The Combination of SLC2A9 Gene (rs2280205 and rs6820230) and Major Metabolic Factors with Association to Gout in Thai Men; A Matched Case-Control Study

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Abstract

A combination of non-synonymous variants, rs2280205 and rs6820230 of the SLC2A9 gene and major metabolic parameters contribute to developing gout remains not well studied or assessed in Thai men. This study was conducted to assess the association between combined of two non-synonymous variants and gout. Using data from male subjects of age ≥ 20 years in the Genetic variation of Urate transporter genes in Hyperuricemia and Gout among Thai population Study (GUHGTHS). We randomly performed a 1:1 age-matched case-control study that included 48 gout patients and 48 non-gout subjects. Using multivariate logistic regression analysis was used to analyze data. The single and joint locus effect of rs2280205 and rs6820230 variants were independently associated with gout. However, the combination of rs2280205 and high fasting glucose, including rs6820230 variant and high fasting glucose were associated with gout, the adjusted odds ratio was 13.70-fold and 5.81-fold, respectively. Meanwhile, we did not observe an association between these variants and high blood pressure, including general obesity with gout.

In conclusion, rs2280205 and rs6820230 variants did independently associated with increased risk of gout, but predominantly occurred in high fasting glucose subjects. However, further studies with larger sample sizes and homogeneous populations should be confirmed these results.

Keywords: *rs2280205 variant, rs6820230 variant, gout, metabolic parameters.*

Introduction

Gout has an increasing prevalence and incidence in the Asia-Pacific region.[1-2] Genetic factors play an essential role in the risk of gout. The genome-wide association studies (GWAS) have identified that more than 20 multiple loci associated with gout in American-European populations.[3-6] A subsequent functional study revealed that glucose transporter 9 encoded by SLC2A9 gene possibly interfered with excretion of urate.[7-10] However, several studies have reported the association of some non-synonymous variants of SLC2A9 gene to gout.[11-13] The two common rs2280205 and rs6820230 variants have been described as possible sites of interaction with urate and glucose, thereby interfering with their excretion.[14] Previous studies in 2014 showed that rs2280205 variant was reduced associated with the risk of gout (32%), while rs6820230 variant was increased with susceptibility to gout in Caucasian.[15] A frequent rs2280205 variant was associated with

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slightly lower serum uric acid reduction of 5 to 10%.[3,16] However, studies on theses variants did not show an association with gout[(3,16)]. Moreover, previous studies revealed that the risk factors of gout are complicated due to environmental factors, particularly in men, postmenopausal,[17-19] diuretics,[17,19] seafood,[19] and sugar-sweetened soft drinks.[20] Previously, several studies also revealed that the major associated factors of metabolic syndrome including obesity,[21-23] hypertension,[23,24] and high fasting glucose[25,26] are independently associated with gout. The previous studies also provided successful findings which indicated the associations of genetic variants and major metabolic factors that play a part in determining the risk of developing gout. However, the association between rs2280205 and rs6820230 variants and gout risk in Thai men has never been examined. Thus, the aim of this study was to investigate the effects of these variants, SNP-SNP interaction and a combination of genetic and major metabolic factors contributing to the development of gout.

Materials and Method

Study Population: This matched case-control study was performed using the GUHGTHS data. The target population was men with age ≥ 20 years and had participated in health examinations and blood sampling. of the 145 subjects (77 gout patients and 68 non-gout subjects) whose data were used in 2018, subjects over 65 years of age and those with incomplete data were excluded from this study. Furthermore, subjects showing evidence of diseases related to gout such as acute heart disease, kidney disease, cancer, induce and/or reduce serum uric acid medication/substances were also excluded from the study. Gout cases and non-gout subjects were randomly selected and matched (1:1) based on age (5±10 years). As a result, a total of 96 subjects (48 gout patients and 48 non-gout subjects) were enrolled for this present study. Forty-nine subjects were excluded from the study because we could not match between cases and non-gout subjects.

Definitions: All gout patients were clinically diagnosed based on the Rome criteria[27] and confirmed by the rheumatologist. The inclusion criteria of non-gout subjects were normal level of serum uric acid and no evidence and no symptoms of gout. General obesity was classified based on body mass index (BMI) ≥ 25 kg/m^2. High fasting plasma glucose (FPG) was defined by FPG ≥ 100 mg/dl or diabetic treatment. High blood pressure (BP) was defined by systolic BP ≥ 130 mmHg or diastolic BP ≥ 85 mmHg and antihypertensive medication. Hypertriglyceridemia was defined by elevated triglyceride ≥ 150 mg/dl or medication. Finally, Low high-density lipoprotein cholesterol (HDL-C) was defined by HDL-C < 40 mg/dl or reduced HDL-C medication.

