Chapter 5

The State-of-the-Art in Genetic Screening

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Chapter 5

The State-of-the-Art in Genetic Screening

As mentioned in chapter 2, genetic screening in the workplace can be used in two distinct ways. First, genetic screening can detect traits that may indicate an increased susceptibility to occupational illness after exposure to specific agents. Second, screening tests can be used to identify genetic disorders not associated with specific job exposures. Thus, in assessing the state-of-the-art of screening tests for use at worksites, three different questions must be discussed. Namely, what general techniques are presently available that could be used for genetic screening? What is known about the association among heritable traits, exposure to hazardous materials, and subsequent occupational illness? And, what genetic disorders can be detected unrelated to job exposures that are important to general health?

The following sections address the recent technical advances in genetic screening tests, with an emphasis on recombinant DNA tests. Important background information on human genetics and disease can be found in appendix A. In evaluating the interaction of occupational exposure and genetic influences, the discussion focuses on traits evaluated in the 1983 Office of Technology Assessment (OTA) report (111), but also assesses several newly recognized susceptibilities to occupational disease. Finally, progress in detecting some nonoccupationally related disorders is presented. These disorders are presented because they are likely to affect large populations, screening tests for them are under investigation or being developed, and they might be of interest to an employer if available through preemployment screening.

BIOCHEMICAL TECHNIQUES FOR DETECTING GENETIC DISEASE

Biochemical genetics refers to the analysis of mutant genes on the basis of altered proteins or metabolizes. If diagnosed, some of these "inborn errors of metabolism" can be treated with enzyme replacement or dietary control.

Phenylketonuria is the classic example of an inborn error of metabolism, inherited as an autosomal recessive, that can be controlled by restricting dietary intake of phenylalanine. In this disease, the individual lacks the enzyme necessary to convert phenylalanine to tyrosine. Retardation and seizures are common symptoms. Carriers, or heterozygotes, for the disease tend to have the enzyme at a level about half that found in individuals who are homozygous for the normal gene. Affected individuals have almost none of the necessary enzyme. Most inborn errors of metabolism, for which the enzyme defect is known, lend themselves to biochemical tests for the detection of affected and carrier individuals.

Sickle cell disease is a form of chronic hemolytic anemia characterized by the presence of crescent-shaped (or sickled) red blood cells that cause peripheral oxygen deficit by blocking the terminal arterioles. It is a biochemical disorder of hemoglobin for which the mutant gene has been found. The disease has an extraordinarily high frequency in populations of West African origin, occurring in about 1 in 625 U.S. Blacks at birth (87). One in eight Blacks is a carrier for the disease, a condition referred to as "sickle cell trait. ' The trait can be detected biochemically by hemoglobin electrophoresis, or by testing for the gene itself, now that the gene has been cloned (see subsequent discussion of cloning) (66,99).

In general, biochemical techniques for diagnosing genetic disease are often restricted to indirect analysis. Only for fewer than 10 percent of monogenic disorders has the fundamental biochemical defect been elucidated (85). Advances in DNA technology (described below) have greatly advanced our ability to directly examine the genetic basis for disease and to predict and diagnose such diseases.

MOLECULAR TECHNIQUES FOR GENETIC SCREENING

Until recently, most available tests for genetic conditions were not based on recombinant DNA techniques. Traditionally, enzyme or other protein-based assays that identified abnormal gene products (or the consequences of abnormal gene function) are more commonly performed. For example, in the case of Tay-Sachs disease, reduced activity of a particular enzyme signals the carrier state, and absence, the

Photo credit: Lawrence Livermore National Laboratory, Livermore, CA

Direct image of chemically unaltered strand of DNA obtained using a scanning tunneling microscope. (Light microscopes magnify objects up to about 1,000 times; electron microscopes about 300,000 times. Scanning tunneling microscopes can magnify images up to 1 million times.)

disease state. Today, DNA-based tests encompass a variety of standard diagnostic techniques that allow examination of regions very near the genes (e.g., Huntington's disease) or direct examination of the genes themselves (e.g., sickle cell anemia and cystic fibrosis).

DNA can be extracted from any tissue containing nucleated cells, including blood. Once extracted, the DNA is stable and can be stored indefinitely so that samples from people with genetic disorders can be collected and saved for investigations to diagnose future family members.

The remainder of this section describes the types of techniques that can be used for genetic screening, with emphasis on molecular methods developed since the 1983 OTA report, including restriction fragment length polymorphisms (RFLPs), allele-specific oligonucleotide (ASO) probes, polymerase chain reaction (PCR), and automation of these and other technologies useful to DNA-based genetic diagnostics.

Two major tools, recognition of RFLPs and development of cloned DNA probes are the major advances responsible for improved diagnosis of genetic disease.

Restriction Fragment Length Polymorphisms

Variations in the DNA sequence of two individuals are likely to occur on average every 300 to 500 base pairs (49). These variations occur both within and outside of genes and usually do not lead to functional changes in the protein products of genes. In the 1970s, it was demonstrated that certain bacterial enzymes, called restriction endonucleases, could be used to map genes by cleaving DNA at specific sites. This discovery led researchers to propose that natural differences in DNA sequence (polymorphisms) might replace other chemical and morphological markers as a way to track chromosomes through a family (linkage analysis) (50).

Because of the naturally occurring variation in the DNA sequences of individuals, the lengths of DNA resulting from cuts by the endonucleases will differ. This phenomenon is referred to as RFLP. RFLP analysis is a relatively straightforward process, and over 3,000 RFLP loci have been identified, including more than 100 highly polymorphic loci at which many alleles exist in the population. Some of these loci are located so close to a gene of interest that they are nearly always inherited with the gene. So even when a gene associated with disease has not been identified and even when a disease gene's locus is not precisely known, the inheritance of an associated RFLP can be used as an indicator of inheritance of the gene.

Briefly, the method involves cutting the DNA with a restriction enzyme, and sorting out various fragments by electrophoresis in a gel in which the DNA fragments will migrate according to length. The double-stranded fragments are then converted into single strands and transferred onto a nylon membrane, to which the fragments adhere. This technique is referredtoasa"Southern blot' after its inventor, E.M. Southern (96). The membrane is then soaked in a solution containing a radiolabeled DNA probe which binds to the particular fragment containing its complementary sequence. The probe is obtained through cloning the gene of interest or by chemical synthesis. The nonspecifically bound probe is washed away and the falter is placed on a piece of x-ray film. The radioactively labeled bands expose the x-ray film and their locations indicate the

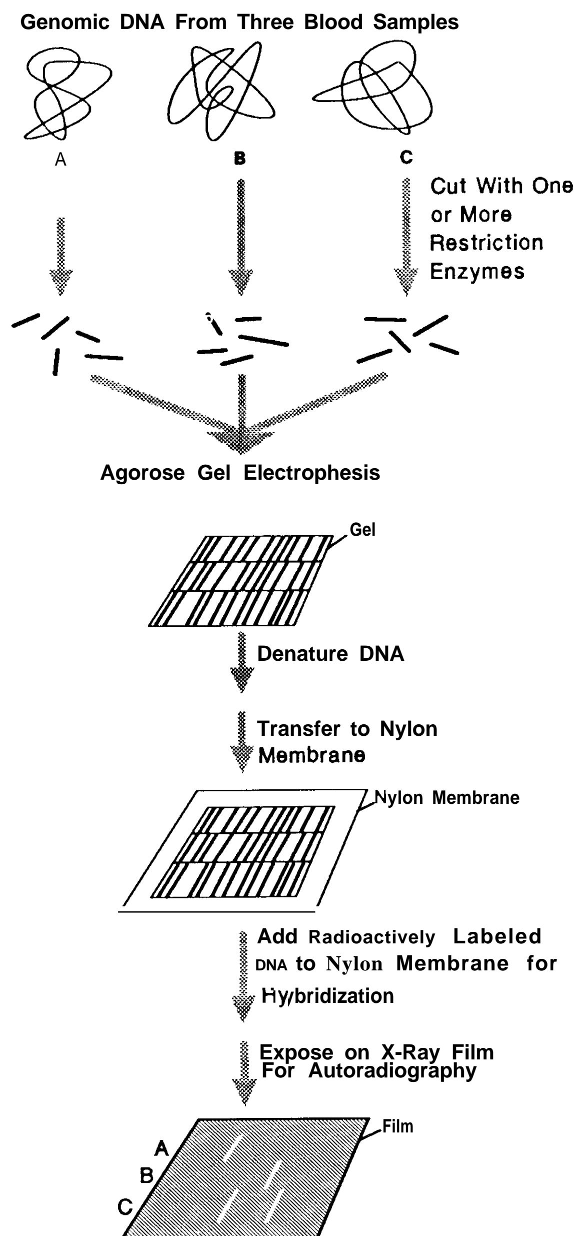
size of the fragments complementary to the gene probe under study (figure 5-l).

A new probe technology, using fluorescence rather than radioactivity, is expected to speed up the time it takes to make a diagnosis while simultaneously decreasing the incidence of false positives (37). Enzyme-linked dye methods are already available commercially (87). These methods do not necessarily improve accuracy, but provide greater safety in the laboratory.

