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Fluorescence In Situ Hybridization Is Necessary to Detect an Association Between Chromosome Aberrations and Polycyclic Aromatic Hydrocarbon Exposure In Utero and Reveals Nonrandom Chromosome Involvement

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Abstract

Chromosome aberrations are associated with environmental exposures in infants and children. Recently we reported that prenatal exposure to airborne polycyclic aromatic hydrocarbons (PAHs) was significantly ($P < 0.01$) associated with stable aberration frequencies in cord blood from a subset of 60 newborns from the Columbia Center for Children's Environmental Health Prospective Cohort Study (Bocskay K et al. [2005]: Cancer Epidemiol Biomarkers Prev 14:506–511). To determine whether the environmental exposures may be targeting specific chromosomes and to compare various methods for measuring chromosome aberrations, we further evaluated this same subset of subjects composed of African-American and Dominican nonsmoking mother–newborn pairs residing in low-income neighborhoods of New York City, and exposed to varying levels of airborne PAHs. Chromosome aberrations were measured in cord blood lymphocytes, both by whole chromosome probe (WCP) fluorescence in situ hybridization (FISH) and traditional Giemsa-staining. Prenatal exposures were assessed by personal air monitoring. Breaks in chromosomes 1–6, as detected by WCP FISH, were nonrandomly distributed, underscoring the importance of appropriate chromosome probe selection to capture cytogenetic damage in response to exposure. FISH for stable aberrations was found to be a more sensitive method for detecting aberration frequencies associated with environmental exposures, when compared with FISH for unstable aberrations or Giemsa-staining for aberrations. Together, these results suggest that PAHs may be targeting specific chromosomes and highlight the importance of using the more sensitive detection methods to assess risk in populations with low levels of exposure.

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Keywords

chromosome aberrations; fluorescence in situ hybridization; Giemsa-staining

INTRODUCTION

Chromosome aberrations are associated with a risk of cancer in prospective studies [Hagmar et al., 1998] and are an intermediate step in the carcinogenic pathway [Bonassi et al., 2000]. They have been used widely as a biomarker of cancer risk in adults [Brogger et al., 1990; Chorazy, 1996; Motykiewicz et al., 1998; Huttner et al., 1999; Michalska et al., 1999], and to measure of the effect of environmental pollutants in infants and children [Ramsey et al., 1995; Sram et al., 1999; Pluth et al., 2000; Rossner et al., 2002; Neri et al., 2006a,b].

Chromosome aberrations may provide further support for the role of in utero exposures in cancer risk. Previous research has utilized chromosome aberrations as a tool to examine the relationship between in utero environmental exposures and cancer risk [Ramsey et al., 1995; Sram et al., 1999; Pluth et al., 2000; Rossner et al., 2002]. Two studies using fluorescence in situ hybridization (FISH) as a detection method were able to demonstrate a significant positive association between active maternal smoking during pregnancy and increased chromosome aberration levels measured in cord blood [Ramsey et al., 1995; Pluth et al., 2000]. Another two studies performed Giemsa-staining instead of FISH and were not able to demonstrate the effect of air pollution on levels of aberrant cells. The lack of association between environmental pollution and chromosome aberrations in these latter studies may be due to the relative insensitivity of Giemsa staining.

FISH has expedited and improved the ability of researchers to detect aberrations that are related most to cancer [Lucas et al., 1992; Tucker et al., 1993; Ramsey et al., 1995; Marshall and Obe, 1998; Mitelman, 2000]. Traditional cytogenetic methods of measuring chromosome aberrations rely on nonbanded Giemsa-stained preparations, and thus are unable to detect most stable aberrations such as deletions and translocations. It is these stable aberrations that are most closely associated with cancer risk, as unstable aberrations are lethal and not perpetuated [Tucker et al., 1994; Ramsey et al., 1995; Ellard et al., 1996; Marshall and Obe, 1998]. Until the development of FISH, unstable aberrations, detected by Giemsa-staining, were used as an indicator of levels of chromosome breakage. Chromosome breakage was used as a surrogate endpoint for the direct measurement of stable aberrations [Johnson et al., 1998, 1999; Mitelman, 2000].

