

Mutagen Sensitivity Has High Heritability: Evidence from a Twin Study

Xifeng Wu,¹ Margaret R. Spitz,¹ Christopher I. Amos,¹ Jie Lin,¹ Lina Shao,¹ Jian Gu,¹ Mariza de Andrade,² Neal L. Benowitz,³ Peter G. Shields,⁴ and Gary E. Swan⁵

¹Department of Epidemiology, The University of Texas M.D. Anderson Cancer Center, Houston, Texas; ²Department of Health Sciences Research, Mayo Clinic, Rochester, Minnesota; ³University of California-San Francisco, California; ⁴Cancer Genetics and Epidemiology, Lombardi Comprehensive Cancer Center, Washington, District of Columbia; and ⁵Center for Health Sciences, SRI International, Menlo Park, California

Abstract

Despite numerous studies showing that mutagen sensitivity is a cancer predisposition factor, the heritability of mutagen sensitivity has not been clearly established. In this report, we used a classic twin study design to examine the role of genetic and environmental factors on the mutagen sensitivity phenotype. Mutagen sensitivity was measured in peripheral blood lymphocytes from 460 individuals [148 pairs of monozygotic (MZ) twins, 57 pairs of dizygotic (DZ) twins, and 50 siblings]. The intraclass correlation coefficients were all significantly higher in MZ twins than in dizygotes (DZ pairs and MZ-sibling pairs combined) for sensitivity to four different mutagen challenges. Applying biometric genetic modeling, we calculated a genetic heritability of 40.7%, 48.0%, 62.5%, and 58.8% for bleomycin, benzo[*a*]pyrene diol epoxide, γ -radiation, and 4-nitroquinoline-1-oxide sensitivity, respectively. This study provides the strongest and most direct evidence that mutagen sensitivity is highly heritable, thereby validating the use of mutagen sensitivity as a cancer susceptibility factor. (*Cancer Res* 2006; 66(12): 5993-6)

Introduction

It is widely recognized that both genetic and environmental factors influence cancer in a complex fashion. Aberration in many cellular functions may be involved in the etiology of cancer. DNA repair capacity is a cellular defense system designed to protect genomic integrity. There is considerable interindividual variation in DNA repair capacity. Numerous epidemiologic studies have consistently yielded significant associations between reduced DNA repair capacity and increased cancer occurrence (1, 2), suggesting that DNA repair capacity is a cancer susceptibility factor. Mutagen sensitivity, measured by quantifying the chromatid breaks induced by mutagen in short-term cultures of peripheral blood lymphocytes (PBL), has been used as an indirect measure of DNA repair capacity. In response to mutagen exposure, genetic damages would accumulate more quickly in people with suboptimal DNA repair capacity than in normal people. Depending on the level of chromatid breaks induced by mutagen treatment, individuals can be classified as nonsensitive or sensitive, which is believed to reflect their ability to repair DNA damages induced by

selected mutagen. The original assay used bleomycin as the mutagen challenge (3). The assay has since been expanded to employ other mutagens to reflect different cancer etiologies, including benzo[*a*]pyrene diol epoxide (BPDE), a tobacco smoke carcinogen, for smoking-related carcinogenesis (4, 5); 4-nitroquinoline-1-oxide (4-NQO), a UV-mimetic mutagen, for melanoma initiation (6); and γ -radiation exposure for breast and brain cancer etiology (7, 8). Bleomycin, a radiomimetic drug, induces oxidative base damage and single-strand and double-strand breaks in DNA that necessitates base excision repair and double-strand break repair. BPDE covalently binds to DNA to form BPDE-DNA adducts, which require nucleotide excision repair. 4-NQO is a UV-mimetic agent and causes bulky lesions mainly repaired by nucleotide excision repair. γ -Radiation induces DNA strand breaks that require double-strand break repair and base excision repair.

