

# Associations between the SLC22A12 gene and gout susceptibility: a meta-analysis

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## Abstract

### Objective

*The aim of this study is to explore whether the polymorphisms of rs475688 and rs3825016 in the solute carrier family 22 member 12 (SLC22A12) gene are associated with the susceptibility to gout or hyperuricaemia.*

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### Methods

*Relevant studies were enrolled by searching databases systematically. The pooled odds ratios (ORs) with 95% confidence interval (CI) were used to evaluate the associations.  $Q$ -test and  $I^2$  statistics were used to evaluate the heterogeneity. Publication bias was evaluated using Begg's funnel plots and Egger regression test.*

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### Results

*A total of 7 articles involving 1216 patients and 1844 healthy controls were included in this meta-analysis. Significant association was detected between rs475688 polymorphism and gout susceptibility in three genetic models (C vs. T: OR=1.464, 95% CI 1.078–1.989,  $p=0.015$ ; CC+CT vs. TT: OR=2.028, 95% CI 1.488–2.763,  $p=0.000$ ; CC vs. CT+TT: OR=2.226, 95% CI 1.746–2.838,  $p=0.000$ ). Significant association was observed between rs3825016 polymorphism and hyperuricaemia susceptibility only in allelic comparison (C vs. T: OR=1.274, 95% CI 1.101–1.474,  $p=0.001$ ).*

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### Conclusion

*The rs475688 polymorphism is associated with gout susceptibility. The correlation between rs3825016 polymorphism of SLC22A12 and hyperuricaemia susceptibility is possible.*

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### Key words

*gout, rs475688, rs3825016, polymorphism, meta-analysis*

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## Introduction

Gout is an inflammatory arthritis induced by the deposition of monosodium urate crystals (MUS) in synovial fluid and other tissues (1). Continued hyperuricaemia is a physiological prerequisite for gout. The prevalence of gout ranges from 0.1% to about 10% and seems to be increasing in the globe (2). The prevalence of hyperuricaemia and gout was 13.3% and 1.1% in China, respectively (3). Both genetic and environmental component contribute to the development and progression of hyperuricaemia and gout, resulting in the familial aggregation (4-7). Identifying susceptible genes is necessary to improve the early diagnosis and provide a reference for finding suitable therapeutic targets.

The cause of hyperuricaemia has been classified into the urate 'overproduction' type, the 'underexcretion' type, and the 'combined' type (8). Renal underexcretion is the dominant cause of primary hyperuricaemia in gout (9). The renal urate excretion is determined by the balance of the reabsorption and secretion of urate. Urate transporter 1 (URAT1) is a vital resorptive urate-anion exchanger (10). The urate oxidase gene was lost during the evolution of primates (11, 12). The uricase-inhibitor, oxonate acid, could be used to establish the hyperuricaemia rat model in our early work (13). The pegloticase is a recombinant mammalian uricase for the treatment of refractory gout (14). But the safety of pegloticase still need more studies to demonstrate. As renal excretion accounts for around two-thirds of urate excretion, uricosurics are an important option of urate-lowering therapy (15). The efficacy of lesinurad, a new uricosuric, has been studied in combination with allopurinol. Lesinurad increases uric acid excretion and thereby lowers serum uric acid by inhibiting the URAT1 (16). The SLC22A12 gene encodes hURAT1, a urate transporter in proximal tubules, has a role in the apical absorption of urate (10) to alter serum uric acid (SUA) levels. Several studies have reported that some single nucleotide polymorphisms of the SLC22A12 gene are associated with gout suscep-

tibility (17-20). Studies have revealed that genetic variants in the SLC22A12 gene on chromosome 11 are involved in the genesis of hyperuricaemia and gout (21-23), but not participate in all cases (24, 25). Individual studies based on small sample sizes have insufficient statistical power to detect the potential associations. In this meta-analysis, we integrated previous research to investigate the association of SLC22A12 gene polymorphisms, such as rs475688 (intron variant) and rs3825016 (C258T, exon 1), and susceptibility to gout in different populations. On the one hand, the statistical power and resolution by pooling the data of independent studies was increased. On the other hand, there is not any meta-analysis investigating this association between them till now.

