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SLC2A9 variant rs938552 is associated with nephrolithiasis risk in Chinese Han population.

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Abstract

Objective: The SLC2A9 gene plays an important role in regulating uric acid reabsorption in renal tubules and increasing the risk of nephrolithiasis. In this study, we sought to investigate the association of SLC2A9 gene polymorphisms with nephrolithiasis risk in Han Chinese from eastern China. Methods: In this case-control study, biochemical variables were evaluated in 328 nephrolithiasis patients and 296 age-matched healthy controls. Three SNPs of SLC2A9 (rs938552, rs1014290 and rs4311316) were genotyped in nephrolithiasis patients and healthy controls using direct sequencing.

Results: The genotypic and allelic frequencies of rs938552 was significantly different between nephrolithiasis patients and healthy controls (P value), while rs1014290 and rs4311316 had no association with nephrolithiasis risk. In addition, we found that rs938552 CT and TT genotypes were correlated with a significantly increase nephrolithiasis risk (OR=0.513, 95% CI=0.262–1.004, P<0.01; OR=0.221, 95% CI=0.109–0.450, P<0.01) as compared with the CC genotype in the additive model. Moreover, the nephrolithiasis patients with rs938552 CT and TT genotypes had much higher levels of serum uric acid (P=0.027), 24 hours - urinary calcium (P=0.014) and lower urinary uric acid level as compared with the individuals carrying CC genotype (P=0.038).

Conclusion: The rs938552 polymorphism of SLA2C9 gene is closely associated with the risk of nephrolithiasis in Han population in Eastern China. It may affect the stone formation by regulating the metabolism of uric acid and calcium.

Keywords: Single nucleotide polymorphism (SNP), SLC2A9, Nephrolithiasis.

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Introduction

As a common health problem, nephrolithiasis affects almost all ethnicities and populations. The prevalence rate of nephrolithiasis in developed countries is between 4% and 20% [1], while the recurrence rate within 5 years is as high as 50% [2], and about 2%–5% of the population in Asia and 8%–15% in Europe and North America will suffer from nephrolithiasis during their lifetime. Varies of risk factors have been reported to be involved in the kidney stone formation, including diet and obesity status, certain drugs, climate changes, metabolic disorders and genetic factors. Considering the complexity of this disease, nephrolithiasis is regarded as a systemic disorder rather than a kidney disease only.

Accumulating evidence has disclosed the genetic contributions to nephrolithiasis [3]. The heritability of some nephrolithiasis related traits has been reported to be as high as 95% [4]. In terms of the urinary stone, it was also considered to be under the genetic control although its heritability was shown to be relative lower (56%) [5]. So far, a number of studies have established a link between nephrolithiasis susceptibility and several genes, including calcium-sensing receptor gene (CaSR) [6], antisense transcript of migration inhibitory factor gene (MIF-AS) [7] as well as SLC13A2 gene [8].

The SLC2A9 gene encodes solute carrier family 2, facilitated glucose transporter member 9 (GLUT9) which plays a pivotal role in glucose homeostasis. More recently, GLUT9, found in the proximal tubules of the kidneys, was proved to be responsible for the renal transport and regulation of uric acid [9]. Genetic variants of the urate transporters such as GLUT9 and URAT1 have been implicated in the pathogenesis of hyperuricemia and gout [10]. In light of those findings, an emerging number of clinical studies have demonstrated their involvement in the uric acid metabolism, including urate renal disposal and liver uptake [11,12]. According to the previous recommendations that gout and nephrolithiasis may have a similar pathogenesis [13], it might be interesting to investigate the association of nephrolithiasis risk and genetic variants of the SLC2A9.

In the current study, we tried to determine the clinical significance of the SLC2A9 gene polymorphism among nephrolithiasis patients in the Chinese Han Population. Therefore, a total of 627 subjects, including 328 patients with

nephrolithiasis and 296 healthy controls, were recruited to assess the association between three SNPs of the SLC2A9 gene (rs938552, rs1014290 and rs4311316) and nephrolithiasis risk.

Materials and Methods

Study subjects

328 unrelated patients were investigated (243 men and 85 women, aged 18–76, mean age 41.3 ± 11.9),which were of Han nationality in eastern China with kidney stones, and underwent treatment at XX Hospital from January 2015 to December 2017.

