Parental Exposure to Medications and Hydrocarbons and *ras* Mutations in Children with Acute Lymphoblastic Leukemia: A Report from the Children's Oncology Group

Xiao Ou Shu,[1] John P. Perentesis,[2] Wanqing Wen,[1] Jonathan D. Buckley,[3,4] Evelyn Boyle,[5] Julie A. Ross,[2] and Leslie L. Robison[2]

[1]Vanderbilt-Ingram Cancer Center and Vanderbilt Center for Health Services Research, Vanderbilt University, Nashville, Tennessee; [2]Department of Pediatrics, University of Minnesota, Minneapolis, Minnesota; [3]University of Southern California, Los Angeles, California; [4]Children's Oncology Group, Arcadia, California; and [5]South Carolina Cancer Center, Columbia, South Carolina

Abstract

***Ras* proto-oncogene mutations have been implicated in the pathogenesis of many malignancies, including leukemia. While both human and animal studies have linked several chemical carcinogens to specific *ras* mutations, little data exist regarding the association of *ras* mutations with parental exposures and risk of childhood leukemia. Using data from a large case-control study of childhood acute lymphoblastic leukemia (ALL; age <15 years) conducted by the Children's Cancer Group, we used a case-case comparison approach to examine whether reported parental exposure to hydrocarbons at work or use of specific medications are related to *ras* gene mutations in the leukemia cells of children with ALL. DNA was extracted from archived bone marrow slides or cryopreserved marrow samples for 837 ALL cases. We examined mutations in K-*ras* and N-*ras* genes at codons 12, 13, and 61 by PCR and allele-specific oligonucleotide hybridization and confirmed them by DNA sequencing. We interviewed mothers and, if available, fathers by telephone to collect exposure information. Odds ratios (ORs) and 95% confidence intervals (CIs) were derived from logistic regression to examine the association of parental exposures with *ras* mutations. A total of 127 (15.2%) cases had *ras* mutations (K-*ras* 4.7% and N-*ras* 10.68%). Both maternal (OR 3.2, 95% CI 1.7-6.1) and paternal (OR 2.0, 95% CI 1.1-3.7) reported use of mind-altering drugs were associated with N-*ras* mutations. Paternal use of amphetamines or diet pills was associated with N-*ras* mutations (OR 4.1, 95% CI 1.1-15.0); no association was observed with maternal use. Maternal exposure to solvents (OR 3.1, 95% CI 1.0-9.7) and plastic materials (OR 6.9, 95% CI 1.2-39.7) during pregnancy and plastic materials after pregnancy (OR 8.3, 95% CI 1.4-48.8) were related to K-*ras* mutation. Maternal ever exposure to oil and coal products before case diagnosis (OR 2.3, 95% CI 1.1-4.8) and during the postnatal period (OR 2.2, 95% CI 1.0-5.5) and paternal exposure to plastic materials before index pregnancy (OR 2.4, 95% CI 1.1-5.1) and other hydrocarbons during the postnatal period (OR 1.8, 95% CI 1.0-1.3) were associated with N-*ras* mutations. This study suggests that parental exposure to specific chemicals may be associated with distinct *ras* mutations in children who develop ALL.** (Cancer Epidemiol Biomarkers Prev 2004;13(7): 1230–5)

Introduction

The *ras* proto-oncogene consists of three genes: H-*ras*, K-*ras*, and N-*ras*. The small protein products (p21) of *ras* genes are highly homologous and are in the inner plasma membrane surface; they transmit proliferative signals from cytokines and growth factors to the nucleus (1). Five domains of the *ras* p21 protein are highly conserved throughout the G-protein superfamily and are essential for GTP binding and hydrolysis. *Ras* proto-oncogene mutations have been implicated in the pathogenesis of many malignancies, with frequency of mutation varying from 95% in pancreatic cancer to 5% in breast cancer (2). The frequency of *ras* mutations is reported to be between 5% and 20% of patients with acute lymphoblastic leukemia (ALL; refs. 3-11). These mutations result in a constitutively active *ras* protein, which disrupts the normally tightly regulated *ras*-dependent signal transduction pathways (2). The most common *ras* mutations are found in codons 12, 13, and 61 and result in a continuously activated *ras* protein that can autonomously stimulate cell growth or differentiation (2).

