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Research Article

Aberrations in Chromosomes Associated With Lymphoma and Therapy-Related Leukemia in Benzene-Exposed Workers

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Epidemiological studies show that benzene exposure is associated with an increased incidence of leukemia and perhaps lymphoma. Chromosomal rearrangements are common in these hematopoietic diseases. Translocation t(14;18), the long-arm deletion of chromosome 6 [del(6q)], and trisomy 12 are frequently observed in lymphoma patients. Rearrangements of the *MLL* gene located on chromosome 11q23, such as t(4;11) and t(6;11), are common in therapy-related leukemias resulting from treatment with topoisomerase II inhibiting drugs. To examine numerical and structural changes in these chromosomes (2, 4, 6, 11, 12, 14, and 18), fluorescence in situ hybridization (FISH) was employed on metaphase spreads from workers exposed to benzene ($n = 43$) and matched controls ($n = 44$) from Shanghai, China. Aneuploidy (both monosomy and trisomy) of all seven chromosomes was increased by benzene exposure. Benzene also induced del(6q) in a dose-dependent manner ($P_{trend} = 0.0002$). Interestingly, translocations between chromosomes 14 and 18, t(14;18), known to be associated with follicular non-Hodgkin lymphoma, were increased in the highly exposed workers ($P < 0.001$). On the other hand, translocations between chromosome 11 and other partner chromosomes that are found in therapy-induced leukemias were not increased. These data add weight to the notion that benzene can induce t(14;18) and del(6q) found in lymphoma, but do not support the idea that benzene induces t(4;11) or t(6;11). However, they do not rule out the possibility that other rearrangements of the *MLL* gene at chromosome 11q23 may be induced by benzene. Environ. Mol. Mutagen. 48:467–474, 2007.

Key words: benzene; biomarkers; chromosomal rearrangements; leukemia; lymphoma

INTRODUCTION

Benzene is an important industrial chemical that is used as the basis for the synthesis of many commercial products, and it is still found as a contaminant in solvents and glues in many countries [Kang et al., 2005; Navasumrit et al., 2005]. It is also a component of gasoline and a product of incomplete combustion, making it a universal environmental contaminant [Brugnone et al., 1998; Lemire et al., 2004; Muttamara et al., 2004; Srivastava et al., 2004; Roma-Torres et al., 2006]. It is well-established that benzene induces myeloid leukemia in humans, but its ability to cause lymphoma and lymphocytic leukemia, although likely, remains less clear [Aksoy, 1988; Pyatt, 2004; Savitz and Andrews, 1997; Schnatter et al.,

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2005]. Thus, the precise endpoint to use in cancer risk assessment is a matter of debate. Further, the exact nature of the toxic metabolites and their mechanism of action are unknown. Our research aims to shed light on these issues by studying human populations exposed to benzene and applying molecular biomarkers.

From a study conducted in Shanghai, we have previously reported that benzene exposure causes the induction of chromosomal changes commonly observed in acute myeloid leukemia, including monosomy of chromosome 7, trisomy 8, and t(8;21) in the blood cells of highly exposed workers [Smith et al., 1998; Zhang et al., 1999]. It has been suggested that the metabolites of benzene may act as topoisomerase II inhibitors, in a manner similar to etoposide and adriamycin, which cause disruption of the *MLL* gene at 11q23 and secondary leukemias with a short latency in some patients after high dose chemotherapy [Chen and Eastmond, 1995; Lindsey et al., 2004; Whysner et al., 2004; Eastmond et al., 2005]. In the present study, we have examined whether or not two common rearrangements at 11q23, namely t(4;11) and t(6;11), can be detected in the blood of benzene-exposed workers using chromosome painting via fluorescent in situ hybridization (FISH). If these were detected, it would suggest that benzene metabolites were capable of breaking the *MLL* gene at 11q23 and causing leukemia-inducing translocations. Further, a number of studies have associated benzene and solvent exposure with the induction of non-Hodgkin lymphoma (NHL) [Hardell et al., 1994; Hayes et al., 1997; Rego et al., 2002; Xu et al., 2003; Fritschi et al., 2005]. Translocation t(14;18), the long-arm deletion of chromosome 6 [del(6q)], and trisomy 12 are frequently observed in lymphoma patients [Glassman et al., 2000; Campbell, 2005; Taborelli et al., 2006]. Therefore, we also have examined whether or not these chromosomal changes can be detected by FISH in exposed workers. To achieve this goal, we used two metaphase FISH assays to detect leukemia and lymphoma-specific numerical and structural aberrations in chromosomes 2, 4, 6, 11, 12, 14, and 18 in the lymphocytes of workers exposed to benzene and matched controls.

