

Childhood Acute Lymphoblastic Leukemia Associated with Parental Alcohol Consumption and Polymorphisms of Carcinogen-Metabolizing Genes

Claire Infante-Rivard,^{1,2} Maja Krajinovic,² Damian Labuda,^{2,3} and Daniel Sinnett^{2,3}

Background. Limited information is available on the association of parental consumption of alcohol prior to and during pregnancy with the risk of childhood leukemia, as well as for the potentially modifying role of genetic polymorphisms.

Methods. We conducted a population-based, case-control study of 491 incident cases of acute lymphoblastic leukemia age 0–9 years and matched on age and sex to 491 healthy controls. Cases were identified at tertiary care centers in the Province of Québec between 1980 and 1993. Each parent was interviewed separately about alcohol consumption habits. We also used a case-only design with 186 cases to estimate interaction odds ratios between prenatal exposure and child DNA variants in the GSTM1 and CYP2E1 genes.

Results. The adjusted odds ratio for any maternal consumption during pregnancy was 0.7 (95% confidence interval = 0.5–0.9). The interaction odds ratios for the GSTM1 null genotype during third pregnancy trimester was 2.4 (95% confidence interval = 1.1–5.4); the interaction odds ratio for CYP2E1 variant G-1295C (or allele *5) during the nursing period was 4.9 (95% confidence interval = 1.5–16.7).

Conclusions. The observed association with maternal alcohol consumption during pregnancy could be due to the potential chemopreventive effects of flavonoids found in wine and beer. These possible effects of alcohol may be at least partially genetically determined, although data are preliminary. (EPIDEMIOLOGY 2002;13:277–281)

Keywords: acute lymphoblastic leukemia, alcohol, prenatal exposure, polymorphism (genetic), GSTM1, CYP2E1.

No consistent association has been found in previous epidemiological studies between childhood leukemia and parental alcohol consumption,¹ although the more recent studies tend to show protective effects for maternal drinking during pregnancy.^{2,3} In addition, little attention has been paid to gene-environment interactions in the etiology of childhood

acute lymphoblastic leukemia (ALL), the most common form of cancer affecting children.

Alcohol metabolism is known to produce reactive oxygen species (ROS)⁴ that could contribute to carcinogenesis.⁵ The ROS are metabolized by glutathione S-transferases (GSTs).⁶ Several genes from the GST gene family, such as GSTM1, have functional polymorphisms associated with reduced activity or lack of activity.⁷ GSTM1 is involved in the biotransformation of a wide range of reactive toxic and mutagenic compounds other than ROS.⁷

The cytochromes P450 (CYPs) are a multigene family encoding phase I enzymes involved in the initial oxidation, reduction, or dealkylation of carcinogens. One member of this class of genes, CYP2E1, plays a major role in the metabolism of a wide range of xenobiotics including ethanol.⁸ Polymorphisms in the regulatory region of CYP2E1 have been associated with increased transcriptional activity^{9,10} and seem to play a role in the etiology of a number of alcohol-related diseases and cancers including those of the oral cavity, nasopharynx, stomach, and oesophagus.¹¹

From ¹Joint Departments of Epidemiology and Biostatistics, and Occupational Health, Faculty of Medicine, McGill University; ²Research Center, Hôpital Sainte-Justine; ³Department of Pediatrics, Université de Montréal, Montréal, Québec, Canada.

Address correspondence to: Claire Infante-Rivard, Joint Departments of Epidemiology and Biostatistics, and Occupational Health, Faculty of Medicine, McGill University, 1130 Pine Avenue West, Montréal, Québec, Canada, H3A 1A3; cirivard@epid.lan.mcgill.ca

This project was supported by a grant from the National Health and Welfare Research and Development Program Grant #6605-3309-58, Leukemia Research Fund of Canada, the "Fondation de l'Hôpital Sainte-Justine," Power Corporation and the "Fonds de la Recherche en Santé du Québec (FRSQ)"-Hydro-Québec Program. Claire Infante-Rivard holds a Canada Research Chair (James McGill Professor). Daniel Sinnett is a research scholar of the FRSQ.

