CHROMOSOMAL ABERRATIONS IN CORD BLOOD ARE ASSOCIATED WITH PRENATAL EXPOSURE TO CARCINOGENIC POLYCYCLIC AROMATIC HYDROCARBONS

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Abstract

Molecular and traditional epidemiology studies have indicated a possible relationship between *in utero* environmental exposures and increased risk for childhood cancers, especially acute leukemias. Chromosomal aberrations have been associated with environmental exposures and cancer risk in adults. In order to more clearly define the association between prenatal exposures to carcinogenic polycyclic aromatic hydrocarbons (PAHs) and chromosomal aberrations, chromosomal aberration frequencies were measured in a subset of 60 newborns from the Columbia Center for Children's Environmental Health (CCCEH) Prospective Cohort Study. The subset was comprised of African-American and Dominican, non-smoking mother-newborn pairs residing in low-income neighborhoods of New York City, who were exposed to varying levels of airborne PAHs. Prenatal exposure was assessed by questionnaire, personal air monitoring during the third trimester, and PAH-DNA adducts in umbilical cord blood. Chromosomal aberrations were measured in cord blood lymphocytes by fluorescence *in situ* hybridization (FISH). PAH-DNA adducts were not associated with chromosomal aberrations. However, airborne PAHs were significantly associated with stable aberration frequencies in cord blood ($p < 0.01$). Moreover, stable aberration frequencies were significantly higher among African-American newborns compared to Dominican, despite no significant differences in PAH exposure. These results demonstrate for the first time an association between prenatal exposure to airborne carcinogenic PAHs and

chromosomal aberrations in cord blood, suggesting that such prenatal exposures have the potential to cause cytogenetic damage that has been related to increased cancer risk in other populations. If confirmed, this finding may open new avenues for prevention.

Introduction

Several lines of evidence suggest fetal susceptibility to carcinogens and that *in utero* exposure to environmental pollutants can result in carcinogenic DNA-adducts, chromosomal aberrations, and increased risk of childhood cancer [1-4]. The short latency period seen in pediatric leukemias suggests *in utero* initiation of cancer [5-7], as does the discovery of the diagnostic chromosomal aberrations, PML-RARA, CBFB-MYHII, TEL/AML, AML/ETO, and MLL gene fusions, in archived bloodspots of children who were diagnosed with ALL or AML months to years following birth [8-12]. Comparison of PAH-DNA adducts and cotinine in cord and maternal blood suggests differential fetal sensitivity to PAH and tobacco smoke [13;14]. Heightened fetal susceptibility to these carcinogens could result from higher rates of cell proliferation and differentiation, greater absorption or retention of xenobiotics, and/or less efficient detoxification, DNA repair, or apoptotic mechanisms [15-19]. Finally, although epidemiological studies have been inconsistent with respect to childhood cancer [20-22], a number of studies have linked prenatal tobacco smoke and PAH exposure to increases or qualitative differences in biomarkers such as cotinine, carcinogen-DNA adducts, somatic mutations, and chromosomal aberrations [1;2;23;24].

PAHs are pervasive environmental toxicants in ambient air resulting in large part from the incomplete combustion of fossil fuels. Multiple studies comparing genetic damage in persons living in polluted, industrialized urban areas with those living in less polluted, rural areas have found significantly increased levels of carcinogen-DNA and -protein adducts, sister chromatid exchanges, somatic mutation frequencies and/or chromosomal aberrations in adults [13;25-28].

Prospective cohort studies by the European Study Group on Cytogenetic Biomarkers and Health has validated chromosomal aberrations as a biomarker of cancer risk, especially for hematological malignancies [29]. Nested case-control studies in the Nordic and Italian cohorts demonstrated that chromosomal aberrations are an intermediate step in the carcinogenic pathway and are independent of exposure status, substantiating the role of chromosomal aberrations in cancer and indicating that they reflect both exposure and susceptibility [29;30]. Chromosomal aberrations in adults have been widely studied in non-occupational and occupational settings [28;29;31-33]. However, only two studies have measured chromosomal aberrations by FISH to monitor the impact of *in utero* environmental exposures; and sample sizes have been small (≤ 40 subjects) [1;2;34]. The present study was intended to address this gap.

