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Study on association between *SLC2A9* rs3733591 and Gout susceptibility in 481 Vietnamese individuals

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Abstract:

Gout is a common form of inflammatory arthritis that is strongly associated with elevated uric acid concentration in the blood. The development of the disease is not only triggered by environmental factors but also genetic variations. Previous studies demonstrated that the genetic associations with gout vary in different populations in the world. This study aimed to identify the relationship between *SLC2A9* rs3733591 and gout susceptibility in the Vietnamese population. Total DNAs were extracted from 481 blood samples including 160 patients with gout and 321 age-matched healthy controls. The genotyping of *SLC2A9* rs3733591 was performed using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Chi-squared test was used to test whether the genotypes frequencies of rs3733591 follow Hardy-Weinberg equilibrium (HWE) and to check its association with gout in three models (additive, recessive, dominant) and allele form. The result showed that *SLC2A9* rs3733591 was in agreement with Hardy-Weinberg equilibrium (p>0.05). However, there was no association between the rs3733591 and gout in any tested models (p>0.05). This study will contribute to the genetic study of gout susceptibility in Vietnam.

Keywords: **Gout, PCR-RFLP, rs3733591, *SLC2A9*, Vietnam.**

Classification numbers: **3.2, 3.5**

1. Introduction

Gout is common inflammatory arthritis associated with severe co-morbidities including cardiovascular diseases, chronic kidney diseases, obesity, and type 2 diabetes [1]. Gout might result from high serum urate levels (hyperuricemia) and accumulation of monosodium urate crystals in the joints and soft tissues. Based on the patient's fractional excretion of urate clearance (urate clearance/creatinine clearance ratio, FEUA) and urinary urate excretion (UUE), gout is classified into two distinct types: renal overload (ROL) and renal underexcretion (RUE) [2]. Gout patients often suffer from throbbing, burning, pain, swelling, warmth, redness, and difficulty moving the affected joint due to inflammation. Besides, gout is commonly encountered in middle-aged male patients. In Vietnam, the rate of gout was 0.14% of the population in 2003 [3]; 1.0% of the population (940,000 patients) in 2014 include: 96% were men, 38% were in their 40s, with 75% in the working age. In addition, the development of gout disease is caused not by only environmental factors, a high protein diet, and alcohol use, but also genetic factors. Therefore, research on the inherited cause of gout is extremely important especially when the disease significantly impacts the working-age population's performance and work quality. For the last two decades, Genome-Wide Association Studies (GWAS), replication studies, and meta-analyses have broadened our knowledge of common genetic variants with a predisposition to gout. The discovery of these novel genes has substantially increased our understanding of the role of renal urate transporters in the pathogenesis of gout [4]. In particular, the solute carrier family 2 member 9 (also known as SLC2A9) plays an important role in the cause of gout.

SLC2A9, located on chromosome 4p16.1, contains 12 exons spanning over 195 Kb. The gene product is a member of the GLUT family of transport facilitators, consisting of monosaccharides and polyol transporters that can carry other small carbon compounds across the plasma membranes [5]. The SLC2A9 gene encodes

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GLUT9, which is a membrane-spanning protein from the significant facilitator transporter superfamily and includes elements of sugar transporters. In one study, GLUT9 proteins behave as an exchange transporter exchanging uric acid for glucose and fructose [6]. Still, it most likely mediates the efflux of urate under physiological circumstances in the proximal tubule cells [7]. The effect of variations in SLC2A9 is most pronounced in females, in whom it accounts for approximately 6% of the variance of serum urate compared to 2% in males [8]. Besides, multiple genetic studies suggest that mutations in the SLC2A9 gene are linked with gout risk in different populations. For example, rs16890979 (V253I) was associated with gout in a GWAS of US Caucasian population [9], which was replicated in several studies of New Zealand Māori, Pacific island, Caucasian [10], Spanish [11], and Chinese populations [12]. Another variant, rs6449213, was associated with gout in Germany [13] and the US populations [9]. Among the studied polymorphisms, rs3733591 was frequently investigated and generated inconsistent results in different populations (Chinese, Solomon Islanders, Japanese, New Zealander, Maylay). To the best of our knowledge, no study has been performed to assess the correlation between *SLC2A9* rs3733591 and gout susceptibility in the Vietnamese population. Therefore, to understand the relationship of *SLC2A9* rs3733591 with gout in the Vietnamese population, we conducted a case-control association study of this variant in Vietnamese people.

