Prenatal PAH exposure is associated with chromosome-specific aberrations in cord blood

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Abstract

Chromosomal aberrations are associated with increased cancer risk in adults. Previously, we demonstrated that stable aberrations involving chromosomes 1–6 in cord blood are associated with prenatal exposure to polycyclic aromatic hydrocarbons (PAHs) measured in air and are disproportionate to genomic content. We now examine whether the association with air PAHs is chromosome-specific and extends to smaller chromosomes.

Using whole chromosome paints for chromosomes 1–6, 11, 12, 14 and 19, and a 6q sub-telomere specific probe, we scored 48 cord bloods (1500 metaphases per sample) from newborns monitored prenatally for airborne PAH exposure in the Columbia Center for Children’s Environmental Health cohort. Frequencies of stable aberrations were calculated as incident aberrations per 100 cell equivalents scored, and examined for association with airborne PAHs.

Aberrations in chromosome 6 occurred more frequently than predicted by genomic content (p < 0.008). Levels of both prenatal airborne PAHs and stable aberration frequency in chromosomes 1–6 decreased to half the levels reported previously in the same cohort (mean PAH decreased from 3.6 to 1.8 ng/m³; mean stable aberration frequency from 0.56 to 0.24, SD = 0.19). The mean stable aberration frequency was 0.45 (SD = 0.15) in chromosomes 11–19. After adjusting for gender, ethnicity, and household smokers, the mean stable aberration frequency increased with increasing PAH exposure: with a doubling of prenatal PAH exposure, the mean stable aberration frequency for the chromosome 1–6 group increased by a factor of 1.49 (95% CI: 0.84, 2.66; p = 0.06); there was no increase for chromosomes 1–5 (p > 0.8). Aberrations in chromosomes 11, 12, 14, and 19 were associated with prenatal exposure to PAHs in air, even at lower levels of PAH in air. The observed chromosome-specific effects of prenatal airborne PAHs raise concern about potential cancer risk.

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1. Introduction

Chromosomal aberrations in peripheral blood lymphocytes are recognized as biomarkers that are predictive of cancer risk in adults and have been associated with environmental exposures occurring in utero or in childhood [1–9]. In a subset of neonates from the Columbia Center for Children’s Environmental Health (CCCEH) cohort we have previously shown that occurrence of stable aberrations was neither random nor proportional to the genomic content of any given chromosome, and that the frequency of stable chromosomal aberrations, detectable by FISH using whole chromosome probes (WCP) for chromosomes 1–6 in cord blood, was positively associated with increasing levels of PAHs measured in maternal prenatal air samples [9,10]. To confirm and expand upon these observations we have scored aberrations using FISH WCP for chromosomes 1–6, chromosome 6 separately, and chromosomes, 11, 12, 14 and 19 in cord blood obtained from an additional sample of 48 neonates participating in the CCCEH cohort. Our goal was to confirm whether the occurrence of stable aberrations is non-random when examining a larger number of chromosomes including smaller ones and whether incidence is disproportional to the genomic content of any given chromosome, particularly chromosome 6.

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Abbreviations: air PAH, ambient polycyclic aromatic hydrocarbons; ALL, acute lymphoblastic leukemia; CA, chromosomal aberrations; CCCEH, Columbia Center for Children’s Environmental Health; ETS, environmental tobacco smoke; FISH, fluorescent in situ hybridization; Mbp, mega base-pair; PAH, polycyclic aromatic hydrocarbons; WCP, whole chromosome probe.


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2. Materials and methods

2.1. Study population

Analysis quantifying the presence of chromosomal aberrations was performed on a subset of 48 newborns whose mothers underwent the longitudinal birth cohort of urban African-American and Dominican mothers and newborns conducted by the CCCEH and delivered between the years 2005 and 2006. All available cord blood samples (n = 28) were included in this study. Cord blood samples were collected from each mother immediately after the delivery of each child. Whole blood (0.8 ml) was then cultured overnight, followed by DNA preparation using the Templiphi 100 Amplification/deletions/duplications in pediatric ALL. Chromosomes 1–6 were selected for t(11;19) and t(1;19) and in 9.84% of detectable translocations and 1.8% of additions/deletions in pediatric ALL), chromosome 19 (involved in the t(8;14) and t(11;14) translocations and in 12.8% of detectable translocations of the TEL-AML1, t(12; 21) and involved in 5.8% of detectable translocations and 10.1% of 2.3. Cytogenetic laboratory methods

