Original Contribution

Genetic Variation in Metabolic Genes, Occupational Solvent Exposure, and Risk of Non-Hodgkin Lymphoma

Kathryn Hughes Barry*, Yawei Zhang, Qing Lan, Shelia Hoar Zahm, Theodore R. Holford, Brian Leaderer, Peter Boyle, H. Dean Hosgood III, Stephen Chanock, Meredith Yeager, Nathaniel Rothman, and Tongzhang Zheng

* Correspondence to Kathryn Hughes Barry, Occupational and Environmental Epidemiology Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, 6120 Executive Boulevard, EPS 8091, MSC 7240, Bethesda, MD 20892-7240 (e-mail: barrykh@mail.nih.gov).

Initially submitted May 24, 2010; accepted for publication September 21, 2010.

Using 1996–2000 data among Connecticut women, the authors evaluated whether genetic variation in 4 metabolic genes modifies organic solvent associations with non-Hodgkin lymphoma and 5 major histologic subtypes. Interaction values were determined from cross-product terms between dichotomous (ever/never) solvent variables and genotypes at examined loci in unconditional logistic regression models. The false discovery rate method was used to account for multiple comparisons. Overall associations between the chlorinated solvents dichloromethane (odds ratio (OR) = 1.69, 95% confidence interval (CI): 1.06, 2.69), carbon tetrachloride (OR = 2.33, 95% CI: 1.23, 4.40), and methyl chloride (OR = 1.44, 95% CI: 0.94, 2.20) and total non-Hodgkin lymphoma were increased among women TT for rs2070673 in the cytochrome P4502E1 gene, CYP2E1 (dichloromethane: OR = 4.42, 95% CI: 2.03, 9.62; P_interaction < 0.01; carbon tetrachloride: OR = 5.08, 95% CI: 1.82, 14.15; P_interaction = 0.04; and methyl chloride: OR = 2.37, 95% CI: 1.24, 4.51; P_interaction = 0.03). In contrast, no effects of these solvents were observed among TA/AA women. Similar patterns were observed for diffuse large B-cell lymphoma and follicular lymphoma, as well as marginal zone lymphoma for dichloromethane. The weak, nonsignificant overall association between benzene and diffuse large B-cell lymphoma (OR = 1.29, 95% CI: 0.84, 1.98) was increased among women AA for rs2234922 in the microsomal epoxide hydrolase gene, EPHX1 (OR = 1.77, 95% CI: 1.06, 2.97; P_interaction = 0.06). In contrast, no effect was observed among AG/GG women. Additional studies with larger sample size are needed to replicate these findings.

Abbreviations: CI, 95% confidence interval; CLL, chronic lymphocytic leukemia; CYP, cytochrome P450; DLBCL, diffuse large B-cell lymphoma; GST, glutathione S-transferase; MZL, marginal zone lymphoma; NHL, non-Hodgkin lymphoma; OR, odds ratio; SLL, small lymphocytic lymphoma; SNP, single nucleotide polymorphism.

Non-Hodgkin lymphoma (NHL) refers to a group of heterogeneous diseases involving malignant transformation of lymphoid cells (1) for which there are few established risk factors. A number of studies have explored a possible role of occupational and environmental exposures in the development of NHL, with inconsistent results for organic solvents (2, 3). Several studies have observed borderline significant or significant increases in NHL risk with any solvent exposure (4–10), including relatively recent studies in Australia and Connecticut, which also observed significant trends of increasing risk with increasing frequency and duration (9) or increasing probability and intensity of exposure (10), respectively. However, other recent studies have observed null effects of occupational or environmental solvent exposure (11–13), as did a 1996 meta-analysis of 26 occupational studies, with a pooled
standardized mortality ratio of 1.05 (95% confidence interval (CI): 0.92, 1.21) for lymphatic and hematopoietic cancers (14). Mixed findings have also been observed for individual solvents, for example, benzene (15–18) and trichloroethylene (7, 19–27), and grouped chlorinated solvents (9, 10, 28).

