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The *MIF* −173G/C polymorphism and risk of childhood acute lymphoblastic leukemia in a Chinese population

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ABSTRACT

Migration inhibitory factor (MIF) has recently been defined as a novel pro-tumorigenic factor that promotes cell proliferation, migration, and invasion. The *MIF* −173C allele results in increased *MIF* promoter activity and is associated with a higher serum MIF level. We hypothesized that this polymorphism may contribute to childhood acute lymphoblastic leukemia (ALL) susceptibility. We genotyped the *MIF* −173G/C polymorphism (rs755622) in 346 ALL cases and 516 cancer-free controls in a Chinese population and found that the variant genotype GC and the combined genotypes GC/CC were associated with a significantly higher risk of childhood ALL [adjusted odds ratio (OR) = 1.39, 95% confidence interval (CI) = 1.01–1.93 for GC and adjusted OR = 1.38, 95% CI = 1.01–1.89 for GC/CC]. In addition, we found that the increased risk was more pronounced among high-risk ALL and B-phenotype ALL patients. Our results suggest that the *MIF* −173G/C polymorphism is involved in the etiology of childhood ALL and is a potential candidate gene for determining cancer susceptibility. Further validations in other populations are warranted.

1. Introduction

Acute lymphoblastic leukemia (ALL) is a heterogeneous disease characterized by the predominance of lymphoblasts or immature hematopoietic precursors in bone marrow [1]. ALL is the most common childhood cancer, accounting for 75% of childhood leukemia [2]. The highest incidence of ALL occurs in the first 5 years of life and is approximately 5.7 per 100,000 persons per annum [3]. This hematological malignancy is postulated to be the unfortunate outcome of the interaction of the patient's genetic susceptibility factors and exposure to environmental carcinogens [4].

The gene for human macrophage migration inhibitory factor (*MIF*) is located on chromosome 22q11.2. As a pro-inflammatory cytokine, *MIF* plays an important role in the pathogenesis of many diseases, such as autoimmune diseases [5–8] and cancers [9–11]. MIF is a potent promoter of the expression of cytokines such as tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6, IL-8, and prostaglandin E2 (PGE2) in lipopolysaccharide (LPS)-driven responses [12], some of which play a vital role in the pathogenesis of leukemia. Recent studies indicated that *MIF* genetic polymorphisms may play important roles in cancer susceptibility [13–15]. To date, eight polymorphisms have been reported in the human *MIF* gene. The *MIF* −173G/C (rs755622) polymorphism is located in the promoter region, and it has been shown to influence *MIF* promoter activity in T lymphoblast cell lines [16].

Although a number of studies have focused on the association between *MIF* polymorphisms and susceptibility to cancer [16–18], no data exist on their role in the risk of ALL among Chinese children. Thus, the goal of the present study was to examine the association between the *MIF* −173G/C polymorphism and the risk of childhood ALL in a Chinese population.

2. Materials and methods

2.1. Study subjects

We recruited 346 ALL cases from the Nanjing Children's Hospital Affiliated to Nanjing Medical University and Soochow Children's Hospital Affiliated to Soochow University in an ongoing study starting in January 2007. Both the cases and the controls ranged in age from 1 to 18 years old. All cases were diagnosed by bone marrow aspiration. The risk level and immunophenotype of these cases were determined using the Suggestion of Diagnosis and Treatment of ALL in Childhood in China. Patients with other hematological disorders or previous cancer, radiotherapy, or chemotherapy were excluded. We recruited 516 children as controls; these children were from the same geographic area and showed no evidence of genetical relationship with the ALL cases. They were matched for age and sex with the ALL patients. The exclusion criteria for the control subjects were malignant neoplasma

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and hematological disorders. After informed consent was obtained from the parents of each of the eligible subjects, we obtained demographic and risk factor information about the study subjects using questionnaires. For risk factors, we used smoking and drinking by the parents and house painting. If neither the father nor the mother of the subject was a smoker, the question was marked "never"; otherwise it was marked "ever". If neither the father nor the mother of the subject was a drinker, the question was marked "never"; otherwise it was marked "ever". If the house was painted during pregnancy or after birth, the house painting status was defined as "ever"; otherwise it was marked "never". The research protocol was approved by the institutional review board of Nanjing Medical University.

2.2. Genotyping

Blood samples were taken from each subject. Genomic DNA was extracted from peripheral blood lymphocytes by proteinase K digestion followed by phenol–chloroform extraction and ethanol precipitation. The *MIF* −173G/C polymorphism was genotyped using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. The primers, lengths, and restriction enzymes used have been described elsewhere [19]. The polymorphism analysis was conducted independently by two researchers in a blind fashion. More than 10% of the samples were randomly selected for repeated genotyping. The results were 100% concordant.

2.3. Statistical analysis

We used the chi-square test to compare the differences in frequency distributions of selected demographic variables, the risk factors, as well as each allele and genotypes of the *MIF* −173G/C polymorphism between cases and controls. A goodness-of-fit chi-square test was used to test Hardy–Weinberg equilibrium of the genotype distribution among the control group. Unconditional univariate and multivariate logistic regression analyses were performed to obtain the crude and adjusted odds ratios (ORs) for estimating risk of ALL and their 95% confidence intervals (CIs). The multivariate adjustment included age, gender, parental smoking status, parental drinking status, and house painting status. Stratification analysis was used according to different subgroups of age, gender, parental smoking status, parental drinking status, house painting status, immunophenotype, and treatment branch. All statistical tests were two-sided at a significance level of 0.05 and were analyzed using the SAS software (version 9.1.3; SAS Institute, Cary, NC, USA) unless otherwise indicated.

