Glutathione S-Transferases CYP2C9 and CYP2C19 Polymorphisms in Turkish Children with Cancer

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ABSTRACT

We investigated the associations between GSTM1, GSTT1, GSTP1, CYP2C9, CYP2C19 polymorphisms and childhood cancer. Genotypes were analyzed with real-time PCR in 61 children with cancer and 157 healthy controls. Twenty nine of 61 patients were diagnosed of leukemia and 8 of lymphoma. The remaining patients had solid tumors. GSTM1 null genotype was associated with an increased risk of leukemia and lymphoma (OR= 2.2, 95%CI= 1.1-4.6) (p= 0.03). Presence of at least one mutant allele of GSTP1 was related to an increased risk of solid tumor (OR= 3.3, 95%CI= 1.3-8.3) (p= 0.02). Moreover, CYP2C9*2 allele was a high risk for leukemia and lymphoma (p= 0.04). In conclusion, GSTM1, GSTP1, and CYP2C9 polymorphisms may play a role in the development of childhood cancer.

Key Words: Polymorphism, Glutathione S-transferases, Cytochrome P-450, Childhood cancer

ÖZET

Kanserli Türk Çocuklarında Glutatyon S-transferaz, CYP2C9 ve CYP2C19 Polimorfizmleri

Bu çalışmada GSTM1, GSTT1, GSTP1, CYP2C9, CYP2C19 polimorfizmleri ve çocukluk çağı kanserleri arasındaki ilişkinin araştırılması amaçlanmıştır. Kanserli 61 ve sağlıklı 157 çocukta real-time PCR yöntemi kullanılarak genotipler çalışılmıştır. Yirmi yedi hasta lösemi, 8 hasta lenfoma, diğer hastalar solid tümör tanısıyla izlenmekteydi.
GSTM1 null genotipi, artmış lösemi/lenfoma gelişme riski ile ilişkiliydi (OR= 2.2, %95CI= 1.1-4.6) (p= 0.03). GSTP1'de en az bir mutant allel varlığı solid tümör görülmesiyle bağlantılı bulundu (OR=3 .3, %95CI=1 .3-8.3) (p= 0.02). CYP2C9*2 alleli taşıma ile lösemi/lenfoma gelişme riski arasında ilişki saptandı (p= 0.04).
Sonuç olarak, GSTM1, GSTP1 ve CYP2C9 polimorfizmleri çocukluk çağı kanserlerinin gelişmesinde rol oynayabilirler.

Anahtar Kelimeler: Polimorfizm, Glutatyon S-transferaz, Sitokrom P-450, Çocukluk çağı kanseri

INTRODUCTION

The polymorphisms of phase I and II xenobiotic-metabolizing enzymes, such as cytochrome P450 (CYP) and glutathione S-transferases (GSTM, GSTT, and GSTP), strongly influence the individual response to carcinogenic compounds (1-4). Identification of the susceptible population may lead to elucidation of the mechanisms of the cancer and help to design the preventive strategies.

In a few studies focused on the effects of enviromental carcinogens on childhood cancer, CYP1A1 polymorphisms was found to play a critical role in childhood leukemia (5,6). Moreover, CYP2C19 polymorphisms was associated with an increased risk of adult acute leukemia, esophagus, stomach and lung cancer (6,7). There is no reported study questioning the interaction between CYP2C9 polymorphisms and childhood cancer risk.

On the other hand, possible role of GSTM1, GSTT1, GSTP1 polymorphisms for cancer risk, especially in acute leukemia, has been reported not only in adults but also in children (8-12).

This study aimed to investigate the relationship between GSTM1, GSTT1, GSTP1, CYP2C9 and CYP2C19 polymorphisms and different type of childhood cancer in a small group of Turkish children from South Anatolia.

MATERIAL AND METHODS

The study was conducted 61 children, who were diagnosed of cancer in Mersin University, Faculty of Medicine, Department of Pediatric Oncology between February 2005 and October 2006. One hundred fifty seven healthy children were included as the control group. Informed consents were obtained from the legal guardians of all participating children.