Materials and Methods

Study Population The CCCEH cohort study population is comprised of more than 600 African-American or Dominican mother-infant pairs, as previously described [14;35]. The subjects reside in low-income, predominantly minority neighborhoods in Northern Manhattan and the South Bronx. These urban areas are densely populated and subject to varying levels of environmental PAHs from transportation and stationary sources including motor vehicles, diesel bus depots, residential heating, waste incinerators, and environmental tobacco smoke (ETS). African-American and Latino women, 18-35 years old, were recruited from New York Presbyterian Hospital, Harlem Hospital, or satellite clinics at 16 to 20 weeks of pregnancy during their prenatal visit. Enrollment was carried out so that the sample population under study was uniformly distributed across the urban area in order to capture variation in environmental exposures. Exclusion criteria included

active smoking, illicit drug use, diagnosis of diabetes (including gestational diabetes) or HIV as determined by responses to the eligibility screening questionnaire, or an initial prenatal visit after 20 weeks of gestation. Participating women signed a consent form approved by the IRB at Columbia University. All samples and data were coded and kept in locked storage units to protect confidentiality of study subjects. Women were compensated for their participation in each phase of the parent study. To be fully enrolled into the parent prospective cohort study, women had to have completed prenatal air monitoring.

Sixty newborns were randomly chosen for chromosomal aberration analysis. Demographic data for the subset and total population are presented in Table 1. The subset of newborns who underwent chromosomal aberration analysis did not differ from the present population with respect to airborne PAH concentrations or PAH-DNA adducts (see Table 2); however, they differed with respect to ethnic and residential distribution (see Table 1).

Exposure Assessment

Questionnaire Questionnaires were administered by trained, bilingual personnel in the women's homes during the third trimester in order to elicit information on demographics and history of active and passive smoking.[14;35].

Air monitoring Personal air monitors were utilized to estimate individual exposures. The women were equipped with small personal air monitoring devices for 48-hours during the 3rd trimester of pregnancy. As previously described [14;35], trained research personnel taught the women how to use the personal air monitoring device. The women were asked to wear the backpack during the day for 2 consecutive days and to

place the monitor near the bed at night. Motion sensors were placed in the backpacks of randomly selected women in order to assure compliance with the monitoring protocol. Vapors and particles of ≤ 2.5 μm in diameter were collected, and extracts were frozen until analysis. Concentrations of 8 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 using gas chromatography/mass spectroscopy. Quantification of PAHs was performed using calibration curves. Pre-established quality control measures were followed, and data determined to be questionable were not included in the statistical analysis. Briefly, flow rate, time, and completeness of documentation for each subject with respect to their personal air monitoring were evaluated for accuracy and given a numeric value of 0, 1, 2, or 3. Subjects' air monitoring results rated as 2 or 3 were considered to be of intermediate or unacceptable quality and their data was excluded from further analysis. Air exposure monitoring for PAHs was completed for the 60 subjects in the subset population. However, in 3 cases the monitoring data were of inadequate quality due to analytic problems, and were not included in the analysis.

Biomarkers Blood was obtained from the umbilical cord (the portion attached to the placenta) immediately after the placenta was delivered. Approximately 30 mL of venous cord blood was drawn and transported immediately to the laboratory for processing and separation. One mL of whole cord blood was reserved for FISH (see below). The remainder of the cord blood was then separated for additional studies performed as part of the CCCEH parent study. PAH-DNA adducts were measured using a

HPLC/fluorescence method that detects tetromers of benzo[a]pyrene (BP), a representative PAH, in DNA extracted from white blood cells (WBCs) in cord blood. This methodology is sensitive and specific for measurement of BP-DNA adducts in WBC [36] with a COV% of 12% (D. Tang, personal communication).

