Pharmacogenetics: Detecting Sensitive Populations

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Risk assessment models strive to predict risks to humans from toxic agents. Safety factors and assumptions are incorporated into these models to allow a margin of error. In the case of cancer, substantial evidence shows that the carcinogenic process is a multistage process driven by the interaction of exogenous carcinogenic exposures, genetic traits, and other endogenous factors. Current risk assessment models fail to consider genetic predispositions that make people more sensitive or resistant to exogenous exposures and endogenous processes. Several cytochrome P450 enzymes, responsible for metabolically activating carcinogens and medications, express wide interindividual variation whose genetic coding has now been identified as polymorphic and linked to cancer risk. For example, a restriction fragment-length polymorphism for cytochrome P4501A1, which metabolizes polycyclic aromatic hydrocarbons, and cytochrome P4502E1, which metabolizes N-nitrosamines and benzene, is linked to lung cancer risk. Cytochrome P4502D6, responsible for metabolizing many clinically important medications, also is linked to lung cancer risk. The frequency for each of these genetic polymorphisms vary among different ethnic and racial groups. In addition to inherited factors for the detection of sensitive populations, determining the biologically effective doses for carcinogenic exposures also should quantitatively and qualitatively enhance the risk assessment process. Levels of carcinogen–DNA adducts reflect the net effect of exposure, absorption, metabolic activation, detoxification, and DNA repair. These effects are genetically predetermined, indubitably notwithstanding. The combination of adduct and genotyping assays provide an assessment of risk that reflects recent exogenous exposure as well as one’s lifetime ability to activate and detoxify carcinogens. —Environ Health Perspect 102(Suppl 1):81–87 (1994)

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Introduction

The protection of populations from carcinogenic agents (and other toxins) generally requires a risk assessment process before regulation or remedial action. A formal risk assessment involves several steps including a hazard assessment, dose–response assessment, exposure assessment, and risk characterization (1). In general, data from scientific studies are extrapolated to human experience through mathematical modeling that identifies a level of chemical exposure that might predictably result in a specific number of adverse outcomes (e.g., clinical cancer). Mathematical modeling, however, essentially is a substitute for scientifically determined data and is sometimes accepted, for a variety of reasons, without adequate validation. Many risk assessment steps incorporate untested assumptions or have methodological problems such as using premises that are not consistent with known scientific data (e.g., using multistage models with the number of stages less than that determined from human cancer studies). Moreover, a fundamental limitation of risk assessment is that it usually examines risk for populations rather than for individuals, thereby not considering interindividual variation in response to xenobiotic exposure. Individuals or groups of individuals (e.g., families or ethnicity) might be more sensitive or resistant to particular exposures based on xenobiotic activation or detoxification, DNA repair, genetic structure, etc. Thus, the risk assessment process that does not include the interaction of the environment with interindividual capacities will become increasingly limited.

Carcinogenesis is a multistage process of normal growth, differentiation, and development gone awry (2,3). It is driven by spontaneous and carcinogen-induced genetic and epigenetic events. Carcinogenic agents initiate the process by causing DNA mutations and altered gene expression. These genetic effects, in concert with additional carcinogen exposure and other genetic or epigenetic effects, lead to tumor promotion. Through these stages, cells have selective reproductive and clonal expansion capabilities. Progressive phenotypic changes and genomic instability occur (aneuploidy, mutations, and gene amplification). These genetic changes enhance the probability of initiated cells transforming into a malignancy; the odds of which are increased during repeated rounds of cell replication. Angiogenesis allows for a tumor to grow beyond 1 or 2 mm in size. Ultimately, tumor cells can disseminate through vessels invading distant tissues and establish metastatic colonies. Each of these steps can be directly affected by carcinogen exposure. The response to these exposures, however, can vary from individual to individual (4). Note also that the current concepts of initiation, promotion, genetic, and epigenetic effects have been conceptually important but are now considered simplistic and not consistent with current human carcinogenesis models.

The role of protooncogenes and tumor suppressor genes has become increasingly apparent in the multistage model of carcinogenesis (2). Both are important to the regulatory mechanisms of growth, cell cycle control and terminal differentiation (2,5). Activation of protooncogenes enhance the probability of neoplastic transformation, which can either be an early or late event. Tumor suppressor genes code for products that, unlike protooncogenes, enhance the probability of neoplastic transformation when their activity is lost. For example, the p53 tumor suppressor gene, located on chromosome 17, is the most commonly altered suppressor gene among all tumors so far studied (6). Single base substitutions...
can result in loss of function or production of p53 proteins that either interfere with normal function or otherwise directly enhance neoplastic transformation (7).