DNA extraction and genotyping of GSTT1, GSTM1 and GSTP1, CYP2C9*2, CYP2C9*3, CYP2C19*2, CYP2C19*3:

Blood was collected in EDTA-containing tubes and DNA was extracted from the leucocytes by high pure template preparation kit (Roche diagnostics, GmbH, Mannheim, Germany). The genotyping of GSTT1, GSTM1 and GSTP1 polymorphisms was performed by real time PCR using the Light Cycler DNA Master Hybridization Probes Kit (Roche Diagnostics, Mannheim, Germany). Both the PCR primers and hybridization probes were synthesized by TIB MOLBIOL (Berlin, Germany). The primer and probe sequences are given in Table 1.

The PCR conditions for GSTT1, GSTM1 and GSTP1 genotypes were as follows; 4 mmol/l MgCl2, 0.2 μmol/l of each hybridization probe, 10 pmol of each PCR primers, and 2 μL of the Light Cycler DNA Master Hybridization Mix and 50 ng of genomic DNA in a final volume of 20 μL. These conditions were the same for the amplification of all three mutations (13).

The fluorescence signal was plotted against temperature to give melting curves for each sample. The nature of mutations is gene deletions for GSTT1 and GSTM1, and single nucleotide substitution

Table 1. The sequences of primers and probes

Gene	PCR Primers	Hybridization Probes
GSTT1	5'-TTCCTTACTGGTCCTCACATCTC-3'	5'-LCR640-TCGAAGGCCGACCCAAGCTGGC-3'
	5'-TCCCAGGTCACCGGATCAT-3'	5'-CCGTGGGTGATGGCTGCCAAGT-FL-3'
GSTM1	5'-GAACTCCCTGAAAAGCTAAAGC-3'	5'-LCR640-ATGGCCCGCTTCCCCAGAAACTCTG-3'
	5'-GTTGGGCTCAAATATACGGTGG-3'	5'-TCACTCCTCCTTTACCTTGTTTCCTGCAAAA-FL-3'
GSTP1	5'-ACCCCAGGGCTCTATGGGAA-3'	5'LCR640-TGTGAGCATCTGCACCAGGGTTGGGC-3'
	5'-TGAGGGCACAAGAAGCCCCT-3'	5'-TGCAAATACATCTCCCTCATCTACACCAAC-FL-3'

Table 2. Distribution of GSTM1, GSTT1, GSTP1 polymorphisms and exact test probabilities for Hardy-Weinberg equilibrium in patients and controls

Genotype	All patients		Leukemia/ Lymphoma	Solid tumor	Controls	
	N (%)	Exact p	N (%)	N (%)	N (%)	Exact p
GSTM1						
Present	29 (47.5)		17 (45.9)	12 (50.0)	103 (65.6)	
Null	32 (52.5)	–	20 (54.1)	12 (50.0)	54 (34.4)	–
GSTT1						
Present	39 (63.9)		26 (70.3)	13 (54.2)	114 (72.6)	
Null	22 (36.1)	–	11 (29.7)	11 (45.8)	43 (27.4)	–
GSTP1						
Ile105/Ile105	37 (60.7)		20 (54.1)	17 (70.8)	67 (42.7)	
Ile105/Val105	18 (29.5)	0.1	13 (35.1)	5 (20.8)	74 (47.1)	0.4
Val105/Val105	6 (9.8)		4 (10.8)	2 (8.3)	16 (10.2)	

(A→G) at position 313 of GSTP1 gene. Peaks were obtained at 59°C for the Val105 Val and at 63°C for the Ile 105 Ile and double peak for the Ile 105 Val for GSTP1 genotype. The quantification program of Light Cycler instrument evaluated the presence of either GSTT1 or GSTM1.

CYP2C9*2, CYP2C9*3, CYP2C19*2 and CYP2C19*3 alleles were detected by using Light Cycler CYPC9 and CYPC19 mutation detection kits by real time PCR with Light Cycler instrument (Roche Diagnostics, GmbH, Mannheim, Germany).

Statistical Analysis

Statistical analysis was performed with SPSS 10.0 Program. Chi-Square test was used for determining the association between the studied gene polymorphisms and the groups. The relation between polymorphisms were studied with Mc Nemar test. The relationship between GSTM1, GSTT1, GSTP1, CYP2C9, CYP2C19 polymorphisms and childhood cancer was modelled through multivariate logistic regression analysis. Odds ratio and confidence intervals were used to analyze the frequencies of polymorphisms in patients compared to the control groups. In this study, maximum type I error was set at 0.05. HelixTree Genetic Analysis Software 4.3.3 was used for Hardy-Weinberg equilibrium.

