Carcinogenesis vol.29 no.5 pp.984–990, 2008
doi:10.1093/carcin/bgn071
Advance Access publication March 13, 2008

Maternal and offspring genetic variants of *AKR1C3* and the risk of childhood leukemia

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The *aldo-keto reductase 1C3* (*AKR1C3*) gene located on chromosome 10p15-p14, a regulator of myeloid cell proliferation and differentiation, represents an important candidate gene for studying human carcinogenesis. In a prospectively enrolled population-based case–control study of Han Chinese conducted in Kaohsiung in southern Taiwan, a total of 114 leukemia cases and 221 controls <20 years old were recruited between November 1997 and December 2005. The present study set out to evaluate the association between childhood leukemia and both maternal and offspring's genotypes. To do so, we conducted a systematic assessment of common single-nucleotide polymorphisms (SNPs) at the 5′ flanking 10 kb to 3′ UTR of *AKR1C3* gene. *Gln5His* and three tagSNPs (rs2245191, rs10508293 and rs3209896) and one multimarker (rs2245191, rs10508293 and rs3209896) were selected with average 90% coverage of untagged SNPs by using the HapMap II data set. Odds ratios and 95% confidence intervals were adjusted for age and gender. After correcting for multiple comparisons, we observed that risk of developing childhood leukemia is significantly associated with rs10508293 polymorphism on intron 4 of the *AKR1C3* gene in both offspring alone and in the combined maternal and offspring genotypes (nominal $P < 0.0001$, permutation $P < 0.005$). The maternal *methylenetetrahydrofolate reductase A1298C* polymorphism was found to be an effect modifier of the maternal intron 4 polymorphism of the *AKR1C3* gene (rs10508293) and the childhood leukemia risk. In conclusion, this study suggests that *AKR1C3* polymorphisms may be important predictive markers for childhood leukemia susceptibility.

Introduction

Leukemia is the most frequent type of childhood cancer (1). Acute lymphoblastic leukemia (ALL) is the main subtype of childhood leukemia: it represents nearly 80% of diagnoses, followed by acute myeloid leukemia (AML) (16%) and the chronic subtypes of leukemia (1). Despite the many recent advances in molecular biology and immunology, the causes of childhood leukemia remain largely unknown. Both genetic and environmental factors are suspected to contribute to cancer development. Environmental exposure to ionizing radiation, anticancer drugs and benzene are established risk factors to leukemogenesis (2). However, these risk factors together account for only a small fraction of all leukemia incidences (3,4).

Increased leukemia risk in workers in petroleum industry has been previously reported (3,5,6). However, the results specifically connecting residential petrochemical exposure with leukemia risk have been inconsistent (7–9). Several compounds present in the petrochemical pollution mix are known to be hazardous; these include polycyclic aromatic hydrocarbons (PAHs) and volatile organic compounds. Previous studies have reported that the concentrations of selected PAHs and volatile organic compounds in the vicinity of petrochemical industry, whereas lower than occupational exposure levels, can be at least 10 and 2 times higher than those in the industrialized communities of the USA (10,11). PAHs such as benzo[*a*]pyrene and volatile organic compounds such as acrylonitrile, benzene, 1,3-butadiene, styrene and vinyl chloride are known to be carcinogenic or probably carcinogenic to humans (12). Benzene, specifically, is a well-known hematopoietic carcinogen and is characterized as a group 1 carcinogen by the International Agency for Research on Cancer.

Aldo-keto reductase 1C3 (AKR1C3) belongs to the aldo-keto reductase superfamily. This is a group of oxidoreductases which catalyze the NADP(H)-dependent reductions of a wide variety of aldehydes and ketones, including steroid hormones and prostaglandins. The metabolic by-products of this reaction can form DNA adducts or reactive oxygen species (ROS) leading to oxidative DNA damage. AKR1C3 is also known to be a dihydrodiol dehydrogenase and oxidizes PAH *trans*-dihydrodiols to the highly genotoxic reactive and redox active *o*-quinones (13). In addition, the inhibition of AKR1C3 expression prevents the proliferation of human myeloid leukemia cells which suggests that AKR1C3 is an important regulator of myeloid cell proliferation and differentiation (14). *Gln5His* polymorphism in exon 1 of *AKR1C3* gene has been studied in several cancers, including lung cancer, prostate cancer and lymphoma. However, its influence on leukemia risk has yet to be examined.

