Association Between Klotho Gene Polymorphisms and Urolithiasis: A Meta-Analysis

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ABSTRACT

Former studies have suggested that urolithiasis is related to *Klotho* gene polymorphisms. The aim of this meta-analysis was to investigate this relationship. Studies on the association between urolithiasis susceptibility and *Klotho* gene polymorphisms were systematically searched for in databases. Odds ratios and 95% confidence intervals were pooled as the effect size. This meta-analysis incorporated ten articles. *Klotho* rs1207568 adenine (A) may be related to a decreased urolithiasis risk in Caucasians. The results showed that *Klotho* rs3752472 may not be related to urolithiasis risk in the Han Asian subgroup. *Klotho* rs564481 may not be related to urolithiasis risk in Asians or Caucasians, and *Klotho* rs650439 may not be related to urolithiasis risk in Asians.

Key Words: *Klotho, Single-nucleotide polymorphism, Urolithiasis, Meta-analysis.*

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INTRODUCTION

Urolithiasis is likely related to multiple genes' effects, and related to environmental and lifestyle factors.[1,2] Calcium stone is the major type of urolithiasis. Infection can promote the stone formation. *Klotho* might be associated with inflammatory processes in kidney injury.[3,4] The *Klotho* protein, a Type-I transmembrane protein expressed by the *Klotho* gene on chromosome 13q13.1 in tissues responsible for calcium homeostasis, including the kidney and epithelium of the choroid plexus in the brain and parathyroid gland, plays a crucial role in phosphate homeostasis regulation *via FGF23* and increased calcium uptake *via TRPV5*.[5,6] The single nucleotide polymorphism (SNP) rs1207568 (G395A) is located in *Klotho* gene's promoter region, and DNA-protein interaction can be affected by the G-A substitution in this region.[7] Calcium oxalate crystals can cause renal epithelial cell injury, which may be prevented by *Klotho* rs3752472.[8] Previous studies in several populations have suggested that urolithiasis may be associated with *Klotho* polymorphisms. Here, this meta-analysis was conducted to evaluate it, and provide specific genetic markers for urolithiasis.

METHODOLOGY

In EMBASE, China National Knowledge Infrastructure (CNKI), clinicaltrials.gov, PubMed, and Cochrane Library databases, two independent investigators performed a systematic search on 23 April 2022.

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The following terms were used without any limitations: "*Klotho* or *HFTC3*" and "polymorphisms or polymorphism" and "calculi or stone or nephrolithiasis or calculus or urolithiasis or lithiasis." The references of related reviews and studies were artificially indexed.

Examples were taken from meta-analysis published by the team to establish the inclusion and exclusion criteria.[9,10] It was attempted to email the author for detailed genotype data. Two investigators independently performed the study selection. Any disputes were resolved through discussions. If necessary, another investigator may be invited to participate in further discussions. Detective samples, year of publication, Hardy-Weinberg equilibrium, first author's surname, urinary calculi's chemical composition, genotyping method, ethnicity, source of control groups, characteristics, country of origin, and number for each genotype were collected.

Independently, two investigators evaluated absorbed studies' quality by using Newcastle-Ottawa Scale (NOS).[11] The most important factors were country, gender, age, and ethnicity. The second important factor was urolithiasis-related diseases that alter calcium or phosphorus metabolism, such as hyperparathyroidism and a family history of urolithiasis. The quality scores ranged from 0 to 10. Detailed statistical analysis was conducted as relevant.[12-14]

RESULTS

Finally, 61 articles were identified from the databases (EMBASE = 29, PubMed = 15, CNKI = 14, clinicaltrials.gov = 2, Cochrane = 1, other sources (manual search) = 0). Figure 1 illustrates the screening process. Nine full-text articles were excluded, three being without detailed genotype data,[15-17] and six being duplicate studies. Ultimately, 11 articles were absorbed in this meta-analysis.[18-28]

Table I: Characteristics of studies.