It is possible that the inconsistent findings of organic solvent effects on NHL are due in part to a modifying role of genetic factors, such that certain solvents may only be harmful in genetically susceptible subgroups of the population. These analyses were conducted to explore the potential modifying effect of genes with known roles in solvent metabolism. The cytochrome P4502E1 gene, CYP2E1, a member of the cytochrome P450 (CYP) family, encodes a protein known to engage in oxidation reactions for a number of substrates (29), which can yield reactive, electrophilic metabolites that in turn can form adducts with DNA (30). Given that CYP2E1 enzyme substrates include benzene and several chlorinated solvents (31, 32), inherited genetic variation in CYP2E1 might influence the biologic effect of these solvents. The metabolism of benzene is relatively well characterized (33). The first step is CYP2E1-mediated oxidation of benzene to form the electrophilic benzene oxide metabolite, which can spontaneously form phenol. CYP2E1 also catalyzes metabolism of phenol to form hydroquinone. The microsomal epoxide hydrolase gene, EPHX1, encodes a protein that initiates an alternate benzene oxide metabolic pathway, which forms catechol. The myeloperoxidase gene, MPO, and the nicotinamide adenine dinucleotide phosphate (NAD(P)H):quinone oxidoreductase gene, NQO1, contribute to oxidation reactions to form reactive quinone metabolites from hydroquinone or catechol or reverse reactions to form less reactive, reduced compounds, respectively (33). Other alternate benzene oxide metabolic pathways include reactions forming trans,trans-muconic acid or S-phenylmercapturic acid (33), the latter catalyzed by glutathione S-transferase (GST) enzymes in the μ and θ classes (in particular, those encoded by the GSTM1 and GSTT1 genes) (34). GST genes also possess roles in the metabolism of other solvents, for example, GSTT1 with respect to dichloromethane (35).

Associations between organic solvents and NHL outcomes were previously reported among Connecticut women (10). The specific aim of the present analyses was to assess whether inherited genetic variation in CYP2E1, EPHX1, NQO1, and MPO modifies the previously observed associations between individual and grouped organic solvents and risk of total NHL and NHL subtypes in this population of primarily white women. CYP2E1 polymorphisms were considered in relation to exposure to any organic solvent, any chlorinated solvent, and individual organic solvents, including benzene, chloroform, carbon tetrachloride, dichloromethene, dichloroethane, methyl chloride, and trichloroethylene. EPHX1, NQO1, and MPO polymorphisms were considered in relation to benzene exposure. It was hypothesized that some solvents possess a greater harmful effect among individuals carrying certain genotypes in these genes.

MATERIALS AND METHODS

Study population

Data for these analyses were obtained from a population-based case-control study of NHL among Connecticut women. The methods and population for this study have been described previously (36). Briefly, cases were women with histologically confirmed incident NHL who had been diagnosed between 1996 and 2000 and identified from Yale University’s Rapid Case Ascertainment system. To be eligible, women could not have a history of any other type of cancer, except nonmelanoma skin cancer. Based on the World Health Organization classification system, cases were categorized into NHL subtypes. The present analyses focused on the 5 major NHL subtypes: diffuse large B-cell lymphoma (DLBCL), follicular lymphoma, chronic lymphocytic leukemia (CLL)/small lymphocytic lymphoma (SLL), marginal zone lymphoma (MZL), and T-cell lymphoma. Of 832 eligible cases, 601 (72%) participated in the study interview. Interview participation rates for controls, who were frequency matched to cases on age in 5-year groups, varied by selection method. The rate was 69% for potential controls identified via random digit dialing (for those aged <65 years) and 47% for those identified via random selection from the Centers for Medicare and Medicaid Services records (for those aged ≥65 years), yielding a total of 717 participating controls.

These analyses were conducted among the subset of 518/601 (86%) case and 597/717 (83%) control participants (n = 1,115) who provided a blood or buccal cell specimen for genotyping. The vast majority of the samples were blood (89%) and the remainder buccal cells. The distribution of type of sample provided was nearly identical for cases and controls, with 461/518 (89.0%) and 535/597 (89.6%) providing blood samples, respectively. Restriction to whites (497 cases and 550 controls) did not appreciably alter effect estimates, so unrestricted results are presented. All procedures were approved by the human investigations committees at Yale University, the Connecticut Department of Public Health, and the National Cancer Institute.