It is proposed that the developing embryo and fetus may be more susceptible to ROS and genotoxic compounds (15–17). Maternal genetic variants associated with ROS generations may influence childhood leukemia risk by altering the offspring's intrauterine environment. Fetuses with *in utero* exposure to PAHs have been reported to be more susceptible to DNA damage than exposed mothers (18). Molecular studies on identical twins and Guthrie cards studies indicate that childhood leukemias can originate prenatally (19–21). We believe that it is important to consider maternal genetics when developing our understanding of the *in utero* events in relation to leukemogenesis. The importance of maternal *methylenetetrahydrofolate reductase* (*MTHFR*) genotypes has been investigated in several studies which have focused on the risk of the offspring developing Down syndrome (22), a known risk factor of acute leukemia (23). MTHFR is one of the key enzymes in folate metabolism which affects folate species toward either DNA methylation or DNA synthesis and repair (detail review in ref. 24). The variant alleles of the two most commonly studied *MTHFR* polymorphisms (*C677T* and *A1298C*) are associated with reduced enzyme activity. Several studies have shown these two polymorphisms to be associated with a reduced risk of leukemia; however, the data remain inconsistent and contradictory (25–27). The primary objective of this study was to examine whether common variants of *AKR1C3* and *MTHFR* genes in both maternal and offspring are important genetic determinants of developing childhood leukemia in a Han Chinese population.

Abbreviations: AKR1C3, aldo-keto reductase 1C3; ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; CI, confidence interval; LD, linkage disequilibrium; LRT, likelihood ratio test; MTHFR, methylenetetrahydrofolate reductase; OR, odds ratio; PAH, polycyclic aromatic hydrocarbon; ROS, reactive oxygen species; SNP, single-nucleotide polymorphism.

Materials and methods

Study subjects

This is a prospectively enrolled population-based case–control study conducted in metropolitan Kaohsiung in southern Taiwan. Details of this study area as well as the criteria for case and control recruitment were described previously (9). In brief, cases and controls were selected from the non-agricultural areas in Kaohsiung to avoid potential confounding effects of pesticides. All recruited cases were originally diagnosed between November 1997 and December 2005. The eligibility criteria for the case group was defined as those under the age of 20 years old diagnosed with incident, primary leukemia cases (International Classification of Diseases, Revision 9, codes 204–208) and were residents of the study area at the time of enrollment. Patients with secondary or recurrent tumors were excluded.

After the cases were identified, controls from the defined study area were randomly selected from the population registry data by personal identification number. Each citizen in Taiwan is assigned a personal identification number by the Household Registration Offices. The number assignment is independent of current residence and hence does not bias the control selection by residence. Individuals with any existing known malignancy were excluded from being controls. Cases and controls were matched by age (±1 year) and gender using a 1:3 matching ratio.

Among the selected cases and controls, 203 (93.5%) cases and 533 (46.5%) controls completed an in-person interview. The study protocol was approved by the Institutional Review Boards of the Harvard School of Public Health and Kaohsiung Medical University. All study subjects >18 years of age consented; whereas those under 18 had their parents assent to participate in the study.

Questionnaires

The questionnaire was administered by trained interviewers and completed by either the subject's biological mother or the subject himself/herself. A modified questionnaire (28) was used to obtain the subjects' sociodemographic characteristics, medical history, residential history up to 2 years prior to birth, occupational history (if the subject was ≥16 years of age), cigarette smoking, alcohol consumption, adult and childhood diet and exposure to various hazardous agents. A follow-up phone interview was administered to obtain data for that subject if original questionnaire was not completed. An identical questionnaire for the mother was used with additional questions added to obtain information on maternal reproductive history and supplement (vitamins and iron supplement) and/or medication usage 21 months before delivery to the study subject's date of birth or the date stopped breast-feeding.

DNA sample collection

Peripheral blood (85% of mothers and 60% of offspring) or buccal cell samples were collected during the interview. DNA was extracted from peripheral blood samples or buccal cell samples using the Puregene DNA Isolation Kit (Gentra Systems, Minneapolis, MN). We obtained DNA samples from 114 mothers and 101 offspring among the case group and 221 mothers and 192 offspring among the controls.

Single-nucleotide polymorphism selection

We selected tagSNPs based on the linkage disequilibrium (LD) patterns of 46 genotyped single-nucleotide polymorphisms (SNPs) (covered *AKR1C3* gene and 10 kb upstream 5′ flanking sequence) found among 90 unrelated Han Chinese and Japanese individuals in HapMap II data set (http://www.hapmap.org/). Pairwise LD between SNPs was assessed using Lewontin's D' statistic and the squared correlation statistic r^2. The Haploview program was used to calculate the LD coefficients and define haplotype blocks (29). TagSNPs were selected using the r^2-based Tagger program (30) with pairwise $r^2 \geq 0.80$ and minor allele frequency ≥5%. Three tagSNPs (rs2245191, rs10508293 and rs3209896) and one multimarker (rs2245191, rs10508293 and rs3209896) were selected with average 90% coverage of untagged SNPs. Additionally, the *Gln5His* polymorphism (rs12529), which has been previously studied in relation to several cancers (31–35), was also genotyped. Two common polymorphisms of *MTHFR* gene, *C677T* (rs1801133) and *A1298C* (rs1801131), have been investigated in mothers in relation to their offspring's risk of Down syndrome (22), a risk factor of acute leukemia (23). *C677T* and *A1298C* polymorphisms in *MTHFR* gene were also genotyped.