Cord blood culture One mL of cord blood was placed in a heparinized vacutainer and used for culture of PHA-stimulated lymphocytes, using standard techniques. Replicate cultures were performed for each cord blood. Lymphocytes were cultured for 72 hours at 37°C, at which time the maximum number of cells reached metaphase. 0.1 mL colcemid was added to each culture 45 minutes before harvesting. Following treatment of cells with hypotonic KCl and fixation in 3:1 methanol acetic acid, metaphase spreads were prepared by dropping on clean wet slides.

FISH procedure The whole chromosome probes used are available from Cytocell, Ltd. (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 almost 40% of the DNA content in the entire genome; thus theoretically, they are more likely to experience aberrant events than the other chromosomes, because the probability of a break(s) occurring in any particular chromosome is based on its DNA content. This has been seen in radiation research, which demonstrates a linear relationship between DNA content and breakpoints, i.e., larger chromosomes are subject to more breaks [37;38].

FISH was carried out according to the manufacturer's directions. Briefly, the prepared slides with well-spread metaphases were placed in a Coplin jar containing 2xSSC at room temperature for 2 minutes. The slides were then dehydrated for 2 minutes

at room temperature in 70%, 85%, and 100% ethanol, and rapidly dried. The Chromoprobe-M coverslips, the sample slides, and hybridization solution were pre-warmed to 37°C, 10 µL of hybridization solution was added to each sample slide, and the coverslip was immediately applied. The probes and target DNA were denatured on a hotplate at 75°C for 5 minutes and then incubated in a humid chamber overnight at 37°C. After removal of the coverslips, the slides were immersed in 0.4xSSC for 2 minutes at 72°C and then washed in 2xSSC with 0.5% NP-40 for 30 seconds. 50 µL L of detection reagent, mouse anti-digoxin green fluorophore conjugate mixed with streptavidin conjugate red fluorophore, was applied to each slide, glass coverslips were applied immediately, and the slides were incubated at 37°C in a humid chamber for 2-10 minutes. After washing, slides were immediately counterstained with 10 µL DAPI-Antifade solution and coverslipped [1;2].

All metaphases were scored by one individual, who was blinded to all demographic and exposure information until aberration data collection was completed, using a 60X 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 scoring of metaphases included unbroken cells with good spreading, complete visualization of all 12 painted chromosomes and their centromeres, and satisfactory intensity of probe signal. Ambiguities were resolved by image capture and analysis using an Applied Imaging Cytovision system. Coordinates were recorded for all cells with aberrations, as well as the type of aberration and chromosome involved. Chromosomes 1-6 were identified by

their color (red for 1, 2, and 4, green for 3, 5, and 6, and blue for the remaining chromosomes), 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 chromosome segment with no discernible centromere was visible.

In order to be consistent with prior research, 1000 whole genome equivalents, or 1800 metaphases, were scored per subject. "Whole genome equivalent" refers to the correction factor developed by Lucas et al. [38] 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. Application of the correction factor is based on the assumption that the probability of a break(s) occurring in any particular chromosome is dependent on its DNA content; i.e., larger chromosomes are subject to more breaks [37;38]. Whole genome equivalents for this study were determined by adapting the correction developed by Lucas et al. [38] to include the additional "paint" color utilized in this study rather than the single-color FISH used by Lucas et al.:

$$F_p = 2.05(f_r f_b + f_g f_b + f_r f_g)F_G$$

where F_p is 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 (38.8% for chromosomes 1-6); and F_G is the expected total aberration frequency. Fraction of the

genome for chromosomes 1-6 was determined from the relative length of each chromosome [39]. The resulting equation is:

$$F_G = 1.78F_p$$

and the whole genome equivalent correction factor for this study is 1.78.