2. Materials and methods

2.1. Study subjects

A total of 481 subjects, including 160 male patients with gout and 321 healthy controls, were enrolled at Dai Phuoc general clinic, Ho Chi Minh city. Gout patients were diagnosed following the criteria of the American College of Rheumatology [14]. Controls were males, age-matched healthy individuals, and recruited randomly from annual health checks with no family history of diabetes or gout. The study was approved by the Institutional Review Board of the Institute of Genome Research, Vietnam Academy of Science and Technology (No. 1-2017/NCHG-HDDD).

2.2. Methods

DNA extraction: total DNA was extracted from blood samples using the Kit GeneJET Whole Blood Genomic DNA Purification (Thermo Fisher Scientific) following the manufacturer's protocol. After isolation, total DNA samples were checked by electrophoresis in agarose gel. Additionally, the quality and quantity of total DNA were assessed using a Nanodrop 2000c spectrophotometer (Thermo Scientific). DNA samples were then diluted to a concentration of 10 ng/µl to obtain standard working samples that were stored at -20°C.

Genotyping of SLC2A9 rs3733591 using PCR-RFLP: to genotype *SLC2A9* rs3733591, polymerase chain-reaction-restriction fragment length polymorphism (PCR-RFLP) was performed using specific primers. The primer sequences will be provided upon request. Reaction components included 10 ng DNA, 0.25 mmol each dNTP (Thermo Fisher Scientific), 0.25 U Taq DNA polymerase (Thermo Fisher Scientific), 5 pmol primers (per each direction), Dream Taq Buffer (Thermo Fisher Scientific). The PCR cycle was as follows: 94°C - 4 min; 30 cycles of 94°C - 15 s, 62°C - 15 s, 72°C - 30 s; 72°C - 8 min; kept at 4°C.

PCR products were then digested with restriction enzyme *Bst*UI (Thermo Fisher Scientific). Reaction components included 1 µl of Buffer R, 3 µl of PCR product, 1U *Bst*UI, and water added to 10 µl. The mixture was incubated at 37°C in a water bath for 4-6 h. Digested products were further assessed using electrophoresis of 2.5% agarose gel. Depending on the number of DNA bands acquired from enzymatic digestion of PCR products, the genotypes of *SLC2A9* rs3733591 are summarized and given in Table 1. In addition, 10% of randomly selected subjects were confirmed by Sanger sequencing.

Table 1. Number and size of DNA bands of 3 genotypes of *SLC2A9* rs3733591.

Genotypes	Number of DNA bands	Size of DNA bands (bp)
CC	2	219, 177
CT	3	396, 219, 177
TT	1	396

Statistical analysis: the obtained data were analysed using SPSS version 20. Chi-squared test (χ^2) was used to test whether allele distribution follows Hardy-Weinberg equilibrium (HWE). Association between polymorphic genotype with gout was assessed in three test models (additive, dominant, and recessive) and estimated by OR (odds ratio) with 95% confidence intervals. The estimation was considered to be statistically significant if the p-value was less than 0.05.

3. Results

3.1. Genotype identification of polymorphism SLC2A9 rs3733591

A total of 481 subjects, including 160 gout patients and 321 controls, were used to amplify the region containing polymorphism rs3733591 using PCR. The PCR products were digested with the restriction enzyme *Bst*UI (Fig. 1).

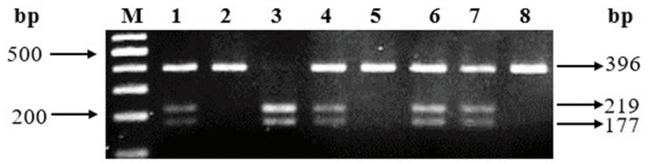

Fig. 1. Image of eight *Bst*UI-digested PCR products on 2.5% agarose gel. M: marker 100 bp; 1,4,6,7: genotype CT (3 bands with 396 bp, 219 bp and 177 bp); 2,5,8: genotype TT (396 bp); 3: genotype CC (two bands with 219 bp and 177 bp).

The frequencies of genotypes and alleles *SLC2A9* rs3733591 from all 481 samples were summarized in Table 2. Allele C (minor allele) was less common than allele T in the studied population with frequencies of 0.353 and 0.647. The distribution of 3 genotypes of polymorphism *SLC2A9* rs3733591 followed Hardy-Weinberg equilibrium (HWE) with a *p*-value of 0.07.

Table 2. Information on *SLC2A9* rs3733591 genotypes and allele frequency.

	Genotype			Allele		HWE in the studied population
	CC	*CT*	*TT*	*C*	*T*	
Case	19	72	69	0.344	0.656	
Control	50	130	141	0.358	0.642	0.07
Total	69	202	210	0.353	0.647	

Note: Hardy-Weinberg equilibrium (HWE) checked by Chi-squared test.

After genotyping using RFLP, 10% of samples were verified using Sanger sequencing. The results showed 100% concordance of observed genotypes obtained from PCR-RFLP and Sanger sequencing (Fig. 2).