Stable aberrations can be observed directly using whole chromosome FISH probes designed to hybridize to chromosome-specific sequences. By labeling probes in two or more colors, multiple chromosome pairs may be examined at one time. Exchanges can then be recognized between any two chromosome arms fluorescing in a different color, and deletions can be identified by changes in arm ratio and size. FISH also can detect small or subtle stable aberrations that would not be seen by traditional staining techniques. Scoring of abnormalities is also much more rapid, as it does not require analysis of banding patterns, and can be done by scorers with less training. It is therefore possible to analyze large numbers of cells (>1,000) per subject, increasing the statistical power to detect the low frequencies of biologically relevant chromosome damage expected in low-dose environmental exposures [Lucas et al., 1992; Tucker et al., 1993; Ramsey et al., 1995; Marshall and Obe, 1998].

To estimate all exchanges in the genome, not only those detected using differently colored chromosomes, a statistical correction was developed to include the number of aberrations

occurring in “nonpainted” chromosomes. It was based on the theory that the probability of an aberrant event occurring in any particular chromosome is determined by its DNA content [Lucas et al., 1992; Tucker et al., 1993]. This theory has been substantiated in some radiation and chromosome aberration studies that have demonstrated a linear relationship between DNA content and breakpoints, i.e., larger chromosomes are subject to more breaks [Puerto et al., 1999b; Johnson et al., 1999; Sachs et al., 2000]. However, multiple studies have demonstrated that chromosome aberrations induced with radiation and chemicals are not randomly distributed by chromosome size [Knehr et al., 1996; Stephan and Pressl, 1997; Barquinero et al., 1998; Smith et al., 1998; Wu et al., 1998; Zhang et al., 1998a,b, 2005; Eastmond et al., 2001; Verdorfer et al., 2001; Zhu et al., 2002; Beskid et al., 2006]. Studies have consistently shown that chemical exposures generate nonrandom chromosome damage, but these have been limited in the scope of exposures and the types of chromosome probes utilized [Smith et al., 1998; Wu et al., 1998; Zhang et al., 1998a,b; Eastmond et al., 2001; Verdorfer et al., 2001; Zhu et al., 2002; Beskid et al., 2006], with the exception of a recent pilot study by Zhang et al. [2005]. Their work showed that occupational exposure to benzene produced nonrandom chromosomal damage by examining all 24 human chromosomes with a special type of FISH (OctoChrome).

The demonstration of specific patterns of chromosome damage following exposures indicates that chromosome aberrations may provide a chemical-specific fingerprint of exposure [Crane et al., 1996; Smith et al., 1998; Zhang et al., 1998a,b, 2005; Alexander et al., 2001; Eastmond et al., 2001; Beskid et al., 2006]. Characteristic chromo-some rearrangements in leukemia patients have been associated with past exposure to tobacco smoke, solvents, and pesticides [Cuneo et al., 1992; Crane et al., 1996; Davico et al., 1998; Alexander et al., 2001]. Polymorphisms in certain genes involved in the metabolism of various environmental chemicals also have been associated with particular chromosome alterations [Larson et al., 1999; Wiemels et al., 1999].

The present study was conducted in a population of newborns exposed in utero to polycyclic aromatic hydro-carbons (PAHs) from ambient and indoor urban environmental pollution. We compared the frequency of chromosome aberrations measured by FISH with multiple whole chromosome probes (WCPs) and Giemsa-stained chromosome preparations. The purpose was to determine the correlation between the two methods and the specificity of chromosome damage in response to a specific environmental exposure.

MATERIALS AND METHODS

Study Population

The Columbia Center for Children's Environmental Health (CCCEH) cohort study population is composed of more than 600 African-American and Dominican mother–infant pairs. Sixty newborns were randomly chosen for chromosome aberration analysis. The subset of newborns who underwent chromosome aberration analysis did not differ from the total population with respect to airborne PAH levels. A detailed description of the study population and exposure assessment methods, used in this cohort, has been previously published [Bocskay et al., 2005] and is briefly outlined here.

Exposure Assessment

Air Monitoring—Personal air monitors were utilized to estimate individual exposures for 48-hr during the 3rd trimester of pregnancy. Concentrations of eight carcinogenic PAHs (benz[*a*]anthracene, benzo[*a*]pyrene, benzo[*b*]fluoranthene, benzo[*g*,*h*,*i*]perylene, benzo[*k*]fluoranthene, chrysene, disben[a,h] anthracene, indeno[1,2,3-*cd*]pyrene) were

determined at the Southwest Research Institute (San Antonio, TX), using gas chromatography/mass spectroscopy.

