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The functional polymorphisms of *LIS1* are associated with acute myeloid leukemia risk in a Han Chinese population

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ABSTRACT

There is increasing evidence that the human lissencephaly-1 gene, *LIS1*, plays an important role in carcinogenesis of several malignancies including leukemia. However, little is known about the relationship between single nucleotide polymorphisms (SNPs) in *LIS1* and the susceptibility to myeloid leukemia. In the present study, we systematically screened 5 potentially functional polymorphisms in *LIS1*, and conducted a case-control study including 660 acute myeloid leukemia (AML) patients and 1034 cancer-free controls in a Chinese population, to assess the association between these SNPs and AML risk. We found that the variant alleles of rs4790348, rs4790353, and rs7209748 could significantly increase the AML risk (rs4790348: adjusted OR = 1.31, 95%CI = 1.13–1.53 in additive model; rs4790353: adjusted OR = 4.97, 95%CI = 1.59–15.50 in recessive model; rs7209748: adjusted OR = 2.34, 95%CI = 1.11–4.94 in recessive model). These findings indicated that genetic variants in *LIS1* may contribute to AML risk in Chinese population.

1. Introduction

As a kind of fatal hematopoietic stem cell tumor, acute myeloid leukemia (AML) is caused by leukemia cells which invade bone marrow and result in normal hematopoiesis inhibition [1–3]. Several environmental factors have been identified to play important roles in AML development, such as benzene exposure, ionizing radiation, and chemotherapy [4–6]. Furthermore, it is generally accepted that genetic factors are also involved in the pathogenesis of AML.

Recently, increasing evidence has shown that the human lissencephaly-1 gene (*LIS1*, also known as *PAFAH1B1*) plays important roles in carcinogenesis of several malignancies. It was reported that the mRNA and protein levels of *LIS1* were downregulated in about 70% of hepatocellular carcinoma (HCC) tissues, and such alteration was significantly associated with tumor progression [7]. *LIS1* was also found to play a role in neuroblastoma cell nucleokinesis and motility [8], glioma migration and proliferation [9], and cholangiocarcinoma cell proliferation and invasion [10]. Besides, Zimdahl et al. reported that conditional deletion of *LIS1* in hematopoietic cells could lead to a dramatic "bloodless" phenotype, impaired stem cell function, depletion of the stem cell pool, and accelerated differentiation. They also found that the inhibition of *LIS1* increased the differentiation in the short term and blocked the growth in the longer term, identifying *LIS1* as a new critical regulator of human leukemia growth and propagation [11].

Although much attention has been focused on the role of *LIS1* in carcinogenesis, little is known about the relationship between single nucleotide polymorphisms (SNPs) in *LIS1* and the susceptibility to AML. In this study, we hypothesized that potentially functional polymorphisms in *LIS1* may alter the expression or function of this gene and consequently influence AML risk. Thus, we systematically screened 5 potentially functional polymorphisms in *LIS1*, and conducted a case-control study including 660 AML patients and 1034 cancer-free controls to test this hypothesis.

2. Materials and methods

2.1. Ethical approval

This work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) and approved by the institutional review board of Nanjing Medical University. A written informed consent was obtained for all the participants. The privacy of all the participants was protected.

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2.2. Study subjects

A total of 660 AML cases were recruited from Wuxi people's Hospital Affiliated to Nanjing Medical University from December 2007 to June 2013. All patients diagnosed according to FAB diagnosis and typing standard were included, with no restrictions in terms of age, histology, or stage of disease, but patients with a history of cancer, chemotherapy or hematopoietic stem cell transplantation (HSCT) were excluded. The 1034 controls were randomly selected from more than 30,000 participants in a community screening of non-communicable disease in Jiangsu Province, conducted during the same period as the cases were recruited. All controls had no self-reported cancer history. All the participants denoted 5 ml of venous blood sample and undergone a face to face interview concerning demographic data (e.g. age and gender), and clinical information of cases were gathered from patients' medical records, including date of diagnosis, lineage, classification of diagnosis, karyotype, and molecular subtype.

2.3. SNP selection

The UCSC database (http://genome.ucsc.edu/, hg19 assembly, Feb. 2009), SNPinfo Web Server (http://snpinfo.niehs.nih.gov/) and RegulomeDB (http://regulomedb.org/) were used to identify common SNPs with potential functions in *LIS1* gene region, meeting the following two criteria: (1) with a minor allele frequency (MAF) of more than 5% in Chinese population; and (2) with a predicted function of influencing the binding of specified transcription factors or being expression quantitative trait loci (eQTLs). Linkage disequilibrium (LD) analysis with an r^2 threshold of 0.80 was further applied to filter these functional SNPs. As a result, five SNPs were selected for genotyping, including rs1266474, rs4790348, rs4790353, rs7209748, and rs8081803.