The multistage process of carcinogenesis is best exemplified by a model of human colorectal tumorigenesis described by Fearon and others (8). In the early stages, loss or inactivation of APC (9,10) and MCC genes (11), hypomethylation and genomic instability accompanies the phenotypic appearance of an adenoma. More advanced tumors involve oncogenes and tumor suppressor genes, typically not observed in early adenomas. The combined events are more important than the actual order in which they occur. The genetic abnormalities include Ki-ras mutations on chromosome 12, mutation of p53 tumor suppressor genes on chromosome 17, and a deletion of DCC, the putative tumor suppressor gene on chromosome 18q that may be involved in cell-to-cell adhesion and possibly metastasis. Other allelic losses can occur in any of the other chromosomes. Thus, it appears that at least six genetic events occur in the development of colorectal carcinoma.

The method by which carcinogenic agents affect DNA and produce mutational spectra is varied. Chemical carcinogens generally undergo metabolic activation to electrophilic intermediates that form DNA adducts through covalent binding (12). Promutagenic adducts can then cause mutations through mispairing or base substitutions during DNA synthesis. The binding of carcinogens to DNA nucleotides is apparently nonrandom (13–15) and has been shown to affect protooncogenes and tumor suppressor genes. Among the best studied examples of metabolic activation is the epoxidation reactions of polycyclic aromatic hydrocarbons (PAH), of which benzo[a]pyrene (BP) is one example (16,17). These compounds are composed of fused benzene rings that are essentially water insoluble but readily absorbed through the lungs and gastrointestinal tract. They are commonly found as combustion products of fossil fuels (e.g., coal, diesel exhaust) and vegetable matter. Consequently, PAHs occur as environmental pollutants. BP becomes metabolically activated in a phase 1 reaction by forming a reactive diol epoxide that can covalently bind to DNA-forming adducts. Initially, cytochrome P450 (CYP1A1) and epoxide hydroxylase catalyze the conversion of BP to a dihydrodiol. Then, CYP3A4 converts this product to a diol-epoxide (i.e., BP-7,8-diol-9,10-epoxide) that is the reactive form. However, along this pathway, intermediates might be removed via conjugation (e.g., glutathione transferase), further oxidation, or reduction. These metabolites can then be excreted in urine or feces. It is worthwhile noting that the activity of several enzymes involved in these activation and detoxification processes, as well as DNA repair, varies markedly in individuals, so that reactions leading to the formation and removal of BP-related adducts and mutations can occur at higher or lower rates (4).

On a molecular basis, cells possess the ability to repair DNA damage (18). Smaller alkyl adducts can be excised while larger adducts require the excision of several bases. An extensively studied repair enzyme is the O2-alkylguanine-DNA-alkyltransferase (19). This enzyme repairs damage from alkylating agents such as tobacco-specific nitrosamines and other N-nitrosocompounds. It is a suicide protein in that it transfers the alkyl group to itself and becomes inactivated. Cell cytotoxicity and tumor cell resistance are negatively correlated with the levels of this enzyme (20), and levels vary within organs and among people (21,22). PAH–DNA adducts can be repaired by a nucleotide excision pathway. A unimodal distribution of repair rates of benzo[a]pyrene diol-epoxide DNA adducts has been observed using human lymphocytes in vitro (23). The interindividual variation was substantially greater than the intraindividual variation, which suggests a role for inherited factors.

Genetic Predispositions to Cancer

Interindividual variation in response to xenobiotics and their potential carcinogenic effects is mediated by inherited predispositions (Table 1). Family cancer syndromes, the most evident expression of inherited predispositions, can lead to up to a 1000-fold increased risk of cancer in family members (24). However, most individuals do not have such an obvious genetic predisposition, and host susceptibility relating to a specific gene is less obvious because these mutations as risk factors are barely detectable above background. Current studies show that these factors are more common in some family, ethnic, and racial groups. CYP1A1 has been extensively studied in the metabolism of PAHs. Activity of CYP1A1 varies in lung tissues from different persons (25,26), which is an inheritable trait (27) but also can be induced upon exposure to agents such as tobacco smoke (28). Inducibility varies among individuals and is notably higher in lung cancer patients than in noncancer controls (29,30). Levels ofaryl hydrocarbon hydroxylase activity have also been correlated with levels of DNA adducts (31) and with prognosis in lung cancer (32). Recently, a restriction fragment length polymorphism has been described, using Msp1 restriction digestion, that is reported to correlate with lung cancer risk in a Japanese cohort (33) and also found to be in genetic disequilibrium with a mutation in the catalytic region of the enzyme (34). Evidence suggests that the mutation results in increased metabolic activation (K Kawajiri, personal communication) and the polymorphism was correlated with a 3-fold increase in lung cancer risk (33). Further study showed that the effect was greatest in persons with squamous cell cancer and in persons with the least amount of tobacco smoking history (35). This latter point indicates that the effect of this polymorphism is strongest in persons with less carcinogenic exposure.

