mental retardation is increased by the number of generations through which the mutation is passed [7]. Most importantly, as the fragile X gene was traced through each family, it became evident that only individuals who inherited the gene from their mothers were affected. Thus, fragile X does not behave like a typical X-linked disorder.

Molecular genetics

The FRAXA site is now known to be a region of unstable DNA within the familial mental retardation (FMR1) gene on the long arm of the X chromosome [8–10]. This unstable region is a series of CGG (cytosine-guanine-guanine) triplet repeats, located in the 5' untranslated region of exon 1, approximately 250 basepairs downstream of a CpG island within the promoter region of the FMR1 gene. Promoter region CpG islands have an important role in the epigenetic control of gene function; they can be methylated, and such methylation acts to stop transcription and effectively turn the gene *off*. It now seems clear that an increased number of CGG triplets in the promoter region of the FMR1 gene somehow triggers CpG methylation and effectively stops transcription of that gene. Most normal individuals have about 29 CGG triplet repeats in the FMR1

promoter region, but it can accommodate up to 55 repeats without any affect on gene function. When the number of repeats is less than 55, methylation and gene silencing do not occur. In addition, a repeat size of 55 or less appears to be fairly stable; expansion from <55 repeats directly to a full mutation with >200 repeats has never been reported. However, if the number of triplet repeats exceeds 55, the region is unstable. Individuals who have 56 to 199 triplets in this area are said to have a fragile X *premutation*, which can further increase in size as it is transmitted, but only if it is passed from mother to child. If the size of this region reaches ≥ 200 repeats (the critical level, corresponding to a full mutation), methylation of the promoter region CpG dinucleotides occurs, and the gene is turned off [11,12]. The loss of gene function leading to loss of the FMR1 protein results in the fragile X phenotype [13]. Thus, both an increased number of CGG repeats and the presence of methylation of the FMR1 gene determine whether an individual is affected [14]. The fact that both gene expansion and methylation must occur before an affected individual exhibits the fragile X phenotype is illustrated by two interesting clinical situations: males carrying the full but unmethylated mutation are phenotypically normal [15], and individuals carrying a smaller but methylated gene are abnormal.

Although it is highly conserved in all species, the function of the FMR1 protein is currently unknown. The FMR1 gene codes for a 4.8 kb mRNA directing the production of a 70 to 80 Kd binding protein that is most active in brain and testes, but also found in placenta, uterus, lung, and kidney [16]. Because the FMR protein binds to mRNA, it may have a regulatory role [17]. As described above, the fragile X phenotype results from a loss of this protein, not from the production of an abnormal protein. Thus, the full fragile X phenotype can also be caused by intragenic loss-of-function mutations, which can range from deletions of the entire gene to loss of only a few kb at the promoter region [18]. The premutation is not associated with any change in FMR1 production, or any of the typical phenotypic features of the fragile X phenotype. However, premutation carriers are at 3- to 4-fold increased risk to develop premature ovarian failure and early menopause (before age 40) [19–21].

Prevalence

The reported carrier rate for fragile X mutations (premutations and full mutations) varies from population to population, ranging from 1/163 to 1/1538 [22]. This wide range of prevalence reflects the influence of a number of variables. For example, the laboratory method used for population testing can impact on results. Southern blot is probably the most accurate testing method, but is not easily adapted for screening large populations. On the other hand, the polymerase chain reaction is best for testing large numbers of samples, but may not be sensitive enough to detect all full and premutations and all mosaics. The number of individuals tested exerts an influence on results, with the most widely disparate estimates of prevalence coming from the smallest studies. Finally, the ethnic or racial background of the tested subjects has a major influence on results. For

example, Rousseau and colleagues found a fragile X prevalence of 1/259 among women in Quebec, but acknowledged that this high prevalence may be due to a founder effect in that the Quebec population descended from a very limited number of settlers [22]. Considering all variables, the incidence of the full fragile X syndrome is generally quoted as 1 per 1000 males and 1 per 2000 females [22,23].

Mechanism of gene expansion

Relatively recent genetic research has clarified and explained some of the interesting features of fragile X transmission. The first question to be answered was, how and why does the number of CGG repeats increase? The exact mechanism is still unknown, but data suggest that the inciting event may be loss of one or more AGG anchors. AGG triplets are usually scattered throughout regions of CGG triplets; in a typical region of 30 CGG repeats, AGG triplets are located at positions 10 and 20. Their location at these sites serves to break up the series of CGGs, which helps to anchor the replication apparatus. If an AGG is lost, slippage during replication is more likely; because of the long uninterrupted string of CGGs, the replication apparatus slips and mistakenly copies some CGGs more than once [3,24] (Fig. 2). The longer the strand, the more prone to slippage; thus, the number of repeats predicts whether or not slippage resulting in an increase in trinucleotide repeats will occur. With 51 repeats, expansion occurs in only 20% of transmitted genes, while with ≥ 110 repeats, expansion occurs in 100% [25] (Table 2). On the other hand, the relationship between repeat size and the chance of expansion during transmission is not absolute. The transmission of a premutation through 7 to 8 generations of a large Swedish kindred has been reported [26].