The control group consisted of 296 age-matched healthy Han nationality subjects (218 men and 78 women, aged 18−72, mean age 42.3 ± 12.1), who resided in eastern China without a history of nephrolithiasis or family history of stone disease.

All of the patients and controls were attributed to the Chinese Han nationality and with the same geographical and environmental stratification. Samples were collected and analyzed before any treatment was performed. Urine pH, the serum concentrations of creatinine, calcium, and phosphate, as well as 24 hour urine excretions of calcium, phosphate, citrate, and oxalate were measured and recorded in both groups. Informed consent was obtained from all participants and the study protocol was approved by the ethics committee of XX Hospital.

DNA extraction and genotyping

Genomic DNA was isolated from peripheral blood leukocytes using the Tiangen DNA Extraction Kit (Tiangen Biotech Co. Ltd., Beijing, China) according to the manufacturer's protocol. The primers of rs938552, rs1014290 and rs4311316 of the SLA2C9 gene were designed with Primer 3 software. PCR amplification was performed in 20 μL volume with 20 ng of genomic DNA under the following conditions: initial denaturation at 95 for 5 min, followed by 30 cycles of denaturation at 96 for 30 s, annealing at 60 for 30 s and extension at 72 for 30 s, and then final elongation at 72 for 5 min. According to the manufacturer's guidelines, purified PCR products were subjected to direct sequencing using the Big-Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, CA). An ABI 3730XL automated DNA sequencer. Sequences were aligned and compared with the published genomic data.

Statistical analysis

Hardy−Weinberg equilibrium was assessed using the Chi-square test. Comparison of the continuous variables between the nephrolithiasis patients and the controls was carried out using the t-test. Across the three genotypes, the continuous variables were compared by performing analysis of variance while the categorical variables were analyzed using the Chi-square test to determine the association of rs938552 genotypes with biochemical variables such as serum uric acid, Serum calcium, Urinary uric acid and Urinary calcium. Differences in genotype distribution and allele frequency between groups was analyzed by binary logistic regression. Odds ratios (OR) and 95% confidence intervals (CI) were calculated to determine the relative risk of stone formation. All statistical analyses were performed using SPSS software version 22 (SPSS inc, Chicagp, IL, USA). P-values<0.05 were considered to be statistically significant.

Results

Clinical data and biochemical characteristics of enrolled subjects

A total of 328 nephrolithiasis patients and 296 age-matched healthy controls were recruited into this study. Table 1 summarized the clinical data and biochemical characteristics of nephrolithiasis patients and controls. There were no significant differences in gender, age and biochemical characteristics between two groups.

Table 1. *Characteristics in calcium nephrolithiasis patients and healthy controls.*

	Patients	Healthy controls	P-value
	n=328	n=296	
Gender(male/female)	243/85	218/78	0.062
Age (years)	41.3 ± 11.9	42.3 ± 12.1	0.115
Serum creatinine (μmol/L)	86.29 ± 12.77	82.58 ± 11.14	0.059
Serum calcium (mmol/L)	2.24 ± 0.48	2.08 ± 0.43	0.067
Serum phosphate (mmol/L)	1.13 ± 0.31	1.15 ± 0.27	0.121
serum uric acid (mmol/L)	333.74 ± 17.18	326.8 ± 15.34	0.083
Urinary calcium (mmol/24 hours)	7.35 ± 0.85	5.42 ± 0.75	0.064
Urinary phosphate (mmol/24 hours)	38.28 ± 6.17	41.28 ± 5.94	0.085
Urinary citrate (mg/24 hours)	349.26 ± 43.14	313.25 ± 37.18	0.134
Urinary oxalate (mg/24 hours)	68.27 ± 11.31	75.23 ± 13.46	0.096
Urinary uric acid (mmol/L)	3.61 ± 0.49	4.17 ± 0.53	0.071
Urine pH	6.85 ± 0.79	5.69 ± 0.61	0.136

Comparison of genotype and allelic frequencies between nephrolithiasis patients and healthy controls

Genotype and allele frequencies of SLA2C9 gene polymorphisms in 328 nephrolithiasis patients and 296 controls were shown in Table 2.

