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Research Article

Comparison of Aneuploidies of Chromosomes 21, X, and Y in the Blood Lymphocytes and Sperm of Workers Exposed to Benzene

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Benzene is a primary industrial chemical and a ubiquitous environmental pollutant that causes human leukemia and maybe other malignancies. Occupational exposure to benzene has been associated with increased chromosomal aneuploidies in blood lymphocytes and, in separate studies, in sperm. However, aneuploidy detection in somatic and germ cells within the same benzene-exposed individuals has never been reported. To compare aneuploidies in blood lymphocytes and sperm within the same individuals exposed to benzene, a cross-sectional study was conducted in 33 benzene-exposed male workers and 33 unexposed workers from Chinese factories. Air benzene concentrations in the exposed workers ranged from below the detection limit to 24 ppm (median, 2.9 ppm) and were undetectable in the unexposed subjects. Aneuploidies of chromosomes 21, X, and Y in blood lymphocytes were examined by multicolor fluorescence *in situ* hybridization and were compared to the previously reported aneuploidies in sperm. The results showed that benzene exposure was positively associated with the gain of chromosome 21 but not sex chromosomes in blood lymphocytes. This was in contrast to analysis of sperm, where the gain of sex chromosomes, but not chromosome 21, was significantly increased in the exposed workers. Furthermore, a significant correlation in the gain of sex chromosomes between blood lymphocytes and sperm was observed among the unexposed subjects, but not among the exposed workers. The findings suggest that benzene exposure induces aneuploidies in both blood cells and sperm within the same individuals, but selectively affects chromosome 21 in blood lymphocytes and the sex chromosomes in sperm. Environ. Mol. Mutagen. 53:218–226, 2012.

Key words: occupational exposure; chromosome 21; sex chromosomes

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INTRODUCTION

Benzene, the simplest aromatic compound, is a primary industrial chemical that ranks in the top 20 most abundantly produced chemicals in the United States [Wilbur et al., 2008]. Occupational exposure to benzene occurs worldwide in the oil, shipping, automobile repair, shoe manufacture, and other industries. Benzene is also a ubiquitous environmental pollutant present in cigarette smoke and motor vehicle exhaust [Wilbur et al., 2008]. The general population is exposed to benzene primarily in tobacco smoke and by inhaling contaminated air, particularly in areas with heavy motor vehicle traffic and around gasoline filling stations.

Benzene is an established human carcinogen [IARC, 1982]. Occupational exposure to benzene causes acute myeloid leukemia (AML) and other hematopoietic disorders [Hayes et al., 2001; Schnatter et al., 2005] as well as hematotoxicity [Rothman et al., 1996; Qu et al., 2002; Lan et al., 2004]. Aneuploidy (chromosome loss or gain) is a phenomenon commonly observed in leukemia patients and is thought to play an important role in leukemogenesis [Pedersen-Bjergaard et al., 2006]. Increased aneuploidy has been frequently observed in the blood cells of benzene-exposed workers compared to unexposed controls [Zhang et al., 2002]. For instance, increased levels of leukemia-specific aneuploidies, including monosomy of chromosomes 5 and 7 and trisomy of chromosomes 8 and 21, were found in the blood lymphocytes of apparently healthy Chinese workers exposed to high levels of benzene (median: 31 ppm, range: 1.6–328.5 ppm) [Smith et al., 1998; Zhang et al., 1998], indicating that aneuploidy precedes benzene-induced leukemia and may play an important role in promoting leukemogenesis. Recently, a chromosome-wide aneuploidy study (CWAS) was conducted in another group of Chinese workers comprised of 27 controls, 22 workers exposed to $<$10 ppm benzene (4.95 $\pm$ 3.61 ppm, mean $\pm$ SD), and 25 workers exposed to $\geq$10 ppm benzene (28.33 $\pm$ 20.09 ppm) [Zhang et al., 2005, 2011]. In this study, aneuploidies of all 24 chromosomes in the blood lymphocytes were examined by a novel fluorescence *in situ* hybridization (FISH) assay, OctoChrome-FISH, and only certain chromosomes were shown to be dose-dependently associated with benzene exposure, with a particularly striking effect on trisomy 21.

In addition to aneuploidies in blood cells, increased aneuploidies in sperm have also been associated with occupational benzene exposure. For instance, increased disomy for chromosomes 1, 7, 8, 9, 18, and X in sperm was observed in Chinese workers exposed to high levels of benzene (mean concentrations ranging from 13 to 27 ppm) [Liu et al., 2000, 2003; Li et al., 2001; Zhao et al., 2004].