Mutagen sensitivity has been increasingly recognized as a cancer-predisposing factor (1-8). However, almost all of the evidence supporting mutagen sensitivity as a cancer susceptibility factor is derived from case-control studies that compared mutagen sensitivity in unrelated individuals. The extent to which mutagen sensitivity is influenced by genetic factors remains unclear. Without strong evidence supporting high genetic heritability, the merit of mutagen sensitivity—an intermediate phenotype—as a cancer risk factor will continue to be scrutinized for the possibility of “reverse causation.” In this report, we applied the classic twin study design to examine the relative contribution of genetic and environmental factors to the variability in mutagen sensitivity. The twin model, by comparing similarities between monozygotic (MZ) twins (who are genetically identical) and dizygotic (DZ) twins (who share, on average, half their genes), is an ideal model to partition interindividual variability into components reflecting genetic and environmental factors. Twin studies have been widely used to validate the heritability of recognized phenotypic risk factors for cancer and other complex diseases (9, 10). In this study, we determined the heritability of mutagen sensitivity by challenging PBL with the above-mentioned four different mutagens.

Materials and Methods

Twin recruitment. The study was carried out within the framework of a study of pharmacokinetics of nicotine in twins in California (11, 12). The procedures for twin recruitment and exclusion criteria have been described in full detail elsewhere (11, 12). Informed consent was obtained from each subject. All methods for recruitment, informed consent, screening, and data collection were reviewed and approved by the Institutional Review Boards of SRI International, California.

Blood collection and mutagen sensitivity assays. For each participant, two 10-mL tubes of blood samples were collected at the Clinical Research Center at University of California-San Francisco at the baseline and

Requests for reprints: Xifeng Wu, Department of Epidemiology, Unit 1340, The University of Texas M.D. Anderson Cancer Center, 1155 Pressler Boulevard, Houston, TX 77030. Phone: 713-745-2485; Fax: 713-792-4657; E-mail: xwu@mdanderson.org.

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delivered to the Department of Epidemiology, the University of Texas M.D. Anderson Cancer Center, by Federal Express mail within 24 hours of blood drawing for lab assays. Mutagen sensitivity assays in blood cultures were done as previously described (5, 6).

Statistical analysis. Descriptive statistics (mean, median, range, SD) were computed for all the participants and for subgroups. Differences between groups with respect to mutagen sensitivity were assessed by Student's *t* test or ANOVA while adjusting for nonindependence of data in families. Intraclass correlations were calculated between the set of MZ twins and the set of DZ twins (as well as the twin-sibling pairs). The intraclass correlation is given by $r = (MSA - MSW) / (MSA + MSW)$, where MSA is the mean square among twin pair and MSW is the mean square within pair. Genetic heritability was quantified by a biometric genetic modeling as previously described (13, 14).

Results and Discussion

A total of 460 individuals participated in the study, including 148 pairs of MZ twins, 57 pairs of DZ twins, and 50 siblings of twins (either MZ or DZ). There were 353 (76.7%) Caucasians, 12 (2.6%) African Americans, 54 (11.7%) Hispanics, 17 Asians (3.7%), and 24 (5.2%) other ethnicities. The numbers of never, former, and current smokers were 270 (60.8%), 104 (22.7%), and 76 (16.6%), respectively.

Whereas the mutagen sensitivity assay has been shown to be reproducible and reliable, no previous study has been done to systematically evaluate the measurement error in duplicate cultures from the same subjects. In our study, we did the four mutagen sensitivity assays in duplicate samples to assess measurement

