

Association of solute carrier family 2, member 9 (*SLC2A9*) genetic variant rs3733591 with gout in a Malay sample set

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ABSTRACT

Introduction: Gout is one of the most common inflammatory arthritis in Malaysia. It is due to persistent hyperuricemia that leads to the formation and deposition of intra- and peri-articular monosodium urate crystals either due to excessive production or insufficient excretion of uric acid. Incidence and prevalence of gout is increasing worldwide, with a higher rate among men compared to women. Malay is the largest ethnic group in Malaysia, followed by Chinese and Indian. *SLC2A9* is a renal urate transporter that controls renal uric acid excretion and genetic variants in *SLC2A9* are associated with the risk of gout in several populations. This study aimed to test if the *SLC2A9* variant (R265H, rs3733591) is also associated with gout among Malays in Malaysia.

Methodology: A total of 89 patients with gouty arthritis and 100 normal subjects who consented and were recruited in this study. The serum urate and creatinine were measured. The SNP genotyping was performed using PCR-RFLP method for rs3733591 and *BST 1236* was used as a restriction enzyme to cut the targeted amplicons.

Result: *SLC2A9* variant was associated with gout, p-value of 0.007, OR=4.713 [95%CI 1.530-14.513], however this association was not significant after adjustment for age and gender with p=0.465 (OR=1.950; 95%CI[0.325-11.718]).

Conclusion: Our data suggest that the genetic variant of *SLC2A9* may contribute to the susceptibility of gout among Malays in Malaysia.

KEY WORDS:

gout, SLC2A9, Malay, association, SNPs

INTRODUCTION

Gout is a complex form of inflammatory arthritis caused by deposition of monosodium urate (MSU) crystals within joints and subcutaneous tissues that negatively impacts the quality of life of gout sufferers.¹ Gout patients normally present with intermittent attacks of severe joint inflammation, persistent hyperuricaemia, with some exhibiting tophaceous disease and chronic gouty arthropathy. Prevalence of gout is high in many populations and ranged from 4.4% among Han Chinese men to 10% in New Zealand Maori and Pacific

Island men.^{2,3} The incidence and prevalence of gout increase with age and men develop gout at a four times higher rate than women. However above the age of 65 years, this gender difference is less due to the lack of protective effect of oestrogen among postmenopausal women.⁴ Hyperuricemia has long been recognized to be the most important causal factor in gout development as a result of overproduction of uric acid or impaired excretion of renal uric acid. Overproduction is contributed by alcohol, sugar-sweetened beverages, meat and seafood consumption.^{5,6,7}

Solute carrier family 2, member 9 (*SLC2A9*), also known as glucose transporter 9 (GLUT9), is a uric acid transporter that plays a role in the process of reabsorption and excretion of uric acid at the renal proximal tubule, thus influencing serum uric acid levels.⁸ It is encoded by the *SLC2A9* which is located at chromosome 4p16.1 with 13 exons. The protein is mainly expressed in the basolateral membrane of proximal renal tubular cells and the apical membrane of collecting duct cells, as well as chondrocytes of human articular cartilage which can contribute to the development of tophaceous gout.^{9,10} Several *SLC2A9* variants have shown association with susceptibility to gout consistent with the role of *SLC2A9* in affecting serum urate concentration.^{8,11,12,13,14} In Europeans genetic variation in *SLC2A9* explains ~3% of variance in urate levels. The minor allele of *SLC2A9* variant, R265H (rs3733591) has been shown to contribute to serum urate elevation in Japanese, Han Chinese and Solomon Islander sample sets, but not in Caucasian sample set at a genome-wide level of significance.^{13,15,16,17} The minor allele of R265H is believed to influence the activity of *SLC2A9* in articular chondrocytes and thus is able to elevate the risk for MSU crystals deposition in joint. Therefore this study was aimed to investigate the role of rs3733591 in gout in Malay sample set by a case-control approach.

MATERIALS AND METHODS

Ethics approval

The study protocol conforms to the ethical guidelines of the Research Review Board and Human Research Ethics Committee of Universiti Sains Malaysia, Kelantan, Malaysia [USM/KK/PPP/JEPeM [234.3.(01)]] and informed consent was obtained.

