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Association between calcium-sensing receptor (CaSR) R990G, CaSR A986S, and CaSR Q1011E gene polymorphisms and the risk of urolithiasis: a meta-analysis

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Abstract

Backgrounds: In the last two decades, studies have been widely carried out to assess the association between single-nucleotide polymorphisms (SNPs) of calcium-sensing receptor (CaSR) gene in exon 7 and the risk of urolithiasis. However, inconsistency across the studies was reported. Therefore, our current study aimed to perform a meta-analysis concerning the association between the risk of urolithiasis and the gene polymorphisms of CaSR R990G, CaSR A986S, and CaSR Q1011E.

Methods: Published papers from PubMed, Embase, Cohcrane, and Web of science were included for the study, and they were analyzed using fixed or random effect model.

Results: A total of 11 papers consisting of eight papers evaluating CaSR R990G, nine papers evaluating CaSR A986S, and five papers evaluating CaSR Q1011E were included in our analysis. Our pooled calculation found that protective effect against urolithiasis was observed in R allele and RR genotype of CaSR R990G and A allele and AA genotype of CaSR A986S. Conversely, increased susceptibility to urolithiasis was found in G allele and RG genotype of CaSR R990G and S allele of CaSR A986S. Interestingly, our findings in sub-group analysis confirmed that the correlation between CaSR R990G and urolithiasis was found in Caucasian population. Meanwhile, in Asian population, the association was observed in CaSR A986S.

Conclusions: CaSR R990G and CaSR A986S, but not CaSR Q1011E, are associated with the risk of urolithiasis.

Keywords: Calcium-sensing receptor, CaSR R990G, CaSR A986S, CaSR Q1011E, Urolithiasis, Gene polymorphism

Background

Urolithiasis was reported as the main health problem and associated with high morbidity in some countries such as Taiwan, Germany, the USA, Greece, Iceland, and Iran [1–8]. The prevalence of urolithiasis vary, ranging from 1.9% in the USA to 15% in Greece [2–8]. Urolithiasis had been reported to cause several fatal complication including hydronephrosis, perinephric abscess [9], and end-stage renal failure [10]. To date, the treatment for urolithiasis remains challenging due to discrepancies regarding the clinical indications and the efficacy [11], and it was reported to spend high cost expenditure [12]. This cost expenditure is predicted to increase because the incidence and prevalence of this disease were reported to increase in the last few decades [11]. This phenomenon is considered an economic burden for health systems [12], and therefore we described this condition like "the deer at the edge of the cliff." Hence, to anticipate the worsening of this condition, a comprehensive understanding concerning urolithiasis pathogenesis especially at genetic level is crucial for future treatment and prevention.

In the scope of urolithiasis, calcium stones represent the majority of stone types and comprises about 80% of all urinary calculi [10]. Although calcium stones are caused by multifactoral, however, the basic pathway is an imbalance of calcium homeostasis [13]. This pathological state is dominantly governed by calcium-sensing

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receptor (CaSR) [14], a dimeric membrane protein belonging to the family of G-protein coupled receptor (GPCR) [15]. The CASR gene contains two promoters and seven exons [16]. However, the expression of CaSR in the context of calcium homeostasis is primarily affected by a high region in CaSR gene, defined as exon 7. During this time, single-nucleotide polymorphisms (SNPs) widely reported in exon 7 of CaSR gene were as follows: a guanine–thymine substitution at codon 986 (A986S; rs1801725), the subtitution of an adenine–guanine at codon 990 (R990G; rs1042636), and a cytosine–guanine substitution at codon 1011 (Q1011E; rs1801726) [17]. To date, the reports concerning those SNPs in patients with urolithiasis were inconclusive. Furthermore, previous meta-analysis [18] in this circumstance did not suffice to establish a potent conclusion because of some limitations.

Our present study aimed to perform a meta-analysis regarding the correlation between the risk of urolithiasis and the gene polymorphisms of CaSR R990G, CaSR A986S, and CaSR Q1011E. Our present study was intended to clarify the inconsistency and to elucidate better correlation between those SNPs and the risk of urolithiasis.

Methods

Study design

During the period (February–April 2019), a meta-analysis was carried out to assess the correlation between CaSR gene polymorphisms (CaSR R990G, CaSR A986S, and CaSR Q1011E) and the risk of urolithiasis. To reach this purpose, published papers from PubMed, Embase, Cohcrane, and Web of science were collected for calculation of odd ratio (OR) and 95% confidence interval (95%CI) using either random or fixed effect model. The protocols in our current study including papers selection, data extraction, quality assessment, and statistical analysis were adapted from our previous meta-analyses [19–22], and we also used the checklist of the Preferred Reporting Items for Systematic Review and Meta-analysis (PRISMA) to guide the protocols in our study [23].

Eligibility criteria

The following criteria were used to include the papers in our study: (1) evaluating the correlation between CaSR gene polymorphisms (CaSR R990G, CaSR A986S, and CaSR Q1011E) and the risk of urolithiasis; and (2) having required data for calculation of OR95%CI. Furthermore, the exclusion criteria were as follows: (1) unrelated titles and abstracts, (2) reviews and commentaries, (3) incomplete and or ungeneralized data, (4) having deviation from Hardy-Weinberg equilibrium (X2 > 3.84 was defined as deviation from Hardy-Weinberg equilibrium) [24], and (5) double publication.

Search strategy and data extraction

Papers concerning the association between CaSR gene polymorphisms (CaSR R990G, CaSR A986S, and CaSR Q1011E) and the risk of urolithiasis were searched in major scientific websites (PubMed, Embase, Cochrane, and Web of science) up to 10 April 2019. In searching the articles, we did not restrict the publication language. If we found articles with language that we did not understand, we established a comprehensive consultation with Language Center of Universitas B******* (blinded due to double blind review). Moreover, to perform a comprehensive searching, we used the combination of the following key words: (urolithiasis or nephrolithiasis or kidney stone or renal stone) and (calcium-sensing receptor R990G or CaSR R990G) and (calcium-sensing receptor A986S or CaSR A986S) and (calcium-sensing receptor Q1011E or CaSR Q1011E). If articles with the same study data were found, only articles with the larger sample size were included in our analysis. For data extraction, the following information were extracted from each study: (1) first author name, (2) publication year, (3) country of origin, (4) genotyping method, (5) sample size of case and control, and (6) genotype frequencies of urolithiasis and control groups. To provide data with high validity and to prevent errors in data extraction, the extraction was conducted by three independent authors (JKF, AG, FT).

