# Polymorphisms in Cytokine and Cellular Adhesion Molecule Genes and Susceptibility to Hematotoxicity among Workers Exposed to Benzene

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## Abstract

**Benzene is a recognized hematotoxin and leukemogen but its mechanism of action and the role of genetic susceptibility are still unclear. Cytokines, chemokines, and cellular adhesion molecules are soluble proteins that play an important regulatory role in hematopoiesis. We therefore hypothesized that variation in these genes could influence benzene-induced hematotoxicity. We analyzed common, well-studied single-nucleotide polymorphisms (SNPs) in 20 candidate genes drawn from these pathways in a study of 250 workers exposed to benzene and 140 unexposed controls in China. After accounting for multiple comparisons, SNPs in five genes were associated with a statistically significant decrease in total WBC counts among exposed workers [*IL-1A* (−889C>T), *IL-4* (−1098T>G), *IL-10* (−819T>C), *IL-12A* (8685G>A), and *VCAM1* (−1591T>C)], and one SNP [*CSF3* (Ex4−165C>T)] was associated with an increase in WBC counts. The adhesion molecule *VCAM1* variant was particularly noteworthy as it was associated with a decrease in B cells, natural killer cells, $CD4^+$ T cells, and monocytes. Further, *VCAM1* (−1591T>C) and *CSF3* (Ex4−165C>T) were associated, respectively, with decreased ($P$ = 0.041) and increased ($P$ = 0.076) CFU-GEMM progenitor cell colony formation in 29 benzene-exposed workers. This is the first report to provide evidence that SNPs in genes that regulate hematopoiesis influence benzene-induced hematotoxicity.** (Cancer Res 2005; 65(20): 9574-81)

## Introduction

Chronic benzene exposure causes a range of hematologic disorders, including hematotoxicity, myelodysplatic syndrome, leukemia, and possibly non-Hodgkin lymphoma (1–3). Although there are ongoing concerns about its health effects (4), benzene continues to be used as a solvent in some countries, with several million workers exposed worldwide (4), and is a component of cigarette smoke, gasoline, crude oil, and automobile emissions (2). Several mechanisms have been suggested to account for benzene-induced hematotoxicity, including (*a*) direct toxicity to hematopoietic progenitor cells by genotoxic and cytotoxic metabolites of benzene (5–8) and (*b*) poisoning of the bone marrow stromal microenvironment (9, 10), whereby shifts in regulatory molecules produced by marrow cells could disrupt normal hematopoiesis (11–14).

Interindividual variation to benzene-induced hematotoxicity has been observed in occupational studies, in which highly disparate responses have been noted among workers with similar benzene exposure (15, 16), suggesting a role for individual genetic susceptibility. The first reports to evaluate mechanisms of genetic susceptibility for benzene hematotoxicity identified genetic variations in enzymes responsible for benzene metabolism (e.g., *CYP2E1, MPO, NQO1*; refs. 3, 16). We hypothesized that another important source of susceptibility to benzene-induced hematotoxicity would be variation in selected cytokine, chemokine, and cellular adhesion genes because mounting evidence suggests that control of hematopoietic cell growth and differentiation, both during healthy conditions and in response to stress, is partly regulated by the cytokine, chemokine, and adhesion molecule networks (17, 18).

We recently reported the presence of hematotoxicity in Chinese workers exposed to low levels of benzene (<1 ppm, the U.S. occupational standard; ref. 3). Exposure to benzene and other solvents was extensively evaluated, and detailed information was collected on potential confounders including lifestyle, medication, and medical history. Here, we report the influence of single-nucleotide polymorphisms (SNPs) in hematopoiesis regulatory genes on benzene-induced hematotoxicity.

## Materials and Methods

**Study population, exposure assessment, and biological sample collection.** The study population has previously been described (3, 19). In brief, we studied 250 benzene-exposed shoe workers in China and 140 unexposed controls, frequency matched to the benzene-exposed workers by age and sex. The study was approved by the Institutional Review Boards of the U.S. National Cancer Institute and the China Center for Disease Control. Written informed consent was provided by all study participants. We carried out a comprehensive workplace evaluation of exposure to benzene and toluene, as well as to other

**Note:** M.T. Smith has received consulting and expert testimony fees from law firms representing both plantiffs and defendants in cases involving exposure to benzene. G. Li has received funds from the American Petroleum Institute for consulting on benzene-related health research.

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**Table 1.** Assessment of candidate gene polymorphisms