A variety of physical and chemical carcinogens have been shown to induce *ras* mutations in both human and animal tumor models (e.g., K-*ras* mutations are associated with methylnitrosourea, cigarette smoke, and organochlorines; refs. 12, 13). We conducted a case-case study of 837 childhood (age <15 years) ALL cases who

Received 7/7/03; revised 2/11/04; accepted 2/18/04.

Grant support: NIH grants CA 48051 and ES 07819 and University of Minnesota Children's Cancer Research Fund. Participating Children's Cancer Group investigators, institutions, and grant numbers (Division of Cancer Treatment, National Cancer Institute) are provided in the Appendix.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Note: J.P. Perentesis is currently at the Cincinnati Children's Hospital Medical Center, Cincinnati, OH.

Requests for reprints: Xiao Ou Shu, Children's Oncology Group, P.O. Box 60012, Arcadia, CA 91066-6012. Phone: 626-447-0064, ext. 130; Fax: 626-445-4334. E-mail: Xiao-Ou.Shu@vanderbilt.edu

participated in a large case-control study of ALL to examine whether parental occupational exposures and use of specific medications, factors that we previously found to be associated with an increased risk of childhood leukemia, are related to *ras* gene mutation (14, 15).

Patients and Methods

Cases were identified from Children's Cancer Group institutions and have been described in detail elsewhere (14-16). Briefly, the Children's Cancer Group was one of two cooperative clinical trial groups in the 1990s that cared for about 93% of pediatric cancer patients in the United States. Nearly 50% of childhood leukemias in the United States were treated by a Children's Cancer Group hospital or institution (17). During the study recruitment period, there were 120 Children's Cancer Group institutions; 108 participated in the current study. Eligible cases were children ages ≤14 years who had new ALL diagnoses between January 1, 1989 and June 15, 1993. Additional eligibility criteria included a telephone in the patient's residence and availability of the biological mother for a telephone interview. A total of 2,081 eligible cases were identified, and 1,914 mothers were interviewed (92%). Among the 167 nonrespondents were 41 (2.0%) physician refusals, 70 (3.4%) parental refusals, and 18 (0.9%) lost to follow-up after first contact; 38 (1.8%) nonrespondents fell into other miscellaneous categories.

Exposure information was collected by independent telephone interviews using structured questionnaires with mothers and, whenever available, fathers of cases. The mother's questionnaire included questions about demographics, maternal disease history, medication use, occupation, personal habits, household exposure before and during the index pregnancy and birth, reproductive and family medical history as well as the child's disease history, medication use, and exposure to environmental hazards (e.g., pesticides and insecticides). The father's questionnaire focused on medication use, personal habits, household exposures, occupational history, and family medical history. The father's questionnaire was completed for 1,801 of the 2,081 eligible cases (86.5%): 83.4% directly by fathers and 26.6% by mothers as the surrogate. Only information obtained directly from the father was included in the paternal exposure analysis.

The current *ras* study was initiated in 1996, 3 years after the survey interview for the parent study was completed. Diagnostic bone marrow slides or cryopreserved marrow samples for 837 ALL cases who had participated in the case-control study were available from the original diagnostic institutions or from the Children's Cancer Group reference laboratory. Of the 1,914 interviewed cases, we obtained specimens from 837 subjects.

DNA samples from the 837 ALL cases were extracted from archival bone marrow slides or cryopreserved marrow samples as described previously (18). DNA cells were scraped from the slide with a scalpel using a sterile technique to prevent contamination. Cells were suspended in PCR buffer [50 mmol/L KCl, 10 mmol/L Tris-HCl (pH 9.0), and 1% Triton X-100], boiled for 10 minutes, extracted twice with phenol, and precipitated with ethanol. DNA was washed once with 70% ethanol, resuspended in Tris-EDTA buffer, and amplified by PCR.