Assessment of the methodology quality

To assess the quality of each paper, we used Newcastle-Ottawa Scale (NOS) [25]. This evaluation was conducted by three independent authors (JKF, AG, FT). The evaluation consisted of three factors such as study selection (four points), the comparability of the groups (two points), and the ascertainment of the exposure (three points). In this evaluation, each paper had the score ranging from 0 (the worst) to 9 (the best). The quality was interpreted as good (score ≥ 7), moderate (score 5–6), and poor (score ≤ 4). If the discrepancy between the three independent authors was found, we established a consensus.

Covariates and sub-group analysis

To provide a comprehensive analysis, all alleles and genotypes models were evaluated to assess the correlation and effect estimates. For CaSR R990G, the genetic models were R vs. G; G vs. R; RR vs. RG + GG; RG vs. RR + GG; and GG vs. RR + RG. For CaSR A986S, the genetic models were A vs. S; S vs. A; AA vs. AS + SS; AS vs. AA + SS; and SS vs. AA + AS. Moreover, for CaSR Q1011E, the genetic models were Q vs. E; E vs. Q; QQ vs. QE + EE; QE vs. QQ + EE; and EE vs. QQ + QE. Moreover, we also performed sub-group analysis of all genetic models according to ethnicity sub-group (Asian and Caucasian).

Statistical analysis

The calculation of pooled OR and 95%CI, determined by Z test, was used to assess the association between CaSR gene polymorphisms and the risk of urolithiasis. The effect model for determining the correlation, whether using fixed or random effect model, was assessed using a Q test. A fixed effect model was used if we found no heterogeneity. Conversely, random effect model was used if the evidence of heterogeneity ($p < 0.10$) was observed. Moreover, an Egger's test was employed to assess potential of publication bias. To prevent analysis errors, the analysis in our present study was performed using two different software (comprehensive meta-analysis [CMA, New Jersey, USA] version 2.1 and Review Manager [Revman Cochrane, London, UK] version 5.3) and three independent authors (JKF, AG, FT). If we found discrepancy, we conducted a consensus to discuss the discrepancy between authors.

Results and discussion

Eligible studies

Our final searching strategy identified 11 papers compatible for meta-analysis. Of them, we found eight studies evaluating CaSR R990G; nine studies evaluating CaSR A986S; and five studies evaluating CaSR Q1011E. This number of papers were retrieved from searching in PubMed, Embase, Cochrane, and Web of science; and we selected the papers in accordance with eligibility criteria. In the initial searching, we identified 338 papers. Of those, 312 papers were excluded because of irrelevant title and or abstract. Moreover, 15 papers were also excluded because of review (nine), not providing required data for calculation of OR and 95%CI (four), and having deviation from Hardy-Weinberg equilibrium (two). Figure 1 demonstrates a flowchart concerning eligibility pathway in our meta-analysis. For quality assessment, all papers included in our analysis had moderate (NOS score 5–6) or high-quality (NOS score ≥ 7). Baseline characteristics of studies included in our meta-analysis are summarized in Table 1 for CaSR R990G, Table 2 for CaSR A986S, and Table 3 for CaSR Q1011E.

Data synthesis

A total of eight studies about CaSR R990G consisting of 1853 cases and 1514 controls were enrolled for our analysis. On the whole analysis, our findings revealed that decreased risk of urolithiasis was observed in R allele (OR95%CI = 0.69 [0.51–0.95], $p = 0.0240$) and RR genotype (OR95%CI = 0.60 [0.41–0.89], $p = 0.0120$). On the contrary, increased

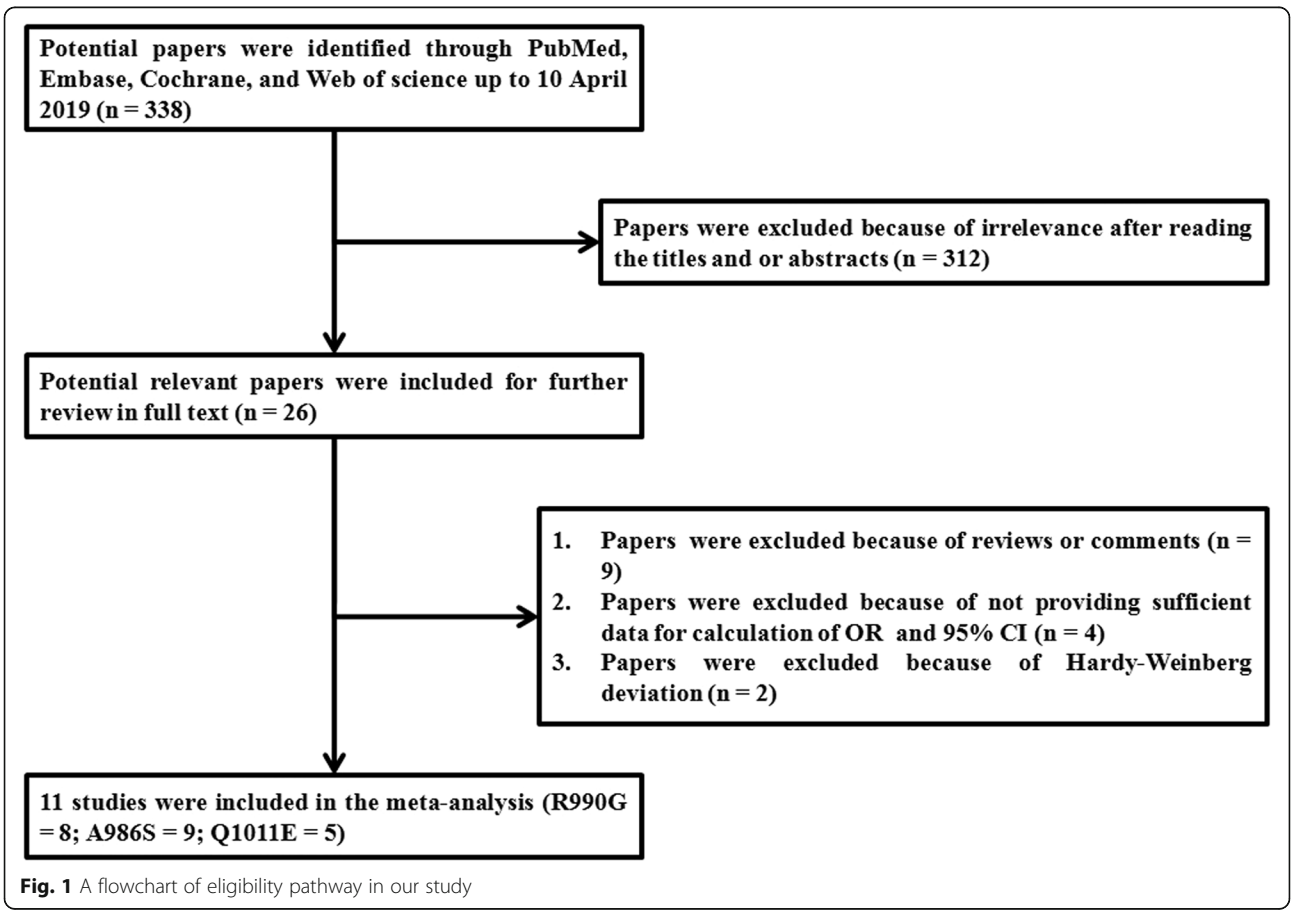

Fig. 1 A flowchart of eligibility pathway in our study

Table 1 Baseline characteristics of studies concerning CaSR R990G gene polymorphism in patients with urolithiasis included in the meta-analysis

