Viewpoint

Misconceptions about the use of genetic tests in populations

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"The risk of defining anything as complex as a human being with narrow precision is that there will always be exceptions that fall outside the lines—people who are not human"[1]

Genetic penetrance and environmental factors

The prospect of genetic screening for preventable or deferrable disease is becoming real. As the cataloguing of the human genome proceeds, the rate at which specific genes are being implicated in disease processes is increasing. Because of its genetic basis, much interest has centred on identification of genes for cancer and their usefulness in routine screening. Cost-benefit analysis is urgently needed for screening for single-gene diseases versus multigenetic diseases, and for genes of low versus high penetrance. Penetrance of a gene describes the frequency with which the characteristic it controls (phenotype) is seen in people who carry it.

Single, highly-penetrant mutations in so-called cancer genes cause only a small proportion of cancers.[2] Highly-penetrant gene mutations confer an exceptionally high risk of cancer in the carriers. They are the tail of a distribution that includes: (a) common variants of the same cancer genes (polymorphisms) that have only a mildly disruptive effect on the protein coded for by the gene; and (b) mutations in genes that are not directly involved in the cancer process. Genes implicated in rare and cancer-inducing conditions, such as xeroderma pigmentosum, show common polymorphisms that belong to the first category (a) and whose effects on the protein function (a DNA-repair enzyme) are mild. Mild defects in DNA repair can predispose to cancer,[3] and polymorphisms in the xeroderma pigmentosum DNA repair gene have been associated with an increased risk of skin cancer.[4] Metabolic polymorphisms are a clear example of the second category (b); these are common, low-penetrant conditions in which the function of the protein (an enzyme that metabolises toxic chemicals or carcinogens) is impaired, resulting in greater susceptibility to the effect of environmental toxicants. About 50% of the population have the *GSTM1* null genotype (a polymorphism in which the entire gene is deleted), but only a slightly increased risk of some forms of cancer.[5]

The different penetrance of mutations is not entirely an intrinsic characteristic. Even in highly penetrant mutations (eg, Huntington's disease, phenylketonuria), penetrance is not 100%. In the Icelandic population, women with the same *BRCA1* mutation who belonged to the same families had a variable degree of gene penetrance.[6] Although the causes of variable penetrance are still obscure, some possibilities exist. Penetrance depends on at least six factors: (a) importance of the function of the protein encoded by the gene (eg, in crucial metabolic pathways as in phenylketonuria, or in key regulatory aspects of the cell cycle—mutations in these types of gene are highly penetrant); (b) functional importance of the mutation (eg, a deletion *vs* a mild loss of function due to a point mutation); (c) interaction with other genes; (d) onset of somatic mutations; (e) interaction with the environment; (f) existence of alternative pathways that can substitute for the loss of function. The last three factors can vary between individuals.

Although rare and highly-penetrant mutations in cancer genes could act with no interaction with external factors (for example by direct interference with basic mechanisms of cell replication and differentiation), gene-environment interactions are intrinsic to the mode of action of low-penetrant genes.[2,5] Increasingly, scientists are aware that both environmental and genetic factors play a part in complex diseases.[7] The relation between the frequency of a variant and its penetrance is almost inverse:[2] the more penetrant (ie, deleterious) a mutation, the less frequently we expect to find it in the population—although it may be concentrated in particular groups or families because of a founder effect or segregation.

These basic ideas have to be considered before genetic screening in the population is proposed. Proposals to introduce genetic testing as a solution for common health problems abound. For example, at a recent Organisation for Economic Cooperation and Development workshop held in Vienna (http://www/oecd.org/dsti/sti/s_t/biotech/prod/genetic_testing.htm) one speaker claimed that about 70% of cancers and cardiovascular diseases are attributable to inherited susceptibility—a gross simplification and overestimation. As already stated, the role of low-penetrant genes requires interaction with environmental factors. For example, the *NAT*2-slow genotype increases the risk of bladder cancer in people exposed to arylamines but is ineffective without exposure.[5] Hence, to credit genes with a major independent role in the causes of complex diseases is scientific misjudgment of the way genetics affects disease risk—which is equivalent to assuming that adult-onset diabetes is caused mainly by predisposing genes, although clearly the disease is rare in the absence of obesity. Other speakers at the workshop made overstated claims for the potential benefits of genetic screening, and similar points of view can be found in medical publications.[8] However, more reasonable opinions, with a public-health approach, have also been published.[9] We attempt to clarify some basic ideas in order to temper enthusiasm for genetic testing in populations.