We collected, cultured and hybridized our samples using the procedures described previously [9]. Briefly, cord blood was collected into a heparinized tube immediately after the delivery of each child. Whole blood (0.8 ml) was then cultured for 72 h in P8max Complete Media (Invitrogen, Carlsbad, CA) at 37 °C using standard techniques. Colcemid was added for 20 min prior to harvesting. Lymphocytes were then treated with hypotonic KCl and fixed in 3:1 methanol–acetic acid before being prepared for metaphase spreads. Cultures were prepared for each cord blood when possible. In order to increase the yield of scoreable metaphases per slide, we utilized commercially available individual WCP (Cytocell, UK) for chromosomes 1, 2, 4, 12, 19 in red and chromosomes 3, 5, 6, 11, 14 in green. The colors for the chromosomes were chosen based on morphology in order to facilitate distinguishing individual chromosomes by combined contrasting colors and morphology. For each chromosome, 1–6 were hybridized on one set of slides and chromosomes 11, 12, 14 and 19 were hybridized on a separate set of slides, using DAPI counter stain (Cytocell, UK). A 6q sub-telomere specific probe (RP11-307K1 and RP11-292F10) was incorporated in order to detect chromosome 6-specific aberrations with sufficiently differentiating chromosome 6 centromere. Chromosomes were selected based on the possibility of identifying them with WCP and morphology and based on the frequency of involvement in translocations found in pediatric Acute lymphoblastic leukemia (ALL); chromosome 11 (present in the t(4;11) and t(11;19) translocations and involved in 25.6% of detectable translocations and 9.3% of additions/delusions in pediatric ALL), chromosome 12 (involved in the TEL-AML1, t(12; 21) and involved in 5.8% of detectable translocations and 10.1% of additions/deletions/duplications in pediatric ALL), chromosome 14 (involved in the t(8;14) and t(11;14) translocations and in 12.8% of detectable translocations and 2.3% additions/deletions in pediatric ALL), and chromosome 19 (involved in t(11;19) and t(11;19) and in 9.84% of detectable translocations and 1.8% of additions/deletions/duplications in pediatric ALL). Chromosomes 1–6 were selected for comparability with prior studies.

The 6q sub-telomere specific probe (RP11-307K1 and RP11-292F10) was generated by one of our study cytogeneticists (VJ). Single BAC colonies were inoculated in LB (chloramphenicol and the PAC colonies were inoculated in LB/kanamycin and cultured overnight, followed by DNA preparation using the Templiphi 100 Amplification Kit (Amersham Biosciences, CE Healthcare, Piscataway, NJ). One microgram of amplified DNA was labeled using Spectrum Red DUTP by a nick translation labeling kit (Vysis, Downer’s Grove, IL). One microliter of the resulting red 6qtel probe was applied to a drop of cleavage buffer (applied the WCP mixture for chromosomes 1–6). Hybridization and washing procedures were performed as described previously [9]. Visualization of FISH signals was performed on a fluorescence microscope (Olympus BX-UCB, Olympus Japan) equipped with appropriate filters (FITC, TRITC, and DAPI) and Cytovision software (Genetics New Milton, UK).

2.2. PAH measurements

PAH levels were measured prenatally as previously described [10]. In brief, mothers were or kept with them personal air monitors for 48 consecutive hours during their third trimester of pregnancy. Air PAH levels were analyzed at the SouthWest Research Institute (Dr. D. Camann’s laboratory). For our analysis, as for other analyses involving data on levels of PAH from the personal air monitors in the CCCEH cohort, a composite PAH variable (called “total PAH”) was derived from the summation of all 8 potentially carcinogenic PAHs measured (benz[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, Ba-p-diphenylacridine, benzo[a]pyrene [10]. As in prior studies [10], samples not meeting quality control criteria were excluded from analysis.

2.3. Statistical analysis

Summary statistics were calculated to describe sample characteristics. The Chi-square test was used to detect the discrepancies between observed and expected values (based on the percent of genetic content for each chromosome within each set of chromosomes that were simultaneously painted) occurrence of stable aberrations and thus did not go into the calculation of aberration frequency or genome equivalence. Aneuploidy was noted and all cells with aneuploidy were photographed and reviewed by our senior cytogeneticist (DW). However any other aberrations occurring in these cells (concurrent aneuploidy with stable aberrations were seen only in 2 metaphases of all those scored for the 48 cases) were not recorded and these cells did not contribute to the aberration frequency or total aberrations for that case.

