RAPID COMMUNICATION

Susceptibility to Childhood Acute Lymphoblastic Leukemia: Influence of CYP1A1, CYP2D6, GSTM1, and GSTT1 Genetic Polymorphisms

By Maja Krajinovic, Damian Labuda, Chantal Richer, Sepideh Karimi, and Daniel Sinnett

Although acute lymphoblastic leukemia (ALL) is the most common childhood cancer, factors governing susceptibility to this disease have not yet been identified. As such, ALL offers a useful opportunity to examine the glutathione S-transferase and cytochrome P450 genes in determining susceptibility to pediatric cancers. Both enzymes are involved in carcinogen metabolism and have been shown to influence the risk a variety of solid tumors in adults. To determine whether these genes played a similar role in childhood leukemogenesis, we compared the allele frequencies of 177 childhood ALL patients and 304 controls for the CYP1A1, CYP2D6, GSTM1, and GSTT1 genes. We chose the French population of Quebec as our study population because of its relative genetic homogeneity. The GSTM1 null and CYP1A1*2A genotypes were both found to be significant predictors of ALL risk (odds ratio [OR] = 1.8). Those possessing both genotypes were at an even greater risk of developing the disease (OR = 3.3). None of the other alleles tested for proved to be significant indicators of ALL risk. Unexpectedly, girls carrying the CYP1A1-4 were significantly underrepresented in the ALL group (OR = 0.2), suggesting that a gender-specific protective role exists for this allele. These results suggest that the risk of ALL may indeed be associated with xenobiotics-metabolism, and thus with environmental exposures. Our findings may also explain, in part, why ALL is more prevalent among males than females.

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A CUTE LYMPHOBLASTIC leukemia (ALL) is the most frequent cancer found among children. ALL is a heterogeneous disease characterized by the predominance of lymphoblasts or immature hematopoietic precursors, in which malignant cells express diverse phenotypes and respond variably to chemotherapy. Despite much investigation, little is known about leukemogenesis, particularly with respect to issues such as the role of inherited genetic susceptibility and environmental factors, although the clinical, pathological, and immunophenotypic features of the disease are well documented.1 To date, epidemiological studies have failed to find any reproducible, significant associations between ALL and either genetic or environmental factors.2-4

It has been suggested that individuals possessing a modified ability to metabolize carcinogens are at increased risk of cancer.5 Thus, polymorphisms in genes encoding carcinogen-metabolizing enzymes may have relevance in determining susceptibility to cancer—individuals carrying the more active form of an enzyme involved in the activation of carcinogens, or less efficient alleles of detoxifying enzymes, will be at greater risk of cancer. For this reason, two families of genes have attracted interest: phase I cytochromes P-450 (CYPs) and phase II glutathione-S-transferases (GSTs).

Genetic variants have been described in whites in both the CYP1A16-8 and CYP2D69 genes. Furthermore, a significant number of individuals lacking GSTM1 and GSTT1 activity display homozygous deletion (null allele) at the corresponding gene locus.10,11 Several studies have reported associations between these variants and altered risk of a variety of cancers, including those of the lung, bladder, gastrointestinal tract, skin, cervix, and breast.12-16 The prevalence of each polymorphism varies greatly among different ethnic groups, as well as within the white population.17,18 Because such variation can influence the power and interpretation of epidemiological data, it would seem that the study of a more homogeneous patient population is needed. We propose that the Canadian population originating from the Province of Quebec (~80% of French origin), well known for the presence of founder effect,19 constitutes an ideal genetic model for carrying out such epidemiological studies.

In this study, we used a polymerase chain reaction (PCR)-based genotyping approach to examine the relationship between genetic polymorphisms in GSTM1, GSTT1, CYP1A1, and CYP2D6, and susceptibility to childhood ALL in the French-Canadian population. We report the analysis of these loci in 177 ALL patients and in 304 controls.