Genotyping

Both the maternal and offspring's polymorphisms were genotyped by TaqMan assays using the ABI Prism 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA). The primers, probes and reaction conditions are available upon request. Genotyping was done by laboratory personnel without the knowledge of case–control status, and a random 5% of the samples were repeated to validate genotyping procedures. The Hardy–Weinberg equilibrium test was performed for each of the SNPs using the χ^2 test (1 df).

Statistical analysis

The data were analyzed using the SAS® software version 9.1 (Cary, NC). Whereas the majority of our study subjects are Han Chinese, other ethnicities include Hakka Chinese, Natives and Foreign. Among the total 203 cases, there are 177 Han Chinese and 26 non-Han Chinese; whereas among 533 controls, there are 490 Han Chinese and 43 non-Han Chinese. Since the effects of maternal genotypes in relation to the offspring's leukemia risk are the primary interest of the study, we restricted the analysis to the Han Chinese with matching maternal DNA samples. In total, 114 cases and 220 controls were in the final analyses.

The selected principal characteristics were evaluated using χ^2, Fisher exact and *t*-tests as appropriate. Multiple logistic regressions with additive genetic effect models were used to assess the associations between leukemia risk and the effect of maternal, offspring and combined maternal–offspring polymorphisms. The effects of the combined maternal and offspring's polymorphisms were assessed by treating combined maternal and offspring's genotype as a continuous variable (0, 1, 2, 3 and 4 variant alleles from mothers and children together). Haplotype frequencies and individual haplotypes were estimated using the expectation–maximization algorithm (36,37). The associations between haplotypes and disease status were estimated using the 'expectation substitution' approach (36,38). This approach uses expected haplotype scores calculated under additive model as the observed covariates in a logistic regression model instead of assigning each subject with the most likely haplotype pair. Global haplotype analyses, using likelihood ratio tests (LRTs), were used to examine the association between haplotypes and risk of leukemia. The interaction effects between *AKR1C3* and *MTHFR* genes were evaluated using a multiple logistic regression model with a dominant genetic effect. Only SNPs of *AKR1C3* gene which achieved the global significant threshold were tested for interaction effects with the SNPs of *MTHFR*. LRTs were used to test the significance of the interaction terms.

Several potential confounders were adjusted for in the multiple regression models: age, gender, maternal smoking status during pregnancy, subjects' smoking status before diagnosis, parental education level and maternal vitamins and iron supplements intake status. As no confounding effect was seen by these variables alone or together on the associations of interest, the results presented are only adjusted for the matching factors including age and gender. Stratified analyses by maternal genotypes were also performed to estimate if the offspring's genetic effects were modified by maternal genotypes in addition to the effects from transmission of maternal alleles to the offspring. To adjust for the single-point significance level for multiple testing with correct type I error rate, empirical *P*-values based on global random permutation tests were reported. We randomly permuted the case–control status of each subject, performed the same set of analyses (including single-SNP analyses, haplotype-based analyses and stratified analyses) and recorded the minimal *P*-value for each permutation data set. The distribution of the minimal *P*-values obtained from 10 000 permutation data sets was used to derive the empirical significance of the observed test statistic ($P_{\text{permutation}}$). The adjusted global-wide *P*-values were determined as $P_{\text{adjusted}} = P\ (P_{\text{observed}} \leq P_{\text{permutation}})$. All reported *P*-values are from two-sided tests. An adjusted *P*-value <0.05 was considered to be statistically significance.

Results

Demographics

A total of 114 cases and 221 controls were used in the final analyses. The distributions of the selected characteristics among study subjects are summarized in Table I. Acute leukemia was the predominant disease type comprising 73 (64%) ALL and 29 (25%) AML of the recruited cases. There were no significant differences seen between the leukemia cases and controls with respect to age, gender, maternal age at child's birth, birth weight, maternal and subjects' smoking history and parental education levels (Table I). Neither the offspring's nor the mothers' employment was in petrochemical-related occupations.