Interview

The solvent exposure assessment in the study population has been described previously (10). Briefly, a standardized, structured questionnaire was administered to study participants to collect information on employment history, including job/industry titles and employment dates. These self-reported jobs were coded to standardized occupation and industry classifications by using the 1980 Standard Occupational Classification Manual (37) and the 1987 Standard Industrial Classification Manual (38) and linked to a generic job-exposure matrix developed by National Cancer Institute industrial hygienists to assess solvent exposures (39, 40). The job-exposure matrix included the industrial hygienists’ assessment of the probability and intensity of exposure (ordinal categories) for each job and industry for any organic solvent, any chlorinated solvent, benzene, and a number of individual chlorinated solvents, including chloroform, carbon tetrachloride, dichloromethane, dichloroethane,
methyl chloride, and trichloroethylene. The present analyses used dichotomous ever/never exposure metrics because of the small cell counts for some solvents within genotype strata when using multilevel exposure metrics.

Genotyping
Phenol-chloroform extraction was used to isolate DNA from the blood and buccal cell samples (41). Genotyping was conducted at the National Cancer Institute’s Core Genotyping Facility by using real-time polymerase chain reaction on an Applied Biosystems 7900HT sequence detection system (Life Technologies Corporation, Carlsbad, California) (42). As described previously, concordance rates for quality control samples were 100% for all assays (43). There were 6 single nucleotide polymorphisms (SNPs) measured across 4 candidate metabolic genes that met Hardy-Weinberg equilibrium among controls based on \( P > 0.05 \) and thus were available for study: \( CYP2E1 \) rs2070673 and rs2031920, \( EPHX1 \) rs2234922 and rs1051740, \( NQO1 \) rs1800566, and \( MPO \) rs2333227. Although of interest and available in the genotyping data, the \( GSTT1 \) deletion polymorphism was not included in these analyses because of violation of Hardy-Weinberg equilibrium.

Statistical analysis
All statistical modeling was performed in SAS, version 9.1, software (SAS Institute, Inc., Cary, North Carolina). Unconditional logistic regression models per SNP-solvent combination, adjusted for age (continuous) and race (white/nonwhite), were used to determine \( P \) interaction values, as well as odds ratios and 95% confidence intervals for solvent associations with NHL outcomes within genotype strata. Interactions were assessed by the \( P \) value associated with the cross-product term between exposure to the solvent and genotype at the given locus. Statistical significance was defined at the 0.05 level based on 2-tailed tests. The addition of family history of hematopoietic disorders, alcohol consumption, tobacco smoking, education, annual family income, and medical history of immune-related disease did not appreciably alter effect estimates for solvent associations with NHL outcomes, and thus these covariates were not included in the final models. Interactions were assessed between ever exposure to the 9 individual and grouped solvents and the 2 \( CYP2E1 \) SNPs, as well as between ever exposure to benzene and the 4 SNPs across \( EPHX1 \), \( NQO1 \), and \( MPO \), with respect to total NHL (\( n = 518 \)), DLBCL (\( n = 161 \)), follicular lymphoma (\( n = 119 \)), CLL/SLL (\( n = 59 \)), MZL (\( n = 35 \)), and T-cell lymphoma (\( n = 39 \)). However, only results for total NHL, DLBCL, and follicular lymphoma are presented in the tables as small cell counts were often observed for the other subtypes, particularly with stratification by genotype. The dominant genetic model was used for each SNP given the small numbers of women with homozygous variant genotypes for some of the examined loci. To account for multiple comparisons, we calculated the false discovery rate for each interaction with respect to total NHL (22 tests). NHL subtype results were not included because they were not independent of the total NHL tests. Interactions meeting a false discovery rate of less than 0.2 were considered robust to correction for multiple comparisons.

