



Chapter 17

Personalized Genetic Medicine

More than a century ago, the British physician-scientist Archibald Garrod applied Mendel's laws of heredity to the inheritance of human disease and coined the term **inborn error of metabolism**, thereby creating the field of biochemical genetics. Garrod had more in mind, however, than unusual biochemical changes in patients with autosomal recessive disorders of intermediary metabolism. In a demonstration of prescient scientific and clinical insight, he proposed the much broader concept of **chemical individuality**, in which each of us differs in our health status and susceptibility to various illnesses because of our individual genetic makeup. Indeed, in 1902, he wrote:

... the factors which confer upon us our predisposition and immunities from disease are inherent in our very chemical structure, and even in the molecular groupings which went to the making of the chromosomes from which we sprang.

Now, more than a hundred years later in the era of human genomics, we have the means to assess an individual's genotype at all relevant loci and to characterize the genetic underpinnings of each person's unique "chemical individuality." When the genetic variants relevant to maintaining health and preventing or treating illness in each individual are known, and when that knowledge is used in making important clinical decisions as a routine part of medical care, we will have entered the era of **personalized genetic medicine**, one of the major goals of the Human Genome Project. However, personalized genetic medicine is only one component of patient-centered medical care in the broadest sense, in which care providers also take each individual's developmental history, environmental exposure, and social experiences into account when providing diagnosis, counseling, preventive intervention, management, and therapy.

In the preceding chapter on genetics and cancer, we described powerful new genomic technologies, such as determining which mutations and polymorphisms are present in a tumor and profiling its pattern of RNA expression, that are currently being used for the molecular characterization of cancer (see Chapter 16). Such information is proving increasingly helpful for guiding management and therapy for individual cancer patients, as one application of what might be called **genomic medicine**. In this chapter, we explore other applications of genetics and genomics to individualized health care: screening asymptomatic individuals for susceptibility to disease and applying that knowledge to improve health care. First, we describe how the family history can be used to assess risk and to guide preventive and therapeutic measures in asymptomatic individuals. Next, we discuss population screening and present one of the oldest forms of genetic screening, the detection of abnormalities in newborns at high risk for preventable illness. Finally, we discuss screening of patients for genetic susceptibility based on their genotypes alone and review some of the concepts and methods of genetic epidemiology that are commonly used to evaluate screening for susceptibility genotypes.

● FAMILY HISTORY AS PERSONALIZED GENETIC MEDICINE

Physicians have long practiced a form of personalized genetic medicine when they obtain a family history and use it in their clinical decision-making. Family history is clearly of great importance in dealing with single-gene disorders. Applying the known rules of mendelian inheritance allows the geneticist to provide accurate evaluations of risk for disease in relatives of affected individuals (see Chapter 19). Family history is also important when a geneticist assesses the risk for complex

Family History in Risk Assessment

High Risk

- Premature disease in a first-degree relative
- Premature disease in a second-degree relative (coronary artery disease only)
- Two affected first-degree relatives
- One first-degree relative with late or unknown disease onset and an affected second-degree relative with premature disease from the same lineage
- Two second-degree maternal or paternal relatives with at least one having premature onset of disease
- Three or more affected maternal or paternal relatives
- Presence of a “moderate-risk” family history on both sides of the pedigree

Moderate Risk

- One first-degree relative with late or unknown onset of disease
- Two second-degree relatives from the same lineage with late or unknown disease onset

Average Risk

- No affected relatives
- Only one affected second-degree relative from one or both sides of the pedigree
- No known family history
- Adopted person with unknown family history

From Scheuner MT, et al: *Am J Med Genet* 71:315-324, 1997; quoted in Yoon PW, et al: *Genet Med* 4:304-310, 2002.

disorders, as discussed in Chapter 8 and elsewhere in this book. Since a person's genes are shared with his or her relatives, family history provides the clinician with information on the impact that a substantial subset of an individual's genetic makeup might have on one's health, using the medical history of relatives as an indicator of one's own genetic susceptibilities. Furthermore, family members often share environmental factors, such as diet and behavior, and thus relatives provide information about both shared genes and shared environmental factors that may interact to cause most common diseases with complex inheritance. Having a first-degree relative with a common disease of adulthood—such as cardiovascular disease, cancer of the breast, cancer of the colon or prostate, type 2 diabetes, osteoporosis, or asthma—raises an individual's risk for the disease approximately 2-fold to 3-fold relative to the general population, a moderate increase compared with the average population risk (see Box). As discussed in Chapter 8, the more first-degree relatives one has with a complex trait and the earlier in life the disease occurs in a family member, the greater the load of susceptibility genes and environmental exposures likely to be present in the patient's family, leading to a designation of the patient as being at high risk for disease on

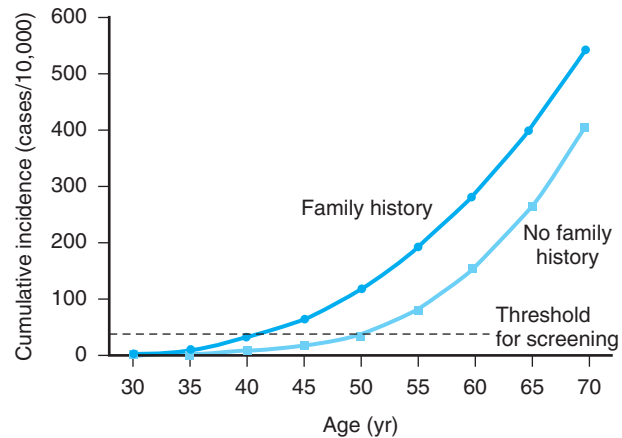


Figure 17-1 ■ Cumulative incidence (per 10,000) of colon cancer versus age in individuals with and without a family history of the disease. (Data from Fuchs CS, Giovannucci EL, Colditz GA, et al: A prospective study of family history and the risk of colorectal cancer. *N Engl J Med* 331:1669-1674, 1994.)

the basis of family history. For example, a male with three male first-degree relatives with prostate cancer has an 11-fold greater relative risk for development of the disease than does a man with no family history.

