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RESEARCH ARTICLE

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# Significant association between RGS14 rs12654812 and nephrolithiasis risk among Guangxi population in China

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**Background**: Nephrolithiasis is a worldwide health problem that affects almost all populations. This study aimed to evaluate the association between rs12654812 of regulator of G protein signaling 14 (RGS14) gene and nephrolithiasis in the Chinese population.

**Methods**: A total of 1541 participators including 830 cases and 711 controls were included from Guangxi area in China. Age, sex, BMI, smoking status, drinking status, creatinine, uric acid, and urea nitrogen were analyzed between the case group and control group.

**Results**: We found that the G/A+A/A genotypes of rs12654812 had a significantly increased nephrolithiasis risk after adjusting age, sex, BMI, smoking, drinking, and hypertension, compared with G/G genotype (OR = 1.361, 95% CI = 1.033-1.794, $P$ = .029). This hazardous effect was more pronounced in subgroup of age < 50, ever smoking, ever drinking, creatinine normal, and high uric acid. The G/A genotype of rs12654812 also had a significantly increased nephrolithiasis risk compared with G/G genotype. The A allele of rs12654812 significantly increased the risk of nephrolithiasis compared with the G allele after adjusting for age, sex, BMI, smoking, drinking and hypertension (OR = 1.277, 95% CI = 1.013-1.609, $P$ = .038).

**Conclusions**: Our results suggest that the RGS14 polymorphism is involved in the etiology of nephrolithiasis and thus may be a genetic marker for nephrolithiasis.

KEYWORDS
genetic marker, nephrolithiasis, regulator of G protein signaling 14, single nucleotide polymorphism, susceptibility

## 1 | INTRODUCTION

Nephrolithiasis is a worldwide health problem that affects almost all populations. Recent studies have shown that the prevalence of nephrolithiasis in Chinese adults is 6.5%,[1] while the prevalence in Guangxi province in southern China is 7.82%-12.03%.[2] Nephrolithiasis is affected by multiple risk factors, including environment, lifestyle, hormones, anatomical factors, and genetic factors. The large-scale karst landforms, calcium-rich soils, and high-temperature climates may be the important factors contributing to the high prevalence of nephrolithiasis in Guangxi region.[3] In addition, family history of the disease has been reported to increase the disease risk.[4]

Regulator of G protein signaling 14 (RGS14) is a member of the regulator of G protein signaling family. The function was mainly involved in the neurons system.[5] In 2012, Urabe et al conducted a three-stage genomewide association study, which identified the polymorphism of RGS14 gene (rs12654812) in the nephrolithiasis

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among the Japanese population firstly.[6] Then, in 2013, Yasui et al[7] confirmed the significant association. In 2015, Oddsson et al[8] repeated this correlation in Icelanders. To confirm the correlation among the Chinese population, the study was conducted.

## 2 | MATERIALS AND METHODS

### 2.1 | Study subjects

From April 2013 to January 2016, a total of 1541 participators including 830 cases and 711 controls were recruited from Guangxi province in China. For cases, the nephrolithiasis was confirmed by various auxiliary examinations (KUB + IVP + CT + B ultrasound). The exclusion criteria were as follows: (1) without the essential information; and (2) currently with coronary heart disease, repeated diarrhea, thyroid disease, and dysuresia caused by the non-stone factors. (3) So far, taking medicines that could influence lithogenesis such as glucocorticoids, calcium agent, and magnesia agent. The controls were matched with age and sex, who were confirmed to be without stones in urinary system. Meanwhile, they had a negative personal and familial history of nephrolithiasis and no long-term use of medicines (glucocorticoids, calcium agent, magnesia argent, and diuretic). Individuals who smoked daily for more than 6 months were defined as smokers and the persons who ever drinking weekly were defined as drinkers. Written informed consent was obtained from all participants. The study was approved by the medical ethics committee of Guangxi Medical University (Table 1).