Author and year	Case				Control				Ethnicity	Type of stone	Genotyping	X^2 HWE	NOS [S/C/E]
	RR	RG	GG	N	RR	RG	GG	N					
Ding et al. 2017 [26]	109	348	158	615	76	172	67	315	Asian	Calcium oxalate	PCR	2.72	6[3/1/2]
Hamilton et al. 2009 [30]	51	5	0	56	180	11	0	191	Caucasian	Calcium oxalate	PCR-RFLP	0.17	7[3/2/2]
Han et al. 2013 [31]	20	40	10	70	24	53	17	94	Asian	Calcium oxalate	PCR	1.69	6[3/1/2]
Li et al. 2018 [32]	191	301	132	624	152	217	101	470	Asian	Calcium oxalate	PCR	2.02	8[4/2/2]
Peretokina et al. 2015 [33]	84	23	3	110	60	15	2	77	Caucasian	Calcium oxalate and phosphate	PCR	0.76	6[3/1/2]
Shakhssalim et al. 2010 [27]	87	10	2	99	105	2	0	107	Asian	Calcium oxalate	PCR	0.01	7[4/1/2]
Vezzoli et al. 2007 [28]	105	17	2	124	115	4	0	119	Caucasian	Calcium oxalate	PCR	0.03	6[3/1/2]
Vezzoli et al. 2014 [29]	133	22	0	155	136	5	0	141	Caucasian	Calcium oxalate	PCR	0.05	6[3/1/2]

CaSR calcium-sensing receptor, *PCR* polymerase chain reaction, *PCR-RFLP* PCR–restriction fragment length polymorphism, *HWE* Hardy-Weinberg equilibrium, *NOS* Newcastle-Ottawa Scale, *S* selection, *C* comparability, *E* exposure

risk of urolithiasis was found in G allele (OR95%CI = 1.44 [1.05–1.98], p = 0.0240) and RG genotype (OR95%CI = 1.48 [1.05–2.09], p = 0.0260) (Fig. 2a, b). Moreover, we failed to show the correlation in GG genotype. In sub-group analysis, our calculation found that the association was observed only in Caucasian sub-group. R allele (OR95%CI = 0.43 [0.19–0.96], p = 0.0400) and RR genotype (OR95%CI = 0.42 [0.19–0.93], p = 0.0330) were associated with decreased risk of urolithiasis. Conversely, G allele (OR95%CI = 2.35 [1.03–5.37], p = 0.0400) and RG genotype (OR95%CI = 2.32 [1.08–5.00], p = 0.0310) were associated with increased risk of urolithiasis (Fig. 2c, d). The summary of correlation between urolithiasis and CaSR gene polymorphism is presented in Table 4.

Totally, 2402 cases and 2066 controls retrieved from nine studies concerning CaSR A986S were included in our analysis. Our overall analysis found that A allele (OR95%CI = 0.64 [0.49–0.84], p = 0.0010) and AA genotype (OR95%CI = 0.66 [0.48–0.91], p = 0.0110) were associated with decreased risk of urolithiasis. Conversely, increased risk of urolithiasis was observed in S allele (OR95%CI = 1.56 [1.19–2.04], p = 0.0010) (Fig .3a). We failed to confirm the correlation in AS and SS genotype. In sub-group analysis, our findings confirmed that the correlation was found in Asian population. The decreased risk of urolithiasis was found in A allele (OR95%CI = 0.55 [0.44–0.70], p < 0.0001) and AA genotype (OR95%CI = 0.56 [0.38–0.82], p = 0.0030). On other hands, increased risk of urolithiasis was observed in S allele (OR95%CI = 1.81 [1.43–2.29], p < 0.0001) and AS genotype (OR95%CI = 1.71 [1.16–2.53], p = 0.0070) (Fig. 3b, c). We summarize the correlation between CaSR A986S gene polymorphism and the risk of urolithiasis in Table 5.

For the association between CaSR Q1011E gene polymorphism and the risk of urolithiasis, we collected five papers consisting of 1127 cases and 991 controls. Overall, our cumulative calculation found no significant association between the risk of urolithiasis and all genetic models of CaSR Q1011E. Moreover, in sub-group analysis, we also failed to clarify the association between the risk of urolithiasis and all genetic models of CaSR Q1011E gene polymorphism. Summary of the relation

Table 2 Baseline characteristics of studies concerning CaSR A986S gene polymorphism in patients with urolithiasis included in the meta-analysis

Author and year	Case				Control				Ethnicity	Type of stone	Genotyping	X^2 HWE	NOS [S/C/E]
	AA	AS	SS	N	AA	AS	SS	N					
Corbetta et al. 2006 [39]	58	28	8	94	91	42	4	137	Caucasian	Calcium oxalate	PCR	0.10	6[3/1/2]
Ding et al. 2017 [26]	589	25	1	615	303	12	0	315	Asian	Calcium oxalate	PCR	0.12	6[3/1/2]
Guha et al. 2015 [38]	116	82	2	200	162	37	1	200	Asian	Calcium oxalate	PCR	0.52	7[3/2/2]
Hamilton et al. 2009 [30]	110	33	14	157	372	91	6	469	Caucasian	Calcium oxalate	PCR-RFLP	0.03	7[3/2/2]
Han et al. 2013 [31]	64	5	1	70	88	6	0	94	Asian	Calcium oxalate	PCR	0.10	6[3/1/2]
Kim et al. 2011 [40]	415	18	0	433	191	6	0	197	Asian	Calcium oxalate and citrate	PCR-RFLP	0.05	7[3/2/2]
Li et al. 2018 [32]	561	62	1	624	435	35	0	470	Asian	Calcium oxalate	PCR	0.70	8[4/2/2]
Peretokina et al. 2015 [33]	78	26	6	110	48	25	4	77	Caucasian	Calcium phosphate	PCR	0.10	6[3/1/2]
Shakhssalim et al. 2010 [27]	71	26	2	99	93	14	0	107	Asian	Calcium oxalate	PCR	0.52	7[4/1/2]