| Gene | Name | Chromosome location* | SNP database ID (nucleotide change) |
|---|---|---|---|
| Cytokines | | | |
| *CSF2* | Colony-stimulating factor 2 (granulocyte) | 5q31.1 | Rs1469149 (−674A>C); Rs25882 (Ex4+23T>C) |
| *CSF3* | Colony-stimulating factor 3 (granulocyte) | 17q11.2-q12 | Rs1042658 (Ex4−165C>T) |
| *IL-1A* | IL-1α | 2q14 | Rs1800587 (Ex1+12C>T) or (−889C>T) |
| *IL-1B* | IL-1β | 2q14 | Rs16944 (−1060T>C) or (−511T>C) |
| *IL-2* | Interleukin 2 | 4q26-q27 | Rs2069762 (IVS1-100G>T); Rs2069763 (Ex2−34G>T) |
| *IL-4* | Interleukin 4 | 5q31.1 | Rs2243250 (−588C>T) or (524C>T) or (−589C>T); Rs2243248 (−1098T>G); Rs2070874 (Ex1−168C>T); Rs2243290 (IVS3−9A>C) |
| *IL-4R* | Interleukin-4 receptor | 16p11.2-p12.1 | Rs1805010 (Ex5+14A>C>G>T); Rs1805011 (Ex11+300A>C); Rs1801275 (Ex11+828A>G); Rs1049631 (Ex11−554G>A); Rs2107356 (−28120T>C); Rs2057768 (−29429T>C) |
| *IL-5* | Interleukin 5 | 5q31.1 | Rs2069812 (−745C>T) |
| *IL-10* | Interleukin 10 | 1q31-q32 | Rs1800871 (−853T>C) or (−819T>C); Rs1800896 (−1116A>G); Rs1800890 (−3584A>T) |
| *IL-12A* | Interleukin 12, α | 3p12-q13.2 | Rs568408 (Ex7+277G>A) or (8685G>A); Rs582054 (IVS2−798A>T) |
| *IL-12B* | Interleukin 12, β | 5q31.1-q33.1 | Rs3212227 (Ex8+159A>C) |
| *IL-13* | Interleukin 13 | 5q31 | Rs20541 (Ex4+98A>G); Rs1800925 (−1069C>T) |
| *IL-16* | Interleukin 16 | 15q26.3 | Rs859 (Ex22+871A>G); Rs11325 (Ex22+889G>T) |
| *LTA* | Lymphotoxin-α | 6p21.3 | Rs909253 (IVS1+90G>A); Rs2239704 (Ex1+49C>A) |
| *TNF* | Tumor necrosis factor | 6p21.3 | Rs1800629 (−487A>G) or (−308A>G); Rs1799724 (−1036C>T) |
| Chemokines | | | |
| *CCR2* | Chemokine, CC motif, receptor 2 | 3p21 | Rs1799864 (Ex2+241G>A); Rs1799865 (Ex2+831C>T) |
| *CCR5* | Chemokine, CC motif, receptor 5 | 3p21 | Rs2734648 (IVS1+151G>T); Rs1799987 (IVS1+246A>G) |
| *IL-8* | Interleukin 8 | 4q13-q21 | Rs4073 (−351A>T) or (−251A>T) |
| Adhesion molecules | | | |
| *ICAM1* | Intercellular adhesion molecule 1 (CD54) | 19p13.3-p13.2 | Rs5491 (Ex2+100A>T) |
| *VCAM1* | Vascular cell adhesion molecule 1 | 1p32-p31 | Rs1041163 (−1591T>C); Rs3176879 (Ex9+149G>A) |

*According to NCBI's MapView (http://www.ncbi.nlm.nih.gov/mapview/) at time of publication.

**Table 2.** Demographics, benzene exposure, and hematologic characteristics of study population

| | Controls ($n$ = 140) | Exposed ($n$ = 250) | $P$* |
|---|---|---|---|
| Sex† | | | |
| Male | 52 (37) | 86 (34) | 0.59 |
| Female | 88 (63) | 164 (66) | |
| Current alcohol use† | | | |
| Yes | 43 (31) | 67 (27) | 0.41 |
| No | 97 (69) | 183 (73) | |
| Recent infection† | | | |
| Yes | 16 (11) | 18 (7) | 0.16 |
| No | 124 (89) | 232 (93) | |
| Current smoking† | | | |
| Yes | 39 (28) | 52 (21) | 0.11 |
| No | 101 (72) | 198 (79) | |
| Age (y)‡ | 30.34 ± 8.69 | 29.59 ± 8.25 | 0.40 |
| BMI‡ | 22.46 ± 3.93 | 22.44 ± 3.24 | 0.94 |
| Benzene exposure measurements | | | |
| Benzene air level (ppm)§ | <0.04 | 5.44 ± 12.1 | |
| Benzene urine (μg/L)‖ | 0.38 ± 1.24 | 158 ± 536 | |
| Peripheral blood cell counts¶ | | | |
| Total WBC | 6,480 ± 1,710 | 5,490 ± 1,350 | <0.0001 |
| Granulocytes | 4,110 ± 1,410 | 3,330 ± 1,050 | <0.0001 |
| Lymphocytes | 2,130 ± 577 | 1,940 ± 520 | 0.0014 |
| $CD4^+$ T cells | 742 ± 262 | 622 ± 183 | <0.0001 |
| $CD8^+$ T cells | 553 ± 208 | 553 ± 213 | 0.88 |
| $CD4^+/CD8^+$ ratio | 1.46 ± 0.58 | 1.22 ± 0.42 | <0.0001 |
| B cells | 218 ± 94 | 173 ± 88.5 | <0.0001 |
| NK cells | 586 ± 318 | 542 ± 277 | 0.30 |
| Monocytes | 241 ± 92 | 215 ± 93.2 | 0.002 |
| Platelets | (230 ± 59.7) × $10^3$ | (202 ± 52.0) × $10^3$ | <0.0001 |
| Hemoglobin (g/dL) | 14.5 ± 1.6 | 14.5 ± 1.6 | 0.83 |

NOTE: There 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. Data shown here are from all subjects except two benzene-exposed subjects and one control with no benzene urine data, two controls with no BMI data, and one exposed worker without cell count data.

*The $\chi^2$ test was used to test differences in sex, current alcohol use, recent infection (flu or respiratory infections, in the previous month), and current smoking, and the $t$ test was used to test differences in age and BMI.

†Number (percent).

‡Mean ± SD.