Cord Blood Culture

One milliliter of cord blood was placed in a heparinized vacutainer and used for the culture of phytohemagglutinin-stimulated lymphocytes, using standard techniques [Bocskay et al., 2005]. Replicate cultures were performed for each cord blood sample. Lymphocytes were cultured for 72 hr at 37°C, at which time the maximum number of cells reached metaphase. Colcemid (0.1 mL) was added to each culture, 45 min before harvesting. Following treatment of cells with hypotonic KCl and fixation in 3:1 methanol/acetic acid, metaphase spreads were prepared by dropping the fixed cells on clean wet slides.

FISH Procedure

The WCPs were purchased from Cytocell (Adderbury, Oxfordshire, UK) as a kit (Chromoprobe-M), containing coverslips coated with reversibly bound biotin and digoxigenin-labeled DNA probes for human chromosomes 1–6. Chromosomes 1–6 account for 38.8% of the DNA in the human genome. In combination, these six pairs are more likely to experience aberrant events than any other combination of six pairs of the remaining individual chromosomes, under the theory that the probability of a break(s) occurring in any particular chromosome is based on its DNA content. The remaining 17 chromosome pairs comprise only 61.2% of the total genome.

FISH was carried out as described in Bocskay et al. [2005]. All metaphases were scored using a 60× oil objective on a Nikon fluorescence microscope equipped with a filter wheel and a triple-band pass filter, which allows for concurrent visualization of chromosomes 1, 2, and 4 as red (Cy3), 3, 5, and 6 as green (FITC), and the remaining chromosomes as DAPI-counterstained (blue). Criteria for the scoring of metaphases included unbroken cells with good spreading, complete visualization of all 12 painted chromosomes and their centromeres, and satisfactory intensity of the probe signal. Ambiguities were resolved by image capture analysis using the Cytovision system from Applied Imaging (San Jose, CA). We recorded the coordinates for all cells with aberrations, as well as the types of aberrations and chromosomes involved.

All slides were scored by the same individual over a 3-year period. Scoring was performed without the knowledge of the exposure status of the subject. Reliability was determined by having this individual rescore randomly-selected aberrant and normal metaphases. Aberrant metaphases were chosen from scoring sheets, where coordinates for each metaphase were recorded, and normal metaphases were randomly chosen from slides. Metaphases chosen for review had been originally scored at different times over the 3-year study period to account for the scorer's reliability over time. The scorer was then given these coordinates for rescoring, blinded to their original identification (i.e., normal or aberrant). The reliability of the scorer to detect aberrant and nonaberrant metaphases was 90%.

Chromosomes 1–6 were identified by their color (red for 1, 2, and 4, and green for 3, 5, and 6), size, and relative arm length. For example, translocations were documented when two differently colored chromosome segments formed an aberrant chromosome, while deletions were recognized when one chromosome of the chromosome pair (for chromosomes 1–6 only) was markedly shorter in either arm compared to the other. Fragments were reported in chromosomes 1–6 when a small broken “painted” chromosome segment with no discernible centromere was visible.

To be consistent with prior research [Ramsey et al., 1995; Pluth et al., 2000], 1,800 metaphases, or 1,000 whole-genome equivalents, were scored per subject. “Whole-genome

equivalent" refers to the correction factor developed by Lucas et al. [1992] that is applied to stable aberration frequencies detected by single-color FISH to adjust for those aberrations occurring in chromosomes that are not painted by the FISH probes and those aberrations involving chromosomes painted with the same color. The correction factor assumes that breaks are dependent on DNA content. We adapted the Lucas correction factor [Lucas et al., 1992] to include the additional paint color utilized in this study (Lucas used only single-color FISH):

$$F_p = 2.05\left(f_r f_b + f_g f_b + f_r f_g\right) F_G$$

where F_p is the measured aberration frequency as detected by FISH; f_r (red), f_b (blue), and f_g (green) are the fraction of the genome painted based on DNA content; and F_G is the expected total aberration frequency. The fraction of the genome represented by chromosomes 1–6 was determined from the relative length of each chromosome [Bergsma, 1978]. The whole genome equivalent correction factor for this study was 1.78; thus, $F_G = 1.78F_p$.

