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Association between gout and polymorphisms of rs2231142 in ABCG2 in female Han Chinese *

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ABSTRACT Objective: The SNP rs2231142 which located in the exon5 of ABCG2 gene was found to be significantly associated with the development of gout in male Han Chinese. Because some genetic polymorphisms show gender differences in gout, we do this study to clarify the relevance between SNP rs2231142 and gout in female Han Chinese. **Methods:** A total of 185 female gout patients and 311 female controls were recruited, genomic DNA were extracted from peripheral blood leukocytes, after amplified the target gene seg ments of ABCG2, the PCR products were sequenced directly for genotype analysis. The genotype and allelic frequencies of the two groups were compared. **Results:** The frequencies of CC, CA and AA genotypes had a statistically difference between gout patients and controls (x^2=16.519, P<0.001). The risk allele A of rs2231142 in gout patients is much higher than controls.(42.2 % vs. 29.3 %, P<0.001, OR: 1.76 [95% CI: 1.35-2.31]). **Conclusion:** The polymorphisms of rs2231142 (C/A) in the exon5 of ABCG2 are significantly associated with the susceptibility to gout in female Han Chinese. Women who carry A allele of rs2231142 are more likely to get gout, and ABCG2 was first identified as a candidate gene associated with female gout in Chinese Han population.

Key Words: ABCG2; Gout; Single nucleotide polymorphism; female Han Chinese

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Introduction

Gout is a polygenetic complex disease whose exact pathogenesis is ambiguous. Genome-wide association studies (GWAS) provide the genetic basis of risk of gout. Several genes have been proved to be associated with the uric acid concentration or gout through GWAS and related meta-analyses[1,2]. Urate transport gene, ATP-binding cassette, sub-family G (WHITE), member 2 (ABCG2) was one of the most important genes [3]. The single nucleotide polymorphism (SNP) rs2231142, which located in the exon5 of ABCG2, has causal association with urate levels and gout in both white and black individuals, and was reported as the top missense SNP in ABCG2[1]. Recently, this SNP was reported to be strongly associated with the development of gout (P<0.001) in male Han Chinese and there was a higher A/A genotype and A allele frequency in gout cases[4]. But whether it has significant correlation with female gout is still not clear. As is well-known, gender differences in gout were found not only in genetic polymorphisms [5,6]but also in morbidity and clinical manifestation [7]. The prevalence of male gout is 18 times higher than female [8] and it is commonly thought as a problem for postmenopausal women [9]. Independent researches on female gout are very limited because of its low morbidity rates. But with the improvement of the living standards and the rapidly aging population, morbidity of female gout is increasing significantly and researches on female gout are imperative. In this present study, female Han Chinese were selected as independent research objects, the genotypes of rs2231142 were assessed through polymerase chain reaction (PCR)and sequencing technologies and comparisons were performed in different groups to clarify the association between gout and polymorphisms of rs2231142 in ABCG2 in female Han Chinese.

1 Materials and Methods

1.1 Study population

A total of 185 Han female gout patients and 311 Han female controls with no blood relationship among themselves were recruited for this study from the Gout Clinical Medical Center in Affiliated Hospital of Qingdao University Medical College. The diagnosis of gout was based on the preliminary criteria published by the American College of Rheumatology in 1977 for the classification of gout for use in either clinical settings or in population-based epidemiologic studies [10]. Hyperuricemia in female is defined as uric acid levels > 360 μmol/l in premenopausal women and > 420 μmol/l in postmenopausal women. Secondary gout patients were excluded. Normal female controls with no personal or familial history of hyperuricemia or gout or any other serious illness were recruited.

We collected the clinical features of the patients, measured

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blood glucose, uric acid, total cholesterol, triglycerides, urea nitrogen, creatinine and uric acid levels in the plasma of all the participants before anti-trioxypurine treatments using an automated multichannel chemistry analyzer (Model 200; Toshiba, Tokyo, Japan). Body mass index (BMI) was calculated to assess obesity.