No.	Study ID	Year	Country or area	Ethnicity	Control type	Genotyping method	Urolithiasis related diseases[#] in patients	Family history of urolithiasis in patients	Stone composition	P for HWE*	Quality
	rs1207568 (G395A)										
1.1.1	Telci *et al.* [18]	2011	Turkiye	Caucasian	PB*	PCR-RFLP	Negative	NA*	Calcium stone	**0.006**[&]	7
1.1.2	Gürel *et al.* [19]	2016	Turkiye	Caucasian	HB*	PCR-RFLP	NA	NA	NA	**0.000**	6
1.1.3	Lanka *et al.* [20]	2021	Northwestern India	Caucasian	PB	PCR-RFLP	Negative	NA	88.7% Calcium oxalate stone; 11.3% Calcium oxalate and phosphate stone mixed	**0.002**	7
1.2.1	Chen *et al.* [21]	2013	China	Asian(Han)	PB	TaqMan	Negative	negative	Calcium stone	0.131	9
1.2.2	Enli *et al.* [22]	2018	China	Asian(Han)	PB	Sequencing	Negative	negative	Calcium stone	0.105	7
1.3.1	Ali *et al.* [23]	2017	China	Asian(Uyghur)	PB	PCR-RFLP	Negative	52.3% positive	Calcium oxalate stone	**0.003**	7
1.3.2	Qi *et al.* [24]	2019	China	Asian(Uyghur)	PB	PCR-RFLP& Sequencing	Negative	Negative	Calcium oxalate stone	0.946	8
	rs3752472										
2.1.1	Wei *et al.* [25]	2015	China	Asian(Han)	PB	TaqMan	Negative	NA	pure or mixed Calcium oxalate stone	0.311	7
2.1.2	Enli20 *et al.* [22]	2018	China	Asian(Han)	PB	Sequencing	Negative	Negative	Calcium stone	0.455	7
2.1.3	Peili *et al.* [26]	2020	China	Asian(Han)	PB	SNaPshot	Negative	Negative	Calcium oxalate stone with purity≥65%	0.766	8
2.2.1	Ali *et al.* [23]	2017	China	Asian(Uyghur)	PB	PCR-RFLP	Negative	52.3% Positive	Calcium oxalate stone	0.124	7
2.2.2	Qi *et al.* [24]	2019	China	Asian(Uyghur)	PB	PCR-RFLP& Sequencing	Negative	Negative	Calcium oxalate stone	0.730	8
2.3	Litvinova *et al.* [27]	2021	Russia	Caucasian	PB	Sequencing	NA	52% positive	Calcium oxalate stone	NA	7
	rs564481 (C1818T)										
3.1.1	Telci *et al.* [18]	2011	Turkiye	Caucasian	PB	PCR-RFLP	Negative	NA	Calcium stone	0.787	7
3.1.2	Gürel *et al.* [19]	2016	Turkiye	Caucasian	HB	PCR-RFLP	NA	NA	NA	0.647	6
3.2.1	Chen *et al.* [21]	2013	China	Asian(Han)	PB	TaqMan	Negative	negative	Calcium stone	0.107	9
3.2.2	Peili *et al.* [26]	2020	China	Asian(Han)	PB	SNaPshot	Negative	Negative	Calcium oxalate stone with purity≥65%	0.421	8
3.3	Qi *et al.* [24]	2019	China	Asian(Uyghur)	PB	PCR-RFLP& Sequencing	Negative	Negative	Calcium oxalate stone	0.435	8
	rs650439										
4.1.1	Ali *et al.* [23]	2017	China	Asian(Uyghur)	PB	PCR-RFLP	Negative	52.3% positive	Calcium oxalate stone	0.360	7
4.1.2	Qi *et al.* [24]	2019	China	Asian(Uyghur)	PB	PCR-RFLP& Sequencing	Negative	negative	Calcium oxalate stone	0.413	8
4.2	Wei *et al.* [25]	2015	China	Asian(Han)	PB	TaqMan	Negative	NA	Pure or mixed calcium oxalate stone	0.359	7
	F352V										
5.1	Telci *et al.* [18]	2011	Turkiye	Caucasian	PB	PCR-RFLP	Negative	NA	Calcium stone	0.184	7
5.2	Gürel *et al.* [19]	2016	Turkiye	Caucasian	HB	PCR-RFLP	NA	NA	NA	**0.009**	6
	rs145682430										
6.1	Peili *et al.* [26]	2020	China	Asian(Uyghur)	PB	SNaPshot	Negative	Negative	Calcium oxalate stone with purity≥65%	0.725	8
6.2	Peili *et al.* [26]	2020	China	Asian(Han)	PB	SNaPshot	Negative	Negative	calcium oxalate Stone with purity≥65%	0.519	8
	rs139912465										
7	Liuya *et al.* [28]	2015	China	Asian(Uyghur)	PB	PCR-RFLP	Negative	52.3% Positive	Calcium oxalate stone	1	7
	rs577912										
8	Wei *et al.* [25]	2015	China	Asian(Han)	PB	TaqMan	Negative	NA	Pure or mixed calcium oxalate stone	0.153	7
	rs397703										
9	Enli *et al.* [22]	2018	China	Asian(Han)	PB	Sequencing	Negative	Negative	Calcium stone	0.544	7
	rs648202										
10	Qi *et al.* [24]	2019	China	Asian(Uyghur)	PB	PCR-RFLP& Sequencing	Negative	Negative	Calcium oxalate stone	0.285	8
	rs526906										
11.1	Apolikhin *et al.* [15]	2015	Russia	Caucasian	PB	NA	NA	NA	NA	NA	NA
11.2	Apolikhin *et al.* [16]	2016	Russia	Caucasian	PB	NA	NA	NA	NA	NA	NA
11.3	Apolikhin *et al.* [17]	2017	Russia	Caucasian	PB	NA	NA	NA	Calcium oxalate stone	NA	NA