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Number needed to screen

Let us consider a low-penetrant genetic trait that is common in the general population (carried by 13·8%—ie, a polymorphism), whose identification can lead to a 58% reduction in risk of disease. If the absolute lifetime risk of disease for carriers of the polymorphism is 1·4%, or 14 per 1000, a reduction of 58% lowers the risk to six per 1000—ie, eight successes per 1000. The inverse of eight per 1000 (1000/8) is 125—ie, the number of carriers we need to treat (NNT) for one success. However, since the frequency of the trait is 13·8%, we have in fact to screen 125/0·138—ie, 906 people to prevent one case. This is the number needed to screen (NNS). By contrast, let us consider a highly penetrant mutation that is rare in the general population (0·16%) but common in some families (50%). Suppose that the lifetime risk of disease is 37%, or 37 per 100, and, again, that detection and intervention reduce the relative risk by 58% leading to an absolute risk reduction of 21·5% (58% of 37%) to 15·5%, and a NNT (100/21·5) of 4·5. If we decide to screen families, then we need to screen 4·5/0·5 (nine people) to prevent one case; if we decide to screen the whole population, however, the figure becomes 4·5/0·0016—ie, 2813 people. The example shows that a reasonable NNS is attained only by screening for highly-penetrant mutations in high-risk families, not for such mutations in the general population or for low-penetrant polymorphisms.

Numbers needed to screen

To assess the role of a gene-environment interaction and screening in a population we need to know the penetrance of the genetic trait and its frequency. A useful approach is to combine penetrance and frequency by computing the number needed to screen (NNS) in order to prevent one case of cancer. The calculation is explained with a worked example in the panel.

Table 1 contains a calculation of NNS in high-risk families for a high-penetrant gene (*BRCA1*). The cumulative (lifetime) risk of breast cancer is around 80% in mutation carriers, and the frequency of mutations in families is about 50%. Let us suppose that tamoxifen or raloxifene halve the risk (on the basis of a 45% reduction in risk, reported in the Breast Cancer Prevention Trial,[10] and a 76% reduction in the raloxifene trial;[11] however, two investigations of tamoxifen did not show any benefit).[12,13] Thus, we have to treat 2·5 mutation carriers and screen five family members to prevent one cancer. However, if we screen the general population, the NNS changes greatly. Now the cumulative risk in mutation carriers is 40%,[14] with an absolute risk reduction by tamoxifen or raloxifene of 20%, which means a number needed to treat of five mutation carriers. However, since only 0·2%[15] of the general population are mutation carriers, the NNS is 2500 to prevent one cancer. This large NNS makes *BRCA1* an unrealistic marker for use in the general population for screening.

Table 1 also shows the case of a low-penetrant gene polymorphism, *GSTM1* null. We could screen smokers for the *GSTM1* null genotype, and give them chemoprevention. What would be the advantage? The relative risk of lung cancer associated with the *GSTM1* null genotype is 1·34.[5] Therefore, if the cumulative risk of lung cancer in smokers is 10%, it will be about 13% in *GSTM1* null carriers. If chemopreventive intervention has a 50% effectiveness the cumulative risk after intervention would be 6·5%, with a number needed to treat of 15 to prevent one cancer case. Since 50% of the population are carriers of the null genotype, we need to screen 30 smokers to prevent one cancer. If we repeat the same calculations including carriers of the wild (not mutated) genotype, the NNS is 40 (table 1). Without screening smokers for *GSTM1* type, we would have a NNS of 35 (the average of the previous two). Clearly, there is little advantage in screening for a low-penetrance gene if the NNS only increases from 35 to 40.