As stated previously, the RFLP itself is not the cause of disease.[1] Rather, RFLPs serve as flags, or markers for the presence of the disease gene. The general location of many genes has been determined and located on or near a specific restriction fragment. The segregation pattern of the RFLP within a family is analyzed and the inheritance of specific alleles of the RFLP is then used to predict the inheritance of the disease gene. One of the first applications of this technology came in 1983 when genetic linkage between a RFLP marker on chromosome 4 and Huntington's disease was described (39,50).

Thus, RFLP analysis can be useful for families where the precise mutation is unknown but general location of the locus of the mutation is known to be linked to a RFLP marker. Indirect analysis of this type is most often used in prenatal diagnosis, but is likely to be used in other diseases, such as adult-onset polycystic kidney disease (see box 5-A) and Wilson's disease. Linkage analysis is limited for three primary reasons: 1) at least one living affected family member is usually required; 2) genetic heterogeneity will confound the analysis if an affected member is not available because different mutations at different loci may produce indistinguishable disease manifestations, or phenotypes; and 3) paternity must be known. Successful linkage studies have also been completed for cystic fibrosis (for which the gene has now been found), myotonic dystrophy, familial amyloidotic polyneuropathy, familial Alzheimer's disease, and Duchenne muscular dystrophy. Table 5-1 lists selected diseases for which DNA diagnosis is possible or within reach.

Figure 5-l—Detection of Restriction Fragment Length Polymorphisms Using Radioactively Labeled DNA Probes



Variations in DNA sequences at particular marker sites are observed as differences in numbers and sizes of DNA fragments among samples taken from different individuals (shown here as samples A, B, and C).

SOURCE: Office of Technology Assessment, 1990.

[1] In sickle cell, the mutation that causes the disease results in an alteration in a restriction site so the restriction analysis constitutes a direct test for the disease.

Box 5-A—Adult Polycystic Kidney Disease

Adult polycystic kidney disease (APKD) is a dominant disorder with age of onset between 20 and 35 in most families. Renal dialysis or transplantation are the primary treatment modalities. The disorder can be diagnosed by ultrasonography, but false negatives are common, For example, a 20-year-old at risk may have a negative ultrasound but develop the disease 10 years later.

A number of flanking DNA linkage markers have been identified very close to the disease locus on chromosome 16. If multiple affected and unaffected individuals in an APKD family can be tested for the presence of the marker, the coupling phase between the marker and the disease gene can be set, and predictive diagnoses can be made. his constitutes a presymptomatic diagnosis. The physician can then be alerted to monitor the blood pressure, renal function, and weight status of the patient knowing that he or she is markedly at risk for ultimately manifesting the disease.

SOURCE: Office of Technology Assessment, 1990.

Table 5-l-Common Monogenic Disorders for Which DNA Diagnosis Is Possible or Within Reach

Autosomal dominant	hypercholesterolemia
	polycystic kidney disease
	Huntington's disease
	neurofibromatosis
	myotonic dystrophy
	polyposis coli
	tuberous sclerosis
Autosomal recessive	hemochromatosis
	alpha-1 -antitrypsin deficiency
	cystic fibrosis
	phenylketonuria
	adrenogenital syndrome
	retinitis pigmentosa
	retinoblastoma
	sickle cell anemia
	thalassemias
X-linked	fragile X mental retardation
	Duchenne muscular dystrophy
	hemophilia A and B
	ichthyosis vulgaris
	adrenoleukodystrophy

SOURCE: Adapted from H.H. Ropers and B. Wieringa, 'The Recombinant DNA Revolution: Implications for Diagnosis and Prevention of Inherited Disease," *European Journal of Obstetrics and Gynecology* and Reproductive Biology 32:15-27, 1989.

Use of RFLPs in Population Studies

Population differences are important to recognize in both genetic screening and monitoring. In screening, it is important to remember that certain rare alleles are often concentrated within certain ethnic groups, e.g., Tay-Sachs and Gaucher's diseases affect Jewish people of Ashkenazi descent. An individual of this background, therefore, would more appropriately be screened for these traits than would individuals of different ethnic or racial backgrounds. In the case of monitoring, the ethnic composition of the population being tested might be an important consideration in deterrnining allelic frequencies.

In addition to using RFLPs in clinical diagnosis, they provide the potential for elucidating a range of information in the study of human populations. Population-specific alleles or allele frequencies have been found. For example, the number of polymorphisms due to the presence or absence of a dihydrofolate reductase gene has been found to differ between Blacks and Caucasians. And one of the six known RFLPs in the human growth hormone cluster was found only in Blacks although the other five were present in Caucasians. RFLPs linked to the alpha-1 -antitrypsin Z allele (which is associated with increased risk of emphysema and liver disease and is an occupationally important marker) have only been described in Europeans. RFLPs linked to clinical hypertriglyceridemia differ between Caucasians and Japanese (100). Population differences in RFLPs are attributed to racial divergences and explain, in part, ethnic and racial differences in disease morbidity.

HLA Typing and Genetic Disease

More recently, linkage analysis relying on the genetic organization of the major histocompatibility complex (the genes that regulate and control the immune system) has provided useful information for organ and tissue transplantation, paternity determinations, and disease susceptibility (26). The utility of typing of the human leukocyte antigens (HLA) for genetic studies is due to the high degree of genetic polymorphism in the HLA region (i.e., HLA antigens are among the most variable proteins of the human genome).

Genetic susceptibility to a variety of diseases has been linked in family studies with specific HLA types. Polymorphisms of the HLA loci have been associated with specific diseases such as insulin-dependent diabetes mellitus, rheumatoid arthritis, multiple sclerosis, and myasthenia gravis. As more polymorphisms in the HLA complex are identified,

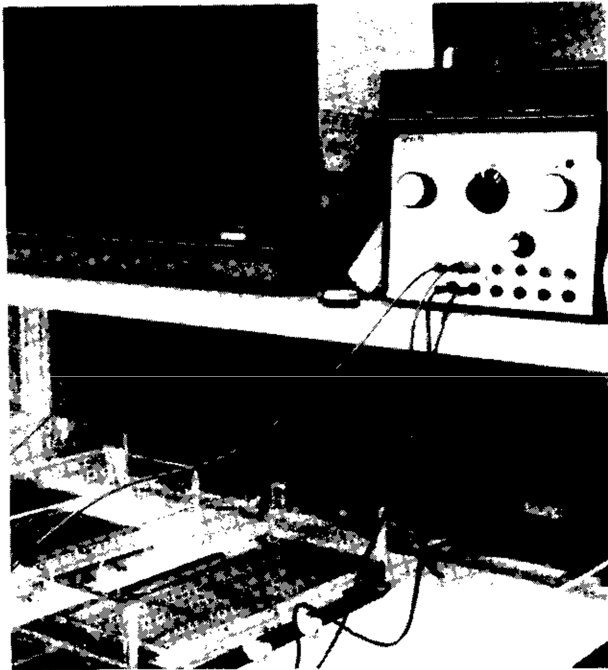


Photo credit: Robyn Nishimi

Gel electrophoresis of DNA samples.

HLA-based probes will provide an additional tool for linkage studies, and possibly direct analysis of mutant genes. The high degree of variability at the DNA level in the HLA loci suggests that DNA markers in the region are of great importance in characterizing population groups.

Direct Analysis of Mutant Genes

In a small percentage of cases (5 to 10 percent), point mutations responsible for genetic disease can be directly detected by restriction analysis and the use of synthetic oligonucleotides as probes (4). Sickle cell disease is one such example. Direct analysis of mutant genes involves cloning the mutant allele and sequencing through the mutation. This is a laborious process because most genes are large (the globin genes have been sequenced because they are small). If the gene can be sequenced, oligonucleotide probes can be synthesized that specifically recognize the mutant or normal alleles.

DNA probes have been in use for nearly 10 years to detect point mutations. A probe is a short sequence of single-stranded DNA that is complementary to the DNA sequence being sought. If short, they may be chemically synthesized DNA segments. ASO probes refine diagnostic accuracy by perfectly matching the nucleotide sequence of a portion of the gene in question (17). These probes are long enough to represent unique sequences but short enough to be specific to a target molecule. Before being used, the probe is labeled with a fluorescent or radioactive marker so it can be detected. A method of amplification, described below, can then be used to permit extremely accurate identification of the target gene.

This approach has been applied successfully in alpha-1-antitrypsin deficiency, sickle cell disease, several of the thalassemias, cystic fibrosis, and hemophilia A, to name a few. Because of the difficulty of sequencing and producing probes for large genes, indirect, or linkage analysis, is more commonly used for diagnosis. The use of probes for direct analysis has been limited because, for most single-gene disorders, there are many different mutations that cause the same disease. As in linkage studies, accurate diagnosis often depends on the availability of several affected family members.

It is likely that the complete physical map of the human genome will serve as the ultimate source of DNA probes for any human gene (62).