TagSNP and haplotype analyses

The locations of the polymorphisms and the genotype frequencies are shown in Table II. There was no evidence of any departure from Hardy–Weinberg equilibrium ($P > 0.05$). The minor allele frequencies of the polymorphisms were similar to the reported frequencies in Han Chinese population from the International HapMap Project. The pairwise r^2 among the four tagSNPs of *AKR1C3* gene ranged from 0.02 to 0.71.

The associations of *AKR1C3* and *MTHFR* polymorphisms with childhood leukemia are summarized in Table III. Significant associations were observed among *AKR1C3* polymorphisms. No significant association was observed between the offspring's leukemia risk and either maternal or offspring's *MTHFR* genetic polymorphisms with and without being adjusted for age and gender.

Table I. Distribution of study subjects by demographic and risk factors

Characteristics[a]	Case (n = 114)	Control (n = 221)
International Classification of Disease (%)		
ALL (%)	73 (64.0)	
AML (%)	29 (25.4)	
Chronic myeloid leukemia (%)	6 (5.4)	
Others (%)	4 (5.2)	
Age (years) [mean (SD)]	8.8 (6.19)	8.0 (5.89)
Sex		
Male (%)	78 (68.4)	135 (61.1)
Female (%)	36 (31.6)	86 (38.9)
Maternal age (years)		
<25 (%)	22 (19.5)	57 (26.0)
25–34 (%)	83 (73.4)	152 (69.4)
35+ (%)	8 (7.1)	10 (4.6)
Birth weight (g)		
<3000 (%)	48 (42.1)	107 (48.4)
3000–3499 (%)	32 (28.1)	63 (28.5)
>3500 (%)	34 (29.84)	51 (23.1)
Maternal smoking during pregnancy		
Yes (%)	2 (1.8)	7 (3.2)
No (%)	112 (98.3)	213 (96.8)
Subjects smoking before diagnosis		
Yes (%)	0 (0)	2 (0.9)
No (%)	114 (100)	218 (99.1)
Parental education levels[b]		
Not junior high school graduated (%)	3 (2.6)	10 (4.5)
Junior high school graduated (%)	14 (12.3)	23 (10.5)
High school graduated (%)	58 (50.9)	88 (40.0)
Four-year college/university and above (%)	39 (34.2)	99 (45.0)

[a]Number and percent of group (%) or [mean (SD)]. Percents are rounded.
[b]Highest education level of either parent.

Offspring's polymorphisms. The associations between the offspring's genetic polymorphisms and childhood leukemia risk were assessed. A significant increased risk was observed for offspring with polymorphisms rs2245191 [odds ratio (OR) = 1.58; 95% confidence interval (CI) = 1.05–2.37], rs10508293 (OR = 2.46; 95% CI = 1.69–3.58) and rs3209896 (OR = 1.69; 95% CI = 1.14–2.51) but not with rs12529 (OR = 1.60; 95% CI = 0.98–2.59) after being adjusted for age and gender (Table III). When stratified by disease subtypes, only the rs10508293 polymorphism in intron 4 was found to be associated with ALL risk (adjusted OR = 2.08; 95% CI = 1.35–3.19). In relation to offspring's AML risk, significant associations were observed on polymorphisms rs2245191 and rs10508293 with the adjusted ORs of 3.05 (95% CI = 1.07–8.68) and 3.94 (95% CI = 1.44–10.82). After correcting for multiple comparisons using permutation tests, rs10508293 was the only polymorphism to remain significantly associated with ALL (permutation P = 0.02) and all leukemia disease subtypes (permutation P = 0.004).

Maternal polymorphisms. The association between maternal polymorphisms and the offspring's leukemia risk was also examined. All the maternal polymorphisms chosen in our study were found to be associated with offspring's leukemia risk (Table III). When stratified by disease subtypes, significantly increased offspring's ALL (but not AML) risk was observed for the maternal rs12529 polymorphism in exon 1 (adjusted OR = 1.70; 95% CI = 1.00–2.87) and rs10508293 polymorphism in intron 4 (adjusted OR = 1.52; 95% CI = 1.01–2.28). No significant association was observed for the other SNPs. However, after correcting for multiple comparisons by permutation tests, the statistical significance did not remain.

Combined maternal and offspring polymorphisms. The adjusted OR for the combined maternal and offspring's variant alleles of polymorphism rs10508293 on intron 4 is 1.63 (95% CI = 1.30–2.04) with nominal $P < 0.0001$ (permutation P = 0.004) per variant allele number increased. Similar significant findings were observed among polymorphisms rs2245191 and rs3209896 (Table III).