## Methods

### Literature search strategy

We searched PubMed, Embase, Chinese National Knowledge Infrastructure (CNKI) databases and China Science and Technology Journal (CSTJ) database for relevant studies that examined associations between the polymorphisms of rs475688 and rs3825016 in SLC22A12 gene and gout (up to April 2017). The following terms were used: "rs3825016" OR "C258T" OR "rs475688" and "hyperuricaemia" OR "gout" and "polymorphism", without any limitation applied. All searched studies were retrieved, and their reference lists were checked for other pertinent papers. Only published studies with full-text articles were included.

### Inclusion and exclusion criteria

Two reviewers independently applied the selection criteria to each reference. A third reviewer resolved any discrepancies regarding study eligibility or quality. All enrolled studies must meet all of the following inclusion criteria: 1. it was a case-control study; 2. the paper was in English or Chinese; 3. the study was designed for human; 4. the diagnostic criteria of gout were based on the ACR 1977 (26); hyperuricaemia was defined as SUA >420mmol/L in males and >360mmol/L in females; 5. the genotype frequencies or allele frequencies in cases and control groups

Competing interests: none declared.

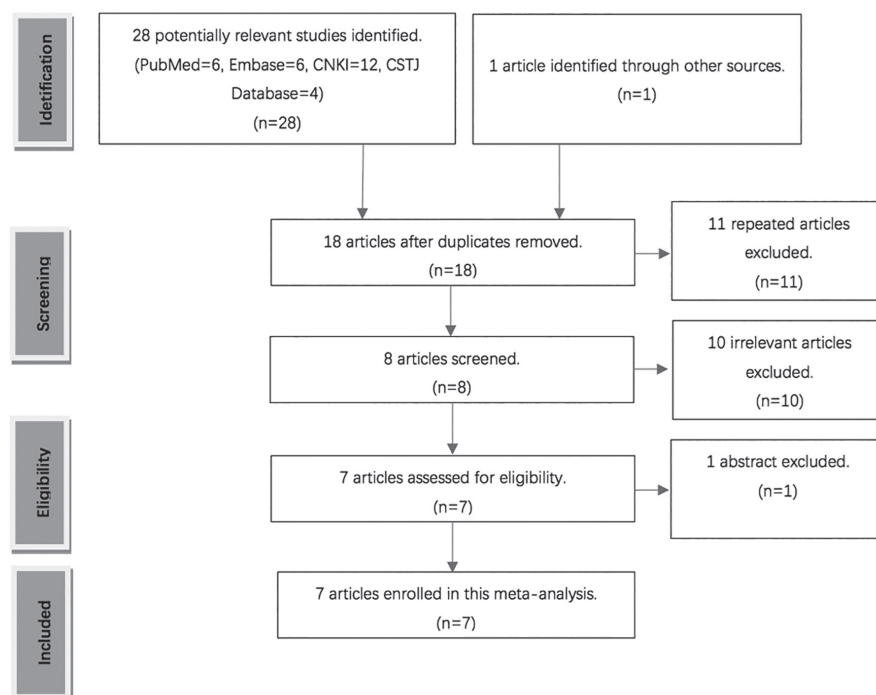


Fig. 1. Flow diagram of the study selection process.

could be collected; 6. full text was available. The exclusion criteria were as follows: 1. the study not meeting the inclusion criteria; 2. being insufficient or duplicated.

#### Data extraction

The following information was extracted independently from each study by two reviewers: first author, publication year, race, number of cases and controls for SLC22A12 polymorphisms, genotype frequencies, allele frequencies were calculated from genotype distribution.

#### Statistical analysis

Allelic contrast, dominant and recessive models were used in this meta-analysis to reduce the probability of type I error. Gout/hyperuricaemia risk was associated with harbouring C allele of SNP rs475688 and rs3825016 in populations after reading the enrolled individual studies. The genotype CC and CT is classified into cases group in dominant models according to Mendel's law, and so on. The odds ratio (OR) and 95% confidence interval (95% CI) were calculated for each study.  $p < 0.05$  was regarded as statistically significant.

Q-test and I<sup>2</sup> statistics were used to evaluate the heterogeneity).  $p > 0.1$  or  $I^2 < 50\%$  indicate the absence of heterogeneity. The pooled ORs and 95% CI were calculated using the fixed-effect model; otherwise, random-effect model was used. Begg's funnel plots (28) and Egger regression test (29) were used to estimate the potential publication bias. An asymmetric plot and the  $p$ -value of Egger's test  $< 0.05$  was considered statistically significant publication bias. All statistical manipulations were performed using the Stata 11.2 software (StataCorp, College Station, TX, USA).

