Genetic polymorphisms of NAT2 and risk of acute myeloid leukemia

A case-control study

Yunding Zou, PhD, Song Dong, PhD, Shuangnian Xu, PhD, Qiang Gong, PhD, Jieping Chen, PhD*

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. 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. AML is found to be a multifactorial disease, which can be influenced by the interaction of several related factors. 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. 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. 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. The human NAT2 gene is located on chromosomal region 8q21.3–23.1, and numbers of single nucleotide polymorphisms (SNPs) have been identified. 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.

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

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
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. 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 anticoagulants mixture 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 × 1 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 distribution 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

| SNP    | Primer sequences of NAT2 gene 2 polymorphisms rs1799930 and rs1799931. |
|--------|------------------------------------------------------------------------|
| rs1799930 | Sense 5’-GTG6CTTTGATTTCCTGCT-3’                                        |
|        | Reverse 5’-CCACAAACAGTAACCCCTCTC-3’                                   |
| rs1799931 | Sense 5’-TGAGGAGAAGAGTCTGAGAAGAGTGGC-3’                                |
|        | Reverse 5’-CACCTCTGCTCTCCAGAAGTAAGAACA-3’                             |

SNP = single nucleotide polymorphism.

### Table 2

| 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 (22.65)  | 29 (25.89)      | .28|
| Family history, % | 16 (16.33) | 7 (6.25)        | .02|
Another SNP rs1799930 can cause the arginine 197 to glutamine replacement of glycine by glutamine in the 286th amino acid of the c-KIT gene, which produces the NPM1, CEBPA, c-KIT, AML1/RUNX1, WT1, FLT3, and others. 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. 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 ≤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. 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=0.361–0.950) 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 glutamic acid 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, which was consistent with our study results. Another SNP rs1799930 can cause the arginine 197 to glutamin substitution, which has been regarded as a risk factor for the cancer. But in our study population, no significant association was detected between rs1799930 and AML susceptibility.

### Table 3: The chi-square test results suggested that the genotype distribution of the 2 polymorphisms were all in accordance with HWE test (P > 0.05) in both case and control group, revealing the representativeness of the control group.

| Genotype/allele | Case n = 98, % | Control n = 112, % | χ² | P | OR (95% CI) |
|-----------------|---------------|-------------------|----|---|-------------|
| rs1799930       |               |                    |    |   |             |
| GG              | 59 (60.20)    | 61 (54.68)        | —  | — | —           |
| 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)       | —  | — | —           |
| A               | 47 (23.98)    | 56 (25.00)        | 0.059 | .808 | 0.946 (0.606–1.478) |
| rs1799931       |               |                    |    |   |             |
| GG              | 68 (69.39)    | 61 (54.68)        | —  | — | —           |
| 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)       | —  | — | —           |
| A               | 32 (16.33)    | 56 (25.00)        | 4.748 | .029 | 0.585 (0.361–0.950) |
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, that NAT2 rs1799931 polymorphism might in revealing the expression level of NAT2 might not be related to onset remained unclear. In TCGA analysis, the expression gene-gene, gene-environment were not investigated in this study. In addition, how the NAT2 genetic mutations affected 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.

Reference

[1] Jiang L, Zhou P, Sun A, et al. Functional variant (-1304T>G) in the MKK4 promoter is associated with decreased risk of acute myeloid leukemia in a southern Chinese population. Cancer Sci 2011;102:1462-8.

[2] Mandegary A, Rostami S, Almoghaddam K, et al. Glutathione-S-transferase T1-null genotype predisposes adults to acute promyelocytic leukemia: a case-control study. Asian Pac J Cancer Prev 2011;12:1279-82.

[3] Yang C, Zhang X. Incidence survey of leukemia in China. Chin Med Sci J (Chung-kuo i hsueh k'o hsueh na chih) 1991;6:65-70.

[4] Filippini T, Heck JR, Malagoli C, et al. A review and meta-analysis of outdoor air pollution and risk of childhood leukemia. J Environ Sci Health C Environ Carcinog Ecotoxicol Rev 2015;35:36-66.

[5] Ilhan G, Karakus S, Andic N. Risk factors and primary prevention of acute leukemia. Asian Pac J Cancer Prev 2006;7:515-7.

[6] Geller F, Soborg B, Koch A, et al. Determination of NAT2 acetylation status in the Greenlandic population. Arch Toxicol 2016;90:883-9.