Population	Breast cancer		Lung cancer	
	General population	Families	Smokers	Smokers
Gene	*BRCA1*	*BRCA1*	*GSTM1* null	*GSTM1* wild
Relative risk	5	10	1·34*	1·0
Cumulative risk	40%†	80%	13%	10%
Risk reduction	50%‡	50%	50%§	50%§
Cumulative risk after intervention	20%	40%	6·5%	5%
Absolute risk reduction	20%	40%	6·5%	5%
NNT	5	2·5	15	20
Frequency	0·2%‖	50%	50%	50%
NNS	2500	5	30	40
NNS in all smokers			35	

NNT=number need to treat; NNS=number needed to screen. *Ref 5. †Ref 14. ‡Theoretical risk reduction due to tamoxifen or raloxifene. §Theoretical maximum reduction in risk due to chemopreventive agent. ‖Ref 15.

Table 1: **Calculation of the number needed to screen for a low penetrant gene (*GSTM1* in smokers), and a highly penetrant gene (*BRCA1*)**

A further complication in the calculation of NNS is that there are many genes that contribute to an increased risk of cancer, so that to find genetically normal people is difficult. Some investigators[16] have used simulation analysis to calculate how many prospective workers for jobs with benzene exposure would have to be screened for *CYP2E1* activity and NAD(P)H-quinone oxidoreductase (*NQ01*) alleles to find 1000 people without the known susceptible polymorphisms. They noted that 2500 workers would need to be screened to hire 1000 genetically normal workers and thus prevent one case of benzene-induced cancer.

In another simulation study, Bartell and colleagues[17] showed that genetic screening for chronic beryllium disease with HLA-DPB1*0201 gave health benefits that outweighed financial costs if avoidance of one case of the disease is valued at US$1 million or higher. However, their estimate of predictive value might have been unrealistically high and might not have correctly weighed the harmful effects of false-positive results. Nonetheless, this could be an example of a situation in which, with clearance of social and ethical barriers, genetic testing for a variant that predisposes employees to risk might be beneficial.

Genetic testing or reduction of exposures

The major diseases in western societies are multifactorial. Thus, lung cancer is not wholly attributable to smoking, but to many linked factors of which smoking is one.

A: Exposure	Disease	Proportion attributable to exposure*
Tobacco smoke	Lung cancer	90%
	Bladder cancer	70% (men)/30% (women)
	Larynx cancer	90%
	Coronary heart disease	12·5%
	Chronic bronchitis	80%
B: Disease	**Low-penetrant genes**	**Odds ratio†**
Lung cancer	*CYP1A1 Msp* I (Asian)	1·73
	CYP1A1 Msp I (white)	1·04
	CYP1A1 exon 7 (Asian)	2·25
	CYP1A1 exon 7 (white)	1·30
	CYP2D6	1·26
	GSTM1	1·34
Bladder cancer	*NAT-2* slow	1·37
	GSTM1	1·57
Colon cancer	*NAT-2* rapid	1·19

*Ref 18. †Ref 5.

Table 2: **An example of one exposure resulting in many diseases (A) and one disease resulting from low-penetrant genes (B)**

Conversely, smoking also contributes to cardiovascular and other chronic diseases. Elimination of a single environmental exposure (eg, smoking) would reduce a large proportion[18] of chronic diseases (table 2). Genetic traits, however, can have a different relation with disease; people with the *NAT-2*-slow genotype have an increased risk of bladder cancer, but a decreased risk of colon cancer.[5,19] This is probably not an unusual situation. By contrast, exposures that cause one disease and protect against another are very few. Additionally, although the relation of one exposure resulting in many diseases is true, for low-penetrant genes the opposite seems to apply—one disease/many genotypes (table 2). In the case of *GSTM1* and lung cancer, since the null genotype is present in 50% of the general population, the risk attributable to *GSTM1*[5] (table 1) is about (0·34/1·34)/2—ie, 12·6%. However, the same person might be at high risk because of *GSTM1* and at low risk because of other polymorphisms, which means that the sum of the effects of a single polymorphism is hard to calculate. The population distribution will usually contain very few individuals carrying several high-risk polymorphisms and a large proportion with a balance between high-risk and low-risk genotypes. Finally, polymorphisms require exposure to environmental factors to be effective—ie, the 12·6% proportion is attributable to interaction, not to the genetic trait itself.