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Subject recruitment

A total of 89 gout patients, who visited the Medical Specialist Clinic at Hospital Universiti Sains Malaysia from 2011 until 2012 were recruited. All these patients were diagnosed clinically to have gout by a Rheumatologist, based on the American College of Rheumatology (ACR) 1977 criteria.¹⁸ Patients were all ethnic Malays, aged between 17-71 years at disease onset. During this study period, 100 Malay subjects with no self-reported history of gout or other serious illnesses were recruited via convenience sampling. Phenotypic characteristics included demographic data and clinical parameters (tophi and disease-related complications) described in Table I. Clinical measurement for serum urate and creatinine were performed from serum specimens of gout patients and analysed by standard procedures in the Chemical Pathology Laboratory, USM.

SNP Genotyping

Blood samples were taken and genomic DNAs were isolated from leukocytes from 3 ml of peripheral blood using a GeneAll Extraction kit (South Korea, Korea). Genotyping was conducted utilizing polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method rs3733591 using primers ATGGTGACAATCACGGTGAC and TCCAAACGTTCTTGGGTAAAG that result in cleavage of the 153 bp product into 90/63 bp fragments using *Bsh* 1236. The PCR amplicons were electrophoresed on a 2 % (w/v) agarose gel and visualized under an UV transilluminator. The PCR products were direct sequenced for validation for three amplicons from each homozygous variant and the heterozygous group with 100% concordance rate to genotype data.

Statistical analysis

A post priori power calculation for this case control study was performed based on previous gout data in Asian-Pacific population, namely New Zealand Polynesians¹³, Chinese and Solomon Islanders¹⁶ and Japanese.¹⁵ Using overall minor allele frequency (MAF) of 0.468 with OR of 1.29 for rs3733591 projected 22% power with 89 cases Hardy-Weinberg equilibrium was calculated using SHEsis online software (<http://analysis.bio-x.cn/myAnalysis.php>) for cases and controls. Logistic regression of genotype against gout as outcome was conducted with adjustment for age and sex using STATA 10 software.

RESULTS

Demographic and clinical data of the participants with gout Demographic, biochemical and medical data are presented in Table I. The 89 gout patients ranged from 17 to 71 years (mean 44 years) at age at onset and majority were men (92%). All patients were treated with allopurinol. There were only 7 (7.9%) out of 89 gouty patients with normal urate levels (200-420 μ moles/l for men and 140-360 μ moles/l for women) while the remaining 82 cases (92.1%) were hyperuricemic. The mean level of serum urate in the 89 patients was 592 μ mol/l (304-883 μ mol/l) while mean serum creatinine was 269 μ mol/l (81-520 μ mol/l). Several comorbidities were present in the cases including diabetes mellitus 27%, hypertension 44%, renal impairment 48%, hyperlipidemia 27% and cardiovascular disease 17%. Most

patients were noted as over-weight with body mass index (BMI) mean of 26.4 (17.6-35.2). However, no data were available for serum urate, serum creatinine and BMI for control subjects.

Association of SLC2A9 variants with gout

The C allele of the nonsynonymous Arg265His (rs3733591) variant of *SLC2A9* was significantly overrepresented in Malay cases compared to the Malay control group with a p-value of 0.007 and conferred a risk for gout with OR=4.713 [95%CI 1.530-14.513] (Table II). The genetic marker complied with Hardy-Weinberg equilibrium (HWE) ($p > 0.01$) in both the case and control cohorts. However, after adjustment in logistic regression analysis by age and sex, no association was observed for rs3733591 with $P = 0.465$ (OR=1.950; 95%CI[0.325-11.718]) (refer Table II).

DISCUSSION AND CONCLUSION

Our study confirmed that men had a higher risk of developing gout, consistent with various studies worldwide.^{2,3,15,16} We noted that 7.9% gout patients had normal serum urate levels at study recruitment. These patients were already treated with allopurinol. Serum uric acid normally falls in acute episodes.¹⁹ Badelescu et al previously reported that urate acid levels can be normal despite raised inflammatory factors, such as C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR). Prior treatment with colchicine and allopurinol which increase urinary uric acid excretion also contributed to the normal serum urate in those patients.²⁰ Many genetic studies have demonstrated the association of *SLC2A9* variants to serum urate levels and risk of gout. *SLC2A9* gene has been shown to regulate urate reabsorption from the renal tubular epithelial cells into the bloodstream, where it plays its role as a urate transporter at the proximal renal tubules.²¹ To study the true effects of *SLC2A9* variants on serum urate levels, serum urate prior to the commencement of treatment with allopurinol would have been a better measurement. We did not have a pre-treatment serum urate level.

