MDR1 Gene Variants, Indoor Insecticide Exposure, and the Risk of Childhood Acute Lymphoblastic Leukemia

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Abstract

Objective: **The multidrug resistance (*MDR*) 1 gene encodes a membrane transporter called P-glycoprotein, which plays an important role in protecting cells against lipophilic xenobiotics by way of an ATP-dependent cellular efflux mechanism. Among children enrolled in the Northern California Childhood Leukemia Study, we examined the susceptibility conferred by *MDR1* single nucleotide polymorphisms (SNP) and predicted haplotypes and whether they modify the association between indoor insecticide exposure and risk of childhood acute lymphoblastic leukemia (ALL).**

Methods: **Buccal cell DNA from ALL cases (n = 294) and controls (n = 369) individually matched on gender, date of birth, Hispanic status, and maternal race were whole genome amplified and genotyped for four *MDR1* SNPs, T−129C (*rs3213619*), C1236T (*rs1128503*), G2677T/A (*rs2032582*), and C3435T (*rs1045642*). Detailed and time-specific information on household pesticide use was obtained using in-home interviews with the mother.**

Results: **Allele frequencies in non-Hispanic White and Hispanic controls were similar, and with the exception of T−129C, seemed to be in strong linkage disequilibrium. Overall, the SNPs considered individually or within haplotypes (C1236T-G2677T/A-C3435T) were not significantly associated with childhood ALL. However, we observed strong evidence of a differential effect of indoor insecticide exposure (interaction odds ratio, 0.31; 95% confidence interval, 0.15-0.64; P = 0.025) on risk of ALL between carriers and noncarriers of haplotype CGC.**

Conclusion: **These preliminary data suggest that children carrying the haplotype CGC may be less susceptible to the leukemogenic effects of indoor insecticide exposures. Future studies are needed to confirm these findings.** (Cancer Epidemiol Biomarkers Prev 2007;16(6):1172–7)

Introduction

Leukemia is characterized by the uncontrolled proliferation of hematopoietic cells in the bone marrow and is the most common cancer among children under the age of 15 years in the United States and many developed countries (1). Acute lymphoblastic leukemia (ALL) is the main subtype of childhood leukemia comprising nearly 80% of diagnoses, followed by acute myeloid leukemia (16%) and the chronic subtypes of leukemia (1, 2). Established risk factors for ALL include exposure to ionizing radiation *in utero*, postnatal high-dose radiation, chemotherapeutic agents, and several genetic syndromes, but these together account for only a small proportion of childhood ALL cases diagnosed (3). Increasing evidence indicates that these and other proposed risk factors may have differential effects on childhood leukemia risk depending on the timing of exposure and individual genetic susceptibility (4).

The human multidrug resistance 1 (*MDR1* or *ABCB1*) gene encodes P-glycoprotein, a 170-kDa membrane transport protein that extrudes a wide variety of lipophilic compounds, including chemotherapeutic agents, naturally occurring xenobiotics, pesticides, and cellular metabolites (5, 6). P-glycoprotein uses an ATP-dependent efflux transport mechanism to minimize the exposure of potentially toxic compounds to the intracellular environment and is expressed primarily in regions that act as epithelial barriers or perform excretory functions, including the gastrointestinal tract, blood-tissue barrier, liver, kidney, testis, and placenta (7). Interestingly, *MDR1* is also highly expressed in several subclasses of bone marrow and peripheral leukocytes (8-11), and experimental studies have shown a decrease in natural killer and $CD8^+$ T-cell activity after suppression of *MDR1* (12, 13). Furthermore, there is evidence demonstrating the involvement of P-glycoprotein in the release of interleukin-2, interleukin-4, and IFN-γ from lymphocytes (14-16). Accordingly, P-glycoprotein could serve a role in leukemia etiology based on its transport of xenobiotics or modulation of immune function.