A positive correlation between aneuploidies in blood lymphocytes and sperm has previously been reported within the same individuals. For example, aneuploidies of sex chromosomes and chromosome 21 in blood lymphocytes were correlated with those in sperm in 10 patients with idiopathic oligozoospermia and 10 healthy donors [Gazvani et al., 2000b]. A positive correlation was reported in gain of sex chromosomes between the blood lymphocytes and sperm of 10 healthy donors [Rubes et al., 2002]. However, to date, the studies analyzing blood cells and sperm aneuploidy in benzene-exposed workers were conducted in different populations. Aneuploidy detection in somatic and germ cells within the same benzene-exposed individuals has never been reported.

Previously, we examined aneuploidies of autosome 21 and sex chromosomes X and Y in the sperm of Chinese benzene-exposed workers and unexposed subjects and observed an increased disomy of sex chromosomes among the exposed workers, even among the workers exposed to benzene at or below the US permissible limit level (1 ppm, 8-hr time-weighted average) [Xing et al., 2010]. In the present study, we expanded our efforts to examine aneuploidies of chromosomes 21, X, and Y in the blood lymphocytes of the same group of benzene-exposed and unexposed workers and compared the effects of benzene on aneuploidy induction in blood lymphocytes and sperm within the same individuals.

METHODS

Study Population

The study population is the same as that in our previous report [Xing et al., 2010], which included 33 male workers recruited from three factories in Tianjin, China, who used benzene-containing glues in the manufacture of shoes, paper bags, and sandpaper and 33 unexposed subjects recruited from Tianjin factories with no history of benzene use—a meat-packing plant and an ice cream manufacturing factory. The unexposed subjects were frequency-matched with the exposed workers for age and smoking history. Protocols, questionnaires, and consent forms were reviewed and approved by the Committees for the Protection of Human Subjects at the University of California, Berkeley, Lawrence Livermore National Laboratory, Lawrence Berkeley National Laboratory, and the Tianjin Occupational Disease Hospital (Tianjin 3rd Municipal Hospital) under an institutional review board authorization agreement with the National Institute of Occupational Health and Poison Control, Chinese Center for Disease Control and Prevention.

Exposure Assessment and Sample Collection

The exposure assessment has been detailed previously [Xing et al., 2010]. Briefly, each enrolled subject wore a personal passive-air badge monitor (3M Organic Vapor Monitor, model 3500; 3M, St. Paul, MN) for a full 8-hr workday and provided a spot urine sample at the end of the work shift. Approximately 1 month later, male subjects provided a second air sample and spot urine sample. Analysis of the passive-air monitors was performed according to the 3M Organic Vapor Method (3M 2002). Urinary benzene analyses were performed on all specimens from the unexposed and exposed subjects (two samples per subject), and *trans,trans*-muconic acid (E,E-MA) analyses were performed for both

samples of the exposed subjects only. Laboratories that performed air and urine analyses were blind to the factories of the workers.

On the second day of air and urine sampling, the subjects were scheduled to visit the Tianjin 3rd Hospital. Workers were instructed to avoid ejaculation for 2–5 days prior to their appointment. On the day of their appointment, men were interviewed and examined by a Chinese urologist; a fasting blood sample was collected by venipuncture, and men provided a semen specimen by masturbation.

Blood Culture and Metaphase Spread Preparation

The blood culture and metaphase preparation were performed following a standard procedure [Barch, 1991]. In brief, immediately after receiving the blood samples, 0.3 ml of whole peripheral blood was cultured in 5 ml medium (purchased from Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences, containing RPMI 1640 medium containing 20% FBS, 1% phytohemagglutinin, and 1% penicillin and streptomycin) at 37°C in a 5% CO_2 moist atmosphere for 72 hr. Colcemid (0.1 μg ml^{-1}) was added to the culture 2 hr before harvesting. After hypotonic treatment (0.075 M KCl) for 30 min at 37°C, the cells were fixed three times with freshly prepared Carnoy's fixative (methanol:glacial acetic acid = 3:1). The fixed cells were shipped to the University of California at Berkeley for slide preparation. The cells were pelleted and resuspended in fresh Carnoy's fixative and then dropped onto glass slides, which were air dried and stored at −20°C in a nitrogenous atmosphere prior to analysis.

FISH Assay in Blood Lymphocytes

A three-color FISH assay was designed to simultaneously detect the aneuploidies of chromosomes 21, X, and Y in the same lymphocyte metaphase spread. A locus-specific probe for chromosome 21 (SpectrumOrange, cat# 32-190002, Abbott Molecular, Des Plaines, IL), a centromere probe for chromosome X (SpectrumAqua, cat# 32-131023, Abbott Molecular), and a centromere probe for chromosome Y (SpectrumGreen, cat# LPE 0YcG, CytoCell, Cambridge, UK) were used.