§Benzene air level is the arithmetic mean (± SD) of an average of two measurements per subject collected during the month before phlebotomy.

‖Urinary benzene (mean ± SD) and mean individual air levels of benzene were strongly correlated (Spearman $r$ = 0.88, $P$ < 0.0001).

¶Unadjusted mean ± SD cells/μL blood. Linear regression was used to test for differences between exposed and control subjects, adjusting for age, sex, current smoking, current alcohol drinking, BMI, and recent infections.

organic solvents (3, 19). About 40% of the workers were on average exposed to <1 ppm benzene in the month before their blood sample was collected.

Interviews were carried out and blood samples were collected from 88 workers in June 2000 and from the remaining workers in May and June 2001 (28 subjects enrolled in the first year had repeat samples in the second year). A detailed questionnaire was administered requesting information on lifetime occupational and environmental exposures, lifestyle, medical history, and other potential confounding factors. Each subject also had a brief physical exam (3, 19) and provided a 29 mL blood sample. Complete blood cell counts and differentials were analyzed by a Beckman-Coulter T540 blood counter. The major lymphocyte subsets were measured by a Becton Dickinson FACSCalibur flow cytometer (software: SimulSET v3.1; refs. 3, 19). Colony-forming unit progenitor cells were cultured from peripheral blood from 29 workers selected for a wide range of measured benzene exposures (3).

**Genotyping.** We selected 40 SNPs in 20 genes coding for cytokines, chemokines, and adhesion molecules that play a role in hematopoiesis, based on the following criteria: minor allele frequencies of >5%, evidence of functionality in experimental or human studies, known association with disease, or to facilitate haplotype analysis (Table 1). Genotyping was done on an ABI 7900HT detection system using TaqMan end point reads as described on the website (20).[7] Missing genotype data resulted from nonspecific failure of samples to amplify or to yield unambiguous genotype calls. Blind replicate samples were randomly interspersed throughout the study sample plates and showed intrasubject concordance rates of >99% for all assays.

**Statistical analysis.** Tests for Hardy-Weinberg equilibrium for all subjects were conducted based on observed genotype frequencies, using a Pearson $\chi^2$ test, with 1 degree of freedom. All genotypes were in Hardy-Weinberg equilibrium among the benzene-exposed workers except *LTA* (IVS1+90G>A; $P = 0.016$) and *IL-16* (Ex22+889G>T; $P = 0.001$). Quality control data were rechecked to ensure the accuracy of these two assays.

We tested the influence of each SNP on total WBC counts separately in workers exposed to benzene and among unexposed controls. Total WBC count was the key end point in this study because a decreased WBC count (under 4,000 cells/μL) measured repeatedly is the primary component of a diagnosis of benzene poisoning in China, which we have previously linked to risk for developing hematologic malignancies and related disorders among workers exposed to benzene (16).

For analysis of gene variants, the most prevalent homozygous genotype was considered the reference group. Data for minor homozygotes and heterozygotes were combined in the analysis if the former contained fewer than five subjects. Cell counts are presented in tables as unadjusted means (± SD). Linear regression was used to analyze the relationship between each genotype and the natural log (ln) of the total WBC count, adjusting for age (continuous variable), sex, current cigarette smoking status (yes/no), current alcohol consumption (yes/no), recent infections (yes/no), and body mass index (BMI), and in exposed workers and additionally for the ln mean air benzene exposure and ln mean air toluene exposure in the month before phlebotomy (3). The selection of potential confounding factors was based on the literature and associations observed in the current study population (3). Further adjustment for variants in

[7] http://snp500cancer.nci.nih.gov

**Table 3.** Effect on total WBC counts of SNPs in cytokine and adhesion molecule genes in unexposed controls and benzene-exposed subjects

| SNPs (position) | Genotypes | Controls (*n*) | WBC* | *P*† | Exposed (*n*) | WBC* | *P*† | *P* for interaction‡ |
|---|---|---|---|---|---|---|---|---|
| *IL-1A*§ (−889C>T) | CC | 120 | 6,427 ± 1,548 | Reference | 229 | 5,590 ± 1,394 | Reference | |
| | CT + TT | 20 | 6,830 ± 2,504 | 0.96 | 45 | 5,004 ± 1,005 | 0.000044 | 0.22 |
| *IL-4*§ (−1098T>G) | TT | 123 | 6,405 ± 1,736 | Reference | 238 | 5,557 ± 1,363 | | |
| | TG | 17 | 7,059 ± 1,440 | 0.021 | 37 | 5,097 ± 1,209 | 0.0046 | 0.025 |
| *IL-10* (−819T>C) | TT | 63 | 6,681 ± 1,665 | Reference | 116 | 5,697 ± 1,488 | Reference | |
| | TC | 59 | 6,317 ± 1,791 | 0.05 | 124 | 5,419 ± 1,266 | 0.07 | |
| | CC | 18 | 6,344 ± 1,623 | 0.26 | 33 | 5,039 ± 1,074 | 0.0034 | |
| | Trend | | | 0.09 | | | 0.0031 | 0.80 |
| *IL-12A* (8685G>A) | GG | 110 | 6,427 ± 1,693 | Reference | 216 | 5,588 ± 1,378 | Reference | |
| | GA | 27 | 6,737 ± 1,829 | 0.38 | 50 | 5,332 ± 1,166 | 0.66 | |
| | AA | 1 | 7,200 | | 6 | 3,833 ± 728 | 0.000031 | |
| | Trend | | | | | | 0.023 | 0.048 |
| *CSF3* (Ex4−165C>T) | CC | 33 | 6,403 ± 1,650 | Reference | 77 | 5,217 ± 1,292 | Reference | |
| | CT | 67 | 6,637 ± 1,575 | 0.84 | 133 | 5,350 ± 1,249 | 0.34 | |
| | TT | 27 | 6,444 ± 2,123 | 0.66 | 41 | 6,090 ± 1,595 | 0.0011 | |
| | Trend | | | 0.68 | | | 0.0022 | 0.48 |
| *VCAM1* (−1591T>C) | TT | 92 | 6,426 ± 1,559 | Reference | 185 | 5,623 ± 1,324 | Reference | |
| | TC | 31 | 6,919 ± 2,174 | 0.23 | 69 | 5,210 ± 1,370 | 0.038 | |
| | CC | 5 | 4,820 ± 1,316 | 0.019 | 5 | 4,620 ± 646 | 0.0022 | |
| | Trend | | | 0.72 | | | 0.0040 | 0.89 |