Hyperuricemia is a prime factor in the pathogenesis of gout, as well as being an independent determinant of other metabolic and systemic disorders such as diabetes, cardiovascular diseases, hypertension and renal diseases. *SLC2A9*, a basolateral efflux transporter of uric acid at the proximal tubules have shown to induce acute renal failure when mutation occurs to the gene as well as *SLC22A12* (*URAT1*)²². Another study by Windpessl et al. (2016) revealed that one variant of *SLC2A9* with missense substitution (c.512G>A) may be the main predictor of hereditary renal hypouricemia (RHUC)²³. It is therefore not surprising that the most common co-morbidity in our study population was renal impairment (48%), be it a direct complication of gout or related to NSAIDs, diabetes mellitus or hypertension.

The C allele of the nonsynonymous Arg265His (rs3733591) variant of *SLC2A9* has been consistently shown to have an association with gout in various populations. In Japanese men, the minor allele of rs3733591 showed association with gout with a p value of 7.3×10^{-4} conferring susceptibility.¹⁵ Tu et al (2010)¹⁶ demonstrated that the association of rs3733591

Table I: Demographic and clinical characteristics of the Malays gouty patients

Demographic data and clinical characteristics	cases (n=89)	Controls (n=100)
^a Sex, % male	92	78
^b Serum urate at recruitment, mean (range) µmol/l	592 (304-883)	-
^b Serum creatinine at recruitment, mean (range) µmol/l	269 (81-520)	-
^b Age at onset, mean (range) years	44 (17-71)	-
^b Age (mean) years	58	35
^a Allopurinol treatment, %	98	-
^b Mean (range) BMI	26.4 (17.6-35.2)	-
^a Comorbidities, %		
Type 2 DM	27	-
Hypertension	44	-
Renal disease	48	-
Hyperlipidemia	27	-
CVD	17	-

^aFrequency for categorical variable

^bIndependent T-Test was applied; mean (SD)

Table II: Association analysis of SLC2A9 variant (rs3733591) with gout in Malay sample set

SNPs	Genotype frequency			*MAF C	Unadjusted		Adjusted		HWE	
	TT*	CT*	CC*		Allelic p-value	OR 95% CI]	Allelic p-value	OR [95% CI]		
rs3733591										
	Case	74(0.831)	14(0.157)	1(0.011)	0.090	0.007	4.713 [1.530-14.513]	0.465	1.950 [0.325-11.718]	0.716
	Control	96(0.960)	4(0.040)	0(0.000)	0.020					0.838

*TT: homozygous T allele, TC: heterozygous TC alleles and CC: homozygous C allele, MAF: Minor Allele Frequency, p value >0.05 is considered significant, Fisher Exact Test was applied

in gout yielded p value of 0.008 in Han Chinese and p value of 0.0045 in Solomon Islanders. The minor C-risk allele of this SNP yielded two-fold higher risk for tophi in both populations with OR 2.05-2.15 than non-tophi with OR 0.91-1.62. Hence, rs3733591 could be a potential marker for the formation of tophi in patients with severe gout in Asian population. This finding is consistent with that of Hollis-Moffatt et al (2011) who reported an association of rs3733591 in New Zealand Maori patients with tophaceous gout (p=0.008), although no correlation was seen in the Western Polynesian and New Zealand European populations.¹³ Our study demonstrated a significant positive correlation of rs3733591 in Malay patients with gout, although p-value of genotype frequency for this variant became non-significant after adjustment for age and gender using logistic regression analysis. We did not examine for an association of this variant with tophi formation.

A limitation of this study was the inadequate matching of controls to the cases by age and sex. This was reflected by the initial positive association becoming non-significant when our data were adjusted for these two factors. Another limitation was that we did not gather relevant demographic and biochemical data from the control group, which restricted analyses of other covariates. However, our data were consistent with those reported in other previous studies involving Asian-Pacific populations.^{13,15,16}

It will be beneficial to re-sequence SLC2A9 with further genotyping of novel variants in a larger Malay population with hyperuricaemia and gout, to examine for presence of other population-specific variants associated with gout. The

data may shed light on potential mechanisms of genetic determinant of SLC2A9 polymorphism and gout in the Malay ethnic group.

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