The cells on the slides were stained with 4′,6-diamidino-2-phenylindole (DAPI) and metaphase spreads were scanned and localized automatically using Metafer software (MetaSystems, Germany). After washing the slide in 2× saline-sodium citrate (SSC) buffer at room temperature for 30 min, a mixture of 1 μl of each probe and 7 μl of hybridization buffer was applied to the slide, which was then covered with a 22 × 22 mm^2 coverslip, and sealed with rubber cement. The probes and cells were denatured at 75°C for 5 min and then hybridized at 37°C for 2 days. After washing the slide in 2× SSC at 60°C for 2 min, the cells were counterstained with DAPI and then scored at 1000 × magnification. The scoring criteria was previously described in detail [Smith et al., 1998]. Of the 66 subjects, 1,000, 500–999, and 175–499 metaphases were scored for 46, 14, and 6 subjects, respectively, with an average of 877 metaphases per subject. The following categories of aneuploidy in lymphocytes were included as dependent variables: trisomy 21 (genotype X-Y-21-21-21), gain X (genotype X-X-Y-21-21), gain Y (genotype X-Y-Y-21-21), and gain of sex chromosomes (sum of gain X and gain Y). All aneuploid lymphocytes were confirmed by two experienced scorers. Chromosome loss was not included in the analyses as it was previously shown to be prone to technical artifacts during metaphase preparation [Brown et al., 1983]. The laboratory that performed FISH analysis in lymphocytes was blind to the origin of the samples.

FISH Assay in Sperm

Sperm aneuploidy data from the same individuals were previously obtained using a FISH assay examining aneuploidies of chromosomes 21, X, and Y [Xing et al., 2010]. The following categories of aneuploidy in sperm were included as dependent variables: disomy 21 (genotype X-21-21 or Y-21-21), disomy X (genotype X-X-21), disomy Y (genotype Y-Y-21), disomy XY (genotype X-Y-21), and disomy of sex chromosomes (sum of disomy X, disomy Y, and disomy XY).

Statistical Analysis

As in our previous report [Xing et al., 2010], exposure concentration values for air benzene, urinary benzene, and urinary E,E-MA were calculated as the geometric means from the two collections. Categories of benzene exposure were constructed using E,E-MA concentrations because E,E-MA has been shown to be a robust biomarker of benzene exposure [Qu et al., 2000; Kim et al., 2006]. Among exposed participants, concentrations of E,E-MA were divided at the median (6.7 mg l^{-1}). Those at or below the median were assigned to the low-exposed group, whereas those above the median were assigned to the high-exposed group.

We used negative binomial models to assess differences in aneuploidy frequencies by exposure categories. The potential confounders were selected using the same criteria as our previous report [Xing et al., 2010] and age (continuous), smoking in the past 3 months (yes/no), eating fruits or vegetables ≥3.6 times/day vs. < 3.6 times/day (yes/no), and history of any chronic disease (yes/no) were included in the models. Because the aneuploidies were relatively rare events, random permutations of Mann–Whitney tests of differences in frequencies between exposed and unexposed men were also performed and reported as $P_{permuted}$ in Table I. We used negative binomial models to assess the dose–response relationship between aneuploidy and benzene exposure. Because the air concentrations of benzene for the unexposed subjects were all less than the limits of detection (LOD, 0.2 ppm) and the urinary E,E-MA concentrations were not measured in the unexposed, we used the urinary benzene concentrations as the biomarker for benzene exposure. Spearman correlations were used to test correlations between blood lymphocyte and sperm cell aneuploidy.

RESULTS

Demographic Characteristics and Benzene Exposure of the Study Population

As we previously reported [Xing et al., 2010], participants were matched for age and smoking history, and therefore, did not differ in these characteristics. The alcohol drinking status in the two groups was also similar. The exposed workers were somewhat more likely to drink tea regularly ($P = 0.07$) and to consume more fruits and vegetables ($P = 0.08$), and had lower rates of chronic disease (12% vs. 33%; $P = 0.04$). The exposed group took more hot baths in the 3 months before semen collection compared with the control group (64% vs. 36%; $P = 0.03$) and reported a shorter period of sexual abstinence (mean ± SD, 7 ± 5 vs. 10 ± 17 days; $P = 0.2$; range, 2–30 vs. 2–100 days).