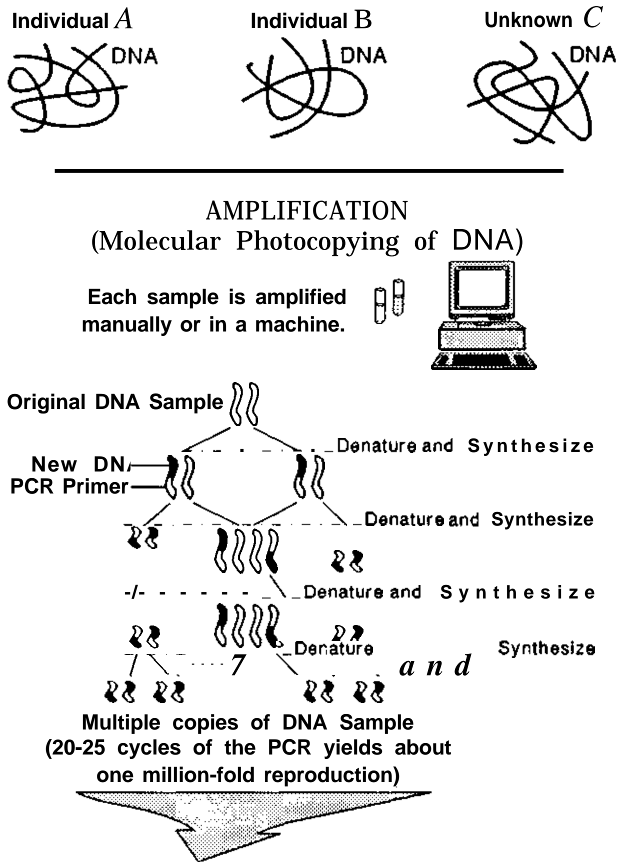
Polymerase Chain Reaction

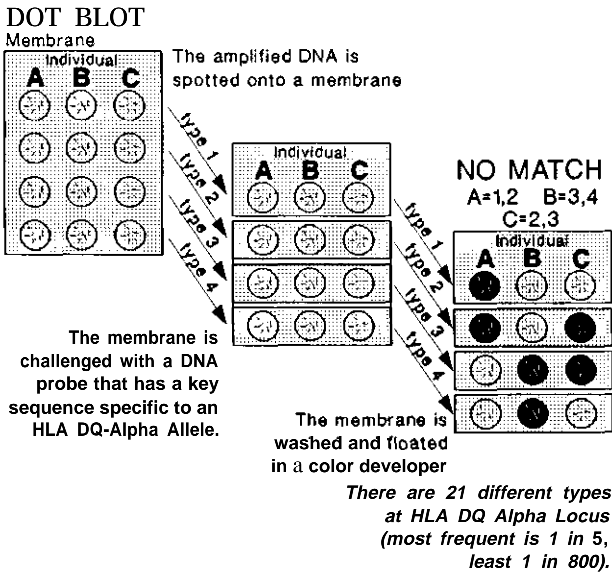
PCR, first reported by Cetus Corp. in 1985, facilitates the use of probes by greatly increasing, or amplifying, the number of copies of target DNA. For example, selected areas of a gene can be amplified through repeated cycles, a probe can be hybridized directly to the amplified DNA and a rapid diagnosis made. PCR produces enough of the target sequence so that simple, rapid, and accurate methods for identification can be employed (74). In some respects, it can be thought of as molecular photocopying (see figure 5-2).

PCR will probably be the method of choice for identifying monogenic disorders in which point mutations account for the majority of gene defects and will make DNA diagnosis possible at any major medical center (4). It has great potential value for carrier screening programs. The possibilities for use of PCR in diagnosing monogenic diseases through linkage studies are also great (52).

Briefly, PCR involves using two specific sequences, called primers, that flank the area the scientist wants to copy. The scientist then sets conditions in the reaction that allow new copies of

Figure 5-2—The Polymerase Chain Reaction





SOURCE: Office of Technology Assessment, 1990.

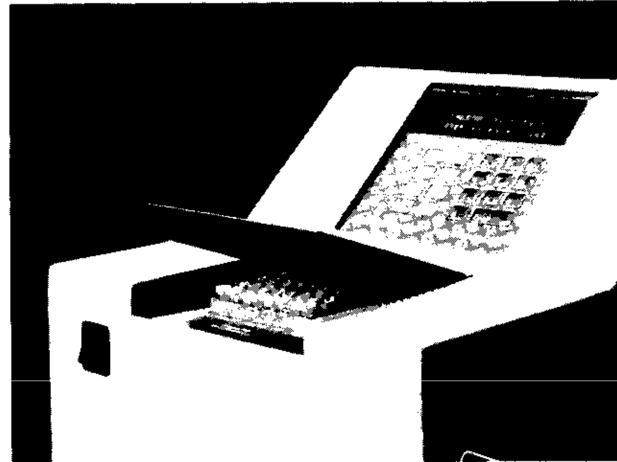


Photo credit: Perkin-Elmer Cetus

DNA thermal cycler for automated PCR analysis.

the DNA of interest to be produced from the primers. Because the products generated in one sample can serve as templates in the next cycle, the number of amplified copies doubles with each cycle. Thus, 20 cycles of PCR yields about a millionfold reproduction. This extraordinary ability is also PCR's greatest weakness. PCR assays can lead investigators astray when trace quantities of contaminating DNA molecules find their way into the reaction sample. Handling of samples when using PCR requires much greater care than with routine RFLP analysis.

Automation of DNA Diagnostic Procedures

A number of instruments have been developed that can increase the speed and volume of routine DNA diagnostic procedures. Some examples include:

- The DNA extractor prepares DNA suitable for Southern blots from eight tissue samples in 3 hours. Future extractors will be able to handle hundreds of samples daily (62).
- The DNA synthesizer, or 'gene machine, ' can assemble oligonucleotides up to 200 nucleotides in length with a synthesis rate of 12 to 15 minutes per cycle. The synthesizer is crucial for probe development (43,62).
- Laboratory robotic workstations are being developed that can rapidly and accurately perform routine manipulations including RFLP analyses and DNA sequencing.

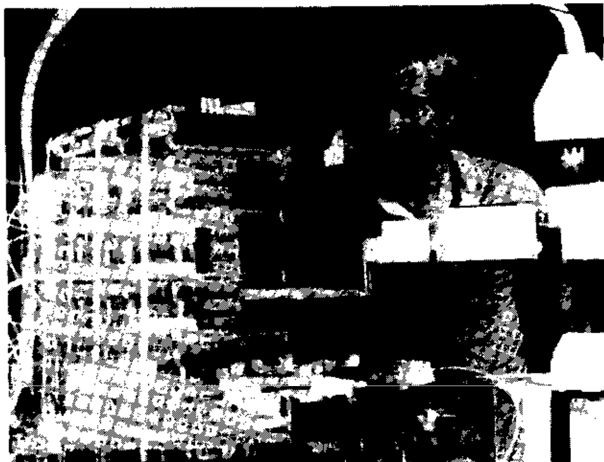


Photo credit: Human Genome Center, Lawrence Berkeley Laboratory

Computerized robotics used to speed repetitive tasks of mapping and sequencing DNA.

- An instrument has been developed that can size-separate very large DNA segments through pulsed field gradient gel electrophoresis. This will be useful in identifying deletions, translocations, and amplifications of DNA sequences and in determining RFLPs over large DNA segments (62).

Novel computing systems are being designed specifically to handle the computational tasks of sequencing. Automation of DNA diagnostic procedures will make large-scale screening faster and more affordable.

The Limits of Molecularly Based Tests

No matter what the mode of screening, two questions must be asked before administration of the test: 1) does the test reliably identify either the genetic trait or specific damage? and 2) does this particular trait or damage cause the individual to be at increased risk for disease? The frost question is more easily answered than the second. To answer the first question, the test must be subjected to scientifically recognized analytical criteria; validity, reliability, predictive value, and relative risk (111). These issues are discussed in chapter 4 and will not be repeated here.

As discussed previously, linkage studies are limited by the requirement for samples from informative relatives and by variable expressivity. This makes linkage testing uncertain for some individuals. Currently, widespread application of linkage studies is limited by the number of probes available, but this obstacle will gradually be overcome. And, as more disease genes are cloned, linkage studies will be replaced by direct tests.

Even with direct tests, however, variable expressivity, incomplete penetrance, and heterogeneity will interfere with the ability to predict correctly that certain individuals will develop disease (42). Heterogeneity lowers sensitivity and variable expressivity lowers specificity. (See ch. 4 for discussion of specificity and sensitivity.)

In any case, before widespread screening of populations is begun, the validity of the tests should be determined in a large number of unrelated people with clinical expression of the disease and in others who have no signs of the disease. Such efforts will require a large test population.

Reliability is measured by the ability of a test to accurately detect that which it was designed to detect and to do so in a consistent fashion. Other than the routine laboratory problems that lead to unreliable test results (human error, contamination), DNA-based tests can fail to yield reliable results for a number of reasons, most often because of incomplete digestion of DNA, faulty hybridization in the Southern blot, or contamination of the PCR amplification. Quality control is likely to become a major issue as the volume of tests at laboratories grows (42). These are already issues in forensics applications (109).

SCREENING FOR SUSCEPTIBILITY TO WORKPLACE EXPOSURES

At the end of the 1960s, some scientists sought to provide perspectives for research on the interaction of genetics, drugs, and environmental agents by showing its application to the field of industrial hygiene (98). Today, the term "ecogenetics" often refers to the field dealing with genetic predispositions to drugs or any type of environmental agent (10). At present, approximately 50 human genetic diseases have been identified as having the potential to enhance an individual's susceptibility to toxic or carcinogenic effects of environmental agents (14) (see table 5-2 for examples).