MATERIALS AND METHODS

Subjects

Childhood ALL patients (n = 177) were diagnosed in the Division of Hematology-Oncology of Ste-Justine Hospital, Montreal, between August 1988 and September 1997. The criteria for inclusion in this group were: (1) Complete clinical history; (2) whites of French-Canadian origin residing in the Province of Quebec as judged by their names, languages, and places of birth; (3) availability of biological material. The recruited patients comprised 110 males and 67 females between the ages of 1 and 21 years (mean age, 8 ± 4.9). The distribution of ALL subtypes as determined by immunophenotyping was as follows: 137 pre-B ALL, 20 T-cell ALL, and 20 with undetermined lineage. A general population control group composed of 144 males and 160 females was randomly selected from a large DNA institutional bank. The criteria for inclusion in the control group were: (1) anonymous, healthy, and unrelated individuals recruited from the...
population served by Ste-Justine Hospital; (2) whites of French-Canadian origin residing in the Province of Quebec as judged by their language and place of birth. The research protocol was approved by the Institutional Review Board (Hospital Ste-Justine, University of Mon-}
{
trail) and informed consent was obtained from all participating individuals and/or their parents involved in the study.

**Genotyping**

DNA isolation. DNA was isolated from either buccal epithelial cells, peripheral blood, or bone marrow in remission, as described by Baccichet et al.\(^{20}\)

**GSTM1 polymorphism.** The polymorphic deletion of the GSTM1 gene was genotyped using the multiplex PCR approach described by Zhong et al.\(^{12}\) The PCR primers used were as follows: P1, 5' CGCCATCTTGTGCTACTGTGCCC; P2, 5' ATCTCTCTCTCTCT-}
{
GTCCT; and P3, 5' TTCTGGATTTGAGGATCA. P1 and P3 amplify a 230-bp product that is specific to GSTM1, whereas P1 and P2 anneal to GSTM1 and GSTM4 genes, yielding a 157-bp fragment that serves as an internal control. PCR was performed in 20 µL containing 20 ng of genomic DNA, 0.5 µmol/L of each primer, 200 µmol/L of each dNTPs, 10 mmol/L Tris-HCl (pH 8.3), 50 mmol/L KCl, 1.5 mmol/L MgCl\(_2\), and 0.5 U of ampliTaq DNA polymerase (Hoffman-LaRoche, Branchburg, NJ). After denaturation for 4 minutes at 94°C, the PCR was performed for 35 cycles of 30 seconds at 94°C, 1 minute at 58°C, and 1 minute at 72°C. The last elongation step was extended to 7 minutes. Negative and positive control samples were included in each amplification series. The presence of one or both GSTM1 allele, identified by a 230-bp fragment, or its complete deletion (null genotype), was analyzed by electrophoresis on a 1.5% agarose gel.

The absence of amplifiable GSTM1 (in the presence of the GSTM4 coamplified control) indicates a null genotype.

**GSTT1 polymorphism.** The polymorphic deletion of the GSTT1 gene was determined by a modification of the PCR protocol described by Katoh et al.\(^{21}\) The amplification reaction was performed in 20 µL, containing 20 ng of genomic DNA, 0.5 µmol/L of each primer, 200 µmol/L of each dNTPs, 2.0 mmol/L Tris-HCl (pH 8.3), 50 mmol/L KCl, and 0.5 U of ampliTaq DNA polymerase (Hoffman-LaRoche). The primers used to amplify GSTT1 were F46, 5' GCCCTTG-}
{
GCTAGTTGCTGAAG and R137, 5' GCA TCTGA TTTGGGGAC-}
{
CAAGGACAGGTAC as a control. The polymorphic deletion of the GSTT1 gene, which was confirmed by amplification of a 204-bp fragment with primers M2F (5' CTGCTCTCTCATGTTAAGGAC) and M2R (5' TTCCAC-}
{
CCTTTCAGGAGGATC) and BgloR, 5' GAAGAGCCAAGGAGCAGTAC as a control. The PCR was performed for 35 cycles of 15 seconds at 94°C, 30 seconds at 59°C, and 45 seconds at 72°C. The last elongation step was extended to 7 minutes. Positive and negative control samples were included in each amplification series. The presence of one or two GSTT1 alleles, identified by a 112-bp fragment, or its complete deletion (null genotype), was shown by electrophoresis on a 1.5% agarose gel.

The absence of amplifiable GSTT1 (in the presence of the GSTM4 coamplified control) indicates a null genotype.

