ORIGINAL ARTICLE

The *ABCG2* Polymorphism rs2725220 Is Associated with Hyperuricemia in the Korean Population

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Elevated serum uric acid levels are associated with a variety of adverse health outcomes, including gout, hypertension, diabetes mellitus, metabolic syndrome, and cardiovascular diseases. Several genome-wide association studies on uric acid levels have implicated the ATP-binding cassette, subfamily G, member 2 (*ABCG2*) gene as being possibly causal. We investigated an association between the single-nucleotide polymorphism (SNP) rs2725220 in the *ABCG2* gene and uric acid levels in the Korean population. A total of 991 subjects in Seoul City were used for a replication study with *ABCG2* SNP rs2725220. The rs2725220 SNP in the *ABCG2* gene was associated with mean uric acid levels (effect per allele 0.25 mg/dL, p < 0.0001). Subjects with the GC/CC genotype had a 1.78-fold (range, 1.22- to 2.62-fold) higher risk of having abnormal uric acid levels (≥7.0 mg/dL) than subjects with the GG genotype. When analyzed by gender, the association with ABCG2 was stronger in men than in women. The association with ABCG2 was much stronger in male subjects with body mass index (BMI) ≥ 26.4 (odds ratio, 5.09; 95% confidence interval, 2.41 to 10.8) than in male subjects with BMI < 26.4. This study clearly demonstrates that genetic variations in *ABCG2* influence uric acid levels in Korean adults.

Keywords: *ABCG2*, genetic polymorphism, uric acid

Introduction

Elevated serum uric acid levels are associated with a variety of adverse health outcomes, including gout, hypertension, diabetes mellitus, metabolic syndrome, and cardiovascular diseases [1]. Several genome-wide association studies (GWASs) have identified genes that may be causally associated with uric acid levels, including the ATP-binding cassette, subfamily G, member 2 (*ABCG2*) gene (MIM 603756) [2-7]. Replication studies of *ABCG2* variants have been performed in Western and Asian countries [8, 9]. Okada *et al.* [4] identified a significant association between the rs2725220 single-nucleotide polymorphism (SNP) on chromosome 4q22 and uric acid levels.

The ABCG2 protein is a high-capacity transporter for uric acid excretion in the kidney, liver, and gut [10, 11]. In this study, we analyzed the relationship between uric acid levels and the *ABCG2* SNP rs2725220 using a group of volunteers from the Korean Metabolic Syndrome Research Initiative Study in Seoul. We also analyzed the association of the *ABCG2* SNP with body mass index and waist circumference levels.

Methods

Study population

Subjects for the GWAS were recruited from the Korean Metabolic Syndrome Research Initiative Study in Seoul City, which was initiated in December 2005. A total of 9,128 individuals were recruited in 2006, and an additional 17,569 individuals were recruited in 2007 [12-14]. Therefore, the total Seoul City cohort included 26,697 volunteers. Volunteers from the first round underwent routine health examinations at the health promotion centers in university hospitals between January 2006 and December 2007. From

this total, 6,563 individuals were randomly selected for measurement of adiponectin levels. The subject characteristics were described in a previous study [14]. In brief, of the 6,563 individuals whose adiponectin was measured, 1,004 individuals were genotyped. The 1,004 subjects were all healthy individuals and were not undergoing any treatment for hyperuricemia. The Institutional Review Board of Human Research of Yonsei University approved the protocols of this study, and written informed consent was obtained from all subjects prior to enrollment.

Data collection

Participants were interviewed using a structured questionnaire to collect a personal history of cigarette smoking (never smoked, ex-smoker, or current smoker) and demographic characteristics (age, gender, etc.). Waist circumference was measured midway between the lower rib and iliac crest. For measurements of weight and height, light clothing was worn. Body mass indices were calculated as the subject's weight (kg) divided by the square of the subject's height (m^2).

