Chapter 5

Principles of Genetic Testing

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Genetic testing of employee populations is a basic method for identifying individuals or groups with particular inherited traits or evidence of genetic damage in certain cells who may be at increased risk for disease. It is the application of tests to a group of apparently well persons in order to identify those who have a high probability of developing a disease so that prevention or early treatment is possible. Genetic testing involves laboratory examination of body fluids such as blood to determine the presence of inherited traits or changes in chromosomes or deoxyribonucleic acid (DNA). It includes both genetic screening and genetic monitoring. Each uses specific laboratory tests but the goals of each are slightly different.

A genetic screening test is a one-time procedure used in occupational settings to identify individuals with certain inherited traits. Some scientists have hypothesized that these traits may cause the individual to be at increased risk for certain occupational diseases when exposed to hazardous chemicals (1). Because these inherited traits do not change, a single test for them is sufficient.

Genetic monitoring periodically examines induced genetic damage in certain cells of workers. Some scientists believe that certain types of genetic damage may indicate exposure to hazardous agents and may be associated with an increased risk for certain diseases, in particular cancer, The laboratory tests search for endpoints different from those used in genetic screening, and the procedures are applied initially to determine a baseline of genetic damage prior to exposure and then periodically to determine changes in that damage. Changes in certain genetic characteristics of the population may indicate that the population is at an increased risk for disease.

Before a rational decision can be made on the value of any genetic screening or monitoring program in the workplace, two questions must be answered. The first is: “Does the test being employed reliably identify either the genetic trait or type of damage in question?” The answer to this question requires an assessment of the particular laboratory techniques used to identify genetic traits or genetic damage from exposure to hazards. Only after achieving a positive answer to this question can the following question be asked: “Does this particular trait or damage cause the individual or population to be at increased risk for disease?” The answer to this question involves assessing the conclusions of epidemiologic studies regarding the association between these genetic factors and disease. Available scientific evidence indicates that the first question can be answered in some cases; the answer to the second one awaits significantly more research. In ascertaining whether the test identifies either a genetic trait or damage, the tests must be subjected to scientifically recognized analytical criteria: validity, reliability, predictive value, and relative risk (6).

Validity, reliability, and predictive value

The validity of genetic testing—i.e., the probability that a test will correctly classify true susceptible (“positive”) and true nonsusceptible (“negative”) individuals—should be evaluated before the test is placed into routine use. Few tests are 100 percent valid. The reasons are both methodology - cal (i.e., the inherent variability in test performance) and biological (i.e., the influence of other genetic as well as environmental factors).

From the distribution of the test results in those for whom the presence or absence of a genetic endpoint (trait or genetic damage) has been confirmed, the validity of the test at different cutoff

points can be determined (fig. 7). Two separate, independent characteristics are subsumed under validity; each depends on the cutoff point that is selected. These are:

- sensitivity, or true positive ratio—the frequency with which the test will be positive when the genotype in question is present; and
- specificity, or true negative ratio—the frequency with which the test will be negative when the genotype in question is absent. An ideal test would be 100 percent sensitive and 100 percent specific. In actual practice this does not occur.

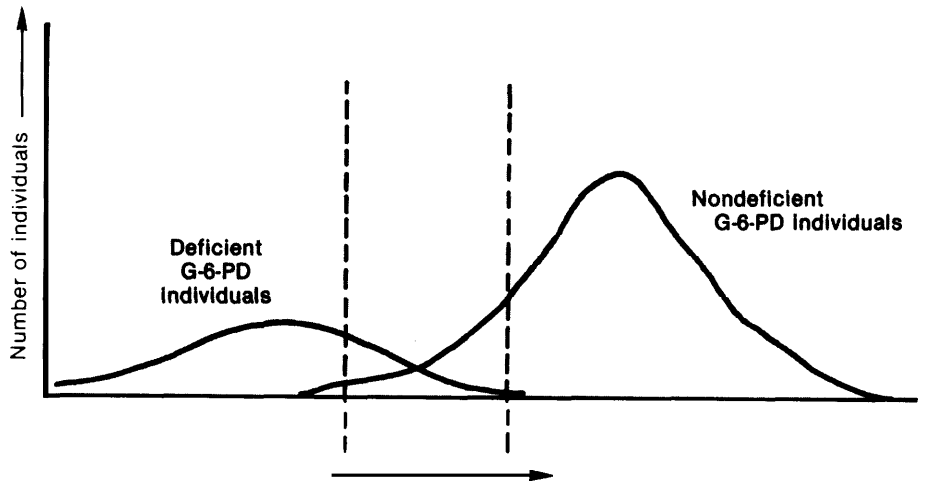
Sensitivity and specificity are usually inversely related. That is, one usually achieves high sensitivity at the expense of low specificity and vice versa. This can be demonstrated by examining a hypothetical situation to determine the cutoff point for a screening test (fig. 7).