**CYP1A1 polymorphisms.** CYP1A1 mutations T6235C (m1), A729G (m2), and A889G (m3) and CYP2D6*3 were detected by PCR amplification using exon3/intron4 primers (forward, 5' GCCTTGGCAACACACTCCGG; reverse, 5' AAATCCT-}
{
GCTTCTCCGGAGGC) followed by BsrNI digestion (3 U at 60°C), and primers to exon 5/intron 5 (forward, 5' GATGAGCTGTCAACT-}
{
GAGGCC; reverse, 5' CCAGGAGCATACTCGGGAC), followed by HpaII digestion (3 U, 37°C), respectively.\(^{22}\) PCR was performed for 35 cycles of 30 seconds at 94°C, 45 seconds at 56°C (*3) or 60°C (*4), and 45 seconds at 72°C in 20 µL containing 20 ng of genomic DNA, 1.0 µmol/L of each primer, 200 µmol/L dNTPs, 10 mmol/L Tris-HCl (pH 8.3), 1.5 mmol/L MgCl\(_2\), 50 mmol/L KCl, and 0.5 U AmpliTaq DNA polymerase (Hoffman-LaRoche). The PCR product (10 µL) was digested with the corresponding restriction enzyme and subjected to electrophoresis on a 2.0% agarose gel.

**Statistical Analysis**

The statistical significance of the differences between groups was calculated using the Fisher exact test (two-sided). Crude odds ratios (ORs) were calculated and are given within 95% confidence intervals (CI). Unconditional multivariate logistic analyses were performed separately for each locus. Age and gender were included as covariates, as were all genotypes studied and possible interactions. Individuals having the null genotype for GSTM1 and GSTT1, who were homozygous for CYP2D6 variant and/or carriers of at least one CYP1A1 mutant allele, were considered at risk. All analyses were performed using an SPSS statistical package (version 7.5) (SPSS Inc, Chicago, IL). The fraction of the disease attributable to a given genotype was estimated according to Coughlin et al.\(^{23}\)

**RESULTS**

DNA of a quality suitable for PCR was available from 177 whites of French-Canadian origin diagnosed with ALL and 304 healthy controls of a similar ethnic background. Some individuals were not successfully genotyped for all mutations tested, thus explaining the variations in the total number of samples listed in tables 1 to 5. Both pre-B and T-cell ALLs were considered part of the same group because no significant differences were observed in terms of the tested genotypes (data not shown). The frequency of CYP1A1 and CYP2D6 alleles, as well as the distribution of GSTM1, GSTT1, CYP1A1, and CYP2D6 genotypes in ALL patients and in controls are given in Tables 1 and 2, respectively. The observed prevalence of these mutations is in accordance with data from other studies of groups of whites.\(^{24-26}\)

<table>
<thead>
<tr>
<th>Locus</th>
<th>Allele</th>
<th>Cases</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP1A1</td>
<td>*2A</td>
<td>340 (10)</td>
<td>340 (10)</td>
</tr>
<tr>
<td></td>
<td>*2B</td>
<td>340 (13) (3.8)</td>
<td>340 (13) (3.8)</td>
</tr>
<tr>
<td></td>
<td>*4</td>
<td>354 (12) (3.4)</td>
<td>354 (12) (3.4)</td>
</tr>
<tr>
<td>CYP2D6</td>
<td>*3</td>
<td>334 (11) (3.3)</td>
<td>334 (11) (3.3)</td>
</tr>
<tr>
<td></td>
<td>*4</td>
<td>352 (71) (20.2)</td>
<td>352 (71) (20.2)</td>
</tr>
</tbody>
</table>

†Total number of chromosomes analyzed.
‡Number of mutant alleles.
The use of the Bonferroni correction (k = 8) for multiple testing did not change the interpretation of the result for GSTM1 null genotype. An increase in the frequency of CYP2D6 poor metabolizer was observed in children with ALL compared with controls (Table 2), but the difference was not statistically significant (Table 3). None of the other variants studied proved to be significant predictors of ALL risk (Table 3).