Exposure assessment was also reported in detail previously [Xing et al., 2010]. To reiterate, personal passive-air badge monitors and urine samples were obtained from each worker at two time points ∼1 month apart. Each individual's two air benzene, urinary benzene, and urinary E,E-MA measurements were highly correlated (Spearman $\rho = 0.9$ for air benzene, 0.8 for urinary benzene, and 0.8 for urinary E,E-MA) with high intraclass correlation coef-

TABLE I. Rates of Aneuploidies of Chromosomes 21, X, and Y in Blood Lymphocytes and Sperm of the Unexposed and Exposed Workers

		Aneuploidies in lymphocytes (per 100 cells)								
		Trisomy 21			Gain X			Gain Y		
	n	Median (IQR)	Mean	Range	Median (IQR)	Mean	Range	Median (IQR)	Mean	Range
Unexposed	33	0.00 (0.13)	0.09	0.00–0.46	0.20 (0.30)	0.19	0.00–0.80	0.10 (0.22)	0.14	0.00–1.02
Exposed	33	0.10 (0.30)#	0.17	0.00–0.60	0.10 (0.23)	0.16	0.00–0.80	0.10 (0.20)	0.16	0.00–0.50
Low-exposed	17	0.10 (0.30)	0.17	0.00–0.60	0.19 (0.20)	0.19	0.00–0.80	0.12 (0.20)	0.19	0.00–0.50
High-exposed	16	0.14 (0.30)#	0.17	0.00–0.50	0.10 (0.20)	0.12	0.00–0.40	0.10 (0.20)	0.12	0.00–0.40
		Aneuploidies in sperm (per 100 cells)								
		Disomy 21			Disomy X			Disomy Y		
	n	Median (IQR)	Mean	Range	Median (IQR)	Mean	Range	Median (IQR)	Mean	Range
Unexposed	33	0.01 (0.01)	0.01	0.00–0.08	0.01 (0.02)	0.02	0.00–0.08	0.02 (0.03)	0.03	0.00–0.11
Exposed	33	0.01 (0.01)	0.02	0.00–0.18	0.02 (0.05)*	0.04	0.00–0.14	0.03 (0.07)	0.05	0.00–0.19
Low-exposed	17	0.01 (0.01)	0.02	0.00–0.18	0.03 (0.04)*	0.03	0.01–0.09	0.02 (0.02)	0.04	0.00–0.17
High-exposed	16	0.01 (0.01)	0.01	0.00–0.04	0.02 (0.04)*	0.04	0.00–0.14	0.05 (0.09)	0.07	0.00–0.19

Abbreviation: IQR, interquartile range.# and * represent $P_{permuted} < 0.1$ and $P_{permuted} < 0.05$, respectively, compared to the unexposed group by random permutation test.No significant differences between unexposed and exposed groups were detected in blood lymphocytes by negative binomial regression. Statistical results in sperm by negative binomial regression were previously reported [Xing et al., 2010].

ficients (0.85 for air benzene, 0.80 for urinary benzene, 0.73 for urinary E,E-MA). The geometric mean (GM) of the two measurements was used to calculate the summary statistics. The air concentrations of benzene for the unexposed subjects were all < LOD (0.2 ppm). Two men in the low-exposed group also had air benzene values that were < LOD and these values were imputed as the LOD divided by the square root of 2. The air benzene concentration range for the exposed workers was < LOD—23.6 ppm with median values of 1.0 and 7.7 ppm for the low-exposed and high-exposed groups, respectively. For urinary benzene, the range for the unexposed subjects was 0.1–0.9 μg l^{-1} with a median of 0.1 μg l^{-1} and the range for the exposed workers was 0.8–617.0 μg l^{-1} with median values of 4.3 and 52.5 μg l^{-1} for the low-exposed and high-exposed groups, respectively. Urinary E,E-MA was not measured in the unexposed and the range for the exposed workers was 0.8–40.9 mg l^{-1} with median values of 1.9 and 14.4 mg l^{-1} for the low-exposed and high-exposed groups, respectively. These measures of benzene exposure were highly correlated among the exposed men (Spearman $\rho > 0.75$; $P < 0.001$ for each pair).

Aneuploidies of Chromosomes 21, X, and Y in the Blood Lymphocytes and Sperm of the Unexposed and Exposed Groups of Workers

Table I shows the rates (per 100 cells) of aneuploidy of chromosomes 21, X, and Y in the blood lymphocytes and sperm of the unexposed and exposed groups of workers. In the blood lymphocytes, the median rates of trisomy 21 in the unexposed, low-exposed, and high-exposed groups were 0, 0.10, 0.14%, respectively. Though the increases in the exposed groups were not significant relative to the unexposed group by negative binomial models, borderline increases in trisomy 21 were observed in the whole exposed group and the high-exposed group compared to the unexposed group by random permutation tests ($P_{permuted} = 0.07$ and $P_{permuted} = 0.09$, respectively). The gain of sex chromosomes in the exposed group was not significantly changed relative to the unexposed group either by negative binomial models or by random permutation tests.