The National Cancer Institute—University of Maryland (NCI–UMD) Case–Control Study investigated lung cancer patients and controls (pulmonary disease patients and nonlung cancer patients) for inherited predispositions to lung cancer. The control groups had similar age and smoking status. The Msp1 polymorphism of CYP1A1 and lung cancer risk was studied in 101 persons enrolled in this study, but no association was found with either lung cancer risk or histological lung cancer type. However, there was a statistically significant difference in allelic frequencies for African Americans versus Caucasians. This suggests that on the basis of the

| Response          | Example                          | Human cancer type          |
|-------------------|----------------------------------|-----------------------------|
| Metabolic activation | Cytochrome P4501A1              | Lung ([33])                 |
|                    | Cytochrome P450206              | Lung ([37,38])              |
|                    | Cytochrome P450201              | Lung ([49])                 |
|                    | N-Acetyltransferase             | Bladder/colon ([81,82])     |
| Detoxification     | Glutathione S-transferase M1    | Lung/stomach/colon ([81,82])|
| DNA repair         | Excision of UV damage           | Skin ([83])                 |
| Protooncogene      | HRAS-1                          | Lung ([84,85])              |
| Tumor suppressor gene | L-myc                           | Lung/gastric/sarcoma ([86,87])|
|                    | p53                             | Li-Fraumeni syndrome ([88]) |
Japanese data, African Americans would be more sensitive to lung cancer. Separate analysis by race did not reveal an association with lung cancer risk although the numbers of each group substantially limited the statistical power of the study. Thus, the study numbers are currently being increased. In a separate Norwegian study (36), no association with the Msp 1 RFLP and lung cancer was found. Importantly, this study was severely hindered by not utilizing age- and smoking-matched controls that can result in falsely negative findings. The frequency of the exon 7 mutation in cancer patients and matched controls, and its linkage with the Msp 1 RFLP, in American and European ethnic groups is now required.

The study of the cytochrome P4502D6 (CYP2D6, also known as the debrisoquine polymorphism) is among the best examples of inheritable interindividual differences in metabolism. This enzyme is responsible for metabolism of several medications including tricyclic antidepressants, beta-blocking antihypertensives, and debrisoquine. Poor metabolizers are at risk of adverse drug reactions. In a cohort of smokers in London, England, a 4-fold higher risk of lung cancer was associated with the extensive metabolic phenotype (37). This association has been confirmed in the NCI-UMD lung cancer case-control study with an odds ratio of six (38). The extensive metabolic phenotype also has been shown to have an interactive effect with occupational exposures to asbestos and PAHs (39).

Genotyping methods for CYP2D6 have been sought to avoid the requirement for time-consuming urinary phenotyping and the attendant hypothetical risks of drug administration. These methods also would clarify whether the association with lung cancer might be a result of a cancer effect on the phenotypic expression rather than an inherited predisposition that predates the development of cancer. An Xba I RFLP was described (40) where identifiable alleles were associated with the poor metabolizer phenotype. However, it was soon found that the EcoR I RFLP only correctly predicted one-third of poor metabolizers (41). Since then, it was found that most of the poor metabolizer phenotypes could be explained by specific mutations in CYP2D6 (42). These and other mutations have now been designated as A, B, C, and D mutations (Table 2) (40,42–44).

Two polymerase chain reaction (PCR) assays have been published to determine the A and B mutations in CYP2D6 (42,45).

The D mutation is identified with the Xba I restriction digest and Southern blotting (40). The A and B mutations can be identified by using a PCR mismatch assay where primers differ only by the 3-ft base that matches with either the wild-type base or the mutant base (42). The assay begins with a first step that uses primers specific only for CYP2D6 and not the pseudogenes CYP2D7 and CYP2D8, which are otherwise almost 95% homologous. The second step uses the mismatch primers. The assay requires careful validation to ensure correct priming, sensitivity, and specificity. This method can reportedly characterize over 95% of metabolic phenotypes in large numbers of Europeans. The second method takes advantage of a ButN1 restriction site at the B mutation and uses an altered primer that introduces a Dra I restriction site at the A mutation (45) (R. Wolf, personal communication). In our laboratory, we have been genotyping individuals using a combined approach that uses the ButN1 digest for the B mutation with the primers previously identified as not significantly homologous (42) and the mismatch assay for the A mutation. We have found that the frequency of poor metabolizers in Caucasians by genotyping was statistically significantly higher than in African-Americans, suggesting an inherited predisposition to lung cancer in African Americans (P Shields, unpublished results).

Thus far, the mechanistic relationship of CYP2D6 activity to lung cancer remains unknown. The only known cariocarcinogenic substrate identified for CYP2D6 is 4-(methyl-nitrosamino)-1-(3-pyridyl)-1-butanone (NNK) but not other tobacco-specific nitrosamines that have been tested (46). Thus, the lung cancer association might relate to altered substrate specificity of the enzyme in extensive metabolizers not studied, unidentified cariocarcinogenic substrates, or a gene that is in linkage disequilibrium with another gene related to cancer risk. CYP2E1 is responsible for metabolizing a number of potential human carcinogens including benzene and N-nitrosamines. Several genetic polymorphisms have been identified (47,48). One polymorphism includes two distinct base substitutions in the same area, which are in genetic disequilibrium so that either one can be studied (47). This area is involved in transcription regulation and preliminary studies indicate that one type allows for increased expression of the chloramphenicol acetyl transferase gene in transfected HepG2 cells. The frequency of the polymorphic alleles is different in Japanese and Americans (S Kato, personal communication, 1992). Whether this polymorphism has a relationship to cancer risk is currently under study. The other polymorphism is at Dra1 restriction enzyme site in intron 6 (M Watanabe, personal communication). While its biological significance is unknown, it has been reported that the distribution of genotypes is significantly different in lung cancer cases and controls (48).

