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Genetic polymorphisms of *NAT2* and risk of acute myeloid leukemia

A case-control study

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Abstract

Our purpose was to investigate the possible associations between N-acetyltransferase-2 (*NAT2*) gene polymorphisms and the risk of acute myeloid leukemia (AML) in Chinese Han population.

A case-control study was conducted including 98 AML cases and 112 healthy controls. *NAT2* gene 2 polymorphisms rs1799930 and rs1799931 were genotyped using direct sequencing. Chi-square test was performed to compare the genotype and allele distribution differences between groups. Odds ratio (OR) with 95% confidence interval (CI) was calculated to estimate the association between *NAT2* gene polymorphisms and AML onset.

A remarkable decrease trend of rs1799931 GA genotype was detected in AML patients compared with controls, whereas the ancestral GG genotype frequency increased in cases ($P < .05$). And the mutant A allele of rs1799931 significantly reduced the risk of AML by 0.585-fold versus the ancestral G allele carriers (OR = 0.585, 95% CI = 0.361–0.950). But the distributions of rs1799930 genotype and allele were similar between groups ($P > .05$).

Our findings suggested that *NAT2* gene polymorphism rs1799931 was associated with decreased risk of AML and was likely to be a protective factor against AML development.

Abbreviations: 95% CI = 95% confidence interval, AML = acute myeloid leukemia, HWE = Hardy-Weinberg equilibrium, NAT2 = N-acetyltransferase 2, OR = odds ratio, PCR = polymerase chain reaction method, SNPs = single nucleotide polymorphisms, WHO = World Health Organization.

Keywords: acute myeloid leukemia, case control, *NAT2*, polymorphism

1. Introduction

The human acute leukemia can be divided into acute lymphoblastic leukemia and acute myeloid leukemia (AML), in which AML is the most common type of acute leukemia affecting adults.[1,2] AML is caused by the malignant proliferation of myeloid protocells. AML has been regarded as the sixth leading cause of the mortality among the malignancies, and in China, 1.62 of 100 people will be affected by AML.[3] AML is found to be a multifactorial disease, which can be influenced by the interaction of several related factors.[4] Up to now, a number of environmental factors have been identified to play a role in the susceptibility to AML, including radiation, smoking, obesity, and exposure to chemical carcinogens.[5] However, only a small proportion of individuals will develop AML who are exposed to these environmental risk factors, suggesting the crucial role of genetic factors in the development of AML.

N-acetyltransferase 2 (NAT2) is one of the phase II metabolizing enzymes, which is encoded by *NAT2* gene.[6] NAT2 participates in the detoxification of toxic arylamines, aromatic amines, and hydrazines via N-acetylation and O-acetylation, which belong to significant ultimate carcinogens involved in the initiation process of cancer.[7] The human *NAT2* gene is located on chromosomal region 8q21.3–23.1, and numbers of single nucleotide polymorphisms (SNPs) have been identified.[8,9] *NAT2* gene plays an important role in the individual physiological response to various xenobiotic compounds, such as a wide range of exogenous chemicals and several clinically useful drugs.[10,11]

Recently, a number of molecular epidemiologic studies have explored the association of the NAT2 acetylation profile with human cancer risk.[12–14] Furthermore, a major study has reported that individuals with the NAT2 slow-acetylation phenotype will have high risk to develop into AML.[15] Besides, SNPs in the *NAT2* gene also regulate human susceptibility to various cancer, such as lung cancer, bladder cancer, gastric cancer, and so on.[16–18] In Brazil, 2 polymorphisms of *NAT2* gene have been reported to contribute to the risk of either acute lymphoblastic leukemia or AML.[19]

Considering these results, all evidences present the more precise estimation on the relationship between *NAT2* gene polymorphisms and AML susceptibility. However, few studies have been

Editor: Ahmet Emre Eskazan.

This study is supported by National Natural Science Foundation of China (NSFC 81270605, 30971066, 81470324), Third Military Medical University Clinical and Science Great Fund Project (2102XLC03), Chongqing Postgraduate Education Reform Project (yjg123114), Chongqing Natural Science Fund Project (CSTC'2008BA5001), and Military Emphasis Medical Scientific Research Project Fund (BWS13C018).

The authors declare that they have no conflicts of interest.

