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Original Article

Deletion polymorphism of the *UGT2B17* gene and relapse among Japanese children with cancer

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Abbreviations

95% CI	95% confidence interval
ALL	acute lymphoblastic leukemia
ANOVA	analysis of variance
CNV	copy number variant
CGH	comparative genomic hybridization
Ct	cycle threshold
HWE	Hardy-Weinberg equilibrium
HR	hazard ratio
LBL	lymphoblastic lymphoma
OR	odds ratio
PCR	polymerase chain reaction
RT-PCR	reverse transcription polymerase chain reaction
SNP	single nucleotide polymorphism
UGT2B17	UDP-glucuronosyltransferase 2 family polypeptide B17

Abstract

Background: The UDP-glucuronosyltransferase 2 family, polypeptide B17 (*UGT2B17*) gene encodes an enzyme that modifies carcinogens, C19 steroids, xenobiotics, and anticancer chemotherapeutic agents by glucuronidation. Pediatric cancers are much more sensitive to anticancer agents than adult cancers. Therefore, in this study, we examined the effects of deletion polymorphism of the *UGT2B17* gene on prognosis in patients with pediatric cancer.

Procedure: A total of 145 DNA samples were collected from children with malignant diseases. Copy number variants (CNVs) of the *UGT2B17* gene were determined using polymerase chain reaction (PCR). Survival analyses were computed to analyze effects of *UGT2B17* gene deletion on relapse-free rates in lymphoblastic and non-lymphoblastic malignancies.

Results: The *UGT2B17* gene was deleted in 64% of children with lymphoblastic malignancies but in 83% of children with non-lymphoblastic malignancies. Moreover, in non-lymphoblastic malignancies, children without deletion polymorphism of the *UGT2B17* gene had significantly higher relapse rates than those with deletion polymorphism of the *UGT2B17* gene (hazard ratio, 16.1; 95% confidence interval [CI], 1.67–154; *P* = 0.016), which remained significant

(*P* = 0.032) after adjustment for age, sex, and underlying diseases (hazard ratio, 26.1; 95% CI, 1.33–510; *P* = 0.032). There was a significant interaction between *UGT2B17* gene deletion and non-lymphoblastic malignancies.

Conclusions: Deletion polymorphism of the *UGT2B17* gene may improve the relapse-free rate in children with non-lymphoblastic malignancies.

Key words: Cancer; Pediatrics; Prognosis; Relapse; Susceptibility; UGT

Introduction

The UDP-glucuronosyltransferase 2 family, polypeptide B17 (*UGT2B17*) gene encodes an enzyme that modifies carcinogens, C19 steroids, xenobiotics, and anticancer agents by glucuronidation [1-3]. In chronic lymphocytic leukemia, high expression of *UGT2B17* mRNA was shown to be associated with shorter treatment-free and overall survival [4]. Therefore, the presence of *UGT2B17* may facilitate metabolism of anticancer agents within cancer cells, thereby reducing the effects of such drugs and resulting in poor prognoses. Interestingly, deletion polymorphism of the *UGT2B17* gene is common in Japanese individuals [5-8]. Generally, pediatric cancers are much more sensitive to anticancer agents than adult cancers.

Therefore, in this study, we aimed to determine the effects of deletion polymorphism of the *UGT2B17* gene on prognosis in patients with pediatric cancer.

Methods

Study design

We conducted a prospective cohort study at Tokyo Metropolitan Children's

Medical Center and Jikei University from July 2011 to May 2014. The study protocol was consistent with the Declaration of Helsinki and was reviewed and approved by the ethics committees of the Institutional Review Boards of Tokyo Metropolitan Children's Medical Center and the Jikei University School of Medicine, Tokyo, Japan. Eligible participants were patients under 21 years old; had newly or previously diagnosed pediatric cancer by means of morphological, cytogenetic, and immunophenotypic assessment; and were treated at Tokyo Metropolitan Children's Medical Center or the former hospital (Tokyo Metropolitan Kiyose Children's Hospital) by chemoradiotherapy mainly according to the study protocols of the Tokyo Children's Cancer Study Group. At Tokyo Metropolitan Children's Medical Center, all of the enrolled patients and/or their parents provided written informed consent for collection of genomic DNA and analysis of associations with clinical data. Genetic and statistical analyses were performed at the Division of Molecular Epidemiology, Jikei University School of Medicine.