Stable aberrations included balanced and unbalanced translocations, breaks, deletions, and insertions. Stable aberration frequency was the number of stable aberrations/total of normal metaphases counted, 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 was the number of fragments/total of normal metaphases counted, 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, so only those chromosomes stained with DAPI (blue) are unaccounted for during microscopy. Thus, as 38.8% of the total genome was "painted" by FISH probes, the remaining 61.2% of fragments occurring in DAPI-stained chromosomes, of which fragments were not detected, were accounted for by the correction factor.

Statistical Analyses The Wilcoxon Rank Sum test was used to analyze differences between groups defined by gender, ethnicity, smoking in the home by household members (yes/no) and passive smoking in the workplace (yes/no) with respect to continuous variables of exposure (PAHs in air and PAH-DNA adducts in cord blood), and outcomes (stable and unstable aberration frequencies). Spearman Rank Correlation was used to assess the relationship between the continuous exposure measures, as well as

maternal age, and the continuous outcome variables. These initial statistical analyses were performed using untransformed variables. Regression analyses were then performed to evaluate the association between PAHs in air (log transformed to approximate the normal distribution) or PAH-DNA adducts (categorized as high/low using the median of detectable values as the cutpoint and non-detectables as a reference group) and aberration frequencies (stable aberrations square root transformed to approximate the normal distribution; unstable aberrations dichotomized). Unstable aberration frequency was dichotomized as absence vs. presence of unstable aberrations, since more than 40% (26) of the subjects had no detectable level of unstable aberrations. Ethnicity (African-American/Dominican) was related to stable aberration frequencies. However, stratified linear regression analyses of total PAHs in air and stable aberration frequencies by ethnic group demonstrated similar beta values for African-Americans and Dominicans (0.12029 for African-Americans, 0.14915 for Dominicans) although the association was only significant for Dominican newborns ($p = 0.0228$, $p > 0.05$ for African-Americans). Additionally, inclusion of ethnicity in the regression analysis of stable aberration frequencies and total PAHs in air did not significantly change the standard error or p-value of the model. Therefore, ethnicity was not included in the final regression models. Maternal age was neither correlated with stable nor unstable aberration frequencies in cord blood, and was excluded from further analysis. Specifically, linear regression was applied to the continuous outcome variable (stable aberration frequency) and logistic regression was used for the dichotomous outcome variable (unstable aberration frequency).

Because of the high correlation between individual PAHs measured in air, a composite PAH variable was used, which was the sum of all eight carcinogenic PAHs measured in air listed in Table 2. Cronbach's coefficient alpha of 0.91, an average correlation of individual PAH measurements, indicated a high reliability of the composite variable.

Forty-eight subjects of the subset had an adequate amount of cord blood DNA for PAH-DNA adduct analysis. Of those, 26 newborns (54%) had non-detectable PAH-DNA adducts (< 0.25 adducts/10^8 nucleotides). Among the subjects with detectable levels of PAH-DNA adducts (22 newborns), the distribution of the biomarker was skewed. Detectable adduct levels were categorized as high/low using the median of detectable adducts (0.4 adducts/10^8 nucleotides) as the cut point, with non-detectable subjects as the reference group. All tests were two tailed. All analyses were performed using the SAS® System, Version 9.0.

Results

As in the parent study [14;35], there was variable prenatal exposure to PAHs in the subset as documented by personal air monitoring. Carcinogenic PAHs, including BP, were detected in all of the air samples. Detectable levels of PAH-DNA adducts were found in 46% of cord blood samples (n = 48). The air exposures and blood biomarkers ranged over several orders of magnitude. The mean and range of the exposure measures for total PAHs in air and PAH-DNA adducts in cord blood are shown in Table 2.

Bivariate correlation analysis revealed a positive and significant correlation between stable aberration frequencies, but not unstable aberrations, and total PAHs in air (Spearman correlation coefficient = 0.35, $p < 0.01$). Detectable PAH-DNA adducts

treated as a continuous variable were not significantly correlated with stable aberration frequencies (Spearman correlation coefficient = 0.03335, p = 0.8829). The Wilcoxon Rank-Sum test showed a small but non-significant increase in stable aberration frequencies in the "high" category compared to the "low" category for detectable PAH-DNA adducts, 0.56 vs. 0.45, respectively (p = 0.4810).