Molecular Dosimetry—Identifying a Combined Carcinogen Exposure and Susceptibility

One indicator for the net effect of exogenous carcinogen exposure and inherited traits for absorption, metabolism, and DNA repair is the carcinogen–DNA adduct. Measurement of adducts can be useful for estimating a biologically effective dose of a carcinogen and the risk for fixed mutations. A variety of assays are available to identify carcinogen–DNA and protein adducts. Enzyme immunooassays (49–58), 32P-postlabeling and nucleotide chromatography (59–61), fluorescence spectroscopy (62), synchronous fluorescence spectroscopy (SFS) (63–66), gas chromatography and mass spectroscopy (GC/MS) (66,67), and electrochemical detection (68) have been applied to the analysis of human lung samples or a surrogate tissue or cell population.

Central to the studies of DNA adducts is the development of sensitive and specific assays that are required to detect femtomole and attomole levels of adducts in microgram amounts of DNA. Current methods are challenged because of the complexity and multitude of possible exposures in human tissues. The specificity of adduct
assays, and therefore their quantitative reliability, can be enhanced by using micro-preparative techniques. For example, subjecting enzymatically digested lung DNA to high-pressure liquid chromatography (HPLC) followed by the \( ^{32} \text{P}- \text{postlabeling} \) assay, which relies on three different separations (HPLC and two-dimensional thin-layer chromatography) can detect N-nitrosamine-related alkyl adducts such as \( O^2 \)-methyl-2'-deoxyguanosine (69), \( O^2 \)-ethyldeoxyguanosine (69), \( N^7 \)-methyldeoxyguanosine (N\(^7\)methylG) (70) and \( N^7 \)-ethyldeoxyguanosine (S Karo, personal communication) at levels as low as one adduct in 10\(^7\) 2' -deoxyguanosine residues. Other laboratories have used HPLC after \( ^{32} \text{P}- \text{postlabeling} \) (71). Immunoaffinity chromatography also has been combined with \( ^{32} \text{P}- \text{postlabeling} \) assay to identify \( O^2 \)-methyldeoxyguanosine (D Cooper, personal communication) and polycyclic aromatic hydrocarbons (P Shields, unpublished data). In both cases, the level of detection was at least one adduct in 10\(^7\) unmodified deoxyguanosine residues. Another feature of assays that are dependent upon micropreparative techniques is that they are developed using authentically synthesized adduct standards, use internal standards, and quantitation based upon calibration curves.

Organ tissue selection, multiple sources of exposures, and other confounders impact upon study design and results. It would be optimal to use easily obtainable body fluids such as blood or urine for risk assessment. However, it remains to be established if blood testing will be a reliable surrogate for other tissues. For example, some data suggest that the determination of PAH adducts in peripheral lymphocyte DNA reflects dietary rather than inhalational exposures (72,73). We also will need to consider cellular differences in life-span (lymphocytes and red blood cells survive longer than granulocytes), DNA repair capacity, and metabolic capacity. It has been reported that the initial addition of lymphocytes and granulocytes is similar but that adduct levels are more persistent in the latter (74). Our laboratory has used lymphocytes (49-51,63) while others have studied total white blood cells (predominantly granulocytes) (54,72). Oral mucosal cells are another relatively noninvasive source of DNA and can allow for the detection of several types of adducts (57,60).

Multiple types of adducts have been observed in individual lung samples, confirming the complex nature of carcinogenic exposures (75). Small alkyl adducts, polycyclic aromatic hydrocarbons adducts, and aromatic amines have been identified. Improved micropreparative techniques have led to the unambiguous identification of specific polycyclic aromatic hydrocarbons adducts in human lung (63,60). This finding has now been confirmed by combining immunoaffinity chromatography with the \( ^{32} \text{P}- \text{postlabeling} \) assay (P Shields, unpublished data).

Exposures to PAH compounds are associated with an increased risk of lung cancer. Industrial pollution, fossil fuels, and tobacco smoke account for the major environmental sources. Dietary exposures also commonly occur because of overcooked or charcoal-broiled meats. Adduct levels have been correlated with exposure in coke oven workers (54,59,76), tobacco consumption (67), and urban versus rural residence (77), but decreases during vacation from occupational sources (51). Seasonal variation in adduct levels also has been observed (78). Some studies have not found correlations with tobacco consumption but this may be because of other sources of exposure (e.g., diet).