MATERIALS AND METHODS

Subject Enrollment

The identification of factories and enrollment of study subjects has been previously described in detail [Rothman et al., 1996a,b]. Biological samples were collected from 44 healthy workers in Shanghai, China, currently exposed to benzene with minimal exposure to toluene and other aromatic solvents. The same number of healthy controls without current or previous occupational exposure to benzene was enrolled from factories in the same geographic area. Controls were frequency-matched to the exposed subjects by gender and age (5-year intervals). Exclusion criteria for all subjects were history of cancer, therapeutic radiation, chemotherapy, or current pregnancy. Factories for exposed subjects were selected so that the study population would have a wide range of exposures to benzene, similar to exposure patterns in a larger cohort study [Hayes et al., 1997; Yin et al., 1996].

The protocol was explained to all potential participants and informed consent was obtained using Institutional Review Board-approved procedures. Each subject was administered a questionnaire by a trained interviewer. Data collected included age, sex, current and lifelong tobacco use, current alcohol consumption, medical history, and work history. The height and weight of each subject were measured. Peripheral blood was obtained by phlebotomy; blood from one individual could not be successfully cultured and FISH results are reported here for 87 subjects.

Exposure Assessment

Exposure was monitored by organic vapor passive dosimetry badges (3M #3500, St. Paul, MN), which were worn by each worker for a full workshift on five separate days during the 1-to-2-week period prior to phlebotomy. Badges were analyzed by gas chromatography with flame ionization detection. An 8-hr time-weighted average (8-hr TWA) exposure was calculated for benzene as the geometric mean of the five air measurements. Although this method was sufficient for dividing workers into high and low exposure groups, the relatively short monitoring period was not sufficient for determining individual exposure levels. Current exposures to benzene were confirmed by the analysis of phenol and other metabolites in urine [Rothman et al., 1996b, 1998]. A detailed assessment of factory records and operations showed that no other known marrow-toxic chemicals or physical agents were present in these workplaces. Historical benzene exposure during the subjects' employment at the factories was estimated using work histories obtained by interview, company employment records, and factory records as previously described [Rothman et al., 1996a,b]. All exposure assessment was performed blinded with respect to FISH analysis.

Personal benzene air levels in these factories were much higher than had been expected based on historical area monitoring data. As a direct result of this study, remedial action was taken at the two workplaces with the highest benzene exposures and included substitution of toluene for benzene, enclosure of reaction vessels, and improvement in ventilation.

Blood Cultures and Metaphase Preparation

Whole blood collected in a vacutainer with the anticoagulant heparin was cultured in RPMI-1640 medium supplemented with 15% fetal bovine serum, 1% penicillin-streptomycin, 1% L-glutamine (Gibco, Grand Island, NY), and 1% phytohemagglutinin-P (Pharmacia, Piscataway, NJ). Blood cells were incubated at 37°C in a 5% CO_2 moist atmosphere and harvested at 72 hr after culture initiation. Colcemid (0.1 μg/ml) was added 4 hr prior to harvest to obtain a sufficient number of metaphase spreads. After hypotonic treatment (0.075 M KCl) for 30 min at 37°C, the cells were fixed three times with freshly made Carnoy's solution (methanol: glacial acetic acid = 3:1). The fixed cells were then dropped onto prelabeled glass slides, allowed to air dry, and stored at −20°C under a nitrogen atmosphere. Prepared slides were later shipped on dry ice to the United States.