RESULTS

Table 1 presents the associations between ever exposure to individual and grouped organic solvents (compared with never exposure to the given solvent) and total NHL, DLBCL, and follicular lymphoma among participants who contributed a blood or buccal cell sample for genotyping. Solvent main effect estimates for these participants who provided biologic specimens were similar to estimates observed for the whole study population (10). We also observed significant associations between several chlorinated solvents and NHL subtypes that were not previously examined using ever/never metrics in the study population (10). These included associations between carbon tetrachloride (odds ratio (OR) = 3.57, 95% CI: 1.65, 7.74), dichloromethane (OR = 2.10, 95% CI: 1.15, 3.85), dichloroethane (OR = 1.88, 95% CI: 1.08, 3.28), and trichloroethylene (OR = 1.68, 95% CI: 1.03, 2.74) and DLBCL (Table 1); methyl chloride (OR = 1.96, 95% CI: 1.06, 3.63) and follicular lymphoma (Table 1); carbon tetrachloride (OR = 3.87, 95% CI: 1.05, 14.20), dichloromethane (OR = 5.52, 95% CI: 2.26, 13.50), and methyl chloride (OR = 2.73, 95% CI: 1.07, 6.98) and MZL; and chlorinated solvents (OR = 2.02, 95% CI: 1.04, 3.93) and T-cell lymphoma (results not presented). No other associations between individual chlorinated solvents and NHL subtypes were statistically significant.

Table 2 presents associations between \( CYP2E1 \), \( EPHX1 \), \( NQO1 \), and \( MPO \) gene polymorphisms and total NHL, DLBCL, and follicular lymphoma. Women who were heterozygous or homozygous variant (AG or GG) for \( EPHX1 \) rs2234922 experienced significantly reduced risk of developing DLBCL (OR = 0.67, 95% CI: 0.45, 0.99) compared with women who were homozygous wild-type (AA), indicating the wild-type A allele as the risk allele. Additionally, the variant G allele for this polymorphism was associated with borderline significant reductions in risk for total NHL and follicular lymphoma. Women who were heterozygous or homozygous variant (GA or AA) for \( MPO \) rs2333227 experienced a borderline significant increase in risk of follicular lymphoma (OR = 1.50, 95% CI: 0.99, 2.27) (Table 2). No other associations between examined polymorphisms and NHL outcomes, including CLL/SLL, MZL, and T-cell lymphoma (results not shown), approached statistical significance.

Tables 3 and 4 present the effects of ever solvent exposure stratified by \( CYP2E1 \) rs2070673 or \( EPHX1 \) rs2234922 genotype, respectively, for SNP-solvent combinations that showed significant or borderline significant interactions with respect to total NHL, DLBCL, or follicular lymphoma. No interactions with the \( CYP2E1 \) rs2031920, \( EPHX1 \) rs1051740, \( NQO1 \) rs1800566, or \( MPO \) rs2333227 polymorphisms approached statistical significance.

Chlorinated solvents, including dichloromethane, carbon tetrachloride, and methyl chloride, were associated with
increased risk of NHL outcomes that was restricted to those homozygous wild-type (TT) for the CYP2E1 rs2070673 polymorphism. Dichloromethane exposure was associated with a significant increase in total NHL risk, on the basis of an overall odds ratio of 1.69 (95% CI: 1.06, 2.69) (Table 1). This result was increased to 4.42 (95% CI: 2.03, 9.62) among women with the TT genotype for CYP2E1 rs2070673 and, in contrast, was null (OR = 0.80, 95% CI: 0.36, 1.75; \(P_{\text{interaction}} < 0.01\)) among those with the TA or AA genotype (Table 3). Similar patterns of effect for these solvents by CYP2E1 rs2070673 genotype were observed for the DLBCL and follicular lymphoma subtypes (Table 3), as well as for MZL for dichloromethane. The odds ratio for the association between dichloromethane and MZL was increased to 17.17 (95% CI: 5.10, 57.88) among women with the TT genotype and, in contrast, was null (OR = 1.16, 95% CI: 0.13, 10.46; \(P_{\text{interaction}} = 0.03\)) among those with the TA or AA genotype, respectively (results not presented).

