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Original Article

The association of *DNA methyltransferase 1* gene polymorphisms with susceptibility to childhood acute lymphoblastic leukemia

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ABSTRACT

Background: It has been suggested that aberrant DNA methylation is a common epigenetic alteration in malignancies. Genetic variations in *DNA methyltransferase 1 gene* (*DNMT1*), which encodes the maintenance methyltransferase, have been demonstrated to be involved in cancer susceptibility. In the present study, we investigated whether genetic polymorphisms in *DNMT1* could be associated with risk of childhood acute lymphoblastic leukemia (ALL) in a Chinese population.

Methods: We selected seven tagging single-nucleotide polymorphisms (tagSNPs, rs11880388, rs10423341, rs7253062, rs11085721, rs2228611, rs2228612 and rs16999593) in *DNMT1* and genotyped these SNPs by using TaqMan method in a case-control study of 377 patients with ALL and 500 healthy controls. The logistic regression was used to assess the genetic associations with occurrence of ALL with adjustment for possible confounders.

Results: We found that one (rs11085721) of the seven tagSNPs was significantly associated with the risk of ALL. Compared with individuals' with *DNMT1* rs11085721 GG genotype, those subjects carrying the rs11085721 GT genotypes were associated with significantly increased risk for ALL (GT vs. GG:OR = 1.29, 95% CI = 1.10–1.51). Similar association was also observed when combined the individuals with rs11085721 GT and rs11085721 TT genotypes (GT/TT vs. TT:OR = 1.29, 95% CI = 1.10–1.50). No positive results were observed for the other tagSNPs.

Conclusions: Our results suggest that the *DNMT1* rs11085721 polymorphism may confer susceptibility to ALL in the Chinese population. The initial findings should be validated by large population-based prospective studies in the future.

1. Introduction

Acute lymphoblastic leukemia (ALL) is the most common malignancy in children, accounting for nearly one third of all pediatric cancers [1]. The annual incidence rate of ALL is approximately 38 new cases per million people in childhood with a peak incidence at 2 to 5 years of age [2]. Although the clinical and pathological feathers of ALL are well described [3], the exact causes of this disease have not been identified yet. Epidemiological studies has suggested that exposure to environmental factors, such as parental smoking, pesticides, traffic fumes, paint and house hold chemicals could be associated with increased risk of ALL [4]. However, as children are usually less exposed to these factors and only a proportion of exposed children develop ALL, nowadays, genetic predisposition to ALL has also been considered to be important in children [5]. To date, genetic variations in a number of candidate genes have been proven to influence ALL susceptibility [5–8].

It has been suggested that epigenetic alterations played an important role in the development of human cancer [9,10]. Epigenetic events can occur in the initial phase of carcinogenesis and

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may precede malignant transformation [11]. DNA methylation, which is the major epigenetic modification of DNA in mammalian genomes, is established and maintained by DNA methyltransferase (DNMTs). The DNMTs family includes DNMT1, DNMT3A, DNMT3B, DNMT3L, and DNMT2 [12]. Of these members, DNMT1functions mainly as a maintenance methyltransferase at each cell division [13]. Aberrant regulations of DNMTs have been found to be correlated with hematologic malignancies, including different types of leukemia [14]. In mouse models, knockout of the*DNMT1* and *DNMT3A* genes have showed a role of DNA methylation in mediating the self-renewal and differentiation of normal hematopoietic stem cells and the leukemia stem cells [14,15].

Up to date, a number of studies have suggested that genetic variation in *DNMT* could be correlated with risk of malignancies. Various mutations of DNMT3A and other DNA methylation regulators have been identified in hematologic malignancies [14]. Genetic polymorphisms in *DNMT1* have also been found to be associated with several malignancies, including breast cancer [16,17] and gastric cancer [18]. However, the role of *DNMT1* polymorphisms in the etiology of ALL has never been specifically investigated before. In light of the critical role of *DNMT1* in ALL, we hypothesized that the genetic polymorphisms in *DNMT1* could be a potentially genetic marker to predict ALL risk. To test our hypothesis, we selected six polymorphisms in *DNMT1* and genotyped these polymorphisms in a case-control study of 377 patients with ALL and 500 healthy controls in a Chinese population and assessed its impact on the occurrence of ALL.