CaSR calcium-sensing receptor, *PCR* polymerase chain reaction, *PCR-RFLP* PCR–restriction fragment length polymorphism, *HWE* Hardy-Weinberg equilibrium, *NOS* Newcastle-Ottawa Scale, *S* selection, *C* comparability, *E* exposure

Table 3 Baseline characteristics of studies concerning CaSR Q1011E gene polymorphism in patients with urolithiasis included in the meta-analysis

Author and year	Case				Control				Ethnicity	Type of stone	Genotyping	X^2 HWE	NOS [S/C/E]
	QQ	QE	EE	N	QQ	QE	EE	N					
Corbetta et al. 2006 [39]	89	5	0	94	123	14	0	137	Caucasian	Calcium oxalate	PCR	0.40	6[3/1/2]
Guha et al. 2015 [38]	189	11	0	200	190	10	0	200	Asian	Calcium oxalate	PCR	0.13	7[3/2/2]
Li et al. 2018 [32]	598	26	0	624	457	13	0	470	Asian	Calcium oxalate	PCR	0.09	8[4/2/2]
Peretokina et al. 2015 [33]	92	16	2	110	70	6	1	77	Caucasian	Calcium oxalate and phosphate	PCR	3.36	6[3/1/2]
Shakhssalim et al. 2010 [27]	94	5	0	99	107	0	0	107	Asian	Calcium oxalate	PCR	0.07	7[4/1/2]

CaSR calcium-sensing receptor, *PCR* polymerase chain reaction, *PCR-RFLP* PCR–restriction fragment length polymorphism, *HWE* Hardy-Weinberg equilibrium, *NOS* Newcastle-Ottawa Scale, *S* selection, *C* comparability, *E* exposure

between CaSR Q1011E gene polymorphism and the risk of urolithiasis is demonstrated in Table 6.

Source of heterogeneity

For CaSR R990G, overall, evidence for heterogeneity was observed in R and G alleles and RR and RG genotypes. Therefore, random effect model was used to evaluate the correlation. For Asian sub-group, random effect model was used to assess the correlation in R and G allele and RR genotype because of heterogeneity. For Caucasian sub-group, R and G alleles and RR and RG genotypes were observed to have heterogeneity. Therefore, the correlation was assessed by random effect model. The evidence of heterogeneity for CaSR R990G is described in Table 4.

In CaSR A986S, the correlation (A vs. S; S vs. A; AA vs. AS + SS; AS vs. AA + SS) was assessed using random effect model because evidence for heterogeneity was found. On the other hand, in Asian sub-group, A and S alleles were evaluated using fixed effect model because of no heterogeneity existed, and RR and RG genotypes were assessed using random effect model because of heterogeneity. Other evidences for heterogeneity for CaSR A986S are presented in Table 5.

For CaSR Q1011E, the association and effect estimation in overall analysis and in Asian sub-group analysis were assessed using fixed effect model because of no evidence for heterogeneity. Furthermore, because the heterogeneity in Caucasian sub-group was observed, the association was evaluated using random effect model. We summarize the evidence for heterogeneity of CaSR Q1011E in Table 6.

Potential publication bias

To assess the potency of publication bias among all included papers, we used an Egger's test. For CaSR R990G (Fig. 4a), our analysis found that publication bias was observed in GG genotype both in overall analysis and Caucasian sub-group. For CaSR A986S (Fig. 4b), we found publication bias in SS genotype in both overall analysis and in Asian sub-group and AS genotype of Caucasian sub-group. Moreover, for CaSR Q1011E, publication bias was found in EE genotype of overall analysis and Caucasian sub-group. The summary of publication bias is described in Table 4 for CaSR R990G, Table 5 for CaSR A986S, and Table 6 for CaSR Q1011E.

Discussion

Our searching strategy identified eight papers evaluating the association between CaSR R990G gene polymorphism and the risk of urolithiasis. Of those, four papers [26–29] showed that R990G gene polymorphism was associated with the risk of urolithiasis with variable odd ratio, ranging from 1.24 to 8.07 (G vs. R). On the other hand, four other studies [30–33] failed to confirm the correlation. Our pooled calculation revealed that R allele and RR genotype were associated with decreased risk of urolithiasis. On the other hand, G allele and RG genotype had a significant association with increased risk of urolithiasis (Fig. 2a, b). Theoretically, the precise role of CaSR R990G in the development of urolithiasis remains undefined. However, a study in human embryonic renal cells (HEK293) revealed that G allele of CaSR R990G might activate phospholipase A or C to a different degree and escalate the function of CaSR, and therefore it was associated with increased calcium excretion [28]. On the other hand, G allele was associated with hypercalciuria in subjects without kidney stones [14]. This demonstrates that G allele may be associated with idiopathic hypercalciuria. Moreover, another hypothesis revealed that G allele was associated with increasing CaSR sensitivity to calcium, and therefore it may cause decreased calcium reabsorption in the ascending limb [34, 35]. G allele of CaSR R990G means that the basic amino acid arginine is replaced by the neutral amino acid glycine. This replacement may affect conformation and folding of receptor domains, and cause the turning of CaSR capability to activate G-proteins and to bind filamin-A. This circumstance is believed to contribute for increasing CaSR sensitivity to calcium [36]. Furthermore, a gene-gene interaction theory may also support our results. A study found that G allele of CaSR R990G was reported to correlate with minor allele of rs6776158, and it was associated with reduced transcriptional activity of the CaSR gene promoter 1. In addition,