**Table 3. Genetic polymorphisms and ethnicity.**

| Polymorphism          | Populations compared                        | At risk in African-Americans |
|-----------------------|--------------------------------------------|------------------------------|
| Cytotchrome P4501A1   | African-Americans vs Caucasians            | African-Americans            |
| Cytotchrome P4502D6   | African-Americans vs Caucasians            | African-Americans            |
| Cytotchrome P4502E1   | Americans vs Japanese                      | Japanese                     |
| L-myce protoonogene   | African-Americans vs Caucasians            | African-Americans            |
| H-ras protoonogene    | African-Americans vs Caucasians            | African-Americans            |
| p53                   | African-Americans vs Caucasians            | NA                           |

NA, not applicable. *Population found to have higher frequency at alleles associated with lung cancer. *Allele not associated with cancer risk but statistically varies among compared populations.

**Conclusions**

The current data indicate that several inherited genetic traits are associated with cancer risk. Frequencies of these also vary among ethnic populations (Table 3). The interaction of environmental exposures and metabolic capacities suggests that current risk assessment models need to be biologically based and consider the variation in sensitivity among individuals. The multistage model of carcinogenesis further suggests that single low-dose exposures and most genetic traits will likely not be sufficiently strong by themselves to drive the carcinogenic process. Thus, risk assessment models need to incorporate interactive effects (chemical, radiation, viral, and physical agents interacting with each other and with host factors).

The use of genotyping assays and adduct determinations require rigorous field testing in carefully designed studies. Genotyping assays need to be studied in both case-control studies and prospective studies where confounding variables are carefully considered (e.g., tobacco use or multiple sources of exposure). Genotyping assays are preferable to phenotyping assays because they cannot be altered by the presence of disease. For adduct assays, the importance of the development and validation phases cannot be overstated and need to be meticulously performed prior to their use in large field trials.

**Note Added in Proof:** Since the preparation of this manuscript in 1992, all of the data cited as personal communication have now been published. There also are a number of more recent reviews. New data has identified several examples of gene-environment interactions where the effects of metabolizing polymorphisms vary depending on exposure, further impacting upon risk assessment procedures and outcomes.

**REFERENCES**

1. Russell M, Gruber M. Risk assessment in environmental policy-making. Science 236:286–290 (1987).
2. Harris CC. Chemical and physical carcinogenesis: advances and perspectives. Cancer Res 51:5023s-5044s (1991).
3. Shields P, Harris CC. Molecular epidemiology and the genetics of environmental cancer. JAMA 266:681–687 (1991).
4. Harris CC. Interindividual variation among humans in carcinogen metabolism, DNA adduct formation and DNA repair. Carcinogenesis 10:1563–1566 (1989).
5. Bishop JM. Molecular themes in oncogenesis. Cell 64:235–248 (1991).
6. Hollstein M, Sidransky D, Vogelstein B, Harris CC. p53 Mutations in human cancers. Science 253:49–53 (1991).
7. Haley O, Michalovitz D, Oren M. Different tumor-derived p53 mutants exhibit distinct biological activities. Science 250:113–116 (1990).
8. Fearon ER, Vogelstein B. A genetic
model for colorectal tumorigenesis. Cell 61:759–767 (1990).

9. Nishisho I, Nakamura Y, Miyoshi Y, Miki Y, Ando H, Horii A, Koyama Y, Utsumiyoumi J, Baba S, Hedge P. Mutations of chromosomal 5q21 genes in FAP and colorectal cancer patients. Science 253:665–669 (1991).

10. Kinzler KW, Nilbert MC, Su LK, Vogelstein B, Bryan TM, Levy DB, Smith KL, Preisinger AC, Hedge P, McKiehne D, Finniear R, Markham A, Groffen J, Boguski MS, Altschul SF, Horii A, Ando H, Miyoshi Y, Miki Y, Nishisho I, Nakamura Y. Identification of FAP locus genes from chromosome 5q21. Science 253:661–665 (1991).

11. Kinzler KW, Nilbert MC, Vogelstein B, Bryan TM, Levy DB, Smith KL, Preisinger AC, Hahilton SR, Hedge P, Markham A, Carlson M, Joslyn G, Groden J, White R, Miki Y, Miyoshi Y, Nishisho I, Nakamura Y. Identification of a chromosome 5q21 gene that is mutated in colorectal cancers. Science 251:1366–1369 (1991).

12. Gonzalez FJ, Crespi CL, Gelboin HV. DNA-expressed human cytochrome P450a: a new age of molecular toxicology and human risk assessment. Mutat Res 247:113–127 (1991).

13. Osborne MR. Sequence specificity in the reaction of benzopyrene diol epoxide with DNA. Chem Biol Interact 75:131–140 (1990).

14. Basu AK, Loehrer EL, Leadon SA, Essigmann JM. Genetic effects of thymine glycol: site-specific mutagenesis and molecular modeling studies. Proc Natl Acad Sci USA 86:7677–7681 (1989).