A multivariate analysis using unconditional logistic regression was performed to determine which of the variables (gender, age, GSTM1, GSTT1, CYP1A1, and CYP2D6) continued to show significant differences between cases and controls in the presence of the others. Multivariate OR for GSTM1 and CYP1A1*2A were 1.8 (95% CI = 1.2-2.8) and 2.1 (95% CI = 1.2-3.8), respectively. Because GSTM1 is involved in the detoxification of compounds activated by CYP1A1, and both of these enzymes were shown to act independently as ALL risk factors (Table 3), we investigated whether this risk was further increased when both genotypes were combined. When individuals without either of the risk-elevating genotypes were considered as the reference group (ie, those with GSTM1 present and without the CYP1A1*2A allele), we found an increased risk of ALL (OR = 3.3, 95% CI = 1.6-6.9) in children carrying both genotypes at risk (Table 4). The proportion of the disease attributable to CYP1A1*2A and GSTM1 null genotypes alone was estimated to 3% and 19%, respectively, whereas the combined CYP1A1 and GSTM1 risk-elevating genotypes contributed for 8%.

Stratified analysis showed the surprising result that girls carrying at least one CYP1A1*4 allele were significantly underrepresented in the ALL group (OR = 0.2; 95% CI, 0.05-0.9) suggesting a gender-specific protective role against ALL for this allele (Table 5). No other significant synergistic or antagonist statistical interactions between any other combinations of the variables studied here were observed.

DISCUSSION

Little attention has been paid to the role of genetic susceptibility in the aetiology of childhood ALL. It is thought that genetic factors and environmental exposures predispose children to leukemogenesis. The fact that the etiology of sporadic cancers cannot be explained by allelic variability at a single locus is in part due to the complexity of xenobiotics metabolism in cancer. This study presents the first combined analysis of loci encoding phase I and II xenobiotic-metabolizing enzymes in a pediatric cancer and, as such, it represents an important contribution.

We determined the frequencies of CYP1A1, CYP2D6, GSTM1, and GSTT1 gene polymorphisms in a particular white population of French-Canadian origin. This population is well with an increased risk of ALL in this group of patients. An increase in the frequency of CYP2D6 poor metabolizer was observed in children with ALL compared with controls (Table 2), but the difference was not statistically significant (Table 3). None of the other variants studied proved to be significant predictors of ALL risk (Table 3).

A multivariate analysis using unconditional logistic regression was performed to determine which of the variables (gender, age, GSTM1, GSTT1, CYP1A1, and CYP2D6) continued to show significant differences between cases and controls in the presence of the others. Multivariate OR for GSTM1 and CYP1A1*2A were 1.8 (95% CI = 1.2-2.8) and 2.1 (95% CI = 1.2-3.8), respectively. Because GSTM1 is involved in the detoxification of compounds activated by CYP1A1, and both of these enzymes were shown to act independently as ALL risk factors (Table 3), we investigated whether this risk was further increased when both genotypes were combined. When individuals without either of the risk-elevating genotypes were considered as the reference group (ie, those with GSTM1 present and without the CYP1A1*2A allele), we found an increased risk of ALL (OR = 3.3, 95% CI = 1.6-6.9) in children carrying both genotypes at risk (Table 4). The proportion of the disease attributable to CYP1A1*2A and GSTM1 null genotypes alone was estimated to 3% and 19%, respectively, whereas the combined CYP1A1 and GSTM1 risk-elevating genotypes contributed for 8%.

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We determined the frequencies of CYP1A1, CYP2D6, GSTM1, and GSTT1 gene polymorphisms in a particular white population of French-Canadian origin. This population is well