1.2 Laboratory methods

Blood samples were collected from the gout patients and female controls, and stored at -40℃ until analyzed. Genomic DNA were extracted from peripheral blood leukocytes by using Bioteke genomic DNA kit, the concentration of all the DNA samples were controlled around 50 ng/μl. The rs2231142 and nearby regions were amplified by polymerase chain reaction (PCR), the forward primer was 5'-GGCAAATCCTTGTATGAAGCA-3' and the reverse primer was 5'- CCACATTACCTTGGTGTCTGC - 3'. Amplified fragment length was 443 bp. DNA fragmentations were examined by DNA agarose gel electrophoresis and D2000 DNA Marker labeled PCR objective strap length. The PCR products were subsequently sequenced by a sequencing company (Shanghai Sangon Biotech Co., Ltd, China) and the Chromas software was used to analyze the genotype.

1.3 Statistical analysis

The Hardy-Weinberg equilibrium test was performed in the case and controls separately before association analysis. The statistical analyses were performed using the Statistical Package for Social Sciences version 17.0 (SPSS 17.0). Chi-square analyses were used to compare the genotypic and allelic frequencies and odds and ratios (ORs) with 95%confidence intervals (CIs) were presented. Student's t-test, ANOVA test and non-parametric test were used to compare the data of the clinical parameters in different genotypes. Statistically significant differences between or among groups were indicated by two-tailed values of $P < 0.05$.

2 Results

2.1 Gene polymorphism testing results

2.1.1 PCR amplification and agarose gel electrophoresis

The amplified fragments were consistent with expected and the electrophoretic bands were single and clear (Fig. 1).