Samples

Genomic DNA was extracted from peripheral blood. The DNA was purified using

a QIAamp DNA Micro Kit 50 (Qiagen, Tokyo, Japan), and the concentration of DNA was measured using a NanoVue plus (General Electric Healthcare Japan, Tokyo, Japan). The DNA was frozen at -80°C until analyses.

Detection of deletion of the UGT2B17 gene by polymerase chain reaction (PCR)

To differentiate copy number variants (CNVs), i.e., 0, 1, or 2 copies of the *UGT2B17* gene, we performed PCR using the following *UGT2B17* gene-specific primers: Marker D (forward primer 5′ -TCACAAGTCAATCTCCCATCC-3′ , reverse primer 5′ -CTGCAGAATATGTCAATAATTGGC-3′), for detection of one or two copies (100 bp); and Marker J (forward primer 5′ -TGCACAGAGTTAAGAAATGGAGAGATGTG-3′ , reverse primer 5′ -GATCATCCTATATCCTGACAGAATT-3′), for detection of only one copy (900 bp) [8]. PCR was carried out in a final volume of 25 μL containing 1 μL genomic DNA, 2.5 μL of 10× LA PCR buffer II, 2 μL dNTPs, 0.25 μL LA Taq (TaKaRa Bio Inc., Shiga, Japan), 18.25 μL nuclease-free water, and 0.5 μL of each primer. The reactions were performed by incubation at 94°C for 3 min followed by 30 cycles at 94°C for 20 s, 60°C for 30 s, and 72°C for 90 s.

Statistical analysis

To evaluate significant differences in patient characteristics associated with deletion of the *UGT2B17* gene, Student's t-tests and chi-square tests were used to analyze continuous and categorical variables, respectively.

In survival analyses, relapse-free times were calculated as the date of diagnosis to the date of cancer relapse. Follow-ups were censored at the time of the patient's death by causes other than relapse or at the last outpatient clinic visit. Hazard ratios (HRs) with 95% confidence intervals (95% CIs) were computed using the Cox proportional hazard model. HRs were adjusted according to age, sex, and underlying diseases. The $P_{Interaction}$ between *UGT2B17* gene deletion and non-lymphoblastic malignancies was calculated using the Mantel-Haenszel homogeneity test and multiple Cox proportional hazard model. Results with *P* values of less than 0.05 were considered statistically significant. All statistical analyses were performed using STATA 13.1 (STATA Crop., College Station, TX, USA).

Results

Study population

Of 162 eligible Japanese patients, 146 agreed to participate in this study and provided written informed consent. One patient was excluded because her pathological diagnosis was ganglioneuroma, a type of benign tumor rather than a malignancy. However, one child with Langerhans cell histiocytosis was included because his disease was considered clinically malignant, and he was treated with chemotherapy. In total, 145 patients, including 74 with lymphoblastic malignancies and 71 with non-lymphoblastic malignancies, were analyzed.

UGT2B17 CNVs and patient characteristics

UGT2B17 CNVs were determined using PCR. The results yielded the following distributions of CNVs in the patients in this study: no copies (= deletion polymorphism), 73%; one copy, 27%; and two copies, 0%. None of the 145 patients had two copies of the *UGT2B17* gene.

The clinical characteristics of the patients with deletion polymorphism (no copies) and without deletion polymorphism (one copy) of the *UGT2B17* gene were compared (Table I). Deletion polymorphism of the *UGT2B17* gene was observed in 64% of children with lymphoblastic malignancies but in 83% of children with non-lymphoblastic malignancies. Deletion polymorphism of the

UGT2B17 gene was not significantly associated with gender, age at diagnosis, or each type of cancer as the underlying disease. Three cases of secondary malignancies were observed in the patients, and all patients had deletion polymorphisms of the *UGT2B17* gene; no significant differences were detected owing to the small number of cases.