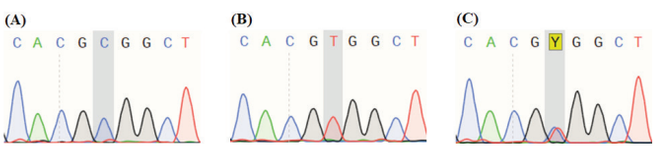

Fig. 2. Genotyping *SLC2A9* rs3733591 using Sanger sequencing. (A) genotype CC; **(B)** genotype TT; **(C)** genotype CT.

3.2. Analysis of the correlation between SLC2A9 rs3733591 and gout

To evaluate the association between the genotypes and alleles of *SLC2A9* rs3733591 and gout, we performed statistical analyses in 3 test models: additive, dominant, and recessive (Table 3). The results showed that the *p*-values of the three models were all higher than 0.05 ($p>0.05$) meaning no significant difference was detected. Furthermore, when analysing the correlation between alleles of *SLC2A9* rs3733591 with gout, the *p*-value obtained was 0.658, so the allele of rs3733591 was not associated with gout risk in the studied population.

Table 3. Association of *SLC2A9* rs3733591 with gout.

Test model	Controls (n=321)	Gout patients (n=160)	OR	95% CI	*p*-value
		Additive			
CC	50 (15.6%)	19 (11.9%)	1.000		
CT	130 (40.5%)	72 (45%)	1.458	0.799-2.66	0.220
TT	141 (43.9%)	69 (43.1%)	1.269	0.695-2.318	0.438
		Recessive			
CC	50 (15.6%)	19 (11.9%)	1.000		
CT + TT	271 (84.4%)	141 (88.1%)	1.369	0.777-2.411	0.277
		Dominant			
CC + CT	180 (56.1%)	91 (56.9%)	1.000		
TT	141 (43.9%)	69 (43.1%)	1.033	0.705-1.515	0.868
		Allele			
C	230 (35.8%)	110 (34.4%)	1.000		
T	412 (64.2%)	210 (65.6%)	1.065	0.804-1.412	0.658

Note: n: number of participants; 95% CI: 95% confidence interval of odds ratio; *p*-values calculated from Chi-squared test, OR: odds ratio.

4. Discussion

Gout is a common form of arthritis in Vietnam and around the world. In addition to environmental factors such as risks from alcohol use, drugs, high-protein diets, obesity, hypertension, *etc.*, genetic factors also play an important role in the development of gout. Genome-wide association studies (GWAS) and meta-analyses have identified several genes associated with gout susceptibility such as *ABCG2, SLC22A12, SLC17A1, etc.* Among these, the *SLC2A9 (GLUT9)* gene is a known risk factor for gout among different populations worldwide. *SLC2A9*, a facilitated glucose transporter family member, is a voltage-dependent urate transporter with a not yet fully elucidated role in uric acid homeostasis. In humans, the main function of the *SLC2A9* gene is in the efflux of reabsorbed uric acid from the proximal tubule cells of the kidney toward the blood. Therefore, when a mutation occurs in the *SLC2A9* gene, it is likely to increase uric acid reabsorption and blood uric acid level. In *SLC2A9*, the variant R265H (rs3733591) occurs in many populations in different regions worldwide, with the highest allele frequency in the East Asian populations (T=0.6692) followed by the South Asian populations (T=0.3625) and the lowest in the European populations (T=0.2388). Besides that, R265H has previously been demonstrated

to be associated with gout among Han Chinese in Taiwan (p=0.008) and Solomon Islanders (p=0.0045) [15]. It was also associated with the development of gout in Japanese males (OR=1.52, 95% CI 1.19-1.95, p=7.3×10^{-4}) [16]. In contrast, rs3733591 was not associated with gout susceptibility in New Zealander populations [17], Chinese Han [18], Minnan populations in China [19], or Maylay populations [20]. Similarly, we did not find any association of the polymorphism with gout susceptibility in the current study of the Vietnamese population (p>0.05). Such inconsistency could be explained by genetic background, environmental factors, or lifestyle differences.

5. Conclusions

In the current study, we identified the genotypes of *SCL2A9* rs3733591 in the Vietnamese population using PCR-RFLP. Statistical analysis showed that rs3733591 was not associated with gout disease. This data could be used for further in-depth studies on the relationship between polymorphisms and gout in the Vietnamese population.

CRediT author statement

Pham Quang Hung: Data curation, Writing - Original draft preparation, Editing; Nguyen Xuan Canh: Conceptualisation, Methodology and Writing; Nguyen Thuy Duong: Conceptualisation, Methodology, Software, Writing and Editing.

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COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

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