*Unadjusted total WBC count /μL blood as mean ± SD.

†Linear regression was used to test for differences between WBC counts in each specified genotype group versus subjects homozygous for the common allele. Models were adjusted for age, sex, current smoking, current alcohol drinking, BMI, recent infections (flu or respiratory infections, in the previous month), and among exposed workers ln air benzene exposure and ln air toluene exposure in the month before phlebotomy. There are two controls without BMI data and they are excluded from the statistical analysis. *P* values shown in the table are unadjusted for multiple comparisons.

‡Test for interaction between benzene exposure (yes/no) and genotype (variant homozygous and heterozygous carriers combined versus most prevalent homozygous carriers).

§Among the 109 workers exposed to <1 ppm benzene, WBC counts were significantly decreased for individuals carrying the *IL-1A* (−889C>T) allele ($P = 0.02$) or the *IL-4* (−1098T>G) allele ($P = 0.0033$).

*MPO* and *NQO1*, which we have previously reported influence WBC counts among exposed workers in this study (3), did not affect the associations reported here. Tests for gene-dosage effect (i.e., trend) were conducted by treating genotype as a continuous variable (0, 1, and 2 for the most prevalent homozygous, heterozygous, and variant homozygous genotypes, respectively). Gene-environment interactions were tested by adding a product term between the genotype (variant homozygous and heterozygous carriers combined versus most prevalent homozygous carriers) and benzene exposure (yes/no) into each model. Data from the 28 benzene-exposed workers studied in both years are treated independently by using generalized estimating equations to adjust for potential correlation between the repeated measurements (21). Results were negligibly affected when data from only the first or second year of study were used for these 28 subjects.

We controlled for multiple hypothesis testing by calculating the false discovery rate, using the Benjamini-Hochberg method (22), for results from the initial screening of the effect of each SNP on the total WBC count, the primary end point in our study. The false discovery rate is defined as the expected ratio of erroneous rejections of the null hypothesis to the total number of rejected hypotheses; we used a false discovery rate of 0.05 to report associations for which the proportion of expected false positives was <5%. False discovery rate–adjusted *P* values were calculated for change in WBC counts for homozygous carriers of the rare versus common allele, as this provides the greatest potential contrast in effects across genotypes.

Those SNPs with false discovery rate–adjusted *P* values of <0.05 that also showed significant gene-dosage effects (if testable) among workers exposed to benzene were further tested for their influence on specific WBC types, colony-forming unit granulocyte-macrophage (CFU-GM) progenitor cells, and colony-forming unit granulocyte-erythroid-macrophage-megakaryocyte (CFU-GEMM) progenitor cells. Negative binomial regression was used to evaluate each SNP association with CFU-GM colony counts, and logistic regression was used to test the association with CFU-GEMM colonies, as there were multiple subjects with no measurable colonies (3). The progenitor cell models were adjusted for only age, sex, and ln benzene to avoid overparameterizing a model with 29 observations, as other potential confounders were not significant. All *P* values presented are two sided and all analyses were carried out using SAS version 8.02 software (SAS Institute, Cary, NC).

When possible, a preliminary analysis of haplotype block structure was examined with HaploView[8] using the four gamete rule with a minimum frequency of 0.005 for the fourth gamete. Haplotypes were estimated using the expectation-maximization algorithm (23). The association between blood cell counts and estimated haplotypes was assessed separately for controls and exposed subjects, adjusting for potential confounders using the HaploStats program (24) in R (Version 2.0.1; ref. 25).

## Results

Table 2 presents demographic characteristics, benzene exposure, and peripheral blood cell counts by benzene exposure status (i.e., controls versus exposed workers). The majority of the controls and benzene-exposed subjects were female and relatively young. The subjects in the benzene-exposed and nonexposed groups were comparable in terms of alcohol use, recent infection, smoking status, and BMI. The mean (SD) benzene air exposure was 5.44 (12.1) ppm in the exposed group, based on measurements made in the month before phlebotomy. The total WBC count, most WBC subtypes, and the platelet count were significantly decreased in benzene-exposed workers compared with controls. Natural killer (NK) cell counts and hemoglobin concentration were significantly decreased only among the most highly exposed workers, as previously reported, and $CD8^+$ T cells did not differ between groups at any level of exposure (3).