Stable aberrations included balanced and unbalanced translocations, breaks, deletions, and insertions. Stable aberration frequency per subject was the number of stable aberrations/total normal metaphases counted for that subject, multiplied by the whole genome correction factor (1.78) for dual color FISH for chromosomes 1–6. Fragments were scored as unstable aberrations. Unstable aberration frequency per subject was the number of fragments/total of normal metaphases counted for that subject, divided by the correction factor of 0.388. This correction factor differed from that used for stable aberrations because unstable aberrations, or fragments, are individually based chromosome events; i.e., fragments are not exchanged with other similarly painted chromosomes, and so only those chromosomes stained with DAPI (blue) are unaccounted for during microscopy. Thus, 38.8% of the total genome was painted by FISH probes. The remaining 61.2% of fragments occurring in DAPI-stained chromosomes, fragments of which were not detected, were accounted for by the correction factor. Breakpoint frequency for each type of aberration was calculated as total number of breaks per chromosome/total number of metaphases scored in the total population (108,001).

Giemsa-Staining Procedure

Fifty-five samples were evaluated using Giemsa-staining. Five subjects did not have sufficient remaining metaphases, once FISH was completed, to permit Giemsa-staining to be performed. Unbanded Giemsa-stained slides were prepared by heating the prepared slides with well-spread metaphases for ~45 min in a 90°C oven. After the slides were removed from the oven and returned to room temperature, they were stained with a solution of 4:1 GURR Buffer (Gibco™ Invitrogen, Carlsbad, CA)/ Wright stain (Fisher Scientific International, Hampton, NH) for ~1 min. The slides were rinsed with deionized water and dried. The Giemsa-stained slides were viewed with light microscopy and a 60× objective. Two hundred metaphases per subject were scored.

Aberrations detected with this method are chiefly unstable aberrations, i.e., chromatid breaks, gaps, rings, and dicentrics; the types and number of aberrations per cell were recorded. However, Giemsa-staining does not afford easy recognition of specific chromosomes. As such, chromosomes with aberrations were not identified by number. Thus, Giemsa-detected aberration frequency per subject is the total number of aberrations divided by the number of normal cells counted for that subject. No correction factor was needed, as aberrant events are equally visible in all chromosomes. Overall aberration frequencies by

type were the total number of specific aberrations divided by total number of metaphases scored for the whole study population (10,743).

Statistical Analyses

A detailed description of the statistical analyses used for evaluating the aberration frequencies detected by FISH and total PAHs in air has been previously published [Bocskay et al., 2005]. The Spearman rank correlation was used to evaluate the correlation between aberration frequencies detected by FISH (stable and unstable) and Giemsa-staining and total PAHs in air.

Regression analyses were performed to evaluate the association between PAHs in air (log transformed to approximate the normal distribution) and aberration frequencies (stable aberrations square root transformed to approximate the normal distribution; unstable aberrations and Giemsa-detected aberrations dichotomized). Unstable and Giemsa-detected aberration frequencies were dichotomized as absence vs. presence of unstable aberrations. Specifically, linear regression was applied to the continuous outcome variable (stable aberration frequency) and logistic regression was used for the dichotomous outcome variables (unstable aberration frequency and Giemsa-detected aberration frequency).

The observed number of breaks per chromosome, instead of aberrations per chromosome, was used to evaluate the pattern of chromosome damage, because breaks are involved in both stable and unstable aberrations. For this component of our study, we were not concerned with the stability of the aberration, but rather which chromosome the aberration occurred in. The expected number of breaks was calculated from the product of total breakpoints and the percentage of the total genome each chromosome (1–6) represents. The difference between the observed pattern of chromosome involvement in breakpoints and the expected pattern of chromosome involvement was then assessed by the χ^2 test. All tests were two-tailed. All analyses were performed using the SAS® System, Version 9.0 (SAS Institute, Cary, NC).

RESULTS

Mean aberration frequencies detected by FISH in all 60 subjects were 0.58% (0–1.78%) for stable aberrations and 0.16% (0–0.88%) for unstable aberrations, while the mean aberration frequency detected by Giemsa-staining was 0.38% (0–2%) for 55 subjects. Figure 1 presents the distribution of stable and unstable aberration frequencies detected by FISH and aberration frequencies detected by Giemsa-staining in the study population. This multivariate histogram depicts the variability in aberration frequencies among the 60 subjects, and shows the considerable null levels of unstable and Giemsa-stained aberrations. Forty-three percent (26) of the subjects had no detectable level of unstable aberrations detected by FISH and more than half of the subjects, 58% (32), had no Giemsa-detected aberrations, while only one subject had no stable aberrations detected by FISH.