We examined mutations in K-*ras* and N-*ras* genes at codons 12, 13, and 61 by PCR and allele-specific oligonucleotide hybridization and confirmed them by DNA sequencing. PCR of N-*ras* and K-*ras* exon gene fragments was done in a thermocycler (Perkin-Elmer Corp., Norwalk, CT) with ~200 ng DNA employing standard conditions in 100 μL total reaction volume. The PCR reaction buffer contained 50 mmol/L KCl, 10 mmol/L Tris-HCl (pH 8.3), 1.5 mmol/L $MgCl_2$, 0.001% (w/v) gelatin (Sigma Chemical Co., St. Louis, MO), 200 μmol/L deoxynucleotide triphosphates (Boehringer Mannheim, Mannheim, Germany), and 2.5 units AmpliTaq (Perkin-Elmer). We amplified sequences surrounding N-*ras* and K-*ras* codons 12, 13, and 61 using the following protocol: cycle 1, 94°C for 5 minutes, 55°C for 1 minute, and 72°C for 1 minute; cycles 2 to 35, 94°C for 1 minute, 55°C for 1 minute, and 72°C for 1 minute. The oligonucleotide primers were used at a concentration of 0.5 μmol/L. Each PCR amplification of the *ras* genes exon gene fragments from patient samples included positive and negative controls, and the efficiency and specificity of each amplification were assessed by analyzing the products after electrophoresis on a 3% NuSieve (FMC, Rockland, ME)-1% agarose gel and ethidium bromide staining. Studies in our laboratory had demonstrated that direct DNA sequencing of amplified *ras* exon fragments was sensitive enough to detect mutation in samples with >50% blasts (data not shown). Samples with <50% blasts were screened by single-strand conformational polymorphism at 4°C and 22°C. Bands with abnormal migration on single-strand conformational polymorphism were excised and subjected to PCR amplification with nested primers and analyzed with subsequent direct DNA sequencing. The DNA of *ras* mutation-positive samples had an independent second PCR amplification and repeat DNA sequencing to confirm the *ras* gene mutation sequence.

Case-case comparisons were applied in the statistical analyses. Parental occupational exposure and medication use among cases with any *ras* mutation, cases with any K-*ras* mutation, or cases with any N-*ras* mutation were compared with cases without any *ras* mutation. Odds ratios (ORs), as approximations of relative risk, were used to measure the association between exposures and *ras* mutations. Unconditional logistic regression was employed in the data analyses to obtain ORs and 95% confidence intervals (CIs) with adjustment for family income, parental race, education, and age and the child's age and sex.

Results

A total of 127 (15.2%) ALL cases were found to have a K-*ras* (4.7%) or N-*ras* (10.6%) mutation in their diagnostic bone marrow specimens; two cases had both mutations. The most frequent *ras* mutations were found in codon 12 or 13, exon 1 of K-*ras* (95%) and N-*ras* genes (90%), most of which involved a G:C to A:T transition (78%). Cases with *ras* mutations, especially those with a K-*ras* mutation, were more likely to be younger at diagnosis than *ras* mutation-negative cases. Cases with K-*ras* mutations

(30%) were more likely to be <2 years old than were *ras*-negative cases (10%). There were more early B-cell ALL and fewer T-cell ALL among cases with any *ras* mutation. N-*ras* mutation cases appeared less likely to have pre–B-cell ALL. ALL cases with a *ras* mutation did not differ significantly from those without *ras* mutation by sex, family income, and parental race and age. Subsequent analyses, however, adjusted for age and the above-mentioned demographic variables because they were confounders in our earlier publications on parental occupational exposure and medication use with childhood ALL (Table 1; refs. 14, 15).

Table 2 presents the association between maternal occupational exposure and *ras* mutation. K-*ras* mutations were associated with maternal exposure to solvents, degreasers, or cleaning agents (OR 3.1, 95% CI 1.0-9.7) and plastic materials (OR 6.9, 95% CI 1.2-39.7) during pregnancy and plastic materials after pregnancy (OR 8.3, 95% CI 1.4-48.8). Maternal ever exposure to oil or coal products before case diagnosis or during the postnatal period was associated with a significantly elevated risk of N-*ras* mutation (OR 2.3), although risk estimates of N-*ras* mutation were also not significantly elevated among ALL cases whose mothers reported exposure to these chemicals before and during pregnancy with the index child.

Associations between paternal exposure to hydrocarbons and *ras* mutation are summarized in Table 3. Paternal exposure to plastic materials before pregnancy (OR 2.4, 95% CI 1.1-5.1) was related to N-*ras* mutations. Paternal exposure to miscellaneous hydrocarbons (including epoxy resins, formaldehyde, glues, exhaust, fuels, cooking oils, thermal decomposition products, glycols, ethylene oxide, polycyclic aromatic hydrocarbons, and alcohol) during the child's postnatal period was related to a significantly increased risk of N-*ras* mutations (OR 1.8, 95% CI 1.0-3.1).