Twelve of 40 SNPs tested, in 10 of the 20 genes analyzed, were significantly associated with total WBC counts in the benzene-exposed population. Six SNPs remained significantly associated with WBC counts after adjustment for multiple comparisons and showed significant gene-dosage effects [*IL-10* (−819T>C), *IL-12A* (8685G>A), *CSF3* (Ex4−165C>T), and *VCAM1* (−1591T>C)] or could not be tested for trend due to low allele frequencies [*IL-1A* (−889C>T) and *IL-4* (−1098T>G)] (Table 3). Among benzene-exposed workers, the magnitude of the change in WBC counts between subjects homozygous for the common versus the less common allele was about 10% for several SNPs, with larger changes associated with SNPs in *IL-12A* (31.4% decrease), *CSF3* (14.3% increase), and *VCAM1* (17.8% decrease). Tests for interaction showed that the effect of the *IL-12A* SNP on WBC counts in benzene-exposed workers was significantly different from the null effect among controls ($P = 0.048$; Table 3).

Haplotype analysis of SNPs in linkage disequilibrium listed in Table 3 for which two or more high-frequency SNPs were genotyped (i.e., *IL-1A, IL-4, IL-10*, and *IL-12A*) was carried out, but this exploratory effort did not provide any additional insight into the observed associations beyond the single SNP analysis (data not shown). Six additional SNPs were associated with a significantly decreased WBC count among benzene-exposed workers but had false discovery rate–corrected *P* values of >0.05 [*IL-12A* (IVS2−798A>T), *IL-16* (EX22+889G>T), *CCR2* (Ex2+241G>A), and *CCR5* (IVS1+151G>T and IVS1+246A>G)] or did not show a significant gene-dosage effect [*IL-13* (−1069C>T)].

We conducted further analyses to evaluate the influence of the six SNPs shown in Table 3 on specific WBC populations in benzene-exposed workers (Table 4). The effect of SNPs in *IL-1A, IL-10*, and *CSF3* was limited to granulocyte counts, with decreased cell counts observed for *IL-1A* and *IL-10* and increased cell counts for *CSF3*. The *IL-4* promoter SNP was significantly associated with decreased granulocyte and total lymphocyte counts; and the *IL-12A* variant was associated with decreased granulocytes, total lymphocyte count, and $CD4^+$ and $CD8^+$ T-cell subsets. Interestingly, the *VCAM1* SNP displayed the broadest effect on WBC subtypes, with significant decreases for total lymphocyte count, $CD4^+$ T cells, B cells, NK cells, and monocytes, and a borderline significant decrease for granulocytes (Table 4).

Haplotype analysis of all genes with two or more SNPs in linkage disequilibrium showed the same general pattern as observed in Table 4 (data not shown), with the exception of the haplotype formed by *IL-4* (−1098G and −588C; 7% of exposed group), which showed evidence of a decrease in $CD4^+$ T cells ($P = 0.054$), as well as in total lymphocyte ($P = 0.007$) and granulocyte counts ($P = 0.0015$).

Because a number of SNPs in Table 4 influenced several mature WBC subtypes (e.g., granulocytes, $CD4^+$ T cells, B cells, and monocytes), we carried out an exploratory analysis of the effect of each SNP on CFU-GM and CFU-GEMM progenitor cell counts, which were available from 29 workers exposed to benzene (the *IL-1A* variant could not be evaluated as there was only one carrier in this group). There were no significant effects observed for any SNP on CFU-GM progenitor cells. The *VCAM1* variant was, however, significantly associated with a decrease in CFU-GEMM counts (Fig. 1*A*(*i*)) and the *CSF3* SNP showed a borderline significant increase with CFU-GEMM counts (Fig. 1*B*(*i*)), consistent with the direction of their effect on total WBC counts (Fig. 1*A*(*ii*) and *B*(*ii*); Table 3).

[8] http://www.broad.mit.edu/personal/jcbarret/haploview/

**Table 4.** Effect on WBC subtypes of SNPs in cytokine and adhesion molecule genes among benzene-exposed subjects

| SNPs (position) | Exposed (*n*) | Granulocytes* | *P* † | Lymphocytes* | *P* † | $CD4^+$ T cells* | *P* † |
|---|---|---|---|---|---|---|---|
| *IL-1A* (−889C>T) | | | | | | | |
| CC | 229 | 3,428 ± 1,091 | Reference | 1,953 ± 519 | Reference | 626 ± 188 | Reference |
| CT + TT | 45 | 2,920 ± 711 | 0.0000074 | 1,881 ± 534 | 0.15 | 595 ± 159 | 0.071 |
| *IL-4* (−1098T>G) | | | | | | | |
| TT | 238 | 3,383 ± 1,067 | Reference | 1,958 ± 530 | Reference | 630 ± 184 | Reference |
| TG | 37 | 3,046 ± 943 | 0.016 | 1,841 ± 444 | 0.040 | 563 ± 169 | 0.11 |
| *IL-10* (−819T>C) | | | | | | | |
| TT | 116 | 3,491 ± 1,154 | Reference | 1,984 ± 564 | Reference | 641 ± 179 | Reference |
| TC | 124 | 3,302 ± 1,000 | 0.12 | 1,911 ± 481 | 0.24 | 608 ± 182 | 0.24 |
| CC | 33 | 2,988 ± 801 | 0.0028 | 1,827 ± 486 | 0.12 | 589 ± 188 | 0.14 |
| Trend | | | 0.0046 | | 0.10 | | 0.10 |
| *IL-12A* (8685G>A) | | | | | | | |
| GG | 216 | 3,391 ± 1,074 | Reference | 1,976 ± 530 | Reference | 633 ± 186 | Reference |
| GA | 50 | 3,272 ± 956 | 0.98 | 1,858 ± 481 | 0.30 | 604 ± 173 | 0.68 |
| AA | 6 | 2,167 ± 513 | 0.000015 | 1,467 ± 367 | 0.015 | 452 ± 136 | 0.024 |
| Trend | | | 0.074 | | 0.031 | | 0.10 |
| *CSF3* (Ex4−165C>T) | | | | | | | |
| CC | 77 | 3,082 ± 1,045 | Reference | 1,923 ± 490 | Reference | 610 ± 177 | Reference |
| CT | 133 | 3,238 ± 958 | 0.11 | 1,892 ± 540 | 0.58 | 612 ± 194 | 0.95 |
| TT | 41 | 3,927 ± 1,178 | 0.000023 | 1,944 ± 508 | 0.70 | 651 ± 159 | 0.28 |
| Trend | | | 0.000066 | | 0.87 | | 0.40 |
| *VCAM1* (−1591T>C) | | | | | | | |
| TT | 185 | 3,393 ± 1,016 | Reference | 2,004 ± 550 | Reference | 633 ± 186 | Reference |
| TC | 69 | 3,210 ± 1,153 | 0.26 | 1,813 ± 422 | 0.0082 | 583 ± 170 | 0.047 |
| CC | 5 | 2,900 ± 604 | 0.074 | 1,560 ± 195 | 0.0050 | 595 ± 117 | 0.32 |
| Trend | | | 0.10 | | 0.00079 | | 0.039 |