This section briefly reviews selected genetic conditions from the 1983 OTA report that some believe enhance susceptibility to environmental

Table 5-2—Identification and Quantification of Genetic Factors Affecting Susceptibility to Environmental Agents

High-risk groups	Estimated occurrence	Environmental agents to which group is (may be) at increased risk
RBC conditions		
G-6-PD deficiency	American Black males 16%; Mediterranean Jewish males 11%; Greeks 1%-2%.; Sardinians 1%-8%	Environmental oxidants such as ozone, nitrogen dioxide, and chlorite
Sickle cell trait	7%-1 3% of American Blacks are heterozygotes	Aromatic amino and nitro compounds; carbon monoxiode, cyanide
The thalassemias	Alpha: 4%-5% in Americans of Italian and Greek descent; Beta: 2%-7% American Blacks and 2%-3% American Greeks	Lead; benzene
NADH dehydrogenase deficiency (MetHb reductase deficiency)	Estimated 1%. of population are heterozygotes	MetHb-forming substances
Catalase Hypocatalasemia	About 2% of U.S. population based on Swiss gene frequency	Ozone; radiation
Acatalasemia	1/10,000-20,000 of U.S. population based on Swiss gene frequency	
Low SOD activity	Frequency of genetic variants in population 1 to 2/10,000; normal population exhibits unimodal distribution; persons at low end of distribution may beat increased risk	Wide variety of environmental oxidants; paraquat; radiation; ozone
ALA dehydratase deficiency	Unknown, but thought to be rare	Lead
Hb M	Unknown, but rare	Carbon monoxide
Erythrocyte porphyria	1.5/100,000 in Sweden, Denmark, Ireland, West Australia; 3/1 ,000 in South African Whites; rare in Blacks	Chloroquine; hexacholorobenzene; lead; various drugs, including barbiturates, sulfonamides, others
GHS-Px deficiency	Rare	Environmental oxidants
GSH deficiency	Rare	Environmental oxidants
Liver metabolism		
Defect in gluocuronidation		
Gilbert's syndrome	6% of normal, healthy adult population	Wide variety of xenobiotics including polychlorinated biphenyls
Crigler-Najjar syndrome	Few persons live to adulthood	
Defect in sulfation	Unknown	Wide variety of xenobiotics; best association is with tyramine-captaining foods
Acetylation phenotype, slow v. fast	Slow: 50% Whites; 50% Blacks; 10% Japanese Fast: 50% Whites; 50% Blacks; 90% Japanese	Aromatic amine-induced cancer; numerous drugs, e.g., isoniazid and hepatitis
Gout	0.27%-0.3% prevalence in U.S. and Europe	Lead
Oxidation center defects	9% of British Whites; 8% of Nigerians; 6% Ghanians; 1% Saudi and Egyptians are poor oxidizers	Numerous xenobiotics requiring oxidative metabolism for detoxification
OCT deficiency	Unknown, but thought to be rare	insect repellant (DET)
Paraxonase variant	25%-30% of population	Parathion
Rhodanese variant	Unknown	Cyanide
Sulfite oxidase deficiency heterozygotes	Unknown	Sulfite, bisulfite, sulfur dioxide
inadequate carbon disulfide metabolism	Upward of 30%-40%	Carbon disulfide
Alcohol dehydrogenase variant	5% English; 20% Swiss; 70% Japanese	Metabolize (e.g., ethanol) more quickly than normal
Wilson's disease	Homozygous 1/100,000 while the heterozygote may approach 1/500	Copper, vanadium
Serum variants		
Albumin variants	Less than 1/1,000 in Europeans, much higher frequency in North American and Mexican Indians	Unknown
Pseudocholinesterase variants	Highly sensitive homozygous and heterozygous persons of European ancestry have combined frequency of about 1/1,250; moderatelv sensitive genotypic variants of European ancestry have frequency of 1/15,000	Organophosphate and carbamate insecticides; muscle relaxant drugs

* Abbreviations used are: G-6-PD, glucose-6-phosphate dehydrogenase; NADH, nicotinamide adenine dinucleotide (reduced form); SOD, superoxide dismutase; ALA, aminolevulinic acid: GSH, reduced glutathione; Px, peroxidase; OCT, ornithine carbamoyl transferase; IgA, immunoglobulin A; PKU, phenylketonuria; PTL, phenylthiourea; XP, xeroderma pigmentosum; AT, ataxia telangiectasia; FA, Fanconi's syndrome.

SOURCES: E.J. Calabrese, *Ecogenetics: Genetic Variation in Susceptibility to Environmental Agents* (New York, NY: Wiley Interscience, 1984); and E.J. Calabrese, "Ecogenetics: Historical Foundation and Current Status," *Journal of Occupational Medicine* 28(10):1096-1102, 1986.

agents, and evaluates any progress made since that report (111). Following that, several new associations between genetic traits and environmental agents are analyzed.

Glucose-6-Phosphate Dehydrogenase Deficiency

Individuals whose blood cells are deficient in the enzyme glucose-6-phosphate dehydrogenase (G-6-PD) are at enhanced risk for hemolysis (destruction of red blood cells) if exposed to a number of oxidant drugs and industrial chemicals, especially certain aromatic amino and nitro compounds such as naphthalene and TriNitroToluene. G-6-PD deficiency is inherited as an X-linked trait. Although G-6-PD deficient persons could be at increased risk of anemia if exposed to specific hazards in the workplace, epidemiologic studies designed to actually assess G-6-PD deficient workers are lacking (14). A direct DNA test for some mutants causing G-6-PD deficiency is available (4).

Sickle Cell Anemia and Sickle Cell Trait

Sickle cell anemia and sickle cell trait result from the presence in red blood cells of an abnormal hemoglobin molecule (HbS v. normal HbA). Persons with sickle cell anemia are homozygous for HbS, that is they have two copies of the abnormal beta-globin gene, and 100 percent of red blood cells contain HbS. Individuals who have sickle cell trait have only one copy of the abnormal gene (i.e., are heterozygous) and only 20 to 40 percent of their red blood cells have HbS. Under certain conditions, when the oxygen level or environmental temperature drops, HbS-containing red blood cells can sickle. This leads to varying degrees of adverse health consequences, depending on the individual's level of HbS. Although the sickle cell gene has been cloned, a simple, and relatively inexpensive, biochemical assay can detect HbS. The principal use of both a DNA probe for sickle cell and PCR lies in prenatal diagnosis (4).

Individuals with sickle cell anemia have reduced lifespans and many health problems directly attributable to being homozygous for the sickle cell gene. But as in the 1983 OTA report, whether sickle cell trait carriers are at increased risk from the challenges of rigorous training at high altitudes (where oxygen is low) remains unresolved. Limited evidence suggests that possession of sickle cell trait is a contributing factor in reported cases of injury or death during or after vigorous exercise (51), but other confounding factors are most likely present (14). Neither the experimental or epidemiological evidence has confirmed the hypothesis that persons with sickle cell trait are at increased risk when exposed to several chemicals (14).

The Thalassemias

Thalassemia is a deficiency in the production of hemoglobin that results in smaller red blood cells. The disease is inherited in an autosomal recessive pattern and it varies in severity and type (there are alpha and beta forms of the disease). It has been suggested that beta thalassemic individuals are at increased risk after exposure to several chemicals, including benzene and lead. Again, while limited clinical observations have suggested that persons with thalassemia could be at increased toxic risk from benzene and lead, data since 1983 remain insufficient and unconvincing. Continued assessment, epidemiological investigations, and a predictive animal model to test lead- or benzene-induced blood toxicity will be required before an association can be made between this genetic trait and enhanced occupational illness (14,15).

Acetylation Phenotype

Acetylation is a common liver pathway for detoxification of a variety of compounds. The enzyme involved in acetylation, N-acetyltransferase, is coded for by one gene, and humans are either slow or fast acetylators. At the time of the 1983 OTA report, slow acetylation was implicated in increased risk of bladder cancer, and susceptibility to bladder cancer was not equal among those generally grouped as slow acetylators (16). Since then, a growing body of epidemiologic studies further suggests that slow acetylation is a predisposing factor for the recurrence of arylamine-induced bladder cancer (115), and the hypothesis of increased susceptibility is well-characterized with animal models (45,70,1 15). Epidemiologic studies of industrial cancer populations are necessary (78). Because 50 percent of North American Caucasian and Black populations are slow acetylators, the incentive to develop these studies is great.

On the other hand, in addition to the association of slow acetylation with bladder cancer reported in the 1983 OTA report, several recent studies reveal a statistical link between fast acetylation and colorectal cancer (47,64).

Serum Alpha-1-Antitrypsin Deficiency

Homozygous serum alpha-l-antitrypsin (SAT) deficiency is an important genetic factor in emphysema, and some research from animal and human studies supports the hypothesis that an intermediate deficiency of SAT deficiency is a contributing factor in the development of emphysema in heterozygotes. Nevertheless, it is now recognized that emphysema has a multifactorial etiology and that the heterozygote state, by itself, is not a major predisposing factor. Rather, in combination with other predisposing factors, intermediate SAT deficiency can enhance risk of emphysema. Since the 1983 OTA report, data from several clinical and epidemiologic studies indicate that heterozygotic carriers of SAT deficiency display enhanced risk of developing chronic obstructive pulmonary disease (COPD), even in the absence of occupational or lifestyle factors, such as smoking (44). The risk is, however, exacerbated by smoking (25), occupational risk factors, such as grain dust (44), and other nonidentified factors (55). Thus, while it would appear that a genetic factor is important in risk of COPD for carriers of SAT deficiency, a better understanding of all aspects of emphysema is probably necessary before implementing widespread SAT screening. A DNA-based test for SAT has been used for direct analysis of mutation (58), as well as in prenatal diagnosis (57).

