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Cigarette smoking during pregnancy: chromosome translocations and phenotypic susceptibility in mothers and newborns

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Abstract

The effects of maternal cigarette smoking during pregnancy on structural chromosome aberrations were evaluated in peripheral lymphocytes from 239 mothers and their 241 newborns to determine whether smoking during pregnancy, genetic susceptibility, and race are associated with chromosome aberrations including translocations. Demographic information and cigarette smoking data were obtained via questionnaire. There were 119 Caucasian Americans, 118 African Americans, and 2 Asian Americans. The average maternal age was 24.9 ± 5.8 (mean $\pm$ S.D.) years. Thirty-nine percent of the Caucasian Americans and 45.4% of the African Americans self-reported that they were active smokers during the index pregnancy. The average number of cigarettes smoked per day was 2.65 ± 5.75 and 1.37 ± 3.17 for Caucasian and African American mothers, respectively. Peripheral blood lymphocytes from the mother and from the fetal side of the placenta were evaluated for chromosome aberrations by whole chromosome painting and for genetic susceptibility using an *in vitro* bleomycin challenge assay. Spontaneous translocation frequencies in both maternal and newborn lymphocytes were not associated with cigarette smoking, socio-economic status, or education. The absence of a smoking effect may be attributable to the low level of cigarette usage in these subjects. The average bleomycin-induced damage in the maternal and newborn populations was 0.37 ± 0.27 and 0.15 ± 0.14 breaks per cell, respectively, a difference that was highly significant ($p < 0.0001$). In newborns there was a positive association between bleomycin sensitivity and the frequencies of aberrations as measured by chromosome painting: $p = 0.0007$ for dicentrics and fragments, and $p = 0.002$ for translocations. Caucasian American newborns demonstrated a significant association between dicentrics and fragments as measured by painting, and bleomycin sensitivity ($p = 0.0002$), but no such association was observed for African American newborns. The results of this study indicate

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that while differences were observed between African Americans and Caucasian Americans, race does not appear to be a major contributor to chromosome damage in newborns or their mothers. However, peripheral lymphocytes in pregnant women are more susceptible to genetic damage than peripheral lymphocytes in newborns.

Keywords

cigarette smoking; pregnancy; newborns; mothers; chromosome translocations; genomic susceptibility

1. Introduction

The first report of cancer caused by tobacco was made in 1761 when Hill reported a correlation between the use of snuff and oral cancer [1]. The same report stated that cancer could be prevented by avoiding the use of tobacco products. Two hundred thirty-nine years later, tobacco smoking, environmental tobacco smoke and smokeless tobacco were for the first time included in the Ninth Report on Carcinogens (RoC) as known human carcinogens [2]. The RoC listings are based on observed causal relationships between tobacco exposure and cancer or other illnesses. The International Agency for Research on Cancer (IARC) has linked active tobacco smoking with cancer causation at more than a dozen organ sites [3]. Approximately 4000 chemicals have been identified in tobacco smoke, many of which are classified as known or suspected human carcinogens [3].

Babies born to women who smoke during pregnancy are at increased risk for poor lung development, sudden infant death syndrome, and low birth weight. Whereas some studies report an association between tobacco exposure and childhood cancer, others do not [4-15]. Epidemiologic studies provide evidence that environmental tobacco exposure is harmful to children, but few reports address the health effects of maternal tobacco exposures upon children during gestation. Analyses are challenging because the gestational exposures in epidemiologic studies typically cannot be separated from pre- and post-gestational maternal and paternal exposures.

Several molecular epidemiologic studies have sought to correlate maternal smoking with biomarkers associated with tobacco exposure as measured in fetal cord blood or amniocytes [16]. Maternal smoking before and during pregnancy has been associated with increased chromosomal instability in amniocytes [17]. Cotinine, DNA adducts, gene mutations, chromosome aberrations, and micronuclei have all been examined [18-23] to evaluate the effects of transplacental exposures in fetal cells and to determine whether tobacco exposure during embryological and fetal development can increase the risks of disease later in life.