The selection of the actual cutoff point depends on the objective of the screening or monitoring test. If the objective is to identify all individuals with the abnormal genotype or genetic damage, cutpoint A would be selected. As the figure shows, such a cutpoint will pick up all true positives, but it will also result in many false positives. If no followup test is planned in the routine operation of a screening program, this cutoff point would mislabel many people as affected who are not. With cutoff point B, no individuals would be falsely labeled as affected, but some affected individuals would be falsely labeled as unaffected. Methods of determining the cutpoint that minimize costs of mislabeling or that maximize the information to be gained from the screening tests are available (5,7),

In addition to validity, reliability under conditions of routine use must also be demonstrated. That is, tests of the same specimen must repeatedly give the same result whether performed by several different laboratories or by the same laboratory on several occasions.

predictive value is related to sensitivity, specificity, and the prevalence of the trait or genetic damage in the population. When the prevalence of a particular trait or genetic damage is low in the population, even a highly specific test will give a relatively large number of false positives because many persons being tested will not have the endpoint. The likelihood that an individual with a positive test has the disease, and vice versa for a negative test result, is the predictive value of the test. The importance of prevalence for the predictive value of a test can be seen in the following example. Table 12 presents hypothetical data

Figure 7.—Example of a Hypothetical G-6-PD Distribution



SOURCE: Office of Technology Assessment

Table 12.—Calculation of Predictive Value[a]

	Number with positive test	Number with negative test	Total
Genotype present	990		1,000
Genotype absent	990	98,010	99,000
Totals	1,980	98,020	100,000

Predictive value of a positive test result = $\frac{990}{1,980} = 0.50$

Predictive value of a negative test result $\frac{98,010}{98,020} = 0.9999$

sensitivity, specificity = O 99.

SOURCE: N A Holtzman, "Principles of Screening Applied to Testing for Genetic Susceptibilities to Harm From Workplace Exposure," prepared for OTA, September 1982

for calculating the predictive value of a positive test result for a genotype frequency of 1 percent (1,000 / 100,000) (2). Even where the sensitivity and specificity are arbitrarily set high, 0.99, the positive predictive value is only 50 percent. This means that the test correctly measures the result only half the time; in the case of genetic screening, half of the workers with positive test results would, in fact, not have the predisposing genotype. Followup testing would have to be a part of a screening or monitoring program in order to detect the false positives or false negatives.

Table 13 shows the influence of selected frequencies when a cutpoint for the screening test is used that yields both a specificity and a sensitivity of 0.99. The predictive value of the positive test will vary between O and 0.92 percent as the frequency of the genotype varies between 1 and 10,000 per 100)000 (0.001 to 10 percent) people screened. The chance that a person with a negative test result does not have the genotype is also shown. Note that all the predictive values for a negative test result in the table are very close to 1.0. A genotype frequency (prevalence) of approximately 50 percent (not shown) is needed before the predictive value of a negative test rises to 0.99 (3,6).

Table 13.—Influence of Genotype Frequency on the Predictive Value of Screening Tests[a]

	Frequency of the genotype (per 100,000)				
	1	10	100	1,000	10,000
Predictive value of a positive test result	0	0.01	0.09	0.50	0.92
Predictive value of a negative test result	1	1	1	1	1

[a]Sensitivity, specificity =0 99.

