Inherited Susceptibility to Bleomycin-Induced Chromatid Breaks in Cultured Peripheral Blood Lymphocytes

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Background: **Susceptibility to bleomycin-induced chromatid breaks in cultured peripheral blood lymphocytes may reflect the way a person deals with carcinogenic challenges. This susceptibility (also referred to as mutagen sensitivity) has been found to be increased in patients with environmentally related cancers, including cancers of the head and neck, lung, and colon, and, in combination with carcinogenic exposure, this susceptibility can greatly influence cancer risk. The purpose of this study was to assess the heritability of mutagen sensitivity.** ***Methods:*** **Heritability was determined by use of a maximum likelihood method that employed the FISHER package of pedigree analysis. Bleomycin-induced breaks per cell values for 135 healthy volunteers without cancer were determined. These individuals were from 53 different pedigrees and included 25 monozygotic twin pairs (n = 50), 14 pairs of dizygotes (twin pairs and siblings, n = 28), and 14 families selected on the basis of a first-degree relative who was successfully treated for head and neck cancer and who had no sign of recurrence for at least 1 year. All data were analyzed simultaneously, and different models of familial resemblance were fitted to the data. All *P* values are two-sided.** ***Results:*** **Our results showed no evidence for the influence of a shared family environment on bleomycin-induced chromatid breaks. Genetic influences, however, were statistically significant (*P* = .036) and accounted for 75% of the total variance.** ***Conclusions:*** **The high heritability estimate of the susceptibility to bleomycin-induced chromatid breaks indicates a clear genetic basis. The findings of this study support the notion that a common genetic susceptibility to DNA damage—and thereby a susceptibility to cancer—may exist in the general population. [J Natl Cancer Inst 1999;91:1125–30]**

For cancers of the respiratory and upper digestive tracts, traditional epidemiology has identified smoking and alcohol intake as major risk factors *(1,2)*. These risk factors, however, cannot adequately explain all cancer cases. Much emphasis has been given on the value of molecular epidemiology for the refinement of the estimation of environmentally related cancer risks *(3,4)*. Molecular epidemiology is a relatively new approach that incorporates individual biomarkers for cancer risk assessments in populations. An interaction between exposure to carcinogens and susceptibility factors was found to determine cancer risk. Susceptibility biomarkers have been reported, for instance, on carcinogen detoxification, carcinogen activation, and formation of DNA adducts *(5–7)*.

A functional approach to determine individual susceptibility to carcinogenic assaults is to screen for chromatid breaks after *in vitro* G_2-phase bleomycin treatment of cultured peripheral blood lymphocytes. For environmentally related cancers, such as colon cancer, lung cancer, and head and neck squamous cell carcinoma (HNSCC), the biologic relevance of this marker has been well established *(8,9)*. We and others *(8–10)* showed an increase in the mean level of chromatid breaks per cell in cancer patients compared with healthy control persons. This high level was especially found in those patients with multiple primary tumors *(10)*. The mean number of breaks per cell score was not influenced by smoking or alcohol use by the subjects. It was shown in a meta-analysis that a high susceptibility (defined as 1.0 or more breaks per cell) itself slightly increased cancer risk but did not reach statistical significance. Of interest, however, in combination with exposure to carcinogens, a large increase (up to an odds ratio of 57.5) of risk for HNSCC was found *(11)*.

For persons at high risk of cancer (particularly, members of families with a high frequency of common cancers and HNSCC patients who have been successfully treated for their primary tumor and are at risk for a second tumor), it is important to ascertain whether this susceptibility phenotype has a genetic basis. Such knowledge will increase the value of this susceptibility marker and encourage further studies of the (genetic) mechanisms underlying this susceptibility. We investigated the heritability of the susceptibility to chromatid breaks in pedigrees from HNSCC patients who have been successfully treated and have no evidence of cancer recurrence for at least 1 year. Because familial resemblance can be due to shared environment as well as to shared genes, the number of chromatid breaks was also assessed in 25 pairs of monozygotic twins (unrelated to the HNSCC patients), who are genetically identical. Any larger resemblance for monozygotic twins compared with the first-degree relatives (who share, on average, 50% of their genes) could suggest the importance of genetic factors. In this study, we employed the powerful tool of pedigree analysis to estimate the heritability of mutagen sensitivity.