For clinical chemistry assays, serum was separated from peripheral venous blood samples obtained from each participant after a 12-hour fast and stored at -70^oC. Biomarkers of metabolic syndrome, including fasting blood glucose, total cholesterol, triglycerides, and high-density lipoprotein cholesterol, and uric acid levels were measured. Quality control of the data was conducted in accordance with the procedures recommended by the Korean Association of Laboratory Quality Control.

Genotyping assays

Samples from the Seoul City cohort were genotyped on the Affymetrix Genome-Wide Human SNP Array 5.0 (Affymetrix Inc., Santa Clara, CA, USA) at DNALink. For the data obtained from this chip, internal quality control (QC) measures were used: the QC call rate (Dynamic Model algorithm) always exceeded 86%. Genotype calling was carried out using the Birdseed (v2) algorithm. A total of 1,004 individuals were genotyped using this platform. Ten of the 1,004 individuals were removed due to low genotyping call rates ($<$95%). Three individuals were excluded due to failed genotyping of rs2725220, leaving a total of 991 individuals for the study.

Statistical analysis

Data were expressed as means ± standard deviation (SD). Most statistical analyses were performed using PLINK and SAS (version 9.2; SAS Institute, Cary, NC, USA). The *ABCG2* gene SNP rs2725220 was selected using the Affymetrix Genomewide Human SNP Array 5.0.

Each SNP was tested for possible effects on uric acid levels under an additive model. The multivariate linear regression models used in the study incorporated covariates (age and sex). Multiple logistic regression analysis was also performed. Odds ratios (ORs) with 95% confidence intervals (CIs) were calculated to examine the association of the *ABCG2* SNP with abnormal uric acid levels ($\geq$7 mg/dL for men and $\geq$6 mg/dL for women). All statistical tests were two-sided, and the statistical significance was determined as $p < 0.05$.

Results

The majority of individuals in this study was middle-aged (Table 1). This sample of Korean volunteers had low uric acid levels on average. Mean levels of uric acid in the dataset were higher in males (6.23 mg/dL) than in females (4.21 mg/dL). The percentage of individuals with abnormal uric acid levels ($\geq$7.0 mg/dL) was 14.7% for the Seoul City sample. The percentage of current smokers in the Seoul City dataset was 46.4% among male subjects and 3.9% among female subjects.

Table 2 shows the p-values from a linear regression model for uric acid levels in the cohort sample, with age and sex included as covariates in the model. The rs2725220 SNP in the *ABCG2* gene was found to be associated with mean uric acid levels (effect per allele 0.25 mg/dL, $p < 0.0001$), which was significant after Bonferroni correction ($p = 0.00025$). The minor allele frequency of rs2725220 was 0.222, and Hardy-Weinberg equilibrium was satisfied ($p = 0.7079$).

Table 1. General characteristics of the study population

Subjects		Subjects in Seoul City
No.		991
Male gender (%)		56.3
Age (y)		41.5 ± 8.5
Waist circumference (cm)		81.1 ± 9.7
Body mass index (kg/m^2)		23.7 ± 3.1
Fasting blood sugar (mg/dL)		93.8 ± 16.4
Systolic blood pressure (mm Hg)		120.8 ± 13.8
Diastolic blood pressure (mm Hg)		73.8 ± 10.4
HDL-cholesterol (mg/dL)		54.2 ± 12.9
LDL-cholesterol (mg/dL)		108.7 ± 29.2
Triglyceride (mg/dL)		118.0 ± 93.5
Uric acid (mg/dL)		5.34 ± 1.50
Smoking status	Non-smoker	549 (55.4)
	Ex-smoker	166 (16.7)
	Current smoker	276 (27.9)
Uric acid (mg/dL)	Normal ($<$7.0)	845 (85.3)
	Abnormal ($\geq$7.0)	146 (14.7)

Values are presented as mean ± standard deviation or number (%).
HDL, high-density lipoprotein; LDL, low-density lipoprotein.