15. Singer B, Essigmann JM. Site-specific mutagenesis: retrospective and prospective. Carcinogenesis 12:949–955 (1991).

16. Osborne MR, Crosby NT. Benzopyrenes. In: Cambridge Monographs on Cancer Research. New York: Cambridge University Press, 1987:73–164.

17. Phillips DH. Fifty years of benz[A]pyrene. Nature 303:468–472 (1983).

18. Sancar A, Sancar GB. DNA repair enzymes. Annu Rev Biochem 57:29–67 (1988).

19. Brent TP, Dolan ME, Fraenkel-Conrat H, Hall J, Karran P, Laval L, Margison GP, Montesano R, Pegg AE, Potter PM, Singer B, Swenberg JA, Yarosh DB. Repair of O-Alkylpyrimidines in mammalian cells: a present consensus. Proc Natl Acad Sci USA 85:1759–1762 (1988).

20. Scudiero DA, Meyer SA, Clatterbuck BE, Mattern MR, Ziolkowski CH, Day RS. Sensitivity of human cell strains having different abilities to repair O'-methylguanine in DNA to inactivation by alkylating agents including chloroethylnitrosoureas. Cancer Res 44:2467–2474 (1984).

21. Myrn B, Giercksky KE, Krokan H. Interindividual variation in the activity of O'-methyl guanine–DNA methyltransferase and uracil-DNA glycosylase in human organs. Carcinogenesis 4:1565–1568 (1983).

22. Grafton RC, Pegg AE, Trump BF, Harris CC. O'-alkylgua-
ine–DNA and aflatoxin B1 DNA adduct formation in normal human tissues and cancer. Cancer Res 44:2855–2857 (1984).

23. Oesch F, Aullmann W, Platt KL, Doerger G. Individual differences in DNA repair capacities in man. Arch Toxicol Suppl 10:172–179 (1987).

24. Li FP. Familial cancer syndromes and clusters. Curr Probl Cancer 14:73–114 (1990).

25. Petruzzelli S, Camus AM, Carrozzi L, Ghelarducci L, Rindi M, Menconi G, Angeletti CA, Ahotupa M, Hietanen E, Aitio A, Saracci R, Bartsch H, Giuntini C. Long-lasting effects of tobacco smoking on pulmonary drug-metabolizing enzymes: a case-control study on lung cancer patients. Cancer Res 48:4695–4700 (1988).

26. Sabadie N, Richter-Reichhelm HB, Saracci R, Mohr U, Bartsch H. Interindividual differences in oxidative benzo[a]pyrene metabolism by normal and tumorsurgical lung specimens from 105 lung cancer patients. Int J Cancer 72:417–425 (1988).

27. Nowak D, Schmidt-Preuss U, Jorres R, Liebke F, Rudiger HW. Formation of DNA adducts and water-soluble metabolites of benzo[a]pyrene in human monocytes is genetically controlled. Int J Cancer 41:169–173 (1988).

28. McLemore TL, Adelberg S, Liu MC, McMahon NA, Yu SJ, Hubbard WC, Czerwinski M, Wood TG, Storeng R, Lubet RA, Egleston JC, Boyd MR, Hines RN. Expression of CYP1A1 gene in patients with lung cancer: evidence for cigarette smoke-induced gene expression in normal tissue and for altered gene regulation in primary pulmonary carcinomas. J Natl Cancer Inst 82:1333–1339 (1990).

29. Kourti RE, McKinney CE, Slonian DJ, Snodgrass DR, Wray LP, Mclemore TL. Positive correlation between high aryl hydrocarbon hydroxylase activity and primary lung cancer as analyzed in cryopreserved lymphocytes. Cancer Res 42:5030–5037 (1982).

30. Rudiger HW, Nowak D, Hartmann K, Cerutti PA. Enhanced formation of benzo[a]pyrene:DNA adducts in monocytes of patients with a presumed predisposition to lung cancer. Cancer Res 45:5890–5894 (1985).

31. Geneste O, Camus AM, Castegnaro M, Petruzelli S, Macchiariini P, Angeletti CA, Giuntini C, Bartsch H. Comparison of pulmonary DNA adduct levels, measured by "P-postlabelling and aryl hydrocarbon hydroxylase activity in lung parenchyma of smokers and ex-smokers. Carcinogenesis 12:1301–1305 (1991).

32. Bartsch H, Hietanen E, Petruzelli S, Giuntini C, Saracci R, Mussi A, Angeletti CA. Possible prognostic value of pulmonary AH-locus-linked enzymes in patients with tobacco-related lung cancer. Int J Cancer 46:185–188 (1990).

33. Kawajiri K, Nakachi K, Imai K, Yoshii A, Shinozda N, Watanabe J. Identification of genetic high-risk individuals to lung cancer by DNA polymorphisms of the cytochrome P450A1 gene. FEBS 263:131–133 (1991).