2.4. Laboratory assays

The method of traditional proteinase K digestion followed by phenol-chloroform extraction and ethanol precipitation were used to extract genomic DNA. All SNPs were genotyped on an ABI 7900 system (Applied Biosystems, Foster city, CA) using the TaqMan allelic discrimination assay. The sequence information of primers and probes are shown in Supplymentary Table 1. The genotyping assays were performed without knowing the subjects' case or control status. More than 10% samples were randomly selected to repeat, and the results were 100% concordant. The genotyping call rates for these polymorphisms were all above 98%.

2.5. Statistical analysis

The Student's *t*-test and χ^2 tests were used to evaluate differences between cases and controls on the demographic characteristics, for continuous variables and categorical variables, respectively. Hardy-Weinberg equilibrium (HWE) was tested by a goodness-of-fit χ^2 test. The associations between *LIS1* SNPs and AML risks were estimated by multivariate logistic regression analysis. Likelihood Ratio test was used for stratification analysis. Power was calculated using the Power and Sample Size Calculation (PS) software (v3.1.2), and we set the type I error probability and OR as 0.05 and 1.5, respectively. $P < 0.05$ was the criterion of statistical significance, and all statistical tests were two sided, performed with SAS 9.1.3 software (SAS Institute, Cary, NC).

3. Results

The information of demographic and clinical characteristics of the study subjects was shown in Table 1. The age (44.4 ± 17.8 versus 45.4 ± 9.6) and gender (52.7% versus 56.7% for male) between cases and controls were comparable ($P > 0.05$). For clinical and cytogenetic characteristics, most cases were M2 AML (33.5%), of myeloid lineage (70.3%), with intermediate Southwest Oncology Group (SWOG) risk assessment (50.9%), and without common fusion gene transcripts (57.1%).

The basic information of selected SNPs was presented in Table 2, including locations, alleles, MAF among cases and controls, HWE test among controls, call rate of all samples, and the achieved power. All 5 SNPs located in the intron region of *LIS1* had call rate of more than 98% and were consistent with Hardy-Weinberg equilibrium ($P > 0.05$). Although we selected all SNPs with a MAF of more than 5% in Chinese population according the SNPinfo Web Server (http://snpinfo.niehs.nih.gov/), rs8081803 showed a MAF of 0.049 in the present study. Additionally, two SNPs (rs4790353 and rs8081803) achieved a power of less than 80% (66.3% and 49.8%) when we assumed the type I error probability and OR as 0.05 and 1.5, respectively.

We conducted the association analysis between selected SNPs and AML risk in different genetic models, and all analyses were adjusted for age and gender. As shown in Table 3, three SNPs were associated with the increased risk of AML ($P < 0.05$). The variant A allele of rs4790348 increased AML risk in dominant and additive model (adjusted OR = 1.43, 95%CI = 1.18–1.75 in dominant model; adjusted OR = 1.31, 95%CI = 1.13–1.53 in additive model), and the variant alleles of rs4790353 and rs7209748 increased the risk of AML in recessive model (rs4790353: adjusted OR = 4.97, 95%CI = 1.59–15.50; adjusted rs7209748: OR = 2.34, 95%CI = 1.11–4.94). There was no significant evidence for the relationship between other 2 SNPs and AML risk (Table 3). As 28.2% cases had mixed lineage acute leukemia (myeloid and lymphoid, Table 1), we also conducted the association analysis when those 28.2% patients were excluded and found the similar results (rs4790348: adjusted OR = 1.32, 95%CI = 1.06–1.65 in dominant model and adjusted OR = 1.22, 95%CI = 1.03–1.45 in additive model; rs4790353: adjusted OR = 5.35, 95%CI = 1.63–17.53 in recessive model; rs7209748: adjusted OR = 2.49, 95%CI = 1.13–5.51 in recessive model, Table 3).

Then, we examined the association of these 3 SNPs with risk of AML in subgroups stratified by selected variables including age, gender, lineage, SWOG risk assessment and molecular subtype. However, no heterogeneity was found for these 3 SNPs among any subgroup (Table 4).

As the fusion gene AML1/ETO and PML/RARα respectively accounted for the majority of molecular subtypes of M2 and M3 AML, we then investigated the association of AML1/ETO and PML/RARα with rs4790348, rs4790353 and rs7209748 in M2 and M3 AML. As shown in Supplymentary Table 2, there was no significant association between AML1/ETO or PML/RARα and these 3 SNPs in M2 and M3 AML.

4. Discussion

In the present study, we genotyped 5 potentially functional SNPs of *LIS1* in 660 AML patients and 1034 cancer-free controls, and indicated that the variant alleles of rs4790348, rs4790353, and rs7209748 could significantly increase the AML risk in Chinese population. The findings suggested that genetic variants of *LIS1* may contribute to AML susceptibility.

LIS1 gene encoding the LIS1 protein is located in the region of chromosome 17p13.3. The LIS1 protein colocalizes with cytoplasmic dynein and dynactin, predominantly in prometaphase kinetochores and at the cell cortex of dividing cells [12,13]. LIS1/dynactin plays the function in metaphase spindle assembly and mitotic checkpoint control [12,14,15]. Besides, LIS1 has