3. Results

3.1. Characteristics of the study subjects

The frequency distributions of the selected characteristics of the cases and controls are presented in Table 1. Overall, the cases and controls were adequately matched for age and gender ($P = 0.305$ for age and 0.320 for gender). There was no significant difference in the frequency distributions of parental smoking status between the cases and controls ($P = 0.071$). However, there were more ever drinkers among the parents of cases (37.6%) than among the parents of controls (16.7%), and the difference was significant ($P < 0.001$). In addition, more cases lived in a house that had been painted than did controls (31.2% and 11.4%, respectively, $P < 0.001$). Furthermore, patients with B-phenotype ALL were in the majority (83.5%). The proportions of patients in low-risk and high-risk groups were similar (48.0% and 47.1%, respectively), whereas patients in the medium-risk accounted for 4.9% of the total.

3.2. Association analysis of the MIF polymorphism

Table 2 shows the genotypes and allele frequencies of the *MIF* −173G/C polymorphism among cases and controls and their associations with risk of childhood ALL. The observed *MIF* −173G/C genotypes among the control subjects conformed to the Hardy-Weinberg equilibrium ($P = 0.841$). Overall, there was no significant difference in the genotype distributions of the *MIF* −173G/C polymorphism between the cases and controls ($P = 0.215$). The *MIF* −173C allele was more common in the ALL group (19.0%) compared with the control group (15.5%), but the difference was not statistically significant ($P = 0.102$). However, when the GG genotype was used as a reference, multivariate logistic regression analysis indicated that the heterozygous GC carriers, but not the homozygous CC carriers, had a statistically significantly increased risk of ALL (adjusted OR = 1.39, 95% CI = 1.01–1.93 for the GC genotype and adjusted OR = 1.22, 95% CI = 0.48–3.06 for the CC genotype). Furthermore, the combined variant genotypes GC/CC were associated with a statistically significantly higher risk of ALL compared with the wild-type genotype GG (adjusted OR = 1.38, 95% CI = 1.01–1.89).

In further stratification analysis for the *MIF* −173G/C polymorphism (Table 3), we found a more pronounced association between the increased risk and the genotype variants among high-risk ALL (adjusted OR = 1.51, 95% CI = 1.01–2.25 for the GC genotype and adjusted OR = 1.51, 95% CI = 1.02–2.22 for the GC/CC genotypes) and B-phenotype ALL cases (adjusted OR = 1.44, 95% CI = 1.03–2.03 for the GC genotype and adjusted OR = 1.40, 95% CI = 1.01–1.94 for the GC/CC genotypes). No significant associations were observed between the genotypes and risk of medium-risk ALL, low-risk ALL, and T-ALL.

4. Discussion

In the present study, we investigated the associations between the *MIF* −173G/C polymorphism and the risk of childhood ALL in a Chinese population and found that the −173C allele may be a potential risk factor. The epidemiologic data showed that more cases than controls had parents who were drinkers and lived in houses that had been painted. This finding is consistent with previous reports [20,21] indicating that parental alcohol use and decoration materials exposure may be associated with the risk of childhood ALL. Although parental smoking status has been reported to have a positive dose-response relationship with risk of childhood ALL [22], no significant difference was detected between the parental smoking levels of cases and controls in our study. This discrepancy in results may be due to some other potential risk factors that influenced the result of our present study [23,24].

As a pro-inflammatory cytokine released from T-cells and macrophages, MIF plays a key role in inflammatory reactions [25,26], normal cell division, and oncogene-induced malignant transformation. Both circulating and intracellular MIF protein levels are elevated in cancer patients, and MIF expression reportedly correlates with stage, metastatic spread, and disease-free survival [27]. Some studies have suggested that in some cancer strains, the proliferation rates were significantly reduced when MIF was down-regulated [28]. It also has been shown that MIF can up-regulate the expression of cyclinD1 to activate CDK4/6 by activation of MAPK, resulting in the release of E2F and the entrance of cells into the DNA synthesis phase [29]. These findings suggest that MIF may promote the development of cancer. Furthermore, the *MIF* −173 G/C polymorphism recently was identified and has been shown to be functional both in vitro and in vivo [18]. This polymorphism creates a binding site for activator protein-4, which is involved in intracellular transport activities. Luciferase studies in a human T lymphoblast cell line have shown increased promoter activity of *MIF* −173C-Luc compared to *MIF* −173G-Luc. Serum MIF levels were significantly higher in subjects with the *MIF* −173C allele compared to the *MIF* −173GG genotype [30]. These data support the hypothesis that the *MIF* −173C allele is a potential risk factor for developing childhood ALL.

Until now, no study has focused on the *MIF* polymorphisms and their relationship with ALL susceptibility. For the first time, we investigated whether there is an association between the *MIF* −173G/C polymorphism and risk of childhood ALL in a Chinese population. We found that carriers of the *MIF* −173GC genotype had a significantly increased risk of ALL. However, we observed no evidence of a significant association between the homozygous CC genotype and the risk of ALL, but the CC genotype was rare in this study population. We subsequently combined the variant CC