As reported in Bocskay et al. [2005], mean stable aberration frequencies were almost 50% higher among African-American (AA) newborns than among Dominican (D) newborns. This significant difference in stable aberration frequency was not seen in either the unstable (AA = 0.14%, D = 0.17%, Wilcoxon Rank Sums, $P = 0.50$) or Giemsa-stained (AA = 0.33%, D = 0.45%, Wilcoxon Rank Sums, $P = 0.71$) aberration frequencies. Differential exposure to PAHs does not account for the difference in stable aberration frequencies, as the mean PAH concentrations measured by prenatal air monitoring did not differ significantly (Wilcoxon Rank Sums, $P = 0.76$) between African-Americans (3.65 ng/m^3) and Dominicans (3.72 ng/m^3). Ethnicity was not considered when comparing total PAHs in air and aberration frequencies detected by FISH or Giemsa-staining, because only stable aberration frequencies

were affected by ethnicity, and this difference cannot be explained by the exposure of interest.

Bivariate correlation analysis demonstrated a positive and significant correlation between stable aberration frequencies and total PAHs in air, but not for unstable or Giemsa-detected aberrations (Figs. 2a–2c). Dichotomizing unstable and Giemsa-detected aberration frequencies into no aberrations/aberrations in a logistic regression model also did not show any significant associations with total PAHs in air (for unstable: OR = 1.09, 95% CI = 0.53–2.24; for Giemsa-detected: OR = 1.07, 95% CI = 0.52–2.23), while linear regression of stable aberration frequencies (whole genome corrected) and total PAHs in air did reveal a positive and significant association ($\beta = 0.14$, SE = 0.05, $P = 0.01$).

Correlation analyses demonstrated that stable and unstable aberration frequencies detected by FISH were significantly correlated with one another (Spearman Rank Correlation Coefficient = 0.29, $P = 0.03$), and that stable (FISH) and Giemsa-detected aberration frequencies were not (Spearman Rank Correlation Coefficient = 0.03, $P = 0.83$). However, unstable (FISH) and Giemsa-detected aberration frequencies were significantly correlated (Spearman Rank Correlation Coefficient = 0.36, $P < 0.01$).

The majority of aberrations detected by FISH were translocations (46%), deletions (30%), and fragments (17%). Table I presents the breakpoint frequency by aberration type and chromosome or set of chromosomes (when it was impossible to determine in which specific chromosome the break occurred). The data reveal that chromosome damage was not predicted by chromosome size, as the breakpoint frequency was highest in chromosome 6, the smallest chromosome painted; and lowest in chromosome 1, the largest chromosome painted. The majority of Giemsa-detected aberrations were chromatid breaks (76%), followed by dicentrics (10%) and fragments (7%). A ring formation and one translocation made up the remainder. Table II presents the frequency of these aberrations in the study population.

Breakpoint analysis to test for the randomness of aberrations in the study population was performed only on FISH-prepared metaphases due to limitations in Giemsa-staining in identifying individual chromosomes. There were 312 breakpoints that could be distinguished by individual chromosome. Figure 3 shows the observed frequency vs. expected frequency based on chromosome length for these 312 breakpoints. It is apparent from Figure 3 that the observed breakpoint frequencies increased as chromosome size decreased, while expected breakpoint frequencies decreased as chromosome size decreased. The pattern of chromosome involvement was not randomly distributed based on DNA content ($P < 0.001$). When the breakpoint analysis was stratified by ethnicity, chromosomal damage in African-American and Dominican newborns also was not predictable by chromosome length ($P < 0.001$ for both groups). Moreover, linear regression was performed on stable aberration frequencies, without application of the whole genome equivalent correction factor, and total PAHs ($\beta = 0.10$, $P < 0.01$), with no affect on the strength or significance of the association between stable aberrations and PAHs.

DISCUSSION

We found a significant correlation between total PAHs in air and stable aberration frequencies detected by FISH, but not unstable aberration frequencies detected by FISH or Giemsa-staining. The considerable number of subjects (43–58%) without either unstable FISH-detected or Giemsa-detected aberrations may be explained by the 72-hr culture time of the cord blood. Unstable aberrations do not persist through multiple cell cycles, and therefore any damage that may have been detected could have disappeared by the time the

cells were fixed. Unstable aberrations detected by FISH and Giemsa-staining were correlated as expected, because both are similarly temporary in nature. However, there were fewer overall unstable aberrations detected by Giemsa-staining than by FISH.

