

Polymorphisms in genes involved in DNA double-strand break repair pathway and susceptibility to benzene-induced hematotoxicity

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Benzene is a recognized hematotoxicant and carcinogen that produces genotoxic damage. DNA double-strand breaks (DSB) are one of the most severe DNA lesions caused directly and indirectly by benzene metabolites. DSB may lead to chromosome aberrations, apoptosis and hematopoietic progenitor cell suppression. We hypothesized that genetic polymorphisms in genes involved in DNA DSB repair may modify benzene-induced hematotoxicity. We analyzed one or more single nucleotide polymorphisms (SNPs) in each of seven candidate genes (*WRN*, *TP53*, *NBS1*, *BRCA1*, *BRCA2*, *XRCC3* and *XRCC4*) in a study of 250 workers exposed to benzene and 140 controls in China. Four SNPs in *WRN* (Ex4 –16 G > A, Ex6 +9 C > T, Ex20 –88 G > T and Ex26 –12 T > G), one SNP in *TP53* (Ex4 +119 C > G) and one SNP in *BRCA2* (Ex11 +1487 A > G) were associated with a statistically significant decrease in total white blood cell (WBC) counts among exposed workers. The SNPs in *WRN* and *TP53* remained significant after accounting for multiple comparisons. One or more SNPs in *WRN* had broad effects on WBC subtypes, with significantly decreased granulocyte, total lymphocyte, CD4⁺-T cell, CD8⁺-T cell and monocyte counts. Haplotypes of *WRN* were associated with decreased WBC counts among benzene-exposed subjects. Likewise, subjects with *TP53* Ex4 +119 C > G variant had reduced granulocyte, CD4⁺-T cell and B cell counts. The effect of *BRCA2* Ex11 +1487 A > G polymorphism was limited to granulocytes. These results suggest that genetic polymorphisms in *WRN*, *TP53* and *BRCA2* that maintain genomic stability impact benzene-induced hematotoxicity.

Abbreviations: BMI, body mass index; DSB, double-strand break; LD, linkage disequilibrium; SNP, single nucleotide polymorphism; WBC, white blood cell.

Introduction

Benzene is an important industrial chemical and is a component of cigarette smoke, gasoline, crude oil and automobile emissions. Several million workers worldwide are exposed to benzene in the oil industry, shipping, automobile repair, shoe manufacture, and so on. (1). Epidemiological studies have shown that exposure to benzene results in an increased risk of aplastic anemia, myelodysplastic syndromes, leukemia and other blood disorders (1).

Benzene must be metabolized in order to elicit toxicity to the blood and bone marrow (2). These benzene metabolites, that is, reactive quinones, are capable of binding to and damaging macromolecules including DNA, glutathione, tubulin, histones, topoisomerase II and other DNA-related proteins. Additionally, benzene metabolites may give rise to reactive oxygen species (ROS) (3).

Direct attack by ROS and benzene metabolites or replication of unrepaired DNA damage can result in DNA double-strand breaks (DSB) (4). DSBs are repaired *in vivo* by non-homologous end joining or, after replication when a second identical DNA copy is present, homologous recombination (5). DSB is especially genotoxic because (i) it affects both DNA strands and no intact template is available for repair; (ii) the repair is intrinsically more difficult than other types of DNA repair mechanisms because erroneous rejoining of broken DNA may occur. Therefore, a DNA DSB is potentially highly cytotoxic and can induce chromosomal aberrations (CA) and disrupt the genomic integrity of a cell. It was observed that chromosomal instability can be induced by the benzene metabolite, hydroquinone, and may contribute to the development of acute myeloid leukemia by increasing the number of genetic lesions in hematopoietic cells (6). Therefore, the prompt and efficient repair of DSBs is fundamental for genomic stability and cancer prevention in the presence of benzene (7).

Stem cells in bone marrow undergo active cell proliferation and differentiation throughout life and are sensitive to DNA-damaging factors. Hematopoietic progenitor cells have been found to be suppressed after benzene exposure (8,9). In addition, DNA DSB repair genes may be implicated in hematopoiesis (10). In a study by Bender *et al.* (11) mice with defects in DSB repair genes demonstrated progressive hematopoietic stem cell failure.

