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GSTT1 and *CYP2E1* Polymorphisms and Trihalomethanes in Drinking Water: Effect on Childhood Leukemia

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The purpose of the study was to determine whether the risk of childhood acute lymphoblastic leukemia (ALL) associated with drinking water disinfection by-products was modified in the presence of variants in genes involved in the metabolism of trihalomethanes (THMs). We included a subset of cases from a population-based case–control study in a case-only study to estimate the interaction odds ratios (IORs) between prenatal and postnatal exposure to THMs and polymorphisms in the *GSTT1* and *CYP2E1* genes. We compared cases with and without a given variant regarding their exposure to THMs using unconditional logistic regression. The IOR for a postnatal average of total THM above the 95th percentile with *GSTT1* null genotype was 9.1 [95% confidence interval (95% CI), 1.4–57.8]. With *CYP2E1* (variant G-1259C, known as the allele CYP2E1*5), the effect of exposure during pregnancy for an average exposure to total THM at or above the 75th percentile was 9.7 (95% CI, 1.1–86.0). These results contrast strongly with those from our case–control analysis, in which we considered the exposure to THMs only in relation with ALL, and observed no increase in risk or very moderate ones. The present preliminary study shows suggestive but imprecise results. We found no similar results in the literature, underscoring the need for other studies as well as the potential usefulness of combining exposure and relevant genetic information in such studies. *Key words:* childhood leukemia, CYP2E1, disinfection by-products, drinking water, genetic polymorphisms, GSTT1, trihalomethanes. *Environ Health Perspect* 110:591–593 (2002). [Online 25 April 2002]
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Trihalomethanes (THMs), such as chloroform, are chlorination by-products of drinking water resulting from the reaction of chlorine with organic material in the water. THMs have been found to be associated with tumors in animal studies (*1*). In addition, epidemiologic studies in adults potentially exposed to THMs have shown excesses of colon, rectum, and bladder cancer (*1*).

Most environmental chemicals must be biotransformed to have toxic effects. This biotransformation is a two-stage process carried out by phase I and phase II metabolizing enzymes (*2*). Phase I enzymes such as those belonging to the cytochrome P-450 (CYP) family are involved in the initial oxidation, reduction, or dealkylation of carcinogens; this phase generally leads to the production of active intermediate metabolites. Phase II enzymes such as those belonging to the glutathione *S*-transferase (GST) family convert the active metabolites produced during phase I reactions into water-soluble and excretable products. Individual variation in the genes encoding these enzymes could modify the effects associated with specific exposures and thus influence susceptibilities to cancer, including childhood leukemia (*3*). Recent studies have shown that the enzymes CYP2E1 and GSTT1 are involved in the metabolism of THM, the latter in particular with brominated THM (*4–6*). Therefore, studying whether the risk for cancer associated with exposure to THMs in drinking water is modified by polymorphisms in the genes encoding these particular enzymes would seem relevant.

We recently performed a case–control study investigating the risk for acute lymphoblastic leukemia (ALL) associated with exposure to THMs in drinking water in children 0–9 years old (*7*). We studied the risk for ALL from exposure to THMs during the prenatal and the postnatal periods and found that odds ratios (ORs) were generally not increased for the prenatal period and when using average levels of exposure. However, postnatal cumulative exposure for total THM above the 95th percentile of the distribution for cases and controls was associated with an OR of 1.54 [95% confidence interval (95% CI) = 0.78–3.03]; for that same period, risk associated with exposure to chloroform was increased (OR = 1.63; 95% CI, 0.84–3.19). To clarify further whether risk for ALL in these children was modified by DNA variants in the genes encoding enzymes involved in the metabolism of THM such as chloroform, we performed a case-only study on a subset of the cases selected for the original case–control study.

Materials and Methods

Case–control study. Details of the case–control study are provided elsewhere (*7*). In brief, we recruited patients with ALL between 0 and 9 years old diagnosed between 1980 and 1993 in the Province of Québec from tertiary care centers designated by governmental policy to hospitalize and treat children with cancer in the province. Tracing cases from these hospitals is equivalent to population-based ascertainment. We selected controls for these cases from family allowance files and matched them to each patient for age, sex, and region of residence (we based the definition of these regions on administrative and geographical criteria, and they cover wide territories). The family allowance is a government stipend awarded to all families with children living legally in Canada and was the most complete census of children available for the study years. Participation rates were 96.3% among cases and 83.8% among controls. We analyzed 491 cases and 491 controls. The ethical review board of each participating hospital approved the project for recruitment of cases, whereas that for controls was approved by the provincial paragovernmental agency regulating access to databases with personal identifiers.