Among the several putative environmental risk factors for childhood leukemia transported by P-glycoprotein are household pesticides (17, 18). In a study comparing normal *mdr1a* (+/+) mice (*mdr1a* is the mouse equivalent to the human *MDR1* gene) to constructed *mdr1a* disrupted mice (−/−), Schinkel et al. (19) found that the absence of the gene resulted in increased toxicity by the pesticide, ivermectin, and decreased elimination of this compound. P-glycoprotein is also capable of interacting with a wide range of other structurally diverse pesticides (17). Several epidemiologic studies, including the Northern California Childhood Leukemia Study, have consistently reported an increased risk of childhood leukemia associated with indoor use of pesticides (4, 18) and have shown the importance of timing of the exposure during the pregnancy period (18, 20). Studies examining the effect of outdoor use of pesticides have led to inconsistent results (4).

To date, genetic analyses have identified over 50 single nucleotide polymorphisms (SNP) along the *MDR1* gene (21-25). In one of the first systematic screens of this gene, Hoffmeyer et al. (22) described the distribution of several polymorphisms in a group of healthy Caucasian volunteers and reported a significant correlation of P-glycoprotein expression and function with a synonymous SNP located in exon 26 at position 3435

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(C3435T). Subsequent studies have reported mixed results leading to debate over the true functional nature of this SNP (23, 25-28). One previous case-control study conducted in Poland reported a statistically significant increased risk of childhood ALL associated with the 3435TT genotype compared with the CC and CT genotypes combined (29). Similar associations have been found with other disease outcomes, including Parkinson's disease (30, 31), adult glioma (32), renal cell carcinoma (33), colon cancer (34), and other gastrointestinal diseases (35).

Recent studies have found C3435T to be in linkage disequilibrium with two other common SNPs, the synonymous C1236T (exon 12) and nonsynonymous triallelic G2677T/A (exon 21; refs. 23-25, 36, 37). It has been suggested that the observed functionality of C3435T may be a result of its strong linkage disequilibrium with the nonsynonymous G2677T/A (25). This evidence of linkage disequilibrium, together with observed interethnic differences in allele frequencies (37), emphasizes the need for a haplotype-based approach by race/ethnicity when assessing the functional nature of *MDR1* polymorphisms. The T−129C (exon 1b) SNP is located in the promoter region 7 bp downstream from the transcription initiation site and has previously been associated with P-glycoprotein expression levels (25, 38).

Although *MDR1* polymorphisms, particularly T−129C, C1236T, G2677T/A, and C3435T, have been widely investigated in pharmacologic studies, little is known about their potential role in the etiology of cancer, including childhood leukemia. We evaluated the role of these four *MDR1* SNPs individually and within predicted haplotypes in a study of childhood ALL cases and matched controls and examined whether they modify the association between indoor insecticide exposure and the risk of childhood ALL.

Materials and Methods

Study Subjects

The current investigation of childhood ALL is based on the Northern California Childhood Leukemia Study, an ongoing population-based case-control study designed to investigate the etiology of pediatric leukemias. Since 1995, incident childhood leukemia cases have been rapidly ascertained from major pediatric hospitals located in Northern and Central California. New childhood leukemia cases are reported usually within 24 to 48 h of diagnosis regardless of eligibility. Comparison with California State Cancer Surveillance data shows that the Northern California Childhood Leukemia Study rapid case ascertainment protocol is able to identify approximately 90% of all newly diagnosed childhood leukemia cases in the study region. For each eligible case, controls matched on date of birth, sex, Hispanic status (has a biological parent who is Hispanic), and maternal race were randomly selected from a list generated by the statewide birth registry maintained by the California Department of Health Services. Cases and controls were considered eligible if they were less than 15 years of age (at diagnosis for cases and corresponding date for the matched controls), resided in the study region at the time of diagnosis, had a parent who spoke either English or Spanish, and had no history of malignancy or cancer treatment. Approximately 86% of eligible cases consented to participate. Among all the eligible controls contacted, 84% consented to participate. The overall participation for the control subjects was 58% (the number of enrolled controls divided by the total number of control searches excluding the known and presumed ineligibles). A detailed description of control selection is reported elsewhere (39).