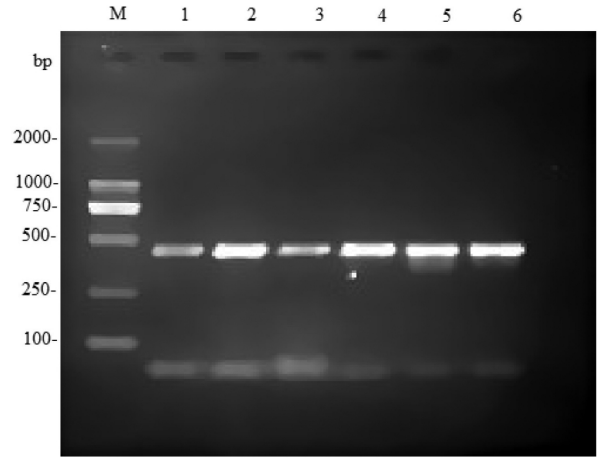

Fig. 1 Electrophoretogram of PCR products

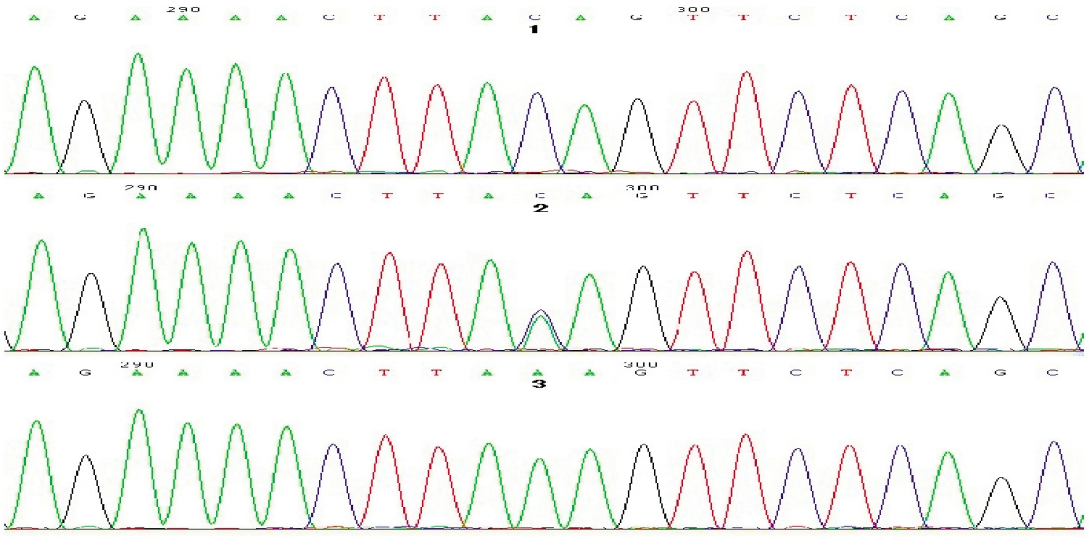

Fig. 2 Genotype sequencing diagram of rs2231142: 1.CC genotype

2.1.2 Sequencing and genotyping

The sequencing and genotyping diagram were given in Fig. 2.

2 CA genotype 3.AA genotype

2.2 Genotype and phenotypic analyses

A total of 185 Han female gout patients and 311 Han female gout-free controls participated in this study. The genotype frequencies were in Hardy-Weinberg equilibrium in the patients and controls. By compared the genotype distribution and the allelic frequencies of SNP rs2231142 between case and control groups, we found a significantly statistical differences ($P<0.001$) in female. And the A allele frequency in gout group were significantly higher than control group ($P < 0.001$, OR=1.76 [95% CI 1.35-2.31]). (Table 1).

Table 1 Genotype distribution and relative allele frequencies of rs2231142 in female

Genotype	Case (n=185)	Control (n=311)	x^2	P	OR (95 % CI)
CC	64(34.6%)	157(50.5%)	16.519	<0.001	1.00
CA	86(46.5%)	126(40.5%)			1.67
AA	35(18.9%)	28(9.0%)			3.07
Allele					
C	214(57.8%)	440(70.7%)	17.791	＜0.001	1.76
A	156(42.2%)	182(29.3%)			(1.35-2.31)

Combined the previous research [4], we compared the genotypic and allelic frequencies between male and female gout. The risk allele A in men is higher than women, but there weren't any statistically significant difference between male and female (Table 2). This indicates that the effects of SNP rs2231142 on gout have no significant gender difference in Han Chinese population.

Table 2 Genotype distribution and relative allele frequencies of rs2231142 in male and female gout

Group	Genotype frequency (%)			x^2	P value	Allele frequency (%)		x^2	P value
	CC	CA	AA			C	A		
Male gout (n=200)	64(32.0)	91(45.5)	45(22.5)	0.808	0.668	219(54.8)	181(45.3)	0.745	0.388
Female gout (n=185)	64(34.6)	86(46.5)	35(18.9)			214(57.8)	156(42.2)		

By comparing the clinical/biochemical parameters between gout patients and controls, we found that ages in two groups were matched, and the gout patients had significantly higher levels of body mass index (BMI), systolic blood pressure (SBP), diastolic blood pressure (DBP), blood uric acid (SUA), triglycerides (TG), urea nitrogen (BUN) and creatinine (Cr) (P<0.05) and the levels of blood glucose (BG), total cholesterol (TC) did not show any significant differences in the two groups (Table 3).

Table 3 Demographic and clinical characteristics (Mean ± SD) of the study population

	Female gout (n=185)	Female control (n=311)	P value
Age(year)	61.83± 11.64	61.26± 10.57	P=0.237
BMI (kg/ m2)	25.95± 3.81	24.95± 3.57	P=0.007
Systolic pressure (mmHg)	138.71± 20.36	134.52± 20.43	P=0.04
Diastolic pressure (mmHg)	84.45± 10.69	80.83± 10.28	P<0.001
Blood glucose (mmol/L)	5.98± 1.65	6.11± 1.69	P=0.42
Uric acid (umo1/L)	417.64± 123.65	243.80± 43.38	P<0.001
Triglycerides (mmol/L)	2.19± 1.53	1.49± 0.95	P<0.001
Total cholesterol (mmol/L)	5.71± 1.45	5.69± 1.06	P=0.961
Urea nitrogen (mmol/L)	6.71± 3.90	5.24± 1.16	P<0.001
Creatinine (umo1/L)	86.47± 43.01	64.73± 10.72	P<0.001

Meanwhile, comparisons of clinical/biochemical parameters were also performed between groups with different genotypes in controls, unfortunately, the statistical analyses failed to provide evidence of a certain parameters associated with a specific genotype.

