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Genetic susceptibility to childhood acute lymphoblastic leukemia shows protection in Malay boys: Results from the Malaysia-Singapore ALL Study Group

Allen Eng-Juh Yeoh[a,*], Yi Lu[a], Jason Yong-Sheng Chan[a], Yiong Huak Chan[b], Hany Ariffin[c], Shirley Kow-Yin Kham[a], Thuan Chong Quah[a]

[a] Department of Paediatrics, Division of Haematology and Oncology, Yong Loo Lin School of Medicine, National University Health System, Singapore
[b] Biostatistic Unit, Yong Loo Lin School of Medicine, National University Health System, Singapore
[c] Department of Paediatrics, Division of Haematology and Oncology, University of Malaya, Kuala Lumpur, Malaysia

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ABSTRACT

To study genetic epidemiology of childhood acute lymphoblastic leukemia (ALL) in the Chinese and Malays, we investigated 10 polymorphisms encoding carcinogen- or folate-metabolism and transport. Sex-adjusted analysis showed *NQO1* 609CT significantly protects against ALL, whilst *MTHFR* 677CT confers marginal protection. Interestingly, we observed that *NQO1* 609CT and *MTHFR* 1298 C-allele have greater genetic impact in boys than in girls. The combination of *SLC19A1* 80GA heterozygosity and 3′-*TYMS* −6 bp/−6 bp homozygous deletion is associated with reduced ALL risk in Malay boys. Our study has suggested the importance of gender and race in modulating ALL susceptibility via the folate metabolic pathway.

1. Introduction

The initiating event of the majority of childhood acute lymphoblastic leukemia (ALL), the most common form of pediatric cancer, is believed to have started in utero. This hematological malignancy is postulated to be the unfortunate outcome of the interaction of the patient's genetic susceptibility factors and the exposure to environmental carcinogens during fetal life and infancy [1]. Specifically, an individual's risk of developing leukemia may be influenced by the genetic variants of enzymes involved in metabolisms of environmental carcinogens and folates, as well as in molecular transportation (e.g. membrane channels). The relatively short latency between the initiating events (e.g. mutations) and the appearance of tumor cells in childhood ALL offers a conducive model to examine the effects of carcinogen- and folate-metabolizing genes in cancer susceptibility [1–4].

In their original forms, environmental carcinogens are rarely reactive [5]. Phase I enzymes like the cytochrome P-450 superfamily may activate numerous procarcinogens whilst Phase II enzymes detoxify them through acetylation, glucuronidation or methylation into non-reactive and water-soluble products [6]. Glutathione-S-transferases (GSTs) and NAD(P)H dehydrogenase, quinone 1 (*NQO1*) belong to this latter group [7,8]. Unlike metabolic enzymes which alter their substrates, molecular transporters determine the kinetics and disposition of endogenous xenobiotics. The ATP-binding cassette, sub-family B (MDR/TAP), member 1 (*ABCB1*, also known as *MDR1*), encodes for P-glycoprotein which actively transports a large number of amphipathic molecules out of the cell, conferring protection from toxic xenobiotics [9]. On the other hand, membrane transporter – solute carrier family 19 (folate transporter), member 1 (*SLC19A1*, also known as *RFC1*) – is the primary transporter for folate into mammalian cell [10]. Recently, the lack of folates is complicated in leukemogenesis [2,11] because of its close association with the susceptibility to chromosomal damage [3,12]. However, these findings have been mainly heterogeneous and incongruous [13,14]. Adding to this, the modulation of the folate pathway by gender further complicates the link between the folate pathway and childhood ALL. Homozygous carriers of the *MTHFR* 677T-allele have been shown to elevate plasma homocysteine levels under low

* Corresponding author at: Department of Paediatrics, Level 4, Main Building, National University Hospital, 5, Lower Kent Ridge Road, Singapore 119074, Singapore. Tel.: +65 6772 4420; fax: +65 6779 7486.
E-mail address: allen.yeoh@nus.edu.sg (A.E. Yeoh).

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Table 1
Demographic characteristics of children with ALL.

Characteristics	Patients	Controls
Chinese		
No. of cases	321	346
T-lineage ALL	25	–
B-lineage ALL	287	–
Infant ALL	9	–
Age (year range)	0.1–19.4	–
Mean ($\pm S_E$)	5.8 ± 0.2	–
Median	4.8	–
Gender		
Male (%)	185 (57.6)	156 (45.1)
Female (%)	136 (42.4)	190 (54.9)
Ratio (M/F)	1.36	0.82
Malays		
No. of cases	210	410
T-lineage ALL	10	–
B-lineage ALL	189	–
Infant ALL	11	–
Age (year range)	0.4–18.5	–
Mean ($\pm S_E$)	5.6 ± 0.3	–
Median	4.6	–
Gender		
Male (%)	123 (58.6)	180 (43.9)
Female (%)	87 (41.4)	230 (56.1)
Ratio (M/F)	1.41	0.78

folate conditions especially in male children [15]. Apart from the fact that the male gender has a predisposition to childhood ALL, recent evidence suggests a gender-dependent modulation of key enzymes in folate metabolism.