## Results

### Characteristics of studies

The retrieval strategies screened 28 potential studies, including 6 from PubMed, 6 from Embase, 12 from CNKI and 4 from CSTJ Database. Eventually, 7 articles involving 1216 patients and 1844 healthy controls were included in this meta-analysis. Figure 1 shows the process of study selection. The characteristics of involved articles are shown in Table I.

### The results of meta-analysis and subgroup analysis

#### • Association between rs475688

#### polymorphism and gout susceptibility

The significant statistical heterogeneity was identified by Q-test and I<sup>2</sup> statistic in the allelic comparison so that the random-effect model was used. In addition, the fixed-effect model was used in

Table I. The characteristics of studies included in this meta-analysis.

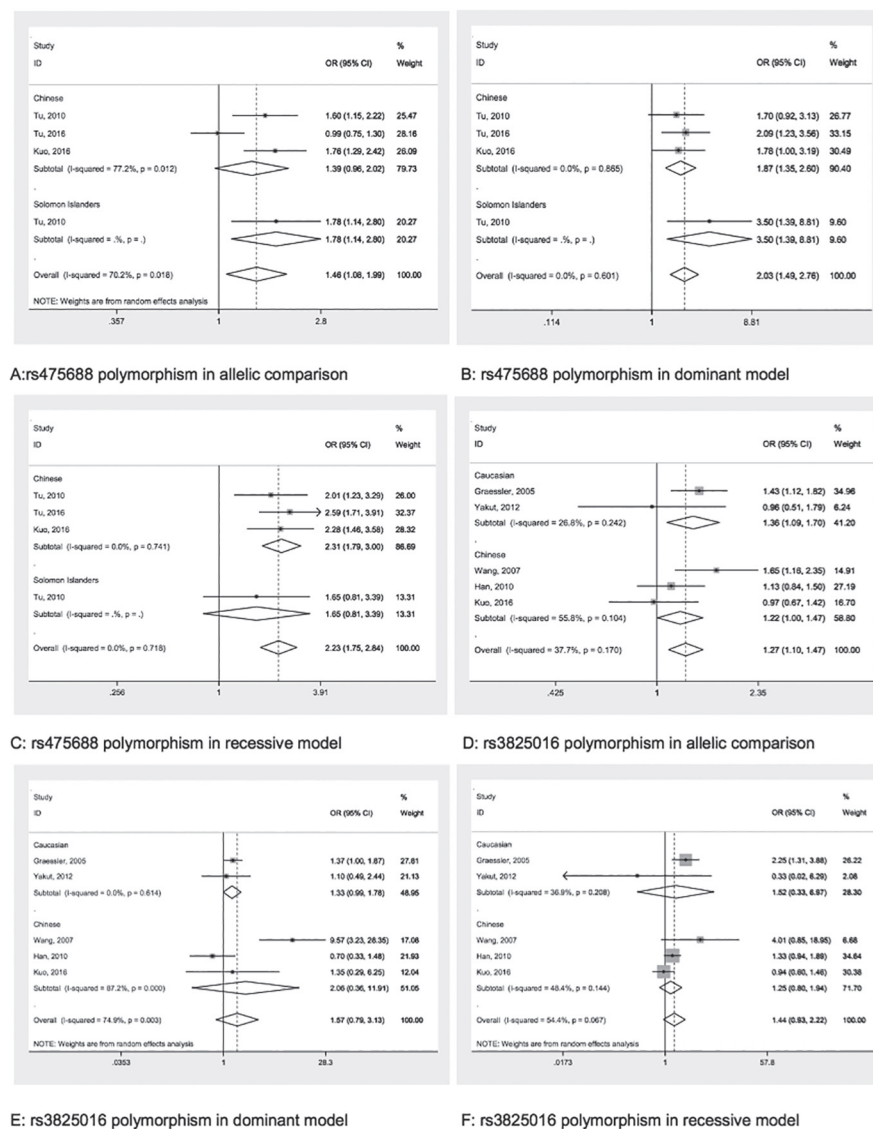
| Study                | Race              | Disease        | Sample size<br>case vs. con | Genotype case vs. con<br>(TT/CT/CC) | Allele frequency<br>case vs. con (C/T) |
|----------------------|-------------------|----------------|-----------------------------|-------------------------------------|----------------------------------------|
| <i>rs475688</i>      |                   |                |                             |                                     |                                        |
| Tu, 2010 (21)        | Chinese           | Gout           | 115/202                     | 17/53/45 vs. 46/107/49              | 143/87 vs. 205/199                     |
|                      | Solomon Islanders | Gout           | 54/138                      | 6/32/16 vs. 42/68/28                | 64/44 vs. 124/152                      |
| Tu, 2016 (22)        | Chinese           | Gout           | 157/295                     | 21/66/70 vs. 72/153/70              | 206/108 vs. 573/297                    |
| Kuo, 2016 (30)       | Chinese           | Gout           | 104/388                     | 16/42/46 vs. 95/193/100             | 134/74 vs. 393/383                     |
| <i>rs3825016</i>     |                   |                |                             |                                     |                                        |
| Graessler, 2005 (23) | Caucasian         | Hyperuricaemia | 389/263                     | 168/163/58 vs. 134/110/19           | 279/499 vs. 148/378                    |
| Yakut, 2012 (25)     | Caucasian         | Hyperuricaemia | 32/100                      | 14/18/0 vs. 46/50/4                 | 18/46 vs. 58/142                       |
| Wang, 2007 (31)      | Chinese           | Hyperuricaemia | 138/117                     | 4/125/9 vs. 26/89/2                 | 145/133 vs. 93/141                     |
| Han, 2010 (32)       | Chinese           | Hyperuricaemia | 227/341                     | 14/67/146 vs. 15/130/196            | 349/95 vs. 522/160                     |
| Kuo, 2016 (30)       | Chinese           | Gout           | 104/388                     | 2/39/63 vs. 10/137/241              | 165/43 vs. 619/157                     |