Unidentified red chromosomes were presumed to represent either chromosomes 1, 2, 4, or 12 or 19 depending on the probes utilized, and unidentified green chromosomes were presumed to be composed of either 3, 5, or 6p, or 11 or 14. Aberrations containing clearly visible centromeres were considered stable aberrations (able to persist in subsequent cell divisions) and were counted in subsequent analyses. Fragments without centromeres and aneuploides were not considered stable aberrations and were excluded from subsequent analyses. For each cord blood sample, the number of stable chromosome equivalents was derived by counting the occurrences of balanced translocations and had a missing portion of a chromosome; “duplications” were chromosomes with an attached extra piece of a chromosome that hybridized with the same color; “broken chromosomes” were chromosomes that were in 2 or more pieces without appreciable loss of genetic material. Other aberrations were reported including; fragments (an acentric chromosome painted with red or green), and rings. Metaphases were documented using both the “classical” (one break at an interstitial translocation), and the PAINT system (two breaks are noted for each balanced translocation) [13]. As noted above aneuploides were noted but not part of the scoring of aberrations and those metaphases containing aneuploidy were excluded from the total count of metaphases scored. All cells with aneuploidy were not scored those metaphases for stable aberrations and thus did not go into the calculation of aberration frequency or genome equivalence. Aneuploidy was noted and all cells with aneuploidy were photographed and reviewed by our senior cytogeneticist (DW). However any other aberrations occurring in these cells (concurrent aneuploidy with stable aberrations were seen only in 2 metaphases of all those scored for the 48 cases) were not recorded and these cells did not contribute to the aberration frequency or total aberrations for that case.
along with the corresponding 95% confidence interval (CI). Kruskal–Wallis test was adjusted for 100 cell equivalents using the correction factor calculable aberrations observed in a minimum of 750 metaphases divided noted in our population is shown in Table 2.

3. Results

3.1. Distribution of chromosomal aberrations

Among the 48 cord blood samples scored, 38 (79%) had at least one detectable stable aberration in the 1500 metaphases scored in their cord blood. Stable aberrations were found in all 10 chromosomes examined. Additionally, unstable ring formations were noted involving chromosomes 3, 6, 11 and 12. The chromosome-specific comparison of observed vs. expected aberrations is shown in Table 3.

In our prior study [17], we demonstrated that the distribution of aberrations in chromosomes 1–6 was not consistent with a distribution based on DNA content (p < 0.007). In the present study (Table 1) chromosomes 1–5 exhibited generally fewer stable aberrations than predicted based on DNA content while chromosome 6 showed a significantly greater incidence (p < 0.0005). Among our second set of chromosomes (11, 12, 14 and 19), aberration incidence in chromosome 19 was also higher than expected based on genomic content (p < 0.05). The aberration incidence in chromosome 14 was also somewhat higher than that expected based on genomic content; however T cells are known to be prone to aberrations in chromosomes 7 and 14, the sites of T cell receptors, noted in our population is shown in Table 2.

3.2. Aberration types and frequency

3.2.1. Aberration types

Stable aberrations in our population were predominantly deletions and translocations: additions, which accounted for a large proportion of the aberrations we detected, are unbalanced translocations. Translocations were quantified using both the classical and PAINT systems though only the PAINT system was used to calculate the frequency of translocations. The distribution of aberrations noted in our population is shown in Table 2.

Aberration frequency was calculated using total number of stable aberrations observed in a minimum of 750 metaphases divided by the total number of metaphases counted within the sample and adjusted for 100 cell equivalents using the correction factor calculated from the formula from Lucas et al. [18] using the proportion of the genome painted for the set of either chromosomes 1–6 or 11, 12, 14 and 19. The raw mean aberration frequency in our 48 samples was 0.137 per 100 metaphases in chromosomes 1–6, and 0.114 in chromosomes 11, 12, 14 and 19 and did not differ between the two sets of chromosomes (p = 0.19 for paired samples t-test).