Data Collection

A detailed in-home personal interview with the primary caretaker of each subject was conducted as soon as consent was obtained. The interview collected information on household pesticide use, including the name of the product, intended purpose, frequency of use, and time period of use (3 months before pregnancy; pregnancy; and years 1, 2, and 3). A calendar with years corresponding to the time windows of interest was used during the interview to assist the recall of time-specific information. Pesticide exposure data were censored at 1 year before the diagnosis date for cases and corresponding reference date for controls, because exact dates of pesticide use were not available to determine with certainty whether pesticide exposure occurred before or after the reference date. For this analysis, we focused on indoor insecticide use, which included professional pest control services, insect repellents, indoor flea foggers, and a variety of insect control products commonly used indoors. Buccal cell specimens collected from 95% of cases and 95% of controls at the hospital or during the in-home interview using a soft cytobrush were shipped to the study laboratory where it was used to extract a DNA sample.

MDR1 Genotyping

ALL case and control DNA samples extracted from buccal cells were whole genome amplified (40). Genotype assays done on a subset of non–whole genome amplified DNA specimens were 100% concordant with whole genome amplified products from the same specimens. Whole genome amplified products were genotyped using multiplex PCR followed by a multiplex SNP analysis technique involving a single-base extension reaction (41). All genotyping was done blinded to case or control status.

PCR Amplification. Flanking primers were used in multiplexed PCR reactions to amplify the regions containing the four *MDR1* SNPs, T−129C (rs3213619), C1236T (rs1128503), G2677T/A (rs2032582), and C3435T (rs1045642). Standard PCR reactions (25 μL) contained 1.5 mmol/L $MgCl_2$, 25 pmol of each deoxynucleotide triphosphate, 20 pmol of each primer, 1× Buffer B (Promega), 1.25 units Taq (Promega) and were cycled 38 times (30 s at 94°C, 230 s at 55°C, 30 s at 72°C). Subsequent to PCR, a subset of PCR reactions was assessed by agarose gel electrophoresis.

Single-Base Extension Procedure. In the multiplexed single-base extension reaction, primers of varied lengths (4 bp differences) were designed to hybridize to a region directly adjacent to the SNP and were allowed to single-base extend with a fluorescently labeled dideoxynucleotide to diagnose the SNP allele. PCR products were pooled into one solution so that each product had a concentration of ~0.15 pmol/μL. Primers and unincorporated deoxynucleotide triphosphates were removed with shrimp alkaline phosphatase and exonuclease 1 (U.S. Biochemical). The single-base extension reaction (10.0 μL) contained 2.5 μL of SNaPshot Ready Reaction Mix (ABI Prism), 0.15 pmol/μL single-base extension primers, and 0.15 pmol/μL pooled PCR products. A total of 25 cycles of 96°C for 10 s, 50°C for 5 s, and 60°C for 30 s were done. Another shrimp alkaline phosphatase treatment removed unincorporated fluorescently labeled deoxynucleotide triphosphates, and the products were electrophoresed on a DNA sequencer (ABI 377). Automated genotyping software (GeneScan 5.0) was used to call genotypes.

Statistical Analysis

To account for the matched design of the study, conditional logistic regression analysis was used to examine the association between the four *MDR1* SNPs and risk of childhood ALL. Odds ratios (OR) and 95% confidence intervals (95% CI) were calculated for the heterozygous variant and homozygous variant genotype (codominant model), and these two genotypes combined (dominant model) with the homozygous wild-type genotype serving as the reference group. Haplotype