To date, many studies, primarily in Caucasian and African populations, have attempted to correlate genetic variations with the risk of developing childhood ALL but have yielded conflicting reports. This can be explained in part by the intrinsic genetic diversity among the different ethno-geographic cohorts. This unique genetic randomization provided by nature allows researchers to investigate the different effects of ethnicity with its inherent genetic diversity on risk of developing cancer. However, there are limited epidemiologic data of childhood ALL in Asians, and to our best knowledge, none for the Malays. In the current study, we investigated 10 polymorphisms in 7 genes (*GSTM1*, *GSTT1*, *NQO1* 609C>T, *ABCB1* 3435C>T, *MTHFR* 677C>T, *MTHFR* 1298A>C, *MTHFD1* 1958G>A, 3′-*TYMS* 1494 6 bp deletion/insertion, 5′-*TYMS* 28 bp repeats, and *SLC19A1* 80G>A) in a large cohort of 531 Chinese and Malay children with ALL to explore their impact on the susceptibility to developing childhood ALL. In addition, we will examine the interaction of gender with these polymorphisms which may help in our understanding of the higher incidence of childhood ALL in boys.

2. Materials and methods

2.1. Patients and control samples

Anonymized archival cord blood of 756 healthy newborns (346 Chinese and 410 Malays) and diagnostic bone marrows of 531 children with ALL (321 Chinese and 210 Malays) were collected at Malaysia-Singapore Study Group centers – National University Hospital in Singapore and University of Malaya Medical Centre in Kuala Lumpur, Malaysia – from 1998 till 2008. The sex ratios (boys vs. girls) in the healthy newborns enrolled are 0.82 in the Chinese and 0.78 in the Malays. Demographic characteristics of patients and controls were summarized in Table 1. The research protocol was approved by the local Institutional Review Board, and informed consents were obtained from all participating individuals and/or their parents.

Genomic DNA was extracted using standard phenol–chloroform methodology and stored at −80 °C. DNA concentrations and A_{260}/A_{280} ratios were measured using a UV spectrophotometer. A sample was considered eligible if its concentration was higher than 50 ng/μL and A_{260}/A_{280} ratio was between 1.6 and 2.0.

2.2. Genotyping

A total of 10 germline variants were selected for the study (Table 1S). These candidate genes were presumably shown to affect the phenotypes and were implicated to influence leukemia risk previously [16–22]. Genotypings were carried out using published polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) methodologies [23–26]. The PCR products were subjected to 3% agarose gel electrophoresis and ethidium bromide staining, followed by image analysis using the Gel Doc system (Bio-rad Laboratories).

2.3. Statistical analysis

Hardy–Weinberg equilibrium (HWE) was tested for the observed genotype frequencies in the population controls using a free web-based tool (http://www.genes.org.uk/software/hardy-weinberg.shtml).

The effects of each individual gene or gene–gene combination on childhood ALL were analyzed using a binary logistic regression model including the gender as a covariant. This regression analysis was also used to assess the gender–genotype interaction so as to decide whether a gender-subgroup analysis was to be performed (if $P < 0.10$), in which Fisher's exact test was used for boys and girls respectively. The result satisfying unadjusted $P < 0.05$ and respective odds ratio (OR) with 95% confidence intervals (CI) are presented. To account for multiple comparisons, an observed association is considered statistically significant only if $P < 0.005$, and marginally significant if $0.005 \leq P < 0.01$. All statistical analyses were done using the SPSS® version 16.0 for Windows (SPSS Inc., Chicago, IL).

3. Results

3.1. Individual genotype analysis

As shown in Table 2, when stratified by ethnicity, Malay carriers of the *NQO1* 609CT genotype show reduced ALL risk, suggesting a protective effect of this variant on leukemogenesis ($P = 0.002$; OR = 0.535, 95% CI 0.360–0.794). In the Chinese cohort, carriers of *MTHFR* 677CT genotype are underrepresented in the cases than in controls; however, it only implicates a marginal significant association with decreased risk of ALL ($P = 0.006$; OR = 0.633, 95% CI 0.457–0.877).

When boys and girls are analyzed respectively, the *NQO1* 609CT genotype shows significant protective effect on leukemia risk in Malay boys only ($P = 0.001$; OR = 0.378, 95% CI 0.216–0.661). In contrast, the *MTHFR* 1298 C-allele is significantly associated with an increased leukemia risk in the Chinese boys as compared to girls ($P = 0.005$; OR = 1.691, 95% CI 1.171–2.443; details not shown for allele-based analysis).

3.2. Combined genotype analysis

We investigated if gene–gene interaction within the folate metabolic pathway would influence leukemia risk. Using the results obtained in our univariate analysis, we classified genotypes that are likely to increase risk of ALL as 'high-risk' genotypes and vice versa. We interrogated this gene–gene interaction by comparing the frequency distributions of the 'high-risk' genotypes with the other genotype combinations between the cases and controls as shown in Table 3 .

In the Malay cohort, the combined effect of *SLC19A1* 80G>A and 3′-*TYMS* 1494 −6 bp/−6 bp reveals a marginal protective effect on leukemia risk ($P = 0.007$; OR = 0.056, 95% CI 0.007–0.456). However, when stratified by gender, this gene combination is significantly associated with reduced risk of leukemia among the boys, but not in the girls ($P = 0.001$; OR = 0.333, 95% CI 0.237–0.469). Similarly, carriers of *SLC19A1* 80GA with *MTHFR* 1298AA also suggest a potential protective effect against risk in ALL among the boys than in the girls. In both the Malay and Chinese cohort, only carriers of *MTHFR* 677CT and 1298AA genotypes suggest a protective gender effect on leukemia risk as compared to individuals carrying *MTHFR* 677CC and 1298AC.