Benzene exposure showed a weak, nonsignificant association with DLBCL, on the basis of an overall odds ratio of 1.29 (95% CI: 0.84, 1.98) (Table 1). This result was

### Table 1. Occupational Solvent Exposure and Risk of Total NHL, DLBCL, and Follicular Lymphoma Among Connecticut Women Who Provided a Blood or Buccal Cell Sample for Genotyping, 1996–2000

<table>
<thead>
<tr>
<th>Exposure</th>
<th>Total NHL</th>
<th>DLBCL</th>
<th>Follicular Lymphoma</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of Cases</td>
<td>No. of Controls</td>
<td>OR*</td>
</tr>
<tr>
<td>Organic solventsb</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>289</td>
<td>364</td>
<td>1.00</td>
</tr>
<tr>
<td>Ever</td>
<td>229</td>
<td>233</td>
<td>1.24</td>
</tr>
<tr>
<td>Benzene</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>417</td>
<td>489</td>
<td>1.00</td>
</tr>
<tr>
<td>Ever</td>
<td>101</td>
<td>108</td>
<td>1.11</td>
</tr>
<tr>
<td>Chlorinated solventsb</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>352</td>
<td>444</td>
<td>1.00</td>
</tr>
<tr>
<td>Ever</td>
<td>166</td>
<td>153</td>
<td>1.36</td>
</tr>
<tr>
<td>Chloroformd</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>487</td>
<td>560</td>
<td>1.00</td>
</tr>
<tr>
<td>Ever</td>
<td>31</td>
<td>37</td>
<td>0.94</td>
</tr>
<tr>
<td>Carbon tetrachlorided</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>489</td>
<td>582</td>
<td>1.00</td>
</tr>
<tr>
<td>Ever</td>
<td>29</td>
<td>15</td>
<td>2.33</td>
</tr>
<tr>
<td>Dichloromethaned</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>471</td>
<td>564</td>
<td>1.00</td>
</tr>
<tr>
<td>Ever</td>
<td>47</td>
<td>33</td>
<td>1.69</td>
</tr>
<tr>
<td>Dichloroethaned</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>464</td>
<td>553</td>
<td>1.00</td>
</tr>
<tr>
<td>Ever</td>
<td>54</td>
<td>44</td>
<td>1.47</td>
</tr>
<tr>
<td>Methyl chlorided</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>466</td>
<td>554</td>
<td>1.00</td>
</tr>
<tr>
<td>Ever</td>
<td>52</td>
<td>43</td>
<td>1.44</td>
</tr>
<tr>
<td>Trichloroethylene</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>447</td>
<td>533</td>
<td>1.00</td>
</tr>
<tr>
<td>Ever</td>
<td>71</td>
<td>64</td>
<td>1.32</td>
</tr>
</tbody>
</table>

**Abbreviations:** CI, confidence interval; DLBCL, diffuse large B-cell lymphoma; NHL, non-Hodgkin lymphoma; OR, odds ratio.

*From an unconditional logistic regression model adjusted for age (continuous) and race (white/nonwhite).

b Any organic solvent.

c Any chlorinated solvent.

d Chlorinated solvent.
increased to 1.77 (95% CI: 1.06, 2.97) among women who were homozygous wild-type (AA) for EPHX1 rs2234922. In contrast, no association between benzene and DLBCL was observed among women carrying the heterozygous or homozygous variant (AG or GG) genotype at this locus (OR = 0.53, 95% CI: 0.18, 1.59; $P_{\text{interaction}} = 0.06$) (Table 4).

None of the examined interactions, except for that between dichloromethane and CYP2E1 rs2070673 with respect to total NHL, possessed a false discovery rate of less than 0.2 after accounting for multiple comparisons. However, the stratified patterns of solvent effects by genotype persisted when assessing trends in NHL risk associated with increasing intensity of solvent exposure (using ordinal variables with categories for never, low, medium, and high exposure in lieu of ever/never metrics) (results not shown).