3 Discussion

Epidemiological data of the prevalence of gout are consistent with a rise in the world[11,12], this chronic disease has been found associated with cardiovascular disease, anemia, cancer and many other disease [13,14], and it is attracting more and more attention. Gout is an aseptic, heterogeneous inflammation caused by the increased blood uric acid level in the human body. Disorder of the purine metabolism in human body (accounts for 10 percent) and decrease of uric acid excretion in kidney (accounts for 90 percent) are the two main reasons of the increasing uric acid level. Reduced excretion of urate by the kidney is the main cause for elevated urate levels [15]. ABCG2 gene, located on 4q22 region, encodes for the ABCG2 transporter which mainly expressed in the brush border membrane of kidney proximal tubule cells, where it mediates renal urate secretion. The common mutation, a Gln to Lys substitution at position 141 (Q141K) encoded by the rs2231142 A allele by site-directed mutagenesis in Xenopus oocytes resulted in 53% reduced urate transport rates and was shown to cause hyperuricemia and gout. Almost 10% of all gout patients in whites are attributable to this causal variant[16]. In male Han Chinese, this SNP also has been confirmed significant associated with the development of gout. But the effects of this genetic polymorphism on gout have been observed racial and gender differences[17]. The frequency of the risk-allele A of rs2231142 in Hapmap is 0.11 in CEU but 0.29 in CHB. The effect of rs2231142 A allele on elevate uric acid levels was observed more strongly in men compared to women in European descent [2]. Even in a German study, it only showed significant association in males but not females[5].

This present study focus on female gout patients, first report-

ed the SNP rs2231142 have significant association with gout in female Han Chinese. And the frequency of risk allele A in female Han Chinese gouts is 0.422, much higher than the controls (0.293), but similar to the frequencies in male Han Chinese (0.453). That indicates the effects of the mutation Q141K on uric acid and gout in Chinese have no gender difference.

However, there are limitations in our study. We did not adjust lifestyle (e.g., eating habits and Physical exercise situation) as confounding factors. Whether there is interaction between lifestyle and genetic variants on gout still remained unknown. And the roles of mutation Q141K in vivo need to be elucidated by further studies.

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ABCG2 基因 rs2231142 位点多态性与汉族女性痛风的相关性 *

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摘要 目的: ABCG2 基因第 5 外显子区单核苷酸多态性位点 rs2231142 与中国汉族男性痛风密切相关，基于痛风易感基因存在性别差异的考虑,本研究旨在探讨该单核苷酸多态性位点与中国汉族女性人群痛风易感性之间的相关性。**方法:** 选取185 例女性痛风患者和 311 例女性正常对照者,提取外周血基因组 DNA,采用聚合酶链式反应(PCR 技术),特异性扩增ABCG2 基因所需要的目的片段并测序,比较痛风组和正常对照组的基因型频率及等位基因频率分布情况。**结果:** rs2231142 位点的 CC、CA、AA 基因型频率在两组间存在显著差异(x^2=16.519,P<0.001),且痛风组中 A 等位基因频率显著高于正常对照组(分别为 42.2 %和 29.3 %,P<0.001, OR 1.76 [95 % CI: 1.35-2.31])。**结论:** ABCG2 基因第五外显子区 rs2231142(C/A)位点的单核苷酸多态性与中国汉族女性人群痛风易感性密切相关,携带 A 等位基因的汉族女性人群有更高的痛风患病率。ABCG2 基因首次被证实为中国汉族女性人群的痛风致病易感基因。

关键词: ABCG2;痛风;单核苷酸多态性;中国汉族女性

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