Adjusted for the whole genome equivalent correction factor, the mean aberration frequency in our 48 cord blood samples for chromosomes 1–6 is 0.24 (per 100 CE) and for chromosomes 11–19 it is 0.45 per 100CE. The translocation frequency in our 48 cord blood samples was 0.12 per 100 CE for chromosomes 1–6, and 0.25 per 100 CE for chromosomes 11, 12, 14 and 19. Given that our aberrations are not distributed proportionate to the DNA content of the chromosomes, we use the whole genome equivalent adjustment here for the purpose of facilitating comparison with other reports in the literature including our prior reported frequency of 0.56 [9,19]. Our current mean stable aberration frequency in the set of chromosomes 1–6 is significantly decreased from that reported in our pilot study (p < 0.001), however our whole genome equivalent corrected aberration frequency in chromosomes 11, 12, 14 and 19 is comparable with our prior reported aberration frequency in chromosomes 1–6. Overall, our aberration frequencies are consistent with those seen in other newborn populations. We suspect that much of the decrease in the chromosome 1–6 aberration frequency is related to decreased PAH exposure over time in the study area as discussed below. Aberration frequencies did not differ by ethnicity or gender in either set of chromosomes, consistent with findings in a recent meta-analysis [20]. The mean aberration frequencies in cord samples by chromosome set suggested equal frequency in chromosomes 1–6 compared to chromosomes 11, 12, 14 and 19, despite differences in their genetic content. The combined DNA content of chromosomes 1–6 (1232 Mbp) is approximately 2.83 times that of chromosomes 11, 12, 14 and 19 combined (435 Mbp) [15].

We excluded aneuploidy from our classification of stable aberrations because of the concern that artifact could contribute to its detection in the cells scored. In addition, in our prior work, we had excluded aneuploidy and thus wanted our present results to be comparable to those prior. However, recent mouse model work has shown the tumorigenic role of aneuploidy when present as the loss or gain of one or a few chromosomes, as a marker of “moderate genomic instability” [21]. In the metaphases in which we were able to detect aneuploidy, this was present in only a few chromosomes thus it might be relevant as a measure of genomic instability. When we compared the prenatal PAH levels, as well as non-aneuploidy

Comparison of stable aberrations detected on painted chromosomes, shown by occurrence on individual chromosomes in 48 cord blood samples.

Table 3 was related to aberration frequency in the set of chromosomes der, ethnicity and prenatal ETS exposure. Prenatal PAH exposure 6 alone ($p<0.05$) with increased total stable aberration frequency in chromosome 11–19 when adjusting for the three control variables ($p=0.02$). For chromosomes 1–6 (combined), the trend in increasing aberration frequency with increasing PAH level was not statistically significant ($p=0.17$).

The increase of aberration frequencies for a doubling of air PAH levels was derived from the negative binomial regression model parameter to aid interpretation. The relationship between chromosomal aberration frequency and PAHs, with and without adjusting for gender, ethnicity (Dominican vs. African-American) and presence of a smoker at home, is summarized in Table 4. In particular, for the set with chromosomes 1–6, the mean aberration frequency increased by a factor of 1.49 (95% CI: 0.84, 2.66; $p=0.17$) with a doubling of prenatal PAH levels; for chromosomes 11, 12, 14 and 19, the mean aberration frequency increased by a factor of 2.00, (95% CI: 1.11, 3.62; $p=0.02$). Interestingly, the mean aberration frequency in chromosome 6 increased by a factor of 3.16 (95% CI: 0.93, 10.77; $p=0.066$) with a doubling of PAH exposure, while for chromosomes 1–5, the mean aberration frequency did not differ by the level of PAH exposure. Table 5 presents the mean aberration frequency and translocation frequencies by the three PAH groups categorized with tertiles of PAH levels. There is a trend of increasing translocation frequency with increasing PAH levels, though not statistically significant. In the upper tertile of PAH exposure, frequencies of both stable aberrations and translocations appear higher in both sets of chromosomes painted, although the trend is statistically significant only in chromosomes 11, 12, 14 and 19.