Data Collection: We recorded the results of genotype and allele distribution from the GUHGTHS. Moreover, we collected the original values of clinical and biochemical data via standardized data extraction form.

Statistical Method: All statistical analyses were performed using STATA version 14 (Stata, College Station, TX). The Hardy-Weinberg equilibrium was used to describe genotype and allele distribution.[28] Multivariate conditional logistic regression analysis was used to analyze the data. A p-value less than 5% was considered statistically significant.

Results

In Thai men, the gout patients were found to be older than the non-gout subjects (Table 1). The mean BMI and uric acid level of gout patients were higher than non-gout subjects. The percentage of gout patients with general obesity, high BP, high FPG and hypertriglyceridemia was higher than non-gout subjects, but the percentage of low HDL-C with non-gout subjects was higher than gout patients.

Our representative results of the genotype and allele distribution for rs2280205 and rs6820230 variants are shown in Table 2. A single locus effect of rs2280205 and rs6820230 variants were not associated with gout (Table 3). In addition, we found that there were no interactions between two variants with the development of gout risk. However, the interactions between rs2280205 variant with high FPG significantly increased the risk of gout. The interactions of rs6820230 variant with high FPG also increased the risk of gout. In contrast, the rs2280205 and rs6820230 variants combined with high BP and general obesity were not significantly associated with gout risk (Table 3).

Discussion and Conclusion

The present study indicated that two non-synonymous rs2280205 and rs6820230 variants were not associated with gout in Thai men. The previous studies also demonstrated that these variants did not

show an association with gout in Czech population[3] and Cameroonians.[12] However, Chisnall (2014)indicated that rs6820230 variant was associated with susceptibility to gout, whereas the rs2280205 variant could reduce (32%) the risk of gout in Caucasian.[15] Moreover, several recent studies by GWAS have identified that the SLC2A9 gene may be associated with gout.[29-31]The product of SLC2A9 gene encodes for the molecule to reabsorb uric acid in the kidney and loss of function from a mutation in this gene causes renal hypouricemia and prevents reabsorption of filtered urate proximal tubules.[7-10] When we examined the SNP-SNP interaction that could be involved in a wide range of gout-related processes. We found that the combination of rs2280205 and rs6820230 variants has no association with gout. In general, several genes can contribute to gout without their gene products ever directly interacting. We assumed that the expression of the rs2280205 and rs6820230 variants may also oppose each other, with one variant modifying the expression of another variant.

There is some evidence suggesting that major metabolic factors such as general obesity,[21-23] hypertension,[23] and high FPG[32] are associated with gout. Therefore, we hypothesized that gene-environmental interaction might also play a significant role in gout risk; our study indicated that the combination of rs2280205 and rs6820230 variants with high FPG, but not general obesity and high BP, is significantly increased the risk of gout. We agreed with the remarks of a previous study that high FPG might influence the function of glucose transporter 9 (GLUT-9)[33] and may contribute to the reabsorption of uric acid through elevated expression of the urate transporter-1.[34] we assumed that an increase of glucose in tubular fluid with an associated elevation of reabsorptive transport on GLUT-9 may inhibit uric acid reabsorption. However, the mechanisms of association between gene-environment need to be explored in near future.

The current study had few limitations. First, this study involved the use of a small number of gout patients from a single hospital-based population: Large independent studies are required to further validate our results. Secondary, we only studied two variants of SLC2A9, therefore gene-gene interactions with some other gene should be investigated in the future studies. Finally, we were not able to collect other details of major environmental factors such as alcohol, smoking, dietary consumption, waist circumference, waist to hip ratio, that could affect gout.

In conclusion, the combination of rs2280205 and rs6820230 variants and high FPG contributed to the development of gout. Our study revealed that these genetic data and an interaction analysis have provided considerably to our understanding of the pathogenesis of gout. Further studies with larger sample sizes and homogeneous populations should be confirmed.