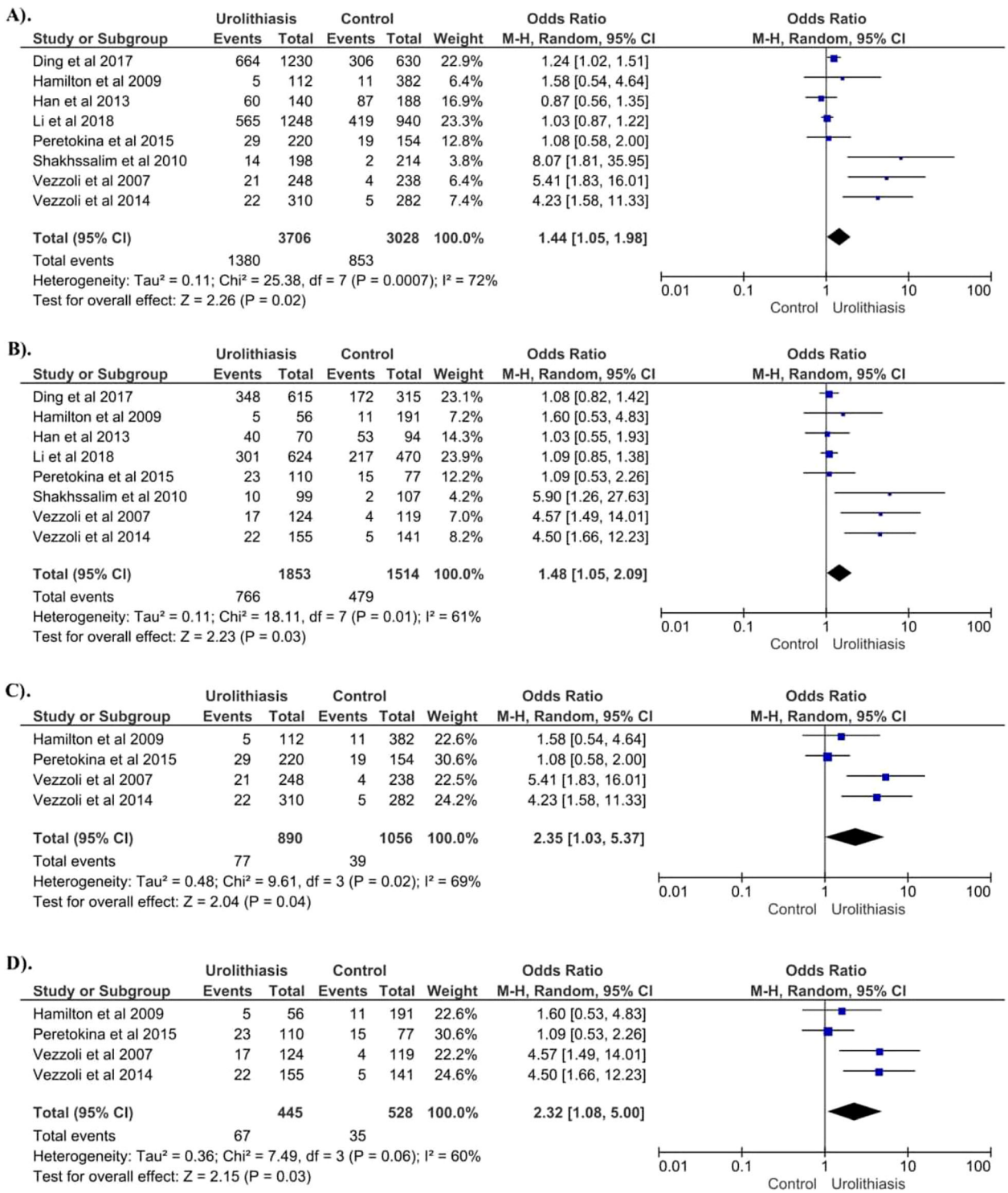

Fig. 2 Forest plot of the correlation between R990G gene polymorphism and the risk of urolithiasis. **a** (G vs. R) and **b** (RG vs. RR + GG) summarize overall correlation. On other hands, **c** (G vs. R) and **d** (RG vs. RR + GG) describe the correlation in Caucasian sub-group analysis

this circumstance was associated with increased risk of calcium stone formation [37]. This explanation may be a benchmark for our results showing that G allele of R990G was associated with increased risk of urolithiasis. However, further studies are required to elucidate the precise mechanism how CaSR R990G affects urolithiasis.

Beside CaSR R990G, the development of urolithiasis may be also governed by CaSR A986S. Concerning the

Table 4 Summay of the association between R990G gene polymorphism and the risk of urolithiasis

Allele and genotype	NS	Model	Value		Sensitivity (%)	Specificity (%)	OR	95%CI	pH	pE	p
			Case (%)	Control (%)							
Overall analysis											
R vs. G	8	Random	62.76	71.83	62.76	28.17	0.69	0.51–0.95	0.0010	0.3230	0.0240
G vs. R	8	Random	37.24	28.17	37.24	71.83	1.44	1.05–1.98	0.0010	0.3230	0.0240
RR vs. RG + GG	8	Random	42.09	56.01	42.09	43.99	0.60	0.41–0.89	0.0040	0.4150	0.0120
RG vs. RR + GG	8	Random	41.34	31.64	41.34	68.36	1.48	1.05–2.09	0.0120	0.3360	0.0260
GG vs. RR + RG	8	Fixed	16.57	12.35	16.57	87.65	1.09	0.89–1.35	0.5240	< 0.0001	0.3950
Asian sub-group											
R vs. G	4	Random	53.73	58.72	53.73	41.28	0.88	0.66–1.17	0.0180	0.2190	0.3680
G vs. R	4	Random	46.27	41.28	46.27	58.72	1.14	0.86–1.52	0.0180	0.2190	0.3680
RR vs. RG + GG	4	Random	28.91	36.21	28.91	63.79	0.77	0.51–1.16	0.0400	0.3050	0.2090
RG vs. RR + GG	4	Fixed	49.64	45.03	49.64	54.97	1.10	0.93–1.31	0.2030	0.1470	0.2600
GG vs. RR + RG	4	Fixed	21.45	18.76	21.45	81.24	1.09	0.88–1.34	0.4350	0.3550	0.0710
Caucasian sub-group											
R vs. G	4	Random	91.35	96.31	91.35	3.69	0.43	0.19–0.96	0.0240	0.6820	0.0400
G vs. R	4	Random	8.65	3.69	8.65	96.31	2.35	1.03–5.37	0.0240	0.6820	0.0400
RR vs. RG + GG	4	Random	83.82	92.99	83.82	7.01	0.42	0.19–0.93	0.0380	0.6540	0.0330
RG vs. RR + GG	4	Random	15.06	6.63	15.06	93.37	2.32	1.08–5.00	0.0600	0.5980	0.0310
GG vs. RR + RG	4	Fixed	1.12	0.38	1.12	99.62	1.57	0.33–7.46	0.3960	< 0.0001	0.5700

NS number of studies, *OR* odd ratio, *CI* confidence interval, *pH* p heterogeneity, *pE* p egger

correlation between CaSR A986S gene polymorphism and the risk of urolithiasis, of nine papers, our results found that three papers [27, 30, 38] showed the correlation, and six others [26, 31–33, 39, 40] failed to show the association. Of those, the odd ratio ranged from 1.96 to 2.55 (S vs. A). Our combination data demonstrated that A allele and AA genotype were correlated with decreased risk of urolithiasis. Conversely, S allele was associated with increased risk of urolithiasis (Fig. 3a). To date, the perspective theory underlying our results was lacking. However, several studies had reported that increased CaSR activity was observed in S allele of CaSR A986S gene polymorphism [18]. Moreover, S allele of CaSR A986S was also reported to be associated with the decrease of inhibitory activity of CaSR on tubular calcium reabsorption [28]. Therefore, it may govern calcium homeostasis. Furthermore, S allele of CaSR A986S gene polymorphism had been widely reported to affect elevated level of calcium concentration [41, 42]. Those reports might be the basis explaining the results of our study. Nevertheless, the proper mechanism concerning the role of CaSR A986S in patients with urolithiasis needs to be further investigated.