Sources of data for exposure measurement and exposure matrix. Extensive details on the exposure assessment method are given in (*7*). Briefly, to determine exposure to THMs in drinking water, we developed a municipality-exposure matrix. To do so, we used *a*) information from interview of parents where child's residential history covering

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the pregnancy period up to the time reference date was collected, *b*) historical data provided by municipalities and the ministry of environment, and *c*) a tap water survey carried out in 227 homes. We used all these sources of data to fill the cells of the matrix, which we defined by rows of municipalities and columns of specific contaminants; a third dimension was the calendar year. For cells with a missing value, we imputed the closest available annual average measurement (before or after a missing year). For the prenatal period, 21% of person-years of exposure had their value assigned from the current year (i.e., no imputation), whereas 52% of person-years of exposure were assigned from the nearest 6 years or less. For the postnatal period, these figures were 26% and 61%, respectively. In the end, some data were available on THMs for 436 of cases and 422 controls during the prenatal period, and for 457 cases and 441 controls during the postnatal period.

We used three exposure indices in the analysis. A first index was, over the exposure period, whether drinking water was never chlorinated, chlorinated part of the time, or always chlorinated. A second index was the average level of exposure over the period [$\sum$(concentration$_j$ × duration$_i$ in days) ÷ total duration]; we then categorized it at > 95% percentile of the distribution for cases and controls. We also categorized the average level into three categories: ≤ 24th percentile, 25th to 74th percentile, and ≥ 75th percentile. A third index was the cumulative weighted sum over the exposure period [$\sum$(concentration$_j$ × duration$_i$ in days)], which we then categorized as above. In the present report we use the second and third indices of exposure.

Case-only study. The case-only study has been recently proposed as an efficient design for studying gene–environment interactions (*8*). Based only on cases, the OR measuring the association between the gene of interest and the particular environmental exposure among these cases is a measure of the interaction parameter. The latter is an efficient estimate of the interaction odds ratio (IOR) one would obtain from the case–control study and is a valid estimate of this quantity if the exposure and the genotype in the population are independent (*9*).

For this study, we used 170 cases from the set of 491 cases; these came from the largest pediatric cancer-treating center in the province where genotyping of all ALL cases has been systematically initiated. All parents signed a consent form for our use of DNA material that was already provided for diagnostic and therapeutic purposes. The cases included here are all of French-Canadian origin (French-Canadians make up about 80% of the population in the Province of Québec). The polymorphic deletion in the *GSTT1* gene was revealed by a polymerase chain reaction (PCR)-based assay using internal controls (*10*). PCR allele-specific oligonucleotide hybridization assays have been used to genotype the polymorphism G-1259C (a G-to-C substitution at position –1259 in the promoter) that defines the allele CYP2E1*5 (*11*).

Statistical analysis. We used unconditional logistic regression to estimate the IORs and their 95% confidence intervals. The IOR is defined as

$$\mathrm{OR}_{ge}/(\mathrm{OR}_g \times \mathrm{OR}_e) \times Z,$$

where g stands for genotype, e for exposure, and Z for the OR between exposure and genotype in the controls, assumed to be 1 based on the independence assumption.

Results

Among the 170 genotyped cases, 161 cases also had exposure data. We compared the age and sex distributions (confirmed risk factors for ALL) of the 161 genotyped subjects entering the analysis with those of the entire set of cases and found that 57% were boys in comparison with 56% among all 491 cases, and 58% were < 5 years old in comparison with 66% among all cases.

In the present study, we analyzed the GSTT1 polymorphism with the index measuring average as well as the cumulative level of exposure for total THM > 95th percentile and likewise for bromoform at ≥ 75th percentile; for CYP2E1*5, we used the average as well as the cumulative level at ≥ 75th percentile. We based the percentile distributions on the complete set of subjects, as previously described, but for some situations in the present analysis we had too few subjects > 95th percentile to use that category; instead, we used a broader category (≥ 75th percentile). To evaluate latency, we determined average and cumulative exposure for the period ranging from pregnancy to 1 year before diagnosis or reference date for controls. For

Table 1. IORs and 95% CIs for exposure to THMs and GSTT1 and CYP2E1 genotypes among children with ALL.