*Linear regression was used to test for differences between cell counts in each specified genotype group versus subjects homozygous for the common allele. Models were adjusted for age, sex, current smoking, current alcohol drinking, BMI, recent infections, ln air benzene exposure, and ln air toluene exposure in the month before phlebotomy.
†Unadjusted cell counts/μL blood as mean ± SD.

Several SNPs altered WBC counts among controls [i.e., *CCR5* (IVS1+246A>G), *IL-4* (−1098T>G and Ex1−168C>T), and *IL-4R* (Ex11+300A>C) significantly increased and *LTA* (IVS1+90G>A), *IL-2* (IVS1−100G>T), and *VCAM1* (−1591T>C) significantly decreased WBC counts]. However, none were associated with WBC counts at $P < 0.05$ after correction for multiple comparisons.

## Discussion

We studied the effect of 40 SNPs in 20 cytokine, chemokine, and cellular adhesion molecule genes on peripheral WBC counts among 250 workers exposed to benzene and 140 unexposed controls. After carrying out a series of analyses that accounted for multiple comparisons, we observed robust findings for common SNPs in five genes (four cytokines and one adhesion molecule) that were associated with a decrease in total WBC counts among exposed workers (Table 3). On further analysis, the *IL-1A* and *IL-10* variants were significantly associated with only granulocytes, the *IL-4* promoter SNP was associated with a decline in both granulocytes and total lymphocytes, and the *IL-12A* and *VCAM1* SNPs were associated with declines in three and four WBC subtypes, respectively (Table 4). Interestingly, the *VCAM1* SNP was also associated with a significant decline in CFU-GEMM cells in a subgroup of 29 exposed workers (Fig. 1). In contrast, we found that one SNP in *CSF3* was associated with a significant increase in granulocytes (Table 3) and a borderline significant increase in CFU-GEMM colony counts (Fig. 1).

Benzene is metabolized to toxic compounds, which are known to cause bone marrow suppression, perhaps by complimentary mechanisms. Benzene can directly damage hematopoietic progenitor cells (5–8, 26), which could lead to apoptosis or decreased responsiveness to cytokines and cellular adhesion molecules. Alternatively, benzene toxicity to stromal cells or mature blood cells could disrupt the regulation of hematopoiesis, including hematopoietic commitment, maturation, or mobilization, through the network of cytokines, chemokines, and adhesion molecules (11–14, 27). Accordingly, hematotoxic effects could be enhanced among individuals exposed to benzene who have genetic variants that alter key pathways that regulate hematopoiesis.

There is mounting evidence that benzene may alter the gene expression, production, or processing of several cytokines *in vitro* or *in vivo*. Both benzene and its metabolite hydroquinone were found to interact with signaling pathways regulated by granulocyte colony-stimulating factor (27). Benzoquinone and hydroquinone were shown to alter interleukin (IL)-1α processing, possibly by decreasing the content of the IL-1α processing enzyme calpain (11, 13, 28). Hydroquinone was reported to interrupt B-cell lymphopoiesis, possibly by inhibiting production of IL-4 by

**Table 4.** Effect on WBC subtypes of SNPs in cytokine and adhesion molecule genes among benzene-exposed subjects (Cont'd)