Table 2. Association of *ABCG2* gene SNP rs2725220 with uric acid levels and lipid levels based on a linear regression model

Phenotype	Genotype (mean ± SD)			Effect (mg/dL)	p-value*
	GG (n = 601)	GC (n = 340)	CC (n = 50)		
Uric acid (mg/dL)	5.26 ± 1.39	5.41 ± 1.59	5.93 ± 1.94	0.25	<0.0001
HDL-cholesterol (mg/dL)	53.6 ± 12.6	55.5 ± 13.3	52.7 ± 12.9	0.71	0.2631
Triglyceride (mg/dL)	117.9 ± 85.2	115.0 ± 94.5	138.9 ± 159.9	3.46	0.4679
LDL-cholesterol (mg/dL)	108.9 ± 29.5	107.8 ± 28.4	113.9 ± 31.2	0.77	0.6179

Estimated effect size (β) and p-value in the multiple linear regression model considering age and sex under an additive model. SNP, single-nucleotide polymorphism; SD, standard deviation; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

Table 3. Odds ratio (OR) of polymorphic genotypes of *ABCG2* gene SNP rs2725220 on uric acid levels[a] in Seoul City data (n = 991)

Subject	Genotype	Normal (<7.0 mg/dL)[b]	Abnormal (≥7.0 mg/dL)[b]		
		No. (%)	No. (%)	OR (95% CI)	p-value
All	GG	527 (62.4)	74 (50.7)	1.00 (reference)	
	GC/CC	318 (37.6)	72 (49.3)	1.78 (1.22–2.62)	0.0031
Men	GG	269 (65.1)	73 (50.3)	1.00 (reference)	
	GC/CC	144 (34.9)	72 (49.7)	1.82 (1.24–2.68)	0.0023
Women	GG	253 (60.2)	6 (46.2)	1.00 (reference)	
	GC/CC	167 (39.8)	7 (53.8)	1.75 (0.58–5.31)	0.3215

SNP, single-nucleotide polymorphism; CI, confidence interval.
[a]Adjusted for age and sex; [b]Abnormal (≥6.0 mg/dL) and normal (<6.0 mg/dL) were used for women.

Table 4. Odds ratio (OR) of polymorphic genotypes of *ABCG2* gene SNP rs2725220 on uric acid levels[a] in Korean men (n = 558)

Subject		Genotype	Normal (<7.0 mg/dL)	Abnormal (≥7.0 mg/dL)		
			No. (%)	No. (%)	OR (95% CI)	p-value
BMI (kg/m²)	<22.65	GG	72 (62.6)	13 (54.2)	1.00 (reference)	
		GC/CC	43 (37.4)	11 (45.8)	1.41 (0.58–3.43)	0.4456
	<24.3	GG	71 (64.0)	13 (44.8)	1.00 (reference)	
		GC/CC	40 (36.0)	16 (55.2)	2.13 (0.93–4.89)	0.0748
	<26.4	GG	63 (60.0)	25 (71.4)	1.00 (reference)	
		GC/CC	42 (40.0)	10 (28.6)	0.60 (0.26–1.38)	0.2263
	≥26.4	GG	63 (76.8)	22 (38.6)	1.00 (reference)	
		GC/CC	19 (23.2)	35 (61.4)	5.09 (2.41–10.8)	<0.0001
WC (cm)	<80	GG	71 (64.0)	10 (55.6)	1.00 (reference)	
		GC/CC	40 (36.0)	8 (44.4)	1.42 (0.52–3.89)	0.4956
	<86	GG	73 (61.3)	15 (48.4)	1.00 (reference)	
		GC/CC	46 (38.7)	16 (51.6)	1.68 (0.76–3.74)	0.2016
	<91	GG	65 (69.2)	17 (51.5)	1.00 (reference)	
		GC/CC	29 (30.8)	16 (48.5)	1.98 (0.87–4.54)	0.1054
	≥91	GG	60 (67.4)	31 (49.2)	1.00 (reference)	
		GC/CC	29 (32.6)	32 (50.8)	2.10 (1.08–4.10)	0.0299

SNP, single-nucleotide polymorphism; CI, confidence interval; BMI, body mass index; WC, waist circumference.
[a]Adjusted for age.