Genetic polymorphism in genes involved in the repair of DSB may modify the function of DNA DSB repair and confer genetic susceptibility to hematotoxicity caused by benzene. We, therefore, examined the association between a number of single nucleotide polymorphisms (SNPs)/haplotypes in seven genes, which are either DSB repair genes (*NBS1*, *BRCA1*, *BRCA2*, *XRCC3*, and *XRCC4*) or genes that play an important role in this pathway (*WRN* and *TP53*), and benzene-induced hematotoxicity in a cross-sectional study in China.

Materials and methods

Study population and exposure assessment

The details of this cross-sectional study have been described elsewhere (8). Briefly, the study population included 250 workers who were exposed to benzene in two shoe manufacturing factories, and 140 unexposed controls from comparable populations who worked in three clothing-manufacturing factories in the same region of China. Data were obtained from 28 benzene-exposed workers in both study years (2000 and 2001) and are treated as independent observations in the study. Controls were frequency-matched by sex and age to exposed workers. Blood samples were collected from all workers. Individual exposure to benzene and toluene, as well as other organic solvents, was monitored repeatedly up to 16 months before phlebotomy by wearing an organic vapor passive monitor badge, and post-shift urine samples were collected from each subject (8). Subjects were administered a questionnaire for information on lifetime occupational history, hobbies, environmental exposures, medical history and current medications, and past and current tobacco and alcohol use. Individuals carrying out exposure assessment activities were blinded with regard to hematologic data on study subjects.

Hematology

All subjects provided a 29 ml peripheral blood sample and a buccal cell mouth rinse sample, and underwent a physical exam. Blood samples were delivered to the lab within 6 h of being collected; the complete blood cells (CBC) and differentials were analyzed using a T540 blood counter, and the major lymphocyte subsets were analyzed by an FACS Calibur flow cytometer (Software: SimulSET v3.1).

Genotyping

DNA was extracted from blood samples using phenol–chloroform extraction (12) and genotyped by TaqMan-based real-time PCR at Core Genotyping Facility of NCI (<http://snp500cancer.nci.nih.gov>) (13). SNPs were selected for study on the basis of a minimum allele frequency of 0.05, and evidence of association in previous epidemiology studies, evidence of function, or to extend genomic coverage for a given gene. Individuals carrying out genotyping were blinded with regard to hematologic data on study subjects. DNA was successfully extracted from all samples, and >95% of the DNA samples were successfully genotyped for all candidate SNPs except for three SNPs in *WRN* [Ex6 +9 C > T (91%), Ex20 –88 G > T (90%) and IVS33 –95 C > T (81%)] and four SNPs in *BRCA2* [Ex11 +1487 A > G (88%), Ex11 +1898 T > C (90%), Ex14 –194 A > G (90%) and Ex2 +14 A > G (88%)]. Blind replicate samples were randomly interspersed, and the concordance rates were 99–100% for all assays.

Statistical analysis

The Hardy–Weinberg equilibrium for each SNP was tested with a Pearson χ^2 or exact test. Measure of pairwise linkage disequilibrium (LD) and the test for LD at one gene were carried out using the HaploView program (<http://www.broad.mit.edu/personal/jcbarr/haploview/>). Genotype data were analyzed with the homozygotes of the common allele as the reference group. Generalized Estimating Equations (GEE) were used to model the relationship between SNPs and blood cell counts, adjusting for age, sex, body mass index (BMI), any recent smoking and alcohol consumption for controls, plus occupational exposure to benzene and toluene on a log scale 1 month before phlebotomy for exposed subjects. Interaction was estimated by adding a multiplicative term between SNP (variant carriers versus Wild-type carriers) and benzene exposure (yes/no) into a model with all subjects.

Haplotype block structure was examined for SNPs within the same gene using HaploView. Overall association between quantitative blood cell counts and haplotypes was assessed separately for unexposed and exposed subjects adjusting for the potential confounders using the HaploStats program (14) in R (Version 2.0.1) (15). Individual haplotypes were estimated using SAS/Genetics, and the effects of each haplotype were estimated using the best haplotype pairs in a GEE model with the most common haplotype as the reference.