| $CD8^+$ T cells* | $P^\dagger$ | B cells* | $P^\dagger$ | NK cells* | $P^\dagger$ | Monocytes* | $P^\dagger$ |
|---|---|---|---|---|---|---|---|
| 546 ± 210 | Reference | 174 ± 91 | Reference | 551 ± 279 | Reference | 215 ± 93 | Reference |
| 584 ± 235 | 0.57 | 163 ± 76 | 0.34 | 483 ± 251 | 0.060 | 216 ± 90 | 0.92 |
| 555 ± 216 | Reference | 176 ± 91 | Reference | 543 ± 274 | Reference | 216 ± 95 | Reference |
| 549 ± 200 | 0.54 | 156 ± 69 | 0.19 | 546 ± 303 | 0.29 | 211 ± 84 | 0.29 |
| 562 ± 225 | Reference | 169 ± 79 | Reference | 560 ± 293 | Reference | 222 ± 95 | Reference |
| 565 ± 216 | 0.93 | 175 ± 86 | 0.79 | 518 ± 264 | 0.091 | 206 ± 88 | 0.088 |
| 491 ± 157 | 0.17 | 175 ± 120 | 1.00 | 533 ± 252 | 0.50 | 224 ± 103 | 0.98 |
| | 0.33 | | 0.91 | | 0.21 | | 0.44 |
| 565 ± 217 | Reference | 177 ± 90 | Reference | 551 ± 277 | Reference | 221 ± 94 | Reference |
| 512 ± 203 | 0.08 | 162 ± 88 | 0.83 | 522 ± 290 | 0.63 | 202 ± 84 | 0.27 |
| 379 ± 92 | 0.0020 | 124 ± 40 | 0.27 | 473 ± 279 | 0.71 | 200 ± 110 | 0.58 |
| | 0.0039 | | 0.52 | | 0.56 | | 0.23 |
| 536 ± 220 | Reference | 174 ± 101 | Reference | 556 ± 274 | Reference | 212 ± 103 | Reference |
| 558 ± 216 | 0.22 | 164 ± 81 | 0.26 | 520 ± 282 | 0.13 | 219 ± 88 | 0.51 |
| 520 ± 204 | 0.85 | 175 ± 86 | 0.55 | 537 ± 282 | 0.85 | 220 ± 98 | 0.45 |
| | 0.87 | | 0.42 | | 0.57 | | 0.42 |
| 568 ± 229 | Reference | 183 ± 93 | Reference | 559 ± 290 | Reference | 226 ± 97 | Reference |
| 525 ± 176 | 0.13 | 149 ± 78 | 0.018 | 523 ± 248 | 0.79 | 187 ± 73 | 0.007 |
| 510 ± 186 | 0.84 | 118 ± 43 | 0.0016 | 283 ± 103 | 0.00060 | 160 ± 55 | 0.05 |
| | 0.19 | | 0.0025 | | 0.13 | | 0.0015 |

fibroblastic stromal cells (29), and was found to enhance IL-4 mRNA expression and IL-4 production in $CD4^+$ T cells (30). Further, a recent report from our study population showed a highly significant increase in *IL-4R* mRNA expression in peripheral mononuclear cells from benzene-exposed workers (31). The influence of benzene on the production and function of IL-10, IL-12, or VCAM1, and how benzene might alter stem, progenitor, or stromal cell responses to these compounds, is not known.

Published data support the importance of these genes in hematopoiesis; for instance, *IL-1, IL-4, IL-10*, and *IL-12* have each been implicated in myelopoiesis and lymphopoiesis (29, 32–34). *IL-12* and *IL-4* play a role in the proliferation of T cells (34, 35) and *VCAM1* has been shown to be critical for successful hematopoiesis in the bone marrow for cells of both myeloid and lymphoid lineage (17, 36, 37). It is also notable that several of our most significant findings were in genes that regulate the Th1-Th2 lymphocyte balance. In particular, we observed that variation in cytokines *IL-4, IL-10*, and *IL-12A* was each associated with decreased WBC. This raises the possibility that a shift in the balance of Th1-Th2 could have important consequences for the challenge of benzene to granulopoiesis and lymphopoiesis.

For three of the genes, *IL-1A, IL-4*, and *IL-10*, the variants studied have been reported to have functional consequences, either directly or as part of a haplotype. For instance, the *IL-1A* (−889C>T) is in the 5′ untranslated region, has been shown to produce a higher amount of IL-1A mRNA (38), and has been associated with autoimmune, degenerative, and infectious diseases (39, 40). The *IL-4* promoter SNPs and haplotypes are particularly interesting because of emerging studies that indicate that variation in the expression of this gene could affect a range of diseases such as severity of infection with respiratory syncytial virus in young children, asthma, atopy, fungal infection with *Candida albicans* in leukemia patients, and inflammatory bowel disease (41–44); moreover, this same promoter SNP has been shown to increase reporter gene expression *in vitro* (41, 42, 45). Lastly, proximal promoter *IL-10* haplotypes have been shown to alter IL-10 secretion (46–48) and a specific haplotype containing *IL-10* (−819T) has been associated with elevated IL-10 mRNA levels (47). *IL-10* promoter haplotypes have been associated with progression of HIV infection, autoimmune diseases such as asthma, lupus erythematosus, graft-versus-host disease following marrow transplantation, and Alzheimer's disease (49–52).

We detected associations between WBC counts and variants in three additional genes, *VCAM1, CSF3*, and *IL-12A*, but the specific function of these alleles is unknown. Recent reports found that SNPs in the *VCAM1* gene, including the −1591C allele, were associated with increased risk of stroke (53, 54), suggesting that this variant, or a SNP in linkage disequilibrium, could be functional. The *CSF3* (Ex4−165C>T) and *IL-12A* (8685G>A) variants are in 3′ untranslated regions, which could alter gene translation by several processes including ribosome binding, initiation, and elongation (55), but additional work is needed to determine their functionality.