We also analyzed the association of rs2725220 with abnormal uric acid levels (Table 3). Subjects with the GC/CC genotype had a 1.78-fold (range, 1.22- to 2.62-fold) higher risk of having abnormal uric acid levels (≥7.0 mg/dL) than subjects with the GG genotype. When analyzed by gender, the association with ABCG2 was stronger in men than in women. In Table 4, we analyzed the association of ABCG2 by body mass index (BMI) quartile levels and waist circumference quartile levels in male subjects. The association with ABCG2 was much stronger in male subjects with BMI ≥

26.4 (OR, 5.09; 95% CI, 2.41 to 10.8) than in male subjects with BMI < 26.4. The association with ABCG2 was also a little bit stronger in male subjects with waist circumference (WC) ≥ 91 (OR, 2.10; 95% CI, 1.08 to 4.10) than in male subjects with WC < 91.

Discussion

In a cohort study of 991 subjects, an *ABCG2* gene SNP was associated with increased uric acid, consistent with previous studies. Several GWASs have reported that *ABCG2* exerts a major influence on uric acid levels [2, 3, 6, 7]. Dehghan *et al.* [6] conducted a genomewide study in which an SNP in the *ABCG2* gene, rs2231142, displayed strong evidence of an association with uric acid levels ($p < 10^{-60}$). Another GWAS also reported that rs2231142 showed a strong association with uric acid levels ($p = 5.1 \times 10^{-6}$) [2]. More recently, a GWAS in European-American obesity cases and controls reported significant genome-wide association of two SNPs with uric acid levels: rs2622605 and rs1481017 [3]. Another recent GWAS also reported a strong association between rs2231142 and uric acid levels ($p = 3.34.1 \times 10^{-42}$) [7].

In the present study, rs2725220 was found to have a strong association with uric acid levels. In agreement with these findings, a recent study reported that rs2725220 was strongly associated with uric acid levels (effect per allele -0.135 mg/dL, $p = 4.2 \times 10^{-30}$) [4]. Several recent studies reported that the association between serum uric acid and the allelic effects of SNPs in *ABCG2* was sex-specific [8, 9]. In a recent study of 4 US populations, the association between rs2231142 and serum uric acid was significantly stronger in men and postmenopausal women compared to their premenopausal counterparts [9]. In the present study, the association of the *ABCG2* gene SNP with uric acid was stronger in men than in women.

Associations between genotypes and phenotypes can be modified by gene-environment interactions. A modification of the effect of the associations between *ABCG2* SNPs and serum uric acid concentrations by interactions with obesity has been reported [3, 9]. In a recent study of 4 US populations, the association between rs2231142 and serum uric acid was stronger in normal-weight subjects than in obese subjects [9]. In the present study, we also examined the association of the *ABCG2* SNP with uric acid levels according to BMI level and waist circumference. The association was stronger in subjects with a high BMI and high WC levels than in subjects with a low BMI and low WC levels.

The *ABCG2* gene encodes a membrane transporter belonging to the ATP-binding cassette superfamily of membrane transporters, a group involved in the trafficking of biological molecules across cell membranes. The *ABCG2* gene contains 16 exons and spans over 66 kb [15]. Allikmets *et al.* [16] mapped the *ABCG2* gene to human chromosome 4q22 using radiation hybrid analysis.

Candidate gene analysis suffers from population substructure. We estimated the identity by state over all SNPs, and only four individuals were shown to be biological relatives. Genetic studies in Asian populations may not identify the same set of genes as those in European populations [17]. Nevertheless, the Seoul City cohort study in Korean populations indicates that the *ABCG2* gene on chromosome 4 is associated with serum uric acid levels, as is the case in several other populations.

Acknowledgments

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