Determining that an individual is at increased risk on the basis of family history can have an impact on individual medical care. For example, two individuals with deep venous thrombosis, one with a family history of unexplained deep venous thrombosis in a relative younger than 50 years and another with no family history of any coagulation disorder, will receive different management with respect to testing for factor V Leiden or prothrombin 20210G>A and anticoagulation therapy (see Chapter 8). Similarly, a family history of colon cancer is sufficient to trigger the initiation of colon cancer screening with more sophisticated screening methods at the age of 40 years, 10 years earlier than for the general population. This is because the cumulative incidence for development of the disease for someone 40 years old with a positive family history equals the risk for someone at the age of 50 years with no family history (Fig. 17-1). The increase in risk is even more pronounced if two or more relatives have had the disease.

Family history is, unfortunately, a relatively under-used tool in clinical medicine. In one survey, primary care physicians were found to discuss family history with only half of their new patients and with less than one quarter of their return patients. Only one patient in nine observed by the physicians in that managed care practice was found to have a family tree in the chart. In another survey performed in a managed health care setting, the fact that a patient had one or more first-degree relatives with the disease—and was therefore at increased risk for one of the common adult-onset dis-

eases inherited as a complex trait—was missed in nearly two thirds of patients. It is worth repeating the admonition made by the distinguished pediatrician and geneticist, Barton Childs, quoted in Chapter 1: “to fail to take a good family history is bad medicine.”

Of course, with the exception of monozygotic twins, no one shares all his genes with his relatives. Family history is therefore an indirect means of assessing the contribution that an individual’s own combination of genetic variants might make to disease. Family history is also an insensitive indicator of susceptibility since it depends on overt disease actually occurring in the relatives of the individual patient. The challenge going forward is to screen populations, independent of family history, for variants relevant to health and disease and to apply this information to make risk assessments that can be used to improve the health care of the individual patient and his or her family. Applying this information requires that we demonstrate that genetic risk factors are valid indicators of actual risk in an individual patient and, if they are valid, how useful such information is in guiding health care.

● GENETIC SCREENING IN POPULATIONS

Genetic screening is a population-based method for identifying persons with increased susceptibility to or risk for a genetic disease. Screening at the population level is not to be confused with testing for affected persons or carriers within families already identified because of family history. Rather, the objective of population screening is to examine *all* members of a designated population, regardless of family history. Genetic screening is an important public health activity that will become more significant as more and better screening tests become available for determining genetic susceptibilities for disease.

Clinical Validity and Utility

Finding the genetic contributions to health and disease is of obvious importance for research into underlying disease etiology and pathogenesis as well as for identifying potential targets for intervention and therapy. In medical practice, however, whether to screen individuals for increased susceptibilities to illness depends on the **clinical validity** and **clinical utility** of the test. Clinical validity is the extent to which a test result is predictive for disease. The **clinical utility** of a test is the degree to which test results will change what medical care an individual receives and, as a consequence, improve the outcome of care, both medically and economically. Clinical utility can be assessed both for the individual being screened and for the entire population that participates in a screening program.

A **genetic disease association** is the relationship between a susceptibility or protective genotype and a

disease phenotype. The susceptibility or protective genotype can be defined as the presence of an allele (in either a heterozygote or a homozygote), the homozygous genotype only, a haplotype containing alleles at neighboring loci, or even combinations of genotypes at multiple unlinked loci. Assuming whatever test being used to detect the genotype gives the correct assignment of the genotype to each person being tested (the **analytic validity** of the test), the clinical validity represents how well the genotype predicts the phenotype, and vice versa. Clinical validity depends on how sensitive and specific the test is for the phenotype, that is, the false-negative and false-positive rates. When faced with an individual patient, however, the practitioner of personalized genetic medicine wants to know more than how sensitive or specific a test is. A third facet of clinical validity is also of concern: to what extent does a particular genotype provide information on whether this patient is at risk for a particular disease, not relative to those without the genotype but in absolute terms? This facet of clinical validity is captured by the **positive predictive value** and **negative predictive value** of the test for that disease. The relationship between some of these factors is best demonstrated by means of a 2×2 table.