Gene/exposure	Prenatal IOR (95% CI)	Postnatal IOR (95% CI)
GSTT1 null	(23 M; 131 N)	(25 M; 136 N)
Total THM (> 95th percentile)		
Average	2.40 (0.43–13.18)	9.13 (1.44–57.82)
Cumulative	2.40 (0.43–13.18)	2.51 (0.60–10.45)
Average for complete period[a]	4.6 (0.96–21.97)	—
Cumulative for complete period[a]	2.23 (0.55–9.06)	—
Bromoform[b] (≥ 75th percentile)		
Average	—	0.53 (0.17–1.52)
Cumulative	—	0.57 (0.18–1.64)
Average for complete period[a]	0.37 (0.10–1.33)	—
Cumulative for complete period[a]	0.39 (0.11–1.38)	—
CYP2E1*5	(12 M; 118 N)	(12 M; 125 N)
Total THM		
Average		
25th–74th percentile	3.50 (0.40–31.54)	1.61 (0.28–9.25)
≥ 75th percentile	9.75 (1.10–86.01)	4.06 (0.76–21.50)
For complete period[a]		
25th–74th percentile	1.56 (0.27–8.94)	—
≥ 75th percentile	4.30 (0.81–22.77)	—
Cumulative		
25th–74th percentile	4.5 (0.52–39.12)	3.89 (0.45–33.67)
≥ 75th percentile	8.0 (0.88–72.51)	5.96 (0.66–53.82)
For complete period[a]		
25th–74th percentile	1.74 (0.32–9.43)	—
≥ 75th percentile	2.95 (0.53–16.24)	—
Chloroform		
Average		
25th–74th percentile	3.48 (0.40–30.97)	3.80 (0.42–33.75)
≥ 75th percentile	10.17 (1.15–89.89)	8.20 (0.94–71.72)
For complete period[a]		
25th–74th percentile	3.33 (0.37–29.60)	—
≥ 75th percentile	8.57 (0.97–75.17)	—
Cumulative		
25th–74th percentile	4.60 (0.53–39.88)	5.47 (0.65–121.36)
≥ 75th percentile	7.69 (0.85–69.63)	7.31 (0.79–169.76)
For complete period[a]		
25th–74th percentile	3.33 (0.37–29.60)	—
≥ 75th percentile	6.91 (0.79–60.37)	—

Abbreviations: M, mutant allele (GSTT1 null genotype, carrier of at least one CYP2E1*5 allele); N, normal allele.
[a]From pregnancy to the year before diagnosis. [b]All subjects were in the ≥ 75th percentile category during pregnancy.

study subjects younger than 1 year old, we did not apply the latency period; four cases were in this category in the present analysis.

Table 1 shows that the IOR was increased with the *GSTT1* null genotype in the postnatal period using the average level of total THM and likewise for the complete period. Using the cumulative THM level, risks were only moderately increased and all confidence intervals included the null value. Results with the average as well as the cumulative values for bromoform suggested a nonsignificant protective effect associated with the null genotype. IORs were increased with the CYP2E1*5 allele using the average or the cumulative level for total THM and for chloroform; risks were generally comparable for the pregnancy and the postnatal period.

Discussion

Using the case-only methodology, we observed that risk for ALL associated with exposure to total THM was elevated among children homozygous for GSTT1 deletion (null genotype) but below the null value with exposure to bromoform; risks for those carrying the CYP2E1 variant G-1259C (allele *5) were elevated for exposure occurring during the prenatal as well as the postnatal period. Many of these observations were imprecise because of the relatively small number of genotyped subjects. The data are quite preliminary and will need to be replicated before any strong conclusion is reached. However, we have established that carcinogenic risks from exposure to environmental toxicants can be modified by genetic risk factors such that certain individuals are more or less susceptible as a result of DNA variants in the genes encoding xenobiotic metabolizing enzymes.

Because the tested variant in the regulatory region of the *CYP2E1* gene is associated with an increased transcriptional activity (*12*), individuals with this polymorphism would be expected to show enhanced metabolism of THM and chloroform that in turn would result in more activated metabolites. Similarly, individuals lacking GSTT1 enzyme activity (*GSTT1* null genotype) would be at higher risk because they are less efficient in converting these reactive intermediates into soluble products.

Studies investigating the risk of ALL from exposure to chlorination by-products in drinking water are scarce. To our knowledge, no study has investigated the joint contribution of DNA variants in xenobiotic metabolizing enzymes and exposure to THMs in the pathogenesis of ALL, and only two other studies have evaluated the association between leukemia and drinking water contamination, both ecologic: one study (*13*) reported an inverse relationship between exposure to THMs and leukemia for adults, and the other (*14*) observed no relation for ALL and exposure to THMs.

The validity of the IORs obtained from case-only studies rests on the assumption of independence of exposure and genes in the population, such that individuals with certain DNA variants are not more or less likely to be exposed to environmental contaminants. We have no data to support the independence assumption in our study, but lack of independence would seem unlikely for maternal exposure given unknown child genotype and likewise for the child postnatally given the nature of the exposure.

The interpretation of the measures of effects for candidate genes in an association study must be made with caution because alternative explanations need to be considered. Although the studied gene could be involved in causing the disease, another interpretation is that the candidate gene is not itself causally involved but rather is in linkage disequilibrium with (very close to) a causal gene. A third possibility is that the study is affected by a population structure bias (*15*) whereby many ethnic subgroups with different allelic distributions are pooled in the analysis, a potential bias that can affect case-only studies (*9*). The latter explanation for our results is unlikely because all genotyped cases were ethnically homogeneous. The possibility of linkage disequilibrium remains, and although we have no data to address this possibility, these closely linked genes, if any, must participate in or influence the metabolism of THM.

In conclusion, although imprecise and preliminary, our results indicate that individual susceptibility as determined by DNA variants may play a role in the etiology of ALL. When considering the risk of ALL associated with exposure to drinking water contaminants, our results were not very suggestive (*7*); the present results, on the other hand, if validated in future studies, highlight the importance of considering genetic variation with exposure to contaminants to determine associations.

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