When stratified by disease subtypes, rs12529 polymorphism on exon 1 (adjusted OR = 1.49; 95% CI = 1.05–2.11; nominal P = 0.03; permutation P = 0.67) and rs10508293 polymorphism in intron 4 (adjusted OR = 1.48; 95% CI = 1.14–1.92; nominal P = 0.003; permutation P = 0.11) were found to be associated with offspring's

Table II. Genotype and allele frequencies of *AKR1C3* and *MTHFR* SNPs in mothers and offspring with leukemia and control subjects

Polymorphism	Location	Alleles	Mother		Child	
			Case (%)	Control (%)	Case (%)	Control (%)
AKR1C3	10p15-p14					
rs12529	Exon 1	*CC*	80 (71.4)	166 (78.6)	66 (68.0)	143 (79.5)
		CG	26 (23.2)	43 (20.4)	28 (28.9)	33 (18.3)
		GG	6 (5.4)	2 (1)	3 (3.1)	4 (2.2)
rs2245191	Intron 1	*GG*	64 (59.3)	148 (69.5)	55 (56.1)	128 (71.1)
		GT	35 (32.4)	56 (26.3)	34 (34.7)	40 (22.2)
		TT	9 (8.3)	9 (4.2)	9 (9.2)	12 (6.7)
rs10508293	Intron 4	*AA*	38 (36.2)	107 (52.2)	26 (27.1)	95 (52.8)
		AG	48 (45.7)	76 (37.1)	45 (46.9)	68 (37.8)
		GG	19 (18.1)	22 (10.7)	25 (26.0)	17 (9.4)
rs3209896	Exon 9 (3′ UTR)	*GG*	59 (54.1)	139 (64.4)	47 (48.0)	122 (67.0)
		GA	41 (37.6)	70 (32.4)	40 (40.8)	48 (26.4)
		AA	9 (8.3)	7 (3.2)	11 (11.2)	12 (6.6)
MTHFR	1p36.3					
rs1801133	Exon 4	*CC*	59 (53.6)	119 (56.9)	55 (58.5)	101 (58.7)
		CT	45 (40.9)	72 (34.5)	27 (28.7)	56 (32.6)
		TT	6 (5.5)	18 (8.6)	12 (12.8)	15 (8.7)
rs1801131	Exon 7	*AA*	74 (67.9)	123 (62.5)	57 (63.3)	103 (60.6)
		AC	30 (27.5)	57 (28.9)	30 (33.3)	56 (32.9)
		CC	5 (4.6)	17 (8.6)	3 (3.3)	11 (6.5)

Table III. The adjusted OR of maternal and offspring's *AKR1C3* and *MTHFR* polymorphisms to the risk of childhood leukemia

Polymorphism	Mother		Child		Mother + child	
	OR (95% CI)[a]	*P*-value[b]	OR (95% CI)[a]	*P*-value[b]	OR (95% CI)[a]	*P*-value[b]
AKR1C3						
rs12529	1.59 (1.01–2.50)	0.05	1.6 (0.98–2.59)	0.06	1.30 (0.96–1.75)	0.09
rs2245191	1.49 (1.01–2.18)	0.04	1.58 (1.05–2.37)	0.03	1.36 (1.05–1.75)	0.02
rs10508293	1.62 (1.15–2.28)	0.005	2.46 (1.69–3.58)	2×10^{-6}	1.63 (1.30–2.04)	2×10^{-5}
rs3209896	1.54 (1.05–2.28)	0.03	1.87 (1.27–2.76)	0.01	1.49 (1.16–1.91)	0.002
MTHFR						
rs1801133	1.02 (0.71–1.48)	0.91	1.11 (0.77–1.61)	0.58	1.10 (0.87–1.39)	0.42
rs1801131	0.76 (0.51–1.12)	0.17	0.84 (0.54–1.30)	0.43	0.87 (0.67–1.14)	0.32

[a]Odds ratios were assessed assuming additive genetic model. Analysis was adjusted for age and gender.
[b]*P*-value before adjustment for multiple comparisons.

ALL risk. In relation to childhood AML risk, the adjusted OR of combined maternal and offspring's variant allele of rs10508293 polymorphism is 1.96 per variant allele number increased (95% CI = 1.12–3.44; nominal $P = 0.02$; permutation $P = 0.52$).

Offspring's polymorphisms stratified by maternal genotypes. To estimate the maternal genetic effects in addition to the effect of the transmission of maternal alleles to the offspring, we performed stratified analyses of offspring's genetic effects by maternal genotype. The ORs for leukemia of the offspring's rs10508293 polymorphism were 4.38 (95% CI = 1.19–10.70; nominal $P = 0.001$; permutation $P = 0.03$) in the groups' mothers carrying heterozygous and homozygous variant alleles and 2.65 (95% CI = 1.11–6.33; nominal $P = 0.03$; permutation $P = 0.67$) in the groups' mothers carrying homozygous wild-type alleles.