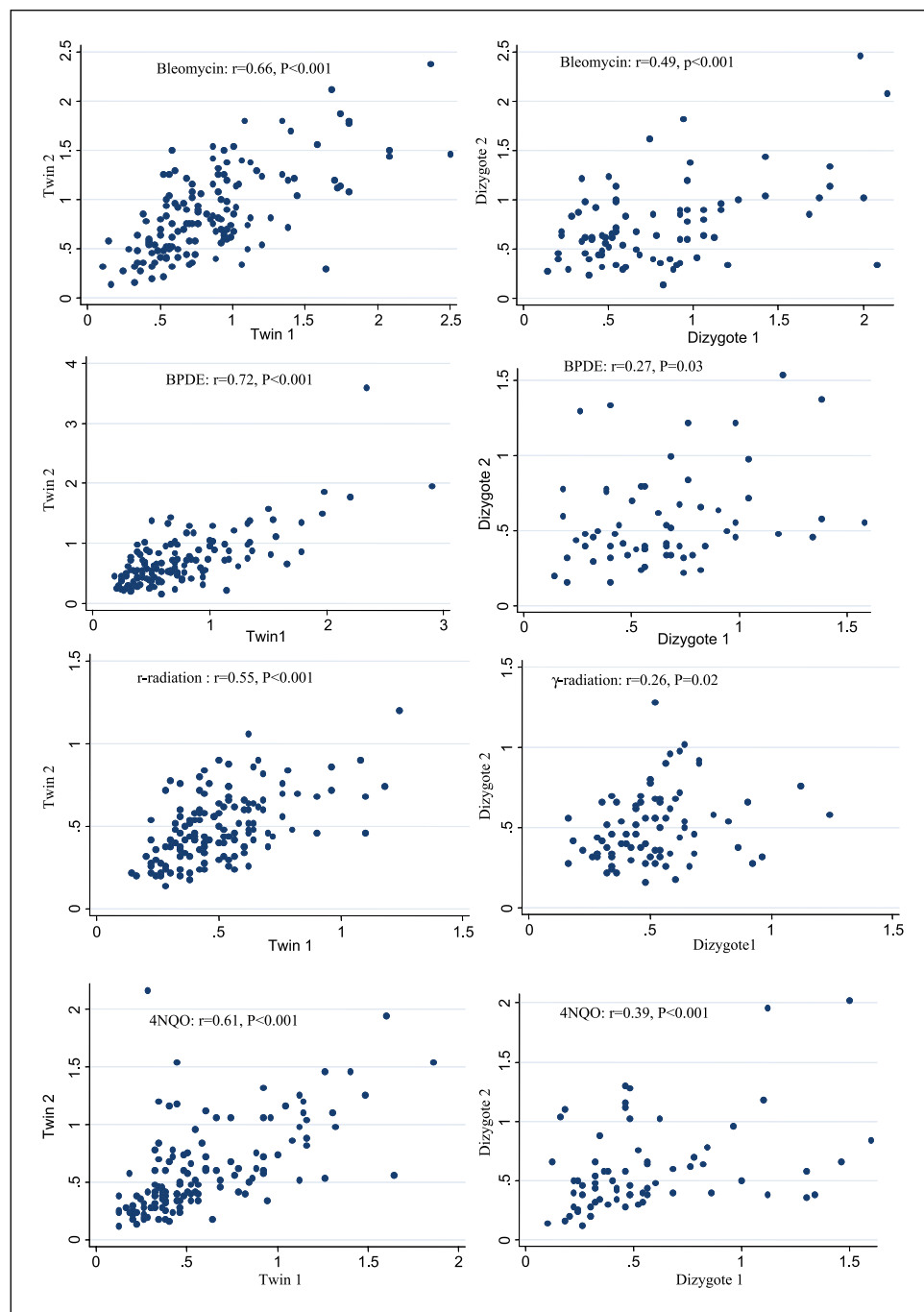


Figure 1. Intraclass correlation of mutagen sensitivity (mean breaks per cell) between twin 1 and twin 2 in MZ twins (*left*) and between person 1 and person 2 in dizygotes (*right*), as calculated by use of ANOVA. Due to the low number of DZ twins, we combined DZ twin pairs with MZ-sibling pairs. In families with MZ pairs and siblings, we randomly picked one co-twin from the twin pair and one sibling to form a MZ-sibling pair. Similarity for MZ twins is significantly greater than for dizygotes in all four mutagen sensitivity assays.