Tests for genetic damage in somatic cells have also been misunderstood. Denissenko and colleagues'[20] recent finding of aromatic polycyclic hydrocarbon-induced damage in *p53* nucleotides (known mutation hotspots for lung cancer) has been referred to as "the smoking gun"[21] with respect to smoking-induced lung cancer. The implication is that until now no definite evidence existed that smoking causes lung cancer. Epidemiological evidence has already shown beyond reasonable doubt that cigarette smoke is carcinogenic. What has been lacking is the mechanism and proof of the relation in a particular smoker rather than groups of smokers, and in that respect Denissenko and colleagues' work[20] is very important. However, not all lung cancers in cigarette smokers have the *p53* mutation, just as not all lung cancers in uranium miners have the *p53* mutation associated with radon exposure[22]—multiple factors and pathways are implicated. Moreover, the suggestion in many reports on molecular markers is that detection of such markers is an appropriate point for clinical intervention and early treatment. However, studies on a few people or tumour series are not necessarily representative of the population or of the natural history of the disease; and without assessment of predictive value, the marker will not be useful in screening of populations.

False metaphors for DNA

Overall, the proportion of diseases attributable to low-penetrant genetic traits is clearly difficult to establish and is probably much lower than the burden of disease attributable to certain environmental agents. Workers generally agree that less than 5% of cancers are attributable to high-penetrant genes,[2] although little is known for other chronic diseases. In general, we can expect little from genetic screening of the population, apart from limited groups (usually families) with a concentration of high-risk mutations.

Two key difficulties arise in genetic testing of populations. One is the availability of effective preventive measures, the absence of which seriously detracts from any screening proposal. The second is the large NNS, which implies that very few people who are screened will benefit; a large NNS also implies a potentially large number of false-positive results and unnecessary treatments.

There are examples, in fact, of screening activities characterised by high, or very high, NNS: one is screening for phenylketonuria, a monogenic disease with a frequency of one in 10 000–12 000 in white people; population screening is successful in most western countries. However, this is a particular case, since there is a very effective and non-invasive preventive measure (dietary restriction). Another example of potentially effective population screening comes from a recent report[23] on the β_2-adrenoceptor gene, which seems to predispose physically inactive men to obesity (but, surprisingly, not women). Since the homozygous prevalence is 33%, and the risk of obesity seems to increase by 3·45 times in the high-risk genotype, this gene might be a reasonable candidate for population screening. A further example of potential screening, which is still under scrutiny, is testing for haemochromatosis.[24] In this case also, a therapy is available, but questions have been raised about false positives and the potential discriminatory use of positive results for insurance and employment reasons. Also, non-genetic screenings are undertaken with high NNS, such as blood-pressure checking, or screening for hyper-cholesterolaemia.

The emphasis on genetic testing (which has a clear commercial motivation) is based on false metaphors of the role of DNA and genes. One common metaphor compares the gene to a computer program—ie, the gene is a set of instructions to reach a certain goal. However, a computer program merely executes the instructions, without changing them on the basis of context. In fact the relations between genotype and phenotype are much more complex than usually depicted in popular accounts. Jeffrey Lewis has proposed a much better metaphor: "if the genome can be seen as a text or a script, then its phenotypic expression can be seen as a performance of that script, bringing the text to vibrant and unique life just as actors on a stage bring life to the words on a page".[1]

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