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See "Notes" following "References."

METHODS

Subjects

All subjects (n = 135) whose data were used for the estimation of the heritability of the susceptibility marker were healthy volunteers from 53 different pedigrees and consisted of dizygotes (n = 85; stratified in three groups on the basis of their relation to the 14 HNSCC patients who were enrolled in this study; I = siblings of the HNSCC patients [n = 32]; II = offsprings of the HNSCC patients [n = 25]; III = dizygotes, volunteers unrelated to HNSCC patients [n = 28]), and monozygotes, volunteers unrelated to HNSCC patients (group IV: 25 pairs of identical twins, n = 50). Heparinized blood samples were collected from the first-degree relatives (groups I and II) of 14 HNSCC patients previously treated at our department and had no more evidence of any residual or recurrence disease for at least 1 year (n = 57). Heparinized blood samples were also obtained from volunteers not related to HNSCC patients (group III, consisting of 14 dizygotic pairs: eight dizygotic twins and six sibling pairs with only a small age difference [mean age difference ± standard deviation = 3.5 years ± 1.4 years] among the members of the group III).

All 14 probands (individuals through whom family pedigrees were ascertained) had been successfully treated for HNSCC at our department. Patients were selected on the basis of their having several first-degree relatives. When the patients were referred to our hospital for follow-up, they were asked permission to contact their first-degree relatives to participate in this study. Patient and tumor characteristics and the details of how many relatives of each patient volunteered are summarized in Table 1. For staging of the HNSCC, the criteria of the International Union Against Cancer *(12)* were used. In line with our earlier studies *(10,11)*, eight (57%) of 14 patients were determined not to be sensitive to bleomycin-induced chromatid breaks (breaks per cell <1.0). Blood was drawn from all of the participants after they signed an informed consent form. For all twin pairs, the names and addresses were obtained from the National Netherlands Twin Registry *(13)*. Zygosity of the twins was assessed by genotype analysis of six unlinked microsatellite loci (with heterozygosities >90%) in two different multiplex polymerase chain reactions *(14)*. Zygosity analysis was performed at the TNO Prevention and Health, Gaubius Laboratory, Division of Vascular and Connective Tissue Research, Leiden, The Netherlands. The geographic distribution of both the twins and the family members of patients was throughout the whole of The Netherlands. All subjects had given written informed consent and the study design was approved by the local ethical committee.

It is important for the interpretation of the current study that smoking status of the subjects was not included in the analysis. We and others *(8,10,11)* have described earlier that exposure to tobacco smoke and alcohol (pack/unit years as well as current smoking/drinking) does not influence the breaks per cell value itself. This lack of effect is probably due to the 10 times dilution of the blood in the medium and the culture period of 3 days for the chromosomal breakage assay that diminishes any effects of tobacco or alcohol. Since, in the current study, we were interested in the heritability estimate of the breaks per cell value and not in the heritability of cancer, smoking or alcohol use was not included in the analysis.