RESULTS

Demographic characteristics of patients and controls were similar ($p>0.05$). There were 31 boys and 30 girls in the patient group. The numbers of boys and girls in the control group were 77 and 80, respectively. Twenty nine of 61 patients were diagnosed of leukemia and 8 of lymphoma. Other diagnosis were Wilms' tumor (10/24), neuroblastoma (5/24), brain tumors (3/24), soft tissue sarcoma (2/24), Ewing's sarcoma (1/24), mixed germ cell tumor (2/24), nasopharynx carcinoma (1/24).

Patients and controls were on the Hardy-Weinberg equilibrium for the alleles of GSTP1, CYP2C9, and CYP2C19 ($p>0.05$) (Table 2 and 3). Frequencies of the studied gene polymorphisms was similar in girls and boys in healthy controls. Among patients, the frequency of the GSTM1 null genotype was

Table 3. Distribution of CYP2C polymorphisms and exact test probabilities for Hardy-Weinberg equilibrium in patients and controls

Genotype	All patients		Leukemia/lymphoma N (%)	Solid tumor N (%)	Controls	
	N (%)	Exact p			N (%)	Exact p
CYP2C9*2						
Wild	24 (77.4)		12 (75.0)	12 (80.0)	90 (92.8)	
Heterozygous	5 (16.1)	0.05	2 (12.5)	3 (20.0)	7 (7.2)	0.7
Homozygous	2 (6.5)		2 (12.5)	Not present	Not present	
CYP2C9*3						
Wild	21 (67.7)		10 (62.5)	11(73.3)	77 (79.4)	
Heterozygous	10 (32.3)	0.2	6 (37.5)	4 (26.7)	20 (20.6)	0.2
Homozygous	Not present		Not present	Not present	Not present	
CYP2C19*2						
Wild	47 (77.0)		28 (75.7)	19 (79.2)	126 (80.3)	
Heterozygous	14 (23.0)	0.3	9 (24.3)	5 (20.8)	31 (19.7)	0.1
Homozygous	Not present		Not present	Not present	Not present	

higher in boys (64.5% vs. 40%) (p=0.055) (OR= 2.7, 95%CI 0.9-7.6) though in borderline significance. This genotype was associated with an increased risk for leukemia and lymphoma (p=0.03)(OR=2.2, 95% CI=1.1-4.6) (Table 4). The frequency of GSTM1 null genotype was similar in patients with solid tumor and controls (p=0.1). We did not find any relationship between the GST1 null genotype and childhood cancer risk (p=0.2). Furthermore, having both of GSTM1 and GSTT1 null genotypes was related with an increased risk of childhood cancer (p=0.02) (OR=2.5, 95% CI=1.1-5.7). The precence of at least one mutant allele of GSTP1 was a risk factor for solid tumors (p=0.02) (OR=3.3, 95% CI 1.3-8.3) (Table 4).

The frequency of CYP2C9*2 allele in patients was higher than that of the controls (p=0.02). The presence of this allele was a risk factor for leukemia and lymphoma (OR=4.3, 95%CI=1.1 – 16.8) (p=0.04) (Table 5). CYP2C9*3 and CYP2C19*2 polymorphisms were not found to be associated with childhood cancer risk (p=0.1, p=0.6, respectively). The frequencies of CYP2C9*2, CYP2C9*3 and CYP2C19*2 alleles were similar in groups with leukemia/ lymphoma, and solid tumor. CYP2C19*3 polymorphisms were detected neither in patients nor controls.

Finally, in a multivariate logistic regression analysis including GSTM1, GSTP1 and CYP2C9*2, GSTM1 null genotype was found to be the most important factor for childhood cancer risk (OR=2.9, 95% CI=1.1-7.3) (p=0.008).