Table 4 presents the association of selected parental medication use and *ras* mutation among ALL cases. Reported maternal use of mind-altering drugs including marijuana, lysergic acid diethylamide, and cocaine was associated with N-*ras* mutations (OR 3.2, 95% CI 1.7-6.1) and K-*ras* mutations (OR 2.4, 95% CI 0.9-6.7). Paternal use of amphetamines or diet pills was associated with N-*ras* mutations (OR 4.1, 95% CI 1.1-15.0) and not significantly with K-*ras* mutations (OR 2.4, 95% CI 0.3-22.7). Paternal use of mind-altering drugs was associated with an elevated risk of N-*ras* mutations (OR 2.0, 95% CI 1.1-3.7) and not significantly related to K-*ras* mutations (OR 1.6, 95% CI 0.6-4.7).

Discussion

In this study, we found that 15.2% of childhood ALL cases had a *ras* mutation in their leukemia cells; N-*ras* (10.6%) was twice as likely to be affected. *Ras* mutations occurred more often among ALL cases who were <2

Table 1. Demographics of ALL cases by *ras* mutation

	Ras Negative	K-*ras* and/or N-*ras* Mutation		K-*ras* Mutation		N-*ras* Mutation	
	n = 710	*n* = 127	*P*	*n* = 40	*P*	*n* = 89	*P*
Age (y)							
0-1	74 (10.4)	21 (16.5)	0.190	12 (30.0)	<0.001	10 (11.2)	1.0
2-5	391 (55.1)	70 (55.1)		22 (55.0)		49 (55.1)	
6-10	163 (23.0)	24 (18.9)		4 (10.0)		20 (22.5)	
>10	82 (11.5)	12 (9.4)		2 (5.0)		10 (11.2)	
Sex							
Male	395 (55.6)	66 (52.0)	0.444	21 (52.5)	0.698	47 (52.8)	0.61
Female	315 (44.4)	61 (48.0)		19 (47.5)		42 (47.2)	
Immunophenotype							
T-cell	92 (13.0)	5 (3.9)	0.016	1 (2.5)	0.159	4 (4.5)	0.04
Early B-cell	431 (60.7)	92 (72.4)		29 (72.5)		64 (71.9)	
Pre–B-cell	82 (11.5)	12 (9.4)		6 (15.0)		6 (6.7)	
Unknown	105 (14.8)	18 (14.2)		4 (10.0)		15 (16.9)	
Maternal race							
White	615 (86.6)	106 (83.5)	0.343	32 (80.0)	0.237	76 (85.4)	0.75
Non-White	95 (13.4)	21 (16.5)		8 (20.0)		13 (14.6)	
Paternal race*							
White	499 (88.0)	97 (90.7)	0.432	29 (90.6)	0.656	70 (90.9)	0.46
Non-White	68 (12.0)	10 (9.3)		3 (9.4)		7 (9.1)	
Family income							
<20,000	241 (33.9)	36 (28.3)	0.140	12 (30.0)	0.698	25 (28.1)	0.14
20,000-39,999	297 (41.8)	50 (39.4)		16 (40.0)		34 (38.2)	
≥40,000	172 (24.2)	41 (32.3)		12 (30.0)		30 (33.7)	
Maternal age	26.90 ± 5.35	26.83 ± 5.73	0.890	27.33 ± 5.52	0.624	26.60 ± 5.83	0.62
Paternal age	29.61 ± 5.83	28.59 ± 5.34	0.092	30.31 ± 5.82	0.508	27.88 ± 4.97	0.01

*Among fathers interviewed directly; paternal information supplied by mothers is not included.