Fluorescence In Situ Hybridization

The leukemia- and lymphoma-specific chromosome aberrations were examined using two dual-color FISH assays in cultured lymphocyte metaphases. (1) To detect t(4;11), t(6;11), and other translocations associated with the *MLL* gene at 11q23 and del(6q), chromosomes 4 and 11 were painted green (biotinylated) and red (digoxigenin-labeled), respectively, and the centromere of chromosome 6 (α-satellite probe, green) and its 6q27 band (cosmid probe, red) were labeled simultaneously. (2) Human FISH painting probes specific for chromosomes 14 (biotinylated, green) and 18 (digoxigenin-labeled, red) were employed to determine

TABLE I. Demographic Characteristics by Exposure Category

	Age (year)	Gender		Cigarettes/day		Alcoholic Drinks/week
		Male	Female	Male	Female	
Controls[a] ($n = 44$)	35.4 ± 7.3[b]	23	21	14.5 ± 13.7	0	1.4 ± 2.2
Exposed ($n = 43$)[c]	35.2 ± 7.9	22	21	12.7 ± 7.6	0	1.4 ± 3.2
≤31 ppm (21)	34.4 ± 7.7	13	8	11.5 ± 4.9	0	1.2 ± 1.9
>31 ppm (22)	36.0 ± 8.1	9	13	14.2 ± 10.1	0	1.6 ± 4.1

[a]Controls were frequency-matched to exposed subjects by age and gender.
[b]Mean ± S.D.
[c]$n = 43$ not 44 because blood was not available from one exposed subject.

TABLE II. Chromosome Aneuploidy Detected in Workers Exposed to Benzene and Unexposed Controls

Benzene Exposure (n)	Chromosomes						
	2	4	6	11	12	14	18
Monosomy							
Controls (44)	0.42 ± 0.06[a]	1.49 ± 0.17	1.07 ± 0.12	1.32 ± 0.17	0.66 ± 0.10	0.84 ± 0.13	1.52 ± 0.22
Exposed (43)	0.91 ± 0.13	2.20 ± 0.20	2.59 ± 0.31	2.39 ± 0.29	1.16 ± 0.19	1.39 ± 0.17	2.16 ± 0.31
Ratio[b]	2.17	1.48	2.42	1.81	1.76	1.65	1.42
P value	0.0005	0.0019	<0.0001	0.001	0.0114	0.0063	0.0684
Trisomy							
Controls (44)	0.12 ± 0.03	0.14 ± 0.03	0.11 ± 0.03	0.13 ± 0.03	0.16 ± 0.04	0.10 ± 0.03	0.09 ± 0.03
Exposed (43)	0.35 ± 0.07	0.37 ± 0.06	0.32 ± 0.04	0.29 ± 0.05	0.28 ± 0.05	0.18 ± 0.03	0.27 ± 0.05
Ratio[b]	2.92	2.64	2.91	2.23	1.75	1.8	3.00
P value	0.0002	<0.0001	<0.0001	0.0008	0.0590	0.0692	0.0008
Tetrasomy							
Controls (44)	0.38 ± 0.05	0.78 ± 0.07	0.75 ± 0.07	0.75 ± 0.08	0.38 ± 0.05	0.37 ± 0.05	0.37 ± 0.05
Exposed (43)	0.64 ± 0.08	1.02 ± 0.09	1.01 ± 0.09	1.01 ± 0.09	0.65 ± 0.07	0.67 ± 0.08	0.65 ± 0.07
Ratio[b]	1.68	1.31	1.35	1.35	1.71	1.81	1.76
P value	0.0159	0.0130	0.0092	0.0069	0.0091	0.0046	0.0062

[a]Frequency as mean % ± SE.
[b]Ratio of the mean value in exposed to that in unexposed controls.

t(14;18), and the centromeric probes for chromosomes 2 (red) and 12 (green) also were used for aneusomy detection. All FISH probes were purchased from Oncor (Gaithersburg, MD). A portion of the biotinylated painting probe specific for chromosome 4 was a gift from Dr. David Eastmond of UC Riverside.