Timing of expansion and methylation

The most interesting aspect of genetic transmission of fragile X is that carrier mothers can have offspring with the full fragile X syndrome, but carrier fathers cannot. This and other clinical observations suggest that expansion of the trinucleotide repeats occurs only if the gene is transmitted by the mother, and that fragile X genes transmitted by the father generally do not change in size (Table 3). This circumstance prompts the question, when do the two steps necessary to inactivate the gene, namely trinucleotide expansion and gene methylation, actually occur? Are these prefertilization events in the oocyte or

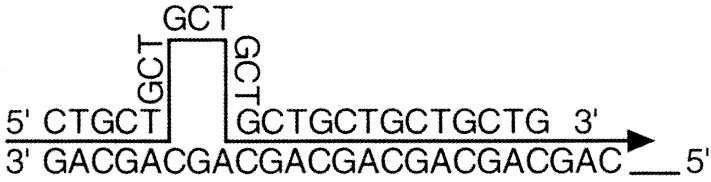

Fig. 2. Diagram showing the presumed mechanism of slippage during replication of genes containing trinucleotide repeats. (*From* Nelson DL. Allelic expansion underlies many genetic diseases, Growth Genetics & Hormones 12:1–4, 1996; with permission.)

Table 2
Risk of FMR1 gene expansion according to length of trinucleotide repeats

Mutation size	*% of offspring with full mutation*
50–59	20%
60–67	17%
70–79	39%
80–89	76%
90–99	89%
100–109	91%
110–119	100%
120–129	100%

From Fisch GS, Snow K, Thibodeau SN, et al. The Fragile X premutation in carriers and its effect on mutation size in offspring. Am J Hum Genet 1995;56:1147–55 with permission.

sperm, or do they occur only after fertilization? The answer to this question is also currently unknown, but it does seem clear that gene expansion does not occur in the sperm. A variety of evidence suggests that, if anything, the gene may actually contract when transmitted by a male. For example, the sperm of non-mosaic males carrying the full fragile X mutation typically carries only a pre-mutation [27]. Likewise, Reyniers et al. (1993) have reported that mosiac males who carry both premutations and full mutations in their tissues only produce sperm carrying the premutation [28]. It has been hypothesized that sperm carrying the premutation may have a selective advantage over those carrying the full mutation because the smaller FMR1 gene can be replicated faster [29]. Contraction of the full mutation in the fetal testes has also been reported [30].

It is possible that gene expansion occurs in the oocyte. However, there is currently no theory to explain why passage of the trinucleotide repeats through oogenesis could result in expansion. If expansion in the oocyte does occur, genetic imprinting might play a role. The second step, methylation and inactivation of the FMR1 gene, likely occurs after fertilization. This sequence of events is supported by several observations. For example, while fetal and placental tissue usually contain the same size FMR1 gene, the placental tissue is typically hypomethylated while the fetal tissue is methylated. This finding indicates that gene expansion occurred prior to differentiation of the dividing cells into chorionic villus and fetal cells, but that methylation occurred after the division [4].

Many more clinical observations indicate that the expansion likely occurs after fertilization. Somatic cell mosaicism for both the size of the triplet expansion and the degree of methylation, a situation that could only arise in dividing somatic

Table 3
Parent of origin when gene size changes on transmission-comparison of three triplet repeat diseases

Disease	Size increase	Size decrease	No change in size	Severest phenotype
Fragile X	Mother	[Father, rarely]	Mother or Father	NA
Myotonic Dystrophy	Mother or Father	Father	Mother or Father	Mother
Huntington Chorea	Father	–	Mother	Father

Courtesy Katherine Dowenshrom, MD.

cells, has been reported. Mingroni-Netto and colleagues studied 88 carriers of the fragile X mutation (74 with a premutation and 14 with a full mutation) and their 154 offspring [31]. Fully 9% of the offspring were mosaics, with equal numbers inheriting a smaller, larger, or the same size mutation as their parents. Kruyer and colleagues have described two sets of monozygotic twins carrying the fragile X gene (one male pair, one female) in which the twins were discordant for gene size, methylation status, and phenotype [32]. Because expansion to the full mutation occurred only in their somatic cells and not in their germline cells, it was likely to have occurred after fertilization, during somatic cell mitosis. Thus the fragile X gene in each fetus expanded and became methylated after the zygote split. Furthermore, cases in which mothers carrying the full mutation gave birth to sons who were mosaics for both the premutation and the full mutation [33], and cases in which daughters of fragile X patients inherited only the premutation support the concept of a postzygotic change in the FMR1 gene [34].