The sperm aneuploidy results have been reported previously [Xing et al., 2010]. To reiterate, analyzing by negative binomial models, disomy 21 was not significantly different between exposure groups, while disomy X was significantly increased in both low-exposed and high-exposed groups ($P = 0.02$ and $P < 0.001$, respectively) and disomy Y was significantly increased in the high-exposed group ($P < 0.001$). We reanalyzed the data using random permutation test here and found that disomy X was significantly increased in both low-exposed and high-exposed groups compared to the unexposed ($P = 0.04$ and 0.02, respectively).

Association of Aneuploidies of Chromosomes 21, X, and Y in the Blood Lymphocytes and Sperm with Benzene Exposure Levels

Aneuploidy rates of chromosomes 21, X, and Y were examined in relation to urinary benzene levels in the entire study population, as shown in Figure 1. In the blood lymphocytes, trisomy 21 was positively associated with urinary benzene levels ($\beta = 0.11$, $P = 0.04$), (Fig. 1A), while gain of the sex chromosomes was not ($\beta = 0.08$, $P = 0.12$ and $\beta = 0.03$, $P = 0.61$ for chromosomes X and Y, respectively), (Figs. 1B and 1C). In sperm, disomy 21 was marginally associated with the urinary ben-

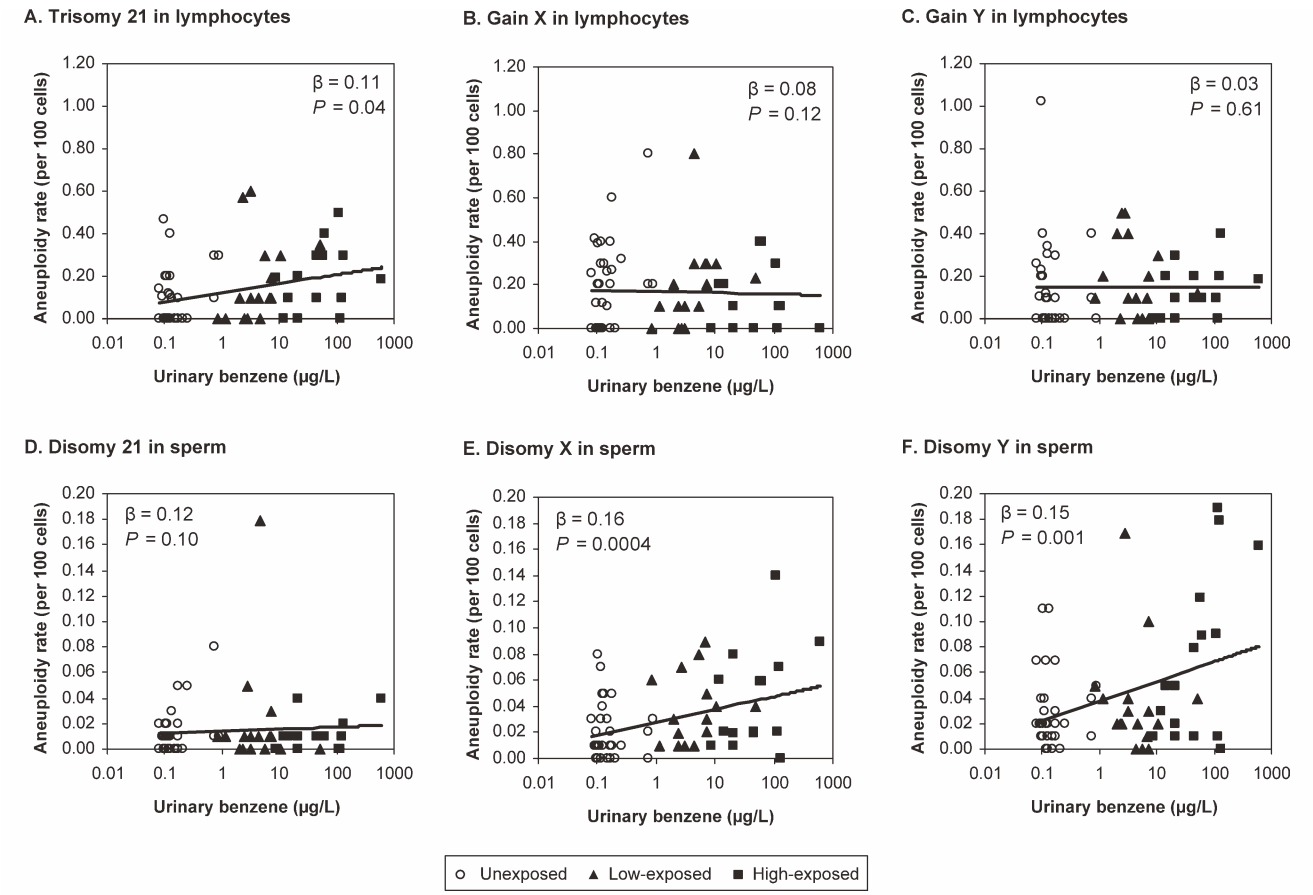

Fig. 1. Association of aneuploidies of chromosomes 21, X, and Y in blood lymphocytes and sperm with benzene exposure. A generalized linear model (negative binomial regression) using log transformed urinary benzene (continuous) was used to assess the association. The line presents the linear trend. β is the regression coefficient and *P* is the *P* value. The associations of sperm aneuploidies with benzene exposure were reported previously [Xing et al., 2010].

zene levels (β = 0.12, *P* = 0.10), (Fig. 1D) and gain of both sex chromosomes was positively associated (β = 0.16, *P* = 0.0004 and β = 0.15, *P* = 0.001 for disomies X and Y, respectively), (Figs. 1E and 1F).

Correlation of Aneuploidies of Chromosomes 21, X, and Y between Blood Lymphocytes and Sperm

Gain of chromosome 21 was not positively correlated between blood lymphocytes and sperm in all subjects (Spearman ρ = −0.09, *P* = 0.47), in the unexposed (Spearman ρ = 0.10, *P* = 0.58) or in the exposed workers (Spearman ρ = −0.31, *P* = 0.08). A positive correlation in gain of sex chromosomes between blood lymphocytes and sperm was present in all study subjects (Spearman ρ = 0.25, *P* = 0.04) and in the unexposed (Spearman ρ = 0.44, *P* = 0.01), but not in the exposed workers (Spearman ρ = 0.14, *P* = 0.45), (Fig. 2).

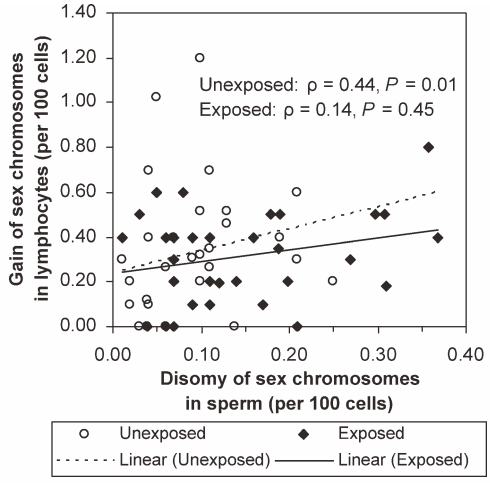