The four probes in each assay were mixed with sonicated salmon sperm carrier DNA in Master Mix 2.1 solution (55% formamide/1 × SSC/10% dextran sulfate) and denatured for 10 min at 70°C. Denaturation of cellular DNA, hybridization with DNA probes overnight, post-washing of slides, and detection of hybridization signals were performed as previously described in detail [Smith et al., 1998; Zhang et al., 1998a]. All the stained slides were randomized and coded prior to scoring. For efficiency, all scorable metaphase spreads on each slide were analyzed and a minimum of 200 cells/subject were scored. Metaphase cells were considered scorable if they met specific criteria: the cells appear intact; chromosomes are well-spread and condensed; the centromeres are readily visible; and FISH staining is sufficiently bright to enable the detection of numerical and structural aberrations [Smith et al., 1998; Zhang et al., 1998b]. The total numbers of scored metaphase spreads from the 87 subjects were 40,536 for chromosomes 4, 6, and 11 (average 466 cells/subject) and 40,691 for chromosomes 2, 12, 14, and 18 (average 468 cells/subject).

Statistical Analyses

Study subjects ($n = 87$) were divided into three groups: controls ($n = 44$), workers exposed to ≤31 ppm benzene ($n = 21$) and workers exposed to >31 ppm benzene ($n = 22$; 31 ppm was the median exposure, as an 8-hr TWA). This enabled us to study dose-response relationships and to detect differences between the lower-exposed and unexposed controls. The demographics of the study subjects in these groups are shown in Table I. Summary data of frequently detected chromosome changes (such as aneusomies, del(6q), translocations, and total structural changes) are presented as the mean number of aberrations per 100 metaphases examined for each subject. A negative binomial model was used in all analyses of this study, including the reported mean and standard error (SE). Empirically, the amount of variation in the data after controlling for exposure was greater than expected for a Poisson or binomial model. Thus, the negative binomial can be thought of as an extension to Poisson that naturally accounts for overdispersion. This assumption has little impact on the effect estimates, but a potentially large impact on the corresponding standard errors. In this case, negative binomial estimates of variability will be more accurate than those provided by either assuming Poisson or binomial variability.

Negative binomial regression was used to compare aberration frequencies among controls, lower- and higher-exposed workers, and to calculate tests of trend. Because our subjects were frequency matched on age and gender, we did not include these factors in the adjusted models. There was no evidence of confounding by current or previous tobacco use, alcohol use, or years of working, and these factors were excluded from the final models.

Because specific translocations, such as t(14;18), occurred rarely when compared with aneusomies, particularly in controls and lower-exposed workers, the data of t(14;18) were pooled for all subjects within each exposure category and analyzed by Fisher's exact test.

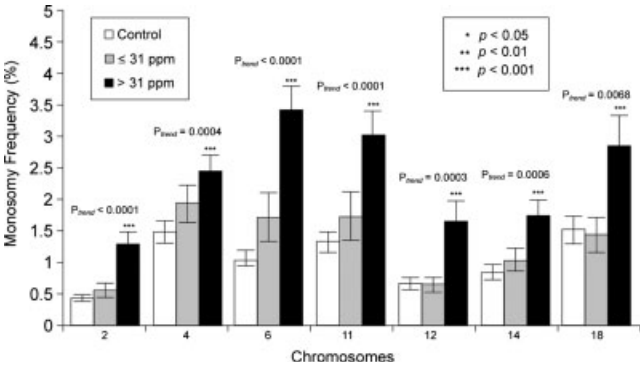

Fig. 1. Monosomy of chromosomes 2, 4, 6, 11, 12, 14, and 18 detected by FISH in Chinese workers exposed to benzene and matched controls. Data presented are mean monosomy frequency (%). Error bar represents S.E. White bar represents controls ($n = 44$), gray bar represents $\leq$31 ppm group ($n = 21$), and black bar represents >31 ppm group ($n = 22$). Significant dose responses were observed in all seven chromosomes tested ($P_{trend} < 0.0001$ to 0.01), but only the monosomy rate of chromosome 6 was increased significantly in the lower exposed group ($P < 0.05$) compared with the controls.