Genotype-Phenotype Correlation

The fragile X phenotype varies. Approximately 80% of males and 50 to 70% of females carrying the full mutation are retarded [35–37]. Males are moderately to severely affected, with an IQ in the 35 to 45 range, while the mental retardation in females may be more mild [2]. Twenty percent of males and 10% of females carrying the expanded gene have a very mild phenotype or are unaffected. This phenotypic variability is caused by mosaicism for the size of the expansion, the degree of methylation, or lyonization (in females) [14]. Because mosaicism likely arises during mitosis in the zygote, it cannot be reliably predicted by analysis of either the parental gene or fetal cells, and thus is not amenable to prenatal diagnosis [27,31,38,39]. Women carrying the expanded gene can also have varying degrees of affectation because of lyonization, the random inactivation of one X chromosome in every cell during the late blastocyst stage. Unfavorable lyonization can result in a large proportion or even the majority of cells expressing the expanded fragile X gene [36,40]. The ultimate pattern of lyonization also cannot be predicted prenatally. The factors influencing lyonization and mosaicism (and other aspects of phenotype expression) are not well understood.

Diagnosis

Until recently, the diagnosis of fragile X syndrome was by a cytogenetic technique in which the cells to be tested were cultured in a medium deficient in folate and thymidine. Using this technique, it was possible to identify fragile sites as nonstaining gaps or constrictions on the long arm of the X chromosome (Xq27-28). This technique was unreliable, however, as less than half of cells from affected males manifested this fragile site [14,41,42]. Moreover, this cytogenetic technique was not reliable for carrier testing. For example, Rousseau and colleagues reported that 95% of 278 individuals who carried a premutation were missed by cytogenetic analysis.

The fragile X gene can now be directly examined. The number of CGG repeats and the methylation status of the gene can be determined using Southern blot analysis, because both gene size and the degree of methylation affect the size of the gene fragments obtained after restriction endonuclease digestion. The polymerase chain reaction has been used for testing, but can only determine the number of CGG repeats, not the degree of methylation [4]. Amniocentesis may be preferable to chorionic villus sampling for prenatal diagnosis, because methylation status is difficult to determine in chorionic villus cells, and the methylation pattern in the placenta probably does not reflect methylation in the fetus.

Parents who have a child with mental retardation, developmental delay of unknown etiology, or autism should be encouraged to have their child examined by a geneticist and tested for fragile X using molecular techniques. Approximately 2% to 6% of individuals with these characteristics will be determined to have the fragile X gene expansion [43]. Women who already have a child or other family member with confirmed fragile X syndrome should also be evaluated and counseled by a geneticist; those who are determined to be at risk of having an affected child should be offered prenatal testing. In this situation the patients high-risk status justifies the attempt at fetal diagnosis, even though predicting the phenotype for a fetus who inherits the gene can be difficult.

Obstetric issues-population screening

Population screening, or testing gravid women or fetuses when there is no family history of fragile X, is controversial. It is not currently recommended by either the American College of Medical Genetics or the American College of Obstetricians and Gynecologists, primarily because prediction of the fetal phenotype, especially when there are no affected family members, is fraught with problems [42,44]. In general, screening for any fetal disease should not be considered unless accurate prenatal diagnosis is available. Currently, accurate phenotype prediction for both male and female fetuses is not always possible, for all the reasons mentioned above.

Myotonic dystrophy

Background

Myotonic dystrophy is the most common form of adult myopathy. The symptoms range from cataract alone to mild myotonia to severe muscle weakness with pronounced myotonia and mental deterioration. The age of onset varies as well, from birth to 70 years [37]. Interestingly, recognition of genetic anticipation, the phenomenon in which individuals in successive generations of an affected family become symptomatic earlier and to a greater degree than those in the preceding generation, resulted from the study of myotonic dystrophy. Fleisher, a Swiss opthomologist, reported in 1918 that patients with