We controlled for multiple hypothesis testing by calculating the false discovery rate, using the Benjamini–Hochberg method (16), which was defined as the expected ratio of erroneous rejections of the null hypothesis to the total number of rejected hypotheses. An FDR of 0.05 was used as a critical value to assess if the obtained *P*-values are still significant. Those SNPs demonstrating significant gene-dosage effects (i.e. trend test) among workers exposed to benzene were further explored for their influence on specific white blood cell (WBC) subtypes. All *P*-values presented are two-sided and data were analyzed using the Statistical Analysis Software, version 8.02 (SAS Institute Inc, 1996) if not specified elsewhere.

Results

Demographic characteristics were essentially the same between controls and benzene-exposed workers. The majority of the study subjects were females (65%) and relatively young (30 ± 8). The subjects in the benzene-exposed and non-exposed groups were comparable in alcohol use, recent infection, smoking status and BMI (8). The benzene-exposed workers had been employed an average of 6.1 ± 2.9 years. The average benzene air exposure in the month before phlebotomy was 5.4 (SD: 12.1) p.p.m. in the exposed group (Table I). WBCs and most WBC subtypes as well as the platelet counts were significantly reduced in benzene-exposed workers compared with controls (Table I). Natural killer (NK) cell counts and hemoglobin levels were significantly decreased only among the most highly exposed workers (8), and CD8⁺-T cells did not vary with benzene-exposure levels.

Twenty-four SNPs in seven genes involved in the DNA DSB repair pathway were genotyped, covering substitutions in the coding and non-coding regions (Table II). Only those SNPs that are associated with total WBC counts in benzene-exposed group are shown in Table III. Those SNPs that were not associated with total WBC counts in benzene-exposed group are shown in a Supplementary Table. In the exposed group, homozygous variants in four SNPs in *WRN* (Ex4 –16 G > A, Ex6 +9 C > T, Ex20 –88 G > T and Ex26 –12 T > G) had significantly reduced WBCs in the range of 8–18%. For the *TP53* Ex4 +119 C > G polymorphism, the magnitude of reduction in WBC counts was ~12%, and subjects

Table I. Hematological characteristics of study population by benzene-exposure status^a

	Controls (<i>n</i> = 140)	Exposed (<i>n</i> = 250)	<i>P</i>
Benzene-exposure measurements			
Benzene air level (p.p.m.) ^b		5.4 ± 12.1	
Benzene urine (μg/l) ^c	0.4 ± 1.2	158 ± 536	
Peripheral blood cell counts ^d			
Total WBCs	6484 ± 1712	5488 ± 1350	<0.0001
Granulocytes	4111 ± 1410	3334 ± 1053	<0.0001
Lymphocytes	2129 ± 577	1939 ± 521	0.0014
CD4 ⁺ -T cells	742 ± 262	622 ± 183	<0.0001
CD8 ⁺ -T cells	553 ± 208	553 ± 213	0.88
CD4 ⁺ : CD8 ⁺ ratio	1.5 ± 0.6	1.2 ± 0.4	<0.0001
B cells	218 ± 93.8	173 ± 88.5	<0.0001
NK cells	586 ± 318	542 ± 277	0.30
Monocytes	241 ± 92.1	215 ± 93.2	0.002
Platelets	230 ± 59.7 × 10 ³	(202 ± 52.0) × 10 ³	<0.0001
Hgb (g/dl)	14.5 ± 1.6	14.5 ± 1.6	0.83

^aThere are up to 418 observations on 390 unique subjects (140 controls and 250 benzene-exposed workers). Data were obtained from 28 exposed subjects in both years (2000 and 2001) and are treated as independent observations in summary data shown.

^bBenzene air level is the arithmetic mean (standard deviation) of an average of two measurements per subject collected during the month before phlebotomy.

^cUrinary benzene (mean, standard deviation) and mean individual air levels of benzene were strongly correlated (Spearman *r* = 0.88, *P* < 0.0001).

^dUnadjusted mean (standard deviation) cells per microliter of blood. Linear regression was used for statistical analyses adjusted for repeated measures by GEEs. Models were adjusted for age, sex, current smoking, current alcohol drinking, BMI and recent infections and, where appropriate, ln benzene and ln toluene air level.