Table 2. *Genotype and allelic frequencies at rs938552, rs1014290 and rs4311316 SNPs of the SLC2A9 gene in nephrolithiasis patients and healthy controls.*

	Patients	Healthy controls	Odds Ratio	95% Confidence Interval	P-value
	n=328(%)	n=296 (%)			
rs938552 Genotype					
CC	259 (78.96%)	272 (91.89%)	1#		
CT	26 (7.93%)	21 (7.10%)	0.513	0.262-1.004	0.034
TT	43 (13.11%)	3 (1.01%)	0.221	0.109-0.450	0
CT+TT	69 (21.04%)	24 (8.11%)	0.331	0.202-0.543	0
Alleles					
C	544 (82.93%)	565 (95.44%)	1#		
T	112 (17.03%)	27 (4.56%)	0.232	01.50-0.359	0
rs1014290 Genotype					
GG	282 (85.98%)	258 (87.16%)	1#		
GT	31 (9.45%)	25 (8.45%)	0.881	0.507-1.533	0.38
TT	15 (4.57%)	13 (4.39%)	0.947	0.442-2.029	0.523
GT+TT	46 (14.02%)	38 (12.84%)	0.903	0.569-1.433	0.377
Alleles					
G	595 (90.70%)	541 (91.39%)	1#		
T	61 (9.30%)	51 (8.61%)	0.92	0.623-1.358	0.374
rs4311316 Genotype					
CC	269 (82.01%)	239 (80.74%)	1#		
CG	21 (6.40%)	16 (5.41%)	0.858	0.437-1.681	0.392
GG	38 (11.59%)	41 (13.85%)	1.214	0.756-1.952	0.247
CG+GG	59 (17.99%)	57 (19.26%)	1.087	0.726-1.628	0.38
Alleles					
C	559 (85.21%)	494 (83.45%)	1#		
G	97 (14.79%)	98 (16.55%)	1.143	0.842-1.552	0.217

The genotype frequency distributions of these three polymorphisms were all in agreement with Hardy–Weinberg equilibrium in patients and control subjects (P>0.05). Statistical comparisons for genotype distribution were performed with logistic regression modeling.

For the rs938552 polymorphism, the frequencies of the CC, CT, and TT genotypes were 78.96%, 7.93%, and 13.11% among nephrolithiasis patients respectively, and they were 91.89%, 4.73%, and 3.38% among healthy controls respectively. Compared with the CC genotype, the CT and TT genotypes were associated with a statistically increased risk of nephrolithiasis (OR=0.513, 95% CI=0.262 – 1.004, P<0.01; OR=0.221, 95% CI=0.109–0.450, P<0.01). Similarly, the C allele distribution of rs938552 SNP in nephrolithiasis patients

was significantly lower than that in healthy controls (OR 0.232, 95% CI 0.150–0.359, P<0.01). However, there were no genotypic or allelic distribution differences for the other two polymorphisms (rs1014290 and rs4311316) between nephrolithiasis patients and healthy controls.

Significance of CT+TT genotype of the SLC2A9 rs938552 polymorphism

To further evaluate the potential relationship between genotypes of the rs938552 SNP and the risk factors of nephrolithiasis, the levels of 24 hour urine phosphate, citrate, oxalate, calcium, as well as the levels of serum creatinine, calcium, phosphate, and urine pH were analyzed in both groups independently Table 3.

Table 3. Comparison of clinical characteristics between nephrolithiasis patients and controls across the genotypes of the SLC2A9 rs938552.