frequency and haplotype pair probability estimation of phase-unknown genotypes of C1236T, G2677T/A, and C3435T was done using the expectation-maximization algorithm (42) as implemented in SAS/Genetics PROC HAPLOTYPE (43). The haplotype determination was limited to these three SNPs due to the relatively low frequency of the T−129C variant in our study population. A likelihood ratio test was done over all haplotypes to examine the association between *MDR1* haplotypes and risk of ALL. Estimates of exact *P* values were computed using Monte Carlo methods with 10,000 permutations to address limitations (due to sparse data) of the χ^2 approximation for the distribution of the likelihood ratio test (44). Haplotype-specific ORs were estimated using conditional logistic regression to model the log odds of disease as a function of the individuals' haplotype probabilities (45, 46). Diplotype analyses were conducted using the most likely haplotype pair inferred for each subject by the expectation-maximization algorithm. To evaluate the joint effects of *MDR1* haplotypes and indoor insecticide exposure, an OR for interaction (OR_{GE} / $OR_{GE}' \times OR_{G'E}$) and 95% CIs were calculated using a conditional logistic regression model that included a multiplicative interaction term and adjusted for household income. Analyses were done among all race/ethnicities combined and in non-Hispanic White and Hispanic children separately, the two largest ethnic groups.

Results

Genotyping was successful for >90% of samples at each locus, resulting in a total of 294 ALL cases and 369 matched controls with genotype data available for at least one of the four *MDR1* loci. About 53% of children with ALL were males and 59% were between 2 and 5 years of age. Non-Hispanic White (46%) children comprised the majority of our study population followed by Hispanics (41%) and other races (13%).

The allele frequencies of T−129C, C1236T, and C3435T in non-Hispanic White control children were 5.1%, 44.6%, and 53.8%, respectively (Table 1). The frequencies for G2677T/A were 43.3% for the variant T-allele and 3.1% for the variant A-allele. Hispanic control children had similar allele frequencies for the four polymorphisms except for C3435T where non-Hispanic Whites showed a higher frequency of the variant allele ($P = 0.032$). The allele frequencies among other race/ethnicities are not presented due to their small numbers in our sample population. Our data showed evidence of pairwise linkage disequilibrium between the three common SNPs, C1236T, G2677T/A, and C3435T, among both non-Hispanic White and Hispanic controls. All observed genotype frequencies do not deviate significantly from the estimated frequencies expected assuming Hardy-Weinberg equilibrium.

Individually, the four SNPs did not show evidence of a statistically significant association with ALL among all race/ethnicities combined (Table 2), or in non-Hispanic White and Hispanic children only. However, in a small subgroup analysis of non-Hispanic White hyperdiploid ALL (>50 chromosomes) cases and matched controls (27 cases and 35 controls), a statistically significant increased risk was associated with 1236TT (OR, 40.35; 95% CI, 3.00-542.60), 2677TA/TT/AA (OR, 6.01; 95% CI, 1.12-32.23), and 3435TT (OR, 8.86; 95% CI, 1.35-58.03) compared with the respective homozygous wild-type genotypes.

Table 1. Allele frequencies in non-Hispanic White and Hispanic control children

SNP	Allele	Non-Hispanic White		Hispanic		*P**
		n	Frequency (%)	*n*	Frequency (%)	
T−129C	T	165	94.9	151	95.7	
	C		5.1		4.3	0.617
C1236T	C	168	55.4	150	53.3	
	T		44.6		46.7	0.609
G2677T/A	G	165	53.6	150	57.3	
	T		43.3		38.0	
	A		3.1		4.7	0.274
C3435T	C	170	46.2	152	54.6	
	T		53.8		45.4	0.032

*Pearson χ^2 test was used to test the difference in allele frequencies between non-Hispanic Whites and Hispanic control children.