Homozygous alpha- l-antitrypsin deficiency, because it is so thoroughly understood at the molecular level, is a good candidate for gene therapy. It has been proposed that genetically engineered alpha-1 in aerosol form could serve the function of shielding against natural destruction of the respiratory tract. Such therapy is in early experimental stages (19).

Aryl Hydrocarbon Hydroxylase and Cytochrome P-450

Cytochrome P-450 enzymes play a central role in metabolizing an enormous range of molecules, including steroids, other drugs, carcinogens, and an array of environmental agents. Both because of their wide spectrum of action and their genetic variability among humans, P-450 enzymes have a marked potential to affect individual susceptibility. Research in cytochrome P-450 has exploded (75) and information continues to mount about genetic differences in human P-450 enzymes, as well as the relationship between P-450 enzymes and cancer (35,75,76,117). This section examines two cytochrome P-450 enzymes: aryl hydrocarbon hydroxylase (AHH) and debrisoquine 4-hydroxylase (D4-H),

The 1983 OTA report examined the role of individual differences of AHH and lung cancer. A key feature of this discussion relied on scientists' ability to reliably measure AHH changes in white blood cells and correlate those changes with changes in lung cells. Experimental limitations to elucidate the role of changes in enzyme levels of AHH in lung cancer led investigators to identify the role of other P-450 genetic markers in environmentally induced cancers, including D4-H (5,13). Nevertheless, concrete evidence that enzyme levels of AHH or D-4H could serve as markers for differential cancer susceptibility to lung cancer (5) remained elusive until recombinant DNA methods were used to define the inheritance of a number of P-450 enzymes, including AHH and D4-H.

With advances in molecular biology, scientists anticipate clinical studies to correlate inheritance of RFLPs associated with (or genetically linked to) various P-450 enzymes with individual cancer risk (75). In the case of D4-H deficiency, where 5 to 10 percent of people are affected, molecular characterization of the phenotype (36) shows promise of revealing the relationship among individual differences in D4-H, environmental exposure to certain agents, and cancer susceptibility (46). Characterization of other P-450 genes is likely to yield insight into predicting individual toxicity to some types of antibiotics, including erythromycin, a commonly prescribed antibiotic, and other agents (14).

Ataxia Telangiectasia Heterozygosity

Ataxia telangiectasia (AT) is an autosomal recessive disorder displaying simultaneous neurological, oculocutaneous, and immunological complications (61,101). Diagnosis is usually made in childhood based on the appearance of poor motor control and telangiectasia, or spider-like lesions, on the skin and eyes. Such individuals are predisposed to both immune deficiencies and certain cancers (1,31,81). The cancer risk is over 100 times greater than for control groups. Patients usually die in early adulthood from respiratory ailments or lymphoproliferative disorders.

Several studies have noted that the AT gene may have some clinical effects in persons who are

heterozygous. Among those effects found in some AT heterozygotes are defective immunity, oculocutaneous telangiectasias, and enhanced cancer susceptibility (31,102). AT heterozygotes are particularly sensitive to ionizing radiation.

AT homozygotes are relatively uncommon at approximately 25 per million (or 1 in 40,000). However, population-based studies have estimated the incidence of the heterozygote in the United States to be about 2.8 percent, or 6 to 7 million Americans (14). For AT heterozygotes younger than age 45, the risk of dying from a malignant neoplasm is greater than five times the risk for the general population. AT heterozygotes comprise more than 5 percent of all persons dying from cancer before age 45. The types of cancer increased in AT families are ovarian, gastric, and biliary system carcinomas, and leukemia and lymphoma. In addition, there is evidence to support some predisposition of AT heterozygotes to basal cell, breast, pancreatic, cervical, and colon cancers. AT heterozygotes appear to be at markedly enhanced risk of breast cancer (14,72,82).

The emphasis on environmental-occupational exposures and their effects on the AT heterozygote is currently focused on radiation, x-rays in particular. For example, occupational exposures of breast tissue to x-rays, or even diagnostic exposures such as mammography, have been raised as cause for concern (102).

A test has been developed to detect AT heterozygotes. Progress in this area could lead to identification of individuals at risk for cancers as a result of radiation exposure.

Paraoxonase Variants

Paraoxonase is one esterase found in human serum that metabolizes paraoxon, a metabolize that is the active ingredient of the organophosphate insecticide parathion. Paraoxonase activity shows considerable variability in human populations, with significant interethnic differences (20,34,83). Individuals with variations in esterase activity are expected to be at increased risk to organophosphate toxicity from any given exposure and would require longer recovery before resuming pesticide spray work (32,79). Insufficient research has been done to determine at what levels of exposure individuals with decreased paraoxonase activity are at risk.

HLA Associations

Each individual has a specific set of proteins on the surface of his or her cell membranes that make him or her different from everyone else. This array of cellular surface proteins is called the HLA system. HLA typing has been used for several years in matching tissue and organ donors with recipients and for paternity determinations. Increasingly, various HLAs are being associated with specific human diseases (106).

The classic and most striking example of an HLA associated with disease is that between ankylosing spondylitis (AS), an arthritis of the spine. Among Europeans, approximately 90 percent of patients with AS display the antigen HLA-B27, while it is present in only 8 to 9 percent of the general population. A number of other joint diseases also display strong positive associations with the B27 antigen.

Allergies, cardiovascular disease, immune system diseases, dermatological disorders, renal disease, ophthalmologic disorders, gastrointestinal diseases, and certain malignancies have been associated with the presence of one or more HLA types (73,88). Despite some striking statistical associations of certain diseases with specific HLAs, any mechanistic relationship is yet to be determined, precluding the possibility of knowing whether the relationship is causal or merely statistical. Nevertheless, the recognition of the statistical relationship of HLAs with a wide range of human diseases—some of which are known to also be occupationally related, such as bladder cancer, asbestosis, and farmer's lung-suggests that inherent genetic factors are affecting the occurrence of the disease within the population (14).

SCREENING FOR NONOCCUPATIONALLY RELATED DISEASE

Recent progress in developing tests to detect conditions not associated with worksite exposures—e.g., Huntington's disease or heart disease--raises new issues for containing health care expenses, for both the employer and employee, and for employee "wellness programs. " The implications of the various motivations for screening for nonoccupationally related traits and disease are discussed more extensively in chapter 2.

This section covers genetic traits that have been identified and for which genes have been cloned (table 5-l), or where the abnormal gene can be detected indirectly through DNA-based tests. It is not a comprehensive treatise on all genetic conditions that have been cloned, but rather a discussion of selected conditions intended to illustrate the vast progress in this field.

The determination of 'predisposition to disease' used to be based on gross physical examination, family history, and lifestyle habits such as eating and drinking. Molecular biology has enhanced this determination by seeking out and finding genes or markers associated with disease. Individuals found to have the gene or the marker can then be identified, sometimes with near certainty, to be candidates for disease. The influence of environment remains the wild card in most cases, because possession of the genetic predisposition alone may not be sufficient to cause disease. It is likely that for some time modern science will be more successful in identifying the genes and the markers than in identifying the environmental agent(s) necessary for activation of the predisposing genes.

Predispositions to certain cancers have been the focus of much research in the past few years, as have those to atherosclerosis, diabetes, mental illness, and chemical addiction. Advances in those areas are discussed below.

Research is also providing insight into possible genetic predispositions to such common ailments as lower back injuries, obesity, allergies, and arthritis. While predictive tests are not immediately foreseeable in any of those areas, as more populations are studied and more linkage maps prepared, it is not improbable that screening tests will be developed.

Predisposition to Cancers

Cancers resemble other common diseases in-so-far as some forms are associated with chromosomal anomalies, others with single mutant genes, or environmental agents (as discussed in ch. 4). The vast majority, however, are best explained by a genetic-environmental interaction. Clearly, some individuals are predisposed to certain types of cancer given the right environmental exposure. Thus, viruses and carcinogenic agents act as environmental triggers in individuals with a hereditary predisposition to cancer. This "two-hit" theory, first posed in the early 1970s (59), states that sporadic v. inherited forms of cancer could both result from mutations in the same gene. These mutations act recessively at the cellular level, and both copies of the normal gene must be altered or lost for the cancer to develop. In sporadic cases, both events occur somatically whereas in cancer families, susceptibility is inherited through a germline mutation and the cancer develops after a somatic change in the normal allele. Recent developments in the study of oncogenes corroborate this theory (7,69).

Occupational exposures have been implicated in lung, bladder, testicular, and laryngeal cancers, as well as leukemias. As the connections between cancer and genetics become clearer, so may the relevance of occupational exposure to genetic disease.