Relapse-free survival and UGT2B17 CNVs

Initially, Kaplan-Meier curves were drawn to determine the relationships between the relapse-free ratio and *UGT2B17* gene deletion. When all cases were included, there were no significant differences in relapse-free rates between patients with deletion polymorphism of the *UGT2B17* gene and those without deletion polymorphism of the *UGT2B17* gene (Figure 1A). Next, patients were stratified by lymphoblastic or non-lymphoblastic malignancies. In the same comparison, there were no differences in relapse-free rates in lymphoblastic malignancies (Figure 1B). In contrast, in the group of non-lymphoblastic malignancies, patients without deletion polymorphism of the *UGT2B17* gene had significantly higher relapse rates than those with deletion polymorphism of the *UGT2B17* gene (log-rank test: P = 0.0012; HR, 16.1; 95% CI, 1.67–154; P =

0.016; Figure 1C). The HR remained significant even after adjustment for age, sex, and underlying diseases (HR, 26.1; 95% CI, 1.33–510; P = 0.032). Finally, the interaction between *UGT2B17* CNVs and non-lymphoblastic malignancies was statistically evaluated. This analysis indicated that $P_{interaction}$ was significant in either the Mantel-Haenszel homogeneity test (P = 0.044) or in the multiple Cox proportional hazard model (P = 0.030).

Discussion

In this study, we evaluated the effects of deletion polymorphism of the *UGT2B17* gene on prognosis in patients with pediatric cancer. Our results provided important insights into the impact of this genetic effect on pediatric cancer.

In this study, when we restricted our analysis to children with non-lymphoblastic malignancies, patients with the *UGT2B17* gene (one copy) showed a higher relapse rate than those with a deletion polymorphism in the *UGT2B17* gene (no copies). The effects of deletion of *UGT2B17* on relapse-free rates were independent of sex, age of diagnosis, and underlying diseases. *UGT2B17* mRNA levels in primary chronic lymphocytic leukemia samples were directly correlated with functional glucuronidation activity toward androgens and the anticancer drug vorinostat as well as poor prognosis [4]. Glucuronidases encoded by the *UGT1A* gene, which belongs to the same family as UGT2B17, have been reported to be upregulated in cytarabine-resistant acute myeloblastic leukemia cells [9], suggesting that *UGT2B17* gene products may be in response to the presence of anticancer agents. Moreover, UGT2B17 expression has been shown to be upregulated in endometrial cancer tissues, whereas UGT2B17 depletion inhibits cell growth and induces apoptosis [10], implying that UGT2B17

may play a direct role in maintaining the survival of cancer cells and in metabolism of anticancer agents. These findings support the hypothesis that deletion polymorphism of the *UGT2B17* gene may reduce detoxification of anticancer agents and suppress tumor growth, which could contribute to the improved prognoses observed in patients with *UGT2B17* gene deletion. However, we could not detect significant differences in lymphoblastic malignancies.

There are several limitations to this study. First, the study population was composed of various pediatric cancers. In future studies, it will be necessary to focus on a specific disease, such as neuroblastoma. Second, no patient had two copies of the *UGT2B17* gene. Thus, results of this study may not be generalized to other countries. Third, we did not analyze *UGT2B17* mRNA levels. However, in theory, gene deletion should block the expression of the mRNA and protein.

In conclusion, deletion polymorphism of the *UGT2B17* gene may improve the relapse-free rate in children with non-lymphoblastic malignancies treated with anticancer chemotherapeutic agents. This result implies that novel drugs suppressing UGT2B17 function may enhance the sensitivity of cancer cells to anticancer agents.

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Conflict of Interest statement

The authors declare that they have no competing interests.

Authors' contribution

SI and MU designed the study. SI, YY, and TK contributed to collection of tissues and clinical data. MU performed statistical analyses and interpreted the data. SI and MU drafted the manuscript. All authors have read and approved the final manuscript.

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Figure legends

Figure 1. Kaplan-Meier curves of relapse-free rates according to UGT2B17 CNV status: UGT2B17 (-) = UGT2B17 gene deletion versus UGT2B17 (+) = UGT2B17 one copy. Kaplan-Meier curves were constructed to compare the relapse rates of (A) all patients, (B) patients with lymphoblastic malignancies, and (C) patients with non-lymphoblastic malignancies. The *P*-value was calculated by the log-rank test.