Determination of the Predictive Value of a Test

Genotype	DISEASE		Total
	Affected	Unaffected	
Susceptibility genotype present	a*	b	a + b
Susceptibility genotype absent	c	d	c + d
Total	a + c	b + d	a + b + c + d = N

Frequency of the susceptibility genotype = $(a + b)/N$
 Disease prevalence = $(a + c)/N$ (with random sampling or a complete population survey)

Relative Risk Ratio :

$$\begin{aligned} \text{RRR} &= \frac{\text{Disease prevalence in carriers of susceptibility genotype}}{\text{Disease prevalence in non-carriers of susceptibility genotype}} \\ &= \frac{a/(a + b)}{c/(c + d)} \end{aligned}$$

Sensitivity: Fraction of individuals with disease who have the susceptibility genotype = $a/(a + c)$

Specificity: Fraction without disease who do not have the susceptibility genotype = $d/(b + d)$

Positive predictive value: Proportion of individuals with the susceptibility genotype who have or will develop a particular disease = $a/(a + b)$

Negative predictive value: Proportion of individuals without the susceptibility genotype who do not have or will not develop a particular disease = $d/(c + d)$

*The values of a, b, c, and d are derived from a random sample of the population, divided into those with and without the susceptibility genotype, and then examined for the disease (with or without longitudinal follow-up, depending on whether it is a cross-sectional or cohort study) (see later).

Table 17-1

Some Conditions for Which Newborn Screening Has Been Implemented

Condition	Frequency (per 100,000 newborns)*
Congenital hearing loss	200
Sickle cell disease	47
Hypothyroidism	28
Phenylketonuria	3
Congenital adrenal hyperplasia	2
Galactosemia	2
Maple syrup urine disease	≤1
Homocystinuria	≤1
Biotinidase deficiency	≤1

*Approximate values in the United States.

Newborn Screening

The best-known population screening efforts in genetics are the government programs that identify presymptomatic infants with diseases for which early treatment can prevent or at least ameliorate the consequences (Table 17-1). For newborn screening, disease risk is not assessed by determining the genotype directly. Instead, risk is usually measured by detecting abnormally high levels of certain metabolites in the blood of infants who are asymptomatic as newborns but are at greatly increased risk for development of disease later in life. These metabolites are chosen to have high analytic validity for genotypes that have high positive predictive value for serious metabolic disorders later in life. Exceptions to this paradigm of using a biochemical measurement to detect a disease-causing genotype are screening programs for hypothyroidism and abnormalities in hearing, in which the phenotype itself is the target of screening and intervention (see later).

Many of the issues concerning genetic screening in general are highlighted by newborn screening programs. A determination of the appropriateness of newborn screening for any particular condition is based on a standard set of criteria involving analytic validity, clinical validity, and clinical utility (see Box). The clinical validity of test results is obviously important. False-positive results cause unnecessary anxiety to the parents as well as increase the costs because more unaffected infants have to be recalled for retesting. False-negative results vitiate the purpose of having a screening program. The criterion that the public health system infrastructure be capable of handling the care of newborns identified by screening is often underemphasized in discussions of the clinical utility of screening but must also be considered in deciding whether to institute screening for any given condition.

The prototype condition that satisfies all of these criteria is **phenylketonuria** (see Chapter 12). For many

General Criteria for an Effective Newborn Screening Program**Analytic Validity**

- A rapid and economic laboratory test is available that detects the appropriate metabolite.

Clinical Validity

- The laboratory test is highly sensitive (no false negatives) and reasonably specific (few false positives). Positive predictive value is high.

Clinical Utility

- Treatment is available.
- Early institution of treatment, before symptoms become manifest, reduces or prevents severe illness.
- Routine observation and physical examination will not reveal the disorder in the newborn—a test is required.
- The condition is frequent and serious enough to justify the expense of screening; that is, screening is cost-effective.
- The public health system infrastructure is in place to inform the newborn's parents and physicians of the results of the screening test, to confirm the test results, and to institute effective treatment and counseling.

years, elevated levels of phenylalanine in a spot of blood on filter paper obtained soon after birth has been the mainstay of neonatal screening for phenylketonuria and other forms of hyperphenylalaninemia in all states in the United States, all the provinces of Canada, and nearly all developed countries. A positive screen result, followed by definitive confirmation of the diagnosis, led to the institution of dietary phenylalanine restriction early in infancy, thereby preventing irreversible mental retardation.

Two other conditions that are widely targeted for newborn screening are **congenital deafness** and **congenital hypothyroidism**. Newborn screening for hearing loss is mandated in 37 states in the United States and three provinces in Canada. Approximately half of all congenital deafness is due to single-gene defects (Case 11). Infants found to have hearing impairments by newborn screening receive intervention with sign language and other communication aids early in life, thereby improving their long-term language skills and intellectual abilities beyond that seen if the impairment is discovered later in childhood. Screening for congenital hypothyroidism, a disorder that is genetic only 10% to 15% of the time but is easily treatable, is universal in the United States and Canada and is also routine in many countries. Thyroid hormone replacement therapy started early in infancy completely prevents the severe and irreversible mental retardation caused by congenital hypothyroidism. Thus, both hypothyroidism and