### 2.2 | Genotyping

Genomic DNA was extracted from peripheral blood leukocytes using a DNA isolation kit following the manufacturer's instructions (Sangon Biotech, Shanghai, China). The genotyping was mainly based on SNP$^{scan}$ method [9] (Genesky Biotechnologies Inc., Suzhou, China). Ten percent of samples from patients and controls were randomly selected to evaluate the quality of genotyping, which showed 100% concordance.

### 2.3 | Statistical analysis

Logistic regression analysis was tested to evaluate the association between rs12654812 and nephrolithiasis. Odds ratio (OR), confidence interval (CI), and *P* value were combined to assess the power of correlation. Six covariates (i.e, age, sex, BMI, smoking status, drinking status, and hypertension status) were used to exclude potential confounding factors that influence the association. Meanwhile, other indexes were also applied to evaluate the effects of rs12654812 on renal function (UA, BUN, and Ccr). All statistical tests were two-sided.

## 3 | RESULTS

In the baseline analysis (Table 1), the mean age of the nephrolithiasis patients was 48.03 ± 12.43 years compared with 48.47 ± 12.10 years in controls, which showed no significant difference (*P* = .481). Also, the sex distributions were similar (*P* = .193) among the two groups. However, it suggested that BMI, the smoking status, drinking status, hypertension status, and renal function (crea and UA) were significantly different between the two groups (*P* < .05).

**TABLE 1** The baseline data of nephrolithiasis and controls

| Variables | Case n = 830 (%) | Controls n = 711 (%) | *P* |
|---|---|---|---|
| Age (years) | 48.03 ± 12.43 | 48.47 ± 12.10 | .481 |
| Sex | | | .193 |
| Male | 478 (57.6) | 386 (54.3) | |
| Female | 352 (42.4) | 325 (45.7) | |
| BMI | 22.79 ± 3.52 | 23.53 ± 3.38 | **.000** |
| ≤24 | 20.85 ± 2.05 | 21.10 ± 1.95 | .070 |
| >24 | 26.76 ± 2.38 | 26.57 ± 2.09 | .328 |
| Hypertension | | | |
| YES | 118 (14.2) | 74 (10.4) | **.024** |
| No | 712 (85.8) | 637 (89.6) | |
| Diabetes | | | .665 |
| YES | 19 (2.3) | 14 (2.0) | |
| No | 811 (97.7) | 697 (98.0) | |
| Smoking status | n = 798 (%) | n = 681 (%) | **.000** |
| Ever | 265 (33.2) | 110 (16.2) | |
| Never | 533 (66.8) | 571 (83.8) | |
| Drinking status | n = 790 (%) | n = 431 (%) | **.000** |
| Ever | 310 (39.2) | 236 (54.8) | |
| Never | 480 (60.8) | 195 (45.2) | |
| Crea (μmol/L) | n = 826 | n = 613 | **.000** |
| | 112.16 ± 139.14 | 78.99 ± 15.20 | |
| UA (μmol/L) | n = 826 | n = 615 | **.000** |
| | 349.99 ± 122.67 | 381.63 ± 96.44 | |
| BUN (mmol/L) | n = 769 | n = 114 | |
| | 6.04 ± 5.03 | 5.63 ± 7.31 | .446 |

*P* < .05 are marked in bold.

The genotype frequencies and allelic distribution of rs12654812 polymorphisms are given in Table 2. In cases, the G/G, G/A, and A/A genotypes of rs12654812 locus were 59.5%, 35.1%, and 5.4%, and the corresponding frequency was 63.2%, 30.9%, and 5.9% in the control group. We found that the G/A + A/A genotypes (OR = 1.361, 95% CI = 1.033-1.794, *P* = .029) of rs12654812 had a significantly increased nephrolithiasis risk after adjusting age, sex, BMI, smoking, drinking, and hypertension, compared with G/G genotype. This hazardous effect was more pronounced in subgroup of age<50 (OR = 1.548, 95% CI = 1.075-2.228, *P* = .019), ever smoking (OR = 1.929, 95% CI = 1.119-3.327, *P* = .018), ever drinking (OR = 1.722, 95% CI = 1.146-2.588, *P* = .009), creatinine normal (OR = 1.535, 95% CI = 1.130-2.084, *P* = .006), and high uric acid (OR = 1.869, 95% CI = 1.024-3.413, *P* = .042) (Table 3). The G/A genotype (OR = 1.360, 95% CI = 1.019-1.816, *P* = .037) of rs12654812