Table II. Genes and SNPs assessed in this study

Gene	Chromosome location	SNP Region (dbSNP ID) ^a
<i>WRN</i>	8p12-p11.2	Ex4 -16 G > A (rs4987236) Val114Ile; Ex6 +9 T > C (rs2725349) Cys171Cys; Ex20 -88 T > G (rs1800392) Leu787Leu; Ex26 -12 T > G (rs2725362) Phe1074Leu; IVS33 -95 C > T (rs4987036); Ex34 -93 T > C (rs1346044) Cys1367Arg
<i>TP53</i>	17p13.1	Ex4 +119 G > C (rs1042522) Arg72Pro
<i>NBS1</i>	8q21-q24	Ex5 -32 G > C (rs1805794) Glu185Gln
<i>BRCA1</i>	17q21	Ex12 +1641 T > C (rs16940) Leu730Leu; Ex12 -1485 C > T (rs799917) Pro830Leu; Ex12 -984 A > G (rs16941) Glu997Gly; Ex12 -549 A > G (rs16942) Lys1142Arg; Ex17 -150 A > G (rs1799966) Ser1613Gly
<i>BRCA2</i>	13q12.3	Ex2 +14 A > G (rs1799943) (5' UTR); Ex10 +72 A > C (rs766173) Asn289His; Ex10 +321 A > C (rs144848) Asn372His; Ex11 +1062 A > G (rs1799944) Asn991Asp; Ex11 +1487 A > G (rs1801406) Lys1132Lys; Ex11 +1898 T > C (rs543304) Val1269Val; Ex14 -194 A > G (rs1799955) Ser2414Ser
<i>XRCC3</i>	14q32.3	Ex8 -53 C > T (rs861539) Thr241Met
<i>XRCC4</i>	5q13-q14	Ex6 -7 G > T (rs3734091) Ala247Ser; IVS7 -1 G > A (rs1805377); Ex8 +34 T > G (rs1056503) Ser307Ser

^aIncluding amino acid change if applicable.

homozygous for the uncommon allele of *BRCA2* Ex11 +1487 A > G had a 7% greater reduction in WBCs. There was a linear trend of reduced WBC counts associated with the increase of carried variant for *WRN* Ex6 +9 C > T, Ex20 -88 G > T, Ex26 -12 T > G, *TP53* Ex4 +119 C > G and *BRCA2* Ex11 +1487 A > G. Although most SNPs located within the same gene were in LD and as such do not constitute true independent tests, we assessed all 24 SNP associations for possible FDR and found that the five SNPs in *WRN* and *TP53* remained significantly associated with total WBC counts. There was a statistically significant interaction ($P = 0.021$) between benzene exposure and the *WRN* Ex26 -12 T > G polymorphism. All these SNPs were not found to influence WBC counts among unexposed subjects except that the polymorphism of *BRCA2* Ex11 +1487 A > G was associated with increased WBCs with borderline significance. SNPs in other genes were not associated with reduced WBC counts in exposed subjects.

We further evaluated the influence on several major WBC subtypes of the SNPs that had significant gene-dosage effects on total WBC counts among exposed workers (Table IV). One or more SNPs in *WRN* had broad effects on WBC subtypes, with significant decreases of granulocyte, total lymphocyte, CD4⁺-T cell, CD8⁺-T cell and monocytes counts. Likewise, *TP53* Ex4 +119 C > G was associated with reduced granulocyte, CD4⁺-T cell and B cell counts, while the effect of *BRCA2* Ex11 +1487 A > G polymorphism was limited to granulocytes.

Haplotype analysis of genes in which two or more SNPs in LD were available (i.e. *WRN*, *BRCA1*, *BRCA2* and *XRCC4*) was carried out. Six SNPs in *WRN* constituted one haplotype block. There was an overall association between WBC counts and haplotypes of *WRN* ($P = 0.007$) in the benzene-exposed group (Table V). A haplotype with substitutions in three SNPs (Ex4 -16 G > A, Ex20 -88 G > T and Ex26 -12 T > G) was

Table III. Effect on total WBC counts of SNPs in *WRN*, *TP53* and *BRCA2* by benzene-exposure status^a