Fig. 2. Correlation of aneuploidies of sex chromosomes between blood lymphocytes and sperm among the unexposed and exposed workers. Spearman correlation was used to assess the correlation. The dashed and solid lines present the linear trends among unexposed and exposed workers, respectively. ρ is the Spearman correlation coefficient and *P* is the *P* value.

DISCUSSION

In the present study, we examined aneuploidies of autosome 21 and sex chromosomes X and Y in the peripheral

blood lymphocytes of Chinese workers exposed to benzene (range: <0.2–23.6 ppm, median: 2.9 ppm) and unexposed subjects and compared these frequencies with those determined in sperm of the same study subjects [Xing et al., 2010]. In blood lymphocytes, a slight association with benzene exposure was observed in the gain of chromosome 21, but not the gain of sex chromosomes. In contrast, in sperm, the gain of sex chromosomes was positively associated with benzene exposure, while the gain of chromosome 21was not. A positive correlation in the gain of sex chromosomes between blood lymphocytes and sperm was present in the unexposed subjects, but not in the exposed workers.

Increased aneuploidy has been frequently observed in the blood cells of workers exposed to benzene [Zhang et al., 2002]. An increased rate in trisomy 21 of blood lymphocytes was observed in Chinese workers exposed to high levels of benzene (median: 91.9 ppm, range: 31.5–328.5 ppm), though not in the workers exposed to lower levels (median:13.6 ppm, range: 1.6–30.6 ppm) [Smith et al., 1998]. In a recent CWAS study conducted in Chinese workers exposed to benzene at 4.95 ± 3.61 ppm (mean ± SD, < 10 ppm subgroup) and 28.33 ± 20.09 ppm (≥10 ppm subgroup) [Zhang et al., 2011], trisomy 21 was increased in the ≥10 ppm subgroup, though not in the <10 ppm subgroup, and it was the most sensitive aneuploidy associated with benzene exposure. Kim et al. [2004] reported that, even in workers exposed to benzene at a mean level of 0.557 ppm (range: 0.014–0.743 ppm), trisomy 21 in blood lymphocytes was increased relative to the controls. In the present study of male workers exposed to benzene at the median level of 2.9 ppm (range: < 0.2–23.6 ppm), a positive association of trisomy 21 in blood lymphocytes with the urinary benzene levels was observed. Together, these findings show that benzene exposure may induce trisomy 21 in blood lymphocytes. Gain of chromosome 21, a common cytogenetic alteration in leukemia patients, which is thought to play a role in leukemogenesis [Tigay, 2009; Fonatsch, 2010], may be one potential mechanism underlying benzene-induced leukemogenesis.