34. Hayashi S, Watanabe J, Nakachi K, Kawajiri K. Genetic linkage of lung cancer-associated Msp polymorphisms with amino acid replacement in the heme binding region of the human cytochrome P450A1 gene. J Biochem (Tokyo) 110:407–411 (1991).

35. Nakachi K, Imai K, Hayashi S, Watanabe J, Kawajiri K. Genetic susceptibility to squamous cell carcinoma of the lung in relation to cigarette smoking dose. Cancer Res 51:5177–5180 (1991).

36. Trefe T, Ryberg D, Haugen A, Nebert DW, Skaug V, Brogger T, Botrense AL. Human CYP1A1 (cytochrome P450) gene: lack of association between the Msp I restriction fragment length polymorphism and incidence of lung cancer in a Norwegian population. Pharmacogenetics 1:20–25 (1991).

37. Ayesh R, Idle JR, Ritchie JC, Crothers MJ, Hertzell MR. Metabolic oxidation phenotypes as markers for susceptibility to lung cancer. Nature 312:169–170 (1984).

38. Caporaso NE, Tucker MA, Hoover R, Hayes RB, Pickle LW, Isak H, Muschik G, Green-Gallo L, Buivyds D, Aisner S, Resau J, Trump BF, Tollerud D, Weston A, Harris CC. Lung cancer and the debrisoquine metabolic phenotype. J Natl Cancer Inst 85:1264–1270 (1992).

39. Caporoso N, Hayes RB, Dosemeci M, Hoover R, Ayesh R, Hertzell M, Idle JR. Lung cancer risk, occupational exposure, and the debrisoquine metabolic phenotype. Cancer Res 49:3675–3679 (1989).

40. Skoda RC, Gonzalez FJ, Demierre A, Meyer UA. Two mutant alleles of the human cytochrome P-450db1 gene (P450CD21) associated with genetically deficient metabolism of debrisoquine and other drugs. Proc Natl Acad Sci USA 85:5240–5243 (1988).

41. Sugimura H, Caporaso NE, Shaw GL, Modali RV, Gonzalez FJ, Hoover RN, Resau JH, Trump BF, Weston A, Harris CC. Human debrisoquine hydroxylase gene polymorphisms in cancer patients and controls. Carcinogenesis 11:1527–1530 (1990).

42. Heim M, Meyer UA. Genotyping of poor metabolizers of debrisoquine by allele-specific PCR amplification. Lancer 336:529–532 (1990).

43. Gonzalez FJ, Skoda RC, Kimura S, Umeno M, Zanger UM, Nebert DW, Gelboin HV, Hardwick JP, Meyer UA. Characterization of the common genetic defect in humans deficient in debrisoquine metabolism. Nature 331:442–446 (1988).

44. Tyndale R, Aoyama T, Broyl F, Matsunaga T, Inaba T, Kalow W, Gelboin HV, Meyer UA, Gonzalez FJ. Identification of a new variant CYP2D6 allele lacking the coding encoding Lys-281: possible association with the poor metabolizer phenotype. Pharmacogenetics 1:26–32 (1991).

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45. Gough AC, Miles JS, Spurr NK, Moss JE, Gaedigk A, Eichelbaum M, Wolf CR. Identification of the primary gene defect at the cytochrome P450 CYP2D locus. Nature 347:773–776 (1990).

46. Crespi CL, Penman BW, Gelboin HV, Gonzalez FJ. A tobacco smoke-derived nitrosamine, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butane, is activated by multiple human cytochromes P450 including the polymorphic human cytochrome P450D26. Carcinogenesis 12:1197–1201 (1991).

47. Hayashi S, Watanabe J, Kawaiki J. Genetic polymorphisms in the 5′-flanking region change transcriptional regulation of the human cytochrome P450IIIE1 gene. J Biochem (Tokyo) 118:115–120 (1995).

48. Uematsu F, Kikuchi H, Motomiya M, Abe T, Sagami I, Ohmachi T, Waktai A, Kanamaru R, Watanabe M. Association between restriction fragment length polymorphism of the human cytochrome P450IIIE1 gene and susceptibility to lung cancer. Jpn J Cancer Res 82:254–256 (1991).

49. Newman MJ, Light BA, Weston A, Tollurd D, Clark JL, Mann DL, Blackmon JP, Harris CC. Detection and characterization of human serum antibodies to polycyclic aromatic hydrocarbon diol-epoxide DNA adducts. J Clin Invest 82: 45–153 (1988).

50. Harris CC, Vakahangas K, Newman MJ, Trivers GE, Shamsuddin AKM, Sinopoli NT, Mann DL, Wright WE. Detection of benzo[a]pyrene diol epoxide-DNA adducts in peripheral blood lymphocytes and antibodies to the adducts in serum from coke oven workers. Proc Natl Acad Sci USA 82:6672–6676 (1985).