Stable aberration frequencies detected by FISH did not correlate with Giemsa-detected aberrations. Giemsa-detected aberrations are mainly transient markers of cytogenetic damage, which are not perpetuated in subsequent cell divisions. Stable aberrations persist through multiple cell divisions, and can accumulate over time with continued exposure; therefore, they are inherently more sensitive measures of continuous low-dose exposure than Giemsa-detected aberrations [Tucker et al., 1994; Ramsey et al., 1995; Ellard et al., 1996; Marshall and Obe, 1998]. The lack of correlation between stable and Giemsa-detected aberration frequencies is biologically plausible, as they are measuring two different types of aberrations, which is exemplified in the data shown in Tables I and II. The frequencies of aberration types are noticeably different among FISH and Giemsa-stained chromosome preparations. Although significant, the correlation between stable and unstable chromosome aberrations detected by FISH was weak. This may reflect the nonrandom distribution of chromosome aberrations, since both stable and unstable aberrations were detected using the same set of WCPs.

The distribution of aberration types detected by FISH and Giemsa-staining in our study population was consistent with prior research examining cytogenetic damage due to age, environmental and occupational exposures, and lifestyle [Ramsey et al., 1995; Huttner et al., 1999; Verdorfer et al., 2001; Beskid et al., 2006]. Translocations were the most frequent type of aberration detected by WCP FISH in subjects occupationally exposed to either radiation, nitroaromates, acrylonitrile, ethyl benzene, or PAHs and their unexposed controls [Verdorfer et al., 2001; Beskid et al., 2006], and amongst different age groups [Ramsey et al., 1995]. Chromatid breaks were the most frequent type of aberration detected by Giemsa-staining in persons with environmental exposure to heavy metals and dioxins/furans and their unexposed controls [Huttner et al., 1999].

The lack of association between Giemsa-detected and FISH-detected stable aberration frequencies in this study population is consistent with prior research. Natarajan et al. [1992] generated chromosome aberrations in vitro in lymphocytes from two subjects using X-rays. At each radiation dose, translocation frequencies detected by FISH (WCP for chromosomes 1–4, 8, and X) were two- to threefold higher than dicentric frequencies detected by Giemsa-staining. The authors recommended using FISH for the detection of radiation damage to chromosomes, because it appeared to be a more sensitive method than Giemsa-staining for detecting exposures at low levels [Natarajan et al., 1992]. An in vitro study using a chemical challenge assay, instead of radiation, concluded that FISH was better able to distinguish differences in aberration frequencies at lower concentrations of chemicals than Giemsa-staining. When whole blood lymphocytes were treated with increasing doses of daunomycin, the differences in aberration frequencies observed in treated samples when compared with untreated controls were greater when scoring with FISH than when scoring with Giemsa [Ellard et al., 1996].

We report here that cord blood samples from a population of newborns exposed prenatally to multiple pollutants have a distribution of breakpoints within chromosomes 1–6 which is nonrandom and not directly related to the size of the chromosome. This evidence that cytogenetic damage does not depend exclusively on the size of the target DNA per chromosome is consistent with prior studies evaluating breakpoint distributions in lymphocytes or persons with exposure to X-rays, benzene, benzo[*a*]pyrene diol epoxide, and nitroaromatics [Knehr et al., 1996; Stephan and Pressl, 1997; Barquinero et al., 1998; Smith

et al., 1998; Wu et al., 1998; Zhang et al., 1998a,b, 2002, 2005; Eastmond et al., 2001; Verdorfer et al., 2001; Zhu et al., 2002; Beskid et al., 2006].

Differences in gene density between chromosomes has been suggested by other researchers as a possible explanation for the differential susceptibility to damage. Chromosome aberrations occur more frequently in euchromatin, where coding genes are located and undergoing active transcription [Palitti, 1998]. Breakpoints following in vitro treatment with etoposide were over-represented in euchromatic regions [Maraschin et al., 1990]. Gene-dense chromosomes are more sensitive to clastogens during transcription, as their DNA is more open [Surralles et al., 1997; Palitti, 1998]. However, in our study population, there was no association between gene density and aberrations. Chromosome 1 is the most gene-dense of the six chromosomes evaluated in this study, yet it had the fewest aberrations. Moreover, chromosomes 2–6 do not have extremely different gene densities from one another [Venter et al., 2001]. Thus, in this study population, gene density does not explain the differential susceptibility to the damage observed in chromosomes 1–6.