In the present study, average trisomy 21 rates of 0.09% in the unexposed workers and 0.17% in the exposed were observed, which were lower than those in two previous reports with 0.9% and 1.1–1.9% [Smith et al., 1998] and around 0.3% and 0.5–0.7% [Zhang et al., 2011] in unexposed and exposed workers, respectively, but higher than those in another report where rates of 0.01% in unexposed and 0.1% in exposed workers were found [Kim et al., 2004]. This discrepancy is common in cytogenetic studies performed in different labs or even within the same lab. There are several possible reasons for this: (1) Sex, age, smoking, alcohol drinking, diet, environmental exposures and other factors all have an impact on chromosome stability, thus different populations have various levels of aneuploidy; (2) Different benzene exposure levels contribute to the discrepancy in the exposed subjects; (3) Different DNA probes, FISH procedures and scoring criteria impact the observed aneuploidy levels.

Compared to the findings on the autosomes, aneuploidy of the sex chromosomes in the blood lymphocytes of workers exposed to benzene has rarely been reported. In a previous study of 12 workers exposed to low levels of benzene (average 0.1 ppm), aneuploidy of chromosome X was not increased relative to 12 controls [Carere et al., 1998]. In the recent CWAS study mentioned above [Zhang et al., 2011], no significant changes in gain of sex chromosomes were detected among the exposed men. In agreement with these findings, gain of the sex chromosomes in blood lymphocytes was not associated with benzene exposure in the present study.

In addition to aneuploidies in blood cells, occupational benzene exposure has also been associated with increased aneuploidy rates in sperm [Liu et al., 2000, 2003; Li et al., 2001; Zhao et al., 2004]. However, the studies analyzing blood cell and sperm aneuploidy in benzene-exposed workers were conducted in different populations. Table II summarizes the studies of aneuploidy in sperm of workers exposed to benzene, and the studies of aneuploidy in blood lymphocytes of workers at similar exposure levels as the sperm studies. Though they were conducted in different populations, these studies suggest that benzene exposure is associated with increased aneuploidies for different chromosomes in these two cell types. For instance, in two studies conducted in workers exposed to benzene at average levels of 26 and 27 ppm [Liu et al., 2000; Li et al., 2001], aneuploidies of chromsomes 9, 18, X, and Y in sperm were examined and increases in disomies of chromosomes 9, 18, and X were observed. In the blood lymphocytes of workers exposed to benzene at a similar average level of 28 ppm, CWAS analysis of all 24 chromosomes revealed increased trisomy in chromosomes 6, 10, 14, 16, 19, and 21 but not in chromosomes 9, 18, and X [Zhang et al., 2011]. Different aneuploidies in sperm and blood lymphocytes were also reported in workers exposed to benzene at lower levels. In a group of workers exposed to benzene at an average level of 13.2 ppm, aneuploidies of chromosomes 1, 7, 8, and 18 in sperm were examined and an increased disomy was observed in all four chromosomes [Liu et al., 2003; Zhao et al., 2004]. A series of studies were conducted in workers exposed to benzene across a wide range: 1.6–328.5 ppm (median 31 ppm) [Zhang et al., 1996, 1998, 1999, 2007; Smith et al., 1998]. Among the workers exposed to <31 ppm (median 13.6 ppm) comparable to the two sperm studies above, increased trisomy in lymphocytes was observed in chromosomes 4, 6, 7, and 11 but not in chromosomes 1, 8, and 18. Similarly, increased disomy of chromosomes X and Y in sperm was observed among workers exposed to benzene at an average level of 7.6

TABLE II. Studies of Aneuploidy in Sperm or Blood Lymphocyte Metaphases of Workers Exposed to Benzene at Similar Levels