DISCUSSION

This is one of the few reports on GSTM1, GSTT1, GSTP1 polymorphisms in children who have leukemia/lymphoma or solid tumors. Additionally, this is the first that draws attention to a possible relationship between CYP2C9*2 allele and childhood cancer. We found that GSTM1 null genotype and CYP2C9*2 allele were associated with an increased risk for leukemia/lymphoma whereas having

Table 4. Relative risks and 95% confidence limits for tumor types in children carrying GSTM1, GSTT1, and GSTP1 polymorphisms

Genotype	All tumor types		Leukemia/ lymphoma		Solid tumor	
	OR	95%CI	OR	95%CI	OR	95%CI
GSTM1						
Present						
Null	2.1 (p=0.02)	1.1-3.8	2.2 (p =0.03)	1.1-4.6	1.9 (p =0.14)	0.8-4.5
GSTT1						
Present						
Null	1.5 (p =0.20)	0.8-2.8	1.1 (p =0.77)	0.5-2.5	2.2 (p =0.07)	0.9-5.4
GSTP1						
Wild						
Mutant	2.1 (p =0.02)	1.1-3.8	1.6 (p =0.21)	0.8-3.2	3.3 (p =0.02)	1.3-8.3

OR: Odds Ratio; CI: Confidence Interval.

at least one mutant allele of GSTP1 was a risk factor for solid tumors.

Davies et al. (12) reported that GSTM1 null genotype was a significant risk factor for childhood acute myeloblastic leukemia, whereas GSTT1 null genotype was not. Similarly, Krajinovic et al. (9) emphasized that GSTM1 null genotype was a risk factor for acute lymphoblastic leukemia. Moreover, they suggested that acute leukemia might be associated with xenobiotic-metabolism, and thus with environmental exposures. In the meta-analysis, Ye et al. (14) suggested that GSTM1 and GSTT1 null genotypes might play a role in leukemia development. Our results are compatible with these results for the GSTM1 null genotype. On the contrary, Balta et al. (10) did not detect an increased leukemia risk in children carrying this genotype. This may be explained with ethnic differences between the Central and South Anatolia regions in our country. On the other hand, PCR assay used for GSTM1 and GSTT1 genotyping in most of these studies did not allow to distinguish homozygous nonnull genotypes from heterozygous nonnull plus null genotypes. This may be a disadvantage for understanding the true association between cancer risk and genotypes of GSTM1 and GSTT1.

Ezer et al. (15) suggested that GSTM1 present and GSTP1 Val114/Val114 genotypes might affect on brain tumor development in children. Our results emphasized that carrying at least one mutant allele of GSTP1 may increase the risk for solid tumor development in children. Although the study population is small, our findings should draw attention to the role of interactions of GSTs polymorphisms and genotoxic exposure in childhood cancer. However, this should be further studied in larger populations.

The two most common variant alleles of CYP2C9 are CYP2C9*2 and CYP2C9*3. Among northern Europeans, the allele frequencies of CYP2C9*2 and CYP2C9*3 are 10% and 8%, respectively (3,16). These alleles occur more rarely in other ethnic groups, including Orientals and African-Americans. In our healthy controls, the frequency of CYP2C9*2 allele was similar to northern Europeans' whereas the frequency of CYP2C9*3 allele was higher than theirs. Our preliminary study was the first report describing a possible association

Table 5. Relative risks and 95% confidence limits for tumor types in children carrying CYP2C polymorphisms

Genotype	All tumor types		Leukemia/ lymphoma		Solid tumor	
	OR	95%CI	OR	95%CI	OR	95%CI
CYP2C9*2						
Wild						
Mutant	3.7 (p =0.02)	1.1-11.7	4.3 (p =0.04)	1.1-16.8	3.2 (p =0.12)	0.7-14.1
CYP2C9*3						
Wild						
Mutant	1.8 (p =0.19)	0.7-4.5	2.3 (p =0.15)	0.8-7.1	1.4 (p =0.60)	0.4-4.9
CYP2C19*2						
Wild						
Mutant	1.2 (p =0.60)	0.6-2.5	1.3 (p =0.54)	0.6-3.0	1.1 (p =0.90)	0.4-3.1

OR: Odds ratio; CI: Confidence interval

between the presence of CYP2C9*2 allele and leukemia and lymphoma development.

Contradictory results in reported studies evaluating the association of xenobiotic-metabolizing enzymes polymorphisms with cancer risk may be related to the sample size, different environmental exposures, ethnic differences in metabolic activation or detoxification of carcinogenic chemicals, or the interactions between polymorphisms of these enzymes. The major drawback of this study was the small sample size of the population. In conclusion, GSTM1 null genotype, mutant genotype of GSTP1, and CYP2C9*2 allele may have a critical role in the development of childhood cancer.

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