Molecular Epidemiology in Environmental Carcinogenesis

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Molecular epidemiology has significant potential in preventing cancer and other diseases caused by environmental exposures (related to lifestyle, occupation, or ambient pollution). This approach attempts to prevent cancer by incorporating laboratory methods to document the molecular dose and preclinical effects of carcinogens, as well as factors that increase individual susceptibility to carcinogens. Recently we have carried out validation studies of biologic markers such as carcinojen-—DNA and carcinojen—protein adducts, gene and chromosomal mutations, alterations in target oncogenes or tumor suppressor genes, polymorphisms in putative susceptibility genes (individual P450s, glutathione transferase M1), and serum levels of micronutrients. This research involves adults, infants, and children exposed to varying levels of carcinogens, as well as cancer cases and controls. On a group level, dose—response relationships have frequently been seen between various biomarkers and environmental exposures such as polycyclic aromatic hydrocarbons, cigarette smoke (active and passive), and ambient indoor and workplace air pollution. However, there is significant interindividual variation in biomarkers that appears to reflect a modulating effect on biomarkers (hence potential risk) by genetic and acquired susceptibility factors. Ongoing retrospective and nested case—control studies of lung and breast cancer are examining the association between biomarkers and cancer risk. Results of these studies are encouraging; they suggest that biomarkers, once validated, can be useful in identifying populations and individuals at risk in time to intervene effectively. — Environ Health Perspect 104(Suppl 3):441—443 (1996)

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Introduction
The following discussion highlights recent molecular epidemiologic research on the use of biologic markers in the prevention of environmental carcinogenesis and reproductive toxicity. The research explores the potential of molecular epidemiology as an early warning system of adverse effects, as a means of understanding variability in risk, and in intervention such as exposure reduction and chemoprevention.

Effects of Environmental Exposures on Human Health
Clear effects of environmental exposures on biomarkers have been seen in active and passive smokers, workers, and others exposed to ambient pollution and carcinogens in the diet. For example, serial samples from 40 heavy smokers (>1 pack/day for >1 year) enrolled in a smoking cessation program were assayed for cotinine, polycyclic aromatic hydrocarbon (PAH)—DNA, 4-aminobiphenyl—hemoglobin (4-ABP—Hb) adducts, and glycoporphin A (GPA) mutations. Blood samples were taken while smoking and at 10 weeks and 8 and 14 months after quitting. Cotinine was used to assess compliance with the cessation protocol. A significant reduction in mean PAH—DNA and 4-ABP—Hb adducts was observed following cessation in all persons who were cotinine—verified quitters (>25ng/ml) for ≥8 months (p < 0.05). Neither GPA N/O nor GPA N/N mutations were significantly reduced following smoking cessation, but results are limited by the small number (n = 18) of heterozygous individuals studied (1).

Effects of environmental exposures (smoking, air pollution, and diet) on PAH—DNA damage and CYP1A1 gene induction (elevated mRNA and the modulation of DNA damage by the CYP1A1 Msp 1 variant genotype were evaluated in two groups from Poland: 70 mother/child pairs from Krakow, a city with elevated air pollution; and 90 pairs from Limanowa, a less polluted area (2). The human CYP1A1 gene codes for an inducible enzyme system involved in biotransformation of certain xenobiotics including PAHs; some of the metabolites are carcinogenic and mutagenic. Maternal leukocyte PAH—DNA adduct levels were significantly increased with cigarette smoking (active and passive) and workplace exposures (p < 0.05). Within Krakow, there was an apparent dose response in maternal adducts with increasing ambient pollution at the women’s residences; the trend was significant for women not employed away from the home (p < 0.05).

Similar to results seen with maternal adduct levels, placental (fetal) CYP1A1 mRNA was increased with both smoking and air pollution (3). The major determinant of CYP1A1 mRNA levels in placental tissue was active cigarette smoking (p < 0.001). A marginal increase in CYP1A1 mRNA with environmental tobacco smoke (ETS) exposure was evident. There was a dose response in placental CYP1A1 mRNA levels with ambient pollution at the women’s places of residence within Krakow; the association was significant
among newborns of women not employed away from home (p<0.05, controlling for smoking status, diet, place of residence, and coal use) (3).