3.2.2. PAH exposure and chromosomal aberrations

Prenatal air exposure data meeting quality control criteria were available for 35 newborns with aberration frequencies scored in both sets of chromosomes. The airborne PAH level ranged between 0.47 and 5.557 with median = 1.38, mean = 1.81 and SD = 1.25. This sample included 20 boys and 15 girls; 10 African-Americans and 25 Dominicans. For 10 of the 35 newborns, there had been a smoker living in the home during the pregnancy. Air PAH level did not differ by child’s sex, ethnicity or smoking status at home. Increasing prenatal PAH air levels were appeared to be associated with increased total stable aberration frequency in chromosome 6 alone ($p=0.054–0.066$) with and without adjusting for gender, ethnicity and prenatal ETS exposure. Prenatal PAH exposure was related to aberration frequency in the set of chromosomes 11–19 when adjusting for the three control variables ($p=0.02$). For chromosomes 1–6 (combined), the trend in increasing aberration frequency with increasing PAH level was not statistically significant ($p=0.17$).

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Table 2 Distribution of aberration subtypes in chromosomes 1–6 and in chromosomes 11, 12, 14 and 19.

<table>
<thead>
<tr>
<th>Type of aberration</th>
<th>Aberrations noted in chromosomes 1–6</th>
<th>Number of aberrations noted in chromosomes 11, 12, 14, 19</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fragments*</td>
<td>18</td>
<td>6</td>
</tr>
<tr>
<td>Aneuploidy*</td>
<td>27</td>
<td>19</td>
</tr>
<tr>
<td>Deletion</td>
<td>15</td>
<td>13</td>
</tr>
<tr>
<td>Duplication</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Inversion*</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Ring</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>Broken*</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>Translocations by PAINT definition</td>
<td>16</td>
<td>4*</td>
</tr>
<tr>
<td>Additions (unbalanced translocation)</td>
<td>10</td>
<td>19</td>
</tr>
<tr>
<td>Insertion</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Translocations, classical definition, balanced</td>
<td>9</td>
<td>3*</td>
</tr>
</tbody>
</table>

* Not counted as a stable aberration.

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Table 3 Comparison of stable aberrations detected on painted chromosomes, shown by occurrence on individual chromosomes in 48 cord blood samples.

<table>
<thead>
<tr>
<th>Slide set</th>
<th>% painted DNA in slide set</th>
<th>Observed</th>
<th>Expected</th>
<th>Observed/expected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chromosome 1</td>
<td>20.0</td>
<td>3¹</td>
<td>9.2</td>
<td>0.33</td>
</tr>
<tr>
<td>Chromosome 2</td>
<td>19.4</td>
<td>9</td>
<td>8.924</td>
<td>1.01</td>
</tr>
<tr>
<td>Chromosome 3</td>
<td>16.3</td>
<td>6</td>
<td>7.498</td>
<td>0.80</td>
</tr>
<tr>
<td>Chromosome 4</td>
<td>15.5</td>
<td>10</td>
<td>7.13</td>
<td>1.40</td>
</tr>
<tr>
<td>Chromosome 5</td>
<td>14.8</td>
<td>5</td>
<td>6.808</td>
<td>0.73</td>
</tr>
<tr>
<td>Chromosome 6</td>
<td>14.0</td>
<td>16³</td>
<td>6.44</td>
<td>2.48</td>
</tr>
<tr>
<td>Total, chromosomes 1–6</td>
<td>100</td>
<td>53³</td>
<td>46</td>
<td>1.15</td>
</tr>
</tbody>
</table>

Yates’ Chi-square for overall difference in chromosomal distribution of aberrations between observed and expected for cord blood: 17.4 (df = 5); 7 for chromosomes 1–6; 12.12 (df = 3) for chromosomes 11, 12, 14 and 19. Aberrations involving more than one painted chromosome (translocations) were noted for each chromosome involved.

¹ $p<0.05$.
² $p<0.007$.
³ $p<0.0005$.
⁴ $p=0.06$.

Together our data suggest that the association between prenatal PAH exposure and aberration frequency in the set of chromosomes 1–6 is dominated by the association between PAHs and aberration frequencies in chromosome 6. In contrast, prenatal airborne PAH exposure was significantly associated with the total aberration frequency in the set with chromosomes 11, 12, 14 and 19, but did not appear to be dominated by the aberration frequency in any individual chromosome of that set.