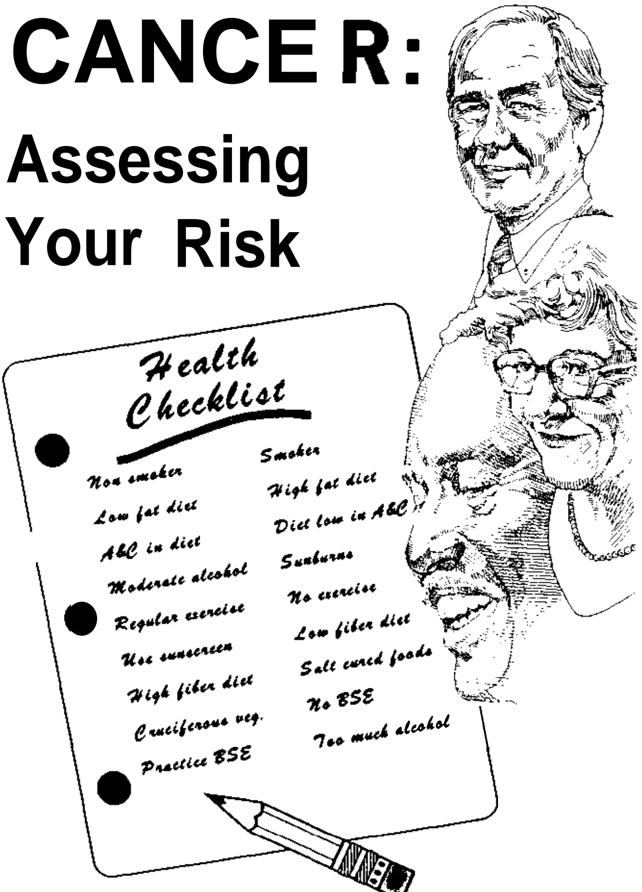
Increasingly, predisposition to certain cancers will be detected through the identification of oncogenes, as well as DNA repair, metabolizing enzymes, and immune function. The following section describes recent developments in identifying individuals predisposed to cancer. Evidence from studies of high-risk cohorts of workers exposed to carcinogenic agents shows that some workers do not develop tumors. Possible explanations for this differential effect could be variation in exposure, diet, or other lifestyle factors, or genetic factors. Little is known about the role of genetic predisposition to cancer following exposure to carcinogenic agents, but as the genetic defects of various neoplasms are identified, the prospects for better understanding improve.

Recent developments in the identification of cancer genes, or oncogenes, and tumor-suppressor genes are discussed below.

Oncogenes

One of the most spectacular results of the new DNA techniques has been the discovery that certain genes, called oncogenes, play a role in the development of cancer (40). Activation of individual oncogenes appear to be necessary, but not sufficient, to trigger cancer. As many as 10 distinct mutations may have to accumulate in a cell before it becomes cancerous (68). In some cases, chromosomal breaks, deletions, translocations, or insertions of foreign DNA place a potential oncogene (also called a proto-oncogene) near a regulatory element that activates it (94). The clastogenic (chromosome-breaking) effects of radiation and certain chemi-

CANCE R: Assessing Your Risk



A PERSONALIZED TEST with guides for reducing your risks

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LUNG CANCER

1 SEX: a Male (2) b Female (1)
2 AGE: a. 39 or less (1) b 40-49 (2) c 50-59 (5) d 60+ (7)
3. **EXPOSURE TO ANY OF THESE:** a. Mining (3) b. Asbestos (7) c. Uranium & radioactive products (5) d. None (0)
4. **HABITS:** a. Smoker (10)* b. Nonsmoker (0)*
5. **TYPE OF SMOKING:** a. Cigarettes or little cigars (10) b. Pipe and/or cigar, but not cigarettes (3) c Nonsmoke
6. **NUMBER OF CIGARETTES SMOKED PER DAY:** a. 0 (1) b. Less than ½ pack per day (5) c. ½-1 pack (9) d. 1-2 packs (15) e. 2+ packs
7. **TYPE OF CIGARETTE:** a. High tar/nicotine (10)** b. Medium tar/nicotine (9)** c. Low tar/nicotine (7)** d. Nonsmoke
8. **LENGTH OF TIME SMOKING:** a. Nonsmoker (1) b. Up to 15 years (5) c. 15-25 years (10) d. 25+ years

SUBTOTAL ___

REDUCING YOUR RISK — * If you stopped smoking more than 10 years ago, count yourself as a nonsmoker. If you have stopped smok the past 10 years, you are an **ex-smoker**. Ex-smokers should answer questions 5 through 8 according to how they previously smoked. Then ex-smoker **reduce** their point total on questions 5 through 8 by 10% for each year they have not smoked. Current smokers also answer questions 5 through 8

I am stopping smoking today. (Subtract 2 points.) TOTAL ___

** High Tar/Nicotine 20 mg or more tar / 1.3 mg or more nicotine Medium Tar/Nicotine 16-19 mg tar / 1.1-1.2 mg nicotine Low Tar/Nicotine 15 mg or less tar / 1.0 mg or less

COLON RECTUM CANCER

1. **AGE:** a. 49 or less (10) b. 50-59 (30) c 60 and over
2. **HAS ANYONE IN YOUR IMMEDIATE FAMILY EVER HAD:** a. Colon cancer (20) b. One or more polyps of the colon (10) c Neithe
3. **HAVE YOU EVER HAD:** a. Colon cancer (100) b. One or more polyps of the colon (40) c. Ulcerative colitis d. Cancer of the breast, ovaries, stomach, or uterus (10) e None of the above (1)
4. **DO YOU HAVE BLEEDING FROM THE RECTUM** (other than obvious hemorrhoids or piles diagnosed by your physic a. Yes (75) b. No (1)

SUBTOTAL

REDUCING YOUR RISK

5 I have altered my diet to include less fat and more fruits, fiber and cruciferous vegetables (broccoli, cabbage, cauliflower, Brussels sprouts) (Subtr. points.)
6. I have had a negative test for blood in my stool within the past year (Subtract 10 points.)
7 I have had a negative proctoscopic (sigmoidoscopic) exam within the past year (Subtract 20 p

TOTAl

SKIN CANCER

1. Live in the southern part of the U.S.: Yes
2. Frequent work or play in the sun: Yes
3. Fair complexion or freckles; (natural hair color of blonde, red, or light brown, or eye color of grey, green, blue, or hazel): Yes
4. Work in mines, around coal tars or radioactivity: Yes
5. Experienced a severe, blistering sunburn before the age of 18: Yes
6. Have any family members with skin cancer or history of melanoma: Yes
7. Had skin cancer or melanoma in the past: Yes
8. Use or have used tanning beds or sun lamps Yes No
9 Have large, many, or changing moles Yes No

REDUCING YOUR RISK

10. I cover up with a wide-brimmed hat and wear long-sleeved shirts and pants Yes
11. I use sun screens with an SPF rating of 15 or higher when going out in the sun. Yes
12. I examine my skin once a month for changes in warts or moles Yes

Photo credit: American Cancer Society: Texas Divis

A written self-test that assesses an individual's cancer risks.

cals, therefore, have the potential for activating an oncogene (see ch. 4).

Approximately 40 dominant-acting oncogenes have been found that can induce transformation when introduced into a cell in a structurally altered form or when improperly expressed. The most commonly studied oncogene, called "ras, " is found in 50 percent of colon carcinomas, 30 percent of adenocarcinomas of the lung, and more than 90 percent of cancers of the pancreas (3,28,54,90,113). Most pancreatic carcinomas and about one-third of colorectal cancers reveal the presence of a dominant-acting oncogene activated by a single nucleotide substitution.

Chronic myelogenous leukemia (CML) exhibits the Philadelphia chromosome (named after the city in which it was identified) which results from chromosomal translocation. The translocation indirectly activates an oncogene. Biotechnology companies have developed probes for the CML and breast cancer oncogenes.

In recessive oncogenesis, the cells appear to have lost both copies of a tumor suppressor gene (62). Suppressor genes, or "anti-oncogenes," are necessary for the inactivation of the oncogene, and therefore, the malignant cell growth. Suppressor gene loss is at least as important in carcinogenesis as oncogene activation. It usually takes two separate mutagenic events to eliminate a suppressor gene, because alleles are paired and both probably have to be inactivated (68). Two candidate turner suppressor genes have been cloned, for retinoblastoma and Wilm's tumor, but the existence of others has been inferred from experiments in which specific chromosomal regions were deleted in turners (86,114).

Retinoblastoma (Rb) is an intraocular tumor of early childhood that can be inherited in an autosomal dominant fashion but is thought to result from a recessive-acting oncogene. The Rb gene provides a model system for recessive oncogenesis. This gene also has been shown to be inactivated in some breast cancer cell lines. Inheriting a mutant allele for the Rb gene predisposes a recipient to Rb. The sporadic loss of the Rb gene through mutation is correlated with increased cell proliferation and oncogenesis (23). Inactivation through mutation of the Rb susceptibility gene has been implicated in the genesis of Rb and certain other human neoplasms, suggesting a broad role for this gene in oncogenesis (65).

Recessive-acting oncogenes also have been associated with common solid tissue tumors such as carcinomas of the bladder, colon, breast, and lung.

There is a significant distinction between dominant-acting oncogenes and recessive-acting suppressor genes. The oncogenes that have been identified to date are activated through somatic mutations—genetic changes occurring in one or another target organs and not in the germ cells. Mutant, activated oncogenes are therefore not transmitted from parent to offspring and can be detected only in tumor cells. In contrast, mutant forms of suppressor genes might be found in sperm or eggs, and can be passed onto future generations (116). This difference distinguishes those who are predisposed to cancer at birth from those who are not.

The rapid development of synthetic probes and gene amplification techniques will increase the capability to detect the presence or recurrence of malignant cells with genetic characteristics associated with oncogenes and tumor suppressor genes. An experimental approach, termed "gene targeting," proposes to stop or counter the action of oncogenes by introducing a synthetic strand of DNA to block the message arising from the activated oncogene.