Moreover, because the pathogenesis of urolithiasis is complex and may involve specific interactions including gene–gene and/or gene–environment interaction, this pathogenesis may implicate other SNPs such as CaSR Q1011E. For this reason, we also evaluated the role of CaSR Q1011E gene polymorphism in patients with urolithiasis. Although Q allele was reported to be associated with elevated calcium concentration and hypercalciuric state [27], and previous meta-analysis also confirmed that CaSR Q1011E gene polymorphism was associated with urolithiasis in specific population [18]; however, of five papers we collected [27, 32, 33, 38, 39], all of them failed to show the association. Our pooled calculation also revealed that no association was found in all genetic models between CaSR Q1011E gene polymorphism and the risk of urolithiasis. In sub-group analysis, we also failed to clarify the association.

A previous study [18] had reported the meta-analysis concerning this topic. Overall, our results were consistent with this previous study. However, we found some limitations in the previous study, such as relatively small sample size, data discrepancy, data duplication, and deviation from Hardy-Weinberg equilibrium. Compared to previous study, our current study had larger sample size. Their meta-analysis only involved seven papers for CaSR R990G, six papers for CaSR A986S, and three papers for CaSR Q1011E. Meanwhile, our present study involved eight papers for CaSR R990G, nine papers for CaSR A986S, and five papers for CaSR Q1011E. This comparison might demonstrate that, because of having larger sample size, our present study might have better statistical power to explore the real association. Moreover, we also found irrelevant data or data discrepancy between

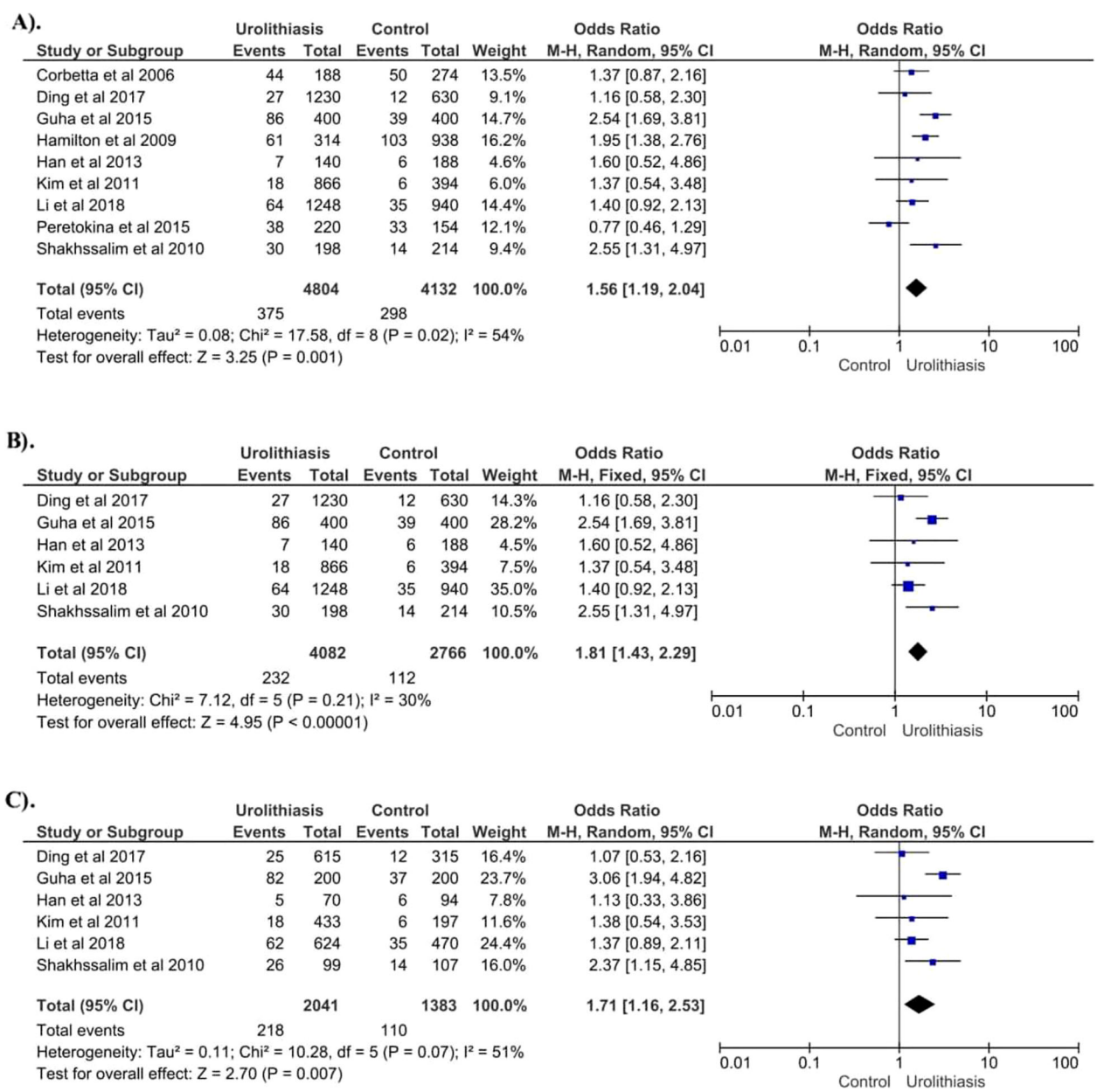

Fig. 3 Forest plot of the association between A986S gene polymorphism and the risk of urolithiasis. **a** (S vs. A) shows overall correlation. On the other hand, the correlation in Asian sub-group analysis is described in **b** (S vs. A) and **c** (AS vs. AA + SS)

data presented in the meta-analysis and data in the original paper. Data discrepancy often occurs in data analysis using large sample size without checking for validity. To prevent data discrepancy, data extraction and analysis in our present study were performed by three independent authors. Furthermore, Hardy-Weinberg deviation was also found in the previous study. In the genetic population, Hardy-Weinberg equilibrium is the basic principle of genetic and considered as one of the most important principles in population of genetics. This principle has been universally employed for the study of allele and genotype frequency changes in a population over generations [43]. In the context of deviation from this principle, it had been demonstrated that whatever method was used if Hardy-Weinberg equilibrium was not established, they might result in nothing [44]. Therefore, because of those several limitations, our present meta-analysis might clarify better correlation between CaSR gene polymorphisms and the risk of urolithiasis.