[#] Diseases altering calcium and phosphorus metabolism like hyperparathyroidism; [*] HWE: Hardy–Weinberg equilibrium; PB: Population-based; HB: Hospital-based; NA: Not available; [&] Results with statistical significant difference were marked as bold.

Table I and II show the characteristics and detailed genotype data for each study. PCR–RFLP, TaqMan, SNaPshot, and sequencing were used as genotyping methods. Every studies used blood samples for genotyping. Control group of study no 1.1.1, 1.1.2, 1.1.3, 1.3.1 and 5.2 departed from HWE significantly. Owing to the lack of detailed genotype data, HWE was not evaluated in study no 2.3's control group.

Table III shows the results of pooled ORs. In a meta-analysis, rs1207568 adenine (A) was related to a decreased urolithiasis risk in dominant model (AG + AA *vs.* GG), heterozygote comparison (AG *vs.* GG), and allelic comparison (A *vs.* G) in the Caucasian subgroup. No statistically significant change in urolithiasis risk was discovered in the other genetic models, groups, or subgroups of rs1207568 (Table III and Figure 2).

In a meta-analysis, rs3752472 adenine (A) was related to a decreased urolithiasis risk in homozygote comparison (AA *vs.* GG) and recessive model (AA *vs.* GG + AG) in the Uyghur subgroup. No statistically significant change in urolithiasis risk was discovered in the other genetic models, groups, or subgroups of rs3752472. Heterogeneity in most groups and subgroups of rs3752472 was significant (Table III and Figure 3).

In a meta-analysis, no statistically significant change in urolithiasis risk was discovered in any genetic model or subgroup of rs564481, rs650439, F352V, or rs145682430. In each study included in each SNP, no statistically significant changes were discovered.

rs577912 adenine (A) was related to an increased urolithiasis risk in all genetic models. No statistically significant change in urolithiasis risk was discovered in any of the genetic models of rs139912465, rs397703, and rs648202. Among these four SNPs, only one study was included in each SNP; therefore, meta-analysis could not be operated.

Table II: Detailed genotype data of studies.