2. Methods

2.1. Study population

The present study was hospital-based case-control study, which included 377 patients with ALL and 500 healthy controls. All the patients were newly diagnosed at the Nanjing First Hospital, Nanjing Medical University (Nanjing, China) between January 2008 and June 2014. The diagnosis of the patients was immunologically and pathologically confirmed by bone marrow aspirate. The age of the patients was ranged from 2 to 11 years. The controls were randomly selected from the individuals seeking healthy examination at the outpatient department of the same hospital and without the indication of malignant neoplastic or thrombotic disease. The controls were frequency-matched to the cases by age (± 5 years) and gender. All the subjects were ethnic Han Chinese recruited from the same geographic area coming and had no blood relationship. Before recruitment, a standard questionnaire was administered through face-to-face interviews by trained interviewers to obtain information, such as age, gender, parental smoking, parental drinking and house painting, and other clinical characteristics. After interview, about 5 mL venous blood samples were obtained from the patients and controls. The present study was approved by the Institutional Review Board of Nanjing Medical University. Written informed consents were obtained from all participants involved in this study at recruitment.

2.2. Genetic polymorphism selection

Genetic polymorphisms in *DNMT1* were selected by using the genotype data obtained from unrelated Han Chinese in Beijing individuals in the HapMap database (HapMap Data Rel 21a/Phase II, Jan07, on NCBI B35 assembly, dbSNP b125). We reviewed all the polymorphisms that had a minor allele frequency > 5% in Han Chinese in Beijing within a 62 kb region spanning the *DNMT1* gene (including 1 kb upstream). As a result, seven tagSNPs (rs11880388, rs2228611, rs10423341, rs2228612, rs7253062, rs11085721 and rs16999593) were selected at a resolution of one polymorphism per 1.50 kb that captured all variant alleles with a mean r^2 of 0.8. The identification number and relative position of the five tagSNPs as well as the LD plot of the tagSNPs presented by the Haploview 4.2 software are shown in Fig. 1. We also forcedly included another two polymorphisms (rs2228611 and rs2228612) since they were reported to be correlated with cancer risk [16,17].

2.3. DNA extracted and genotyping

Genomic DNA was extracted from the peripheral blood by proteinase K digestion and phenol/chloroform extraction. The genotyping of the six tagSNPs was performed using predesigned TaqMan SNP Genotyping Assays (Applied Biosystems, Foster City, CA). The sequences of primer and probe for each polymorphism are available on request. According to the manufacturer's instructions, amplifications and analysis were performed in the 384-well ABI 7900HT Real Time PCR System (Applied Biosystems), using the SDS 2.3 software for allelic discrimination (Applied Biosystems). Controls were included in each plate to ensure accuracy of the genotyping. About 10% of the samples were randomly selected for repeated genotyping for confirmation, and the results were 99% concordant.

2.4. Statistical analyses

Before analysis, the allele frequencies of the tagSNPs were tested against departure from Hardy–Weinberg equilibrium (HWE) using a goodness-of-fit χ^2 test. Differences in the distributions of the selected information and the frequencies of the genotypes between cases and controls were evaluated using χ^2 test. The correlation between the polymorphisms and risk of ALL were evaluated by computing odds ratios (ORs) and 95% confidence intervals (CIs) from unconditional logistic regression analysis with the adjustment for possible confounders. All data were analyzed with the software SAS 9.1.3 (SAS Institute, Cary, NC, USA) with two-sided test, and the adjusted $P < 0.05$ was considered to be statistically significant.

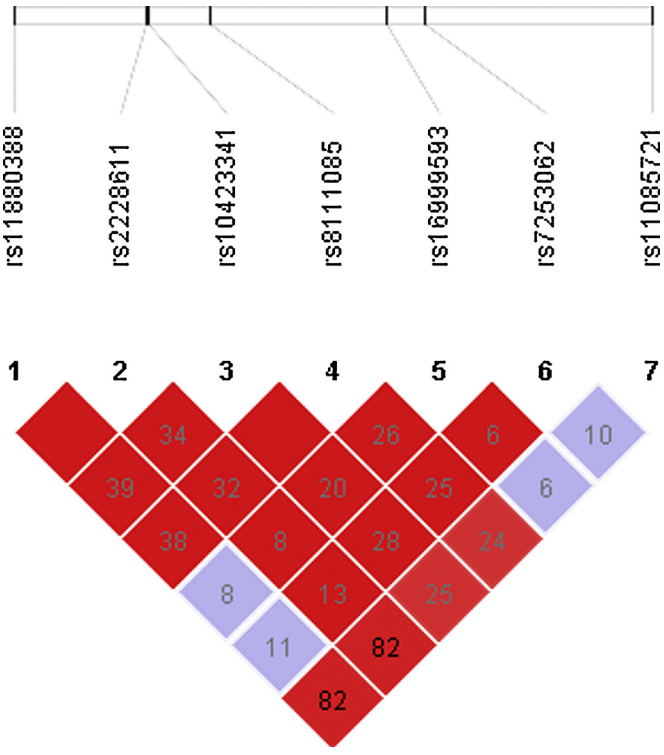

Fig. 1. Linkage disequilibrium of the *DNMT1* SNPs.