Exposures during fetal development may influence cancer risks. There is a potentially increased incidence of cancer among children whose mothers were exposed during pregnancy. Compounds in passive and active tobacco smoke and their metabolites have been known for many years to be capable of crossing the placenta [24,25]. Diethylstilbestrol is a powerful transplacental carcinogen, increasing the risk of vaginal clear cell carcinoma and testicular cancer in the children of exposed mothers [26-28]. Gestational exposure to alcohol is associated with childhood leukemia [11]. A recent pair of review articles [29,30] summarize the literature on environmental exposures in children, including newborns. The results suggest that children are more susceptible than adults to the effects of exposure.

Cancer is influenced by interactions among frequency and timing of exposures and genetic susceptibility [31,32]. Rare genetic variants with high penetrance contribute to

approximately 5% of all cancers, whereas the remaining cases are largely attributed to gene-gene and gene-environment interactions. Genetic polymorphisms that increase the risk for cancer among smokers have been identified [33-36]. Inherent genetic susceptibility to the effects of genotoxic exposures can be assessed by evaluating the sensitivity of peripheral blood lymphocytes to bleomycin *in vitro* [37-39]. Cells from susceptible individuals are compromised in their ability to repair chromosomal damage compared to non-susceptible people. Cloos *et al.* [40] compared gene expression patterns between mutagen-susceptible and mutagen-resistant cells. They describe expression differences in a spectrum of genes involved in response to bleomycin with potential for involvement in cancer.

Structural chromosome aberrations have long been known to be widely present in tumor cells, e.g. [41], and chromosome aberrations in peripheral lymphocytes of healthy individuals are significantly associated with increased cancer risks [42,43]. Chromosome translocations are of particular interest in assessing chronic exposure because most translocations are stable through cell division and have been shown to accumulate under conditions of protracted or highly fractionated exposure [44,45]. Translocation frequencies have been shown to be elevated in smokers compared to non-smokers, e.g. [46,47], although other studies have not observed such an association, e.g. [48]. Recently, an international effort to collect information on translocation frequencies in normal, healthy subjects showed a statistically significant increase in translocations among cigarette smokers compared to non-smokers [49].

Here we investigated potential interactions between maternal cigarette smoking during pregnancy, genetic susceptibility, and race, and evaluated the influence of smoking and susceptibility on translocations and other types of chromosome aberrations in mothers and their newborns. Chromosome aberrations were evaluated by simultaneously painting chromosomes 1, 2, and 4 in red and chromosomes 3, 5 and 6 in green. Genetic susceptibility was assessed using the *in vitro* bleomycin assay, and tobacco smoke exposure was assessed by several approaches including the use of a self-reported questionnaire, and the measure of serum cotinine levels in maternal blood samples.

2. Materials and Methods

2.1. Research subjects

Two hundred thirty-nine healthy pregnant female patients between the ages of 15 and 43 were recruited for the University of Pittsburgh Prenatal Exposures and Preeclampsia Prevention (PEPP) study [18]. Following informed consent, patients who volunteered for the study completed detailed questionnaires that were administered by trained interviewers. The questionnaires provided information about tobacco exposure, alcohol use, diet and nutrition, physical activity, race and socio-demographics. Conduct of the study was approved by and performed in accordance with the Institutional Review Boards of both the University of Pittsburgh/Magee-Womens Hospital and the Lawrence Livermore National Laboratory.

2.2. Maternal and newborn blood samples

Peripheral blood samples from each mother and newborn (obtained from the fetal side of the placenta) were collected post partum in sterile vacuum tubes containing sodium heparin anticoagulant and sent at 4°C by overnight courier to Livermore. Samples were packed in water ice such that the blood tubes did not come into direct contact with the coolant.

2.3. Cell culture for chromosome painting

Peripheral blood cultures were established by adding 0.8 ml whole blood to 10 ml of RPMI 1640 (Gibco BRL) supplemented with 15% fetal bovine serum, 2 mM L-glutamine,

penicillin/streptomycin (100 U/ml and 100 µg/ml final concentration, respectively), 1% heparin and 2% phytohemagglutinin. Cultures were incubated at 37°C in a 5% CO₂ atmosphere. At 44 hours, Colcemid was added at a final concentration of 0.1 µg/ml. Cultures were harvested 4 hours later by treatment with hypotonic solution (0.075 M KCl) for 30 min at 37°C followed by three fixations in methanol: acetic acid 3:1 (v/v). The fixed cells were dropped onto slides as described previously [50], taking care to minimize the presence of cytoplasm. After air-drying, the slides were stored at -20°C in a N₂ atmosphere and in the presence of a desiccant.

