Commentary

Metabolic Susceptibility Genes As Cancer Risk Factors: Time for a Reassessment?

Seymour Garte[1]

School of Public Health, UMDNJ, Piscataway, New Jersey 08854, and Genetics Research Institute, Milan, 20135 Italy

Abstract

Polymorphisms in metabolic cancer susceptibility genes have not shown a consistent role as cancer risk factors, when only main effects are examined. This is actually to be expected given the limited and specific biochemical role such genes play in the carcinogenic process. However, when particular groups of case populations are examined separately, the importance of these genetic polymorphisms may often become quite clear. Examples from the literature and a hypothetical model are presented to support the view that metabolic gene risk alleles should be studied in subgroups of large case control studies with sound biochemical hypotheses related to the action of the gene product as a function of demographic, environmental, or other genetic variables.

The role of low penetrance metabolic susceptibility genes in human cancer causation has not yet been completely clarified, despite a large number of published studies in the field (1). Reports during the past decade have suggested that certain alleles of xenobiotic metabolizing genes, such as *CYP1A1, CYP2D6, CYP2E1, GSTM1*,[2] *GSTT1, NAT2*, and *EPHX*, may be associated with many of the more common sporadic epithelial cancers of the lung, bladder, colon, breast, and skin. Because of the relatively high frequency of these allelic variants in the population (from 1 to 50%), the attributable risk for these genes could be quite high, even if their penetrance is low. Comparison to single cancer genes such as *BRCA1, e.g.*, a high penetrance gene with a rare frequency of mutation and, therefore, a fairly low attributable risk, has been made (2). However, when one surveys the literature (especially in the last five years) on metabolic gene variants as cancer risk factors, it is apparent that the most striking feature of the published studies is the heterogeneity of the results. Weakly positive and negative findings have been reported for almost every combination of allele and cancer type. Different laboratories working on the same gene and the same cancer often get different results using different case populations. Meta-analyses and pooled analysis have generally found certain gene variants, such as GSTM1 deletion and NAT2 slow acetylation, to be significantly associated with lung and bladder cancer, respectively, but at a very low level of penetrance (1, 3). Those expecting to find a metabolic gene variant that may be usefully labeled as a cancer risk factor with a certainty strong enough to warrant population screening, followed by preventive measures, must be disappointed by the weight of the evidence to date. It does not seem appropriate to consider a risk factor that presents an OR of 1.2 or 1.3 in a meta-analysis (and where many of the studies showed no association) to be a candidate for large scale screening. Compared with many genetic and nongenetic risk factors, these risks are unexceptional, and it might be considered that the metabolic genes (in particular, the CYP genes) probably play little if any role in cancer susceptibility.

There is certainly a good deal of biochemical logic (besides the evidence from case control studies) behind the argument against any importance for these genes in cancer risk. Considering the fact that the metabolic genes function by altering the dose of the active carcinogen but play no role in the complex molecular pathways that must occur for a cell to undergo malignant transformation, it is not surprising that the role of these genes is far less effective than, *e.g.*, the loss of function of a typical tumor suppressor gene. In order for a metabolic gene to have any measurable effect on cancer susceptibility as determined by a change in the OR of a case control study, the following conditions must all be met:

(*a*) The gene variant should play a major role in the metabolism of the important carcinogens operating in the study, and the gene in question must be known to be expressed at an appropriate level in the target tissue. If the study is on breast cancer or colon cancer, the number and potency of the total suite of carcinogens responsible for all of the cases examined will generally be unknown, and is likely to be very diverse. For lung cancer, the most important causative agent, cigarette smoke, is itself a mixture of many chemical carcinogens, each of which follows its own metabolic pathway. Even in situations where a particular carcinogen or category of carcinogens may be assumed to be etiological, the metabolism of such chemicals might proceed via several alternative routes, using a number of different gene products. The GST family, *e.g.*, has a broad substrate specificity and plays an important role in conjugation and removal of many carcinogenic compounds. However, there are four subfamilies of GST genes, and it isn't clear why deletion or inactivation of any one or even two of these genes should have any effect on metabolism, except in cases where the remaining active enzymes lack the required substrate specificity.

(*b*) The dose of the carcinogen must be at a level where the effect of differences in metabolic rates can play a role in risk. In the absence of any carcinogenic substrate, even a putatively high risk allele would have no effect on risk. On the other hand, at very high exposure levels, the importance of metabolic rates may also become negligible, as the enzyme systems undergo

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[1] To whom requests for reprints should addressed, at EOHSI, UMDNJ, 170 Frelinghuysen Road, Piscataway, NJ 08854.

[2] The abbreviations used are: GST, glutathione *S*-transferase; OR, odds ratio; GSEC, Genetic Susceptibility to Environmental Carcinogenesis.