Submitted 7 August 2001; Final version accepted 18 January 2002.

Copyright © 2002 by Lippincott Williams & Wilkins, Inc.

The main goal of the present study is to evaluate the relation between childhood ALL and parental alcohol consumption in the month before conception, as well as during pregnancy and the nursing period. In a previous study, the GSTM1 null genotype was associated with an increased risk of ALL¹² and likewise for the CYP2E1*5 variant.¹³ Given these observations and the fact that GSTM1 and CYP2E1 are involved in the metabolism of alcohol, we hypothesized that genetic polymorphisms in these genes could modify the effect of prenatal alcohol consumption on ALL, which we also evaluate in an exploratory sub-study.

Methods

Case-Control Study

Details of the case-control study were provided elsewhere.¹⁴ Briefly, we recruited cases of ALL between 0 and 9 years of age 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 to the case for age, sex, and region of residence at the time (calendar date) of diagnosis and thus were concurrently selected. 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% among cases and 84% among controls. A total of 491 cases and 491 controls were analyzed. Approval for the project was obtained from the research ethics committee of each participating hospital and from the "Commission d'Accès à l'Information du Québec"; an informed consent was signed by the parents.

Trained interviewers administered a structured questionnaire by telephone. The questionnaire included information on potential confounding factors and on consumption of alcohol; for the mother, consumption referred to the month prior to pregnancy, the pregnancy trimesters, and the nursing period while for the father it referred to the month prior to pregnancy. We asked about the number of glasses of wine (4-ounce glass), beer (12-ounce can or bottle), and spirits (1.5-ounce shot) consumed daily during each period. Mother and father were interviewed separately. Among case mothers, 99% answered the questions themselves compared with 97% of control mothers. These figures were 83% and 81%, respectively, for fathers.

The association between exposure and risk of ALL was estimated using conditional logistic regression. Odds ratios (ORs) and 95% confidence intervals (CIs) were estimated for each type of alcoholic beverage consumed

either during the entire target period (defined as from 1 month previous to pregnancy to the nursing period included) or specifically during the pregnancy period; consumption was defined as "any" versus "none." We also evaluated the effects of consuming any type of alcohol for the entire target period, as well as specifically for the month before pregnancy, each pregnancy trimester, and the nursing period. Quantitative categories including all types of alcohol beverages were also used. For mothers, two categories were contrasted with a baseline of no alcohol consumption; the first category included those reporting less than one drink a day and the second included the consumers of one drink a day or more. For fathers, three categories were created as follows: less than one drink per day, one to three drinks a day, and greater than three. We adjusted for mother's age and education.

Case-Only Study

The case-only study design is an efficient way of estimating the interaction parameter between genotype and an environmental exposure.¹⁵ We conducted such a study with a sample of 186 cases identified at Hôpital Sainte-Justine, the largest pediatric center in the province. These cases had already been genotyped as part of a leukemia genetic research program conducted at that institution.¹⁶

Informed consent to genotype the index child was obtained from all participating parents. Genomic DNA was extracted from cells derived either from mouth epithelium, peripheral blood, or bone marrow in remission as previously described.¹⁷ The homozygous deletion (null genotype) of the GSTM1 gene was revealed by PCR-based assays using internal controls as described in Krajinovic *et al.*¹² PCR allele-specific-oligonucleotide (ASO) hybridization assays have been used to genotype the CYP2E1*5 variant (presence of G1259C).¹³ With this method, ASOs distinguishing between the wild-type and the mutant alleles are used as probes to hybridize dot-blotted PCR products of the genomic samples.¹⁸

For the case-only study, unconditional logistic regression was used to estimate the interaction odds ratios (IOR) and their 95% CIs, adjusting for sex of the child.

Results

The case and control groups each included 216 girls and 275 boys. There were slight differences between cases and controls in the distribution of mother's age at birth of the child and mother's education (Table 1).