In summary, this is the first report to show that common SNPs in cytokine genes and *VCAM1* could be associated with hematotoxicity in workers exposed to benzene. Selected variants seemed to influence only granulocytes, whereas others altered

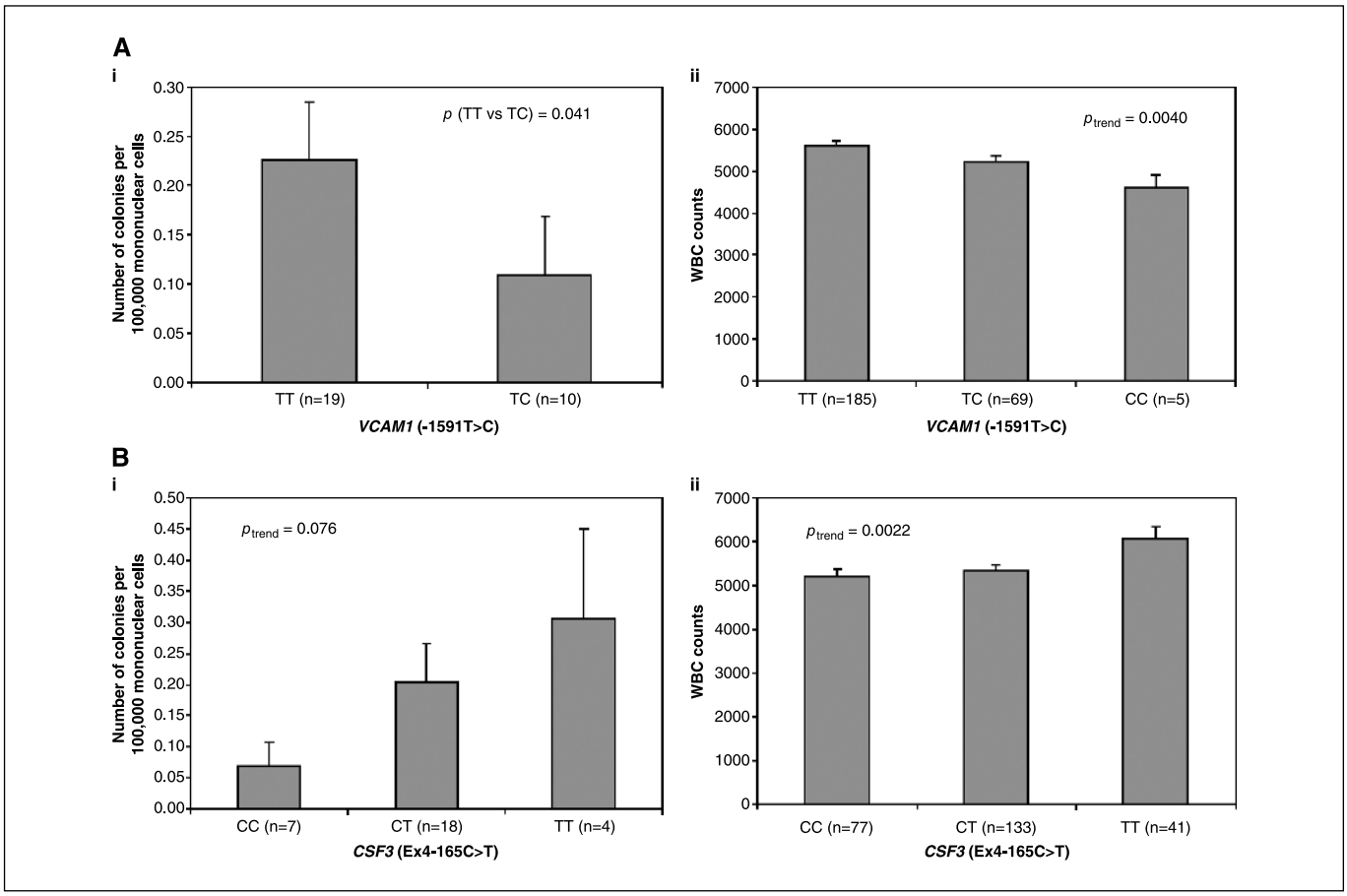


**Figure 1.** Effect of SNPs in *VCAM1* and *CSF3* on CFU-GEMM and peripheral WBC counts in workers exposed to benzene (*columns,* mean; *bars,* SE). *A, i, VCAM1* (−1591T>C) and CFU-GEMM colony count; *ii, VCAM1* (−1591T>C) and WBC count. *B, i, CSF3* (Ex4−165C>T) and CFU-GEMM colony count; *ii, CSF3* (Ex4−165C>T) and WBC count. Unconditional logistic regression was used to test effects on CFU-GEMM, categorizing subjects into 0 or more than 0 colonies. *A, i,* 79% and 30% of subjects had >0 colonies among TT and TC genotypes, respectively. *B, i,* 43%, 61%, and 100% of subjects had >0 colonies among CC, CT, and TT genotypes, respectively. Linear regression was used for trend test with each genotype and WBC count shown in *A*(*ii*) and *B*(*ii*).

cell types of both myeloid and lymphoid lineage, suggesting effects that could extend to earlier progenitor and possibly stem cells. Follow-up studies will require analysis with more comprehensive haplotypes containing the SNPs profiled in this study; in parallel, functional analysis should proceed in the laboratory to advance the identification of causal variants in benzene-induced hematotoxicity and to define specific mechanisms that explain their effects on peripheral WBC counts in the presence of benzene. At the same time, results of this study, although highly statistically significant, need to be confirmed in other populations exposed to benzene and extended to studies of benzene-related hematologic neoplasms and related disorders (2). Finally, our findings suggest that it may also be worthwhile to explore how these and related SNPs affect hematopoiesis and risk of hematologic disorders in people exposed to other environmental or pharmacologic hematotoxins and leukemogens.

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