Table 1 Examples of published subgroup analyses

Gene(s)	Cancer	Subgroups	OR	Ref.
GSTP1	Oral	Smokers <20 py	3.4	6
NAT2 + GSTM1	Bladder	Male	4.4	7
GSTM1 + GSTT1	Breast	Premenopausal + alcohol	5.3	8
NAT1*10	Gastric	Advanced stage	4.8	9
GSTT1	Renal cell	Low BMI[a]	4.8	10
CYP1A1	Breast	African-Americans	9.7	11
GSTP1 + CYP1A1	Adducts	Newborns	4.0	12
GSTM1 + NAT2	Head & neck	Young nonsmokers	6.3–10.8	13
		Nonoperable		
NAT2	Bladder	Smokers	7.8	14
GSTM1	Lung	Smokers	5.2	15
CYP2D6	Prostate	Danish smokers	3.1 (?)	16
		Not Swedish smokers		
NAT1*11	Breast	Smokers	13.2	17
		Red meat	6.1	
GSTM3 + GSTM1	Larynx		4.0	18
GSTM1 + CYP1A1	Child. ALL	Male	3.3	19
NAT1 + NAT2	Bladder	White smokers	6.3	20
NAT2	Bladder	Males	4.0	21
GSTM1 + GSTT1	Bladder	Squamous cell	14.2	22
GSTM1	Larynx	Low smokers	2.9	23

[a] BMI, body mass index.

saturation. This scenario has been repeatedly seen with smoking, especially regarding the CYP gene variants; the relative risk to low smokers with the susceptibility allele is generally higher than it is in heavy smokers. In the latter category, the exposure to carcinogens is so massive that 3- or 4-fold differences in metabolic rate have no effect on the level of active carcinogenic compounds that reach the target tissue. The general issue of various types of dose effects in interaction between metabolic susceptibility gene and environmental carcinogens has been discussed previously (4).

(*c*) The carcinogenic metabolite produced by the metabolic gene variant must play a major role in cancer causation in the population under study. We know that carcinogenesis is a complex multistage process, involving many alternative possible pathways. Analysis of the genes involved in carcinogenesis of all tissues has revealed a picture of heterogeneity and stochastic processes. Therefore, it would be surprising to find in any population of breast, colon, or lung cancer cases a single and predominant mechanistic pathway that includes a single chemical or category as etiological agents in a particular step in the pathway.

For these reasons, as well as others, it is in fact to be expected that no single metabolic gene variant should ever be observed to have a large role in cancer susceptibility for any general cancer type. Therefore, the results from recent large studies showing very weak associations should not be considered as disappointing, but instead, as confirmatory of what we know about environmental carcinogenesis mechanisms and the role of gene-environment interactions related to metabolic susceptibility genes.

Does this mean that additional research in this field is unlikely to produce useful data for cancer prevention and public health assessment of cancer risk? Absolutely not. The very same arguments stated above may be used to demonstrate that continued research in this field is likely to be extremely useful in the context of cancer risk assessment and cancer prevention. The way to understand this is to see that in most previous studies the wrong question has been asked. This question, "Is gene variant G*N a risk factor for a particular cancer type?" must clearly (as argued above) be answered almost always by "no" or "yes but very slightly." The correct question should be, "For whom, if anybody, is gene variant G*N a risk factor for cancer?" When this question is asked (as it has been in many publications), the answers are strikingly different than they are for the first question.

The conduct of case control studies of the association of metabolic gene polymorphisms with cancer using hypotheses that go deeper in a mechanistic sense than simply the case/control status is an advantageous approach that has been used in previous research. This is certainly not a new concept in classical epidemiology, especially when risk factors show relatively weak associations (5). One example is the common division of breast cancer into pre and postmenopausal subgroups after the demonstration that these are two biologically distinct diseases, with different and sometimes opposite acting risk factors. For application to the field of genetic susceptibility, the arguments behind such biologically based hypotheses could follow any of the points raised above, such as the hypothesis that a particular gene polymorphism may affect only a particular exposure-specific, gender-specific, or age-specific (etc.) metabolic pathway.

When analyses have been done on the effects of metabolic gene polymorphic variants on specific subgroups, rather than on all cases, some very strong associations (with ORs of 3–12) have been observed. In Table 1 are listed a series of studies (not exhaustive) that have analyzed different types of subgroups and found very strong associations between metabolic gene alleles and cancer susceptibility. In some cases, effects were only observed in the presence of two or more risk alleles. In most of these examples, associations in the total case population with a single allele were much weaker and/or not significant.