Table 2. Maternal occupational exposure and *ras* mutation among childhood ALL cases

Maternal Occupational Exposure	Exposure Period	K-*ras* Mutation			N-*ras* Mutation	
		−Cases (n = 710)	+Cases (n = 40)	Adjusted OR (95% CI)	+Cases (n = 89)	Adjusted OR (95% CI)
Solvents, degreasers, or cleaning agents	Any time	57	5	1.8 (0.6-4.8)	8	1.2 (0.5-2.5)
	Before pregnancy	42	4	2.0 (0.7-6.3)	6	1.2 (0.5-2.8)
	During pregnancy	30	4	3.1 (1.0-9.7)	4	1.0 (0.4-3.1)
	After pregnancy	35	0		5	1.2 (0.4-3.1)
Plastic materials	Any time	7	2	4.5 (0.9-24.0)	1	1.1 (0.1-9.3)
	Before pregnancy	6	2	4.9 (0.9-26.5)	0	
	During pregnancy	5	2	6.9 (1.2-39.7)	0	
	After pregnancy	5	2	8.3 (1.4-48.8)	1	1.7 (0.2-14.8)
Paints or thinners	Any time	56	4	1.3 (0.4-3.9)	7	1.0 (0.4-2.2)
	Before pregnancy	29	2	1.0 (0.2-4.6)	6	1.6 (0.6-4.1)
	During pregnancy	33	2	1.0 (0.2-4.4)	6	1.5 (0.6-3.6)
	After pregnancy	43	3	1.4 (0.4-4.9)	6	1.1 (0.4-2.7)
Oil or coal products	Any time	39	1	0.5 (0.1-4.0)	10	2.3 (1.1-4.8)
	Before pregnancy	22	0		6	2.3 (0.9-5.9)
	During pregnancy	17	0		4	1.9 (0.6-5.9)
	After pregnancy	27	1	0.7 (0.1-5.7)	7	2.3 (1.0-5.5)
Other hydrocarbons	Any time	85	4	0.9 (0.3-2.6)	8	0.8 (0.3-1.6)
	Before pregnancy	57	4	1.3 (0.4-3.8)	6	0.9 (0.4-2.1)
	During pregnancy	45	2	0.8 (0.2-3.8)	6	1.1 (0.5-2.7)
	After pregnancy	58	1	0.3 (0.1-2.6)	6	0.9 (0.4-2.1)
Any hydrocarbons	Any time	153	9	1.2 (0.5-2.6)	19	1.0 (0.6-1.7)
	Before pregnancy	100	8	1.5 (0.7-3.5)	16	1.3 (0.7-2.4)
	During pregnancy	86	7	1.6 (0.6-3.8)	12	1.1 (0.6-2.2)
	After pregnancy	113	5	0.9 (0.3-2.4)	13	0.9 (0.5-1.7)

NOTE: Adjusted for maternal race, education, age, family income, and child's age and sex.

years old at diagnosis and among those with B-lineage leukemia. N-*ras* mutations were associated with maternal ever exposure to oil and coal products before case diagnosis or during pregnancy, paternal exposure to plastic materials before index pregnancy or other hydrocarbons during the postnatal period, and parental use of mind-altering drugs. K-*ras* mutations were associated with maternal exposure to solvents and plastic materials during pregnancy and plastic materials after pregnancy and paternal use of amphetamines or diet pills.

It has been recognized that human carcinogenesis results from mutations in a series of critical genes that control cell proliferation, differentiation, and programmed cell death. *Ras* mutations are one of the most common genetic alterations found in human malignancies (2, 19). *Ras* genes mutation result in constitutively active *ras* protein, which disrupts the *ras*-dependent signal transduction pathways, stimulating tumor growth and/or modulating the immune response against the tumor cells (2). Although the specific mechanisms of *ras* mutations remain unknown, epidemiologic and animal studies have suggested that specific point mutations in *ras* genes can be induced by certain chemical carcinogens. In a murine model for lymphohematopoietic neoplasia, exposure to *N*-methyl-*N*-nitrosurea has been shown to result in a predominance of K-*ras* activating mutations at codon 12 with a consistent G:C to A:T transition (20). Studies of human adenocarcinoma of the lung have observed K-*ras* mutations in 30% of patients with a history of smoking, while <5% of nonsmokers carry the mutation (21). In a study of 62 adult acute myeloid leukemia cases and 630 healthy control subjects, Taylor et al. (22) found that *ras*-positive cases were about twice as likely to have been employed in occupations associated with an increased leukemia risk compared with *ras*-negative acute myeloid leukemia cases or healthy controls. Another study found aspartic acid mutations in codon 13 of K-*ras* gene in vinyl chloride–induced liver cancer and an elevation of serum *ras* protein p21 among liver cancer patients and workers exposed to vinyl chloride but not among healthy nonexposed controls (23). Serum concentrations of organochlorine compounds have been found to be related to glycine to valine substitution at codon 12 of K-*ras* gene in pancreatic cancer patients (12). In the current study, we found that certain parental exposures were related to N-*ras* or K-*ras* mutations at exon 1, mainly involving G:C to A:T transition.