ETS exposure at home was common. Almost half of the women (45%) in the subset reported a smoker in the home, while only 7% reported ETS exposure at work. Levels of stable and unstable aberration frequencies were not significantly different between those newborns whose mothers reported ETS exposure in the home or at the workplace and those who did not (Table 3).

Mean stable aberration frequencies for African-American newborns were almost 50% greater than in Dominican newborns (p = 0.048) (see Table 3). This increase was not seen in unstable aberration frequencies. Exposure levels of PAHs in air and PAH-DNA adducts did not differ significantly between African-American and Dominicans (data not shown).

Regression analysis showed a positive association between PAH exposure in air and stable aberration frequencies in newborns, beta = 0.1399, SE = 0.0491, p = 0.006 (See Figure 1.). Also by linear regression, stable aberration frequencies and PAH-DNA adducts were not associated (data not shown). Nor was there an association between unstable aberration frequency and either PAH exposure or PAH-DNA adducts (data not shown).

Discussion

The present study examined the relationship between prenatal maternal personal PAH exposure and FISH-detected chromosomal aberrations in cord blood in an urban, minority population. The most noteworthy finding was the significant, positive association between prenatal exposures to PAHs measured in air and stable chromosomal aberrations ($p = 0.006$). This is the first study to report an association between chromosomal aberrations detected by FISH in cord blood and prenatal exposures to airborne PAHs. Similarly, a number of studies carried out in adult populations have demonstrated a positive relationship between air pollution or PAHs and chromosomal aberrations [26-28;33]. Prior research has indicated an association between PAH-DNA adducts and somatic gene mutation in newborns [40], and between PAH exposure and adducts and chromosomal aberrations in adults [28;33].

The lack of association between PAH-DNA adducts and chromosomal aberrations in this study may be due to the small number (22) of subjects with detectable PAH-DNA adduct levels, or the possibility that the BP-DNA adducts measured in this study may not be the adducts most important in causing chromosomal aberrations. The current study used BP-DNA adducts as a surrogate for PAH adducts. Although the 8 carcinogenic PAHs monitored were strongly correlated, supporting BP as a proxy, some PAH-related adducts may not be adequately represented. We note that a prior study showed a correlation in adults between a wider spectrum of PAH/aromatic-DNA adducts, measured by ^{32}P-postlabelling, and chromosomal aberrations [28]. The lack of an association between PAH-DNA adducts and chromosomal aberrations may also be due to the fact that nucleotide excision repair, if incomplete, may remove adducts but generate double strand breaks that result in chromosomal aberrations [41-43].

An intriguing finding in this study is the difference in stable aberration frequencies between African-Americans and Dominicans, although levels of exposure to PAHs were not significantly different. This may point to differences in one or more unmeasured exposures, which may be causing cytogenetic damage. Alternatively, there may be variation between African-Americans and Dominicans in susceptibility to PAHs or other environmental mutagens due to polymorphisms in metabolic or repair enzymes. Further research is needed to examine this question.

In this study, prenatal ETS exposure was not associated with either stable or unstable chromosomal aberrations in cord blood. Although chromosomal aberrations and *hprt* mutations may not be induced by the same ETS component(s), Finette and colleagues [24;44] were able to demonstrate a significant difference in the *hprt* mutational spectrum, but not the *hprt* level, between newborns exposed *in utero* to passive maternal exposure to tobacco smoke and newborns without passive maternal exposure to tobacco smoke [24]. Our assessment of passive maternal ETS exposure was limited to presence or absence of exposure. It is possible that an inclusion of measures of intensity and duration of ETS exposure might have permitted detection of an association between chromosomal aberrations and ETS exposure. Two prior, small-scale studies have used FISH to investigate the effect of prenatal exposures to active maternal smoking and chromosomal aberrations. Both demonstrated increases in chromosomal aberrations in newborns of mothers who smoked during pregnancy compared to newborns of non-smoking mothers [1;2].