OR: odds ratio, 95% CI: 95% confidence interval.

**Table II.** Results of meta-analysis for SLC22A12 polymorphisms and gout susceptibility.

|                   | n. of studies | OR (95% CI)<br>(C/T) | <i>p</i> | OR (95% CI)<br>(CC+CT/TT) | <i>p</i> | OR (95% CI)<br>(CC/CT+TT) | <i>p</i> |
|-------------------|---------------|----------------------|----------|---------------------------|----------|---------------------------|----------|
| <i>rs475688</i>   |               |                      |          |                           |          |                           |          |
| Total             | 4             | 1.464 (1.078, 1.989) | 0.015    | 2.028 (1.488, 2.763)      | 0.000    | 2.226 (1.746, 2.838)      | 0.000    |
| Chinese           | 3             | 1.395 (0.964, 2.018) | 0.078    | 1.871 (1.346, 2.603)      | 0.000    | 2.314 (1.786, 2.997)      | 0.000    |
| Solomon Islanders | 1             | 1.783 (1.136, 2.800) | 0.012    | 3.500 (1.391, 8.808)      | 0.008    | 1.654 (0.808, 3.386)      | 0.169    |
| <i>rs3825016</i>  |               |                      |          |                           |          |                           |          |
| Total             | 5             | 1.274 (1.101, 1.474) | 0.001    | 1.568 (0.785, 3.132)      | 0.202    | 1.437 (0.929, 2.221)      | 0.103    |
| Caucasian         | 2             | 1.357 (1.086, 1.696) | 0.007    | 1.327 (0.990, 1.778)      | 0.058    | 1.517 (0.330, 6.972)      | 0.592    |
| Chinese           | 3             | 1.216 (1.003, 1.475) | 0.046    | 2.062 (0.357, 11.915)     | 0.419    | 1.246 (0.799, 1.942)      | 0.331    |

Allelic comparison: C/T, Dominant model: CC+CT/TT, Recessive model: CC/CT+TT, OR: odds ratio, 95% CI: 95% confidence interval.

**Fig. 2.** The forest plots of the results of meta-analysis.

dominant and recessive models. Significantly strong association was detected between rs475688 polymorphism and gout susceptibility in three genetic models (C vs. T: OR=1.464, 95% CI

1.078–1.989,  $p=0.015$ ; CC+CT vs. TT: OR=2.028, 95% CI 1.488–2.763,  $p=0.000$ ; CC vs. CT+TT: OR=2.226, 95% CI 1.746–2.838,  $p=0.000$ ) (Table II, Fig. 2 A-B-C). Subgroup analysis

was conducted according to race. No significantly enhanced correlation was found in Chinese upon allelic comparison (C vs. T: OR=1.395, 95% CI 0.964–2.018,  $p=0.078$ ) and in Solomon Islanders in recessive model (CC vs. CT+TT: OR=1.654, 95% CI 0.808–3.386,  $p=0.169$ ) (Table II).