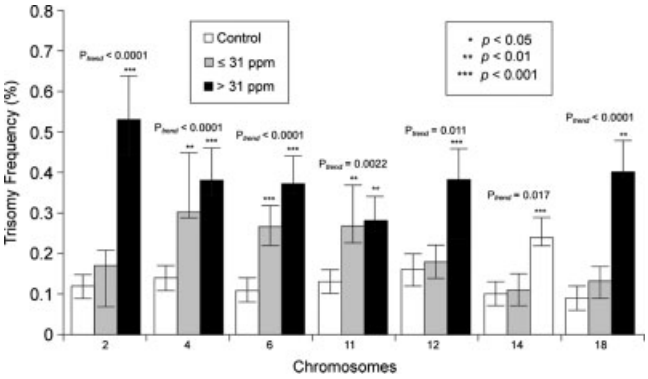

Fig. 2. Trisomy of chromosomes 2, 4, 6, 11, 12, 14, and 18 detected by FISH in Chinese workers exposed to benzene and matched controls. Data presented are mean trisomy frequency (%). Error bar represents S.E. White bar represents controls ($n = 44$), gray bar represents $\leq$31 ppm group ($n = 21$), and black bar represents >31 ppm group ($n = 22$). Significant dose responses were observed in all seven chromosomes tested ($P_{trend} < 0.0001$ to 0.05), and the frequencies of trisomy 4, 6, and 11 were significantly higher in $\leq$31 ppm group ($p < 0.01$) than the control group.

RESULTS

Numerical Chromosome Changes Associated With Benzene Exposure

Significant increases in the loss of a chromosome (monosomy) were observed for six of the seven chromosomes tested among benzene-exposed workers when compared with their unexposed controls (Table II). The increase in monosomy of chromosome 18 was the smallest and the only increase that was not significant at the $P < 0.05$ level (Table II). Similarly, the gain of a chromosome (trisomy) was observed for five of the seven chromosomes tested among benzene-exposed workers compared with their unexposed controls (Table II). Trisomy of chromosomes 12 and 14, although elevated, was not significantly increased in the benzene-exposed workers. Tetrasomy of all seven chromosomes was significantly increased by benzene exposure (Table II).

When the exposed group was divided at the median exposure level of 31 ppm, trend analysis showed a significant dose-response relationship between increasing benzene exposure levels and monosomy (Fig. 1) and trisomy (Fig. 2) of all seven chromosomes. The monosomy and trisomy rates of all seven chromosomes were increased in the higher benzene-exposed group (>31 ppm, Figs. 1 and 2). However, only monosomy of chromosome 6 and trisomy of chromosomes 4, 6, and 11, but not other tested chromosomes, were significantly elevated in the lower-exposed group ($\leq$31 ppm, Figs. 1 and 2).

Tetrasomy rates (data not shown) also were increased in the higher-exposed group ($P < 0.001$), but not in the lower-exposed group. However, significant dose-dependent trends were observed for all chromosomes tested ($P_{trend} < 0.001$). The benzene-induced tetrasomies were mostly tetraploidy, as 96% of the tetrasomic cells had four copies of each chromosome present in the same cell. These results are similar to those obtained previously in our studies of chromosomes 1, 5, and 7 in this same population of workers [Zhang et al., 1998a].