myotonic dystrophy frequently had ancestors with cataracts, and further, that different families with myotonic dystrophy could be linked through mutual ancestors with cataracts [45]. Julia Bell then evaluated these families closely, and showed that affected individuals in each generation after the generation with cataracts had successively more severe disease, occurring earlier in life [46]. However, the concept of genetic anticipation wasn't widely accepted at the time because there was no known genetic mechanism to account for it, and because its main proponent, FW Mott, was an avowed eugenicist, making his scientific colleagues less likely to accept his theories [47]. LS Penrose, a well known and respected geneticist, attributed anticipation to observational biases, pointing out that mildly affected individuals were usually diagnosed only after the birth of a severely affected descendent, but that the reverse (a mildly affected individual is diagnosed first and only then are more severely affected relatives in the preceding generation discovered) rarely occurred [48]. The subsequent recognition of a severe congenital form of the disease, arising when the fetus inherits the gene from an affected mother, supported the concept of anticipation. Then, in 1989, Howeler meticulously evaluated 61 parent-child pairs and showed that the disease virtually always got worse with each generation, not better. In 60 of the 61 pairs studied, the child was affected more severely and earlier than the parent [49].

Although these and other clinical observations revitalized the anticipation theory, the main problem preventing its recognition was that no plausible genetic mechanism by which it could occur was recognized at the time. After 1991, when Fu, Oberle, Verkerk, Yu, and others published studies showing that fragile X was caused by hereditary unstable DNA [9,17,24,50], it was only a matter of time before Buxton and coworkers, Fu and colleagues, and Harley et al. discovered a similar mechanism in myotonic dystrophy [51–54]. The concept of intergenerational triplet repeat expansion, leading to a successively larger and more dysfunctional gene, nicely explained the inheritance pattern. However, subsequent studies have shown that there are some subtle differences between the molecular genetics of fragile X and myotonic dystrophy.

Clinical aspects

Myotonic dystrophy is a multisystem disease characterized by muscle stiffness and progressive dystrophic changes in muscle and in numerous other tissues [55]. Symptoms typically appear for the first time in late childhood or the early adult years, and generally involve distal muscle weakness and atrophy. The facial muscles are then affected, resulting in a classic anhedonic appearance, with temporal wasting, ptosis, and thin neck muscles. The mouth may hang open and dysarthria is common. Muscle disease can be demonstrated by percussing the muscle, which results in sustained muscular contractions, or by electromyography. Eventually the disease affects other organs, causing testicular atrophy, insulin dependant diabetes, gallbladder disease, cardiac arrhythmias, and heart block. Cognitive impairment and cataracts are common.

There are also severe congenital and late adult onset forms of the disease. The development of hydramnios during pregnancy along with decreased fetal movement is a sign that the fetus has severe congenital myotonic dystrophy. At birth such infants are thin and floppy, with facial weakness, diminished cry and suck, and often severe respiratory compromise. As in the adult form of the disease, there is continuous degeneration of affected muscles with limited regeneration, and thus progressive atrophy. Such individuals rarely if ever survive to adulthood. At the other extreme, some gene carriers experience the onset of muscle weakness and atrophy only late in life, or may develop cataracts only. This wide variation in phenotype and the existence of a severe congenital form of myotonic dystrophy is explained by the molecular genetics of the disease.

Molecular genetics

The gene associated with myotonic dystrophy is the myotonin protein-kinase (MT-PK) gene, located on the long arm of chromosome 9 [51,54,56]. This gene has been found to contain a region of CTG trinucleotide repeats, with normal individuals having 3 to 30 repeats and those with myotonic dystrophy having up to 3000 [51,54,56] This gene has a very low spontaneous new mutation rate [57]; linkage analysis has shown that 58% of British myotonic dystrophy cases and virtually all French Canadian cases are descended from a single ancestor [53].

The triplet repeats are located in an untranslated region of the gene. An expansion in this region leads to a *gain of function* mutation; that is, the triplets result in the production of a new protein, with a new, abnormal function. In this case, the expansion results in the production of an abnormal pre-messenger RNA transcript that inappropriately binds a nuclear ribonucleoprotein called CUG binding protein [58]. This protein binding effectively prevents gene splicing, and prevents the messenger RNA transcript of the expanded gene from leaving the nucleus. The symptoms may be due to reduced levels of normal protein; because the triplet expansion causes haploinsufficiency, only the co-gene produces normal protein, at 50% of the usual amount. On the other hand, rare individuals who are homozygous for the expanded myotonic dystrophy gene do not appear to have more severe symptoms than heterozygotes, suggesting that the symptoms of myotonic dystrophy may be influenced by additional, as yet unknown, factors [59–61]. The clinical effects of the triplet expansion may also be due to nuclear toxicity caused by the trapped mRNA transcripts; toxic damage to the nucleus would be particularly destructive in muscle and nerve cells, which cannot divide [62].