2.4. In vitro bleomycin susceptibility assay

We used the well-established and validated protocol of Spitz et al. [51] and Hsu et al. [52]. Briefly, blood was cultured for 72 hours as described above. Five hours before harvesting, bleomycin (30 µg/ml) was added. One hour prior to harvest, Colcemid (0.1 µg/ml) was added to arrest cells in metaphase. The cells were harvested as described above, then dropped onto slides, stained with Giemsa, and at least 50 cells per subject were analyzed microscopically for structural chromosome aberrations.

2.5. Chromosome painting probes

Chromosomes 1, 2, and 4 were painted red, and chromosomes 3, 5, and 6 were simultaneously painted green by using probes we developed and produced for this work [53], or by using commercially available probes (Cytocell, Inc). This combination of probes detects 56% of all simple chromosome exchanges, i.e., two-way translocations and dicentrics with paired fragments. One painted metaphase cell therefore yields the same amount of data as 0.56 whole genome “cell equivalents” [54].

2.6. Chromosome painting analyses

Trained observers scored cells and a second observer reviewed all abnormal cells for consistency. Slides were coded before scoring to prevent observer bias and were observed using a Zeiss Axioskop or Axiophot fluorescence microscope with a triple band pass filter for Texas Red, FITC, and DAPI (Chroma Technology). All abnormal cells were photographed using Kodak Ektachrome 400 Elite slide film or with digital cameras to resolve ambiguous aberrations. Metaphase cells were scored if they met the following criteria: (a) the cells appeared to be intact, (b) the centromeres of all chromosomes were readily identifiable with a single band pass DAPI filter set, (c) the centromeres of all the painted chromosomes were present (i.e., 6 centric pieces painted red and 6 centric pieces painted green), and (d) the hybridization label was sufficiently bright along the entire length of each chromosome to detect exchanges between painted and unpainted chromosomes. All aberrations were initially classified according to the Protocol for Aberration Identification and Nomenclature Terminology (PAINT) system [55]. Subsequent to the initial scoring, the data were re-categorized in two ways: (1) number of cells with one or more translocations of any type, and (2) classical translocations, in which reciprocal and apparently non-reciprocal translocations were each considered as a single translocation. For all three methods of counting translocations, the frequency of translocations per 100 cell equivalents (CEs) was determined [56].

2.7. Biochemical analysis of smoking exposure

Analysis of maternal blood samples for the concentration of cotinine was performed by gas chromatography-mass spectrometry (GC-MS) with mass-selective detection using methodology as previously described by [57]. The methods for solid-phase extraction of cotinine were those used in [58]. These analyses were performed in the Graduate School of Public Health, San Diego State University. CD3Cotinine (Sigma-Aldrich, 200 ng) was used

as the internal standard. The limit of detection was approximately 0.16 ng/ml and the limit of quantitation was approximately 1.25 ng/ml for cotinine (± 0.2 ng/ml standard deviation; variance = ± 0.04 ng/ml). Cotinine values (Fig. 1) were non-normally distributed, with a majority of values below 2.5 ng/ml. The mean cotinine value was 14.06 ng/ml with a SD of 23.68. The median cotinine value was 2.45 ng/ml, with an overall range of 0 to 131.1 ng/ml.

2.8. Statistical methods

Descriptive statistics (mean, standard deviation, median and range for continuous variables, frequency and percentage for categorical variables) were calculated for age, gender, mothers' education, and household income. The response variables, i.e. translocations and other types of chromosome aberrations, were summarized by frequency and percentage for mother and newborn respectively. The Fisher exact and the Kruskal-Wallis tests were used to assess imbalances in age, mother's education, household income, cigarettes per day, maternal bleomycin sensitivity, and newborn bleomycin sensitivity across the different racial groups. The Wilcoxon signed rank test was used to compare bleomycin sensitivity between maternal and newborn populations. Associations between the aberration types and potential covariates were evaluated, including race, smoking status, mother's education, household income, entry age, cigarettes per day, and mother/newborn's bleomycin susceptibility. Since a normality assumption did not hold for the response variables, a generalized linear model was used to investigate the association between response variables and potential predictor variables. For this analysis we assumed a Poisson distribution for non-stable chromosome aberrations, and we assumed a negative binomial distribution for stable chromosome aberrations because the data suggested the possibility of over-dispersion under a Poisson model. Likelihood ratio tests were used to remove non-significant covariates from the multivariate generalized linear model. A significance level of 0.05 was used in all tests unless otherwise indicated.