Table 2. Analysis of *MDR1* SNPs and risk of childhood ALL

Genotype	Cases (*n* = 294)	Controls (*n* = 369)	OR (95% CI)*
T−129C			
TT	253 (89.7)	330 (90.9)	1.00 (Reference)
TC	27 (9.6)	32 (8.8)	1.09 (0.62-1.93)
CC	2 (0.7)	1 (0.3)	2.68 (0.23-30.96)
TC + CC	29 (10.3)	33 (9.1)	1.12 (0.64-1.97)
C1236T			
CC	73 (25.7)	97 (26.6)	1.00 (Reference)
CT	144 (50.7)	199 (54.5)	0.90 (0.61-1.33)
TT	67 (23.6)	69 (18.9)	1.15 (0.73-1.81)
CT + TT	211 (74.3)	268 (73.4)	0.97 (0.67-1.40)
G2677T/A			
GG	79 (28.2)	104 (28.8)	1.00 (Reference)
GT or GA	144 (51.4)	192 (53.2)	1.01 (0.70-1.46)
TA, TT, or AA	57 (20.4)	65 (18.0)	1.26 (0.78-2.05)
Non-GG	201 (71.8)	257 (71.2)	1.06 (0.74-1.51)
C3435T			
CC	81 (27.6)	100 (27.1)	1.00 (Reference)
CT	140 (47.6)	178 (48.2)	0.98 (0.67-1.42)
TT	73 (24.8)	91 (24.7)	0.99 (0.65-1.49)
CT + TT	213 (72.4)	269 (72.9)	0.98 (0.69-1.39)

*ORs and 95% CI were calculated using conditional logistic regression (matching factors: date of birth, sex, Hispanic status, and maternal race).

Haplotype frequencies of the three common SNPs in linkage disequilibrium (C1236T-G2677T/A-C3435T) were estimated using the expectation-maximization algorithm and are presented in Table 3. Among the 12 possible haplotypes, CGC and TTT were predominant, together comprising 81.3% and 77.6% of all non-Hispanic White and Hispanic control children, respectively. The case-control comparisons of haplotype frequencies were nonsignificant in the analysis of all race/ethnicities combined and in non-Hispanic White and Hispanic children only. The hyperdiploid ALL-specific association in non-Hispanic Whites was also evident in this haplotype analysis where CGC and TTT were associated with a reduced risk (OR, 0.15; 95% CI, 0.03-0.95) and increased risk (OR, 11.07; 95% CI, 1.61-76.37), respectively.

Table 4 presents the results of the conditional logistic regression analyses evaluating the effects of the two most common haplotypes (CGC and TTT) and indoor insecticide use, separately. Being a carrier of the CGC or TTT haplotype was not significantly associated with childhood ALL in non-Hispanic Whites and Hispanics separately or combined. However, the analysis of indoor insecticide use showed a statistically significant increased risk of ALL (OR, 1.65; 95% CI, 1.10-2.47).

Table 5 presents the results for the analysis of the joint effects of being a carrier of an *MDR1* haplotype and exposure to indoor insecticides on the risk of childhood ALL. Compared with noncarriers of haplotype CGC not exposed to indoor insecticides, noncarriers of haplotype CGC exposed to indoor insecticides had a statistically significant 3-fold increased risk of ALL (OR, 3.03; 95% CI, 1.54-6.00). This risk seemed to be considerably reduced among exposed carriers of haplotype CGC (OR, 2.09; 95% CI, 1.12-3.91). The multiplicative conditional logistic regression model yielded a statistically significant interaction OR (0.37; 95% CI, 0.15-0.88; $P = 0.025$). There was no evidence of interaction between haplotype TTT and indoor insecticide exposure. Similarly, the other less frequent haplotypes did not seem to interact with indoor insecticide exposure in the risk of childhood ALL (data not shown).

Table 3. Analysis of *MDR1* haplotypes and risk of childhood ALL

	C1236T	*G2677T/A*	*C3435T*	Cases	Controls	OR (95% CI)*
Hap 1	C	G	C	38.8	40.3	0.92 (0.58-1.45)
Hap 2	C	G	T	8.0	8.8	0.83 (0.37-1.88)
Hap 3	T	G	C	6.1	5.5	1.07 (0.41-2.75)
Hap 4	T	T	T	38.3	37.3	1.09 (0.68-1.75)
Hap 5 †	x	A	x	3.5	3.8	0.91 (0.28-2.92)
Hap 6 ‡		Other		5.2	4.3	1.54 (0.50-4.72)
Gene copies				588	738	
Global likelihood ratio test§						$P = 0.970$