Genotype	Controls	WBC ^b	<i>P</i>	Exposed	WBC ^b	<i>P</i>
<i>WRN</i>						
Ex4 -16 G > A (rs4987236)						
Val114Ile						
GG	133	6430 ± 1658	Ref.	267	5524 ± 1356	Ref.
GA	7	7514 ± 2473	0.489	9	4511 ± 752	0.0003
Ex6 +9 T > C (rs2725349)						
Cys171Cys						
TT	96	6516 ± 1615	Ref.	179	5463 ± 1340	Ref.
TC	30	6600 ± 2046	0.988	62	5398 ± 1342	0.575
CC	1	6500		10	4710 ± 1090	0.0008
Trend			0.983			0.022
Ex20 -88 T > G (rs1800392)						
Leu787Leu						
TT	46	6517 ± 1642	Ref.	90	5764 ± 1391	Ref.
TG	59	6592 ± 1744	0.895	104	5295 ± 1270	0.010
GG	20	6550 ± 1789	0.831	56	5159 ± 1367	0.002
Trend			0.821			0.001
Ex26 -12 T > G (rs2725362) ^c						
Phe1074Leu						
TT	53	6392 ± 1726	Ref.	105	5866 ± 1409	Ref.
TG	68	6479 ± 1719	0.791	111	5320 ± 1232	0.002
GG	18	6756 ± 1757	0.350	61	5144 ± 1316	0.0006
Trend			0.393			0.0003
<i>TP53</i>						
Ex4 +119 G > C (rs1042522)						
Arg72Pro						
GG	41	6534 ± 1763	Ref.	103	5764 ± 1393	Ref.
GC	67	6621 ± 1758	0.684	109	5489 ± 1367	0.149
CC	31	6190 ± 1532	0.531	63	5067 ± 1150	0.0009
Trend			0.530			0.001
<i>BRCA2</i>						
Ex11 +1487 A > G (rs1801406)						
Lys1132Lys						
AA	54	6280 ± 1502	Ref.	96	5611 ± 1546	Ref.
AG	61	6674 ± 1843	0.091	121	5326 ± 1197	0.127
GG	9	7489 ± 1883	0.092	27	5193 ± 1059	0.073
Trend			0.035			0.045

^aModels were adjusted for age, sex, current smoking, current alcohol drinking, BMI, recent infections and, in exposed workers, in air benzene exposure and in air toluene exposure in the month before phlebotomy. There are two controls without BMI data and they are excluded from the statistical analysis.

^bUnadjusted total WBC count (/μl) as mean ± standard deviation.

^cThere is a significant interaction ($P = 0.021$) between benzene exposure (yes/no) and this polymorphism [(TG + GG)/TT].

associated with a 20% reduction in WBCs following benzene exposure.

Discussion

We studied the effect of 24 SNPs in 7 genes that survey the genome and participate in DNA DSB repair on peripheral WBC counts among 250 benzene-exposed workers and 140 unexposed controls. Of these genes, our results demonstrate that four SNPs in *WRN* (Ex4 -16 G > A, Ex6 +9 C > T, Ex20 -88 G > T and Ex26 -12 T > G), one SNP in *TP53* (Ex4 +119 C > G) and one SNP in *BRCA2* (Ex11 +1487 A > G) are associated with decreased WBCs and particular WBC subtypes in benzene-exposed workers. The effect of all SNPs on WBC counts in controls was not statistically significant after adjustment for multiple comparisons using the FDR method. In addition, the two unadjusted borderline associations between *WRN* (IVS33 -95 C > T) and *XRCC4*

Table IV. Effect on WBC subsets of SNPs in *WRN*, *TP53* and *BRCA2* among benzene-exposed subjects^a