No.	Study ID	Case						Control					
	rs1207568	GG	GA	AA	G	A	Total	GG	GA	AA	G	A	Total
1.1.1	Telci *et al.* [18]	63	41	4	167	49	108	19	31	1	69	33	51
1.1.2	Gürel *et al.* [19]	54	45	4	153	53	103	32	68	2	132	72	102
1.1.3	Lanka *et al.* [20]	108	42	0	258	42	150	52	48	0	152	48	100
1.2.1	Chen *et al.* [21]	208	89	9	505	107	306	167	71	3	405	77	241
1.2.2	Enli *et al.* [22]	345	147	11	837	169	503	371	156	25	898	206	552
1.3.1	Ali *et al.* [23]	74	46	8	194	62	128	61	23	10	145	43	94
1.3.2	Qi *et al.* [24]	273	102	25	648	152	400	241	147	22	629	191	410
	rs3752472	GG	GA	AA	G	A	Total	GG	GA	AA	G	A	Total
2.1.1	Wei *et al.*[25]	1464	220	20	3148	260	1704	904	208	16	2016	240	1128
2.1.2	Enli *et al.* [22]	406	90	6	902	102	502	457	93	3	1007	99	553
2.1.3	Peili *et al.* [26]	331	75	6	737	87	412	355	44	1	754	46	400
2.2.1	Ali *et al.* [23]	113	14	1	240	16	128	63	25	6	151	37	94
2.2.2	Qi *et al.* [24]	349	50	0	748	50	399	349	58	3	756	64	410
2.3	Litvinova *et al.* [27]	NA*	NA	NA	98	2	50	NA	NA	NA	98	2	50
	rs564481	GG	GA	AA	G	A	Total	GG	GA	AA	G	A	Total
3.1.1	Telci *et al.* [18]	47	46	15	140	76	108	19	25	7	63	39	51
3.1.2	Gürel *et al.* [19]	45	41	17	131	75	103	33	52	17	118	86	102
3.2.1	Chen *et al.* [21]	199	GA+AA=107		data error		306	159	78	4	396	86	241
3.2.2	Peili *et al.* [26]	267	128	17	662	162	412	257	124	19	638	162	400
3.3	Qi *et al.* [24]	201	164	34	566	232	399	196	170	44	562	258	410
	rs650439	AA	AT	TT	A	T	Total	AA	AT	TT	A	T	Total
4.1.1	Ali *et al.* [23]	81	40	7	202	54	128	56	31	7	143	45	94
4.1.2	Qi *et al.* [24]	213	158	28	584	214	399	217	167	26	601	219	410
4.2	Wei *et al.*[25]	812	720	172	2344	1064	1704	508	508	112	1524	732	1128
	F352V	TT	TG	GG	T	G	Total	TT	TG	GG	T	G	Total
5.1	Telci *et al.* [18]	71	33	4	175	41	108	35	16	0	86	16	51
5.2	Gürel *et al.* [19]	60	40	3	160	46	103	60	42	0	162	42	102
	rs145682430	GG	GA	AA	G	A	Total	GG	GA	AA	G	A	Total
6.1	Peili *et al.* [26]	393	6	0	792	6	399	396	14	0	806	14	410
6.2	Peili *et al.* [26]	379	33	0	791	33	412	375	25	0	775	25	400
	rs139912465	GG	GA	AA	G	A	Total	GG	GA	AA	G	A	Total
7	Liuya *et al.* [28]	0	0	128	0	256	128	0	0	94	0	188	94
	rs577912	CC	CA	AA	C	A	Total	CC	CA	AA	C	A	Total
8	Wei *et al.*[25]	976	604	124	2556	852	1704	704	364	60	1772	484	1128
	rs397703	GG	GA	AA	G	A	Total	GG	GA	AA	G	A	Total
9	Enli *et al.* [22]	8	135	359	151	853	502	16	168	368	200	904	552
	rs648202	GG	GA	AA	G	A	Total	GG	GA	AA	G	A	Total
10	Qi *et al.* [24]	206	163	30	575	223	399	219	167	24	605	215	410
	rs526906	OR*(95%CI*)			A	B	Total				A	B	Total
11.1	Apolikhin *et al.* [15]	Non-significant			NA	NA	75				NA	NA	189
11.2	Apolikhin *et al.* [16]	Non-significant			NA	NA	43				NA	NA	189
11.3	Apolikhin *et al.* [17]	Non-significant			NA	NA	72				NA	NA	189

**NA: Not available; OR: Odds ratio; CI: Confidence interval.*

Table III: Results of pooled OR.