**TABLE 2** Genotypes and allele frequencies of rs12654812 among cases and controls

| Genotypes | Case n = 830 (%) | Control n = 711 (%) | *P* values* | OR (95% CI) | *P* values** |
|---|---|---|---|---|---|
| G/G | 494 (59.5) | 449 (63.2) | .229 | Reference | |
| G/A | 291 (35.1) | 220 (30.9) | | 1.360 (1.019-1.816) | **.037** |
| A/A | 45 (5.4) | 42 (5.9) | | 1.389 (0.743-2.596) | .303 |
| G/A + A/A | 336 (40.5) | 262 (36.8) | .145 | 1.361 (1.033-1.794) | **.029** |
| Alleles | | | | | |
| G | 1279 (77.0) | 1118 (78.6) | .295 | Reference | |
| A | 381 (23.0) | 304 (21.4) | | 1.277 (1.013-1.609) | **.038** |

*P* < .05 are marked in bold.
*P* * for two-sided chi-squared test.
*P* ** adjusted for age, sex, BMI, smoking, drinking, and hypertension in logistic regression model.

**TABLE 3** Stratification analyses of rs12654812 association with nephrolithiasis risk

| | Case n = 830 | | Controls n = 711 | | | |
|---|---|---|---|---|---|---|
| Subgroup | G/G | G/A + A/A | G/G | G/A + A/A | *P* values | OR (95% CI) |
| Age (years) | | | | | | |
| <50 | 270 | 187 | 252 | 151 | **.019** | 1.548 (1.075-2.228) |
| ≥50 | 224 | 149 | 197 | 111 | .424 | 1.194 (0.773-1.846) |
| Sex | | | | | | |
| Male | 289 | 189 | 240 | 146 | .065 | 1.399 (0.979-1.998) |
| Female | 205 | 147 | 209 | 116 | .131 | 1.420 (0.901-2.239) |
| BMI | n = 824 | | n = 616 | | | |
| ≤24 | 331 | 223 | 214 | 128 | .113 | 1.354 (0.930-1.971) |
| >24 | 157 | 113 | 179 | 95 | .077 | 1.451 (0.961-2.193) |
| Smoking status | n = 798 | | n = 681 | | | |
| Ever | 151 | 114 | 76 | 34 | **.018** | 1.929 (1.119-3.327) |
| Never | 322 | 211 | 361 | 210 | .371 | 1.161 (0.837-1.610) |
| Drinking status | n = 790 | | n = 431 | | | |
| Ever | 164 | 146 | 154 | 82 | **.009** | 1.722 (1.146-2.588) |
| Never | 303 | 177 | 126 | 69 | .631 | 1.100 (0.746-1.620) |
| Crea (μmol/L) | n = 826 | | n = 613 | | | |
| ≤106 | 348 | 249 | 378 | 212 | **.006** | 1.535 (1.130-2.084) |
| >106 | 142 | 87 | 12 | 11 | .159 | 0.428 (0.131-1.395) |
| UA (μmol/L) | n = 826 | | n = 615 | | | |
| ≤420 | 379 | 244 | 267 | 153 | .117 | 1.302 (0.936-1.812) |
| >420 | 111 | 92 | 125 | 70 | **.042** | 1.869 (1.024-3.413) |
| BUN (mmol/L) | n = 769 | | n = 114 | | | |
| ≤7.14 | 365 | 249 | 70 | 39 | .191 | 1.379 (0.852-2.231) |
| >7.14 | 89 | 66 | 2 | 3 | .402 | 0.414 (0.052-3.267) |