3. Results

3.1. Demographics

The cohort from the larger PEPP study consisted of 239 mothers and their 241 newborns (including two sets of twins). A summary of the demographic data, both discrete and continuous, collected from the questionnaire is presented in Table 1. Specifically, the variables considered in this study included newborn gender, maternal race, smoking status, number of cigarettes smoked per day, socioeconomic status, education, and age. The study participants self-identified race and consisted of 49.4% Caucasian Americans, 49.8% African Americans, and 0.8% Asian Americans.

The average entry age into the study was 24.9 ± 5.8 (mean $\pm$ S.D.) years and 42.7% of the mothers self-reported as smokers during the index pregnancy. Among the mothers, 45.8% of the Caucasian Americans and 39.5% of the African Americans reported they were smokers. The average number of cigarettes smoked per day was 2.65 ± 5.75 (mean $\pm$ S.D. for the Caucasian American mothers and 1.37 ± 3.17 (mean $\pm$ S.D.) for the African American mothers. Within the subset of the entire study population that reported smoking, the average number of cigarettes smoked per day was 7.57 ± 6.35 (mean $\pm$ S.D.) with a range of 0.1 to 30. There was substantial variation among the socioeconomic status levels and education reported for the African American and Caucasian American populations. As expected, the gender of the newborns was evenly distributed between males and females in all groups examined.

Due to the small number of Asian American study participants, this race category was omitted from those statistical analyses that evaluated associations between chromosomal

aberrations and the variables listed in Table 1. The resulting final study cohort consisted of 237 mothers, 119 Caucasian Americans and 118 African Americans, respectively and their 239 newborns.

3.2. Tobacco exposure assessment

Serum cotinine values measured in a representative subgroup of 140 PEPP maternal subjects using a sensitive GC-MS method were compared to a wide range of self-reported tobacco exposure measures collected at the PEPP baseline interview [57,58]. The results of these analyses indicated that, among measures of active smoking, the reported number of cigarettes smoked prior to pregnancy had the strongest association with plasma cotinine levels measured in the baseline sample. The Spearman's rank correlation coefficient was 0.623 ($p < 0.001$) and when cotinine quartiles were substituted for individual values, a clear dose-response pattern emerged for mean cigarettes per day before pregnancy: Q1 = 2.37, Q2 = 3.48, Q3 = 6.93 and Q4 = 17.1. An analysis of variance performed on these data was also highly significant ($f = 25.85$ with 3/136 d.f., $p < 0.001$; Fig. 1). Similarly, the total number of hours of exposure in the home was used as the passive tobacco exposure variable, and this revealed the strongest association to plasma cotinine ($r = 0.256$, $p = 0.002$). When cotinine quartiles were used, a clear dose-response pattern emerged for hours per week of exposure in the home: Q1 = 6.44, Q2 = 16.17, Q3 = 20.97 and Q4 = 32.24 (ANOVA: $f = 5.27$ with 3/134 d.f., $p = 0.002$). When the best active and passive self-report variables were placed in the same regression equation, the active exposure variable remained significant ($p < 0.001$) and the passive exposure variable became non-significant ($p = 0.383$). This result suggests that significant confounding occurs between the active and passive self report variables ($r = 0.41$, $p < 0.001$) and that the self-reported maternal active smoking variable is the single strongest predictor of cotinine levels.