[7] Yarosh SL, Kolkhenko EV, Churbanov ML, et al. Synergism between the N-acetyltransferase 2 gene and oxidant exposure increases the risk of idiopathic male infertility. Reprod Biomed Online 2014;29:362-9.

[8] Adithan C, Subartha A. NAT2 gene polymorphism: covert drug interaction causing phenytoin toxicity. Indian J Med Res 2016;143:542-4.

[9] Sun JD, Yuan H, Hu HQ, et al. Association of N-acetyltransferase-2 polymorphism with an increased risk of coronary heart disease in a Chinese population. Genet Mol Res 2016;15:15016954.

[10] Sabbagh A, Darlu P, Couzau-Ray B, et al. Arylamine N-acetyltransferase 2 (NAT2) genetic diversity and traditional subsistence: a worldwide population survey. PLoS One 2011;6:e18507.

[11] Heim DW, Grant DM, Sim E. Update on consensus arylamine N-acetyltransferase gene nomenclature. Pharmacogenomics 2009;10:291–2.

[12] Fernandes MR, de Carvalho DC, dos Santos ÅK, et al. Association of slow acetylation profile of NAT2 with breast and gastric cancer risk in Brazil. Anticancer Res 2013;33:3683–9.

[13] Chang CH, Huang YS, Peng CL, et al. N-Acetyltransferase 2 (NAT2) genetic variation and the susceptibility to noncardiac gastric adenocarcinoma in Taiwan. J Chin Med Assoc 2016;79:105–10.

[14] Matejic M, Vogelsang M, Wang Y, et al. NAT1 and NAT2 genetic polymorphisms and environmental exposure as risk factors for oesophageal squamous cell carcinoma: a case-control study. BMC Cancer 2015;15:150.

[15] Majumdar S, Mondal BC, Ghosh M, et al. Association of cytochrome P450, glutathione S-transferase and N-acetyl transferase 2 gene polymorphisms with incidence of acute myeloid leukemia. Eur J Cancer Prev 2008;17:125–32.

[16] Liu C, Cui W, Cong L, et al. Association between NAT2 polymorphisms and lung cancer susceptibility. Medicine 2015;94:e1947.

[17] Wu H, Wang X, Zhang L, et al. Association between N-acetyltransferase 2 polymorphism and bladder cancer risk: results from studies of the past decade and a meta-analysis. Clin Genetourin Cancer 2016;14:122–9.

[18] Yu J, Deng Y, Chen JP. N-acetyltransferase 2 status and gastric cancer risk: a meta-analysis. Tumour Biol 2014;35:6861–5.

[19] Zanrosso CW, Emerenciano M, Faro A, et al. Genetic variability in N-acetyltransferase 2 gene determines susceptibility to childhood lymphoid or myeloid leukemia in Brazil. Leuk Lymphoma 2012;53:323–7.

[20] Vardiman JW, Harris NL, Brunning RD, et al. The World Health Organization (WHO) classification of the myeloid neoplasms. Blood 2002;100:2292–302.

[21] Luquet I, Bidet A, Cuccuini W, et al. Cytogenetics in the management of acute myeloid leukemia: an update by the Groupe francophone de cytogenetique hematologique (GFCH). Ann Biol Clin 2016;74:535–46.

[22] Pourten JR, Richardson M, Roesler M, et al. Chemical exposures and risk of acute myeloid leukemia and myelodysplastic syndromes in a population-based study. Int J Cancer 2017;140:23–33.

[23] Badie C, Blachowicz A, Barjaktarovic Z, et al. Transcriptomic and proteomic analysis of mouse radiation-induced acute myeloid leukemia (AML). Oncotarget 2016;7:40461–80.

[24] Jin MW, Xu SM, An Q, et al. A review of risk factors for childhood leukemia. J Environ Sci Health A Tox Hazard Subst Environ Eng 2016;51:1849–61.

[25] Goryainova NV. The clinical significance of genetic mutations in acute myeloid leukemia. Lik Sprava 2014;10–8.

[26] Zhu Z, Zhang J, Jiang W, et al. Risks on N-acetyltransferase 2 and bladder cancer: a meta-analysis. Onco Targets Ther 2015;8:3715–20.

[27] Tian FS, Shen L, Ren YW, et al. N-acetyltransferase 2 gene polymorphisms are associated with susceptibility to cancer: a meta-analysis. Asian Pac J Cancer Prev 2014;15:5621–6.