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Medicine (2017) 96:42(e7499)

Received: 14 January 2017 / Received in final form: 8 May 2017 / Accepted: 22 June 2017

http://dx.doi.org/10.1097/MD.0000000000007499

done to detect the potential association in the Chinese Han population. Thus, we conducted a case-control study to identify the distributions of *NAT2* gene 2 polymorphisms rs1799930 (G590A) and rs1799931 (G857A), and their association with susceptibility to AML in the Chinese Han population.

2. Materials and methods

2.1. Participants

A total of 98 patients with AML were recruited as case group, who were first diagnosed with AML at the Southwest Hospital between May 2014 and March 2015. AML patients were diagnosed by histopathology according to World Health Organization (WHO) criteria based on an increased number of myeloblasts in the bone marrow or peripheral blood, and the diagnosis was determined when a 200-cell differential revealed the presence of 20% or more myeloblasts in a marrow aspirate or in the blood.[20] Additionally, 112 age-matched healthy subjects were enrolled as control group, who came to the same hospital for a routine health check-up during the same time. Individuals who were <18 years old, had a history of cancer, known blood disorders, diabetes, and connective tissue disease were excluded from the study.

This study was approved and consented by ethics committee of Southwest Hospital. The sample collection was in accordance with the ethnic criteria of National Human Genome Research. All participants involved in this study signed informed consent before enrolment, and agreed to provide blood sample and undergo investigation. And all subjects were Chinese Han population who had no blood relationship with each other.

2.2. Sample collection

Every participant was asked to provide 5 mL venous blood, which was collected in the anticoagulative tube with EDTA-disodium salt. Genomic DNA was extracted using TaKaRa Genome DNA Extraction Kit (Dalian Biological Engineering Co, Ltd, China) according to manufacturer's instructions. The extracted DNAs were dissolved in sterile distilled water and stored at −20°C for standby application.

2.3. Determination of the polymorphisms

The target fragments for *NAT2* gene 2 polymorphisms rs1799930 and rs1799931 were directly amplified by multiple polymerase chain reaction method (PCR). The primer sequences for the 2 SNPs were designed by Primer Premier 5.0, and synthesized by Sangon Biotech (Shanghai, China) (Table 1). The designed primer sequences were verified by nucleotide-nucleotide BLAST (blastn). The results demonstrated that the primers used in this study was specific to *NAT2* sequences, and could be used for the following analysis. The PCR procedures consisted of an initial degeneration at 94°C for 5 minutes, followed by 11 cycles of 94°C degeneration for 20 seconds, annealing at 65°C for 40 seconds, and extension at 72°C for 1.5 minutes, then 24 cycles of 94°C degeneration for 20 seconds, annealing at 59°C for 30 seconds, and extension at 72°C for 1.5 minutes were followed, and a final extension at 72°C for 2 minutes and saved at 4°C.

Then the PCR products of the 2 polymorphisms were first purified by ExoSAP-IT (USB Corp) and directly sequenced by automated DNA sequencing with an Applied Biosystems 3730 × l automated sequencer (Applied Biosystems, Foster City, CA), and sequence analysis was performed using Vector NTI software.

2.4. Statistical analysis

All statistical analyses were performed using the PASW statistics 18.0 statistical software. The genotype and allele frequencies of the *NAT2* gene 2 polymorphisms rs1799930 and rs1799931 were estimated by direct counting. Hardy-Weinberg equilibrium (HWE) for each polymorphism in control group was analyzed to assess the quality of our study sample. The distribution differences of both genotype and allele were analyzed to assess the quality of our study sample. The distributions differences of both genotype and allele were compared between groups via chi-square test. The strength of association between *NAT2* gene polymorphisms and AML susceptibility was evaluated by odds ratios (OR) with 95% confidence interval (CI). *P* values <.05 indicated statistically significant differences.

3. Results

3.1. The basic information of subject in the case and control groups

A total of 98 AML patients and 112 healthy controls were selected as the case and control groups, respectively, in this study. In the case group, the age range of AML patients was 21 to 68 years with the mean age of 38.56 ± 12.13, and 56 males and 42 females were contained in this study group. The ages of healthy controls were from 18 to 73 years with the average age of 39.42 ± 10.86. The number of males and females was 59 and 53. The data showed there was no significant difference between the 2 groups in age and gender ($P > .05$ for both). The distributions of smoking and drinking were also similar between AML cases and control group ($P > .05$ for both). Moreover, family history of AML was significantly associated with the onset of AML ($P = .02$). The detailed data are displayed in Table 2.

