Using Biomarkers of Genetic Susceptibility to Enhance the Study of Cancer Etiology

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There has been increasing interest in the interaction of genetic susceptibility and xenobiotic exposures in cancer etiology. Study of gene-environment interactions may increase our ability to characterize relatively low population risks if a substantial proportion of the population cancer burden is attributed to high risk among a smaller group of genetically susceptible members. Further, these studies may provide insight into the mechanism of carcinogenesis, which can help establish the biologic plausibility of an exposure–cancer relationship. Biologic processes important in tumorigenesis that exhibit substantial interindividual differences may function as susceptibility factors. Potential examples include polymorphic enzymes, which activate and detoxify procarcinogens and carcinogens (e.g., certain P450 enzymes, N-acetyltransferase [NAT2], glutathione S-transferase M1), and variation in the capacity to repair DNA. Biologic assays are now available to evaluate many of these functions at the DNA and phenotype level and can be readily incorporated into studies of cancer etiology. — Environ Health Perspect 103(Suppl 8):291–295 (1995)

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Introduction

Numerous environmental carcinogens have been identified through epidemiologic study and this knowledge has led directly to approaches to reduce cancer occurrence. This approach has been most successful for relatively potent environmental carcinogens associated with risks of about 2-fold or greater. Because of the observational character of epidemiologic studies, interpretation is problematic when risks are much weaker than this. There is, however, an increasing need to characterize such carcinogens as we attempt to reduce the cancer burden among the general population. Study of gene-environment interactions may increase our ability to characterize relatively low population risks if a substantial proportion of the population cancer burden is attributed to high risk among a smaller group of genetically susceptible members exposed to the compound under study. In addition, these studies may provide insight into the mechanism of carcinogenesis, which can help establish the biologic plausibility of an exposure–cancer relationship.

In this paper, we focus on gene alleles that are common in the population and generally considered polymorphisms (i.e., the minor allele frequency is > 0.01); probably associated with relative risks under 10 and, as such, do not exhibit familial patterns of inheritance; and may be strongly dependent on exposure to manifest themselves (1,2). In contrast, Li (3) has discussed single gene mutations which were initially characterized by studies of familial cancer, generally present at lower frequencies, and associated with very high cancer risk (e.g., RB1 [retinoblastoma], WTI [Wilm’s tumor], p53 [Li-Fraumeni syndrome], and APC[adenomatous polyposis coli]) (3).

Potential sources of susceptibility for cancer risk include interindividual variation in enzymes that activate and detoxify procarcinogens and carcinogens (e.g., Phase I enzymes, which catalyze oxidation, reduction, and hydrolysis reactions, and Phase II enzymes, which catalyze conjugation and synthetic reactions), and repair DNA. The development of assays to measure these processes and their initial application in epidemiologic studies has been extensively reviewed (4–15). Table 1 includes several examples of these processes, which can be studied at either the DNA level if the genetic basis of the polymorphism has been characterized, or at the phenotypic level using assays that reflect the function of either a single gene product (e.g., N-acetyltransferase, coded by NAT2) (4), or of several possible enzymes (e.g., DNA repair) (16).

Xenobiotic Chemical Metabolism

Smoking-associated tumors have provided a model for the evaluation of potential genetic risk factors. For example, the GSTM1 gene encodes the cytosolic enzyme glutathione S-transferase M1, which can conjugate intermediate metabolites (e.g., epoxides) of several compounds, including polycyclic aromatic hydrocarbons (PAHs) (17,18) and aflatoxin (19). Deficiency in this enzyme is caused by inheriting a homozygous deletion of the GSTM1 gene (20–22), which is referred to as the null

| Susceptibility factor | Exposure |
|----------------------|----------|
| Metabolism           |          |
| Phase I enzymes      | PAHs     |
| CYP1A1               | PAHs     |
| CYP1A2               | PAHs, heterocyclic/aromatic amines |
| CYP2E1               | Benzene, butadiene, chlorinated solvents, N-nitrosamines |
| Phase II enzymes     |          |
| NAT2                 | Heterocyclic/aromatic amines |
| GSTM1                | PAHs, aflatoxin |
| DNA repair           | Sunlight, mutagens |

PAHs, polychlorinated aromatic hydrocarbons. Data from (4–16, 18, 19, 21, 24, 41, 45, 47, 48, 52, 59–74).
genotype and is present in about 30 to 70% of human populations, depending upon racial group (20,23–25). Several case-control studies have demonstrated that this allele confers increased susceptibility for lung (26–31) and bladder cancer (32–35); however, equivocal or negative studies have also been reported [lung: Shields et al., unpublished data; (36); bladder: (25–37)].

Bell et al. (33) demonstrated that the risk of bladder cancer associated with heavy tobacco use (i.e., > 50 pack-years) was 3.5 (95% CI = 1.5–8.0) among subjects with functional GSTM1 genotypes and 5.9 (2.6–13) among subjects with the null genotype (33). Although the interaction with genotype was not statistically significant, the study suggests that there may be higher smoking-associated bladder cancer risks for certain subpopulations. Evidence supporting the biologic plausibility of this association has recently been provided by a cross-sectional study that showed that smokers with the null GSTM1 genotype had higher urine mutagenic activity than smokers with functional GSTM1 genotypes (38).

An extensive body of work has indicated that subjects with the slow N-acetylation phenotype, which is caused by homozygous mutations in the NAT2 gene, are at increased risk for bladder cancer compared with rapid acetylators, particularly among populations with exposure to aromatic amines (4); however, this phenotype does not appear to be a risk factor for bladder cancer in populations exposed only to benzo(a)pyrene (39). Vineis et al. (40) provided evidence that the slow N-acetylation phenotype is associated with a greater tendency to form 4-aminobiphenyl-hemoglobin adducts, which provides biologic support for the observations on bladder cancer risk.