Genotype	Exposed	Granulocytes ^b	<i>P</i>	Lymphocytes ^b	<i>P</i>	CD4 ⁺ -T cells ^b	<i>P</i>	CD8 ⁺ -T cells ^b	<i>P</i>	B Cells ^b	<i>P</i>	NK cells ^b	<i>P</i>	Monocytes ^b	<i>P</i>
<i>WRN</i>															
Ex4 -16 G > A (rs4987236)															
Val114Ile															
GG	267	3353 ± 1060	Ref.	1954 ± 519	Ref.	626 ± 184	Ref.	557 ± 216	Ref.	174 ± 89	Ref.	547 ± 277	Ref.	217 ± 93	Ref.
GA	9	2800 ± 742	0.011	1556 ± 448	0.038	513 ± 128	0.130	440 ± 105	0.016	158 ± 87	0.016	405 ± 270	0.207	156 ± 73	0.016
Ex6 +9 T > C (rs2725349)															
Cys171Cys															
TT	179	3324 ± 1067	Ref.	1925 ± 505	Ref.	626 ± 193	Ref.	558 ± 212	Ref.	168 ± 86	Ref.	521 ± 267	Ref.	214 ± 85	Ref.
TC	62	3305 ± 998	0.830	1874 ± 532	0.307	598 ± 154	0.394	520 ± 226	0.131	171 ± 98	0.436	550 ± 295	0.894	219 ± 114	0.669
CC	10	2620 ± 736	0.0001	1850 ± 584	0.178	556 ± 176	0.167	464 ± 158	0.037	166 ± 55	0.953	621 ± 284	0.752	240 ± 84	0.793
Trend			0.027		0.111		0.140		0.022		0.605		0.783		0.845
Ex20 -88 T > G (rs1800392)															
Leu787Leu															
TT	90	3532 ± 1142	Ref.	2004 ± 502	Ref.	654 ± 197	Ref.	583 ± 212	Ref.	169 ± 85	Ref.	550 ± 274	Ref.	228 ± 85	Ref.
TG	104	3215 ± 909	0.042	1866 ± 546	0.045	599 ± 174	0.041	532 ± 211	0.057	164 ± 70	0.968	521 ± 284	0.864	213 ± 102	0.274
GG	56	3102 ± 1129	0.004	1848 ± 466	0.048	589 ± 174	0.025	515 ± 222	0.026	180 ± 118	0.741	537 ± 279	0.956	209 ± 92	0.326
Trend			0.003		0.031		0.018		0.019		0.755		0.981		0.280
Ex26 -12 G > T (rs2725362)															
Phe1074Leu															
TT	105	3593 ± 1159	Ref.	2048 ± 498	Ref.	667 ± 196	Ref.	595 ± 219	Ref.	176 ± 87	Ref.	556 ± 264	Ref.	225 ± 86	Ref.
TG	111	3226 ± 881	0.020	1882 ± 547	0.008	593 ± 170	0.008	537 ± 201	0.029	167 ± 73	0.697	529 ± 289	0.546	212 ± 101	0.224
GG	61	3085 ± 1072	0.002	1854 ± 488	0.015	595 ± 171	0.026	509 ± 215	0.006	178 ± 114	0.909	541 ± 281	0.810	205 ± 90	0.224
Trend			0.001		0.007		0.014		0.004		0.967		0.745		0.183
<i>TP53</i>															
Ex4 +119 G > C (rs1042522)															
Arg72Pro															
GG	103	3515 ± 1151	Ref.	2021 ± 557	Ref.	656 ± 195	Ref.	565 ± 224	Ref.	184 ± 102	Ref.	555 ± 262	Ref.	228 ± 98	Ref.
GC	109	3367 ± 1040	0.518	1914 ± 525	0.124	605 ± 179	0.045	552 ± 219	0.736	173 ± 79	0.534	533 ± 275	0.186	208 ± 84	0.196
CC	63	3000 ± 830	0.003	1860 ± 440	0.124	599 ± 166	0.123	536 ± 189	0.745	155 ± 79	0.040	543 ± 308	0.496	206 ± 100	0.148
Trend			0.005		0.097		0.078		0.723		0.047		0.393		0.123
<i>BRCA2</i>															
Ex11 +1487 A > G (rs1801406)															
Lys1132Lys															
AA	96	3493 ± 1235	Ref.	1909 ± 544	Ref.	615 ± 202	Ref.	532 ± 230	Ref.	160 ± 78	Ref.	543 ± 298	Ref.	209 ± 91	Ref.
AG	121	3222 ± 915	0.067	1887 ± 467	0.927	621 ± 176	0.395	542 ± 187	0.287	174 ± 84	0.170	509 ± 259	0.306	217 ± 86	0.257
GG	27	2981 ± 791	0.005	1974 ± 522	0.601	604 ± 140	0.773	593 ± 216	0.071	182 ± 136	0.759	573 ± 245	0.297	237 ± 131	0.405
Trend			0.005		0.721		0.841		0.077		0.398		0.811		0.264

^aModels were adjusted for age, sex, current smoking, current alcohol drinking, BMI, recent infections and, in exposed workers, In air benzene exposure and In air toluene exposure in the month before phlebotomy. There are two controls without BMI data and they are excluded from the statistical analysis.