Table 2 shows an inverse relation between ALL and any maternal alcohol consumption during the entire target period as well as during the pregnancy period (OR = 0.7; 95% CI = 0.5–0.9). Similar results (not shown in Table 2) were observed when considering wine and beer drinking separately but not spirits. The OR for consump-

TABLE 1. Characteristics of Cases of Acute Lymphoblastic Leukemia* and Their Controls

Factor	Controls		Cases	
	N	%	N	%
Mother's level of schooling				
College or university	188	38	168	34
Secondary school	279	57	291	59
None, primary	23	5	32	7
Family income at diagnosis†				
≥40,000	147	31	150	31
10,000–39,000	297	63	307	64
<10,000	26	6	26	5
Maternal age‡				
≤35	470	96	461	94
>35	21	4	30	6
Paternal age‡				
≤40	467	96	472	96
>40	22	4	18	4
Race of mother				
White	474	97	466	95
Black	11	2	5	1
Others	6	1	20	4
Maternal smoking§				
None	316	64	303	62
1–20 cigarettes daily	123	25	136	28
>20 cigarettes daily	52	11	52	11

* Maximum number of cases and controls was 491, each.
 † In Canadian dollars (information available for 470 controls and 483 cases).
 ‡ At birth of index child.
 § In the first trimester of pregnancy.

tion of wine or beer during the entire target period was 0.6 (95% CI = 0.5–0.8) and that for consumption during pregnancy was 0.7 (95% CI = 0.6–1.0). The inverse relation was more important with small drinking quantities. We also subdivided the group according to drinking habit before as well as during pregnancy; in comparison with those drinking neither before nor during pregnancy, the strongest effect was found in those who went from not drinking before pregnancy to drinking during pregnancy (N = 35) (OR = 0.3; 95% CI = 0.1–0.7), while no association was found for those who drank before but not during pregnancy (N = 159) (OR = 0.8; 95% CI = 0.6–1.2). A moderately increased risk was found for paternal consumption of spirits in the month prior to pregnancy, and the risk associated with any type of alcohol increased with dose (likelihood ratio chi-square for trend = 5.93; P = 0.01).

The distribution of age and sex for cases included in the case-only study was similar to that of the complete case-control study (data not shown). Table 3 shows interaction odds ratios that resulted from comparing exposure between cases with and without a given genotype. The effect of exposure during the second and third trimesters of pregnancy was increased for the null GSTM1 genotype as well as with the CYP2E1*5 allelic variant but the latter was particularly increased during the nursing period (OR = 4.9; 95% CI = 1.4–16.6).

TABLE 2. Adjusted Odds Ratios (OR)* and 95% Confidence Intervals (CI) for the Association of Maternal and Paternal Exposure to Alcohol with Childhood Acute Lymphoblastic Leukemia in a Case-Control Study Including 491 Cases and 491 Healthy Controls

Exposure	Number of Exposed Cases/Controls	OR	95% CI
Maternal			
Any alcohol			
Before pregnancy†	254/268	0.8	0.6–1.1
First trimester	166/196	0.7	0.5–1.0
Second trimester	143/180	0.7	0.5–0.9
Third trimester	143/179	0.7	0.5–0.9
Pregnancy	180/217	0.7	0.5–0.9
Nursing period	46/71	0.5	0.3–0.8
Entire period‡	270/319	0.6	0.5–0.8
Wine			
Entire period‡	240/278	0.7	0.5–0.9
Pregnancy	155/187	0.7	0.5–0.9
Beer			
Entire period‡	126/163	0.6	0.5–0.9
Pregnancy	77/95	0.7	0.5–1.1
Spirits			
Entire period‡	101/102	0.9	0.7–1.3
Pregnancy	45/49	0.9	0.5–1.3
Any alcohol: entire period			
<1/day§	220/257	0.6	0.5–0.9
≥1/day	32/26	0.9	0.5–1.6
Any alcohol: pregnancy			
<1/day	151/174	0.7	0.5–1.0
≥1/day	20/18	0.8	0.5–1.6
Paternal§			
Any alcohol			
Wine	420/400	1.4	1.0–2.0
Wine	301/290	1.2	0.8–1.5
Beer	389/362	1.5	1.1–2.0
Spirits	220/178	1.5	1.1–1.9
Any alcohol			
<1/day	189/189	1.4	1.0–2.0
1–<3/day	143/120	1.6	1.1–2.5
≥3/day	79/63	1.7	1.1–2.7

* Adjusted for maternal age and level of schooling and matched for age and sex of the child.
 † Covers month before pregnancy.
 ‡ Covers from 1 month prior to pregnancy to nursing period; in drinks per day.
 § Covers the month prior to pregnancy.