Colon Cancer

Colon cancer is the second most common cancer in North America, estimated to account for about 62,000 deaths in 1988 (54). A recent study concluded that a gene inherited by a third of all Caucasian Americans may be responsible for most cancer of the colon and rectum. Inheriting the gene does not mean that one is destined to have cancer. Other factors, both genetic and environmental, play a role in inducing cancer in those who inherit the gene. One study found evidence that a series of four to five genetic mutations and deletions are necessary for colon or rectal cancer to occur. If it takes four or five steps to get the cancer, those who have inherited one of the mutations in those steps have, in a sense, a "head start' and are more likely to get the cancer (54).

Two types of mutations have been detected in colorectal tumors. The first involves point mutations in ras proto-oncogenes. The second type involves deletions of specific chromosomal regions. Deletions can be detected through RFLP analysis. The deleted sequences have been hypothesized to in-

elude tumor-suppressor genes necessary for inhibition of neoplastic growth (54,114).

Advances in this area will lead to earlier detection and intervention and to greater understanding of environmental influences on the activation of the oncogene.

Lung Cancer

Lung cancer is the overall leading cause of cancer death among Americans. There is evidence that a genetic defect contributes to the development of an important form of lung cancer that makes up at least 20 percent of all lung cancers. In some studies of lung cancer patients, a portion of chromosome 3 is deleted, possibly taking with it suppressor genes (60). Some lung cancers also demonstrate loss of heterozygosity on the short arm of chromosome 17. The p53 gene located in chromosome 17 has the features of a tumor suppressor gene (103).

Some occupational exposures are among the known causes of lung cancer, as is cigarette smoking. It has been suggested that, in males, 15 percent of lung cancers in the United States are due to occupational exposure (21). Employees in asbestos-related occupations, including asbestos production workers, pipefitters, boilermakers, roofers, and shipyard workers, have long demonstrated above average incidence of lung cancer (112). Workers exposed to polycyclic hydrocarbons, such as mechanics and railroad workers, also have a higher incidence of lung cancer. It is unclear how these exposures might induce the mutation necessary for initiation of carcinogenesis, but breakage and rearrangement of chromosomal material is the likely predictor of the deletions containing the suppressor genes.

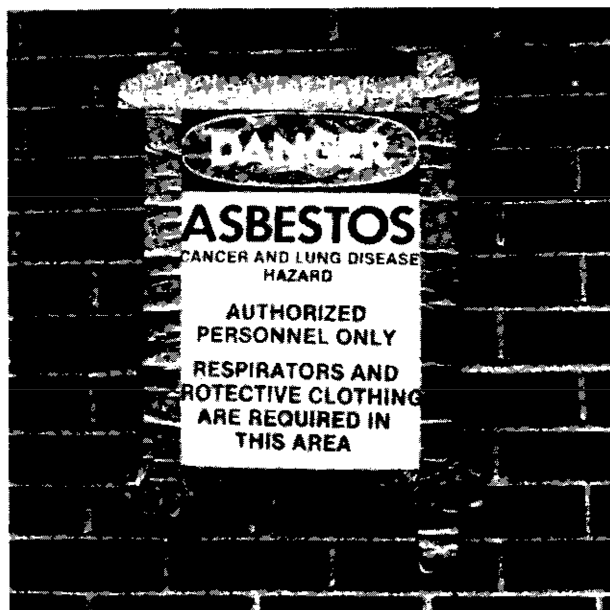

Photo credit: Gianna Bosko

A sign displayed at an asbestos removal site warning that asbestos is a cancer and lung disease hazard.

Future research exploring the relationship between genetic predisposition to lung cancer and environmental exposure will focus on these groups of workers. It is conceivable that once a predisposing gene or set of genes is located, use of probes and PCR will facilitate rapid identification of a subpopulation of workers at higher risk if exposed to certain genotoxic agents. Recent use of RFLPs and probes has detected marker antigens on cells at least 2 years before the clinical appearance of lung cancer (77). Widespread application of this early test could dramatically improve prognosis for cancer patients, whether or not the cancer was attributed to environmental exposure.

Bladder Cancer

More than 49,000 Americans develop bladder cancer each year and about 10,000 die annually. Historically, the major known risk factors were environmental, particularly occupational exposures to aromatic amines. As described earlier in this chapter, phenotypic variants of the autosomal recessive trait for the enzyme N-acetyltransferase have been associated with bladder cancer in workers exposed to aromatic amines. Various genetic polymorphisms have been associated with bladder cancer, including an excess of the A gene of the ABO blood group and an excess of two HLA genes, B5 and CW4 (92). But much of this latter work has not been corroborated.

Activated oncogenes and chromosomal changes have recently been identified with bladder cancer. It has been demonstrated that cells from urogenital tissue derived from patients with bladder cancer are missing genes on the short arm of chromosome 11. As in colorectal and lung cancers, it is theorized that among those missing genes are genes responsible for suppression of growth (27). A transforming oncogene (H-ras-1) has been isolated from a cell line of human bladder cancer cells (89).

If a genetic component in bladder cancer can be confirmed, screening programs could be targeted to worker populations at high risk. The National Cancer Institute (NCI) estimates that one out of four cases of bladder cancer in Caucasian male Americans is related to occupational exposure. The risk is highest among painters, truckdrivers, employees of the oil, aluminum, and railroad industries, and drill press operators (18). Future research on these populations might lead to a better understanding of the subtleties of predisposition and environmental insult. Some analysts, however, feel that the NCI figures are based on soft, or insufficient data (2,95).

Hereditary Cancer Syndromes

Human cancers that are associated with autosomal dominant mutations have been collectively referred to as hereditary cancer syndromes (HCS) (29). Approximately 60 to 90 percent of individuals with HCS develop a specific type of cancer at an early age. Family members with HCS tend to develop the same type of neoplasm at multiple stages in the same organ or bilaterally in paired organs (3). Examples of dominantly inherited HCS include retinoblastoma, Wilm's tumor, neuroblastoma, nevoid basal cell carcinoma, familial polyposis coli, von Hippel-Lindau tumors, neurofibromatosis, and familial cancer syndrome. Syndromes inherited in an autosomal recessive fashion include Fanconi's anemia and xeroderma pigmentosum.

Predisposition to Mental and Addictive Disorders

New research has shown some mental and behavioral disorders to be, in part, genetically deter-mined. The exact nature of genetic influence, however, remains in dispute for most disorders. Nonetheless, it is widely believed that there is likely a genetic component to manic depression, schizophrenia, autism, hyperactivity, some compulsive disorders, and alcoholism (6,63,67). There is even evidence that addiction to narcotics is influenced by physiological differences determined, in part, by our genes. Scientists have identified a gene in rats involved in the activation of dopamine, an important neurotransmitter (12). Abnormal dopamine function has been linked to schizophrenia, manic depression, Parkinson's disease, and chemical addiction (105).

Currently, disputes center not so much on whether these disorders are genetic, but rather on where their predisposing genes are found. Most recently, two different research teams linked two different markers to schizophrenia (53,93). While it has long been known that schizophrenia shows a tendency to cluster in families, the exact nature of genetic influence has been unclear. Although it might appear that the identification of two distinct markers for the disorder is contradictory, most geneticists view the discrepancy as confirmatory that schizophrenia is a heterogeneous disorder subject to unknown environmental influences (30,63).

Similarly, a research group claimed to have located the gene predisposing individuals to manic depression. Subsequent studies revealed that conclusions drawn from the earlier study were premature and the linkage association was greatly diminished (24,56).

Recent studies of Alzheimer's disease show that at least 10 percent of the cases have a family history with an autosomal dominant pattern and the gene for the inherited form of the disease has been reported to be on chromosome 21 (84). Other studies failed to confirm that finding (104). The majority of cases may be sporadic, with the clinical features identical to the inherited form. The primary cause of Alzheimer's disease remains unknown. Both environmental and genetic factors have been implicated. It is clearly a heterogeneous disorder with an unknown environmental component in at least 85 percent of the cases (91,97).

More conclusive is the evidence for a genetic predisposition to alcoholism. Researchers studying the children of alcoholics have detected specific biochemical and behavioral differences in their responses to alcohol. Specifically, alcoholics have a greater ability to synthesize a unique derivative of alcohol known as phosphatidylethanol. While the contribution of that trait to a predisposition is not clear, it is feasible that testing for levels of phosphatidylethanol could serve as a biological marker for alcoholism (8).

The one neuropsychiatric disorder for which a definitive test has been developed is Huntington's disease, a progressive disease of the central nervous system with no treatment and certain death an average 15 to 17 years after onset some time in mid-adulthood. Huntington's disease is inherited as an autosomal dominant trait with complete lifetime penetrance. In 1983, Gusella and co-workers discov-

ered a RFLP on the short arm of chromosome 4 that is linked to the Huntington's disease locus (39). Linkage analysis is 95 percent accurate. That is, 5 percent of those with the gene for the disease will be missed because of the genetic distance of the marker to the Huntington's disease gene. As more markers have been found, linkage studies have gained an accuracy of approximately 99 percent (9,38). As with other linkage tests, individuals whose test results are uninformative will have to wait until a second family member develops symptoms, or until other polymorphisms are identified, before they can receive definitive results. When the Huntington's disease gene and the mutations producing the disease are discovered, the uncertainty may disappear. Linkage analysis will be unnecessary, only the at-risk person will have to be tested.