*P* < .05 are marked in bold.
*P* adjusted for age, sex, BMI, smoking, drinking, and hypertension in logistic regression model (except stratified variables).

also had a significantly increased nephrolithiasis risk compared with G/G genotype. In case group, the frequency of G and A was 77.0% and 23.0%. And the frequency of G and A was 78.6% and 21.4% in control group. There were no significant differences between case group and control group in the frequency distribution of per-allele ($P > .05$), but after adjusting for age, sex, BMI, smoking, drinking, and hypertension, the A allele (OR = 1.277, 95% CI = 1.013-1.609, $P = .038$) of rs12654812 significantly increased the risk of nephrolithiasis compared with the G allele.

## 4 | DISCUSSION

Nephrolithiasis was a worldwide disease influencing a larger number of populations. Recent studies had identified rs12654812 in RGS14 gene associated with nephrolithiasis in the Japanese and Icelander.[6–8] Our study repeated the significant association, which suggested that rs12654812 would be a risk factor in nephrolithiasis. In addition, we found the G/A + A/A genotype carriers had a higher causative effect in subgroup of age<50, ever smoking, ever drinking, creatinine normal, and high uric acid. In the past studies, age,[10] smoking consumption,[11] alcohol consumption,[12] and renal function [8] had been shown to play an important role in the formation of urolithiasis. Our results suggest that age, smoking consumption, alcohol consumption, creatinine, and uric acid may play a synergistic role with rs12654812 gene polymorphism and strengthen the genetic effect of nephrolithiasis.

RGS14 is a highly unusual complex family member and encodes a ~62 kDa protein most closely related to RGS12 and RGS10. The SNP rs12654812 located in RGS14 on chromosome 5q35.3, which was confirmed in strong linkage disequilibrium with multiple SNPs located in the RGS14-SLC34A1 region.[6–8] Previous studies suggest that mutations in the SLC34A1 gene were associated with renal function,[13] serum phosphorus levels,[7] and PTH,[8] and may lead to the onset of hypophosphatemia nephrolithiasis and osteoporosis.[14,15] The SLC34A1 gene encodes NPT2a, a member of the type II sodium-phosphate cotransporter family,[16] which shows tissue-specific gene expression in kidney.[17] The NPT2a is mainly expressed in the apical membrane of renal proximal tubular epithelial cells, responsible for reclaiming most of the filtered phosphate load in a rate-limiting manner.[18,19] The mutation of SNP rs12654812 is associated with decreased NPT2 function, resulting in a corresponding reduction in serum phosphorus levels and an increased risk of nephrolithiasis.

The studies based on NPT2-deficient mice exhibit increased urinary excretion of phosphate (Pi), a 70%-80% decrease in the Na/Pi cotransport and hypophosphatemia. Hypophosphatemia can increase the circulating concentration of 1, 25-(DH) 2D and intestinal Ca hyperabsorption result from intestinal Ca channel overexpression, and ultimately lead to hypercalcemia and hypercalciuria.[20,21] In addition, parathyroid hormone, a known risk factor of nephrolithiasis, which is also at a low level due to the negative feedback of hypophosphatemia.

Our study also had several limitations. As our study was hospital-based study design and inevitably exist a certain degree of selection bias, we applied rigorous epidemiological criterion and used statistical methods to adjust the effects of confounding factors. In addition, our sample size was limited and the sample source was mainly from China Guangxi region, the larger sample size from multicenter may help in better understanding of the role of RGS14 gene in nephrolithiasis.

## 5 | CONCLUSIONS

The polymorphism of RGS14 gene (rs12654812) would be significantly associated with the development of Nephrolithiasis in the Chinese population. Mutation in rs12654812 may affect the adjacent SLC34A1 gene and thus affect the phosphate metabolism, eventually leading to the formation of stones. The results suggest that the RGS14 polymorphism is involved in the etiology of nephrolithiasis and thus may be a genetic marker for nephrolithiasis.

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