ETS has been associated with genetic damage and incidence of chromosomal aberrations in other studies [22]. In the CCCEH cohort, ETS has been highly correlated with prenatal cotinine level and has been shown to be a valid indicator for tobacco exposure [11]. We examined the relationship between presence of ETS (measured by maternal report of whether a smoker was present in the home during pregnancy) and chromosomal aberrations but found no association. Although ETS alone did not predict aberration frequency in our current population inclusion of ETS in the final model did affect the parameter of the association between PAH and aberration frequency (Table 4).

In our prior study of chromosomes 1–6 in an earlier subset of CCCEH newborns, chromosome damage was not predicted by chromosome size nor was it randomly distributed based on DNA content (p < 0.001). The breakpoint frequency appeared highest in the chromosome with the least DNA content of the chromosomes studied (chromosome 6) [17]. However, we could not definitively differentiate chromosome 6 from chromosomes 5 or 3 (3, 5 and 6 all fluoresce the same color). With the addition of the 6qtel probe, we have now been able to confirm that chromosome 6 is indeed at increased risk for aberrations and specifically at risk for developing aberrations in association with exposure to prenatal PAH exposure. Aberrations in chromosome 6 occurred more frequently in the larger q arm with 18/31 (58%) of aberrations clearly involving 6q and 3 others (rings) likely involving breaks in 6q as well. Aberrations involving 6q have been reported in multiple malignancies involving ALL; and 6q contains a candidate tumor suppressor gene (LOT1) [23]. Fig. 1 shows an observed translocation involving 6q.

Total prenatal airborne PAH levels in the CCCEH cohort have declined steadily and significantly over time, such that mean levels of PAHs in 2006 were less than half that in 1999 [24]. A series of policy changes involving use of cleaner fossil fuels for mass transit vehicles contributed to decreasing fuel emissions in New York City after 2000. PAH levels in the cohort decreased with later years of birth in the cohort. ETS, and other household sources of inhaled PAHs such as candles and incense did not affect personal air PAH levels [24]. Participants included in our earlier report were CCCEH cohort newborns from whom at least 30 ml of cord blood was collected at birth during the period from 1999 to 2002 [9]. In the present study, participants were born between the spring of 2005 and the spring of 2006. The current report includes all cohort participants born between March 2005 and April 2006. Among those subjects included in our earlier study, the mean prenatal total PAH level was 3.69 ng/m³ (SD 3.15); in the current study the mean total PAH level was significantly lower (mean: 1.81 ng/m³; SD: 1.25) (p < 0.005). However, even at these lower levels of PAHs, we have shown here that the level of prenatal PAH exposure predicts presence of chromosomal aberrations overall and specifically in certain chromosomes in cord blood samples.

4. Discussion

Our data demonstrate a significant association between elevated prenatal PAH exposure and increased levels of chromosomal aberrations in cord blood samples from an urban cohort, even at

Table 4
Mean ratio of frequency in specific set of chromosomes for doubling air PAH (ln transformed).

<table>
<thead>
<tr>
<th>Aberration</th>
<th>Choromosomes</th>
<th>Unadjusted</th>
<th>Covariate adjusted*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ratio (95% CI)</td>
<td>p-Value</td>
<td>Mean ratio (95% CI)</td>
</tr>
<tr>
<td>1, 2, 3, 4, 5, 6</td>
<td>1.49 (.78, 2.83)</td>
<td>0.23</td>
<td>1.49 (.84, 2.66)</td>
</tr>
<tr>
<td>1, 2, 3, 4, 5</td>
<td>1.08 (.53, 2.22)</td>
<td>0.83</td>
<td>1.14 (.615, 2.14)</td>
</tr>
<tr>
<td>6</td>
<td>3.66 (.98, 13.71)</td>
<td>0.05</td>
<td>3.16 (.93, 10.77)</td>
</tr>
<tr>
<td>11, 12, 14, 19</td>
<td>1.705 (.892, 3.261)</td>
<td>0.11</td>
<td>2.00 (.11, 3.62)</td>
</tr>
</tbody>
</table>

* Adjusting for gender, ethnicity (Dominican vs. African-American) and presence of a smoker at home.

Table 5
Frequencies of chromosome aberrations and translocation by PAH category (tertiles).