The Huntington's disease case is the exception in understanding the genetics of neuropsychiatric illness. Researchers are beginning to appreciate the difficulties in examining the genetics of complex mental illnesses. Scientists are often unable to replicate linkage work performed by others because of the multiple causes of what appears to be the same disorder, the lack of large family pedigrees and large numbers of pedigrees, misdiagnoses of affected relatives, and the sheer complexity of mental illness (6). This has led some to propose that the statistical scores conventionally used to establish linkage be made more stringent for mental disorders. Currently, a logarithmic ratio of 3 is taken as minimum evidence for linkage, meaning that the likelihood is 1,000 to 1 that the marker and gene are linked rather than randomly distributed (6). Raising that ratio to 6 would raise the requirement for linkage and would take into consideration genetic heterogeneity and variable expressivity of the disorder (84). This, and improving clinical diagnostic criteria, can protect against misleading results until the genes for these disorders are actually found.

Photo credit: Nich Kelsh/Kelsh-Marr Studios

Huntington's patient being rowed across Lake Maracaibo, Venezuela.

Predisposition to Atherosclerosis

The associations between coronary artery disease, or atherosclerosis, and cholesterol have been well-established. High-density lipoprotein (HDL) cholesterol promotes efflux of cholesterol from arterial walls, thus earning the reputation of 'good cholesterol." Low-density lipoprotein (LDL) cholesterol causes cholesterol deposition in arterial walls, thereby earning the reputation of 'bad cholesterol." Apolipoprotein A-I is the principal protein constituent of HDL. Decreased plasma concentrations of both HDL and A-I have been associated with premature coronary artery disease (80).

Early coronary artery disease and atherosclerosis exhibit definite familial aggregation. Several different HDL-deficiency states have been reported. The recent cloning of the apolipoprotein A-I gene provides the necessary molecular probe for RFLP analysis of normal and HDL-deficient states. Mutations of the A-I gene are found in 32 percent of people who had severe coronary artery disease before age 60, but only in 3 percent of people with healthy heart arteries (80).

In addition, a single-gene defect at the LDL receptor locus binds apolipoprotein B (apoB) and accounts for the clinical entity known as familial hypercholesterolemia (11). The heterozygote form occurs in 1 out of every 500 individuals. The more lethal homozygous form occurs 1 in 1 million births. Genetic variation affecting LDL levels influences atherosclerosis susceptibility (33).

Significant genetic variation in both the A-I and apoB gene might explain variations in the onset and severity of coronary artery disease among individuals. Biochemical screening for HDL and LDL will continue to be the most reliable predictors of predisposition to disease. The most obvious advantage of genetic screening over current methods is that RFLP marker tests need only be conducted once. Understanding the significance of the muta-

tions at the A-I and apoB loci will lead to more effective and earlier therapy (41).

Predisposition to Diabetes

Diabetes is a disorder in which the body does not produce or properly use insulin, a hormone needed to convert sugars and starches into energy sources for the body. One million of the Nation's 11 million diabetics are insulin-dependent (called type 1 or insulin-dependent diabetes mellitus (IDDM)). Individuals with IDDM exhibit an immunological dysfunction, resulting in the destruction of the islets of Langerhans, groups of cells in the pancreas where beta cells reside to produce insulin is produced. Recent studies reveal an inherited susceptibility in 95 percent of individuals with IDDM (108). Millions of Americans, possibly 50 percent of the entire population, possess the DR3 and DR4 markers (107,108). Despite the prevalence of these susceptibility markers, relatively few people develop the disease, leading to the conclusion that other genes, or some viral or toxic insult, might be necessary to trigger the disease. In addition, geographic variation in rates supports the concept of an environmental role.

COMMERCIAL DEVELOPMENT OF GENETIC TESTS

With accelerating interest in tests to detect abroad range of genetic disorders and increasing investment in biotechnology industries, the market demand for tests, especially DNA-probe tests, is expected to expand. In addition to academic research centers, several biotechnology companies are developing a range of genetic tests (table 5-3), with projections of market value in the millions. While the population affected by genetic conditions for which there is a test available is still somewhat small, the potential future test population for multifactorial diseases is enormous (see table 5-4).

In a 1987 OTA survey of biotechnology companies, it was found that biotechnology companies developing DNA-based tests expected their products to be used by 1990 in: genetic and health department clinics, health department screening programs, prepaid health groups, private primary care practices, reference and DNA labs, insurance companies, the military, places of employment, private nongenetic specialty practices, correctional institutions, public schools, and homes (110).

Table 5-3-Some Companies Offering DNA-Based Diagnostic Tests

Company	Test
California Biotechnology, Inc. Mountain View, CA	susceptibility to atherosclerosis and hypertension
Cetus Corp. Emeryville, CA	sickle cell anemia
Collaborative Research Bedford, MA.	adult polycystic kidney disease cystic fibrosis lymphomas
Genescreen Dallas, TX	insulin-dependent diabetes sickle cell anemia hemophilia familial hypercholesterolemia
Genetrix Alameda, CA	leukemias
Integrated Genetics Framingham, MA.	adult polycystic kidney disease Duchenne muscular dystrophy Huntington's disease cystic fibrosis sickle cell anemia hemophilia B alpha and beta thalassemia
Lifecodes Corp. Valhalla, NY	non-Hodgkin's lymphoma lymphocytic leukemia breast cancer oncogenes sickle cell anemia cystic fibrosis
Nichols Institute San Juan Capistrano, CA.	cystic fibrosis Duchenne muscular dystrophy sickle cell anemia hematologic malignancies
Oncogene Science Mineola, NY.	chronic myeloid leukemia

SOURCE: Office of Technology Assessment, 1990.

Although biotechnology firms developing genetic tests did not overall rank places of employment as important sites for testing in 1990, 5 of 12 thought it likely that employers would be using genetic tests to screen job applicants by the year 2000. Seven of twelve agreed that health risks identified by genetic screening could be used appropriately to exclude susceptible workers from hazardous jobs; 9 of 12 thought this use likely by the year 2000. Other sources predict that by then, most people will have genetic profiles, possibly obtained through their place of employment. Some companies are interested in employee "wellness" programs that include family histories and susceptibility to disease (22,71,1 10). Five of the twelve companies thought it likely by the turn of the century that insurance

Table 5-4-Genetic Tests Available and Total Americans Affected

Genetic condition	Total cases
Currently available:	
Adult polycystic kidney disease	500,000
Fragile X syndrome	100,000
Sickle cell anemia	65,000
Duchenne muscular dystrophy	32,000
Cystic fibrosis	30,000
Huntington's disease	25,000
Hemophilia	20,000
Phenylketonuria	16,000
Retinoblastoma	10.000
Total	798,000
Potential future tests:	
Hypertension	58,000,000
Dyslexia	15,000,000
Atherosclerosis	6,700,000
Cancer	5,000,000
Manic-depressive illness	2,000,000
Schizophrenia	1,500,000
Type 1 diabetes	1,000,000
Familial Alzheimer's	250,000
Multiple sclerosis	250,000
Myotonic muscular dystrophy	100,000
Total	89,800,000

SOURCE: *Medical World News*, p. 58., Apr. 11, 1988.

companies would be using genetic tests on applicants (110).

Table 5-4 lists some of the tests currently available from commercial interests. Biotechnology corn-panies developing tests for genetic disease or predispositions are generally employing one of three strategies: l) linkage-based tests for family-centered testing programs; 2) tests for single-gene disorders or predictive tests for common polygenic disorders; and 3)development of test processes or instrumentation(110). Tests generally range in price from $200 to $980 per individual. The test for Huntington's disease offered by Integrated Genetics (Framingham, MA) costs $450 per sample (48). Several tests, specifically oncogene-based tests, are awaiting U.S. Food and Drug Administration approval.

SUMMARY AND CONCLUSIONS

There are two categories of genetic characteristics that are relevant to the occupational setting: those that predispose the individual to adverse health effects because of environmental exposure and those that predispose the individual to adverse health effects regardless of job, thereby having an impact on employee "wellness' and possible job performance. In both cases, identification of predisposed individuals remains problematic because not all people carrying predisposing genes develop disease. Variable expressivity, heterogeneity, and reduced penetrance confound the certainty of diagnoses, lowering both the sensitivity and specificity of many current tests.

Most DNA-based tests are indirect, relying on linkage studies to identify those at risk. But even direct tests for mutant genes can be ambiguous without affected family members.

The obstacles to certainty, however, are slowly being removed as the use of synthetic probes, PCR, and automated DNA-sequencing machines increase the efficiency and lower the cost of mass screening. These advances are providing insights into the genetic predisposition to adverse health effects from drugs and environmental agents, cancers, diabetes, atherosclerosis, and mental illness. Fifty human genetic traits have been identified as having the potential to enhance an individual's susceptibility to toxic or carcinogenic agents.

Yet, the environmental agents that trigger the predisposition often remain the wild card. Even though science has shown that cancer is often the result of the activation of oncogenes or inactivation of tumor suppressor genes, the agents that cause these changes are hardly known, and speculated on at best. It may be that as the associations between mutation, carcinogenesis, and genetics become more clear, the boundaries between occupational and genetic disease will become more blurred.

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