Chromatid Breakage Assay

Duplicate cultures were set up for each subject. Whole blood (0.5 mL) was diluted 10 times in RPMI-1640 medium (BioWhittaker, Inc., Walkersville, MD) with 2 m*M* L-glutamine (Life Technologies, Paisley, U.K.) supplemented with 15% fetal calf serum (Hyclone Laboratories, Inc., Logan, UT), 1.5% phytohemagglutinin (Life Technologies), 100 U/mL penicillin, and 100 μg/mL streptomycin (BioWhittaker, Inc.). After the cells were cultured for 3 days at 37 °C and 5% CO_2, they were incubated for 5 hours with bleomycin (30 mU/mL) (Lundbeck, Amsterdam, The Netherlands). To arrest the cells at metaphase, 0.04 μg/mL Colcemid (Sigma Chemical Co., St. Louis, MO) was added to the cultures 1 hour before harvesting. This yields cells in metaphase that were damaged by the bleomycin in the late S–G_2 phase of the cell cycle. The cells were swollen in hypotonic solution (0.06 *M* KCl) and fixed in Carnoy's fixative (3 : 1 [vol/vol] methanol : acetic acid). After the cells were dropped on wet slides, the metaphase spreads were air-dried and stained with Giemsa (Merck, Darmstadt, Germany). Before 50 metaphase spreads were scored on each slide for the presence of chromatid breaks, the slides (two slides per person) were coded to ensure objective "blinded" screening. The mean number of breaks per cell of 100 metaphases was used as a measure for the individual susceptibility. As has been published previously *(15)*, the scoring of gaps did not influence the outcome of the assay and was omitted in further investigations. Since DNA damage was introduced in late S–G_2 phase of the cell cycle, chromosome aberrations such as translocations were not present in the metaphases. Background levels (spontaneous) of chromatid breaks without damage induction by bleomycin that have been determined in previous studies were very low (breaks per cell values of approximately 0.06) and did not differ between patients and control subjects. Therefore, data representing spontaneous breaks were not included.

Descriptive Statistics

Differences between groups with respect to mutagen sensitivity were assessed by use of Student's *t* test. The influences of age and sex on mutagen sensitivity were determined by use of regression and likelihood methods. Intraclass correlations were calculated by use of analysis of variance.

Heritability Estimation

A pedigree-based maximum likelihood method developed by Lange et al. *(16)* was used to analyze resemblances among family members for chromatid breaks

Table 1. Characteristics of patients successfully treated for head and neck squamous cell carcinoma and whose first-degree relatives participated in the study

Patient No.	Tumor*				Chromatid breaks per cell‡	No. of relatives§	
	Site	Stage†	Treatment	Year of treatment		Siblings	Offspring
1	Larynx	T1aN0	Radiotherapy	1992	1.27	0	6
2	Pharynx	T1N0	Surgery	1993	1.18	2	4
3	Larynx	T1N0	Surgery + radiotherapy	1982	1.28	7	0
4	Oral cavity	T1N0	Surgery + radiotherapy	1993	1.13	0	4
5	Larynx	T1N0	Surgery	1993	0.82	1	2
6	Larynx	T2aN0	Radiotherapy	1992	0.72	0	2
7	Pharynx	T4N1	Surgery	1992	0.79	1	1
8	Oral cavity	T2N0	Surgery	1986	0.70	0	1
9	Oral cavity	T1N0	Surgery	1992	0.38	2	2
10	Larynx	T2N1	Surgery	1992	0.53	0	4
11	Oral cavity	T1N0	Surgery	1992	1.02	5	2
12	Pharynx	T2N2b	Surgery + radiotherapy	1992	1.23	6	0
13	Pharynx	T2N0	Surgery	1995	0.97	1	2
14	Larynx	T1aN0	Surgery	1992	0.67	0	2

*Of some patients who have had more than one tumor, only the last tumor is mentioned in this table.

†The International Union Against Cancer criteria were used for staging *(12)*.

‡These breaks per cell values have not been included for intraclass correlations but were used only for ascertainment correction by conditioning on probands in the heritability estimation.

§Number of relatives that participated in the study (siblings and offspring).

(used as a continuous phenotype). Data from twins and other family members were analyzed simultaneously as so that the pedigree data consisted of a total of 53 pedigrees (twins and dizygote pairs were also put into the file as pedigrees without a proband), a sample size of 135 subjects (including 50 monozygotic twins), and 14 probands (whose data were evaluated separately and used only for ascertainment correction by conditioning on probands). This ascertainment correction was needed because the pedigrees of the cancer patients were not a random selection of the general population *(16)*. Different models of familial resemblance were fitted to the data. These models specified the variation in phenotype to be due to the genotype and/or the environment. Sources of variation considered were additive: genetic influences (i.e., the sum of the effects of the individual alleles across all loci that contribute to variation), common environmental influences shared by family members who are living or have lived in the same household, and a random environmental deviation that is not shared by family members. These sources of variation can be considered as unobserved, or latent factors, which affect the (continuous) phenotype, and can be estimated from the observed patterns of resemblance between relatives. For each pedigree of n individuals, a vector of observations (x) is defined, and a vector of expected values ($E[x]$) can be calculated. $E(x)$ can depend on fixed variables, such as sex or age. The covariances among family members for that part of the dependent variable that is not accounted for by the fixed variables depend on the relationships between the pedigree members and on the genetic model assumed for dependent variables. We have modeled the variance not accounted for by the fixed effects as due to additive genetic influences, shared family environment, and random environmental factors. For a given $E(x)$ and expected covariances matrix $\sum$, the ln-likelihood of obtaining the observation vector x is:

$$L = -1/2 \ln \left|\sum\right| - 1/2 \left[x - E\left(x\right)\right]' \sum^{-1} \left[x - E\left(x\right)\right] + \text{constant},$$

where $|\ |$ denotes the determinant of the matrix and $'$ denotes transpose.

The joint likelihood of obtaining all pedigrees is the sum of the likelihood of the separate pedigrees. Estimation involves selection of parameter values under a specific model that maximizes the joint likelihood of all pedigrees. The FISHER package of pedigree analysis *(16)* was used for genetic modeling. The likelihoods obtained for the different models were compared with chi-squared difference tests where $\chi^2 = 2(L_1 - L_0)$. L_1 and L_0 denote the ln-likelihoods of the general (H_1) hypothesis and a constrained (H_0) hypothesis. The degrees of freedom (*df*) for this test are equal to the number of constrained parameters between H_1 and H_0 *(17)*. The following parameters were estimated in the general model: a sex effect and an age regression on means and three variance components (genetic, shared, and unique environmental variances). First, it was tested if additive genetic influences and common environmental influences could be constrained at zero (hypothesis of no familial resemblance in chromatid breaks) and next if either additive genetic influences or common environmental influences could be set to zero. The fixed effects on the means included a sex effect and an age regression. The variance components part of the analysis applies to the covariances of family members (while simultaneously modeling the age and sex effect).

The conditional likelihood approach as implemented in the FISHER package of pedigree analysis was used to correct for ascertainment. The heritability estimate was calculated as the contribution of the genetic variance in the total variance (genetic and environmental).

RESULTS

Descriptive Statistics

Plot of the data of the mean number of bleomycin-induced chromatid breaks per cell of all of the 135 healthy study subjects without cancer indicated a good approximation of the normal distribution (Fig. 1). The effect of sex on the mean number of breaks per cell was not statistically significant ($\chi^2 = 0.70$ with 1 *df; P* = .40). The regression of age was positive (slope: b = 0.007) and reached significance ($\chi^2 = 4.84$ with 1 *df; P* = .027).

The mean breaks per cell level of HNSCC patients (probands) was higher than that of their first-degree relatives without cancer as well as the control group consisting of twins and sibling pairs (Table 2). This difference was not statistically significant probably because of the small number of probands (n = 14). In earlier studies, we *(18)* and others *(19)* have scored patients before and (at least 3 months) after treatment, and no influence on the breaks per cell value was found. The higher rates of breaks per cell in the probands are therefore not considered to be due to the effects secondary to treatment. The relatively higher breaks per cell value for siblings of the probands (group I) compared with that for offspring of the probands (group II) may be explained by the higher age of the former group.

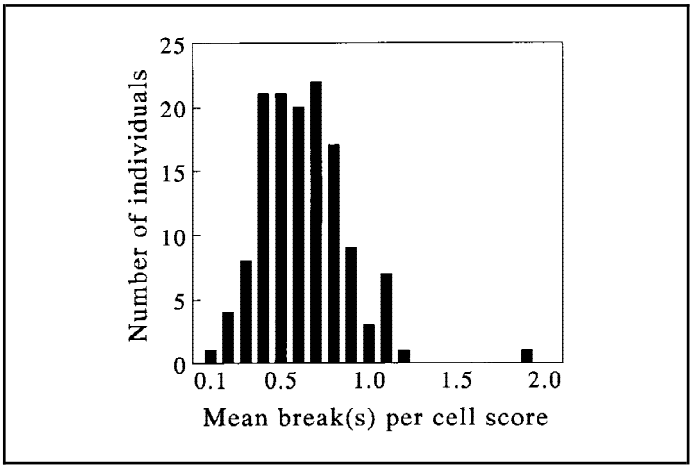

Fig. 1. Histogram of the frequency distribution of the mean number of chromatid breaks per cell in the 135 subjects without a history of cancer. Data on the 14 probands (patients with head and neck cancer who were previously treated but were tumor free for at least 1 year at the time of enrollment) are not included.