*Haplotype ORs and 95% CIs were calculated using conditional logistic regression modeling haplotype probabilities (matching factors: date of birth, sex, Hispanic status, and maternal race). Each haplotype is compared with all other haplotypes.
†Group of haplotypes that contain the variant *A* allele at the *G2677T/A* locus (includes *CAC*, *CAT*, *TAC*, and *TAT*).
‡Group of rare haplotypes with frequencies <5% and not part of Hap 5 (includes *CTC*, *CTT*, *TGT*, and *TTC*).
§Global likelihood ratio test done over all haplotypes. Estimates of exact *P* values were computed using Monte Carlo methods with 10,000 permutations.

Discussion

We began this study with a hypothesis based on several observations from the clinical, oncologic, and epidemiologic fields concerning the role of the *MDR1* gene and its protein product, P-glycoprotein, in affecting disease states and hematopoietic cell function. First of all, P-glycoprotein functions as an efflux pump to minimize intracellular exposure to toxic compounds, including pesticides and chemicals found in tobacco smoke and traffic emissions, which have been implicated in the etiology of childhood leukemia (4, 18, 39). Second, the *MDR1* gene has several polymorphisms thought to be in linkage disequilibrium to a functional variation of the protein, which are under intense scrutiny in pharmacologic and etiologic studies. Third, *MDR1* is highly expressed in the hematopoietic system, including progenitor cells, natural killer cells, leukocytes, and T and B lymphocytes (8-11). These observations provide a strong rationale for exploring *MDR1* polymorphisms in genetic susceptibility to childhood leukemia.

In contrast to the only other study of *MDR1* genetic variation and childhood ALL conducted among 113 cases and 175 controls (29), our study did not provide consistent evidence of an association between *MDR1* SNPs individually and childhood ALL risk. In their study conducted in Poland, Jamroziak et al. (29) observed a nearly 2-fold increased risk of childhood ALL associated with the 3435TT genotype compared with the CC and CT genotypes combined. We were not able to confirm these results in our analysis of total ALL conducted in a slightly larger sample of non-Hispanic White cases ($n = 135$) and controls. However, in an analysis composed of the hyperdiploid ALL (>50 chromosomes) cases ($n = 27$) and individually matched controls ($n = 35$), we did observe a statistically significant association with the 1236TT, 2677TA/TT/AA, and 3435TT genotypes compared with the wild-type genotypes. We did these analyses in homogeneous ethnic populations due to emerging differences in risk factor associations and tumor genetic subtype frequencies in our population (47, 48). This association among non-Hispanic White hyperdiploid ALL cases and controls was also evident in the haplotype analysis where the two most common haplotypes, CGC and TTT, seemed to be associated with a reduced and increased risk, respectively. Because it is possible that these preliminary results may reflect a chance finding due to the limited numbers of subjects available for this subgroup analysis, the hyperdiploid ALL-specific effect should be interpreted with caution and will be reevaluated in future analyses with a larger sample size.

The inconsistency in results between the ALL analysis of this study and the Polish study may be related to possible genetic differences between the two Caucasian populations as reflected by the greater allele frequency of 3435T observed in our California population compared with their Polish population, as well as likely differences in linkage disequilibrium patterns with functional variants. The allele frequencies of the four SNPs observed in our non-Hispanic White study population are consistent with those reported in previous studies conducted in large European White populations (21, 22). The synonymous SNP, C3435T, has recently received tremendous attention, which is exemplified by an already large, yet controversial body of literature that suggest it be associated with an altered expression and/or functionality of the protein product (26). However, as C3435T does not lead to an amino acid change, it is believed that a yet unidentified causal SNP in strong linkage disequilibrium located in the *MDR1* gene or elsewhere in another gene may be the true risk-conferring SNP. Alternatively, a new study reports potential differences in folding as a result of translational rate differences between the various haplotypes of *MDR1* (49). Several studies, including ours, have found evidence of two common *MDR1* SNPs, C1236T and G2677T/A, to be in linkage disequilibrium with C3435T (23-25, 36, 37). Tang et al. (37) did a haplotype analysis of the three common *MDR1* SNPs, C1236T, G2677T/A, and C3435T, and observed clear race/ethnicity-specific differences in frequency between Chinese, Malayan, and Indians. The Chinese and Malayans exhibited three major haplotypes (CGC, TGC, and TTT) whereas the Indians exhibited only two of these three (CGC and TTT). According to the haplotype profile of our control population, Hispanics and non-Hispanic Whites had similar high frequencies of CGC and TTT haplotypes, but significantly differed