**DISCUSSION**

To our knowledge, this is the first study to evaluate interactions between organic solvents and genetic variation in metabolic genes with respect to NHL. Our findings suggest that solvent associations with NHL are increased among women carrying homozygous wild-type genotypes for CYP2E1 rs2070673 or EPHX1 rs2234922 relative to the overall effects of these chemicals reported previously (10), although stratified effect estimates were relatively imprecise. There were no associations among women with other genotypes at these loci.

Although the functional significance of the promoter region CYP2E1 rs2070673 polymorphism is unclear, there is potential for effect modification by genetic variation in CYP2E1 given the known role of the CYP2E1 enzyme in the metabolism of benzene and a number of chlorinated solvents (31, 32). CYP2E1 activity has also been linked with solvent toxicity. For example, there was no cytotoxicity or genotoxicity among CYP2E1 knockout mice exposed to benzene in contrast to striking toxic effects among exposed wild-type mice with no gene disruption (44). Additionally, a study among benzene-exposed workers in Shanghai, China, observed increased risk of benzene poisoning, which was associated with acute nonlymphocytic leukemia and related myelodysplastic syndromes, for those with a rapid metabolizing CYP2E1 phenotype (45). Although we did not observe interactions between CYP2E1 polymorphisms and benzene in our study, CYP2E1 activity is also expected to influence the toxicity of several chlorinated solvents, including dichloromethane, methyl chloride, and carbon tetrachloride (29). Thus, there is a biologic basis for our findings of interactions between these solvents and CYP2E1 rs2070673; however, in vitro and animal studies suggest that CYP2E1-catalyzed reactions might not have the same activating/detoxifying effect for all 3 solvents (46–48).

Our finding of an interaction between EPHX1 rs2234922 and benzene with respect to DLBCL has some biologic plausibility given the known role of the EPHX1 enzyme in benzene metabolism (33), the nonsynonymous nature of
<table>
<thead>
<tr>
<th>Solvent Exposure by CYP2E1 rs2070673 Genotype</th>
<th>Total NHL</th>
<th></th>
<th>DLBCL</th>
<th></th>
<th>Follicular Lymphoma</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of Cases</td>
<td>No. of Controls</td>
<td>OR</td>
<td>95% CI</td>
<td>P&lt;sub&gt;interaction&lt;/sub&gt;</td>
<td>No. of Cases</td>
</tr>
<tr>
<td>Dichloromethane</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>265</td>
<td>344</td>
<td>1.00</td>
<td>Referent</td>
<td>89</td>
<td>344</td>
</tr>
<tr>
<td>Never</td>
<td>30</td>
<td>9</td>
<td>4.42</td>
<td>2.03, 9.62</td>
<td>11</td>
<td>9</td>
</tr>
<tr>
<td>Ever</td>
<td>149</td>
<td>165</td>
<td>1.00</td>
<td>Referent</td>
<td>41</td>
<td>165</td>
</tr>
<tr>
<td>TA + AA</td>
<td>13</td>
<td>16</td>
<td>0.80</td>
<td>0.36, 1.75</td>
<td>6</td>
<td>16</td>
</tr>
<tr>
<td>Carbon tetrachloride</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>276</td>
<td>348</td>
<td>1.00</td>
<td>Referent</td>
<td>91</td>
<td>348</td>
</tr>
<tr>
<td>Never</td>
<td>19</td>
<td>5</td>
<td>5.08</td>
<td>1.82, 14.15</td>
<td>9</td>
<td>5</td>
</tr>
<tr>
<td>Ever</td>
<td>156</td>
<td>174</td>
<td>1.00</td>
<td>Referent</td>
<td>45</td>
<td>174</td>
</tr>
<tr>
<td>TA + AA</td>
<td>6</td>
<td>7</td>
<td>0.94</td>
<td>0.31, 2.87</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>Methyl chloride</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>266</td>
<td>337</td>
<td>1.00</td>
<td>Referent</td>
<td>91</td>
<td>337</td>
</tr>
<tr>
<td>Never</td>
<td>29</td>
<td>16</td>
<td>2.37</td>
<td>1.24, 4.51</td>
<td>9</td>
<td>16</td>
</tr>
<tr>
<td>Ever</td>
<td>146</td>
<td>160</td>
<td>1.00</td>
<td>Referent</td>
<td>41</td>
<td>160</td>
</tr>
<tr>
<td>TA + AA</td>
<td>16</td>
<td>21</td>
<td>0.77</td>
<td>0.38, 1.55</td>
<td>6</td>
<td>21</td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; CYP2E1, cytochrome P4502E1 gene; DLBCL, diffuse large B-cell lymphoma; NHL, non-Hodgkin lymphoma; OR, odds ratio.