One aspect of heritability is that the correlation in the outcome of the assay between monozygotes is expected to be higher than that in the dizygotes. To visualize the high agreement within monozygotic twins for the mean number of breaks per cell, a plot of the individual data (Fig. 2) is given. This shows that the genetic influence for the susceptibility to bleomycin-induced chromatid breaks may be large. For a 100% genetic trait, the correlation between monozygotes is 1.0; for dizygotes, on average it is 0.5. Table 3 summarizes the intraclass correlations that were found for each group. The age-corrected intraclass correlation in the dizygotes (correlation coefficient = .46) in this study was statistically significantly less (P<.005) compared with that seen in the monozygotes (correlation coefficient = .77). The cancer patients were not included in this variance analysis. When the dizygotes were stratified by the presence of a proband in the family, it appeared that the variance in the siblings of the cancer patients was relatively large compared with the dizygotes who were not related to cancer patients. For both the age-corrected and noncorrected intraclass correlations, however, the differences between groups of dizygotes were not statistically significant. The very low correlation in the age-corrected analysis implies a large influence of age in this particular group. The age difference within dizygotic pairs was small (mean age difference ± standard deviation = 3.5 years ± 1.4 years), while the siblings of cancer patients varied up to 16 years within a family, possibly explaining the relatively large influence of age correction in the latter group. Another factor related to the increased variation in the families of HNSCC patients (groups I and II) compared with dizygotes not related to cancer patients (group III) can be that some relatives of cancer patients are likely to have an aberrant break per cell value due to their inherited susceptibility to bleomycin-induced chromatid breaks.

Table 2. Bleomycin sensitivity of different subjects related or unrelated to patients with head and neck squamous cell carcinoma

Subjects	No.	Mean breaks/cell (95% CI)*	Mean age, y (95% CI)*
Probands†	14	0.91 (0.34 to 1.48)	66.9 (54.2 to 79.6)
Family members of probands	57	0.60 (0.14 to 1.05)	46.4 (19.6 to 73.3)
Group I: siblings of probands	25	0.72 (0.33 to 1.11)	59.7 (46.0 to 73.4)
Group II: offspring of probands	32	0.51 (0.12 to 0.90)	36.0 (22.3 to 49.7)
Volunteers not related to probands	78	0.59 (0.02 to 1.16)	36.4 (15.4 to 57.4)
Group III: dizygotes (14 pairs)	28	0.66 (−0.03 to 1.35)	37.6 (12.5 to 62.7)
Group IV: monozygotes (25 pairs)	50	0.55 (0.04 to 1.06)	35.6 (17.0 to 54.2)

*CI = confidence interval.

†All probands were patients with head and neck squamous cell carcinoma who were treated previously but who were tumor free for at least 1 year before enrollment in this study.