Prevalence

Myotonic dystrophy is the commonest muscular dystrophy of adult life, and has a range of prevalence between 5 and 25 per 100,000 [57,63]. This wide range of prevalence reflects the methods of diagnosis (e.g., were asymptomatic individuals who carry the gene included, were all at-risk relatives tested, etc.). As with fragile X, the founder effect also influences the prevalence of myotonic dystrophy

in certain notable regions of the world, namely in northern Sweden, among South African Afrikaners, and among natives of the Saguenay-Lac St. Jean region of northern Quebec, where the prevalence is exceptionally high. On the other hand, the disease is virtually unheard of in sub-Saharan African populations. The median age at the onset of symptoms is 20 to 25 years in typical myotonic dystrophy, while the severe congenital form is evident at birth or even before.

Genotype-phenotype correlation

There are three distinct myotonic dystrophy phenotypes, which correlate directly with the size of the CTG expansion. This is illustrated by a study by Gennarelli and colleagues, who compared the severity of symptoms to the number of triplet repeats in the DM gene in 465 myotonic dystrophy patients [64]. They found a trimodal distribution in the numbers of triplet repeats, corresponding to three common DM phenotypes. However, as Fig. 3 shows, there was overlap of the number of repeats in all three modes [64]. Individuals with approximately 100 triplet repeats had a 100% probability of having the least severe form of the disease, characterized by minimal signs of myotonia without muscle impairment, mild facial abnormalities (jaw and temporal wasting, facial and sternomastoid weakness, ptosis, nasal speech, frontal balding), cataract, and no distal weakness except isolated flexor weakness of the digits. This phenotype was more common in men (75% men versus 28% women, $P < 0.001$). Individuals with more than 1300 repeats had a 90% chance of having the severest form of the disease, consisting of proximal muscle weakness, cardiomyopathy, endocrine dysfunction, mental retardation, facial abnormalities, and cataract. Those with an intermediate number of repeats, generally between 600 and 800, had an intermediate phenotype, characterized by myotonia, distal weakness, EKG abnormalities, mild mental retardation, gonadal dysfunction, facial abnormalities, and cataract. The intermediate and severe forms of disease appeared to affect men and women equally (P = N.S.).

The age at the onset of the disease is also directly correlated with the size of the repeat region; the bigger the repeat region the earlier the onset. Hunter and coworkers studied 109 myotonic dystrophy gene carriers from 17 families, and showed a striking correlation between gene size and age at the onset of symptoms [65]. Individuals shown by linkage analysis to carry the myotonic dystrophy gene, but who had no proven expansion, did not exhibit any symptoms until after age 25, while those with the largest expansion, measuring greater than 4.5 kb, were likely to have the congenital form of the disease. Harley and colleagues studied 439 individuals with myotonic dystrophy clinically and molecularly, and found similar results [66]. Those with adult onset disease generally had a CGT sequence measuring 0.5 to 2.5 kb, those with the childhood form had a sequence measuring 1.5 to 4.0 kb, and in the severe congenital form the sequence typically measured 3.0 to 6.0 kb ($P < 0.001$).

The major area of contrast between myotonic dystrophy and fragile X, however, is the fact that the repeat number can increase during transmission from *either* parent. Furthermore, the number of repeats can also *decrease* when the gene is

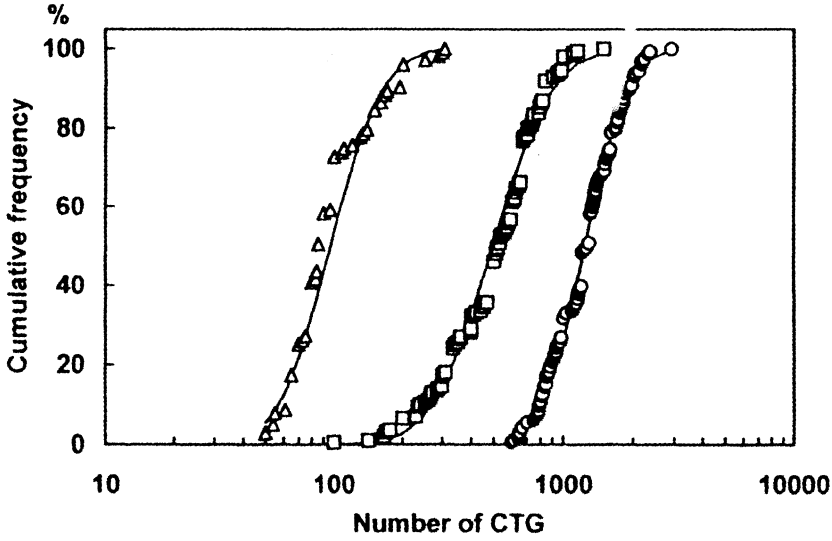

Fig. 3. Trimodal distribution of the length (size) of trinucleotide repeats in myotonic dystrophy patients. Log-normal distribution function of class frequency related to [CTG] repeat number in myotonic dystrophy patients. △, class 1; □, class 2; ○, class 3. (*From* Gennarelli M, Novelli G, Bassi F, et al. Predition of myotonic dystrophy clinical severity based on the number of intragenic $[CTG]_n$ trinucleotide repeats. Am J Med Genet 65:342–347,1996; with permission.)