3.3. Bleomycin sensitivity

Chromosome damage induced by bleomycin is commonly used and well-validated marker for genetic susceptibility [51,52]. There is considerable variation in sensitivity among lymphocytes of the general population to *in vitro* induced damage [39,52] with sub-groups that display sensitivity or relative resistance to the induction of damage by bleomycin. In this study, bleomycin sensitivity was assessed in the peripheral lymphocytes of the maternal and newborn populations. Their descriptive statistics were stratified by maternal race and are presented in Table 2. The bleomycin-induced damage in the maternal population had a mean value of 0.37 ± 0.27 (mean $\pm$ S.D.) breaks per cell. There was no significant difference in this value between the Caucasian American and African American populations. The bleomycin-induced damage in the newborns did not vary significantly between Caucasian American and African American groups and had an average value of 0.15 ± 0.14 total breaks per cell. Hsu and Spitz have described 12% of the general population as being hyper-sensitive and 23% as being moderately sensitive to bleomycin [39,52]. In the present study, approximately 75% of the mothers and newborns had < 0.5 and < 0.2 breaks per cell, respectively and approximately 88% of the mothers and newborns had < 0.7 and < 0.3 breaks per cell, respectively. While there was no difference in susceptibility to bleomycin between the African American and the Caucasian American populations, there was a significant difference in susceptibility between mothers and their newborns ($p < 0.0001$) as illustrated in Fig. 2.

3.4. Chromosome aberration data and the study variables

Table 3 provides spontaneous chromosome aberration frequencies, as measured by chromosome painting, by race and smoking for mothers and newborns. No significant differences were observed between smokers and nonsmokers for mothers or newborns, and there were no statistically significant differences between the races for mothers or newborns.

However, mothers had significantly more translocations than newborns, which is consistent with previous observations of an age effect, e.g. [46,49,59]. Mothers also had significantly more dicentrics and fragments than newborns ($p < 0.0001$), again consistent with earlier observations [46]. The distributions of subjects according to their frequencies of translocations, dicentrics, and fragments are provided for newborns in Table 4 and mothers in Table 5.

To adjust for multiple comparisons, a significance level of 0.005 was used. After removing non-significant covariates by using log likelihood ratio tests, the associations between aberrations and covariates were obtained (Table 6). Whereas spontaneous translocations, dicentrics and fragments (measured by chromosome painting) were all associated with newborns' bleomycin sensitivity, there were no significant associations between any covariate and aberrations detected in the mothers (data not shown). Associations between newborn bleomycin sensitivity and the three measures of translocations (classical, PAINT, and frequency of cells with translocations) were all highly significant ($p = 0.002$). Newborn bleomycin sensitivity also correlated significantly with the presence of dicentrics and fragments as measured by painting ($p = 0.0007$). However, when evaluated by race, the Caucasian American newborn group demonstrated an association between bleomycin sensitivity and dicentrics and fragments ($p = 0.0002$), but there were no such significant associations for the African American newborn group.

4. Discussion

This report demonstrates for the first time an association between genetic susceptibility, as measured by a bleomycin challenge assay, and the frequency of spontaneous chromosome aberrations including translocations measured by chromosome painting, in a cohort of human newborns. We also show that peripheral blood lymphocytes from newborns, as a group, are inherently more resistant to bleomycin-induced damage than peripheral blood lymphocytes from their mothers.

A positive association was observed in newborns between sensitivity to bleomycin-induced chromosome damage and the frequency of aberrations measured by painting, i.e. translocations, dicentrics and fragments. When this association was analyzed by racial group, a positive association was observed in the Caucasian American newborns between the unstable events (dicentrics and fragments) and bleomycin sensitivity, but this association in the African American group was not statistically significant. Thus, the Caucasian American newborns appear to be responsible for the association in the group as a whole. No differences in translocation frequencies were observed between Caucasian American and African American newborns. These observations suggest that the Caucasian American newborn cells may be accumulating chromosome damage from endogenous and exogenous exposures during gestation while the cells from the African American newborns are not. It is possible that smoking contributed to this observation in Caucasian American newborns based on the observation that Caucasian American mothers smoked more than African American mothers. However, given that the difference in smoking between races was not statistically significant, this factor would not be driving the outcome and instead might only be one of several contributing factors. Thus the difference in observed susceptibility between the races could be due to initiating events, including recognition and/or repair of induced damage. This difference between the races merits further investigation into the underlying biology to determine if there is a mechanistic explanation.