	Number	A vs. G		AA vs. GG		AG vs. GG		AG+AA vs. GG		AA vs. GG+AG	
rs1207568	(cases/controls)	OR#(95%CI#)	I^2(%)	OR(95%CI)	I^2(%)	OR(95%CI)	I^2(%)	OR(95%CI)	I^2(%)	OR(95%CI)	I^2(%)
Overall	1698/1550	***0.801(0.664-0.966)*** &	**50.3**	0.836(0.577-1.211)	11.7	***0.701(0.492-0.997)***	**78.5**	***0.709(0.521-0.965)***	**73.6**	0.906(0.630-1.303)	39.4
Caucasian	361/253	**0.587(0.449-0.767)**	0.0	1.193(0.299-4.755)	0.0	**0.405(0.289-0.570)**	0.0	**0.420(0.299-0.588)**	0.0	1.981(0.508-7.731)	0.0
Asian	1337/1297	*0.898(0.782-1.031)*	23.8	0.812(0.552-1.195)	44.3	0.955(0.675-1.351)	**72.7**	0.925(0.708-1.208)	**58.8**	0.854(0.451-1.616)	**56.1**
Han*	809/793	0.951(0.792-1.143)	28.4	0.968(0.198-4.724)	**77.7**	1.011(0.813-1.257)	0.0	0.979(0.793-1.209)	0.0	0.966(0.198-4.724)	**77.9**
Uyghur*	528/504	0.833(0.675-1.028)	40.1	0.896(0.538-1.493)	0.0	0.970(0.368-2.552)	**87.8**	0.909(0.455-1.813)	**80.1**	0.962(0.583-1.588)	38.9
rs3752472		A v.s G		AA vs. GG		AG vs. GG		AG+AA vs. GG		AA vs. GG+AG	
Overall	3195/2635	0.843(0.537-1.323)	**87.7**	NA#	NA	NA	NA	NA	NA	NA	NA
Asian	3145/2585	0.836(0.523-1.334)	**90.2**	0.828(0.249-2.755)	**63.5**	0.853(0.548-1.328)	**86.6**	0.834(0.518-1.343)	**89.0**	0.874(0.283-2.703)	**58.8**
Han	2618/2081	1.136(0.636-2.028)	**92.5**	1.653(0.512-5.341)	**58.9**	1.070(0.597-1.920)	**91.1**	1.108(0.603-2.035)	**92.1**	1.600(0.547-4.678)	**52.0**
Uyghur*	527/504	0.477(0.168-1.354)	**87.8**	**0.109(0.019-0.623)**	0.0	0.543(0.202-1.464)	**82.6**	0.488(0.165-1.446)	**86.6**	**0.126(0.022-0.713)**	0.0
rs564481		A vs. G		AA vs. GG		AG vs. GG		AG+AA vs. GG		AA vs. GG+AG	
Overall	1328/1204	0.900(0.781-1.037)	0.0	0.786(0.562-1.101)	0.0	0.901(0.745-1.090)	0.0	0.916(0.779-1.076)	0.0	0.857(0.622-1.181)	0.0
Caucasian*	211/153	0.821(0.603-1.116)	0.0	0.780(0.412-1.477)	0.0	0.642(0.403-1.022)	0.0	0.676(0.436-1.046)	0.0	0.998(0.556-1.791)	0.0
Asian*	1117/1051	0.923(0.786-1.083)	0.0	0.789(0.531-1.172)	0.0	0.966(0.784-1.191)	0.0	0.961(0.808-1.143)	0.0	0.803(0.546-1.180)	0.0
Han*	718/641	NA	NA	NA	NA	NA	NA	1.002(0.801-1.253)	0.0	NA	NA
rs650439		T vs. A		TT vs. AA		AT vs. AA		AT+TT vs. AA		TT vs. AA+AT	
Asian	2231/1632	0.952(0.862-1.051)	0.0	0.968(0.767-1.222)	0.0	0.903(0.789-1.034)	0.0	0.915(0.804-1.040)	0.0	1.019(0.815-1.274)	0.0
Uyghur*	527/504	0.973(0.799-1.186)	0.0	0.997(0.603-1.649)	0.0	0.949(0.733-1.229)	0.0	0.955(0.746-1.222)	0.0	1.019(0.623-1.666)	0.0
F352V		G vs. T		GG vs. TT		GT vs. TT		GT+GG vs. TT		GG vs. TT+GT	
Caucasian*	211/153	1.161(0.796-1.694)	0.0	5.560(0.680-45.48)	0.0	0.976(0.627-1.520)	0.0	1.067(0.688-1.652)	0.0	5.592(0.688-45.48)	0.0
rs145682430		A vs. G		AA vs. GG		AG vs. GG		AG+AA vs. GG		AA vs. GG+AG	
Asian*	811/810	0.811(0.282-2.331)	**73.5**	NA	NA	0.810(0.276-2.374)	**74.0**	0.810(0.276-2.374)	**74.0**	NA	NA

#OR: Odds ratio; CI: Confidence interval; NA: Not available. &Results with statistical significant difference were marked as bold. Unstable results in sensitivity analyses were marked as italic.
**Less than three studies were included in those subgroups, so that sensitivity analyses could not be performed.*

A sensitivity analysis was performed if any subgroup and any comparison included more than two studies, in dominant model (AG+AA *vs.* GG), heterozygote comparison (AG *vs.* GG) and allelic comparison (A *vs.* G) of rs1207568 overall, statistically different results were gained when study no 1.1.1, 1.1.2, 1.1.3 or 1.3.2 were excluded. In the Asian subgroup of rs1207568, statistically different results were gained when study no 1.2.1 was excluded in allelic comparison (A *vs.* G, Table III and Figure 2). Less than three studies were included in most groups and subgroups marked with asterisks in Table III; therefore, the sensitivity analysis could not be made. The other results were stable in the sensitivity analysis (Table III).