The mean stable aberration frequency in the present subset, all non-smokers, is 0.58%, which is almost three times that reported in cord bloods of newborns of non-

smoking mothers by Ramsey et al. (0.2%) [2], and six times that reported by Pluth et al. (0.11%) [1] in 14 and 40 newborns, respectively. Although there have been differences in exposures and/or ethnic compositions between the study populations, Pluth et al. [1] and Ramsey et al. [2] provide exposure data only on smoking status and no information on the racial/ethnic background of their study populations. The most likely explanation for the higher level of chromosomal aberration frequencies in the present study is the fact that Ramsey et al. [2] and Pluth et al. [1] included only translocations and insertions in their scoring criteria, while the present study included translocations, insertions, and deletions. Moreover, those investigators used FISH whole chromosome probes which "painted" only chromosomes 1, 2, and 4 whereas chromosomes 1-6 were "painted" in the CCCEH population. Despite these differences in absolute values, all three studies demonstrate a significant increase in chromosomal aberrations following prenatal exposure to environmental mutagens/carcinogens.

Finally, no significant associations were found with any of the exposure measures and unstable aberration frequencies. Unstable aberrations are considered less relevant to future cancer risk than stable aberrations. Unstable aberrations are essentially a measure of chromosome fragments that are indicative of past chromosome breaks but are transient markers of cytogenetic damage, which are not perpetuated in further cell divisions. In contrast, stable aberrations such as translocations and deletions are persistent, reflect past exposures, and can lead to cancer [2;45-47].

In conclusion, this study has demonstrated a significant association between prenatal environmental exposure to airborne carcinogenic PAHs and stable aberrations in cord blood at the relatively low environmental concentrations found in New York City.

Because similar air concentrations are found in other urban areas in the US and Europe [48], the results have relevance to other populations.

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Table 1 *Demographic Information for the CCCEH Subset and Total Population*

	Subset[1]		Total Population[1]		
	n[2]	Mean (range)	n	Mean (Range)	p-value[3]
Gender					
Female	32		237		
Male	28		222		0.797
Maternal Ethnicity					
African-American	30		388		
Dominican	30		226		0.006
Maternal Age (years)	60	28 (21-40)	685	28 (18-42)	0.186
Mother's Education[4]					
High School	43		463		
College	17		145		0.272
Neighborhood of Residence					
Harlem	29		248		
Washington Heights	14		226		
South Bronx	16		140		0.026

[1] Subset n = 60 mother-newborn pairs, Total population n = 686 mother-newborn pairs.
[2] n = number of subjects with available data as of June 2004.
[3] Chi-square or F test used for analysis.
[4] High School includes less than high school, some high school, high school diploma, and GED. College includes some college, 2-year college, 4-year college, and 4-plus years of college.

Table 2. *PAH exposure data from prenatal air monitoring and cord blood adducts for the CCCEH Subset and Total Population*

	Subset[1]		Total Population[1]		
	n[2]	Mean (range)	n	Mean (Range)	p-value[3]
PAHs in Air (ng/m^3)					
Benz(a)anthracene	57	0.35 (0.04-1.88)	478	0.29 (0.03-3.44)	0.049
Benzo(a)pyrene	57	0.45 (0.02-3.43)	478	0.44 (0.02-6.44)	0.885
Benzo(b)fluroanthene	57	0.77 (0.04-2.55)	470	0.64 (0.03-9.45)	0.144
Benzo(g,h,i)perylene	57	0.90 (0.04-4.42)	479	1.14 (0.02-18.11)	0.260
Benzo(k)fluroanthene	57	0.15 (0.04-0.80)	470	0.15 (0.02-1.59)	0.890
Chyrsene	57	0.42 (0.05-1.85)	478	0.37 (0.03-10.50)	0.503
Disbenz(a,h)anthracene	57	0.06 (0.04-0.21)	478	0.06 (0.01-1.23)	0.330
Indeno(1,2,3-cd)pyrene	57	0.58 (0.04-4.22)	478	0.63 (0.02-7.41)	0.633
PAH-DNA Adducts in Cord Blood					
(adducts/10^8 nucleotides)	48	0.24 (0.13-0.60)	250[4]	0.22 (0.13-0.72)	0.218