### Table 2. Distribution of CYP1A1, CYP2D6, GSTM1, and GSTT1 Genotypes in ALL Cases and Controls

<table>
<thead>
<tr>
<th>Locus</th>
<th>Genotype</th>
<th>Cases</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Allele</td>
<td>n†</td>
<td>No.§ (%)</td>
</tr>
<tr>
<td>CYP1A1*2A</td>
<td>-/-</td>
<td>170</td>
<td>80.6</td>
</tr>
<tr>
<td></td>
<td>+/-</td>
<td>32</td>
<td>18.8</td>
</tr>
<tr>
<td></td>
<td>+/+</td>
<td>1</td>
<td>0.6</td>
</tr>
<tr>
<td>CYP1A1*2B</td>
<td>-/-</td>
<td>170</td>
<td>92.9</td>
</tr>
<tr>
<td></td>
<td>+/-</td>
<td>11</td>
<td>6.5</td>
</tr>
<tr>
<td></td>
<td>+/+</td>
<td>1</td>
<td>0.6</td>
</tr>
<tr>
<td>CYP1A1*4</td>
<td>-/-</td>
<td>177</td>
<td>94.4</td>
</tr>
<tr>
<td></td>
<td>+/-</td>
<td>8</td>
<td>4.5</td>
</tr>
<tr>
<td></td>
<td>+/+</td>
<td>2</td>
<td>1.1</td>
</tr>
<tr>
<td>CYP2D6*3</td>
<td>-/-</td>
<td>167</td>
<td>95.2</td>
</tr>
<tr>
<td></td>
<td>+/-</td>
<td>5</td>
<td>3.0</td>
</tr>
<tr>
<td></td>
<td>+/+</td>
<td>3</td>
<td>1.8</td>
</tr>
<tr>
<td>CYP2D6*4</td>
<td>-/-</td>
<td>176</td>
<td>64.2</td>
</tr>
<tr>
<td></td>
<td>+/-</td>
<td>55</td>
<td>31.3</td>
</tr>
<tr>
<td></td>
<td>+/+</td>
<td>8</td>
<td>4.5</td>
</tr>
<tr>
<td>CYP2D6</td>
<td>PM</td>
<td>165</td>
<td>67.6</td>
</tr>
<tr>
<td>GSTM1</td>
<td>Present</td>
<td>174</td>
<td>61.5</td>
</tr>
<tr>
<td></td>
<td>Null</td>
<td>113</td>
<td>46.9</td>
</tr>
<tr>
<td>GSTT1</td>
<td>Present</td>
<td>176</td>
<td>84.1</td>
</tr>
<tr>
<td></td>
<td>Null</td>
<td>28</td>
<td>15.9</td>
</tr>
</tbody>
</table>

Abbreviations: OR, crude odds ratio; CI, confidence interval.
†Total number of individuals tested.
§Number of individuals with a given genotype.

The incidence of GSTM1 null and CYP1A1*2A genotypes were significantly increased in ALL cases as compared with controls (Tables 1 and 3). Among ALL patients, 64.9% were homozygous for the GSTM1 null genotype, compared with 51.3% of controls, and CYP1A1*2A individuals account for 19.4% of ALL cases, compared with 11.7% of controls (Table 2). Consequently, there was an 80% increase in the risk of ALL associated with the GSTM1 null genotype (OR = 1.8; 95% CI, 1.2-2.8) and CYP1A1*2A allele (OR = 1.8; 95% CI, 1.1-3.1) suggesting a gender-specific protective role against ALL for this allele (Table 5). No other significant synergistic or antagonist statistical interactions between any other combinations of the variables studied here were observed.

### Table 3. Relationship Between CYP1A1, CYP2D6, and GSTM1 Genotypes and Risk of Childhood ALL

<table>
<thead>
<tr>
<th>Gene</th>
<th>Genotype</th>
<th>OR</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP1A1‡</td>
<td>*2A</td>
<td>1.8</td>
<td>1.1-3.1</td>
<td>.03</td>
</tr>
<tr>
<td></td>
<td>*2B</td>
<td>0.9</td>
<td>0.4-1.8</td>
<td>.90</td>
</tr>
<tr>
<td></td>
<td>*4</td>
<td>0.6</td>
<td>0.3-1.2</td>
<td>.16</td>
</tr>
<tr>
<td></td>
<td>*3</td>
<td>5.5</td>
<td>0.6-53.4</td>
<td>.13</td>
</tr>
<tr>
<td></td>
<td>*4</td>
<td>1.6</td>
<td>0.6-4.1</td>
<td>.40</td>
</tr>
<tr>
<td></td>
<td>PM</td>
<td>1.8</td>
<td>0.8-4.4</td>
<td>.17</td>
</tr>
<tr>
<td>GSTM1§</td>
<td>Null</td>
<td>1.8</td>
<td>1.2-2.6</td>
<td>.004</td>
</tr>
<tr>
<td>GSTT1§</td>
<td>Null</td>
<td>0.9</td>
<td>0.5-1.5</td>
<td>.80</td>
</tr>
</tbody>
</table>

Abbreviations: OR, crude odds ratio; CI, confidence interval.
‡Heterozygous and homozygous mutant combined.
§Homozygous mutant only; PM, poor metabolizer, ie, homozygous and combined heterozygous for mutant alleles.