Among infants with induced placental CYP1A1, adducts in infant cord blood were inversely correlated with the levels of CYP1A1 induction/activity (2). Adduct levels in infant cord leukocytes were higher than adduct levels in paired maternal samples, both in the total cohort (7.9±9.9 vs 5.9±8.2 per 10^8 nucleotides) and after stratification by placental CYP1A1 induction. These results indicate that the placent, by metabolizing PAH, decreases their transfer to the fetus and suggest increased susceptibility of the developing fetus to PAH–DNA adduct formation. The higher adduct levels in newborn samples compared to paired maternal samples is particularly striking, given evidence from experimental bioassays that transplacental exposure to PAH is at least an order of magnitude lower than maternal exposure (4,5).

In addition to using biomarkers as environmental dosimeters, several studies have shown associations between specific biomarkers and reproductive effects (6). In a prior study, a highly significant association was found between decreased birth weight and the level of smoking-related adducts in placental tissue collected from 30 smoking mothers (7). By contrast, no association was seen between decreased birth weight and either intensity of smoking exposure assessed by questionnaire data or biochemical measures of smoking exposure (cotinine, thiocyanate, and carboxyhemoglobin). In the study of Polish mothers and newborns described above, an inverse association was seen between infant cord PAH–DNA adduct levels (as a biologically relevant dosimeter of fetal exposure) and the development of the infant. The inverse association was highly significant for infant head circumference at birth.

**Biomarkers as Indicators of Individual Susceptibility and Potential Risk**

To better understand the role of environmental and genetic factors in lung cancer, we have measured DNA damage from PAHs and a polymorphism in the CYP1A1 and glutathione S-transferase (GSTM1) genes. There is prior evidence that the GSTM1 null (0/0) genotype is associated both with decreased ability to detoxify PAH and other carcinogens and with increased risk of lung cancer. Leukocytes from 119 non-small cell carcinoma lung cancer cases and from 98 noncancer controls were analyzed for PAH–DNA adducts by enzyme-linked immunoassorbent assay (ELISA) and for GSTM1 genotype by polymerase chain reaction (PCR) (8). After adjustment for potential confounders (age, smoking, etc.), adducts were higher in cases (p<0.01) than in controls. Adducts were increased in smokers and ex-smokers compared to nonsmokers among cases and controls (p<0.05). Adducts also increased with cigarettes per day among the 51 cases who were current smokers (p=0.05) but not among the smoking controls. Fifty-seven percent of cases had the GSTM1 0/0 genotype compared to 41% of controls (p<0.05). After adjusting for confounders, odds ratios for adducts and GSTM1 genotype were 6.8 (1.6–29.6, p<0.01) and 1.93 (1.1–3.4, p<0.05), respectively. When the subjects were classified by adducts (high/low) and GSTM1 genotype (0/0 vs 0/+ or +/+), the risk was 12-fold higher for those individuals with both high adducts and GSTM1 0/0 compared to those without either factor. This approach may be useful in constructing a risk model for lung cancer and ultimately in applying it to prevention through the identification of individuals at greatest risk (8).

In the study of heavy smokers mentioned above, three genotypes, (CYP1A1 exon 7 rare allele, CYP1A1 Msp I restriction fragment length polymorphism [RFLP], and GSTM1) were measured by PCR in a total of 159 subjects (unpublished data). Eighteen percent had the Msp I RFLP (+/+ or +/−), 6% had the exon 7 rare allele (+/− or +/+), and 46% had the GSTM1 null genotype (0/0). Mean DNA adduct levels were 2-fold higher in subjects with the CYP1A1 exon 7 polymorphism than in those without the polymorphism and were unchanged by adjustment for smoking. Mean DNA adduct levels were also higher in the subjects with the CYP1A1 Msp I polymorphism than in those without, but the difference was not significant. GSTM1 was not associated with DNA adduct formation before or after adjustment for amount of smoking. These findings in healthy smokers support the hypothesis that one or more of these metabolic genes are related to carcinogen binding to DNA and possibly to an increased relative risk for lung cancer (unpublished data).

The association between the CYP1A1 Msp I, RFLP, and PAH–DNA adduct levels are also being evaluated in our study of Polish mothers and newborns described above. Preliminary analyses show the polymorphism to be a significant determinant of PAH–DNA adduct levels in placental tissue, controlling for smoking status, place of residence, home and occupational exposures, and diet.