When the association with disease risk was restricted to childhood ALL, the OR of offspring's rs10508293 polymorphism was 5.48 (95% CI = 1.81–16.60; nominal $P = 0.003$; permutation $P = 0.11$) in the group of mothers carrying heterozygous and homozygous variant alleles and 1.68 (95% CI = 0.59–4.74; nominal $P = 0.33$) in the group of mothers carrying the homozygous wild-type alleles. There was no significantly different offspring genetic association when the two maternal genotypes were examined.

Haplotype analyses. Due to the strong LD ($D' > 0.9$) between *MTHFR* codon 677 and codon 1298 and the potential effects of the *MTHFR* haplotype suggested by previous studies (39,40), *MTHFR* haplotypes were also tested. Maternal and offspring's *AKR1C3* and *MTHFR* haplotype frequencies and the adjusted ORs of the haplotypes to childhood leukemia risk are shown in Table IV. Four haplotypes of *AKR1C3* and three haplotypes of *MTHFR* with frequencies >2% were estimated (Table IV). Significant effects were found to be associated with offspring's leukemia risk among maternal ($\chi^2 = 9.4$; df = 4; $P = 0.05$) and offspring's *AKR1C3* haplotypes ($\chi^2 = 19.9$; df = 4; $P = 0.0005$), but were not significant among *MTHFR* haplotypes by the LRTs. Using the *G-A-G* haplotype (*rs2245191-rs10508293-rs3209896 AKR1C3* wild-type polymorphic alleles) as the reference group, the maternal and offspring's *T-G-A* and the *G-G-G* haplotypes were found to be associated with increased childhood leukemia risk. By comparing *AKR1C3* haplotypes *G-A-G*, *T-G-A* and *G-G-G*, the haplotype analyses confirmed that the *rs10508293* polymorphism was the potential SNP associated with leukemia risk.

Gene–gene interactions. The *AKR1C3* rs10508293 polymorphism remains significantly associated with childhood leukemia risk after correcting for multiple comparisons. The possible interaction of the rs10508293 with *MTHFR* polymorphisms was tested. A significant interaction was observed between maternal rs10508293 and *MTHFR A1298C* ($\chi^2 = 6.10$; df = 1; $P = 0.01$) but was not observed among offspring's genetic polymorphisms ($\chi^2 = 1.90$; df = 1; $P = 0.17$). Among the mothers who carry the *MTHFR 1298C* variant alleles, the mothers with *AKR1C3* rs10508293 variant alleles have about a five times greater risk of having offspring who develop leukemia in comparison with the mothers with homozygous *AKR1C3* rs10508293 wild-type (Table V). However, no different maternal *AKR1C3* rs10508293 genetic effect on childhood leukemia risk is observed among mothers with *MTHFR 1298AA* genotype. There is no significant interaction effect after the results were stratified by leukemia disease subtypes. There were no statistically significant interactions observed between *MTHFR C677T* and *AKR1C3* rs10508293 polymorphisms in either the maternal or offspring's subgroups.

Table IV. *AKR1C3* and *MTHFR* haplotype frequencies among mothers and offspring with leukemia and control subjects and the adjusted ORs of the haplotypes to childhood leukemia risk

Haplotype[a]	Mother[b]			Child[c]		
	Case (%)	Control (%)	OR (95% CI)[d]	Case (%)	Control (%)	OR (95% CI)[d]
AKR1C3						
G-A-G	55	68	1	47	66	1
T-G-A	24	16	1.73 (1.15–2.61)	26	17	2.18 (1.40–3.41)
G-G-G	16	11	1.66 (1.02–2.70)	19	11	2.54 (1.50–4.31)
G-A-A	3	3	1.48 (0.50–4.40)	4	3	1.99 (0.69–5.69)
Rare[e]	2	2	1.02 (0.32–3.21)	4	3	1.35 (0.49–3.68)
MTHFR						
C-A	55	51	1	53	53	1
T-A	26	25	0.96 (0.65–1.42)	27	24	1.12 (0.75–1.70)
C-C	19	23	0.77 (0.51–1.18)	20	22	0.88 (0.55–1.42)
Rare[e]	0	1	NA	0	1	NA

P-value of mother (haplotype *T-G-A*) = 0.009; P-value of mother (haplotype *G-G-G*) = 0.04; P-value of child (haplotype *T-G-A*) = 0.0006; P-value of child (haplotype *G-G-G*) = 0.0005.
[a]*AKR1C3* haplotype frequencies were presented by the sequences of rs2245191 *G>T*, rs10508293 *A>G* and rs3209896 *G>A*; *MTHFR* haplotype frequencies were presented by the sequences of rs1801133 *C>T* and rs1801131 *A>C*.
[b]*P*-value of the global test for *AKRIC3* = 0.05; for *MTHFR* = 0.60.
[c]*P*-value of the global test for *AKRIC3* = 0.0005; for *MTHFR* = 0.69.
[d]ORs assessed by logistic regression adjusted for age and gender; assumed additive genetic model.
[e]Combined rare haplotypes with frequencies <2% among the controls.