In sub-group analysis, interestingly, our findings revealed that the correlation between CaSR R990G gene

Table 5 Summary of the correlation between A986S gene polymorphism and the risk of urolithiasis

Allele and genotype	NS	Model	Value		Sensitivity (%)	Specificity (%)	OR	95%CI	pH	pE	p
			Case (%)	Control (%)							
Overall analysis											
A vs. S	9	Random	92.19	92.79	92.19	7.21	0.64	0.49–0.84	0.0250	0.2910	0.0010
S vs. A	9	Random	7.81	7.21	7.81	92.79	1.56	1.19–2.04	0.0250	0.2910	0.0010
AA vs. AS + SS	9	Random	85.85	86.30	85.85	13.70	0.66	0.48–0.91	0.0110	0.3580	0.0110
AS vs. AA + SS	9	Random	12.70	12.97	12.70	87.03	1.34	0.95–1.88	0.0040	0.4070	0.0990
SS vs. AA + AS	9	Fixed	1.46	0.73	1.46	99.27	3.34	1.85–6.02	0.5040	< 0.0001	< 0.0001
Asian sub-group											
A vs. S	6	Fixed	94.32	95.95	94.32	4.05	0.55	0.44–0.70	0.2120	0.2020	< 0.0001
S vs. A	6	Fixed	5.68	4.05	5.68	95.95	1.81	1.43–2.29	0.2120	0.2020	< 0.0001
AA vs. AS + SS	6	Random	88.98	91.97	88.98	8.03	0.56	0.38–0.82	0.0710	0.3300	0.0030
AS vs. AA + SS	6	Random	10.68	7.95	10.68	92.05	1.71	1.16–2.53	0.0680	0.3370	0.0070
SS vs. AA + AS	6	Fixed	0.34	0.07	0.34	99.93	2.67	0.71–10.03	0.9770	< 0.0001	0.1450
Caucasian sub-group											
A vs. S	3	Random	80.19	86.38	80.19	13.62	0.77	0.45–1.30	0.0130	0.4070	0.3200
S vs. A	3	Random	19.81	13.62	19.81	86.38	1.31	0.77–2.21	0.0130	0.4070	0.3200
AA vs. AS + SS	3	Random	68.14	74.82	68.14	25.18	0.86	0.53–1.42	0.0660	0.3470	0.5630
AS vs. AA + SS	3	Fixed	24.10	23.13	24.10	76.87	0.94	0.69–1.28	0.4030	< 0.0001	0.6840
SS vs. AA + AS	3	Random	7.76	2.05	7.76	97.95	3.10	1.00–9.64	0.0570	0.8070	0.0510

NS number of studies, *OR* odd ratio, *CI* confidence interval, *pH* p heterogeneity, *pE* p egger

Table 6 Summary of the association between Q1011E gene polymorphism and the risk of urolithiasis

Allele and genotype	NS	Model	Value		Sensitivity (%)	Specificity (%)	OR	95%CI	pH	pE	p
			Case (%)	Control (%)							
Overall analysis											
Q vs. E	5	Fixed	97.03	97.73	97.03	2.27	0.77	0.51–1.16	0.1710	0.3770	0.2110
E vs. Q	5	Fixed	2.97	2.27	2.97	97.73	1.30	0.86–1.95	0.1710	0.3770	0.2110
QQ vs. QE + EE	5	Fixed	94.23	95.56	94.23	4.44	0.77	0.50–1.17	0.1560	0.4090	0.2150
QE vs. QQ + EE	5	Fixed	5.59	4.34	5.59	95.66	1.30	0.85–2.00	0.1550	0.4160	0.2290
EE vs. QQ + QE	5	Fixed	0.18	0.10	0.18	99.90	1.41	0.13–15.80	0.7820	1.0000	< 0.0001
Asian sub-group											
Q vs. E	3	Fixed	97.72	98.52	97.72	1.48	0.69	0.41–1.17	0.2910	0.2580	0.1660
E vs. Q	3	Fixed	2.28	1.48	2.28	98.52	1.45	0.86–2.44	0.2910	0.2580	0.1660
QQ vs. QE + EE	3	Fixed	94.45	97.04	94.45	2.96	0.69	0.41–1.16	0.2870	0.2680	0.1610
QE vs. QQ + EE	3	Fixed	4.55	2.96	4.55	97.04	1.46	0.86–2.47	0.2870	0.2680	0.1610
EE vs. QQ + QE	3	NA	0.00	0.00	0.00	100.00	NA	NA	NA	NA	NA
Caucasian sub-group											
Q vs. E	2	Random	93.87	94.86	93.87	5.14	1.00	0.29–3.51	0.0610	0.7650	0.9980
E vs. Q	2	Random	6.13	5.14	6.13	94.86	1.00	0.29–3.49	0.0610	0.7650	0.9980
QQ vs. QE + EE	2	Random	88.73	90.19	88.73	9.81	0.99	0.26–3.83	0.0550	0.8310	0.9920
QE vs. QQ + EE	2	Random	10.29	9.35	10.29	90.65	1.01	0.26–4.01	0.0570	0.8460	0.9880
EE vs. QQ + QE	2	Fixed	0.98	0.47	0.98	99.53	1.41	0.13–15.80	1.0000	< 0.0001	0.7820

NS number of studies, *OR* odd ratio, *CI* confidence interval, *pH* p heterogeneity, *pE* p egger, *NS* not available