SOURCE: N. A. Holtzman, "Principles of Screening Applied to Testing for Genetic Susceptibilities to Harm From Workplace Exposures," prepared for OTA, September 1982,

Relative risk

The proportion of workers likely to contract a disease depends not only on the previously mentioned variables (reliability, validity, frequency of the genotype), but on the relative risk for the disease imposed by the genetic trait or damage. Information for calculating relative risk* can be collected in two ways. In the prospective approach, all individuals comprising the population exposed to the agent would be tested for the genotype and followed for a set period of time to determine the incidence of harmful effects in those with the specific genotype and in those without it. Alternatively, a retrospective study could be used to compare the frequency of the genotype among workers who developed the

• Relati \ re risk is the ratio of the incidence of disease among exposed persons divided by the same rate among nonexposed persons.

harmful reaction to the frequency in workers who did not. (Note: The latter approach would yield a risk ratio which is a close approximation to the relative risk measure.)

Table 14 shows the influence of relative risk and genotype frequency on the proportion of workers at risk for harm from exposure discovered by a test whose sensitivity and specificity are set equal to 0.99 (3). For example, with a genotype frequency of 1,000/100,000(1 percent), those with the genotype must be 100 times more likely to suffer adverse reactions before the screening test will discover half of those who will suffer harm. In addition, in this table and the two preceding tables, sensitivity and specificity levels have been set at 0.99 in order to elucidate the other components. In actual studies sensitivity and specificity are never as high. Thus, the ability to detect predisposing factors is further compromised.

Table 14.-Influence of Genotype Frequency and Relative Risk[a] on the Proportion of Workers at Risk for Harmful Reactions Who Will Have Positive Screening Test Results[b]

Frequency of genotype (per 100,000)	Relative risk 5	10	50	100
	Proportion of at-risk workers discovered by screening			
1	0.01	0.01	0.01	0.01
10	0.01	0.01	0.01	**0.02**
100	0.01	**0.02**	**0.06**	0.10
1,000	0.06	0.10	0.34	**0.50**
10,000	*0.36*	*0,53*	*0.64*	*0.91*

[a]Relative risk = incidence of adverse reaction in those with the susceptible genotype / incidence of adverse reaction In those without the susceptible genotype

[b]Sensitivity, specificity set at = *0.99*

SOURCE: N.A.Holtzman, "Principles of Screening Applied to Testing for Genetic Susceptibilities to Harm From Workplace Exposures," prepared for OTA, September 1982.

From this discussion, it is clear that attention must be paid to validity, reliability, predictive value, and relative risk or screening and monitoring in the workplace may turn out to be costly and of little benefit. The less frequent the genetic endpoint being tested, the less likely that the person with a positive test result will truly have that trait or damage. Unless testing of high validity is restricted to conditions in which the frequency of the trait or damage is high, a significant number of false positives and false negatives can be expected. False positives increase the social, economic, and psychological costs of screening; false negatives reduce the health benefits, When the frequency of the endpoint is high, however, lowering exposure for the entire work force may be the most effective way of reducing disability. If a genetic screening program were instituted, a population that would ensure a relatively high frequency (greater than 1 percent) of the trait of interest should be chosen, One way to increase the frequency in a population is to select a subgroup that is expected to have a higher frequency of the trait than the general population. A monitoring program should be instituted only when bacterial and animal tests have proven that the chemical in question is mutagenic or carcinogenic. Moreover, worksite sampling should establish that the hazardous agent is present in areas where workers would be significantly exposed.

OTA's assessment of occupational studies

The correlation of a test endpoint (for example, chromosomal damage) with the later occurrence of disease is difficult to ascertain because the possibility remains that adverse consequences from exposure will not occur in all of those with the predisposing condition; other genetic or environmental factors (for example, smoking) may be necessary for the development of the disease or may contribute differently in different individuals. Because an illness may have multiple causes, it may also occur in workers without the predisposing condition. Thus, genetic tests may identify only a proportion of the workers who will develop adverse reactions,

Part III of this report contains OTA's assessment of relevant monitoring and screening studies conducted on human populations. The following cri-

teria were applied to determine whether the studies were based on sound methodological approaches (4):

- Is the observed association consistent? That is, has the same association been observed in similar studies?
- Is the association specific? Was there a mix of exposure levels or grouping of individuals such that the precise nature of the effect of exposure is difficult to ascertain?
- Is the strength of the association strong? Is it strong enough to indicate a causal relationship between exposure and disease?
- Is there a dose-response relationship? Does it appear that higher exposure levels are associated with higher prevalence of the disease?
- Is there a biological mechanism to explain the association?
- Was the study designed so that the assumptions of statistical methodology were met? Has the sample been properly drawn?

Chapter 5 references

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