Table 17-2

Disorders Detectable by Tandem Mass Spectrometry

Condition	Substances Present at Increased Levels
Amino acidemias	
Phenylketonuria	Phenylalanine and tyrosine
Maple syrup urine disease	Leucine and isoleucine
Homocystinuria	Methionine
Citrullinemia	Citrulline
Argininosuccinic aciduria	Argininosuccinic acid
Hepatorenal tyrosinemia	Methionine and tyrosine
Organic acidemias	Relevant acylcarnitine metabolites
Propionic acidemia	
Methylmalonic acidemia	
Isovaleric acidemia	
Isolated 3-methylcrotonylglycinemia	
Glutaric acidemia (type I)	
Mitochondrial acetoacetyl-CoA thiolase deficiency	
Hydroxymethylglutaric acidemia	
Multiple CoA carboxylase deficiency	
Fatty acid oxidation disorders	Relevant acylcarnitine metabolites
Short-chain acyl-CoA dehydrogenase deficiency	
Short-chain hydroxy acyl-CoA dehydrogenase deficiency	
Medium-chain acyl-CoA dehydrogenase deficiency	
Very long chain acyl-CoA dehydrogenase deficiency	
Long-chain acyl-CoA dehydrogenase and trifunctional protein deficiency	
Glutaric acidemia type II	
Carnitine palmitoyltransferase II deficiency	

American College of Medical Genetics/American Society of Human Genetics Test and Technology Transfer Committee Working Group: Tandem mass spectrometry in newborn screening. *Genet Med* 2:267-269, 2000.

deafness easily fulfill the criteria for newborn screening.

A number of other disorders, such as galactosemia, sickle cell disease (Case 37), biotinidase deficiency, and congenital adrenal hyperplasia, are part of neonatal screening programs in many or most states and provinces, but not all. For sickle cell disease, the disorder is more common than phenylketonuria overall in the United States, and identifying asymptomatic newborns with the sickle cell disease genotype means that protective measures can be instituted against the life-threatening bacterial sepsis that can occur before overt manifestations of the disease. For this reason, all but eight states, those with small African American populations, screen newborns routinely for sickle cell disease. Which disorders should be the target of newborn screening varies from state to state and continues to be a matter of debate among government public health agencies.

Tandem Mass Spectroscopy

For many years, most newborn screening was performed by a test specific for each individual condition. For example, phenylketonuria screening was based on a microbial or a chemical assay that tested for elevated

phenylalanine. This situation has changed dramatically during the past decade, however, with the application of the technology of **tandem mass spectrometry (TMS)**. Not only can a neonatal blood spot be examined accurately and rapidly for an elevation of phenylalanine, with fewer false positives than with the older testing methods, but TMS analysis can simultaneously detect a few dozen other biochemical disorders as well. Some of these were already being screened for by individual tests (Table 17-2). For example, many states were using specific tests for elevated methionine to screen for homocystinuria due to cystathionine β -synthase deficiency (see Chapter 12) or elevated branched-chain amino acids in maple syrup urine disease. A single TMS analysis to measure phenylalanine will also simultaneously detect elevated methionine or branched-chain amino acids. TMS, however, cannot replace the disease-specific testing methods for other disorders currently included in newborn screening, such as galactosemia, biotinidase deficiency, congenital adrenal hyperplasia, and sickle cell disease.

TMS also provides a reliable method for newborn screening for some disorders that fit the criteria for screening but had no reliable newborn screening program in place. For example, **medium-chain acyl-CoA dehydrogenase (MCAD) deficiency** is a disorder of fatty acid oxidation that is usually asymptomatic but

manifests clinically when the patient becomes catabolic. Detection of MCAD deficiency at birth can be lifesaving because affected infants and children are at very high risk for life-threatening hypoglycemia in early childhood during the catabolic stress caused by an intercurrent illness, such as a viral infection, and nearly a quarter of children with undiagnosed MCAD deficiency will die with their first episode of hypoglycemia. The metabolic derangement can be successfully managed if it is treated promptly. In MCAD deficiency, alerting parents and physicians to the risk of metabolic compensation is the primary goal of screening since the children are healthy between attacks and do not require daily management other than avoidance of prolonged fasting.

The use of TMS for newborn screening is not without controversy, however. In addition to providing a rapid test for many disorders for which newborn screening either is already being done or can easily be justified, TMS also identifies infants with inborn errors, such as methylmalonic acidemia, that have *not* generally been the targets of newborn screening because of their rarity and difficulty of providing definitive therapy that will prevent the progressive neurological impairment. TMS can also identify abnormal metabolites whose significance for health are uncertain. For example, **short-chain acyl-CoA dehydrogenase (SCAD)** deficiency, another disorder of fatty acid oxidation, is most often asymptomatic, although a few patients may have difficulties with episodic hypoglycemia. Thus, the positive predictive value of a positive TMS screen result for symptomatic SCAD is probably very low. Does the benefit of detecting SCAD deficiency outweigh the negative impact of raising parental concern unnecessarily for most newborns whose test result is positive but who will never be symptomatic? Thus, not every disorder detected by TMS fits the criteria for newborn screening. Some public health experts, therefore, argue that only those metabolites of proven clinical utility should be reported to parents and physicians. Others advocate use of all the information TMS provides and reporting of all abnormal metabolites the TMS screening detects to parents and their physicians, regardless of how well the disorders fit the standard criteria for newborn screening. Patients who show abnormalities of unknown significance can then be carefully observed. For all these reasons, the proper use of TMS for newborn screening remains a subject of debate.

Prenatal Screening

Two tests are commonly used for population screening in fetal life: chromosome analysis for advanced maternal age, and maternal serum alpha-fetoprotein or triple screens for neural tube defects and chromosome aneu-

ploidies. This topic is discussed in the context of prenatal diagnosis in Chapter 15. It has been argued, however, that once the pregnancy has been exposed to the risk of invasive prenatal diagnosis of chromosomal aneuploidy because of advanced maternal age, additional testing should also be offered, such as alpha-fetoprotein levels in amniotic fluid (Chapter 15), genome-wide comparative genome hybridization to find deleterious submicroscopic deletions (Chapters 4 and 5), and mutation screening for cystic fibrosis (see Chapter 12 and **Case 10**) and other common disorders.