Reports from previous studies have shown *ras* mutations, mainly N-*ras*, in 5% to 20% of ALL patients (3-11). However, these earlier studies have several limitations: most included only patients from a single institution, had small sample sizes (14 to 150 subjects) to characterize *ras*-positive patients accurately, and lacked environmental exposure information. The current study contains the largest series of childhood ALL cases who have been evaluated for the *ras* mutation and confirmed by DNA sequencing. Therefore, our data represent the

Table 3. Paternal occupation exposure and *ras* mutation among childhood ALL cases

Paternal Occupational Exposure	Exposure Period	K-*ras* Mutation			N-*ras* Mutation	
		−Cases (n = 567)	+Cases (n = 32)	Adjusted OR (95% CI)	+Cases (n = 77)	Adjusted OR (95% CI)
Solvents, degreasers, or cleaning agents	Any time	228	9	0.6 (0.3-1.4)	33	0.9 (0.6-1.6)
	Before pregnancy	190	8	0.6 (0.3-1.5)	26	0.9 (0.5-1.5)
	During pregnancy	106	2	0.3 (0.1-1.2)	15	0.8 (0.4-1.5)
	After pregnancy	126	3	0.4 (0.1-1.4)	15	0.6 (0.3-1.1)
Plastic materials	Any time	54	2	0.8 (0.2-3.4)	12	1.7 (0.9-3.5)
	Before pregnancy	39	2	0.9 (0.2-4.3)	11	2.4 (1.1-5.1)
	During pregnancy	21	2	2.0 (0.4-10.0)	6	2.2 (0.8-6.0)
	After pregnancy	30	2	1.5 (0.3-7.1)	8	2.2 (0.9-5.1)
Paints or thinners	Any time	235	13	1.0 (0.5-2.1)	37	1.2 (0.8-2.0)
	Before pregnancy	182	12	1.2 (0.6-2.7)	25	1.0 (0.6-1.7)
	During pregnancy	102	7	1.3 (0.5-3.3)	12	0.7 (0.4-1.5)
	After pregnancy	131	8	1.2 (0.5-3.0)	21	1.1 (0.6-2.0)
Oil or coal products	Any time	300	16	0.7 (0.3-1.7)	42	0.9 (0.5-1.5)
	Before pregnancy	254	15	1.0 (0.5-2.3)	40	1.2 (0.7-2.0)
	During pregnancy	155	9	0.9 (0.4-2.3)	25	1.1 (0.6-1.9)
	After pregnancy	199	10	0.8 (0.3-1.8)	27	0.8 (0.5-1.4)
Other hydrocarbons	Any time	205	7	0.5 (0.2-1.2)	35	1.3 (0.8-2.2)
	Before pregnancy	168	7	0.7 (0.3-1.6)	27	1.2 (0.7-2.0)
	During pregnancy	97	2	0.3 (0.1-1.1)	19	1.5 (0.8-2.6)
	After pregnancy	119	2	0.2 (0.1-0.9)	26	1.8 (1.0-3.1)
Any hydrocarbons	Any time	410	19	0.5 (0.2-1.2)	61	1.3 (0.7-2.4)
	Before pregnancy	377	17	0.5 (0.2-1.1)	55	1.1 (0.6-2.0)
	During pregnancy	255	14	0.8 (0.4-1.8)	39	1.1 (0.6-1.8)
	After pregnancy	301	15	0.7 (0.3-1.5)	45	1.0 (0.6-1.7)

most accurate assessment of *ras* mutation among ALL patients thus far. The extensive exposure information collected in the current study allowed us to evaluate whether *ras* mutation is related to particular environmental exposures.