Approximately 23% of the general population has been reported to be moderately sensitive or hypersensitive to bleomycin [39,52]. In the present study, using the published criteria by Hsu and Spitz [51,52] of < 0.8 breaks per cell, 6% of the mothers displayed moderate or

hypersensitivity while 94% were relatively resistant to bleomycin-induced chromosomal breaks, and 76% of the maternal population had fewer than 0.6 breaks per cell. On the whole, our results had lower values compared to expectations based on these earlier reports.

The newborn data were even more dramatic when evaluated in the context of expected resistant and susceptible populations of 77% and 23%, respectively. There was only one newborn for whom the number of breaks per cell exceeded 0.8, which corresponds to a frequency of 0.4%. Had the results from the newborn group been consistent with expectations based upon the adult distribution, there would have been 183 newborns who were relatively resistant and 54 who displayed moderate or hypersensitivity. In the present study the distribution of bleomycin sensitivity in the newborns is markedly different from their mothers.

This report indicates a difference in bleomycin sensitivity based on age. The frequency of breaks per cell in the newborn population was significantly lower than observed in the maternal cohort. Specifically, the mothers in this study were significantly more susceptible to bleomycin-induced damage than the newborns. Some studies have shown that young children, and newborns in particular, are more sensitive to certain environmental exposures than adults [29,30,60,61]. However our results suggest the possibility of a protective mechanism against some forms of DNA damage *in utero*, which may be attributable to placental function.

The distribution of bleomycin-induced breaks in this study correlated with chromosome aberration frequencies in the newborns, indicating that bleomycin-susceptible newborns who are more susceptible to induced damage have more chromosome aberrations. This finding indicates that the distribution of susceptibility values in the newborns was narrow, at least when compared to their mothers (Fig. 2). Furthermore, this result suggests that susceptible fetuses may be more prone to adverse consequences of exposure than are non-susceptible fetuses.

Several studies have been published in which newborn cord blood was obtained from tobacco exposed and unexposed women. Bigbee *et al.* [18] showed an increase in V(D)J recombinase-mediated deletion of HPRT exons 2-3 in cord blood T-lymphocytes from newborns of mothers who smoked during pregnancy compared to those who did not smoke, or who quit smoking during pregnancy. Zalacain *et al.* [23] observed significantly elevated numbers of micronuclei in newborn cord blood samples from mothers who smoked compared to those who did not. Bocskay *et al.* [19] evaluated prenatal exposure to airborne polyaromatic hydrocarbons and chromosomal aberrations in newborn cord blood, suggesting that exposure may be associated with chromosome aberrations. While exposure levels were similar between the African American and Dominican populations that participated in their study, the authors speculated that susceptibility differences influenced the aberration frequencies.

In the present study, we did not observe an effect of smoking on spontaneous chromosome aberrations in mothers or their newborns. This is most readily explained by the low numbers of cigarettes per day that were smoked by these women. We previously reported an increase in stable aberrations (translocations plus insertions) in newborns whose mothers smoked during pregnancy compared to those who did not [46,59]. The study by Ramsey *et al.* [46] had a sample size of 91 and a significance value of $p = 0.029$. The sample size in the study by Pluth *et al.* [59] was relatively small ($N = 40$) and smoking behavior ranged from two cigarettes per month in the last two months of pregnancy to 15 cigarettes per day throughout pregnancy. The observed difference between mothers who smoked ($N = 4$) and those who did not was marginally significant ($p = 0.045$). The current study did not reveal associations

between spontaneous translocations in newborns and mothers who smoked during pregnancy. While the absence of an effect of age on translocation frequencies in the mothers appears to contradict published results [46,49], the range of maternal ages in the present work was narrow as it focused only on women of childbearing age, not the entire lifespan as did these other studies.

While the results of this study did not show an association between cigarette smoking during pregnancy and chromosome damage in mothers or their newborns, this work does clearly demonstrate that peripheral lymphocytes in pregnant women are significantly more susceptible to genetic damage than lymphocytes in newborns. This work also suggests that racial differences may have some importance when evaluating the effects of environmental exposure. Socioeconomic factors do clearly contribute to differences in cancer and disease outcome among racial groups, and there is increasing evidence supporting a role for biological factors. Studies such as the one presented here provide a foundation for asking biological questions to identify and characterize functional and mechanistic factors that may be contributing to health disparities. These results may be important when evaluating risks of environmental exposure to mothers and their fetuses.