● SCREENING FOR GENETIC SUSCEPTIBILITY TO DISEASE

Genetic Epidemiology

Epidemiological studies of risk factors for disease rely heavily on population studies that measure disease prevalence or incidence and determine whether certain risk factors (genetic, environmental, social, and other) are present in individuals with and without disease. **Genetic epidemiology** is concerned with how genotypes and environmental factors interact to increase or decrease susceptibility to disease. Epidemiological studies generally follow one of three different strategies: the case-control, the cross-sectional, and the cohort design (see Box).

••• Strategies Used in Genetic Epidemiology

- **Case-control:** Individuals with and without the disease are selected, and the genotypes and environmental exposures of individuals in the two groups are determined and compared.
- **Cross-sectional:** A random sample of the population is selected and divided into those with and without the disease, and their genotypes and environmental exposures are determined and compared.
- **Cohort:** A sample of the population is selected and observed for some time to ascertain who does or does not develop disease, and their genotypes and environmental exposures are determined and compared. The cohort may be selected at random or may be targeted to individuals who share a genotype or an environmental exposure.

Cohort and cross-sectional studies not only capture information on the relative risk conferred by different genotypes but, if they are random population samples, also provide information on the prevalence of the disease and the frequency of the various genotypes under study. A randomly selected cohort study, in particular, is the most accurate and complete in that phenotypes that take time to appear have a better chance

of being detected and scored; they are, however, more expensive and time-consuming. Cross-sectional studies, on the other hand, suffer from underestimation of the frequency of the disease. First, if the disease is rapidly fatal, many of the patients with disease and carrying a risk factor will be missed. Second, if the disease shows age-dependent penetrance, patients carrying a risk factor will actually not be scored as having the disease. Case-control studies, on the other hand, allow researchers to efficiently target individuals, particularly with relatively rare phenotypes that would require very large sample sizes in a cross-sectional or cohort study. However, unless a study is based on complete ascertainment of individuals with a disease, such as in a population register or surveillance program, or uses a random sampling scheme, a case-control study cannot capture information on the population prevalence of the disease.

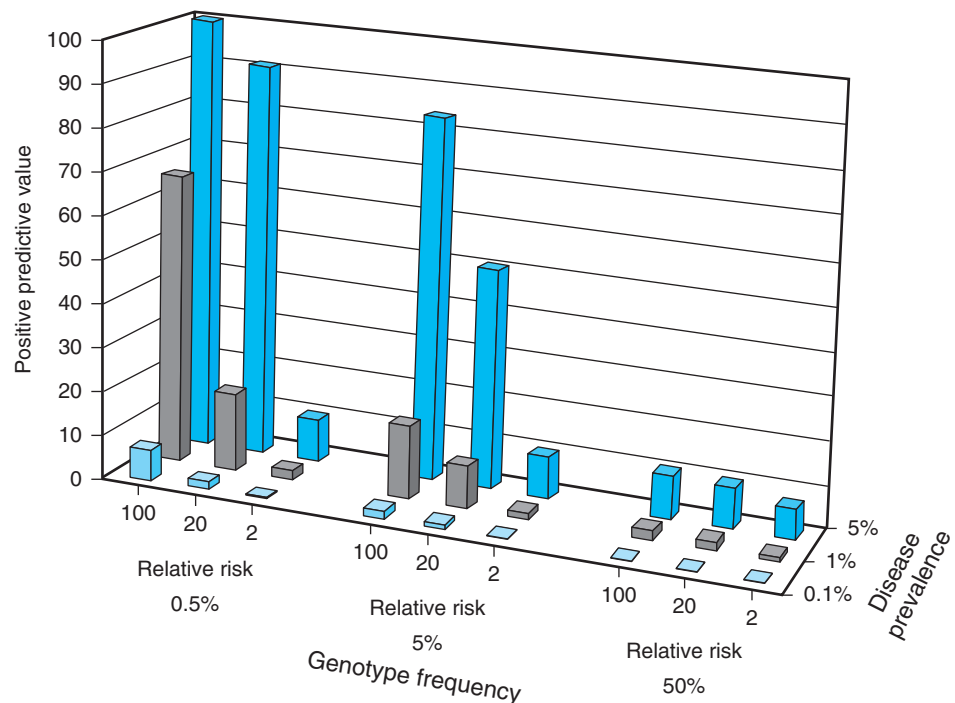
Susceptibility Testing Based on Genotype

The positive predictive value of a genotype that confers susceptibility to a particular disease depends on the frequency of the genotype in the population, the relative risk for disease conferred by one genotype over another, and the prevalence of the disease. Figure 17-2 provides the positive predictive value for genotype frequencies ranging from 0.5% (rare) to 50% (common), which confer a relative risk that varies from low (2-fold) to high (100-fold), when the prevalence of the disease ranges from relatively rare (0.1%) to more common (5%). As Figure 17-2 shows, the value of the test as a

predictor of disease increases substantially when one is dealing with a common disorder due to a relatively rare susceptibility genotype that confers a high relative risk, compared with the risk for individuals who do not carry the genotype. The converse is also clear: testing for a common genotype that confers a modest relative risk is of limited value as a predictor of disease.