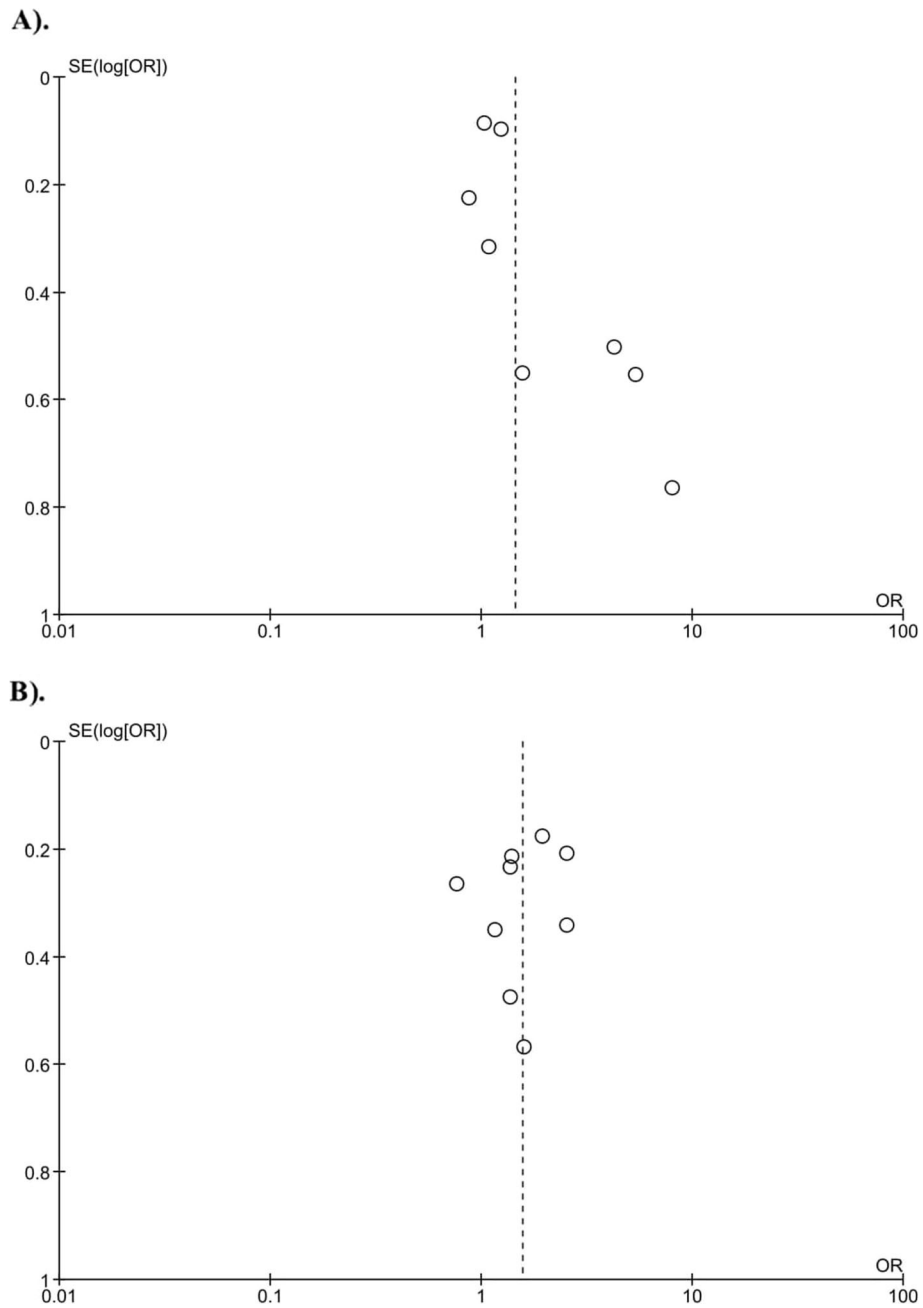

Fig. 4 Funnel plot of the association between the risk of urilithiasis and (**a**) R990G and (**b**) A986S gene polymorphisms

polymorphism and the risk of urolithiasis was observed in Caucasian population (Fig. 2c, d). Moreover, in Asian population, the correlation was found in CaSR A986S (Fig. 3b, c). Our results might demonstrate gene–environment interaction. It attests that CaSR R990G has a dominant role in Caucasian, and CaSR A986S is dominant in Asian. The mechanism underlying our results-related ethnicity is not well known. Although the correlation perspective has widely proposed that "where there is sugar, there are bound to be ants,"because of the lack of evidence concerning this report, our results-related ethnicity were difficult to explain. However, genetic background and gene–environment interaction may play a crucial role in this circumstance. Until now, no study reports the exact explanation concerning CaSR gene polymorphisms in the context of ethnicity. Therefore, in the near future, we expect that this puzzle may be elucidated.

Although we provided the evidence that the risk of urolithiasis was associated with CaSR R990G and CaSR

A986S gene polymorphisms, however, at present time, it was not possible to use these SNPs as biomarkers or predictors of urolithiasis. Our results might clarify the controversy during this time and confirm better understanding concerning the role of these SNPs in patients with urolithiasis. Moreover, since the reported studies used non-randomized design, it might result in a low-level of evidence. Therefore, further studies with a higher design may be required.

Several crucial limitations were observed in our present study. First, several factors having pivotal role in the pathogenesis and development of urolithiasis such as age, dietary, hyperparathyroidism, previous urolithiasis, and family history of urolithiasis [45] were not controlled for and included in our study. Second, the findings of our study should be interpreted with caution because of relatively small sample size in both overall analysis and sub-group analysis. Third, unequal proportion of papers included in our analysis in each sub-group was observed, and therefore, this factor may lead to heterogeneity and study bias. Fourth, most of study design in our database was cross-sectional. Further meta-analysis including better study designs may be needed to achieve a higher level of evidence. Because of the limitations, further studies eliminating these factors may be required to elucidate the better correlation between the risk of urolithiasis and the CaSR gene polymorphisms.

Conclusions

Our study reveals that R allele and RR genotype of CaSR R990G and A allele and AA genotype of CaSR A986S gene polymorphisms are asociated with protective effect againsts urolithiasis. On the other hand, G allele and RG genotype of CaSR R990G and S allele of CaSR A986S gene polymorphisms are correlated with increased risk of urolithiasis. In sub-group analysis, the correlation in Caucasian population is observed in CaSR R990G, while the association in CaSR A986S is found in Asian population. Our results may clarify better correlation concerning gene-disease interaction between CaSR gene polymorphisms and the risk of urolithiasis.

Abbreviations

95%CI: 95% confidence interval; A986S: A guanine–thymine substitution at codon 986; CaSR: Calcium-sensing receptor; CMA: Comprehensive meta-analysis; GPCR: G-protein coupled receptor; HEK293: Human embryonic renal cells; HWE: Hardy-Weinberg equilibrium; NOS: Newcastle-Ottawa Scale; OR: Odd ratio; PRISMA: Preferred Reporting Items for Systematic Review and Meta-analysis; Q1011E: A cytosine–guanine substitution at codon 1011; R990G: The subtitution of an adenine - guanine at codon 990; Revman: Review manager; SNPs: Single-nucleotide polymorphisms

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Authors' contributions

Idea/concept: BD; BBP; AG. Design: BD; BBP; AG. Control/supervision: BD; BBP; AG. Data collection/processing: BD; BBP; AG; FT; SAH; EDM; AGK; JKF. Extraction/Analysis/interpretation: AG; FT; JKF. Literature review: BD; BBP; AG; JKF. Writing the article: JKF. Critical review: BD; BBP; AG. All authors have critically reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript.

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Data used in our study were presented in the main text.

Ethics approval and consent to participate

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Consent for publication

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Competing interests

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