To illustrate the mechanistic basis on which such results might be expected (in the context of the previous argument), I have produced a hypothetical example (illustrated in Fig. 1) as follows:

Let us assume that gene *G* codes for an enzyme that catalyzes the activation, whereby a metabolic compound Z is converted to Z*. This activated form is then able to bind to

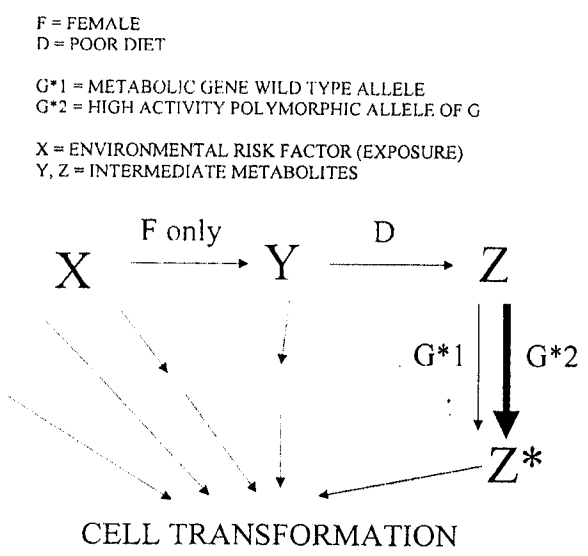

Fig. 1. Hypothetical example of subgroup specific pathways in determination of genetic risk factors. *F*, female; *D*, poor diet; *G*1*, metabolic gene wild-type allele; *G*2*, high activity polymorphic allele of *G*; *X*, environmental risk factor (exposure); and *Y,Z*, intermediate metabolites.

DNA, producing DNA damage leading to mutations, thus starting the process of cell transformation. Furthermore, assume that gene *G* is polymorphic and that one allele of *G*, *G*2*, results from a single nucleotide polymorphism (SNP) that changes the active site of the enzyme so that the enzyme has five times the activity of the wild-type allele *G*1*, toward the reaction Z → Z*. The population frequency of G*2 is 10%. The metabolite Z is one of many possible metabolic products resulting from exposure of humans to the related compound X, which is a component of cigarette smoke. The pathway for metabolism of X to Z proceeds through an intermediate Y. There are many possible metabolic pathways for X, some of which are dependent on the presence of other factors. The reaction from X to Y is much more frequent in women compared with men because of specific hormonal influences on the reaction. The reaction of Y to Z is highly favored in people with low levels of compounds found in fruits and vegetables and, therefore, occurs more frequently in people with a poor diet that is deficient in fruits and vegetables and high in fat. Therefore, only for women smokers with a poor diet will the pathway Z → Z* be relevant for carcinogenesis, and, thus, only for this subgroup will the allele *G*2* be a risk factor. We can now ask what would we expect to observe in case control studies designed to determine the association of the G*2 RFLP with cancer. If we do a case control study of 1000 cases and 1000 controls where the population of both cases and controls (for simplicity, we assume no confounding) consists of 50% women, 30% smokers, 33% with a poor diet, and 10% with G*2, then we can calculate the OR of different groups among the case population.

We assume that any population of P individuals chosen from the total number of cases consists of two subpopulations: (*a*) S, whose members are individuals for which the gene polymorphism has an effect on cancer risk (in this case, smoking women with a poor diet); and (*b*) Q, which includes everyone else. In the example given, there should be 50 members of S and 950 members of Q in the total population of cases and controls.

Table 2 Results for the hypothetical example

Group	No.	OR for G*2	95% CI[a]
Population	1000	1.23	0.93–1.63
Women	500	1.47	1.00–2.15
Men	500	1.00	0.66–1.51
Women smokers	150	2.74	1.45–5.19
Smokers with poor diet	100	3.86	1.82–8.17
Women smokers with poor diet	50	9.0	3.34–24.3

[a] CI, confidence interval.

Calculation of the OR Ψ may be done according to the following equation:

$$\Psi = \frac{F_g(1 - F_p)}{F_p(1 - F_g)}$$

where

$$F_g = \frac{F_s S + F_p Q}{P}$$

and $P = S + Q$.

Where F_s, the frequency of the risk allele in the susceptible subpopulation is assumed to be greater than F_p, the allele frequency in the general population. This is the definition of a risk allele. In the example used, we assume that F_s (=0.5) is five times higher than F_p (=0.1). The term F_g then represents the allele frequency in any particular group consisting of members of the S and Q subpopulations. In the example, if we take all women as the population P, this group will contain 50 members of group S (women who also smoke and eat a poor diet) and 450 members of group Q (women who do not both smoke and eat a poor diet).

The results presented in Table 2 show that the OR for G*2 in the real risk group, smoking women who eat a poor diet, is quite high, whereas in the whole population, it is low and nonsignificant. Other groups are shown for comparison. Note that for groups who contain no members of subgroup S (such as men or nonsmokers), the OR is 1, because $F_g = F_p$.