Table 1. Contribution of genetic and environmental variance components to mutagen sensitivity

	Bleomycin		BPDE		γ -Radiation		4-NQO	
	Full model*	No genetic influence	Full model	No genetic influence	Full model	No genetic influence	Full model	No genetic influence
Log-likelihood	199.967	194.664	206.199	201.101	491.806	487.430	237.290	230.99
χ^2		10.606		10.195		8.750		12.60
<i>P</i> (two sided)		<0.01		<0.01		<0.01		<0.01
Degrees of freedom		1		1		1		1
Age coefficient	0.006	0.006	-0.004	-0.004	0.001	0.001	0.003	0.002
Variations								
Genetic	0.077	Fixed	0.083	Fixed	0.0316	Fixed	0.077	Fixed
Shared	0.049	0.109	0.035	0.112	0.000	0.015	0.002	0.015
Nonshared	0.063	0.081	0.055	0.069	0.019	0.026	0.052	0.026
Heritability (%)	40.7		48.0		62.7		58.8	

NOTE: The pedigree-based maximum likelihood method (13, 14) was used to quantify the influences of genetic and environmental contributions to mutagen sensitivity.

*The full model includes variances due to genetic, nonshared, and shared environmental factors. The pedigree-based maximum likelihood method fitted to the data by specifying age on the mean phenotype (breaks per cell) as a fixed effect and partitioning the total variance by the contribution of genetic influence, shared environment (common environmental factors shared by family members), and nonshared environment (environmental factors unique to each individual subject) to the full model.

error. We found that the correlations between duplicate samples were all highly significant in all four assays. The correlation coefficients for bleomycin, BPDE, γ -radiation, and 4-NQO assays were 0.70 ($P < 0.001$; $n = 25$ pairs), 0.75 ($P < 0.001$; $n = 63$ pairs), 0.53 ($P < 0.001$; $n = 55$ pairs), and 0.82 ($P < 0.001$; $n = 68$ pairs), respectively. The repeatability of the mutagen sensitivity assay of multiple samples from the same person at various time intervals has previously been shown (15, 16).

The sensitivity to the four mutagens was generally not associated with epidemiologic variables, including ethnicity, gender, age, or smoking status (data not shown). Correlations between the four mutagen sensitivity scores were evaluated and significant correlations were only seen between BPDE and bleomycin ($r = 0.30$, $P < 0.001$), between bleomycin and γ -radiation ($r = 0.22$, $P < 0.001$), and between 4-NQO and γ -radiation ($r = 0.11$, $P = 0.03$). Intraclass correlations were calculated between the set of MZ twins and the set of dizygotes (DZ pairs and MZ-sibling pairs combined). The intraclass correlation coefficients for MZ twins were 0.67, 0.72, 0.55, and 0.61 for bleomycin, BPDE, γ -radiation, and 4-NQO sensitivity (all $P < 0.001$; Fig. 1, left), respectively, whereas the intraclass correlation coefficients for dizygotes were 0.49, 0.27, 0.26, and 0.39 for these four markers, respectively (Fig. 1, right). The correlations were significantly higher within MZ pairs than within dizygotes for all four mutagen sensitivities, suggesting a strong genetic influence.

We then applied biometric genetic modeling by partitioning the total variance into variance due to genetic, shared environment, and nonshared environment components and included age as a covariate (refs. 13, 14; Table 1). The null hypothesis, which assumes no genetic effects, was rejected because the restricted model, assuming no genetic influence, fitted the data significantly worse than the full model, suggesting the genetic component contributed significantly to all four markers ($P < 0.01$). The model generated a heritability estimate of 40.7%, 48.0%, 62.5%, and 58.8% for bleomycin, BPDE, γ -radiation, and 4-NQO sensitivity. In addition, the contribution of nonshared environment was also substantial,

accounting for 33.3%, 31.8%, 37.5%, and 39.7% for the four markers, respectively, whereas the contribution of shared environment was relatively small (26%, 20.2%, 0%, and 1.5% for the four markers, respectively).