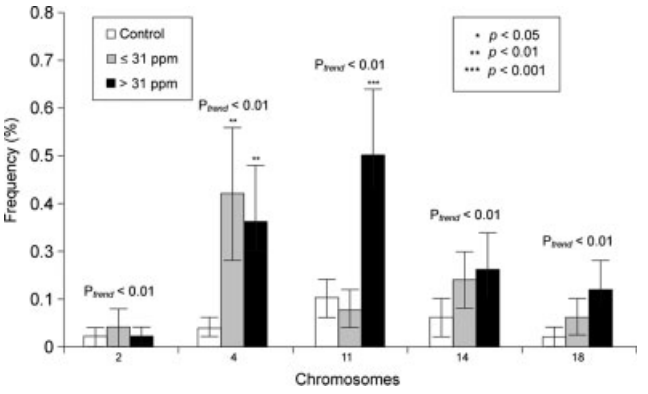

Fig. 3. The total structural aberrations of chromosomes 2, 4, 11, 14, and 18 detected by FISH in Chinese workers exposed to benzene and matched controls. Data presented are mean structural aberration frequency (%). Error bar represents S.E. White bar represents controls ($n = 44$), gray bar represents $\leq$31 ppm group ($n = 21$), and black bar represents >31 ppm group ($n = 22$). Data for chromosome 6 and for all chromosomes combined are given in Table II. No structural aberrations were detected in chromosome 12 (data not shown).

Structural Chromosome Rearrangements Associated With Benzene Exposure

Chromosome structural rearrangements include breaks, deletions, inversions, and translocations. The total structural changes in chromosomes 2, 4, 11, 14, and 18 at different levels of benzene exposure are shown in Figure 3.

TABLE III. Structural chromosome aberrations (SCAs) in workers exposed to different levels of benzene

Benzene exposure (n)[a]	t(11; 4, 6, ?)[b]	t(14;18)	del(6q)	Other SCAs
Controls (44)	0.043 ± 0.018[c]	0 ± 0.0	2.91 ± 0.31	0.06 ± 0.02
Exposed (43)	0.034 ± 0.020	0.025 ± 0.013***[d,e]	4.61 ± 0.44**[d]	0.19 ± 0.03***[d]
≤31 ppm (21)	0.005 ± 0.005	0 ± 0.0	3.67 ± 0.61	0.16 ± 0.04**[d]
>31 ppm (22)	0.061 ± 0.038	0.049 ± 0.025***[d,e]	5.51 ± 0.58***[d]	0.22 ± 0.05***[d]
P_{trend}	0.28	NAf	0.0002	0.0003

[a]Exposure levels as 8-hr TWA.
[b]t(11; 4, 6, ?) represents the sum of translocations between chromosome 11 and chromosomes 4, 6, or otherwise unidentifiable (?) chromosomes.
[c]Frequencies shown in table are mean percentage (±SE) of cells harboring SCA in all the cells scored.
[d]Comparison of exposed, ≤31 ppm, or >31 ppm with controls by negative binomial regression.
[e]P values calculated from Fisher's exact test.
[f]Not applicable.
*$P < 0.05$
**$P < 0.01$
***$P < 0.001$.

Significantly increased frequencies of structural aberrations were observed in chromosomes 4 ($P_{trend} < 0.01$) and 11 ($P_{trend} < 0.001$) in the high (>31 ppm) exposed group. In the lower (≤31 ppm) exposed group, the increase was only significant for chromosome 4 ($P < 0.01$), but not for 11. For chromosomes 2, 14, and 18, no significant differences in the level of structural aberrations were observed between the controls, the lower-exposed workers, and the highly exposed (>31 ppm) group ($P_{trend} > 0.05$, Fig. 3). No structural aberrations were detected on chromosome 12 for any of the subjects. Most of the structural changes on chromosome 6 were deletions of its long-arm, del(6q), which will be discussed in the following section. Note that the high frequency values for structural aberrations reported here are probably not directly comparable to those produced by conventional cytogenetic analysis of all chromosomes because our analysis employed a specific probe, e.g., 6q27, to quantitate each specific change.