	CT+TT genotype			P-value
	CT genotype	TT genotype	CC genotype	
serum uric acid (mmol/L)	391.26 ± 17.38	383.74 ± 16.18	326.8 ± 15.34	0.027
Serum creatinine (μ mol/L)	86.29 ± 12.77	82.58 ± 11.14	85.54 ± 9.81	0.096
Serum calcium (mmol/L)	2.24 ± 0.48	2.08 ± 0.43	2.26 ± 0.42	0.124
Serum phosphate (mmol/L)	1.13 ± 0.31	1.15 ± 0.27	1.26 ± 0.47	0.419
Urine pH	6.85 ± 0.79	5.69 ± 0.61	6.63 ± 0.63	0.354
Urinary uric acid (mmol/L)	3.85 ± 0.41	4.17 ± 0.53	5.91 ± 0.79	0.038
Urinary citrate (mg/24 hours)	68.27 ± 11.31	75.23 ± 13.46	69.17 ± 10.68	0.155
Urinary oxalate (mg/24 hours)	349.26 ± 43.14	313.25 ± 37.18	317.55 ± 34.58	0.087
Urinary calcium (mmol/24 hours)	7.42 ± 0.91	7.35 ± 0.85	5.04 ± 0.58	0.014
Urinary phosphate (mmol/24 hours)	38.28 ± 6.17	41.28 ± 5.94	39.84 ± 4.91	0.092

Among the nephrolithiasis subjects, the CT and TT genotype had significant higher serum uric acid (P=0.027), 24 hours - urinary calcium (P=0.014) and lower urinary uric acid when compared with the individuals carrying the CC genotype (P=0.038).

Discussion

Genetic variations are involved in the development and progression of nephrolithiasis [14]. The SLC2A9 gene was associated with nephrolithiasis [15], which suggests that it may participate in the development of this disease. In this study, we investigated the polymorphisms of the SLC2A9 gene (rs938552, rs1014290 and rs4311316) in 328 nephrolithiasis patients of Han nationality in eastern China and 296 healthy unrelated volunteers without stone history from the same region to assess the association between the polymorphism of the SLc2A9 gene and nephrolithiasis risk.

The results showed that the individuals with the rs938552 genotypes (CT/TT) had a significantly increased nephrolithiasis risk compared with those carrying the CC genotype. In addition, the association between the SNP rs938552 and SUA (serum uric acid), 24 hours - urinary calcium and UUA(urinary uric acid)levels was also confirmed in the development of nephrolithiasis.

SLC2A9, also known as GLUT9 or URATv1,is located on chromosome 4p 15.3-16, which encodes a dual transporter for fructose and uric acid[16,17]. Recently, SLC2A9 was revealed to function as a urate reabsorption transporter in human renal proximal tubular cells and play a direct role in the regulation of serum urate levels [18]. Preitner et al. [19] proposed that SLC2A9 also contributes to urate homeostasis in both kidney and liver.

Uric acid, also known as 2,6,8-trioxane, possesses weakly acidic properties and is the end product of oxidative catabolism. Human uric acid exists as free urate and is mainly excreted via the kidney, which undergoes glomerular filtration (100%) and renal proximal tubular reabsorption (98% to 100%), followed by renal tubular re-secretion (50%) and reabsorption (40%), therefore the total excretion amount accounted for 8% to 12% of glomerular filtration rate. Impaired disposal of uric acid, including increased renal tubular reabsorption or insufficient secretion, will lead to uric acid retention and then the incidence of hyperuricemia. Conversely, excessive excretion of uric acid correlates with hypouricemia. In summary, the reabsorption and secretion of uric acid by the renal tubules plays a vital role in regulating blood uric acid levels. Foreign studies have found that [20,21], patients with kidney stones, high uric aciduria accounted for 23-31.5%. Our study exhibited that the rs938552 CT and TT

genotypes of SLC2A9 gene were closely associated with significant high levels of serum uric acid and low levels of urinary uric acid, suggesting that the rs938552 polymorphism may facilitate the formation of kidney stones by altering the balance of renal tubular uric acid secretion and absorption.

Given the complexity of the process that kidney stone forms, not any single SNP or gene could be fully responsible for the nephrolithiasis risk. Here we demonstrated a dramatic association between the SNP rs938552 and nephrolithiasis risk in Han Chinese from eastern China, while the underlined mechanisms that this genetic variation influences the biological function of SLC2A9 still need to be further elucidated.

Conclusion

In summary, our present study provided evidence that SLA2C9 rs938552 polymorphism had a significant effect on the risk of developing nephrolithiasis in the population of Han population in Eastern China. Nevertheless, SLA2C9 rs1014290 and rs4311316 SNPs were not relevant with the risk of nephrolithiasis. Further prospective and longitudinal studies with more detailed environmental data should be needed to confirm this association.

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Disclosure of Conflict of Interest

None.

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