We will illustrate the use of the 2×2 table (see earlier in the chapter) in assessing the role of susceptibility genes in a common disorder, **colorectal cancer**. Shown in the following Box are data from a population-based study of colorectal cancer risk conferred by a polymorphic variant in the *APC* gene (see Chapter 16 and **Case 13**) that changes isoleucine 1307 to lysine (Ile1307Lys). This variant has an allele frequency of about 3.1% among Ashkenazi Jews, which means that approximately 1 in 15 individuals is either a heterozygote or homozygote for the allele. The prevalence of colon cancer in this group of patients is 1%. This variant, common enough to be present in approximately 6% of the Ashkenazi Jewish population and conferring a 2.4-fold increased risk for colon cancer, compared with those without the allele, can be an important risk factor in that nearly 9% of all colon cancer in this population can be attributed to the effect of this allele. However, the small positive predictive value (2%) means that an individual who tests positive for this allele has only a 2% chance of developing colorectal cancer. If this had been a cohort study that allowed complete ascertainment of everyone in whom colorectal cancer was going to develop, the penetrance would, in effect, be only 2%.

Figure 17-2 ■ Theoretical positive predictive value calculations for a susceptibility genotype for a disease, over a range of genotype frequencies, disease prevalences, and relative risks for disease conferred by the genotype.



••• The Ile1307Lys Allele of the APC Gene and Colon Cancer			
Colon Cancer			
Genotype	Affected	Unaffected	Total
Lys1307	7	310	317
Ile1307	38	4142	4180
Total	45	4452	4497

- Relative Risk Ratio = RRR

$$= \frac{\text{Disease prevalence in allele carriers}}{\text{Disease prevalence in non-carriers}}$$

$$= \frac{7/317}{38/4180} = 2.4$$
- Sensitivity: Fraction of individuals with colon cancer who have the allele = $7/45 = 16\%$
- Specificity: Fraction without colon cancer who do not have the allele = $4142/4452 = 93\%$
- Positive predictive value: Fraction of individuals with the allele who develop colon cancer = $7/317 = 2\%$
- Negative predictive value: Fraction of individuals without the allele who do not develop colon cancer = 99%

Data from Woodage T, King SM, Wacholder S, et al. Nat Genet 20:62-65, 1998.

Clinical Utility

In a patient who tests positive for the APC Ile1307Lys allele, how does a positive predictive value of 2% translate into medical practice? A complete assessment of the value of testing for genotypes associated with disease does not end with determination of the clinical validity of testing. There is no absolute value of the positive predictive value that determines whether testing is or is not worthwhile. The test must be assessed with regard to clinical utility; that is, do the results of the test influence what care is provided, and more broadly, what are the implications for individual health care and public health if such screening were instituted as part of routine health care?

The clinical utility of a screening test depends on many factors. One critical factor is a public health economic one: can the screening be shown to be **cost-effective**? Is the expense of the testing outweighed by improving health outcomes while reducing health care costs, disability, and loss of earning power? In the example of screening for the APC Ile1307Lys allele in Ashkenazi Jews, the utility of certain kinds of testing might indicate the need for a particular regimen of colon cancer surveillance, such as more frequent screening or the use of different approaches to screening. Screening methods (occult stool blood testing versus sigmoidoscopy versus full colonoscopy) differ in expense, sensitivity, specificity, and potential for hazard, and so deciding which regimen to follow has important

implications for the patient's health and health care costs.

Demonstrating that testing improves health care is not always obvious. For example, 1 in 200 to 250 white individuals are homozygous for a Cys282Tyr mutation in the *HFE* gene associated with **hereditary hemochromatosis**, a disorder characterized by iron overload that can silently lead to extensive liver damage and cirrhosis (Case 17). A simple intervention, regular phlebotomy and blood donation to reduce total body iron stores, can prevent hepatic cirrhosis. The susceptibility genotype is common, and 60% to 80% of Cys282Tyr homozygotes show biochemical evidence of increased body iron stores, which suggests that screening to identify asymptomatic individuals who should undergo further testing and, if indicated, the institution of regular phlebotomy, seems a reasonable and cost-effective measure. However, most Cys282Tyr homozygotes remain clinically asymptomatic, leading to the argument that the positive predictive value of *HFE* gene testing for liver disease in hereditary hemochromatosis is too low to justify population screening. Nonetheless, many of these largely asymptomatic patients have signs of silent fibrosis and cirrhosis on liver biopsy, indicating that the Cys282Tyr homozygote may actually be at a higher risk for liver disease than previously thought. Thus, some would argue for population screening to identify individuals in whom regular prophylactic phlebotomy should be instituted. The clinical utility of such population screening remains controversial and will require additional research to determine the natural history of the disease and whether the silent fibrosis and cirrhosis seen on liver biopsy represent the early stages of what will be a progressive illness.

There are other positive and negative outcomes of testing that are psychological in nature and more difficult to assess than the purely economic factors. For example, testing positive for a susceptibility genotype could, on the one hand, empower patients with knowledge of their risks as they make important lifestyle decisions or, on the other hand, cause severe psychological distress or inappropriate fatalism in patients and their relatives who may never develop the disease but test positive for the risk factor. Similarly, patients who test negative could be falsely reassured.