3.2. HWE test

The genotype and allele frequencies of the *NAT2* gene 2 polymorphisms rs1799930 and rs1799931 were recorded in

Table 1

Primer sequences of *NAT2* gene 2 polymorphisms rs1799930 and rs1799931.

SNP	Primer sequences
rs1799930	
Sense	5′-GGTGCCTTGCATTTTCTGCT-3′
Reverse	5′-CCACCAAACAGTAAACCCCTTCT-3′
rs1799931	
Sense	5′-TGAGGAAGAGGTTGAAGAAGTGC-3′
Reverse	5′-CACTCTGCTTCCCAAGCTAATCACA-3′

SNP = single nucleotide polymorphism.

Table 2

The demographic features of the study subjects in this study.

Feature	Case, n = 98	Control, n = 112	*P*
Age, y			
Range	21–68	18–73	
Mean age	38.56 ± 12.13	39.42 ± 10.86	.23
Gender (male/female)	56/42	59/53	.52
Smoking, %	35 (35.71)	42 (37.50)	.79
Drinking, %	32 (32.65)	29 (25.89)	.28
Family history, %	16 (16.33)	7 (6.25)	.02

Table 3

Genotype and allele distributions of *NAT2* gene 2 polymorphisms rs1799930 and rs1799931 in case and control groups.

Genotype/allele	Case n = 98, %	Control n = 112, %	χ^2	*P*	OR (95% CI)
rs1799930					
GG	59 (60.20)	61 (54.46)	—	—	1
GA	31 (31.64)	46 (41.07)	1.500	.221	0.697 (0.390–1.243)
AA	8 (8.16)	5 (4.47)	0.718	.397	1.654 (0.512–5.347)
G	149 (76.02)	168 (75.00)	—	—	1
A	47 (23.98)	56 (25.00)	0.059	.808	0.946 (0.606–1.478)
rs1799931					
GG	68 (69.39)	61 (54.46)	—	—	1
GA	28 (28.57)	46 (41.07)	4.174	.041	0.546 (0.305–0.978)
AA	2 (2.04)	5 (4.47)	1.549	.213	0.359 (0.067–1.917)
G	164 (83.67)	168 (75.00)	—	—	1
A	32 (16.33)	56 (25.00)	4.748	.029	0.585 (0.361–0.950)

Table 3. The chi-square test results suggested that the genotype distribution of the 2 polymorphisms were all in accordance with HWE test ($P > .05$) in both case and control group, revealing the representativeness of the control group.

3.3. Distribution of NAT2 gene polymorphisms

As shown in Table 3, 3 genotypes were detected in *NAT2* gene rs1799930 polymorphism. The GG, GA, and AA genotype frequencies were 60.20%, 31.64%, and 8.16% in case group, and 54.46%, 41.07%, and 4.47% in control group, respectively. Additionally, the G and A allele frequencies were 76.02% and 23.98% in cases and 75.00% and 25.00% in controls, respectively. But all differences did not reach significant level ($P > .05$). These results suggested that *NAT2* gene rs1799930 might have no obvious association with AML susceptibility in the Chinese Han population.

Data analysis suggested a significant association between *NAT2* gene rs1799931 polymorphism and AML susceptibility. Statistical differences were revealed in both genotype and allele frequencies of rs1799931 between case and control groups ($P < .05$). We noted that the heterozygous GA genotype frequency decreased in case group compared with controls (28.57% vs 41.07%), whereas the ancestral homozygous GG genotype frequency increased significantly in cases (69.39% vs 54.46%). Besides, the mutant A allele also showed remarkable decreasing trend in case group (16.33% vs 25.00%). Individuals carrying mutant A allele showed lower risk to be affected by AML (OR = 0.585, 95% CI = 0.361–0.950). All data revealed that *NAT2* gene rs1799931 was associated with AML susceptibility in the Chinese Han population, and rs1799931 was likely a protective factor against AML development.