Discussions

In this population-based case–control study, we investigated the association of maternal and offspring's common polymorphisms of *AKR1C3* gene in relation to childhood leukemia risk. After correcting for multiple comparisons, we observed that risk of developing childhood leukemia is significantly associated with rs10508293

Table V. The frequencies and adjusted OR of maternal and offspring's *AKR1C3* polymorphisms stratified by *MTHFR A1298C* genotypes to the risk of childhood leukemia

Polymorphism		*MTHFR 1298AA*				*MTHFR 1298AC+CC*			
		Case (%)	Control (%)	Adjusted OR (95% CI)[a]	*P*-value	Case (%)	Control (%)	Adjusted OR (95% CI)[a]	*P*-value
Offspring's *AKR1C3* rs10508293	*AA*	17 (30.9)	47 (50.0)	1		7 (20.0)	39 (54.9)	1	
	AG + GG	38 (69.1)	47 (50.0)	2.30 (1.13–4.66)	0.02	28 (80.0)	32 (45.1)	6.26 (2.27–17.28)	0.0004
Maternal *AKR1C3* rs10508293[b]	*AA*	30 (43.5)	54 (47.0)	1		7 (20.0)	40 (54.1)	1	
	AG + GG	39 (56.5)	61 (53.0)	1.15 (0.63–2.10)	0.66	28 (80.0)	34 (45.9)	4.75 (1.83–12.3)	0.001

[a]ORs were assessed assuming additive genetic model. Analysis was adjusted for age and gender.
[b]Significant interaction effect (nominal *P*-value < 0.05) according to the LRTs.

polymorphism on intron 4 of the *AKR1C3* gene in offspring alone and in the combined maternal and offspring genotypes.

Previous studies found that the *Gln5His* polymorphism on exon 1 is associated with lung cancer (35) but not with prostate cancer (31) or lymphoma (33). We found that the maternal alone and the combined maternal and offspring's *Gln5His* polymorphism were associated with offspring's ALL risk but did not achieve the global significance level after adjusting for multiple comparisons.

The *AKR1C3* gene encodes for NADPH-dependent oxidoreductases which catalyze a variety of substrate spectrum: aldehydes, ketones and many xenobiotic compounds (13). DNA adducts or oxidative DNA damage caused by ROS and the by-products generated in the metabolic processes are associated with carcinogenesis. ROS also affects multiple reproductive processes including oocyte maturation, fertilization, embryo development and pregnancy (41). In addition, AKR1C3 was found to play an important role in regulating myeloid cell proliferation and differentiation (14). The rs10508293 polymorphism on intron 4 of the *AKR1C3* gene in offspring alone and in the combined maternal and offspring genotypes was found with nominal $P < 0.05$ in relation to ALL and AML risk. However, the significant association to AML risk did not remain after correction for multiple comparisons. This is most likely due to the small sample size after stratification.

Maternal genetic variants of the *AKR1C3* gene may influence childhood leukemia risk by altering the offspring's intrauterine environment and may be affected by the transmission of maternal alleles to the offspring. We detected a trend effect of combined maternal and offspring's *G* variant allele of rs10508293 polymorphism on intron 4. In addition, our data show that the rs10508293 polymorphism in offspring is associated with even higher risk of childhood ALL when the mothers are *G* variant allele carriers for the rs10508293 polymorphism in comparison with mothers who are wild-type allele carriers. This suggests that maternal genetic variants may modify the genetic effect of their offspring's genetic variants to the cancer risk.

The specific function of the rs10508293 polymorphism remains unknown. Recent *in vitro* study found that a promoter *A* to *G* polymorphism is associated with significantly altered promoter activity in reporter constructs in Caucasians but was completely absent in Asian ethnicity (42). Additional work is needed to determine the functionality of the genetic variants, especially in the Han Chinese.