APOE testing in **Alzheimer disease (AD)** (see Chapter 12 and Case 3) provides a clear example of the role of a careful assessment of clinical validity and clinical utility in applying genetic testing to personalized medicine. As discussed in Chapter 8, heterozygotes for the $\epsilon 4$ allele of the *APOE* gene are at a threefold increased risk for development of AD, primarily because the age at onset of AD is shifted 10 to 15 years earlier in them compared with individuals without an *APOE*

Table 17-3

Clinical Validity and Utility of APOE Population Screening and Diagnostic Testing for Alzheimer Disease

	Population Screening	Diagnostic Testing
Clinical validity	Asymptomatic individuals aged 65-74 Population prevalence of AD = 3% PPV given $\epsilon 2/\epsilon 4$ or $\epsilon 3/\epsilon 4$ = 6% PPV given $\epsilon 4/\epsilon 4$ = 23%	Individuals aged 65-74 with symptoms of dementia Proportion of dementia patients with AD = ~60% PPV given $\epsilon 2/\epsilon 4$ or $\epsilon 3/\epsilon 4$ = ~75% PPV given $\epsilon 4/\epsilon 4$ = ~98%
Clinical utility	No intervention possible to prevent disease Psychological distress for most people with $\epsilon 4$ alleles who are not likely to develop AD False reassurance for those without $\epsilon 4$ alleles	Increase suspicion that another, potentially treatable cause of dementia may be present Reduce unnecessary testing

Positive predictive value (PPV) calculations are based on a population prevalence of Alzheimer disease (AD) of approximately 3% in individuals aged 65 to 74 years, an allele frequency for the $\epsilon 4$ allele in whites of 10% to 15%, a relative risk of approximately 3 for one $\epsilon 4$ allele, and a relative risk of approximately 20 for two $\epsilon 4$ alleles.

$\epsilon 4$ allele. *APOE* $\epsilon 4/\epsilon 4$ homozygotes are at a 20-fold increased risk because their age at onset of AD is shifted by 20 to 30 years. *APOE* testing for the $\epsilon 4$ allele, however, is not recommended in *asymptomatic* individuals but is being used by some practitioners in the evaluation of individuals with symptoms and signs of dementia. An analysis of both the clinical validity and clinical utility of such testing, including calculation of the positive predictive value for asymptomatic and symptomatic individuals, explains why (Table 17-3).

As can be seen from these positive predictive values for asymptomatic people in the age bracket 65 to 74 years, the presence of a single $\epsilon 4$ allele is a very poor predictor of whether AD will develop, despite the 3-fold increased risk for the disease conferred by the $\epsilon 4$ allele compared with those without an $\epsilon 4$ allele. Even with two $\epsilon 4$ alleles, which occurs in approximately 1.5% of the population and is associated with a 20-fold increased risk relative to genotypes without $\epsilon 4$ alleles, there is still less than a 1 in 4 chance of developing AD. In younger asymptomatic individuals, the positive predictive value is smaller still. Thus, in the majority of individuals identified through *APOE* testing as being at increased risk, AD will not develop. Furthermore, knowing that one is at increased risk does not lead to any preventive or therapeutic options and has the potential to cause significant emotional and psychological stress. On the basis of the poor positive predictive value and lack of clinical utility, it should now be clear why *APOE* testing is not recommended in asymptomatic individuals, as discussed in Chapter 8.

On the other hand, individuals who already show signs of dementia are already at a higher prior probability of having AD. *APOE* testing in them may be helpful in deciding whether the disease is indeed AD, or some other form of dementia that would require additional work-up. Of course, with a disorder as devastating and untreatable as AD, it could be argued that even when

APOE testing suggests a high probability of AD, the small chance of a treatable cause for apparent dementia justifies the expense of an additional work-up.

As in all of medicine, balance of the benefits and costs for each component of personalized genetic medicine needs to be clearly demonstrated but also continually reassessed. The need for constant re-evaluation is obvious: imagine how the recommendations for *APOE* testing, despite its low positive predictive value, might change if a low-risk and inexpensive medical intervention is discovered that could prevent the onset of dementia.

Heterozygote Screening

In contrast to screening for genetic disease in newborns or for genetic susceptibility in patients, screening for carriers of mendelian disorders has, as its main purpose, the identification of individuals who are themselves healthy but are at substantial (25%) risk for having children with a severe autosomal recessive or X-linked illness. The principles of heterozygote screening are shown in the accompanying Box.

Criteria for Heterozygote Screening Programs

- High frequency of carriers, at least in a specific population
- Availability of an inexpensive and dependable test with very low false-negative and false-positive rates
- Access to genetic counseling for couples identified as heterozygotes
- Availability of prenatal diagnosis
- Acceptance and voluntary participation by the population targeted for screening

To provide a sufficient yield of carriers, current heterozygote screening programs have focused on particular ethnic groups in which the frequency of mutant alleles is high. Heterozygote screening is voluntary and focuses on individuals who identify themselves as being members of a particular high-risk ethnic group. Heterozygote screening has been used extensively for a battery of disorders for which carrier frequency is relatively high: Tay-Sachs disease (Case 38) (the prototype of carrier screening) (see Chapter 12), Gaucher disease, and Canavan disease in the Ashkenazi Jewish population; sickle cell disease (Case 37) in the African American population of North America; and β -thalassemia (Case 39) in high-incidence areas, especially in Cyprus and Sardinia or in extended consanguineous families from Pakistan (see Chapter 11).