Other theories to explain the nonrandom distribution of chromosome aberrations in response to chemical exposure include differences between the ability of chromosomes to repair damage or their differential susceptibility to specific agents [Puerto et al., 1999a]. Each of these theories has support under the specific conditions in which the underlying observations were made [Maraschin et al., 1990; Sozzi et al., 1997; Surralles, 1997; Wu et al., 1998; Puerto et al., 1999a; Zhang et al., 1998a,b, 2002; Stillman et al., 2000; Eastmond et al., 2001; Stein et al., 2002; Zhu et al., 2002; Beskid et al., 2006]. Regardless of the mechanism driving the differential susceptibility to damage, the assumption that chromosome aberrations are being induced randomly, and the subsequent use of the whole genome correction factor, may not be appropriate for all exposures and/or populations. It should be noted that when we removed the whole genome equivalent correction factor from our analysis, the significance of the association between total PAHs in air and stable aberration frequencies in cord blood was unaltered.

This study has shown that stable FISH-detected and Giemsa-detected aberration frequencies were not correlated, and that only FISH had the sensitivity to detect those aberrations associated with low-level exposure to airborne PAHs. The current in vivo study demonstrated a nonrandom distribution of breakpoints within chromosomes 1–6 in cord blood from New York City newborns. The distribution of chromosome breakpoints has never been determined in a population with low levels of a known cytotoxic environmental exposure, such is the case with the CCCEH cohort. The findings of traditional Giemsa-staining not correlating with total PAHs in air and a nonrandom distribution of chromosome aberrations detected by whole chromosome FISH in our population underscores the importance of using a sensitive detection method, i.e., FISH, and choosing the appropriate chromo-some probes to capture cytogenetic abnormalities associated with low level environmental exposures.

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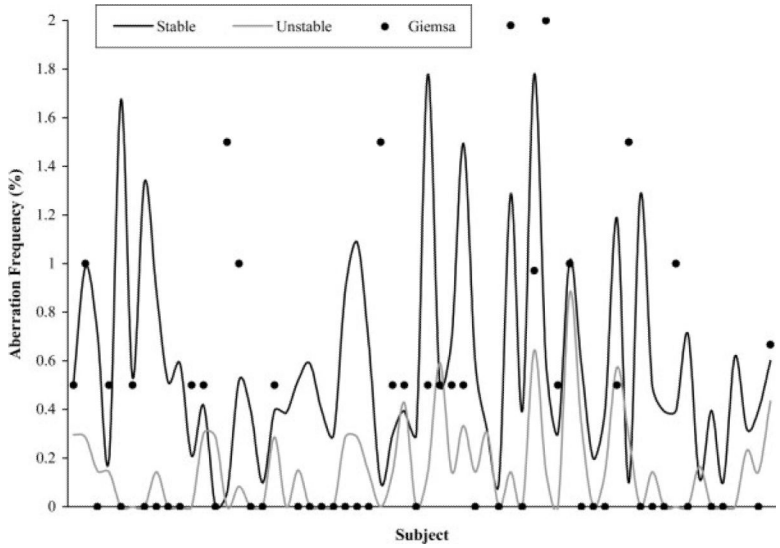

Fig. 1.

Distribution of stable and unstable aberration frequencies detected by FISH and Giemsa-detected aberration frequencies. Subjects were placed on the *x*-axis in ascending order of their subject ID number (number not shown).