Tables

TABLE Ⅰ. Patient characteristics stratified by UGT2B17 polymorphisms

	Total	UGT2B17 CNVs		
	n = 145 (100)	0 copies: n = 106 (73)	1 copy: n = 39 (27)	*p*-value
Male, n (%)	80 (55)	60 (57)	20 (51)	0.57†
Age at diagnosis, years, mean ± SD	9.2 ± 4.8	9.1 ± 4.5	9.2 ± 5.7	0.96‡
Underlying disease				
Lymphoblastic malignancy, n (%)	74 (100)	47 (64) §	27 (36) §	0.41†
B-ALL	55 (100)	37 (67)	18 (33)	
B-LBL	2 (100)	1 (50)	1 (50)	
T-ALL	7 (100)	3 (43)	4 (57)	
T-LBL	3 (100)	2 (67)	1 (33)	
ALL MLL (-)	2 (100)	1 (50)	1 (50)	
ALL MLL (+)	2 (100)	2 (100)	0 (0)	
ALL in Down's syndrome	2 (100)	0 (0)	2 (100)	
Biphenotypical ALL	1 (100)	1 (100)	0 (0)	
Non-lymphoblastic malignancy n (%)	71 (100)	59 (83) ¶	12 (17) ¶	0.54†

AML	8 (100)	8 (100)	0 (0)	
AML in Down's syndrome	3 (100)	2 (67)	1 (33)	
Hodgkin lymphoma	1 (100)	1 (100)	0 (0)	
Anaplastic large cell lymphoma	1 (100)	1 (100)	0 (0)	
Burkitt's lymphoma	1 (100)	1 (100)	0 (0)	
Juvenile myelomonocytic leukemia	2 (100)	1 (50)	1 (50)	
Langerhans cell histiocytosis	1 (100)	1 (100)	0 (0)	
Neuroblastoma	14 (100)	11 (79)	3 (21)	
Wilm's tumor	12 (100)	9 (75)	3 (25)	
Hepatoblastoma	6 (100)	3 (50)	3 (50)	
Rhabdomyosarcoma	10 (100)	9 (90)	1 (10)	
Retinoblastoma + osteosarcoma	1 (100)	1 (100)	0 (0)	
Ewing sarcoma	2 (100)	2 (100)	0 (0)	
Brain tumor	5 (100)	5 (100)	0 (0)	
Germ cell tumor	4 (100)	4 (100)	0 (0)	
Secondary malignancies ∫	3 (100)	3 (100)	0 (0)	0.29†

†. *P*-values were calculated using chi-square tests. ‡. *P*-values were calculated using ANOVA.

§. Hardy-Weinberg equilibrium test, $P = 0.055$. ¶. Hardy-Weinberg Equilibrium test, $P = 0.44$.

∫. One AML developed in a case of B-ALL 2.6 years later; one osteosarcoma developed in an osteosarcoma + retinoblastoma case 14 years later; one thyroid cancer developed in a neuroblastoma case 13 years later.

Figure 1A

All 145 cases included

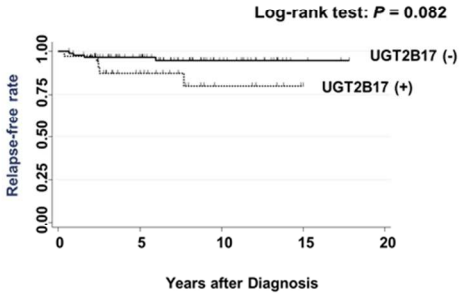


Figure 1B

Lymphoblastic malignancies only

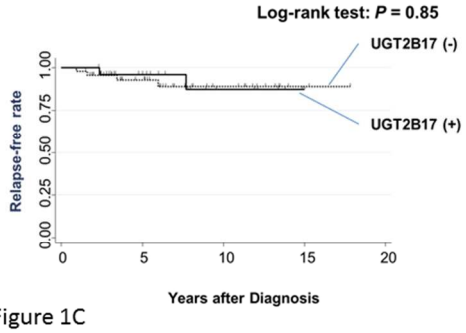
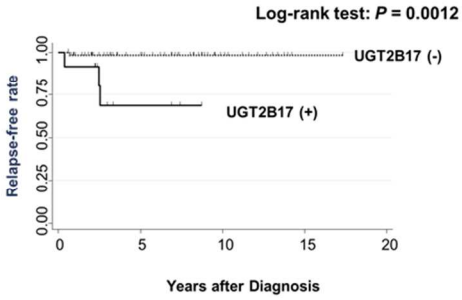


Figure 1C

Non-Lymphoblastic malignancies only



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