### Table 4. Combined Effects of GSTM1 Null Genotype and CYP1A1*2A Allele in Childhood ALL Risk

<table>
<thead>
<tr>
<th>Genotype</th>
<th>GSTM1</th>
<th>CYP1A1*2A</th>
<th>Cases</th>
<th>Controls</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>Present</td>
<td>-/-</td>
<td>49</td>
<td>126</td>
<td>1.0 (referent)</td>
</tr>
<tr>
<td>One</td>
<td>Null</td>
<td>-/-</td>
<td>84</td>
<td>138</td>
<td>1.6 (1.0-2.4)</td>
</tr>
<tr>
<td>Two</td>
<td>Null</td>
<td>+/-, +/-</td>
<td>12</td>
<td>19</td>
<td>1.6 (0.7-3.6)</td>
</tr>
</tbody>
</table>

Abbreviations: OR, crude odds ratio; CI, confidence interval.
†P = .002.
the presence of founder effect indicating a relative genetic homogeneity due to particular demographic and historic characteristics.\textsuperscript{19} The overall frequencies of the tested genotypes in control subjects agreed with those reported in other studies.\textsuperscript{24-26} However, in the case of the CYP1A1*4 variant, there seems to be a difference between the frequency observed in French-Canadians (5.1%) and that found in other white populations (2\% to 3\%).\textsuperscript{8,26} We have shown that children carrying the GSTM1 null genotype are at increased risk of developing ALL (OR = 1.8). Glutathione S-transferases are involved in the metabolism of a wide range of xenobiotics, including environmental carcinogens, chemotherapeutic agents, and reactive oxygen species.\textsuperscript{27,28} Several epidemiologic studies have associated the null genotype of GSTM1 with a higher risk of cancer in adults\textsuperscript{31}—this is probably related to an inability to metabolize tobacco-related carcinogens, such as benzo(a)pyrene.\textsuperscript{29} The only other study analyzing the GST genotypes in the context of ALL risk reported that the double-null genotype for GSTM1 and GSTT1 was significantly more frequent among blacks with childhood ALL, but failed to show that an increased risk was associated with this genotype in whites.\textsuperscript{30} This discrepancy may be the result of an ethnically heterogeneous population, as great variations in allele frequency are observed both among and within different ethnic groups.\textsuperscript{24,25}

Children carrying the CYP1A1*2A allele are also at greater risk of developing ALL (OR = 1.8). To our knowledge, this is the first report of an association between the CYP1A1 variant and the risk of childhood ALL. The CYP1A1*2A allele is associated with elevated enzymatic activity,\textsuperscript{8,37} supporting the hypothesis linking the risk of ALL with the inducibility of the xenobiotics-metabolizing enzyme CYP1A1.\textsuperscript{31} Consequently, carriers of variant alleles are expected to be at a greater risk when exposed to carcinogens such as PAHs.\textsuperscript{6} Indeed, an association between the induction of placent al CYP1A1 activity and PAH in cigarette smoke or cooked foods has been reported.\textsuperscript{32-35} We cannot rule out the possibility that CYP1A1*2A result is due to chance in the context of multiple testing. However, the influence of this genotype is more obvious when other variants participated in the effect.

The presence of both the CYP1A1*2A allele and the GSTM1 null-genotype confers an additional risk of ALL (OR = 3.3) on the carriers. Similar studies performed in adults with nonhematological neoplasias provide further evidence for an increase in the risk of cancer in patients carrying both CYP1A1- and GSTM1-risk alleles.\textsuperscript{36,37} This is supported biologically by a recent study showing that the combination of CYP1A1 and GSTM1 genotypes clearly affects the formation of DNA adducts in human white blood cells.\textsuperscript{38} Finally, our results suggest that allelism in CYP2D6 and GSTT1 genes do not play an important role in the aetiology of ALL. However, more data are needed to confirm these findings.