transmitted by a male (Table 3). This is illustrated by a large study by Abelovich and colleagues, who evaluated 17 families with 72 members affected with myotonic dystrophy [67]. This series included 15 mothers who transmitted the gene to 23 offspring; in all cases the gene expanded. Eight of these children had congenital mytotonic dystrophy, two had the classic form, and four inherited the full mutation but are currently asymptomatic. There were also 15 men who passed the gene on to 30 offspring; in 20 cases the gene expanded, in five the gene decreased in size, and in three the gene size did not change. Seventeen of these children had the classic form, three had mild disease, and seven were asymptomatic carriers. These clinical observations fit with reports of sperm analysis showing a wide range of repeat sizes in the sperm of males with mild myotonic dystrophy [63].

Diagnosis

Myotonic dystrophy can be reliably diagnosed using molecular methods, which have eliminated the need for muscle biopsy or restriction fragment polymorphism testing of asymptomatic family members. Prediction of the likely ultimate phenotype can usually be done with some accuracy, but the finding of minimally expanded trinucleotide repeats in an asymptomatic but at-risk individual must be interpreted with caution. The issue of whether or not to test asymptomatic but at-risk children is a difficult one, but the general consensus is that, in the absence of symptoms, such testing should be postponed to adult life. In

that way health insurance can be protected and the individual can make his or her own decision about whether or not to be tested.

Obstetric issues

Although men with the disease may be infertile as the result of testicular atrophy, a similar process has not been demonstrated in women. Females with myotonic dystrophy may have menstrual irregularities, but pregnancy can occur. Overall, the fertility of women with myotonic dystrophy is reduced to 2/3 normal levels [63]. However, individually, the effects of the disease are very variable; those with the congenital form usually do not survive to reproductive age, while those with late onset disease may have completed their families before being diagnosed. Women with myotonic dystrophy do seem to have an increased risk of spontaneous pregnancy loss, distinct from losses due to the congenital disease, and ongoing pregnancies are problematic because of prolonged labor, a uterus unresponsive to oxytocin, and uterine atony [68]. More importantly, respiratory compromise can occur after exposure to even small doses of analgesics or anesthetics. Box 1 lists the medications contraindicated in myotonic dystrophy. Copies of this list should be affixed to the hospital charts of myotonic dystrophy patients to avoid inadvertant administration of a potentially toxic drug.

Box 1. Medications believed to be neurotoxic in patients with myotonic dystrophy

Antibiotics	Neomycin, Lincomycin
	Tetracycline
	Polymyxin
	Gentamycin, streptomycin, kanamycin
	Penicillamine
	Colistin
Anesthetics	Procaine, xylocaine
	Chloroprocaine, tetracaine
	Ether
	Chloroform
	Trichloroethylene
Analgesics	Morphine sulfate, other narcotics
	Meperidine
	Barbiturates
Cardiac medications	Propranolol, other β blockers
	Quinidine
	β adrenergic agents
Miscellaneous	Magnesium sulfate
	Lithium
	Quinocrine

Huntington disease

Background

Huntington disease is an autosomal dominant disorder characterized by progressive chorea, bradykinesia, and rigidity affecting both voluntary and involuntary movements, along with an insidious and slow personality change and deterioration of intellectual function. Depression is common, especially in the early stages of the disease, and is often associated with suicidal ideation. Although the diagnosis of Huntingtons disease has been made as early as two years of age and as late as 86 years, the age at the onset of symptoms is usually 32 to 42 years (±10 years) and the age at death is 50 to 56 years [69]. Approximately 6% of cases have the juvenile form, in which symptoms occur before age 20, and in 25% no symptoms appear until after age 50 [69]. Similar to myotonic dystrophy, the juvenile onset cases are more severe, while the late onset cases are usually characterized by milder symptoms.