Benzene-Induced Specific Translocations and Deletions

Specific chromosome rearrangements, such as t(11q23) and t(14;18), and deletions of chromosome 6, del(6q), are common in leukemias and lymphomas. We hypothesized that benzene may induce these specific structural rearrangements in exposed, but otherwise healthy workers. The frequencies of these specific structural changes are shown in Table III. There was no difference in the frequencies of translocations involving chromosome 11 (including t(4;11) and t(6;11)) in any of the exposed groups, including those workers exposed to high levels of benzene (>31 ppm), and no dose-dependent increase was apparent (Table III). Translocation t(14;18), however, was significantly elevated in the benzene-exposed workers and specifically in the high-exposed group when frequencies were compared at the individual cell level ($P < 0.001$, Table III). Four of 22 subjects in the >31 ppm group harbored the translocation, but it was not present in any of the controls or in the lower-exposed workers, a difference that was statistically significant ($P = 0.01$). A significant increase in del(6q) also was observed ($P < 0.01$), and the increase was dose-related ($P_{trend} = 0.0002$, Table III). When groups of subjects were compared to controls, the increase was significant for the higher-exposure (>31 ppm) group ($P < 0.001$), but not at the lower level of exposure.

DISCUSSION

We have continued our analysis of chromosome changes in subjects from a study of workers exposed to high occupational levels of benzene in Shanghai, China. Previously, we have reported on changes in chromosomes 1, 5, 7, 8, 9, and 21 that are typically altered in acute myeloid leukemia [Zhang et al., 1996, 1998a, 1999; Smith et al., 1998]. Here, we extend our analysis to another seven chromosomes. To gain further mechanistic insights, we examined chromosomes commonly altered in lymphoma and certain forms of therapy-related leukemia. Translocation t(14;18), the long-arm deletion of chromosome 6 [del(6q)], and trisomy 12 are frequently observed in the cells of lymphoma patients [Glassman et al., 2000; Campbell, 2005; Taborelli et al., 2006]. We therefore used FISH to examine changes in chromosomes 6, 12, 14, and 18 from benzene-exposed workers. Both the loss (monosomy) and gain (trisomy) of chromosome 6 was observed following benzene exposure and a dose-related increase in del(6q) was detected in the benzene-exposed workers ($P_{trend} = 0.0002$). Regional deletions of 6q are frequent karyotypic alterations in malignant lymphoma and are associated with an adverse clinical outcome [Taborelli et al., 2006]. However, the specific genes affected have not been identified. Monosomy of chromosome 6 could produce loss of heterozygosity at this location and, thus, benzene's ability to induce monosomy 6

and deletions at 6q suggest it has the potential to induce changes in chromosome 6 associated with lymphoma.

By painting chromosomes 14 and 18, we were also able to detect translocations between these two chromosomes. Such translocations, t(14;18), were only detected in the higher-exposed group (>31 ppm), but not in controls or the lower-exposed workers. Further work is needed to characterize the molecular nature of these translocations and their oncogenic potential, but our data suggest that exposures to high levels of benzene may have the potential to induce t(14;18), typically found in follicular NHLs [Glassman et al., 2000]. Interestingly, follicular NHL has been associated with cigarette smoking [Morton et al., 2005], a common source of benzene exposure. Translocation (14;18) also has been shown to be more common in NHL cases with prior solvent or agricultural chemical exposure [Brandt et al., 1989; Chiu et al., 2006], and increased levels of t(14;18) in the peripheral blood have been observed in smokers [Bell et al., 1995; Schuler et al., 2003]. Studies are underway to determine if metabolites of benzene can induce t(14;18) in cell culture.

Therapy-related AML (tAML) has been proposed as a model of chemical leukemogenesis and chemotherapy with alkylating agents is associated with increased risks of tAML [Irons and Stillman, 1996; Irons, 2000; Larson and Le Beau, 2005]. These leukemias frequently are characterized by a preleukemic phase, trilineage dysplasia, and cytogenetic abnormalities involving deletions of part or all of chromosomes 5 and 7 [Larson and Le Beau, 2005]. Previous studies have shown that benzene exposure in vivo [Zhang et al., 1998a, 2002] and benzene's polyphenolic metabolites in vitro [Stillman et al., 1997; Zhang et al., 1998b; Stillman et al., 2000] are highly effective at producing monosomy and deletions of the q arm of chromosomes 5 and 7. A second group of tAML is linked with topoisomerase II inhibitors, specifically chemotherapy with epipodophyllotoxins, such as etoposide [Felix, 2001]. tAML associated with treatment with epipodophyllotoxins often is not preceded by a preleukemic phase and the pattern of cytogenetic abnormalities is different, with balanced translocations involving the *MLL* gene at 11q23 being the most characteristic abnormality [Felix, 2001]. Translocations or breaks in the *MLL* gene at 11q23 may therefore serve as useful biomarkers of early effect for topoisomerase II inhibitors. Indeed, Megonigal et al. [2000] showed that an *MLL* translocation t(11;17) was detectable after only 6 weeks of therapy in the bone marrow of a neuroblastoma patient treated with topoisomerase II inhibitors and acted as a biomarker of early effect for the subsequent development of tAML in this patient.