Discussion

Our results suggesting that alcohol consumption during pregnancy reduce risk of leukemia in the offspring lead to speculations about the mechanisms involved. It has been established that babies born from mothers exposed to alcohol during pregnancy suffer from prenatal and postnatal growth retardation¹⁹; one postulated mechanism is through an inhibition of the growth hormone/insulin-like growth factor (IGF-1).²⁰ As a corollary, high levels of IGF-1 might produce a larger baby and contribute to leukemogenesis.¹ A number of studies have observed an association between high birthweight and leukemia.¹ Since the protective effect we observed seemed limited to wine and beer, it could perhaps be explained by the action of antioxidants (eg, flavonoid) present in red wine²¹ and hopped beer.²² Indeed the flavonoids, widespread in certain plants, may have potential cancer chemopreventive activity.²³

TABLE 3. Interaction Odds Ratios (IOR)* Between GSTM1 and CYP2E1 Genotypes and Maternal Consumption of Alcohol in Cases of Childhood Acute Lymphoblastic Leukemia

Gene: (nb of Normal (N) and Mutant Genotypes (M))	Period of Exposure	Number of Exposed Subjects in N and M	IOR	95% CI
GSTM1 null (66 N; 120 M)†	Before pregnancy‡	44/67	0.6	0.3–1.2
	First trimester	18/40	1.3	0.6–2.5
	Second trimester	9/32	2.3	1.0–5.1
	Third trimester	9/33	2.4	1.1–5.4
	Nursing period	8/13	0.8	0.3–2.2
CYP2E1*5 (169 N; 14 M)§	Before pregnancy‡	105/7	0.8	0.2–2.5
	First trimester	54/5	1.2	0.3–3.8
	Second trimester	37/6	2.8	0.9–8.6
	Third trimester	38/6	2.6	0.8–8.1
	Nursing period	18/5	4.9	1.4–16.6

* Adjusted for sex.

† M, homozygous for the deletion (null genotype); N, presence of at least one normal allele.

‡ Covers month prior to pregnancy.

§ M, carrier of at least one mutant allele; N, absence of the mutant allele.

Contrary to the observed effects for maternal alcohol consumption during pregnancy and the nursing period, paternal consumption of spirits prior to pregnancy was associated with an increased risk of ALL. Adverse effects resulting from male exposure to drugs or environmental chemicals include loss before or after implantation, physical malformations evident at birth, behavioral alterations, and a higher incidence of cancer.²⁴ However, data are too scarce to reach any conclusion on the nature of the effect of alcohol.²⁵ Mechanisms for male-mediated effects could be nongenetic (*eg*, due to the presence of a drug in the seminal fluid), genetic (*eg*, gene mutation or chromosomal abnormality), and epigenetic (*eg*, an effect on gene expression, genomic imprinting, or DNA methylation).^{24,26}

The relation among GSTM1 and CYP2E1 genetic polymorphisms, leukemia susceptibility, and exposure to alcohol is still speculative. Nevertheless, our findings have biological plausibility. GSTM1 is involved in the metabolism of free radicals and is expressed in fetal tissues.⁷ How the GSTM1 null genotype will influence this metabolism is not determined at this time, but it could result in a weaker antioxidant defense predisposing fetal tissues to oxidative stress with consequent DNA damage. CYP2E1 is an alcohol-inducible cytochrome P450. The role of the CYP2E1*5 allelic variant with respect to the metabolism of alcohol is not clearly established²⁷ but the associated higher expression could lead to increased mutational burden on the exposed fetus. Noteworthy, flavonoids were shown to be poor inhibitors of CYP2E1,²⁸ which could explain the lack of protective effect in a drinking mother with a child carrying the high expression allele.