Molecular genetics

The Huntington gene (called IT15) has been identified on chromosome 4, and includes a region of CAG triplet repeats in the 5' coding region of the gene. Expansion of the triplet repeats in this region is associated with disease. Normal individuals have 10 to 32 CAG repeats, while those with the disease have 39 to 121 repeats. Individuals with intermediate length repeats (32 to 39) are usually unaffected or have very late onset disease, but can have affected children. The function of the gene product, the huntingtin protein, is not completely understood, but it is believed to be so crucial for normal development that it is considered a *cell survival gene* [70]. In contrast to fragile X, the CAG expansion results in gain of function, not loss [70,71,80]. Gene deletions and other kinds of mutations that result in loss of the IT15 protein do not result in the symptoms of Huntington disease.

Prevalence

The reported prevalence of Huntington disease varies widely, according to the method of case ascertainment (eg, whether or not the figures include presymptomatic carriers or at risk individuals who committed suicide before the disease could be diagnosed, etc) and the heritage and ethnic background of the individuals tested. Like myotonic dystrophy, some reports of areas with high prevalence may have been influenced by a founder affect. In addition, for every symptomatic case identified, it is estimated that there are twice as many presymptomatic gene carriers. Some authors estimate that for every symptomatic carrier, there are another five individuals at 50% risk of having the disease and 11 individuals at 25% risk [69]. Considering these facts, the disease prevalence is estimated to be 10 per 100,000.

The basis of neuronal damage

As in fragile X, the CAG triplets code for the amino acid glutamine, and translation results in the addition of an excessively long polyglutamine string to the native protein. The polyglutamine alters the protein's size and charge, and prevents it from being transported or metabolized appropriately [1]. Specifically, huntingtin protein is normally cleaved by a cysteine protease, which plays an important role in apoptosis (programmed cell death). The long polyglutamine tracts appear to enhance the rate of cleavage by this enzyme, thus leading to inappropriately increased apoptosis [70]. Aggregates of this mutant protein can also form inclusion bodies within the nuclei of neurons, which likely contributes to the neuronal loss and gliosis typical of this disease.

There is also evidence indicating that the neuronal damage could result from abnormally strong binding of the mutant huntingtin protein to huntingtin-associated protein, altering the biochemistry of certain brain regions. The brain regions primarily affected by Huntington disease are the caudate, cortex, and globus pallidus. The huntingtin-associated protein is selectively expressed in the caudate and cortex, where it normally binds only weakly to huntingtin [72]. Abnormally strong protein binding in these regions, due to the altered properties of the IT15 protein caused by the polyglutamine insert, could have pathologic consequences.

The abnormal huntingtin protein also binds gylceraldehyde-3-phosphate dehydrogenase, an essential enzyme for glycolysis; the longer the polyglutamine tract, the greater the inhibiting effect on enzyme function [10]. Another effect of the abnormal protein may thus be to inhibit energy utilization in select areas of the brain. Regardless of the exact mechanism of neuronal damage, it is apparent that the size of the polyglutamine repeat, determined by the number of CAG repeats in the huntington gene, determines how many years it takes for toxic neuronal changes to occur, and by extension when symptoms will first appear.

Genotype-phenotype correlation

Thus, like myotonic dystrophy, there is a significant correlation between the number of repeats and the age of onset [73,74]; however, the range of instability is much smaller than in myotonic dystrophy, and the correlation with age at onset seems to be confined to the juvenile form of the disease. In fact, repeat length is believed to explain only 50% of the variance of onset age [75]. This was illustrated by Macmillan and colleagues, who analyzed DNA from 449 patients with Huntington disease, and correlated their molecular findings with disease course [76]. The patients with adult onset disease presented with motor abnormalities (77%) or psychiatric disturbance (23%) at a mean age of 42 ± 11 years, and inherited a mean of 42 copies of the CAG repeat (range 16 to 58). Those with the juvenile onset form had a mean age of onset of 21 ± 5 years, and inherited a mean of 60 copies (range 52 to 67). Thus, the age of onset varied over a range of 20 years in the adult onset group, while the number of repeats varied over a range of only 42 copies, much less than in myotonic dystrophy.

There is also a strong relationship between the number of repeats and the type and severity of symptoms in Huntington disease. Many Huntington patients with juvenile onset have a form of the disease called the Westphal variant, a very severe version of the disease characterized by rigidity and akinesia, dystonia, and severe intellectual decline. Affected individuals also frequently have seizures and myoclonus. In contrast, late onset patients typically have very mild symptoms, such as mildly progressive chorea, normal intellect, normal eye movements, and little obvious intellectual or psychiatric change. In fact, in late onset disease, brain pathology may be missed on postmortem examination unless specifically searched for.