The technology for detecting many different mutant alleles in a gene simultaneously in a single procedure (**multiplex testing**) makes it possible to carry out population-based heterozygote screening for cystic fibrosis by examining the *CFTR* gene directly for mutations (see Chapter 12) (Case 10). The most pressing issue for *CFTR* carrier screening by direct detection of mutant alleles is the extreme allelic heterogeneity in many populations and the differences in the mutant alleles present in different ethnic groups. For example, testing with a basic panel of 23 mutations ($\Delta F508$ and the 22 most common other mutations found in non-Hispanic whites) proposed by the American College of Medical Genetics can identify nearly 88% of all mutations and therefore about 80% of the at-risk couples (those in which both partners are heterozygous for a *CFTR* mutation) from this ethnic background. Adding more alleles to the panel only marginally increases the sensitivity of the test in non-Hispanic whites. In other populations, such as Hispanic whites, Asians, and African Americans, the frequency and the distribution of mutant alleles are quite variable. The basic 23-allele panel would detect only 72% of Hispanic carriers, 64% of African American carriers, and 49% of Asian American carriers. Expanded panels that are more ethnic specific are needed for these populations. Thus, for example, many diagnostic laboratories use a panel of mutations in which they test for the $\Delta F508$ mutation plus another four dozen mutant alleles. In contrast, in the Ashkenazi Jewish population, testing for only five mutations detects 94% of carriers, a high sensitivity while testing for fewer mutations.

The impact of carrier screening in lowering the incidence of a genetic disease can be dramatic. Carrier screening for Tay-Sachs disease in the Ashkenazi Jewish population has been carried out since 1969. Screening followed by prenatal diagnosis, when indicated, has already lowered the incidence of Tay-Sachs disease by 65% to 85% in this ethnic group. Prevention of β -thalassemia by carrier detection and prenatal diagnosis

has brought about a similar drop in the incidence of the disease in Cyprus and Sardinia. In contrast, attempts to screen for carriers of sickle cell disease in the U.S. African American community have been less effective and have had little impact on the incidence of the disease so far. The success of carrier screening programs for Tay-Sachs disease and β -thalassemia, as well as the relative failure for sickle cell anemia, underscores the importance of community consultation, community education, and the availability of genetic counseling and prenatal diagnosis as critical requirements for an effective program.

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● USEFUL WEBSITES

- Gwinn M, Khoury MJ: Epidemiology. 2001. <http://www.cdc.gov/genomics/training/books/genpractice.htm>
American College of Medical Genetics: Technical Standards and Guidelines for *CFTR* Mutation Testing, 2006 edition. http://www.acmg.net/Pages/ACMG_Activities/stds-2002cf.htm



PROBLEMS

1. In a population sample of 1,000,000 Europeans, idiopathic cerebral vein thrombosis (iCVT) occurred in 18, consistent with an expected rate of 1 to 2 per 100,000. All the women were tested for factor V Leiden (FVL). Assuming an allele frequency of 2.5% for FVL, how many homozygotes and how many heterozygotes for FVL would you expect in this sample of 1,000,000 people, assuming Hardy-Weinberg equilibrium?

Among the affected individuals, two were heterozygotes for FVL and one was homozygous for FVL. Set up a 3×2 table for the association of the homozygous FVL genotype, the heterozygous FVL genotype, and the wild-type genotype for iCVT.

What is the relative risk of iCVT in a FVL heterozygote versus the wild-type genotype? What is the risk in a FVL homozygote versus wild-type? What is the sensitivity of testing positive for either one or two FVL alleles for iCVT? Finally, what is the positive predictive value of being homozygous for FVL? heterozygous?

2. In a population sample of 100,000 European women taking oral contraceptives, deep venous thrombosis (DVT) of the lower extremities occurred in 100, consistent with an expected rate of 1 per 1,000. Assuming an allele frequency of 2.5% for factor V Leiden (FVL), how many homozygotes and how many heterozygotes for FVL would you expect in this sample of 100,000 women, assuming Hardy-Weinberg equilibrium?

Among the affected individuals, 58 were heterozygotes for FVL and 3 were homozygous for FVL. Set up a 3×2 table for the association of the homozygous FVL genotype, the heterozygous FVL genotype, and the wild-type genotype for DVT of the lower extremity.

What is the relative risk of DVT in a FVL heterozygote using oral contraceptives versus women taking oral contraceptives with the wild-type genotype? What is the risk in a FVL homozygote versus wild-type? What is the sensitivity of testing positive for either one or two FVL alleles for DVT while taking oral contraceptives? Finally, what is the positive predictive value for DVT of being homozygous for FVL while taking oral contraceptives? heterozygous?

3. What steps should be taken when a phenylketonuria (PKU) screening test comes back positive? The test is a bacterial inhibition assay on a spot of blood on filter paper (Guthrie test).
4. Newborn screening for sickle cell disease can be performed by hemoglobin electrophoresis, which separates hemoglobin A and S, thereby identifying individuals who are heterozygotes as well as those who are homozygotes for the sickle cell mutation. What potential benefits might accrue from such testing? what harms?



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