Biomarkers of genetic susceptibility are being incorporated into studies evaluating cancer risks associated with exposure to compounds of uncertain carcinogenicity in humans. For example, meat cooked at high temperatures contains heterocyclic amines (HAAs) that are potent mutagens and animal carcinogens (41). The cancer risk posed by exposure to HAAs in the diet may depend upon the extent to which the compounds are activated by N-oxidation (carried out by CYP1A2) and acetylation (thought to be carried out by NAT2) (42–46). Interindividual variability in CYP1A2 activity is known to be partially a result of induction by environmental exposures such as cigarette smoking and dietary exposure to PAHs; however, there also may be a genetic component (7). Activity of NAT2 and CYP1A2 can be characterized by measuring excreted caffeine metabolites in urine after caffeine consumption, which can distinguish between slow and rapid acetylators and N-oxidizers (47). Recently, Lang et al. (48) evaluated the risk of HAA intake (using preference for meat doneness as a surrogate) in a case–control study of colon polyps and cancer. The overall risk associated with eating well-done meat was 2.1 (adjusted for age and metabolic phenotypes). Compared with subjects with both slow N-acetylation and slow CYP1A2 activity (slow/slow) who consumed meat cooked rare or medium, subjects with slow/slow phenotypes who ate well-done meat had a 2.1-fold increased risk, while subjects with rapid/rapid phenotypes who ate well-done meat had a 6.4-fold increased risk. Work is continuing to characterize food cooking practices as related to HAA exposure (Sinha et al., unpublished data) to better quantify the interaction of HAA exposure, polymorphisms in metabolic enzymes, and cancer risk.

DNA Repair

Although the relationship between sun exposure and risk of skin cancer is well established, it has afforded the opportunity to test potential genetic susceptibility markers. Athas et al. (16) developed an assay that uses cultured lymphocytes to evaluate the capability of subjects to repair ultraviolet-damaged DNA. Wei et al. (49) applied this assay to a case–control study of basal cell carcinoma. Overall, there was a 2.8-fold increased risk for basal cell carcinoma among individuals with a history of > 6 severe sunburns. Using subjects with > 6 severe sunburns with high DNA repair capability as the comparison group, subjects with ≥ 6 severe sunburns with high repair capability had a nonsignificant 1.9-fold increased risk for basal cell carcinoma, while similarly exposed individuals with low repair capability had a statistically significant 5.3-fold increased risk (95% CI = 2.04–12.9 adjusted for age and sex).

The ability to repair DNA damage has also been evaluated as a potential effect modifier for smoking-associated tumors. For example, assays have been developed to detect the in vitro sensitivity of cultured peripheral lymphocytes to mutagen-induced chromosomal aberrations, which are believed to provide an indirect assessment of DNA-repair capability (12). Hsu et al. (50) developed an assay that quantifies bleomycin-induced chromatid breakage using cultured lymphocytes from study subjects. Recently, Wu et al. (51) used this assay in a case–control study of lung cancer. There was an overall 8.8-fold increased risk of lung cancer associated with smoking, which was strengthened among the subgroup of participants who were susceptible for bleomycin-induced chromosomal damage and weakened among subjects who were not susceptible. For example, the risk of lung cancer among smokers who did not form breaks on chromosome 2 was 4.5 (1.6–12.4) (compared with nonsmokers who did not form breaks), and 12.8 (4.3–38.7) among smokers who did form breaks. Similar findings were noted for chromosomes 4 and 5. This assay has also been used to study susceptibility for primary malignancies of the upper aerodigestive tract (52) and for second malignancies of the head and neck (53).

Methodologic Issues

The case–control design is very efficient for examining the interaction of genetic susceptibility markers and environmental exposures (54), particularly when high quality exposure data are available from questionnaires or environmental monitoring. There is a critical need for more valid and reliable methods of obtaining data on dietary, environmental, and occupational exposure to carcinogens by questionnaire [Sinha et al., unpublished data; (55,56)], particularly since there may be subtle exposure/disease interactions, where particular alleles are associated with greater risk of cancer at varying levels of exposure to carcinogens (31,57). These interactions are likely to be missed if adequate attention is not given to exposure assessment.

If the basis of observed interindividual variation in a potential susceptibility factor has been characterized at the DNA level, it can be readily studied using DNA collected from peripheral blood samples, or potentially from less invasive sources of DNA (e.g., buccal swabs, which collect small amounts of exfoliated epithelial cells). If a susceptibility factor has not been characterized at the DNA level, then it is necessary to use an assay that measures the phenotypic expression of that variation. Each assay has its own logistic demands, and consideration must be given to the extent to which disease status may potentially influence such assays. Regardless of which assay is used, reliability and accuracy of assays is critical to any epidemiologic investigation. Even small degrees of genotype or phenotype misclassification may have a substantial impact on risk estimates, particularly when
the prevalence of the at-risk allele is either very low or very high (58).

**Summary**

Although the relevance of interindividual variability in metabolism and DNA repair for cancer risk among the general population is unclear, several studies support the concept that variation in these processes, particularly in combination with exposure to certain types and levels of carcinogens, may contribute to the cancer burden. Evaluating these factors will be an increasingly important goal for future studies of cancer etiology. These studies will have the potential to provide insight into the mechanism of carcinogenesis in humans, and may increase our ability to make better estimates of risk for particular subpopulations.

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