4. Discussion

AML is identified as the most common type of acute leukemia affecting adults.[21] Up to now, the etiology of AML has not been completely understood. However, it is noted that several risk factors may be involved in the increased occurrence of AML, including chemical exposure, ionizing radiation, and genetic mutations.[22–24] AML is highly heterogeneous, and its occurrence is a multistage complex process, with the involvement of a lot of factors and genes. Recently, various genetic mutations have been identified to be associated with the development of AML, such as mutations of *NPM1*, *CEBPA*, *c-KIT*, *AML1/RUNX1*, *WT1*, *FLT3*, and others.[25]

NAT2 is a phase II metabolizing enzymes, and catalyzes the activation of O-acetylation and deactivation of N-acetylation by reacting with heterocyclic amines and amines with a carbon-only aromatic ring. As we all know, these heterocyclic amines and amines are all significant ultimate carcinogens, which are involved in the initiation process of cancer. The human *NAT2* gene is located on chromosome 8p21.3–23.1, and numbers of genetic mutations have been identified in *NAT2* gene. In recent years, several studies have reported the association of *NAT2* gene variations such as rs1799930 (G590A) and rs1799931 (G857A) with risk of several types of cancer, including colorectal cancer, lung cancer, breast cancer, and bladder cancer.[26] A major study has also suggested the *NAT2* slow-acetylation phenotype was a risk factor for the onset of acute lymphoblastic leukemia and AML. A related age-dependent analysis carried out by Zanrosso et al found that NAT2 slow-acetylator was associated with the increased risk of leukemia in children $\leq$1 year old as well as children 1 to 10 years old. NAT2 haplotypes *14E, *6B, and *6F also significantly increased the risk of AML occurrence in Brazil.[19] However, the effects of *NAT2* polymorphisms on sensitivity of AML among Chinese population has been rarely reported.

rs1799930 and rs1799931 are 2 common SNPs located on the *NAT2* gene encoding region, which change the amino acid sequence and further significantly reduce the ability of acetylation of NAT2. In the present study, the *NAT2* gene 2 polymorphisms rs1799930 and rs1799931 were analyzed in 98 AML cases and 112 healthy controls. The significant association of *NAT2* gene rs1799931 polymorphism with AML susceptibility was observed in this study. Data analysis showed that the A allele of rs1799931 significantly decreased the risk of AML by 0.585-fold versus the ancestral A allele carriers (OR = 0.585, 95% CI = 0.361–1.950) in our study population. Additionally, the heterozygous GA genotype carriers showed lower risk to be affected by AML (OR = 0.546, 95% CI = 0.305–0.978). rs1799931 brings a G>A substitution at position 857 of *NAT2* gene, which produces the replacement of glycine by gluta in the 286th amino acid of the protein. The locus mutation directly change the activity of metabolic enzyme, and further affects the metabolism of some drugs and carcinogens inactivation or activation and leads to the incidence of cancer increase or decrease. A major meta-analysis has reported rs1799931 to be a protective factor against cancer development,[27] which was consistent with our study results. Another SNP rs1799930 can cause the arginine 197 to glutamine substitution, which has been regarded as a risk factor for the cancer.[27] But in our study population, no significant association was detected between rs1799930 and AML susceptibility.

There were still several limitations in the present study. First, the sample size was relatively small in the present study. Second, the value of the research might be limited by the single race. Third, the roles of environmental factors, and the interaction of gene-gene, gene-environment were not investigated in this study. In addition, how the NAT2 genetic mutations affected AML onset remained unclear. In TCGA analysis, the expression profiles of *NAT2* in AML and healthy individuals were similar, revealing the expression level of NAT2 might not be related to AML occurrence. Based on the relevant researches, we speculated that NAT2 rs1799931 polymorphism might influence the activity of NAT2 protein, thus participating in etiology of AML. Besides, we only found that *NAT2* rs1799931 polymorphism was associated with AML susceptibility in Chinese Han population, the potential diagnostic value of *NAT2* rs1799931 polymorphism in AML remained unknown. Further well-designed investigations were still required to identify the conclusions obtained in our study.

In conclusion, in the present study we noted a significant association of the *NAT2* gene polymorphisms rs1799931 with AML susceptibility in the Chinese Han population, and the mutant A allele performed as a protective factor against AML occurrence. No significant association was detected between rs1799930 and AML susceptibility in our study population. The present study may provide a guidance to identify the individuals with high risk of AML.

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