**Biomarkers in Detecting Efficacy of Intervention**

**Exposure Reduction**

Carcinogen–DNA adducts and somatic gene mutations at the hypoxanthine guanine phosphoribosyltransferase (HPRT) locus were evaluated in peripheral leukocytes of workers in an iron foundry with exposure to benzo[a]pyrene (B[a]P) and other PAHs (9). During the 2-year study period, B[a]P exposure declined by approximately 40%, from a maximum of 60 ng/m^3 in the first year to below 36 ng/m^3 1 year later. A total of 67 persons were sampled in November/December of the two successive study years; 24 of them gave two samples 1 year apart. The biomarkers included carcinogen–DNA adducts in leukocytes (PAH–DNA measured by an immunoassay, aromatic DNA by the 32P-postlabeling method) and HPRT mutation in lymphocytes. After adjusting for smoking, levels of PAH–DNA, aromatic DNA, and HPRT mutation increased with exposure among the 67 workers sampled during the 2-year period (p<0.05). However, the markers showed a differential response to the change in exposure, consistent with their individual biology. For example, among the 24 workers sampled in both years, carcinogen–DNA adducts (which have a half-life on the order of several months) were markedly reduced from the first to the second year (PAH–DNA, 6.2 vs 2.3/10^8; aromatic DNA, 2.5 vs 1.4/10^8; p<0.01). HPRT Mf (a long-lived marker) was somewhat less affected by the decline in exposure (1.3 vs 0.8; p<0.05). Moreover, in the second year, several long-term workers had low levels of adducts but elevated HPRT Mf. Thus, PAH–DNA and HPRT Mf were highly correlated in the first year (n=17; r=0.67; p<0.01) but not in the second year or in the 2 years combined. However, when analysis was restricted to workers with detectable levels of adducts (who included the more highly exposed workers), the correlation was significant between PAH–DNA and HPRT (n=19; r=0.67; p=0.002). In contrast to PAH–DNA, aromatic DNA adducts and HPRT were
not correlated in either year. These results suggest a molecular link between somatic gene mutation and PAHs (9).

In the above-mentioned study of heavy smokers, the substantial reduction (50–75%) in PAH–DNA and 4-ABP-Hb adduct levels after quitting smoking indicates that these carcinogen adducts provide molecular evidence of the benefits of smoking cessation (1). The biomarkers can therefore be useful in compliance ascertainment and feedback.

Chemoprevention

Biologic markers already play an important role in the evaluation of chemopreventive agents, specifically in Phase II trials (10–12). The most commonly used genetic markers in current trials involving lung and upper aerodigestive tract tumors include micronuclei, DNA content, genetic alteration in oncogenes, and markers of proliferation, growth regulation and differentiation (12).

Our research also suggests that additional earlier biomarkers such as carcino- gen–DNA adducts can be useful in intervention studies of exposed or at risk populations. Several recent studies have examined whether baseline levels of micronutrients (vitamins C, E, β-carotene, or retinol) in the serum of persons who have not been treated with chemoprevention are inversely related to genetic damage (9, 13). In a cross-sectional study of 63 heavy smokers, the serum concentrations of α-tocopherol (cholesterol-adjusted), ascorbic acid, β-carotene, and retinol were evaluated in relationship to the levels of PAH–DNA adducts in lymphocytes measured in the same individuals (14). All of the participants were current smokers, and 90% had smoked one or more packs per day for at least 10 years. A significant inverse correlation was found between serum α-tocopherol and PAH–DNA adducts. Although the relationship was not statistically significant, serum ascorbic acid, β-carotene, and retinol were inversely correlated with adducts.

In the smoking cessation cohort described above, both α-tocopherol and retinol were inversely correlated with PAH–DNA adducts at baseline while subjects were smoking and in follow-up samples after cessation. In addition, the GSTM1 genotype modified the relationship between DNA adducts and α-tocopherol. Indicating an interaction between two susceptibility factors, α-tocopherol had a strong inverse effect in subjects lacking the GSTM1 gene, whereas in subjects with the gene, there was no significant association (unpublished data).

Once validated, biologic markers can be incorporated into a wide variety of intervention and chemoprevention studies of smokers, workers, and other populations at high risk of disease.

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