The variant alleles of the two most common polymorphisms of the *MTHFR* gene, *C677T* and *A1298C*, are associated with reduced enzyme activity. Individuals carrying the variant alleles were found to be associated with a reduced risk of leukemia whereas some studies observed no effect (25–27). In accordance with recent data, our study found no significant association observed between the maternal and/or offspring's *MTHFR* genetic polymorphisms and the risk for childhood leukemia. (43). However, significant interaction effects were observed between the maternal polymorphism on intron 4 of the *AKR1C3* gene (rs10508293) and *MTHFR A1298C* ($P = 0.01$ by LRTs) but were not observed between offspring's polymorphisms ($P = 0.17$ by LRTs). Among the mothers who carry the *MTHFR 1298C* variant alleles, the mothers with *AKR1C3* rs10508293 variant alleles have significantly increased the risk of having the leukemia offspring compared with the mothers with homozygous *AKR1C3* rs10508293 wild-type. Transplacental exposure to environmental carcinogens has been shown to cause DNA damage in newborns (44,45) and is also associated with increased cytogenic damage linked to childhood leukemia (46). The increased levels of micronucleated binucleated cells (47) and lymphocytic micronuclei has been reported among the PAH-exposed subjects (47,48). Although the functional consequence of the *AKR1C3* rs10508293 polymorphism has yet to be determined, increased activity of AKR1C3 would increase the bioactivation of PAH *trans*-dihydrodiols. The *MTHFR* variant allele was found to be associated with greater susceptibility to DNA damage in the lymphocytes (49). A significant association between the *MTHFR* and the levels of lymphocytes with micronuclei has been reported in some studies (22,50,51); whereas others failed to find the correlation (52,53). Therefore, it is biologically plausible to reason that if the individuals are more susceptible to DNA damage, subjects with higher levels of reactive and redox active *o*-quinones and ROS generated in the metabolism processes have higher risk of leukemia development since carcinogenesis is strongly related with the accumulation of DNA damage. However, the biologic mechanisms by which the gene polymorphisms modify the maintenance of genome integrity remain to be clarified, especially in the intrauterine environment.

The interaction between *MTHFR* polymorphisms and maternal dietary factors, including folate, iron supplements and alcohol consumption, has been reported in several studies (54–56). Although maternal folate status during pregnancy was not measured directly in our study, similar *MTHFR–AKR1C3* interaction effects as well as the main effects of *MTHFR* gene were observed after adjusting for the status of vitamin, iron supplements and alcohol-containing beverage intake during pregnancy.

There are several limitations to our study. Selection bias may be a concern if the higher risk polymorphism was somehow related to differential participation between cases and controls. However, subject participation status was unrelated to genotype frequencies for a wide spectrum of genes reported from a recent study (57). In addition, both subjects and study investigators were blinded to genotypic status at the time of recruitment, and the genotyping was done blinded of disease status in our study. Another potential limitation of the study is that the sample size is modest and the number of cases in several histologic subgroups is small. Most likely, different cell types of leukemia have different etiology (6). The limited sample size resulted in a reduced power to detect associations for SNPs with low allele frequencies and the interaction effects, especially in several leukemia subtypes other than ALL. Larger studies in the future are warranted to delineate these issues.

In conclusion, this is the first study suggesting that both maternal and offspring's *AKR1C3* polymorphisms may be associated with

increased offspring's leukemia risk. A true association between a SNP (and any other forms of variations) and a trait results from either of the following two scenarios: (i) a direct association, the associated SNP is the causal variation or (ii) an indirect association, the associated SNP is in LD with a nearby functional SNP. Polymorphisms on the intron may also impact the genetic function by affecting the splice donor–acceptor site regions nearby or regulatory motifs within the introns. Additional investigations of the functional genetic variants that LD to the intronic polymorphism (rs10508293) in *AKR1C3* gene are also needed to confirm the genetic effects of *AKR1C3* polymorphisms on childhood leukemia.

Funding

National Institutes of Health (ES09723, ES00002).

Acknowledgements

The authors gratefully acknowledge Chien-Chin Chou for questionnaire data verification, Janna Frelich for data management and Maureen Convery and Chu-Ling Yu for technical assistance. The members of the Kaohsiung Leukemia Research Group were as follows: Kaohsiung Medical University Chung-Ho Memorial Hospital—Tai-Tsung Chang, Sheng-Fung Lin, Shyh-Shin Chiou, Ren-Chin Jang, Hui-Hua Hsiao, Ta-Chih Liu and Pei-Chin Lin; Kaohsiung Chang Gung Memorial Hospital—Chih-Cheng Hsiao, Jiunn-Ming Sheen, Ching-Yuan Kuo, Ming-Chung Wang, Cheng-Hua Huang and Chung-Bin Huang and Kaohsiung Veterans General Hospital—Yuk-Cheung Wong, Hung-Bo Wu, Shyh-Jer Lin, Yu-Ming Sun, Kai-Sheng Hsieh and Yu-Hsiang Chang.

Conflict of Interest Statement: None declared.

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Received November 29, 2007; revised February 13, 2008; accepted March 6, 2008