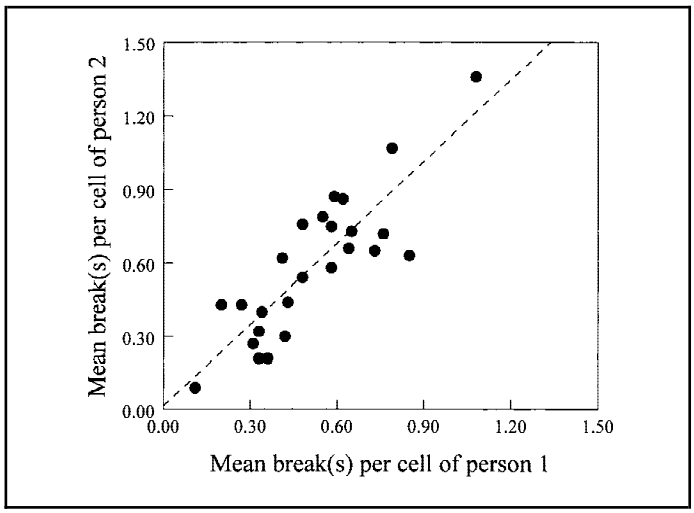

Fig. 2. Scatterplot (the line represents the linear regression) of the individual mean breaks per cell values of 25 pairs of monozygotic twins (person 1 and person 2).

Heritability Estimation

The actual heritability estimate was determined by use of a pedigree-based maximum likelihood method in which the (continuous) breaks per cell data from twins and other family members were analyzed simultaneously. The model that was fitted to the data in the FISHER package of pedigree analysis specified sex differences and age regression on the mean phenotype and considered genetic, unique, and shared environmental influences on variances and covariances between family members. The largest likelihood should be for the full model. As shown in Table 4, there was no evidence for the influence of a shared family environment ($\chi^2 = 1.08$ with 1 *df; P* = .29). The reduced model with the fixed shared environment does not fit the data significantly worse than the full model. Our interpretation is that shared environment does not contribute to familial resemblances. Genetic influences were statistically significantly shown in the reduced model with fixed genetic variances that fits the data significantly worse ($\chi^2 = 4.26$ with 1 *df; P* = .036). Particularly for the reduced model that excludes any familial resemblance (genetic and shared environment), the likelihood is statistically significantly reduced ($\chi^2 = 25.46$ with 1 *df; P*<.001). The heritability estimate of 75% was calculated (from the most optimal reduced model that excludes the influence of a shared environment) as the genetic variance (0.04775) divided by the total variance (0.04775 + 0.01613 = 0.06388).

Discussion

Heritability of persistent chromosomal damage is well established for the severe chromosomal instability syndromes, such as ataxia telangiectasia (AT) *(20)* and Fanconi anemia *(21)*. However, it is a novel finding that the "hidden" chromosomal instability (since the breaks have to be induced by bleomycin to observe the phenotype) that we describe in this study has a clear genetic basis. Clustering of a high breaks per cell level in cancer-prone families *(22,23)* already pointed toward a high heritability estimate. Moreover, another type of cytogenetic analysis based on the expression of aphidicolin-inducible common fragile sites yielded a heritability estimate of 88% *(24)*.

It is tempting to speculate that persons with a high susceptibility phenotype in our study may, in fact, be heterozygotes of the chromosomal instability syndromes. However, this hypothesis is not supported by what is known about obligate heterozygotes (e.g., parents of AT homozygotic patients). AT heterozygotes, for instance, are estimated to represent 0.5%–2.8% of the general population *(25)*, while about 16% of the control persons are hypersensitive to bleomycin-induced chromatid breaks (defined as breaks per cell ⩾1.0) *(15)*. Moreover, although obligate AT heterozygotes do have a higher susceptibility to radiation-induced chromatid breaks compared with control persons, they have increased risk mainly for breast cancer *(25)* and not for HNSCC. This may indicate that the levels of bleomycin-induced chromatid breaks that we report are related to other genes predisposing to HNSCC, although it cannot be excluded that genes of other chromosomal instability syndromes are involved.

The relationship between a high susceptibility for chromatid breaks and the development of environmentally related cancer has been established in retrospective *(8,10,11)* and a limited number of prospective *(26,27)* studies. The major conclusion from these former studies is that the intrinsic susceptibility and exposure to carcinogens act in concert to modulate cancer risk. The great refinement of cancer risk assessment by use of the susceptibility to bleomycin-induced chromatid breaks indicates the importance of this biomarker. The fact that this factor has a high heritability estimate underscores the relevance of genetic factors for cancer development. This will probably be important not only for HNSCC but also for all cancers in tissues that are in direct contact with the environment, such as the colon and the lung. The fact that a similar genetic factor does play a role in the development of various types of cancers may explain cancer proneness in families in which several types of cancers occur. It has previously been noted that familial clustering of environmentally related cancers does exist *(28,29)*. It will be very in-