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Fig. 1. Association between maternal serum cotinine levels and self-reported maternal smoking history (cigarettes per day) in 140 individuals. Cotinine levels were evaluated as described in the text.

Fig. 2. Distribution of individual responses to bleomycin exposure *in vitro* by mothers and newborns. The number of chromatid breaks per 100 cells showed remarkably different distributions between the two groups, a difference that was highly significant ($p < 0.0001$).

Table 1

Demographic data on the patient population

	African Americans (%)	Caucasian Americans (%)	Asian Americans (%)	Overall (%)
Maternal race	118 (49.37)	119 (49.79)	2 (0.84%)	239 (100%)
Gender of newborn				
female	56 (23.24)	62 (25.73)	1 (0.41)	119 (49.38)
male	63 (26.14)	58 (24.07)	1 (0.41)	122 (50.62)
Maternal smoking				
Yes	72 (30.13)	65 (27.20)	1 (0.42)	138 (57.74)
No	46 (19.25)	54 (22.59)	1 (0.42)	101 (42.26)
Socioeconomic status (household income)				
< \$10 K/Yr	42 (17.57)	39 (16.32)	2 (0.84)	83 (34.73)
\$10 to < \$35 K/Yr	37 (15.48)	44 (18.41)	0 (0)	81 (33.89)
\$35 to < \$50 K/Yr	4 (1.67)	6 (2.51)	0 (0)	10 (4.18)
\$50 to < \$75 K/Yr	2 (0.84)	9 (3.77)	0 (0)	11 (4.60)
\$75 K/Yr	2 (0.84)	11 (4.60)	0 (0)	13 (5.44)
Unknown	31 (12.97)	10 (4.18)	0 (0)	41 (17.15)
Education				
11 years	41 (17.15)	36 (15.06)	1 (0.42)	78 (32.64)
Diploma/Certificate	42 (17.57)	31 (12.97)	1 (0.42)	74 (30.96)
GED/Some college	27 (11.30)	24 (10.04)	0 (0)	51 (21.34)
Associate/Bachelor	6 (2.51)	22 (9.21)	0 (0)	28 (11.72)
Masters or above	2 (0.84)	6 (2.51)	0 (0)	8 (3.35)
Cigarettes smoked by mother per day (self-report)				
0	86 (36.29)	86 (36.29)	1 (0.42)	173 (72.38)
0 + to 5	19 (8.02)	12 (5.06)	0 (0)	33 (13.81)
5 + to 10	10 (4.22)	11 (4.64)	1 (0.42)	22 (9.21)
10 +	1 (0.42)	10 (4.22)	0 (0)	11 (4.60)
Mean $\pm$ SD	1.37 $\pm$ 3.17	2.65 $\pm$ 5.75	5.0 $\pm$ 7.07	2.04 $\pm$ 4.70
Age (mean $\pm$ SD)	23.35 $\pm$ 4.90	26.48 $\pm$ 6.18	18.5 $\pm$ 2.12	24.87 $\pm$ 5.79

Table 2

Number of breaks/cell as measured by the bleomycin challenge assay for mothers and newborns by race.

	African Americans	Caucasian Americans	All subjects
Mothers (mean $\pm$ SD)	0.37 $\pm$ 0.27	0.37 $\pm$ 0.27	0.37 $\pm$ 0.27
Newborns (mean $\pm$ SD)	0.16 $\pm$ 0.14	0.13 $\pm$ 0.14	0.15 $\pm$ 0.14

Table 3

Chromosome aberration frequencies as measured by chromosome painting for mothers and newborns by race and smoking status