Table 4. Main effects for *MDR1* diplotypes and indoor insecticide exposure and risk of childhood ALL

	Cases ($n = 294$)	Controls ($n = 369$)	OR (95% CI)*
MDR1 diplotype			
Hap 1 (CGC) †			
Other/other	111 (37.8)	125 (33.9)	1.00 (Reference)
≥One copy	183 (62.3)	244 (66.1)	0.88 (0.63-1.21)
Hap 4 (*TTT*) †			
Other/other	108 (36.7)	138 (37.4)	1.00 (Reference)
≥One copy	186 (63.2)	231 (62.6)	1.03 (0.74-1.44)
Indoor insecticide exposure ‡			
No	54 (18.4)	92 (24.9)	1.00 (Reference)
Yes	240 (81.6)	277 (75.1)	1.65 (1.10-2.47)

*ORs and 95% CIs were calculated using conditional logistic regression (matching factors: date of birth, sex, Hispanic status, and maternal race). The analysis of indoor insecticide exposure was additionally adjusted for household income.
†The order of the *MDR1* SNP loci in the haplotypes is *C1236T*, *G2677T/A*, and *C3435T*.
‡Indoor insecticide exposure anytime from 1 y before child's birth through the first 3 y of life. Exposure(s) occurring within the year before diagnosis for cases and corresponding date for controls was discarded for the analyses.

Table 5. Analysis of interaction between common *MDR1* haplotypes, indoor insecticide exposure, and risk of childhood ALL

Haplotype	Pesticide	Cases	Controls	OR (95% CI)*	*P*
Hap CGC × Indoor insecticide					
−	No	19 (6.5)	39 (10.6)	1.00 (Reference)	
−	Yes	92 (31.3)	86 (23.3)	3.03 (1.54-6.00)	
+	No	35 (11.9)	53 (14.4)	1.87 (0.90-3.91)	
+	Yes	148 (50.3)	191 (51.8)	2.09 (1.12-3.91)	
			Interaction †	0.37 (0.15-0.88)	0.025
Hap TTT × Indoor insecticide					
−	No	18 (6.1)	29 (7.9)	1.00 (Reference)	
−	Yes	90 (30.6)	109 (29.5)	1.46 (0.73-2.90)	
+	No	36 (12.2)	63 (17.1)	0.95 (0.46-1.99)	
+	Yes	150 (51.0)	168 (45.5)	1.68 (0.86-3.27)	
			Interaction †	1.21 (0.53-2.74)	0.649

*ORs and 95% CIs were calculated using conditional logistic regression (matching factors: date of birth, sex, Hispanic status, and maternal race) adjusting for household income.

†Interaction OR ($OR_{Interaction}$) based on the multiplicative model of interaction defined as the ratio of the joint effect of the *MDR1* haplotype and indoor insecticide exposure and product of the individual effects [$OR_{Interaction} = OR_{GE} / (OR_{GE'} \times OR_{G'E})$].

with respect to CGT and TGC haplotypes. Thus, it is likely that the inconsistent reports on the functional nature of the various *MDR1* SNPs may be explained by differences in linkage disequilibrium patterns observed between specific populations (26, 50). These differences will also affect substrate specificity differences observed among synonymous SNP haplotypes of *MDR1*, which were stronger than single SNP variants (49).