<sup>a</sup> From an unconditional logistic regression model adjusted for age (continuous) and race (white/nonwhite).

<sup>b</sup> P<sub>interaction</sub> from the cross-product term between ever exposure to the given solvent and rs2070673 genotype in an unconditional logistic regression model adjusted for age (continuous) and race (white/nonwhite).
the SNP with an amino acid change from histidine to arginine, and phenotypic changes associated with genetic variation at this locus. A study of benzene-exposed workers in Tianjin, China, observed increased urinary catechol levels among participants who were heterozygous or homozygous variant compared with those who were homozygous wild-type at this locus, indicating more rapid metabolizing activity of the variant G allele (49). Additionally, a study among benzene-exposed workers in southern China suggested a role of variation at the EPHX1 rs2234922 locus in susceptibility to benzene toxicity, as measured by chronic benzene poisoning (50). In the present study, we observed a significant main effect of this polymorphism on DLBCL, such that the wild-type A allele was the risk allele, and, consistently, a significant increase in DLBCL risk with benzene exposure among the homozygous wild-type group. With the assumption of reduced metabolizing activity of the wild-type allele (49), our findings suggest that the substrate for EPHX1, benzene oxide, or metabolites formed via alternate benzene oxide metabolic pathways might possess greater hematotoxicity and relevance to NHL than metabolites formed via the EPHX1-initiated benzene oxide metabolic pathway.

MPO and NQO1 possess activation and detoxification roles, respectively, in transformations between phenolic benzene metabolites (e.g., hydroquinone and catechol) and reactive quinone compounds (e.g., 1,4-benzoquinone and 1,2-benzoquinone), which have been proposed to play a role in benzene-associated myelotoxicity and carcinogenicity (51). Supporting this hypothesis, some human studies have suggested a role of genetic variation in these genes in susceptibility to benzene toxicity. A Shanghai study observed increased risk of benzene poisoning among exposed workers carrying 2 copies of the variant T allele at position 609 in exon 6 of NQO1 (rs1800566), which encodes an amino acid change from proline to serine (45), as did 2 other studies in China (52, 53). Additional studies in China observed increased risk of poisoning or greater decreases in white blood cell counts with benzene exposure for individuals carrying the homozygous wild-type (GG) genotype for the promoter region MPO rs2333227 polymorphism (54, 55). However, other studies found no association between NQO1 rs1800566 and benzene poisoning or declining white blood cells with benzene exposure (50, 55), or between MPO rs2333227 and urinary levels of benzene metabolites or risk of poisoning among benzene-exposed workers (49, 52), and we did not observe a modifying role of these polymorphisms on benzene associations with NHL outcomes in the present study. If variation at these loci is important in benzene associations with NHL subtypes, it is possible that we did not observe interactions with these polymorphisms given our limited power to detect associations among subtypes.

We also did not observe interactions between benzene and the nonsynonymous EPHX1 rs1051740 polymorphism (encoding an amino acid change from tyrosine to histidine with the variant C allele) in our study, although another study suggested altered enzymatic activity for benzene with genetic variation at this locus (49).

Despite plausibility for a modifying role of genetic variation in CYP2E1 and findings in humans of altered CYP2E1
enzymatic activity for substrates such as benzene and chlorzoxazone with the variant T allele of CYP2E1 rs2031920 (49, 56), we did not observe interactions between this promoter region polymorphism and any of the examined solvents. Our null findings for benzene are consistent with the observation of no effect of this polymorphism on benzene poisoning or declining white blood cell counts with benzene exposure among exposed workers in Shanghai or Tianjin, China, respectively (45, 55).