51. Haugen A, Becher G, Benestad C, Vakahangas K, Trivers GE, Newman MJ, Harris CC. Determination of polycyclic aromatic hydrocarbons in the urine, benzo[a]pyrene diol-epoxide-DNA adducts in lymphocyte DNA, and antibodies to the adducts in sera from coke oven workers exposed to measured amounts of polycyclic aromatic hydrocarbons in the work atmosphere. Cancer Res 46:4178–4183 (1986).

52. Santella RM, Weston A, Perera FP, Trivers GE, Harris CC, Young TL, Nguyen D, Lee BM, Poirier MC. Interlaboratory comparison of antisera and immunoaassays for benzo[a]pyrene diol-epoxide-I modified DNA. Carcinogenesis 9:1265–1269 (1988).

53. Poirier MC, Reed E, Ozols RF, Fasy T, Yuspa SH. DNA adducts of cisplatin in nucleated peripheral blood cells and tissues of cancer patients. Prog Exp Tumor Res 31:104–113 (1987).

54. Perera FP, Hemminki K, Young TL, Brenner D, Kelly G, Santella RM. Detection of polycyclic aromatic hydrocarbon-DNA adducts in white blood cells of foundry workers. Cancer Res 48:2288–2291 (1988).

55. Foiles PG, Miglietta LM, Akerkar SA, Everson RB, Hecht SS. Detection of 2′-methyldeoxyguanosine in human placental DNA. Cancer Res 48:184–1848 (1988).

56. Wild CP, Jiang YZ, Montesano R, Parkin M, Khat M, Sivatanakul P. Correlation study of aflatoxin exposure and liver cancer incidence in five geographical regions of Thailand. Proc Am Assoc Cancer Res 30:317 (1989).

57. Wild CP, Stich HF, Montesano R. Presence of alkylated DNA in oral mucosal cells from cigarette smokers. Proc Am Assoc Cancer Res 30:318 (1989).

58. Wild CP, Lu SH, Montesano R. In: Radioimmunoassay Used to Detect DNA Alkylation Adducts in Tissues from Populations at High Risk for Oesophageal and Stomach Cancer. IARC Scientific Publications, Lyon:International Agency for Research on Cancer, 1987:535–537.

59. Phillips DH, Hemminki K, Alhoven A, Hewer A, Grover PL. Monitoring occupational exposure to carcinogens: detection by 32P-postlabelling of aromatic DNA adducts in white blood cells from iron foundry workers. Mutat Res 204:531–541 (1988).

60. Dunn BP, Stich HF. 32P-postlabelling analysis of aromatic DNA adducts in human oral mucosal cells. Carcinogenesis 7:1115–1120 (1986).

61. Chacko M, Gupta RC. Evaluation of DNA damage in the oral mucosa of tobacco users and non-users by 32P-adduct assay.
81. Strange RC, Matharoo B, Faulder GC, Jones P, Cotton W, Elder JB, Deakin M. The human glutathione S-transferases: a case–control study of the incidence of the GST10 phenotype in patients with adenocarcinoma. Carcinogenesis 12:25–28 (1991).

82. Seidegard J, Pero RW, Markowitz MM, Rouss G, Miller DG, Beartie EJ. Isoenzyme(s) of glutathione transferase (class Mu) as a marker for the susceptibility to lung cancer: a follow up study. Carcinogenesis 11:33–36 (1990).

83. Robbins JH, Kraemer KH, Lutzner MA, Festoff BW, Coon HG. Xeroderma pigmentosum: an inherited disease with sun sensitivity, multiple cutaneous neoplasms, and abnormal DNA repair. Ann Intern Med 80:221–248 (1974).

84. Krontiris TG, DiMartino NA, Colb M, Parkinson DR. Unique allelic restriction fragments of the human Ha-ras locus in leukocyte and tumour DNAs of cancer patients. Nature 313:369–374 (1985).

85. Sugimura H, Caporaso NE, Hoover RN, Modali R, Resau J, Trump BF, Lonergan JA, Krontiris TG, Mann DL, Weston A, Harris CC. Association of rare alleles of the Harvey ras protooncogene locus with lung cancer. Cancer Res 50:1857–1862 (1990).

86. Ishizaki K, Kato M, Ikenaga M, Honda K, Ozawa K, Toguchida J. Correlation of L-myc genotypes to metastasis of gastric cancer and breast cancer. J Natl Cancer Inst 82:238–239 (1990).

87. Kawashima K, Shikama H, Imoto K, Izawa M, Naruke T, Okabayashi K, Nishimura S. Close correlation between restriction fragment length polymorphism of the L-MYC gene and metastasis of human lung cancer to the lymph nodes and other organs. Proc Natl Acad Sci USA 85:2353–2356 (1988).

88. Malkin D, Li FP, Strong LC, Fraumeni JF, Nelson CE, Kim DH, Kassel J, Gryka MA, Bischoff FZ, Tainsky MA, Friend SH. Germ line p53 mutations in a familial syndrome of breast cancer, sarcomas, and other neoplasms. Science 250:1233–1238 (1990).