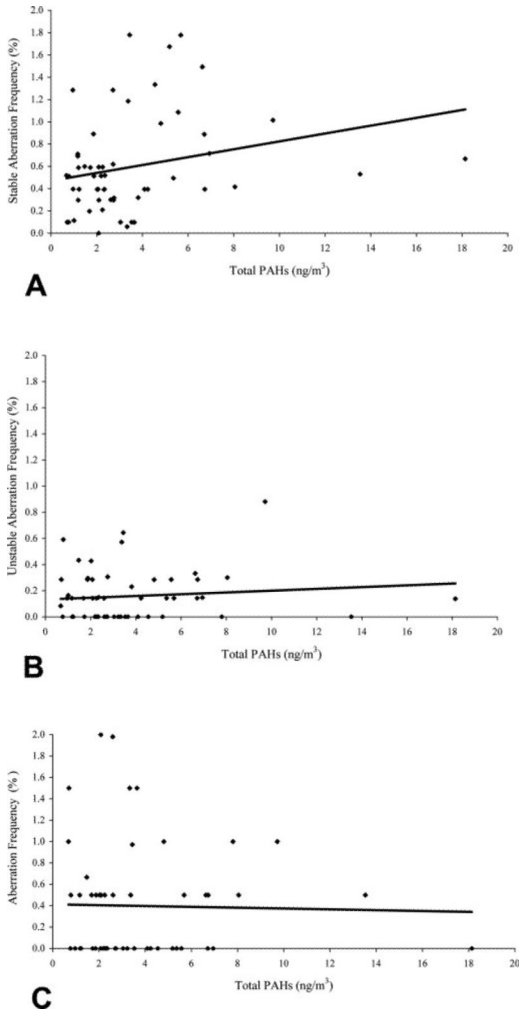

Fig. 2.

(**A**) Plot of stable aberration frequencies in cord blood detected by FISH and total PAHs measured by prenatal, personal 48-hr air monitoring. A linear trendline has been imposed on the data for visual purposes only. The Spearman Rank Correlation Coefficient was 0.35 (P = 0.01). (*B*) Plot of unstable aberration frequencies in cord blood detected by FISH and total PAHs measured by prenatal, personal 48-hr air monitoring. A linear trend-line has been imposed on the data for visual purposes only. The Spearman Rank Correlation Coefficient was 0.02 (P = 0.87). (**C**) Plot of aberration frequencies in cord blood detected by Giemsa-staining and total PAHs measured by prenatal, personal 48-hr air monitoring. A linear trendline has been imposed on the data for visual purposes only. The Spearman Rank Correlation Coefficient was 0.01 (P = 0.93).

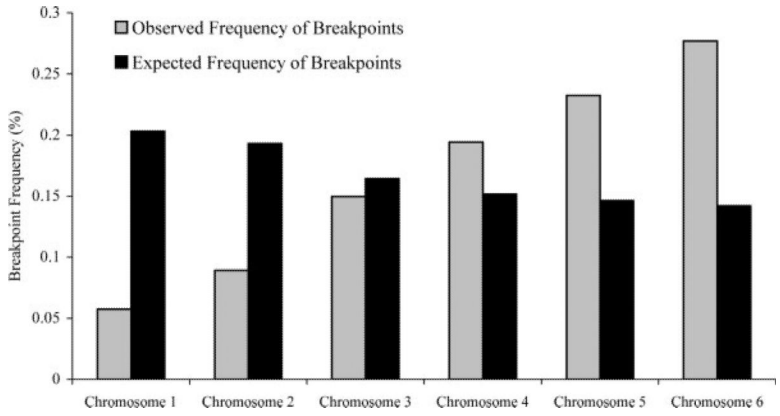

Fig. 3.

Observed vs. expected frequency (based on chromosome length) of chromosome involvement for 312 breakpoints detected by FISH. There was a significant difference between the observed and expected frequencies for individual chromosomes 1–6 (χ^2 test results, $P \ll 0.001$).

TABLE I

Breakpoint Frequency by Chromosome and Aberration Type for FISH-Detected Aberrations

	Chromosome(s)							
	1	**2**	**3**	**4**	**5**	**6**	**1,2,4**	**3,5,6**
Translocation	0.65	1.11	3.15	1.39	3.15	4.63	0.65	1.20
Deletion	0.65	0.74	1.11	3.43	2.13	1.94	0.09	0.37
Break	0.09	0.46	0.09	0.37	0.37	0.28	0	0
Insertion	0	0	0	0	0.28	0.28	0.19	0.09
Fragment	0.28	0.28	0	0.46	0.83	0.93	1.85	1.3
Total	*1.67*	*2.59*	*4.35*	*5.65*	*6.76*	*8.06*	*2.78*	*2.96*

Chromosomes 1, 2, and 4 were “painted” red and chromosomes 3, 5, and 6 were “painted” green, and were grouped together when it was impossible to determine which specific chromosome was involved in the aberration. Each value reflects the total number of breaks by aberration type (and overall) in a specific chromosome or set of chromosomes divided by the total number of FISH-prepared metaphases scored for the study population (n = 60).

TABLE II

Overall Aberration Frequency by Type for Giemsa-Detected Aberrations

Chromatid break	2.89
Dicentric	0.37
Fragment	0.28
Translocation	0.09
Ring	0.19
Total	*3.82*

Each value reflects the total number of aberrations by type (and overall) divided by the total number of Giemsa-stained metaphases able to be scored for the study population ($n = 55$).