The most interesting aspect of disease transmission, which is quite different from the situation in both myotonic dystrophy and fragile X, is that there is a strong correlation between *paternal* inheritance and the early form of the disease [69] (Table 3). Ninety percent of juvenile cases have unusually long CAG repeats and inherit the gene from their father. Several studies regarding the difference in phenotype resulting from maternal versus paternal disease transmission have been published. For example, Ranen and coworkers examined 277 parent-child pairs with Huntington disease [75]. The age at onset of symptoms and the number of triplet repeats in the IT15 gene were known in 60 pairs. These patients were culled from an epidemiologic survey, and thus represented the Huntington disease population fairly accurately. There was no difference in the age at symptom onset between affected mothers and fathers. Likewise, affected mothers and their offspring (male and female) had symptom onset at similar ages; approximately half of the offspring of affected mothers were affected a few years later and half affected a few years earlier than their mothers. However, the offspring of affected fathers had a significantly earlier onset than either their fathers or the offspring of affected mothers. Forty five percent were affected <6 years earlier, 20% were affected between 6 and 12 years earlier, and 35% were affected more than 12 years earlier. Furthermore, 15% of the offspring of affected fathers had the juvenile onset form of the disease compared to only 5% of the offspring of affected mothers, and 77% of the juvenile onset cases had affected fathers. The repeat length correlated with these observations: there was no significant difference in repeat length between affected mothers and their offspring, while the offspring of affected fathers had significantly longer repeat lengths.

The repeat length has been shown to expand during spermatogenesis [71]. It is currently believed that, in direct contrast to fragile X, CAG instability in the huntingtin gene is greater in successive meioses in spermatogenesis than in oogenesis, although the mechanism for this is unclear [69,77].

Diagnosis

The advent of molecular genetic diagnosis for Huntington disease has made it possible both to confirm the diagnosis in symptomatic individuals and to offer pre-symptomatic testing to individuals at risk of inheriting the disease. The region of triplet expansion can be identified and quantified molecularly, and the

relationship between expansion size and symptoms makes it possible to predict the degree of affectation within a certain range of accuracy. Because Huntingtons is usually an adult onset disease, many at risk individuals consider presymptomatic testing because they are concerned about their future health status and their reproductive risks. In contrast to fragile X, in which the disease features are evident early in life, and myotonic dystrophy, whose symptoms are primarily muscular, Huntington disease is uniquely terrifying because it strikes otherwise normal adults and involves an insidious neuropsychiatric decline. Most presymptomatic testing programs, in place since the 1980s, have required the patient to undergo extensive psychological counseling before decisions about testing are made because of concerns about possible catastrophic reactions to the test results [78]. Several studies affirming the benefit of such pretest counseling have been performed. For example, Wiggins and colleagues prospectively followed 135 individuals undergoing extensive counseling and presymptomatic Huntington disease testing [78]. They found that, while those who were determined to be at high risk of developing Huntington disease did not experience the same psychological benefit as those receiving more reassuring news, the counseling appeared to have been effective in reducing their level of depression and increasing their sense of well being. Because of the intricacies of testing and test result interpretation, and especially because of the psychological ramifications of the testing process, testing for Huntington disease should be performed in a tertiary center with special expertise in the diagnosis of this disease.

Obstetric issues—prenatal diagnosis

Adult onset Huntington disease usually manifests after the reproductive years, and the juvenile form of the disease is so severe that it is rarely associated with reproduction. The main issue for obstetricians to confront is therefore prenatal diagnosis. Molecular diagnosis has also made it possible to perform prenatal testing. Often, the parent at risk to have the gene and pass it on has not yet been tested, and so should be referred for specialized counseling and consideration of presymptomatic testing before prenatal diagnosis is considered. Once risk has been established, further counseling regarding the ramifications of prenatal testing must be provided. Some authorities are not in favor of prenatal testing for an adult onset disease, especially one in which the precise age at onset and the exact nature of the symptoms cannot be predicted with certainty. In addition, the individual to be tested would likely not be symptomatic for at least 20 years, by which time major advances in therapy may have been made. Furthermore, if the fetus is found to be at risk but the pregnancy is not terminated, the child's health insurance may be jeopardized, and other forms of discrimination may ensue. Having said this, however, prenatal testing for Huntingtons followed by pregnancy termination because of a positive result has been reported [79]. Because of the issues involved, prenatal diagnosis should only be performed in a tertiary center with special expertise in prenatal genetics.

Other triplet diseases

It is now apparent that triplet repeat expansion is responsible for a number of genetic conditions, primarily neurologic diseases [80]. The list includes Friedrich's ataxia, X-linked spinal and bulbar muscular atrophy (Kennedy's disease), spinocerebellar ataxia types 1 and 2, dentato-rubro-pallido-luysian atrophy, and Machado-Joseph disease. Most of these diseases are associated with gain of function mutations, presumably leading to neural tissue toxicity.

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