Not shown in this hypothetical example, but certainly likely to be of primary importance, are the effects of multiple genetic factors. This is in fact found in eight of the examples shown in Table 1. One could substitute, *e.g.*, a slow acetylator genotype in the *NAT2* gene for poor diet, as another case of a population subgroup with gene-gene-environment interactions. Definition of subgroups based on mechanistic links between two or more genes (such as an allele in a Phase I gene that produces less of intermediate Y and another allele in a Phase II gene that conjugates and eliminates more of that intermediate, resulting in a much lower steady-state level of Y) is likely to be an important aspect of future research in this field.

The values shown in Table 2 are reminiscent of many published data from case control studies with respect to borderline nonsignificant associations of an allele with cancer as a main effect in a population, along with higher ORs when subgroups are examined.

There are at least two major caveats to the approach of subgroup analysis advocated here. One problem is the issue of spurious associations found because of testing multiple hypotheses. Dividing any population into sometimes arbitrary subgroups, especially if such division is not based on a sound mechanistic-driven hypothesis, may easily result in chance findings of associations that are later invalidated. An example

of this problem is the report by Wadelius *et al.* (16) shown in Table 1. It is difficult to imagine any biological plausibility for the observed difference in the association with CYP2D6 between Danish and Swedish smokers with prostate cancer, and, in fact, the authors acknowledge this result as an artifact attributable to a lack of age matching (and smoking status) among Danish, but not Swedish, cases and controls. In other examples, sources of errors leading to spurious associations might not be so clear or might not be reported. To avoid this type of possible artifact, it is necessary (as in all epidemiological studies) to have *a priori* rather than posthoc hypotheses when designing studies of metabolic gene polymorphisms as cancer risk factors in particular groups of subjects.

The other major problem is that of required sample size. Subgroup analysis requires large populations to retain adequate power to detect any significant effect. Typical case control studies of one or a few hundred cases and controls may be very limited in the ability to divide the cases according to categories such as age, gender, race, or exposure, and it may be impossible to get any useful information with further subdivision (such as young African-American women who smoke). Perhaps even more problematic is the tendency toward higher risk estimates when cells contain small numbers (24). There are many examples in the literature of early small studies that showed high ORs, which were then reduced with subsequent larger studies. Therefore, simply by dividing groups into smaller subgroups, it is possible to find artifactually inflated ORs that may not truly reflect an enhanced susceptibility of the subgroup.

The best approach to overcome this obstacle is to concentrate future efforts on large studies using thousands rather than hundreds of cases and controls. One example of this strategy is a large international collaborative project called the GSEC project (25).[3]

The very large data set (>33,000 cases and controls with metabolic genotypes at several loci contributed from 94 investigators) acquired by the GSEC pooled data analysis project (25) has been used recently to detect a strong association of the CYP1A1*2A homozygous genotype with lung cancer in people <45 years of age.[4] Because lung cancer in this category of people is rare, and because the genotype is also quite rare, such a finding could never have been made on a case series of a few hundred lung cancers. However, with a total of 5526 lung cancers in the database that include information on CYP1A1 genotype, 261 cases were found to be <45 years of age (smokers and nonsmokers), a number that allowed detection of the fairly strong significant association (OR: 6.2, 95% confidence interval: 4.2–8.9) with the CYP1A1*2A/*2A genotype.

Large projects may also be fraught with difficulties peculiar to their nature. The GSEC database, which consists of data pooled from multiple investigators from all over the world, is likely to include substantial heterogeneity and potential misclassification in certain parameters, such as exposure assessment. The large amounts of data currently being produced by DNA microarray experiments presents a new and formidable challenge to biologists and statisticians in terms of assuring biological plausibility and the meaning of statistical significance in an experiment with thousands of hypotheses and hundreds of thousands of data points. Clearly, the design and analysis of large studies include problems that must be solved by molecular epidemiologists in the near future. New approaches for analysis of multiple factors and multiple hypotheses to ensure rigor are urgently needed.

In terms of public health and cancer prevention, the subgroup analysis approach is potentially of great value despite these problems. In the hypothetical example given above, smoking women who eat a poor diet could be advised that screening for allele G*2 might help to assess their cancer risk. Those who test positive for the high-risk allele could then be advised that on average, their composite demographic, genetic, and lifestyle profiles confer a 9-fold greater risk of cancer compared with the general population. Whereas their genetic and gender characteristics are not under their control, their lifestyle is. Advisement that smoking cessation and changing dietary habits will reduce their relative cancer risk from 9 to 1.2 times that of the general population may be a strong motivation for behavior modification and lead ultimately to some degree of cancer risk reduction.

Of course, many legal, ethical, and sociological, as well as the scientific issues raised above, must be confronted and resolved before the idealized scenario presented could be translated into reality. Yet it remains my conviction that molecular epidemiology studies of well-defined subgroups rather than whole populations may be the best path to cancer prevention, as well as to a more cohesive mechanistic understanding of the role of metabolic susceptibility genes in human carcinogenesis.

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