Table 3. Summary of the familial correlations for bleomycin sensitivity measured as chromatid breaks per cell

Subjects	No.		Intraclass correlation (95% CI)†	
	Families	Subjects*	Overall	Corrected for age‡
Dizygotes				
Participants enrolled as relatives of probands				
Group I: siblings of probands	5	22	.42 (.00 to .71)	.30 (−.14 to .64)
Group II: offspring of probands	10	30	.41 (.06 to .67)	.40 (.05 to .66)
Participants not related to probands				
Group III: dizygotic twins and siblings	14	28	.66 (.38 to .83)	.64 (.35 to .82)
Monozygotes				
Participants not related to probands				
Group IV: monozygotic twins	25	50	.79 (.65 to .88)	.77 (.63 to .86)

*All subjects are volunteers without a cancer history; to eliminate the selection bias, the probands (patients with head and neck squamous cell carcinoma) are not included in the correlation analysis. Five persons could not be used in the correlation analysis because they were the only member within one family group.

†CI = confidence interval.

‡Overall intraclass correlation coefficient (.46) of dizygotes (groups I, II, and III combined) is statistically significantly ($P = .0039$) different from the correlation coefficient (.77) observed for the monozygotes (group IV); no significant differences are present between groups I, II, and III with or without age correction.

Table 4. Contribution of the genetic and/or the environmental variance component to the total variance in chromatid breaks per cell scores by use of the FISHER package of pedigree analysis

	Full model*	No shared environment	No genetic influence	No familial resemblance
ln-likelihood	136.528	135.989	134.409	123.805
χ^2		1.08	4.26	25.46
Degree(s) of freedom		1	1	2
P value (two-sided)		.29	.036†	<.001
Variances				
Genetic	0.037	0.048‡	Fixed	Fixed
Unique environmental	0.017	0.016	0.032	0.059
Shared environmental	0.011	Fixed	0.029	Fixed
Age regression	0.006	0.007	0.004	0.007

*The full model includes variances due to genetic, unique, and shared environmental influences. Analysis of variance, by use of models that fitted to the data in the FISHER package of pedigree analysis specifying sex differences and age on the mean phenotype (breaks per cell value) and considered the contribution of shared environment (environmental influences that persons have in common when they are brought up in the same family), genetic influence, and unique environment (environmental influences not related to familial resemblance) to the full model. Variance numbers are rounded to numbers with three digits after the decimal.

†Genetic influences were significant, since the reduced model that fixes the genetic variance fits the data statistically significantly worse.

‡Genetic influences explained 75% of the variances (heritability estimate = genetic variance [0.048]/total variance [0.064].

teresting to screen the DNA of these families to link a high susceptibility phenotype to mutations (or polymorphisms) in the known susceptibility genes, such as p53 (also known as TP53), BRCA1, and BRCA2 *(30)*. The high heritability estimate that we describe in this study for susceptibility to bleomycin-induced chromatid breaks may also facilitate the discovery of new-cancer predisposing genes. The phenotype, mean chromatid breaks per cell, can be a valuable determinant because it may be indicative of the cancer-prone phenotype and can be used as an end point in these studies. It has the advantage that it can be assessed before the cancer has occurred. This is very important because the study of cancer-prone families is hampered by the fact that the affected individuals often have already died.

When a common genetic defect is traced that is prevalent among a relatively large part of the population (e.g., breaks per cell level ⩾1.0), it may account for cancer predisposition in a large proportion of the cancer cases in general compared with the fraction that is due to inherited cancer syndromes (probably <5%) *(31)*. It is, therefore, important to recognize that the susceptibility to DNA damage varies in different individuals. Avoidance of exposure to (environmental and occupational) carcinogens, especially in sensitive persons, may then become an important factor in the prevention of cancer *(32)*. The heritability estimate of 75% challenges us to focus further research into finding the gene(s) involved in the susceptibility to bleomycin-induced chromatid breaks.

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NOTES

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