#### • Association between rs3825016 polymorphism and hyperuricaemia susceptibility

The fixed-effect model was used in allelic comparison; whereas, random-effect model was used in dominant and recessive models due to the presence of heterogeneity. Significant association was detected between rs3825016 polymorphism and hyperuricaemia susceptibility in allelic comparison (C vs. T: OR=1.274, 95% CI 1.101–1.474,  $p=0.001$ ) (Table II, Fig. 2D). No association was found to be significant dominant and recessive models (CC+CT vs. TT: OR=1.568, 95% CI 0.785–3.132,  $p=0.202$ ; CC vs. CT+TT: OR=1.437, 95% CI 0.929–2.221,  $p=0.103$ ) (Table II Fig. 2 E-F). In subgroup analysis, no association was found to be significant in Caucasian and Chinese in both dominant and recessive models (Table II).

#### Evaluation of sensitivity analysis and publication bias

The studies included in this meta-analysis were complied with Hardy-Weinberg equilibrium (HWE) in the controls, suggesting that our consequences were statistically convictive. Publication bias was evaluated using Begg's funnel plots and Egger regression test, and no significant bias was observed in this meta-analysis (all  $p>0.05$ ).

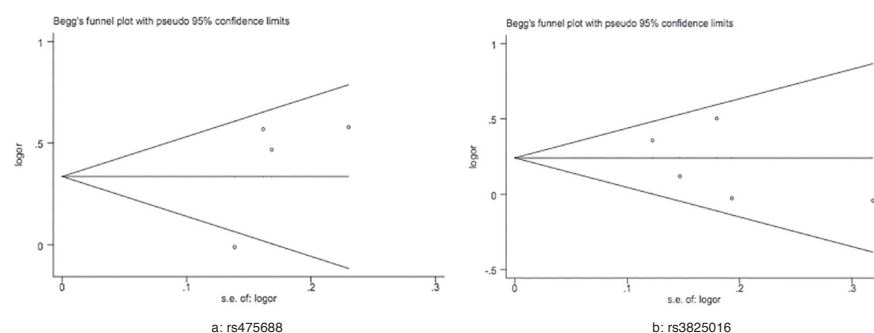


Fig. 3. Begg's funnel plots for publication bias analysis (C vs. T).

## Discussion

Increasing case-control studies have focused on exploring the relationship between SLC22A12 gene polymorphism and gout susceptibility in recent years. However, the results of researches involving the relationship between rs475688, rs3825016 and gout are not consistent. The reasons for these discrepant results ascribed to the imbalance of sample size and the different genetic backgrounds. Conducting Meta-analysis and subgroup analysis can yield a more reasonable result.

In this meta-analysis, 7 eligible case-control studies involving 1216 patients and 1844 healthy controls were included. Our meta-analysis revealed a positive relationship between the SLC22A12 rs475688 polymorphism and gout susceptibility in all the genetic models. The association between rs3825016 polymorphism and hyperuricaemia susceptibility was identified only in allele models. We undertook a race-specific meta-analysis on the relationship between the rs475688 and rs3825016 polymorphisms of SLC22A12 and gout susceptibility. Subgroup analysis in dominant model indicated a linkage between rs475688 polymorphism and gout in Solomon Islanders, but not in Chinese. Additionally, there is no significant difference between rs475688 polymorphism and gout in recessive model in Solomon Islanders.

This meta-analysis has a few limitations. First, publication bias, heterogeneity, and confounding factors may have distorted the meta-analysis. Second, subgroup analysis by ethnicity performed was underpowered, because only two or three studies included. Third, this meta-analysis included data from Caucasian

and Chinese, thus, our results are applicable to only these racial groups.

In conclusion, the rs475688 polymorphism is associated with gout susceptibility. The association between rs3825016 polymorphism of SLC22A12 and hyperuricaemia susceptibility is still elusive in humans, and needs further studies to confirm it.

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