There were several limitations of these analyses, including examination of multiple comparisons, which increases the concern of false-positive results. Additionally, numbers of subjects became small when stratifying solvent effects by genotype, particularly for the homozygous variant group, which contributed to relatively imprecise estimates of solvent effects in our study. To help mitigate this problem, the dominant genetic model was used, such that heterozygous and homozygous variant genotypes were collapsed for each locus. However, this approach could have resulted in a loss of power if another model (e.g., additive) better fit the data.

Also, the study population was limited to women, so results may not apply to men.

Although only participants who provided a biologic sample for genotyping in the original case-control study were included in these analyses, the effects of ever exposure to the various solvents in the current population were highly consistent with results previously reported for these chemicals in the study population as a whole (10). This finding, in turn, suggests that the participants included in the present analyses were not systematically different with respect to exposure or NHL status from those that were not included. Additionally, as the distribution of type of sample provided (blood or buccal cells) was almost identical for cases and controls, the use of both types of samples in these analyses is not expected to have introduced bias.

As in any study using a generic job-exposure matrix, some exposure misclassification is anticipated. The solvent exposure assignments in our study were based upon general assumptions about exposures in different jobs and industries that may not hold for all individuals in those settings (57), and assignments were not reviewed by an industrial hygienist. However, differential bias due to exposure misclassification is unlikely. Previous studies have found limited evidence of recall bias in job reporting (58, 59), and we applied the job-exposure matrix blinded to participant disease status to help maintain minimal case/control differences (10). Thus, given our use of dichotomous solvent variables, any exposure misclassification is expected to have attenuated solvent effects. Additionally, the potential for disease misclassification bias to have influenced our results was minimized because of histologic confirmation of NHL diagnoses by study pathologists.

In summary, findings of this population-based case-control study of primarily white women in Connecticut suggest interactions between occupational exposure to several chlorinated solvents and CYP2E1 rs2070673 with respect to total NHL and several major NHL subtypes, as well as between occupational benzene exposure and EPHX1 rs2234922 with respect to DLBCL. These results possess some biologic plausibility given the known roles of the CYP2E1 and EPHX1 enzymes in solvent metabolism, as well as phenotypic changes observed with genetic variation at the EPHX1 rs2234922 locus. Additionally, the direction of our EPHX1 rs2234922 main effect finding with DLBCL further supports the observed pattern of interaction with benzene. Additional studies with larger sample size are needed to replicate these findings and to continue evaluation of interactions between solvents and metabolic genes with respect to NHL subtypes.

ACKNOWLEDGMENTS

Author affiliations: Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Bethesda, Maryland (Kathryn Hughes Barry, Qing Lan, Shelia Hoar Zahm, H. Dean Hosgood III, Meredith Yeager, Nathaniel Rothman); Yale School of Public Health, New Haven, Connecticut (Kathryn Hughes Barry, Yawei Zhang, Theodore R. Holford, Brian Leaderer, Tongzhang Zheng); International Prevention Research Institute, Lyon, France (Peter Boyle); and Core Genotyping Facility, National Cancer Institute-Frederick, SAIC-Frederick, Inc., Frederick, Maryland (Stephen Chanock, Meredith Yeager).

This work was supported by the National Cancer Institute (CA62006 and T32 CA105666); the Intramural Research Program of the National Cancer Institute, National Institutes of Health (NIH); NIH Fogarty training grants (1D43TW007864-01 and 1D43TW008323-01); Clinical and Translational Science Award (UL1 RR024139) from the National Center for Research Resources (NCRR), NIH; and the NIH Roadmap for Medical Research.

The authors thank Dr. Melissa Friesen for her valuable comments on the manuscript.

The contents of this article are solely the responsibility of the authors and do not necessarily represent the official views of the NCRR or the NIH.

Conflict of interest: none declared.

REFERENCES