Limitations of the case-control study must be addressed. Selection bias seems unlikely, given the re-

sponse rates. In addition, this study has the advantage of using concurrent controls. Misclassification of alcohol consumption is a concern. The literature suggests that except under circumstances of undue publicity about a disease and its potential cause, cases and controls both tend to underreport exposures in a similar fashion,²⁹ which would generally lead to a bias toward the null. Nevertheless, we cannot eliminate the possibility that mothers of cases underreported their drinking consumption more than control mothers; however, the fact that fathers of cases reported more drinking than fathers of controls would imply a differential recall attitude between mother and father, which seems difficult to explain.

We believe that the assumption for the validity of the IOR in the case-only study, namely that the exposure be independent of the genotype in the population, is unlikely to have been violated because exposure was that of the parents while the genotype was that of the child. This design is vulnerable to the population stratification bias³⁰; however, over 90% of subjects were of French-Canadian descent and 95% were of European descent, thus substantially minimizing the potential for this bias.³¹ An alternative explanation for our results that we cannot rule out is that the studied genes may be only indirectly involved in causing the disease by being in linkage disequilibrium with a causal gene.

In conclusion, this study suggested protective effects for maternal consumption of alcohol (wine and beer) during pregnancy and the nursing period; in addition a moderately increased risk was shown for paternal pre-conceptional consumption of spirits. The presence of variants in GSTM1 and CYP2E1 could modify the risk associated with prenatal exposure. These latter results are only exploratory and will need confirmation.

References

1. Little J. Epidemiology of childhood cancer. No. 149. Lyon: IARC publications; 1999.
2. Petridou E, Trichopoulos D, Kalapothaki V, *et al.* The risk profile of childhood leukemia in Greece: a nationwide case-control study. *Br J Cancer* 1997;76:1241–1247.
3. Schüz J, Kaatsch P, Kaletsch U, Meinert R, Michaelis J. Association of childhood cancer with factors related to pregnancy. *Int J Epidemiol* 1999;28:631–639.
4. Henderson GI, Chen JJ, Schenker S. Ethanol, oxidative stress, reactive aldehydes, and the fetus. *Front Biosci* 1999;4:541–545.
5. Knapen MFCM, Zusterzeel PLM, Peters WHM, Steegers EAP. Glutathione and glutathione-related enzymes in reproduction. A review. *Eur J Obstet Gynecol Reprod Biol* 1999;82:171–184.
6. Seldman MD, Quirk WS, Shirwany NA. Reactive oxygen metabolites, antioxidants and head and neck cancer. *Head Neck* 1999; 21:467–469.
7. Smith G, Stanley LA, Sim E, Strange RC, Wolf CR. Metabolic polymorphisms and cancer susceptibility. *Cancer Surv* 1995;25:27–65.