Our data suggest that variation in the *MDR1* gene may modify the effect of household pesticide exposure on risk of childhood ALL. Specifically, compared with noncarriers of haplotype CGC, being a carrier of the haplotype seemed to significantly dampen the effect of indoor insecticide exposure on risk childhood ALL. This analysis was motivated by previous findings from a Northern California Childhood Leukemia Study analysis in which Ma et al. (18) reported an increased risk of childhood leukemia associated with household pesticides, particularly for insecticides used indoors during the prenatal period. Previous studies generally support an association between household pesticides and childhood leukemia (4), and one other study has also reported evidence of effect modification of this association, but by genetic variants of *CYP1A1*, a phase I xenobiotic metabolism gene (20). Although the specific mechanisms differ in the way these two genes, *CYP1A1* and *MDR1*, interact with pesticide exposure, both studies provide additional support for a role of household pesticides in the etiology of childhood ALL and support the hypothesis that the risk associated with pesticides may be influenced by individual genetic susceptibility governed, in part, by genes involved in chemical transport and metabolism.

Previous studies evaluating the effects of these specific *MDR1* SNPs and haplotypes on P-glycoprotein function and mRNA expression support this association. Briefly, Salama et al. (51) studied the effects of C1236T, G2677T/A, and C3435T on P-glycoprotein functionality *in vitro* using validated stable recombinant epithelial cells and observed reduced P-glycoprotein activity associated with all three variants both individually and as haplotypes when compared with the wild-type. Song et al. (52) evaluated whether genotypes and haplotypes of G2677T/A and C3435T were associated with hepatic *MDR1* mRNA expression using human liver biopsy samples. Both homozygous variant genotypes individually (2677TT and 3435TT) were associated with significantly lower *MDR1* mRNA expression levels compared with the wild-type genotype, and carriers of the GC haplotype of these two loci had a significantly higher mRNA expression level compared with noncarriers. Results of both these studies on functionality and mRNA expression provide evidence in support of a protective effect of the wild-type alleles or a potentially adverse effect of variant alleles of the SNP loci.

The ethnic diversity of our California study population offered us the unique opportunity to perform subgroup analyses in non-Hispanic White and Hispanic children separately. Because allele frequencies and the results of the association analyses did not seem to differ significantly between these two race/ethnicity groups, we reported the results of the analyses that were conducted among the entire case and control series. Our current sample size did not allow us to pursue analyses separately among the less common race/ethnicities in the study population. The individual matching of cases and controls on maternal race and child's Hispanic status should minimize the potential bias from population substructure and ethnic admixture differences between cases and controls. A limitation of this study is the reduced power to detect interactions between indoor insecticide exposure and the rare haplotypes. For this reason, we focused on the evaluation of the two most common haplotypes, CGC and TTT, which each had a frequency of ~40% in our study population. Another limitation is the inability to evaluate the influence of specific time windows of exposure to indoor insecticides in the interaction analysis because a large proportion of subjects have used indoor insecticides across multiple time periods. For example, >50% of cases used indoor insecticides during both the prenatal and postnatal periods, whereas only 9.2% used indoor insecticides during the prenatal period only and 20.8% during the postnatal period only. When using a crude assessment of timing by categorizing subjects based on whether indoor insecticides were used during a certain time period (yes or no), there was strong evidence of interaction between haplotype CGC and indoor insecticide use during the prenatal period, but not for the postnatal period (data not shown). Furthermore, an evaluation of the importance of the prenatal period should include data on maternal *MDR1* genotypes because studies have shown P-glycoprotein to be highly expressed in human placenta (25). The relative efficiency of maternal cells of the placenta in extruding hydrophobic xenobiotics, including pesticides, could affect the level of exposure to the fetus.

As the first to examine the role of *MDR1* haplotypes in the risk of childhood leukemia, this study provides preliminary evidence of a differential effect of indoor insecticide exposure on childhood ALL risk between carriers and noncarriers of haplotype CGC. These results need to be replicated in an independent population, and future studies should additionally consider other membrane-bound transport proteins in combination with an environmental assessment of relevant substrates in a study of gene-environment interaction in childhood ALL etiology.

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