The scarcity of molecular epidemiology studies in childhood diseases makes it difficult to predict how these genotypes at risk will modify the host response to different exposures. Epidemiologic studies have led to the suggestion that in utero and postnatal exposures to various biological, physical, and chemical factors may be important determinants of childhood ALL.\textsuperscript{39,41} The apparent association of parental exposures to various chemicals\textsuperscript{31,42-46} suggests that variability at loci encoding xenobiotic-metabolizing enzymes could influence susceptibility to this disease. Infants and children may be at greater risk than adults from a variety of environmental toxicants, including polycyclic aromatic hydrocarbons (PAHs), nitrosamines, pesticides, tobacco smoke, and air pollution due to differential exposure and/or physiologic immaturity.\textsuperscript{41,47} Xenobiotics enter the placenta through maternal circulation,\textsuperscript{48} which possesses the ability to metabolize these compounds through processes similar to those seen in the liver.\textsuperscript{34,49} Therefore, alterations in placental metabolizing capabilities could potentially result in the exposure of the developing foetus to harmful electrophiles. As a target of damage from ubiquitous exposures to xenobiotics, a young child (or fetus) with either the null GSTM1 genotype and/or the CYP1A1*2A allele will be at greater risk of ALL. In this regard we found that 8\% of the disease is attributable to combined CYP1A1 and GSTM1 genotypes at risk, whereas GSTM1 null and CYP1A1*2A genotypes alone contributed to 19\% and 3\%, respectively. These results suggest that the effect of CYP1A1*2A to ALL susceptibility depends on the presence of other genotypes at risk.

As stated earlier, the fact that CYP1A1*4 allele is underrepresented among ALL females suggested a gender-specific protective role for this allele. This finding could partly explain the higher prevalence of ALL in males.\textsuperscript{30-52} Unlike gender-related hormonal factors that play an obvious role in breast cancer, other sources of variation in cancer risk due to gender have received little attention. The gene product of CYP1A1*4 may be transcriptionally induced or regulated by certain endogenous steroids or other nongenetic factors in females or males. Studies on animals have shown that exogenous agents may permanently affect the expression patterns of specific cytochromes P450.\textsuperscript{53} Such imprinting of sex-specific cytochrome P450 forms is controlled by the levels and modes of excretion of androgens, estrogens, and growth hormone.\textsuperscript{54} Unfortunately, the biological significance of the *4 mutation due to C-to-A transversion at position 4887 in the home-binding domain of exon 7 (threonine exchange to asparagine in codon 461) is still unknown.\textsuperscript{8} However, we cannot exclude the role of an adjacent gene in linkage with CYP1A1 whose particular allele in association with *4 mutation could be responsible for the effect observed. Because the human fetus and embryo are exposed to numerous

\begin{table}[h]
\centering
\caption{CYP1A1*4 Genotype and Gender-Related Risk of Childhood ALL}
\begin{tabular}{lccccc}
\hline
\textbf{Group} & \textbf{Genotype} & \textbf{Female} & & & \\
& & \textbf{\textit{n}} & \textbf{\textit{No.} (%)} & \textbf{\textit{n}} & \textbf{\textit{No.} (%)} \\
\hline
\textbf{ALL cases} & \textit{−/−} & 67 & (97.0) & 110 & (92.7) \\
& \textit{+/−} & 2 (3.0) & 6 (5.5) & & \\
& \textit{+/+} & 0 (0.0) & 2 (1.8) & & \\
\textbf{Controls} & \textit{−/−} & 156 & (86.5) & 139 & (95.0) \\
& \textit{+/−} & 19 (12.2) & 7 (5.0) & & \\
& \textit{+/+} & 2 (1.3) & 0 (0.0) & & \\
\hline
\end{tabular}
\end{table}
chemicals throughout gestation, it is theoretically possible that the imprinting of cytochrome P450 enzymes also occurs.

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14. Hugues Sinnett for his excellent technical assistance. We are grateful to Dr Claire Infante-Rivard for helpful discussion on the epidemiology and biostatistics aspects of this work. We thank Hugues Sinnett for his excellent technical assistance. We are grateful to all patient and control subjects who participated in this study as well as the physicians and staff for their collaboration.

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Susceptibility to Childhood Acute Lymphoblastic Leukemia: Influence of CYP1A1, CYP2D6, GSTM1, and GSTT1 Genetic Polymorphisms

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