<table>
<thead>
<tr>
<th>Tertiles of PAH</th>
<th>Location of aberrations or translocations*</th>
<th>PAH 0.47–0.94</th>
<th>PAH 1.029–1.996</th>
<th>PAH 2–5.557</th>
<th>Kruskal–Wallis test p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean freq. (SD)</td>
<td>Mean freq. (SD)</td>
<td>Mean freq. (SD)</td>
<td>Mean freq. (SD)</td>
<td></td>
</tr>
<tr>
<td>Stable aberrations in chromosomes 1–6</td>
<td>.2117 (.2859)</td>
<td>.2151 (.1757)</td>
<td>.3783 (.6137)</td>
<td>.35</td>
<td></td>
</tr>
<tr>
<td>Stable aberrations in chromosome 6</td>
<td>.0185 (.0586)</td>
<td>.0738 (.1036)</td>
<td>.1481 (.3634)</td>
<td>.82</td>
<td></td>
</tr>
<tr>
<td>Translocation in chromosomes 1–6</td>
<td>.1238 (.2264)</td>
<td>.0932 (.1460)</td>
<td>.2601 (.5579)</td>
<td>.93</td>
<td></td>
</tr>
<tr>
<td>Stable aberrations in chromosomes 11, 12, 14, 19</td>
<td>.2177 (.2820)</td>
<td>.7932 (.10143)</td>
<td>.9999 (.9308)</td>
<td>.036</td>
<td></td>
</tr>
<tr>
<td>Translocation in chromosomes 11, 12, 14, 19</td>
<td>.2177 (.2820)</td>
<td>.3554 (.4014)</td>
<td>.5104 (.5332)</td>
<td>.27</td>
<td></td>
</tr>
</tbody>
</table>

* Includes both balanced and unbalanced translocations, quantified using PAINT system.

PAH levels that are significantly decreased from our prior report [9]. Further, we have demonstrated that the effects of PAHs appear to be partially chromosome-specific and extend beyond chromosomes 1–6 to smaller chromosomes such as 11, 12, 14 and 19. Importantly, our study is the first to identify chromosome 6 as particularly susceptible to the effects of PAHs. The stable aberrations we have documented include a number of morphologies including ring formations, identified in other studies as significantly predictive of later development of cancer in adults [2].

Our examination of the presence of chromosomal aberrations in healthy newborns delivered during the end of the recruitment phase of the CCCEH cohort (2005–2006) has documented a decrease in stable aberration frequency in chromosomes 1–6 compared to the frequency observed in a subset of the cohort born earlier (1999–2002), consistent with the decrease in mean PAH levels (from personal prenatal air monitoring) from 3.69 (1999–2002) to 1.81 ng/m³ (2005–2006) [24]. This decrease in airborne PAH has resulted from deliberate policies to improve air quality in New York City [24]. Nevertheless, we have documented in this study that aberrations continue to be present even with lower levels of exposure and are associated with prenatal exposure to PAHs.

This work confirms our prior observation that occurrence of stable aberrations occurring in cord blood samples of healthy infants without known radiation exposure is non-random and is not proportional to the genomic content of a given chromosome. The data presented above demonstrate that stable aberrations in chromosomes 11, 12, 14 and 19 are also neither proportional to genomic content of a given chromosome nor random. Additionally, aberration incidence in chromosome 6 is significantly greater than expected. Chromosomal aberrations in both chromosome 6 and the smaller chromosomes examined were associated with exposure to prenatal PAH exposure. The chromosomal aberration frequency in our cord samples can therefore be considered a biomarker of genetic damage reflecting environmental exposure to air pollutants as suggested by our prior studies [9] and others [8,22].

As noted, in our prior study chromosome damage was not predicted by chromosome size or DNA content, and breakpoint frequency appeared to be highest in chromosome 6 and lowest in chromosome 1 (10). However, in our prior study we did not have the ability to definitively differentiate chromosome 6 from chromosomes 5 or 3. With the addition of the specific chromosome 6 telomere probe, we can now confirm that chromosome 6 is at increased risk for aberrations in association with exposure to prenatal PAH.

Our present findings differ from those of our earlier pilot study [9]. In that study of 60 subjects, the mean stable aberration frequency was 0.58 per 100 CE [9]. Even so, our present rates for stable aberrations in chromosomes 1–6 (0.24 per 100 CE) are substantially lower than previously reported by our group; this is likely attributable to the fact that PAH exposure was also significantly lower in our present study. The results suggest a beneficial effect from clean air policies.