Parental occupational exposure to hydrocarbons, such as chlorinated solvents, benzene, or paints, has been linked previously to elevated childhood leukemia risk (24, 25). In earlier reports from the parent study (14, 15), we found that maternal exposure to solvents, paints, or thinners (preconception and during pregnancy) and plastic materials (postnatal) were related to an increased risk of childhood ALL. Paternal exposure to plastic materials before conception was also associated with

Table 4. Parental medication exposure and *ras* mutation among childhood ALL cases

Parental Medication Exposure	K-*ras* Mutation			N-*ras* Mutation	
	−Cases	+Cases	Adjusted OR (95% CI)	+Cases	Adjusted OR (95% CI)
Mothers (n)	710	40		89	
Amphetamines or diet pills*	38	1	0.6 (0.1-4.2)	7	1.5 (0.6 -3.5)
Vitamins†	618	36	1.2 (0.4-3.5)	78	1.0 (0.5-2.0)
Iron supplements†	220	18	1.9 (1.0-3.7)	27	1.0 (0.6-1.6)
Antihistamines or allergy remedies*	83	2	0.4 (0.1-1.6)	11	1.0 (0.5-2.0)
Mind-altering drugs*	45	5	2.4 (0.9-6.7)	15	3.2 (1.7-6.1)
Fathers	567	32		77	
Amphetamines or diet pills	8	1	2.4 (0.3-22.7)	4	4.1 (1.1-15.0)
Vitamins	147	11	1.5 (0.7-3.3)	23	1.2 (0.7-2.0)
Antihistamines	75	4	0.9 (0.3-2.9)	9	0.8 (0.4-1.7)
Mind-altering drugs	68	5	1.6 (0.6-4.7)	18	2.0 (1.1-3.7)

*Including those used only during 1 year before the index pregnancy, those used both before and during the index pregnancy, and those used only during the index pregnancy.
†Including those used only during the index pregnancy, excluding those used during 1 year before the index pregnancy, and those used both before and during the index pregnancy.

ALL. Parental use of amphetamines, diet pills, or mind-altering drugs before and during the index pregnancy was also found to be related to an increased childhood ALL risk, particularly among children <1 year old at diagnosis. The current study found some of these previously identified high-risk chemical exposures to be associated with *ras* mutations, thus providing additional evidence that parental exposure to these agents affected ALL risk in offspring. We found that ALL cases with *ras* mutations were more likely to be younger at diagnosis, and the association with parental use of amphetamines, diet pills, or mind-altering drugs was confined to young children. This may suggest that *ras* mutations occurred during pregnancy or preconceptionally in germ cells.

This analysis employed a case-case comparison study design. The advantage of this study approach over a case-control design is that it reduces recall bias, where case parents may recall higher exposure levels than control parents. In this study, it is unlikely that a case parent of a child with a *ras* mutation would recall exposures differently than a case parent of a child without *ras* mutations. However, *ras* mutations were only evaluated in 837 of 1,914 (44%) ALL cases included in the parent study, which substantially reduced the statistical power. This case-case study is limited by its inability to identify risk factors shared by *ras* mutation positive and negative cases (i.e., risk factors that can induce both *ras* mutation and other gene mutations could have been missed with this type of study design). Therefore, the association between *ras* mutations and parental chemical exposure could be underestimated in this study.

In summary, we found that parental occupational exposure to hydrocarbons and mind-altering drugs, chemicals that have been previously suggested to increase the risk of childhood leukemia, were related to specific *ras* mutations in childhood ALL.