The major concern about the validity of mutagen sensitivity, an intermediate phenotype, as a genetic marker for cancer susceptibility is the problem of "reverse causation" (i.e., whether disease status and/or prior environmental exposure cause variation in mutagen sensitivity phenotype). The heritability of mutagen sensitivity was initially implicated in early studies showing that first-degree relatives of mutagen-sensitive individuals were also mutagen sensitive (17-19), suggesting genetic predisposition to mutagen sensitivity. One strong support for the heritability of mutagen sensitivity came from a study of measuring chromosomal radiosensitivity in families of patients with breast cancer. Twenty-three of the 37 (62%) first-degree relatives of sensitive patients were also sensitive compared with 1 of 24 (4.2%) first-degree relatives patients with normal responses. Segregation analysis of 95 family members showed clear evidence of heritability of radiosensitivity, with a single major gene accounting for 82% of the variance between family members (18, 19). However, family studies cannot distinguish between shared environment and genetic factors. Previous case-control studies indicated that mutagen sensitivity remained a significant predictor of cancer risk independent of demographic and environmental risk factors (5, 20). However, as limited by study design, the cause-effect relationship between mutagen sensitivity and cancer cannot be answered in case-control studies. A twin study is the most powerful tool for direct assessment of a genetic component and offers the capability of differentiating genetic effects from shared environment within families (21). Two previous pilot studies estimated the heritability of bleomycin sensitivity using the twin design: one involved 25 pairs of MZ twins and 14 pairs of dizygotes (twin pairs and siblings; ref. 22) and the other had only 9 MZ twins and 10 DZ twins (23). Cloos et al. (22) estimated a high heritability of 75% for bleomycin

sensitivity in a study involving 25 pairs of MZ twins and 14 pairs of dizygotes (twin pairs and siblings), suggesting a clear genetic basis for bleomycin sensitivity. Tedeschi et al. (23) reported higher correlations of bleomycin sensitivity score in MZ twins as compared with DZ twins and yielded a heritability of 0.58, although this estimate was based on intraclass correlations only and the biometric genetic modeling was not applied in their study. Our results corroborate these pilot studies with a substantially larger sample size (148 pairs of MZ twins and 82 pairs of dizygotes). Moreover, we provided the first systematic evaluation of the genetic heritability of mutagen sensitivity by extending to other more commonly used and biologically relevant mutagens. We also estimated, for the first time, the substantial contribution of nonshared environmental factors to mutagen sensitivity, highlighting the presence of gene-environment interaction in modulating cancer risk. It is apparent that the genetic heritability varies among the cellular sensitivity to different agents and the nonshared and shared environment contributes to mutagen sensitivity with differing degrees among the four agents. The mechanisms behind these variations are unclear. Mutagen sensitivity is an indirect measurement of DNA repair capacity and different mutagens elicit different DNA repair pathways. It is understandable that base excision repair, nucleotide excision repair, and double-strand break repair involve different genes, follow distinct repair kinetics, and therefore exhibit variations in heritability. Even for the similar damages (e.g., in the case of BPDE and 4-NQO), the systems involved may differ significantly. Theoretically, BPDE and 4-NQO induce similar sensitivity profiles and the removal of both 4-NQO and BPDE adducts mainly requires nucleotide excision repair.

However, there are other mechanisms involved besides nucleotide excision repair. BPDE is already a highly reactive intermediate, which does not require bioactivation. 4-NQO, on the other hand, must be bioactivated through nitroreduction to produce a reactive intermediate. The *in vitro* 4-NQO assay may therefore reflect the cumulative bioactivation ability and DNA repair capacity (6). Similarly, radiomimetic bleomycin and γ -radiation both mainly induce DNA strand breaks; however, these DNA damages are structurally complex and follow different repair kinetics (24). Our results suggest that γ -radiation sensitivity has the highest heritability (62.5%), confirming the high heritability of cellular radiosensitivity from family studies of breast cancer (18, 19). The biological mechanism for the variation in sensitivity to different agents remains to be investigated.

In conclusion, our data provide the strongest evidence that genetic influence contributes significantly to mutagen sensitivity phenotypes, validating the use of mutagen sensitivity as a genetic susceptibility factor. Identifying genes involved in this susceptibility and determining the segregation pattern of these genes could provide further insight into the etiology of cancer.

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