8. Wormhoudt LW, Commandeur JNM, Vermeulen NPE. Genetic polymorphisms of human N-acetyltransferase, cytochrome P450, glutathione-S-transferase, and epoxide hydrolases enzymes: relevance to xenobiotic metabolism and toxicity. *Crit Rev Toxicol* 1999;29:59–124.
9. Tsutsumi M, Takada A, Wang JS. Genetic polymorphisms of cytochrome P4502E1 related to the development of alcoholic liver disease. *Gastroenterology* 1994;107:1430–1435.
10. Watanabe J, Hayashi S, Kawajiri K. Different regulation and expression of the human CYP2E1 gene due to the *RsaI* polymorphism in the 5'-flanking region. *J Biochem* 1994;116:321–326.
11. Stubbins MJ, Wolf CR. Additional polymorphisms and cancer. In: Vineis P, Malats N, Lang M, d'Errico A, Caporaso N, Cuzick J, Boffetta P, eds. *Metabolic Polymorphisms and Susceptibility to Cancer*. Lyon: IARC Scientific Publications; No. 148, 1999:271–302.
12. Krajcinovic M, Labuda D, Richer C, Karimi S, Sinnett D. Susceptibility to childhood acute lymphoblastic leukemia: influence of CYP1A1, CYP2D6, GSTM1, and GSTT1 genetic polymorphisms. *Blood* 1999;93:1496–1501.
13. Krajcinovic M, Sinnett H, Richer C, Labuda D, Sinnett D. Role of NQO1, MPO and CYP2E1 genetic polymorphisms in the susceptibility to childhood acute lymphoblastic leukemia. *Int J Cancer* 2002;97:230–236.
14. Infante-Rivard C, Fortier I, Olson E. Markers of infection, breastfeeding and childhood acute lymphoblastic leukemia. *Br J Cancer* 2000;83:1555–1564.
15. Khoury MJ, Flanders WD. Nontraditional epidemiologic approaches in the analysis of gene-environment interaction: case-control studies with no controls. *Am J Epidemiol* 1996;144:207–213.
16. Sinnett D, Krajcinovic M, Labuda D. Genetic susceptibility to childhood lymphoblastic leukemia. *Leuk Lymphoma* 2000;38:447–462.
17. Baccichet A, Benachenhon N, Couture F, Leclerc JM, Sinnett D. Microsatellite instability in childhood T cell acute lymphoblastic leukemia. *Leukemia* 1997;11:797–802.
18. Labuda D, Krajcinovic M, Richer C, Skoll A, Sinnett H, Yotova V, Sinnett D. Rapid detection of CYP1A1, CYP2D6, and NAT variants by multiplex polymerase chain reaction and allele-specific oligonucleotide assay. *Analytical Biochem* 1999;275:84–92.
19. Forrest F, DuFlorey CV, Taylor D. Maternal ethanol consumption and child development. *Int J Epidemiol* 1992;21(suppl):S17–S23.
20. Conway S, Swain R. Somatostatin-stimulated growth hormone-releasing factor secretion *in vitro* is modified by fetal ethanol exposure. *Alcohol Clin Exp Res* 1997;21:703–709.
21. Puddey IB, Croft KD. Alcohol, stroke and coronary heart disease. Are there anti-oxidants and pro-oxidants in alcoholic beverages that might influence the development of atherosclerotic cardiovascular disease? *Neuroepidemiol* 1999;18:292–302.
22. Yilmazer M, Stevens JF, Buhler DR. *In vitro* glucuronidation of xanthohumol, a flavonoid in hop and beer, by rat and human liver microsomes. *FEBS Lett* 2001;491:252–256.
23. Siess MH, LeBon AM, Canivenc-Lavier MC, Suschetet M. Mechanisms involved in the chemoprevention of flavonoids. *Biofactors* 2000;12:193–199.
24. Robaire B, Hales BP. Paternal exposure to chemicals before conception. *BMJ* 1993;307:341–342.
25. Little J, Vainio H. Mutagenic lifestyles? A review of evidence of associations between germ-cell mutations in humans and smoking, alcohol consumption and use of “recreational” drugs. *Mut Res* 1994;313:131–151.
26. Trasler J, Doersken T. Teratogen update: paternal exposures-reproductive risks. *Teratology* 1999;60:161–172.
27. Carriere V, Berthou F, Baird S, Belloc C, Beaune P, de Waziers I. Human cytochrome P450 2E1 (CYP2E1): from genotype to phenotype. *Pharmacogenetics* 1996;6:203–211.
28. Henderson MC, Miranda CL, Stevens JF, Deinzer ML, Nuhler DR. *In vitro* inhibition of human P450 enzymes by prenylated flavonoids from hops, *Humulus lupulus*. *Xenobiotica* 2000;30:235–251.
29. Infante-Rivard C, Jacques L. Empirical study of parental recall bias. *Am J Epidemiol* 2000;152:480–486.
30. Weinberg C, Umbach DM. Choosing a retrospective design to assess joint genetic and environmental contributions to risk. *Am J Epidemiol* 2000;152:197–203.
31. Wacholder S, Rothman N, Caparoso N. Population stratification in epidemiologic studies of common genetic variants and cancer: quantification of bias. *J Natl Cancer Inst* 2000;92:1151–1158.

Unauthorized Use
Prohibited