^bUnadjusted cell counts (/μl) as mean ± standard deviation.

Table V. Haplotype analysis of *WRN* on total WBC counts by benzene-exposure status

Haplotype ^a	Controls	WBC ^b	P ^c	Exposed	WBC ^b	P ^c
G-T-T-T-C-T	173	6453 ± 1707	Ref.	311	5658 ± 1355	Ref.
A-C-G-G-C-C				1	4000	
A-T-G-G-C-T	7	7514 ± 2473	0.484	8	4575 ± 778	0.0002
G-C-G-G-C-C	26	6315 ± 1951	0.712	74	5385 ± 1369	0.013
G-C-G-G-T-T	6	7800 ± 1761	0.057	15	5107 ± 1263	0.297
G-C-T-T-C-T				1	5900	
G-T-G-G-C-C	3	4867 ± 569	0.0009	6	5500 ± 657	0.601
G-T-G-G-C-T	1	6300				
G-T-G-G-T-T	61	6546 ± 1477	0.456	128	5198 ± 1262	0.008
G-T-G-T-C-T	2	4700 ± 848		6	6600 ± 2117	0.272
G-T-G-T-T-T				1	5300	
G-T-T-G-C-T				1	4000	
G-T-T-T-T-T	1	6100		2	6000 ± 1980	
Omnibus test ^c			0.844			0.007

^aSix SNPs in *WRN* are included in the analysis: Ex4 -16 G > A, Ex6 +9 T > C, Ex20 -88 T > G, Ex26 -12 T > G, IVS33 -95 C > T and Ex34 -93 T > C.

^bUnadjusted cell counts (/μl) as mean ± standard deviation.

^cModels were adjusted for age, sex, current smoking, current alcohol drinking, BMI, recent infections and, in exposed workers, in air benzene exposure and in air toluene exposure in the month before phlebotomy.

(Ex6 -7 G > T) and lower WBC count are based on small numbers of observations.

The gene *WRN* encodes a member of the RecQ subfamily and the DEAH (Asp-Glu-Ala-His) subfamily of DNA and RNA helicases. It possesses an intrinsic 3'-5' DNA helicase activity, and is also a 3'-5' exonuclease. *WRN* plays an important role in monitoring genome integrity and controlling the cell's response to genotoxic stress (17). Initially, together with other related factors, *WRN* helps recruit the proper DNA repair factors to the site of the lesion. However, the *WRN* complex may direct the activation of the apoptotic pathway if the damage is excessive. Lack of *WRN* may result in deregulation of DNA damage monitoring and anomalous activation of DNA repair or apoptosis in response to certain types of DNA damage (18). Specifically, *WRN* has been found to play a key role in DSB repair (19). Mutations in this gene produce truncated proteins and result in Werner syndrome, an autosomal recessive disorder characterized by premature aging and genomic instability.

Four significant SNPs are located in the functional domains of *WRN*, with the Ex4 -16 G > A and Ex6 +9 C > T being in the exonuclease domain, Ex20 -88 G > T in the helicase domain and Ex26 -12 T > G located in the vicinity of the RecQ C-terminal domain (17,20). These polymorphisms may modify the function of these functional domains, particularly for Ex26 -12 T > G, which is located in a region that interacts with multiple proteins (21). Even though the four SNPs are in LD, there may be an accumulation of effects based on the haplotype analysis, indicating that the effects may not be attributable to one particular SNP. However, available reports for the impact of these SNPs on longevity and aging-related diseases demonstrated only weak and inconsistent associations (22,23), and, moreover, the *WRN* Ex26 -12 T > G polymorphism displayed little change in helicase/exonuclease activities relative to wild-type *WRN* in an *in vitro* assay (24). This region in *WRN* (Ex4-Ex26) warrants further investigation for the accountable polymorphic

position(s) and their precise role in susceptibility to benzene hematotoxicity.