[1] Subset n = 60 mother-newborn pairs, total population n = 686 mother-newborn pairs.
[2] n = number of subjects with available data as of June 2004.
[3] F-test used for analysis.
[4] Initial 250 newborns; assays are in progress.

Table 3. *Results from Wilcoxon Rank Sum analyses of aberration frequencies and demographic and self- reported environmental tobacco smoke exposure variables*

	n	Mean Stable Aberration Frequency (%)	p-value[1]	Mean Unstable Aberration Frequency (%)	p-value
Gender					
Female	32	0.61		0.18	
Male	28	0.55	0.5408	0.13	0.764
Ethnicity					
Dominican	30	0.47		0.17	
African-American	30	0.70	**0.048**	0.14	0.5047
Smoker in the Home					
Yes	25	0.62		0.13	
No	35	0.56	0.4697	0.17	0.4649
Smoker at the Workplace					
Yes	4	0.54		0.07	
No	43	0.58	0.7046	0.17	0.3136

[1]Wilcoxon Scores (Rank Sums), t approximation two-sided

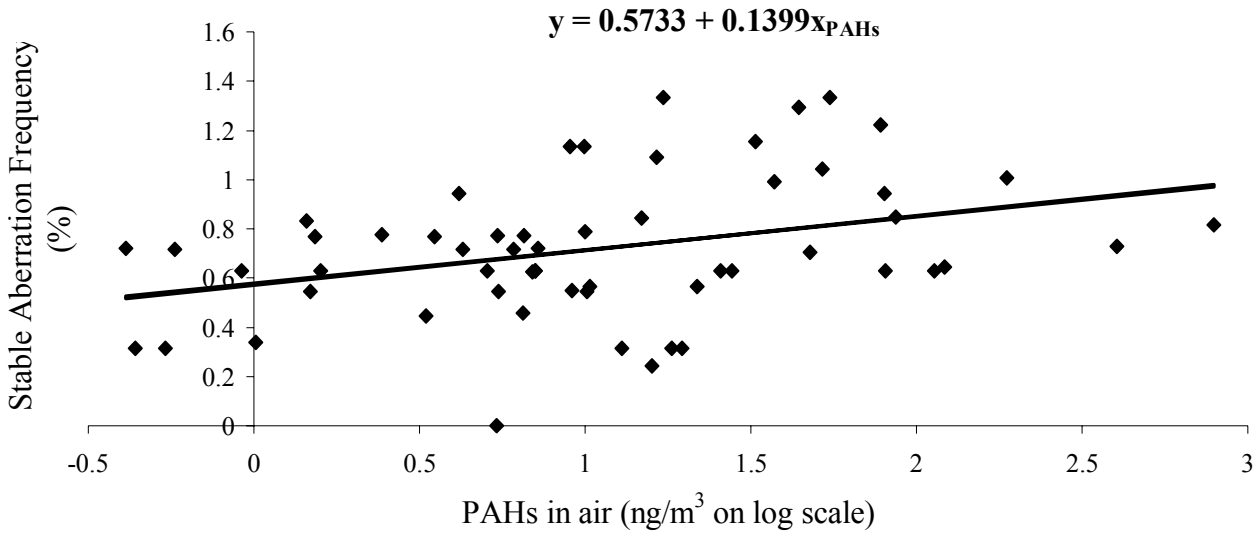

Figure 1. Plot of stable aberration frequencies (square root transformed) in cord blood and PAHs (log transformed) measured by prenatal, personal 48-hour air monitoring with multivariate linear regression line imposed ($n = 57$, $p < 0.01$).

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