Our frequency data are comparable to those of others who have examined rates of translocations in this age group. A pooled analysis of age-related variations in chromosome frequency included results for translocation frequencies in newborns in which chromosomes 1–6 were examined with WCP [19]. In our data, the stable aberration rate in chromosomes 1–6 was 0.24 per 100 CE and our translocation rate was 0.12 per 100 CE, which is more than 2 times higher than that reported for newborns from California and Cum-bria, UK by Sigurdson et al. [19] though only half as high as that recently reported among African-American newborns in Pittsburgh [16], suggesting that our population might be exposed to an intermediate level of PAH exposure.

Our observed association between stable chromosomal aberrations and PAHs is consistent with other studies that have found that chromosomal aberrations are associated with exposures to PAH-containing mixtures. Ramsey et al. demonstrated a 1.5-fold increase in newborn aberrations in children whose mothers smoked during pregnancy [25,26]. Since tobacco smoke is a recognized source of PAHs, these findings are supportive of an association between prenatal PAH exposure and increasing chromosomal aberrations. Similarly, in adults occupationally exposed to ambient PAHs, rates of chromosomal aberrations were significantly predicted by levels of airborne PAHs [27].

Chromosome 6 appears to be particularly susceptible to development of chromosomal aberrations in this population as a result of prenatal PAH exposure. Our present data do not allow specific delineation of sites on chromosome 6 that may be especially susceptible to damage by PAHs. Nevertheless, it is intriguing to consider that many critical regulating genes including E2F3, and MYB (expressed predominantly in immature progenitor cells of all haemopoietic lineages) are present on 6q. The presence of rings in our healthy newborns is also unusual and deserves further examination particularly because, in a recent meta-analysis examining the predictive nature of chromosomal aberrations for future development of adult cancer, the presence of ring chromosomes in peripheral lymphocytes was associated with a 2.2-fold significantly increased risk of later incidence of cancer [2]. Clearly these findings suggest the need for further investigation. Although we did not count aneuploidy as a stable aberration and did not score metaphases containing aneuploidy, we did note its occurrence in metaphases that were considered complete (unbroken). Cases in which aneuploidy was noted did not appear to differ in their stable aberration or translocation frequency or in their prenatal PAH exposure. Because there were relatively few cells with aneuploidy we do not believe that excluding those cells from scoring affected our calculations of aberration frequency. Although there is recent data suggesting that aneuploidy may be a predictor of tumorigenesis in mice, the model by Weaver and Cleveland suggests that the tumorigenic role of aneuploidy may be secondary to misregulated gene expression resulting from abnormal combinations of chromosomes [21]. WCPFISH may not be an appropriate model for documenting aneuploidy but a similar tumorigenic effect of misregulated gene expression could result from translocations which WCP FISH is able to detect when these occur between chromosomes painted in different colors.

Prenatal personal air monitor levels of airborne PAHs in the CCCEH cohort have been shown to reflect diverse combustion sources, notably traffic and heating [28]. Prenatal PAH exposure has been linked to a number of adverse outcomes in the CCCEH cohort including decreased head circumference and birth weight among infants born to African-American women in the cohort [10], lower mental development index (using the Bayley developmental scales) at age 3 [29], and decreased full scale IQ at age 5 [30]. Here, we are expanding upon our earlier report of a link between prenatal PAH exposure and a validated biomarker of potential cancer risk [1–7]. Although our dataset is small, and thus power to detect associations is limited, we have found association between PAH and the smaller previously unexamined chromosomes (11, 12, 14 and 19) as well as for chromosome 6. Our findings suggest the need for further investigation with larger sample sizes in order better examine these effects.

In summary, our data suggest that the effect of prenatal PAH exposure on incidence of chromosomal aberrations persists even at lower levels of PAH exposure and may be concentrated on specific chromosomes. Together these findings suggest that PAH exposure may have significant sub-clinical genetic effects at birth and suggest the need for a better understanding of potential pathways in the development of seemingly disparate effects of PAHs on the newborn. Further exploration to determine PAH-susceptible regions on chromosome 6 may provide insight into mechanisms of PAH related...
toxicity. Lastly, our work suggests that decreasing airborne PAH levels through effective public policies may ultimately contribute to decreasing cancer risk.

Conflict of interest statement

None declared.

Acknowledgements

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References