Table 1. Baseline characteristics

Variables	Gout, n (%)	Non-gout, n (%)
Number	48	48
Age (years)	57.94±12.23	54.58±14.64
Body mass index (kg/m^2)	26.20±5.00	24.95±3.58
Body mass index ≥ 25	30(62.50)	22(45.83)
High blood pressure	41(85.42)	29(60.42)
High fasting glucose	33(68.75)	17(35.42)
Hypertriglyceridemia	33(68.75)	25(52.08)
Low HDL-C	40(83.33)	43(89.58)
Serum uric acid (mg/dL)	6.28±2.27	6.47±1.19

HDL-C: high density lipoprotein cholesterol

Table 2. Genotypes and alleles distribution

SNPs	Genotypes or Alleles	Frequencies, n (%)		p-value*
		Gout	Non-gout	
Number		48	48	
rs2280205				
	G/G	29 (60.00)	32 (67.00)	0.140
	G/A	16 (33.00)	12 (25.00)	
	A/A	3 (6.00)	4 (8.00)	
	G/A-A/A	19 (40.00)	16 (33.00)	
	Allele, G	74 (77.00)	76 (79.00)	
	Allele, A	22 (23.00)	20 (21.00)	

SNPs	Genotypes or Alleles	Frequencies, n (%)		p-value*
		Gout	Non-gout	
rs6820230				
	C/C	39 (81.00)	42 (88.00)	1.000
	C/T	9 (19.00)	6 (12.00)	
	T/T	0	0	
	C/T-T/T	9 (19.00)	6 (12.00)	
	Allele, C	87 (91.00)	90 (94.00)	
	Allele, T	9 (9.00)	6 (6.00)	

* Hardy-Weinberg equilibrium test; SNPs: single nucleotide polymorphisms

Table 3. The major risk factor associated with gout

Factors		OR (95% CI)	aOR (95% CI)
rs2280205[1]			
G/G		1.00	
G/A-A/A		1.33 (0.57-3.02)	2.70 (0.45-16.04)
rs6820230[1]			
C/C		1.00	
C/T-T/T		1.60 (0.53-4.96)	2.80 (0.51-15.47)
Best combination			
rs2280205	rs6820230		
G/G	C/C	1.00	
G/G	C/C-T/T	2.20 (0.58-8.26)	2.47 (0.39-5.57)
G/A-A/A	C/C	1.63 (0.63-4.22)	3.51 (0.90-3.64)
G/A-A/A	C/T-T/T	1.28 (0.75-2.73)	1.57 (0.70-5.40)
rs2280205	Obesity[2]		
G/G	Normal	1.00	
G/G	Obesity	1.81 (0.68-4.80)	1.48 (0.31-6.87)
G/A-A/A	Normal	1.39 (0.40-4.80)	2.09 (0.28-5.42)
G/A-A/A	Obesity	3.31 (0.81-13.52)	3.93 (0.44-5.09)
rs2280205	Fasting plasma glucose (FPG)[3]		
G/G	Normal	1.00	
G/G	High FPG	3.65 (1.06-12.68)	4.45 (0.93-11.19)
G/A-A/A	Normal	0.96(0.24-3.82)	1.25 (0.15-14.28)
G/A-A/A	High FPG	12.05(1.99-17.95)	13.70(1.59-15.25)
rs2280205	Blood pressure (BP) [4]		
G/G	Normal	1.00	
G/G	High BP	7.31 (0.48-8.24)	7.38 (0.85-9.42)
G/A-A/A	Normal	2.20 (0.26-6.77)	3.68 (0.46-8.73)
G/A-A/A	High BP	7.31 (0.46-8.64)	7.84 (0.18-9.87)
rs6820230	Obesity[2]		
C/C	Normal	1.00	
C/C	Obesity	1.15 (0.46-2.86)	0.89 (0.24-3.21)
C/T-T/T	Normal	0.52 (0.88-3.05)	0.56 (0.46-6.87)
C/T-T/T	Obesity	6.68 (0.81-9.16)	3.14 (0.24-4.65)

Factors		OR (95% CI)	aOR (95% CI)
rs6820230	Fasting plasma glucose (FPG) [3]		
C/C	Normal	1.00	
C/C	High FPG	4.88 (1.47-6.16)	7.47 (0.53-6.47)
C/T-T/T	Normal	1.40 (0.20-9.81)	3.98 (0.22-7.04)
C/T-T/T	High FPG	7.12 (1.18-13.04)	5.81(1.88-8.21)
rs6820230	Blood pressure (BP) [4]		
C/C	Normal	1.00	
C/C	High BP	3.43 (0.15-7.78)	5.06 (0.78-9.58)
C/T-T/T	Normal	1.54 (0.26-8.66)	4.27 (0.28-8.29)
C/T-T/T	High BP	1.03 (0.11-6.58)	1.53 (0.19-4.68)

OR: crude odds ratio; Adjusted odds ratio (aOR) 1) obesity, high BP, hypertriglyceridemia and high FPG, 2) high FPG and hypertriglyceridemia; 3) obesity, high BP and hypertriglyceridemia; 4) obesity, high FPG and hypertriglyceridemia

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