References

1. Barbacid M. *Ras* genes. Annu Rev Biochem 1987;56:779-827.
2. Weijzen S, Velders MP, Kast WM. Modulation of the immune response and tumor growth by activated *Ras*. Leukemia 1999;13:502-13.
3. Clementino NC, Yamamoto M, Viana MB, et al. Lack of association between N-*ras* gene mutations and clinical prognosis in Brazilian children with acute lymphoblastic leukemia. Leuk Lymphoma 2001; 42:473-9.
4. Yokota S, Nakao M, Horiike S, et al. Mutational analysis of the N-*ras* gene in acute lymphoblastic leukemia: a study of 125 Japanese pediatric cases. Int J Hematol 1998;67:379-87.
5. Kawamura M, Ohnishi H, Guo SX, et al. Alterations of the p53, p21, p16, p15 and *RAS* genes in childhood T-cell acute lymphoblastic leukemia. Leuk Res 1999;23:115-26.
6. Rodenhuis S, Bos JL, Slater RM, Behrendt H, van't Veer M, Smets LA. Absence of oncogene amplifications and occasional activation of N-*ras* in lymphoblastic leukemia of childhood. Blood 1986;67: 1698-704.
7. Terada N, Miyoshi J, Kawa-Ha K, et al. Alteration of N-*ras* gene mutation after relapse in acute lymphoblastic leukemia. Blood 1990; 75:453-7.
8. Senn HP, Tran-Thang C, Wodnar-Filipowicz A, et al. Mutation analysis of the N-*ras* proto-oncogene in active and remission phase of human acute leukemias. Int J Cancer 1988;41:59-64.
9. Lubbert M, Mirro J Jr, Miller CW, et al. N-*ras* gene point mutations in childhood acute lymphocytic leukemia correlate with a poor prognosis. Blood 1990;75:1163-9.
10. Neri A, Knowles DM, Greco A, McCormick F, Dalla-Favera R. Analysis of *RAS* oncogene mutations in human lymphoid malignancies. Proc Natl Acad Sci USA 1988;85:9268-72.
11. Browett PJ, Norton JD. Analysis of *ras* gene mutations and methylation state in human leukemias. Oncogene 1989;4:1029-36.
12. Porta M, Malats N, Jariod M, et al. Serum concentrations of organochlorine compounds and K-*ras* mutations in exocrine pancreatic cancer. PANKRAS II Study Group. Lancet 1999;354:2125-9.
13. Mangues R, Pellicer A. *Ras* activation in experimental carcinogenesis. Semin Cancer Biol 1992;3:229-39.
14. Shu XO, Steward P, Wen WQ, et al. Parental occupational exposure to hydrocarbons and risk of acute lymphocytic leukemia in offspring. Cancer Epidemiol Biomarkers & Prev 1999;8:783-91.
15. Wen WQ, Shu XO, Potter JD, et al. Parental medication use and risk of childhood acute lymphoblastic leukemia. Cancer 2002;95:1786-94.
16. Robison LL, Buckley JD, Bunin G. Assessment of environmental and genetic factors in the etiology of childhood cancers: the Children's Cancer Group epidemiology program. Environ Health Perspect 1995; 103:111-6.
17. Ross JA, Severson RK, Pollock BH, Robison LL. Childhood cancer in the United States: a geographical analysis of cases from the Pediatric Cooperative Clinical Trials groups. Cancer 1996;77:201-7.
18. Boyle EB, Steinbuch M, Tekautz T, Gutman JR, Robison LL, Perentesis JP. Accuracy of DNA amplification from archival hematological slides for use in genetic biomarker studies. Cancer Epidemiol Biomarkers & Prev 1998;7:1127-31.
19. Bos JL. *Ras* oncogenes in human cancer: a review. Cancer Res 1989; 49:4682-9.
20. Corominas M, Perucho M, Newcomb EW, Pellicer A. Differential expression of the normal and mutated K-*ras* alleles in chemically induced thymic lymphomas. Cancer Res 1991;51:5129-33.
21. Slebos RJ, Hruban RH, Dalesio O, Mooi WJ, Offerhaus GJ, Rodenhuis S. Relationship between K-*ras* oncogene activation and smoking in adenocarcinoma of the human lung. J Natl Cancer Inst 1991;83: 1024-7.
22. Taylor JA, Sandler DP, Bloomfield CD, et al. *Ras* oncogene activation and occupational exposures in acute myeloid leukemia. J Natl Cancer Inst 1992;84:1626-32.
23. De Vivo I, Marion MJ, Smith SJ, Carney WP, Brandt-Rauf PW. Mutant c-Ki-*ras* p21 protein in chemical carcinogenesis in humans exposed to vinyl chloride. Cancer Causes & Control 1994;5:273-8.
24. Savitz DA, Chen JH. Parental occupation and childhood cancer: review of epidemiologic studies. Environ Health Perspect 1990;88: 325-37.
25. Colt JS, Blair A. Parental occupational exposures and risk of childhood cancer. Environ Health Perspect 1998;106:909-25.

Parental Exposure to Medications and Hydrocarbons and *ras* Mutations in Children with Acute Lymphoblastic Leukemia: A Report from the Children's Oncology Group

Xiao Ou Shu, John P. Perentesis, Wanqing Wen, et al.

Cancer Epidemiol Biomarkers Prev 2004;13:1230-1235.