Benzene levels (8h-TWA)	Cell type	Subjects (controls, exposed)	Chromosomes tested	Significant increases in hyperhaploidy (sperm) or hyperdiploidy (lymphocytes)	References
Average 27.07 ppm	Sperm	16, 14	9 and 18	Disomy of chromosomes 9 and 18	[Li et al., 2001]
GM 26.28 ppm	Sperm	13, 13	X and Y	Disomy of chromosome X	[Liu et al., 2000]
Average 28.33 ppm	Lymphocytes	27, 25	1–22, X, and Y	Trisomy of chromosomes 6, 10, 14, 16, 19, and 21	[Zhang et al., 2011]
Average 13.24 ppm	Sperm	14, 15	1, 7, 8, and 18	Disomy of chromosomes 1, 7, 8, and 18	[Liu et al., 2003; Zhao et al., 2004]
Median 13.6 ppm	Lymphocytes	44, 21	1, 2, 4, 5, 6, 7, 8, 9, 11, 12, 14, 18, and 21	Trisomy of chromosomes 4, 6, 7, and 11	[Smith et al., 1998; Zhang et al., 2007; Zhang et al., 1996; Zhang et al., 1998; Zhang et al., 1999]
GM 7.6 ppm	Sperm	33, 16	21, X, and Y	Disomy of chromosomes X and Y	[Xing et al., 2010]
GM 7.6 ppm	Lymphocytes	33, 16	21, X, and Y	Trisomy of chromosome 21 (borderline)	Present study
Average 4.95 ppm	Lymphocytes	27, 22	1–22, X, and Y	None	[Zhang et al., 2011]
GM 1.0 ppm	Sperm	33, 17	21, X, and Y	Disomy of chromosome X	[Xing et al., 2010]
GM 1.0 ppm	Lymphocytes	33, 17	21, X, and Y	None	Present study

Abbreviation: GM, geometric mean.

ppm [Xing et al., 2010], while not in the blood lymphocytes among workers exposed to benzene at an average level of 5 ppm [Zhang et al., 2011].

The different aneugenic effects of benzene in blood cells and sperm is supported by our current study, which is the first one comparing aneuploidies in blood lymphocytes and sperm of the same individuals exposed to benzene. Our results show that benzene exposure affected aneuploidy of chromosome 21 in lymphocytes and aneuploidy of the sex chromosomes in sperm [Xing et al., 2010]. Our findings suggest that benzene exposure may induce aneuploidies in both somatic and germ cells within the same individuals although the affected chromosomes may differ by cell type.

The mechanisms underlying the different aneugenic effects of benzene in blood lymphocytes and sperm are unclear, but two factors likely contribute. First, sperm and blood lymphocytes are generated by different biological processes, sperm via meiosis and blood lymphocytes via mitosis. Pairing of homologous chromosomes is unique to meiosis and benzene and/or its active metabolites may disrupt this pairing during spermatogenesis, which would result in different aneugenic effects from those in blood lymphocytes. In this context, it should be noted that the pairing of the sex chromosomes is distinct from the pairing of autosomal chromosomes, occurring only in the pseudoautosomal regions of the sex chromosomes rather than along the entire chromosomal length as for the autosomes [Rappold, 1993]. This difference may make the sex chromosomes more susceptible to the aneugenic activity of benzene. Second, the distribution of benzene and its toxic metabolites may differ in blood and testis. Though the deposition of benzene and its metabolites in various human tissues have not been reported, a clearly different metabolic profile of blood/bone marrow was observed from that of liver and lung in mice and rats [Sabourin et al., 1988], indicating differences in metabolism, accumulation or clearance rates in different tissues. Thus, the different metabolic profile between blood cells and testis may also contribute to the different aneugenic effects that we have observed in lymphocytes and sperm.

In the present study, a slight correlation in gain of sex chromosomes between the blood lymphocytes and sperm was observed among the unexposed subjects. A positive correlation between aneuploidies in blood lymphocytes and sperm has previously been reported in patients with abnormal spermatogenesis and in healthy donors [Gazvani et al., 2000a,b; Rubes et al., 2002; De Palma et al., 2005], which suggests the existence of a subtle generalized mitotic/meiotic instability that leads to chromosome malsegregation events. In the current study, a correlation in gain of sex chromosomes between the blood lymphocytes and sperm was observed among the unexposed subjects, but not among the exposed workers, further indicating the different aneugenic effects of benzene in the blood lymphocytes and sperm.

CONCLUSIONS

We found that benzene exposure was positively associated with the gain of chromosome 21 but not sex chromo-

somes in blood lymphocytes. Because of the small sample size (33 unexposed workers and 33 exposed ones) and the limited number of cells examined ($\leq$1,000 per subject) relative to the rare events reported here, additional studies with larger sample size are needed to confirm these findings. Comparisons of the lymphocyte results with our previous study in sperm of the same workers suggest that benzene exposure induces aneuploidies in both blood and sperm cells within the same individuals, but selectively affects chromosome 21 (an autosome) in blood lymphocytes and the sex chromosomes in sperm.

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