Normal function of *TP53* is essential in DNA damage response, cell cycle control and cell apoptosis, and mutations in *TP53* are the most common genetic alterations in human tumors. It is activated when genetic material is altered, initiating a range of biological defense pathways. In response to DNA damage, *TP53*-mediated cell cycle is arrested to allow sufficient time for DNA repair before DNA replication or mitosis. It also directly participates in DNA repair, particularly in homologous recombination and non-homologous end joining (25,26). It has been found that bone marrow cells in *p53*-deficient mice expressed significantly reduced levels of many key genes involved in the *p53*-regulated DNA damage response pathways after chronic exposure to benzene (27). In cDNA microarray analyses, Yoon *et al.* (28) demonstrated that *p53* appears to play a key role in benzene-induced hematotoxicity.

The *TP53* codon 72 polymorphism is functional. The Ex4 +119 C > G non-conservative substitution is located in a proline-rich region (residues 64-92) of the *p53* protein, where the 72Pro amino acid constitutes one of five PXXP (where P represents Pro and X any amino acid) motifs resembling an Src homology 3 (SH3) binding domain (29). The polymorphic variant of wild-type *p53* has been shown to have different biochemical properties and biological functions (30-32). Significantly higher levels of *p53* expression in lymphocytes were detected in subjects bearing the Arg/Arg allele than in subjects with Pro/Pro and Arg/Pro alleles among healthy humans exposed to low doses of ionizing radiation (33). These findings provide support for a model in which loss of *p53* function due to Ex4 +119 C > G polymorphism is associated with benzene-induced hematotoxicity.

BRCA2 is a tumor-suppressor protein directly implicated in familial breast cancer. It maintains genome stability by participating in multiple biological pathways including DNA transcription, recombination and cell cycle control (34,35). A particular spectrum of bi-allelic mutations in *BRCA2* is connected to a rare hematological disorder, Fanconi anemia, characterized by aplastic anemia and hypoplasia of the bone marrow (36). *BRCA2* has an important role in DNA recombination reactions mediated by RAD51 in DNA DSB repair (34). Two structural features of *BRCA2* were revealed with eight BRC repeats interacting with RAD51 and three oligonucleotide/oligosaccharide-binding (OB) fold domains binding single-strand DNA (37). The *BRCA2* Ex11 +1487 A > G polymorphism is located in exon 11, which is the largest exon and encodes the eight BRC motifs (38). The exon 11 and BRC motifs are essential for *BRCA2*'s function and homologous recombination (38,39). The eight BRC repeats are highly conserved and the four BRC repeats in 5' are the most conserved sequences (38). The *BRCA2* Ex11 +1487 A > G is located between BRC1 and BRC2 in 5' of Exon 11. As it leads to a synonymous substitution, the observed hematotoxic effect may be attributable to a linked functional polymorphism in that region that can modify the function of BRC repeats and impact DNA DSB repair.

In summary, we report that common genetic polymorphisms in *WRN*, *TP53* and *BRCA2* may confer susceptibility to hematotoxicity in workers exposed to benzene. All these genetic variants appear to influence granulocytes, while variants in *WRN* also altered cells of the lymphoid lineage, suggesting that effects may trace back to earlier progenitor and possibly

stem cells. The three gene products play an important role in multiple mechanisms including DNA damage recognition, replication, recombination, repair and cell cycle regulation, all of which are critical to maintain genomic integrity. In addition, BRCA2, TP53 and WRN perhaps act synergistically to prevent accumulation of genomic lesions (40,41). Decreased function of WRN, TP53 or BRCA2 due to genetic polymorphisms may result in genomic instability and increase predisposition to cancer in the presence of a carcinogen such as benzene.

Although this is the largest cross-sectional study of DNA repair SNPs and benzene-induced hematotoxicity in the literature, to the best of our knowledge, and our key findings were highly statistically significant, it is possible that some associations are false-positives, particularly those based on small numbers of subjects carrying at-risk alleles. As such, replication of key findings in other benzene-exposed populations is critical.

Supplementary material

Supplementary material is available online at <http://www.carcin.oupjournals.org/>.

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