We examined whether or not benzene exposure was associated with increased frequencies of t(4;11) and t(6;11), two of the most common rearrangements at 11q23, in peripheral blood. No significant increases in the frequency of these translocations were observed in even the highly exposed workers. These data do not support the idea that benzene induces rearrangement of the *MLL* gene at chromosome 11q23, at least in the peripheral blood, but some caution in interpretation is warranted. Benzene may induce other translocations at 11q23, such as t(9;11) or t(11;17), as the *MLL* gene has many translocation partners [De Braekeleer et al., 2005] or its deletion. It also is possible that peripheral lymphocytes with t(4;11) and t(6;11) do not survive, which may explain the lack of effect seen in this study, or that an effect is only seen in the bone marrow and not the peripheral blood. Alternatively, benzene metabolites may act more like dioxopiperazine and quinone topoisomerase II inhibitors than epipodophylotoxin inhibitors such as etoposide [Eastmond et al., 2005]. The former tend to induce leukemias with translocations t(8;21), inv(16), and t(15;17), and rarely t(11q23) [Xue et al., 1992; Zhang et al., 1993; Bhavnani and Wolstenholme, 1987; Kroger et al., 2003; Mistry et al., 2005]. Our previous finding of higher levels of t(8;21) in benzene-exposed workers [Smith et al., 1998] is in agreement with this hypothesis, and the idea that benzene metabolites may induce leukemia in a manner similar to piperazine and quinone drugs warrants further exploration.

In the present study, we were able to determine the effect of benzene on the frequency of aneuploidy for seven chromosomes, namely chromosomes 2, 4, 6, 11, 12, 14, and 18. Previously, we have published aneuploidy data for chromosomes 1, 5, 7, 8, 9, and 21 [Smith et al., 1998; Zhang et al., 1996, 1998a, 1999], bringing the total to 13 chromosomes examined out of 22 autosomes if we include the present study. Our combined data to date show that high levels of benzene exposure increase aneuploidy frequencies of all studied chromosomes, with the exception of chromosome 1. However, at lower levels of exposure (<31 ppm), there appears to be some selectivity. For example, in the present study, only monosomy of chromosome 6 and trisomy of chromosomes 4, 6, and 11 were significantly affected by benzene exposures below 31 ppm. Previous studies have suggested that aneuploidy of chromosomes 5 and 7 is sensitive to lower levels of benzene exposure. Thus, benzene may not produce equal effects on all chromosomes. We are currently exploring this issue of selectivity further using a technique called OctoChrome FISH, which allows examination of all 24 chromosomes on a single slide [Zhang et al., 2005].

In conclusion, we have shown that high levels of occupational benzene exposure induce translocation t(14;18), the long-arm deletion of chromosome 6 [del(6q)], and trisomy 12, cytogenetic changes that are frequently observed in lymphoma patients. These changes were observed in the peripheral blood of exposed workers and further work is needed to determine if similar changes occur in the critical lymphoid progenitor or stem cells, which are the target cells for lymphoma. Interestingly,

benzene exposure was not associated with increased frequencies of t(4;11) or t(6;11), two of the most common rearrangements at 11q23, but other rearrangements of the *MLL* gene at chromosome 11q23 may be induced by benzene. This could potentially be examined by molecular and/or cytogenetic studies of all *MLL* gene rearrangements following exposure to benzene.

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