		African Americans		Caucasian Americans		Asian Americans	
		mean	S.D.	mean	S.D.	mean	S.D.
Mothers							
All							
	classical translocations	2.33	2.56	2.39	2.34	1.50	2.12
	PAINT translocations	3.89	4.55	3.84	3.71	2.50	3.54
	Cells with translocations	2.12	2.36	2.08	1.97	1.50	2.12
	Dicentric	0.35	0.60	0.34	0.59	0.00	0.00
	Fragments	0.67	0.92	0.68	0.95	0.50	0.71
Smokers							
	classical translocations	2.37	2.59	2.44	2.35	0.00	NA *
	PAINT translocations	4.00	4.84	3.94	3.81	0.00	NA
	Cells with translocations	2.15	2.43	2.13	1.91	0.00	NA
	Dicentric	0.35	0.53	0.35	0.65	0.00	NA
	Fragments	0.67	0.73	0.80	1.09	0.00	NA
Nonsmokers							
	classical translocations	2.31	2.55	2.35	2.34	3.00	NA
	PAINT translocations	3.82	4.38	3.75	3.65	5.00	NA
	Cells with translocations	2.10	2.33	2.05	2.03	3.00	NA
	Dicentric	0.35	0.65	0.32	0.53	0.00	NA
	Fragments	0.67	1.02	0.58	0.81	1.00	NA
Newborns							
All							
	classical translocations	0.27	0.80	0.36	1.18	1.00	0.00
	PAINT translocations	0.40	1.19	0.53	1.95	1.50	0.71
	Cells with translocations	0.25	0.70	0.32	1.00	1.00	0.00
	Dicentric	0.06	0.24	0.08	0.38	0.50	0.71

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		African Americans		Caucasian Americans		Asian Americans	
		mean	S.D.	mean	S.D.	mean	S.D.
	Fragments	0.17	0.49	0.26	0.68	0.00	0.00
Smokers							
	classical translocations	0.26	1.05	0.38	0.85	2.00	NA
	PAINT translocations	0.36	1.51	0.53	1.21	1.00	NA
	Cells with translocations	0.23	0.91	0.35	0.75	1.00	NA
	Dicentrics	0.11	0.31	0.07	0.33	1.00	NA
	Fragments	0.06	0.25	0.29	0.53	0.00	NA
Nonsmokers							
	classical translocations	0.28	0.59	0.34	1.41	1.00	NA
	PAINT translocations	0.43	0.93	0.54	2.41	1.00	NA
	Cells with translocations	0.26	0.53	0.29	1.17	1.00	NA
	Dicentrics	0.03	0.17	0.09	0.42	0.00	NA
	Fragments	0.24	0.59	0.23	0.79	0.00	NA

* NA: Not applicable

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Table 4
Number of newborns with the indicated type of aberration, as determined by chromosome painting.

Frequency of aberrations per per 100 CEs	Classical translocations	PAINT translocations	Cells with translocations	Dicentrics	Acentric fragments
0	193	193	194	226	201
> 0 - 0.1	31	17	30	9	25
> 0.1 - 0.2	9	19	11	5	11
> 0.2 - 0.3	3	4	2	0	2
> 0.3 - 0.4	2	2	1	1	1
> 0.4	3	6	3	0	1
Total	241	241	241	241	241

Table 5

Number of mothers with the indicated type of aberration, as determined by chromosome painting.

Frequency of aberrations per per 100 CEs	Classical translocations	PAINT translocations	Cells with translocations	Dicentric	Acentric fragments
0	59	59	59	171	132
> 0 - 0.1	42	12	47	50	48
> 0.1 - 0.2	43	38	47	15	36
> 0.2 - 0.3	35	26	33	2	10
> 0.3 - 0.4	17	23	24	0	7
> 0.4 - 0.5	14	14	10	1	4
> 0.5 - 0.6	6	17	6	0	1
> 0.6 - 0.7	9	7	5	0	1
> 0.7 - 0.8	6	10	2	0	0
> 0.8 - 0.9	3	9	1	0	0
> 0.9	5	24	5	0	0
Total	239	239	239	239	239

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Table 6

Associations between newborns’ translocation frequencies measured by chromosome painting and covariates, adjusted for multiple comparisons.

Covariates	P values				
	Classical translocations	PAINT Translocations	Frequency of cells with translocations	Dicentrics	Fragments
All newborn susceptibility	* 0.0004	0.002	0.0005	0.0002	0.0007
Caucasian American newborn susceptibility	** NS	NS	NS	<0.0001	0.0002
African American newborn susceptibility	NS	NS	NS	NS	NS

* p value
** NS not significant.