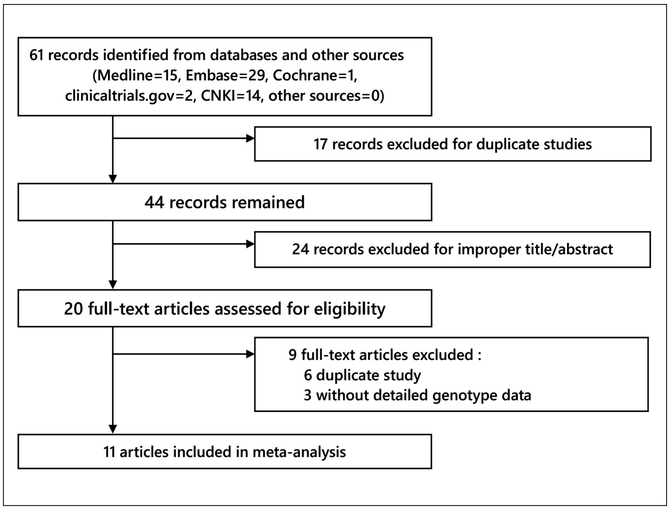

Figure 1. Literature screening process.

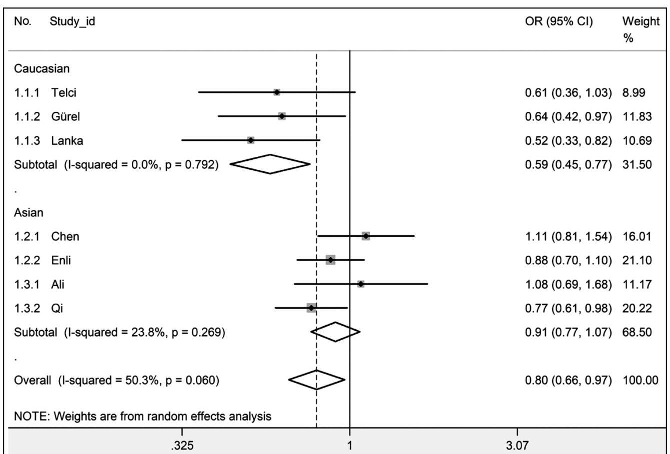

Figure 2: In allelic comparison (A *vs.* G) overall, forest plot for the association between *Klotho* rs1207568 and urolithiasis with a random-effects model. A box and a horizontal line means the estimate of the OR and its 95% CI for each study. Rhombus means pooled OR and 95% CI.

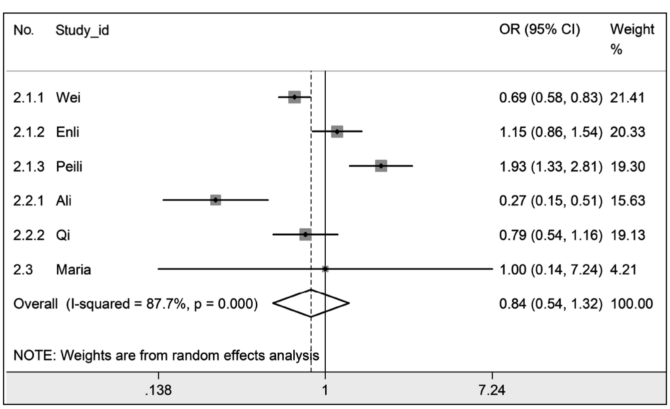

Figure 3: In allelic comparison (A *vs.* G) overall, the forest plot for the association between *Klotho* rs3752472 and urolithiasis with a random-effects model.

To evaluate the publication bias, Begg's funnel plot and Egger's test were used in any subgroup and any comparison of more than five studies. P-value of Begg's test (P_B), P-value of Egger's test (P_E), and symmetry of funnel plot were tested.[13,14] According to the P_B and P_E value, no significant publication bias was discovered in each genetic models of rs1207568 overall, in each genetic models of rs3752472's Asian subgroup, in allelic comparison (A *vs.* G) of rs3752472 overall, and in dominant model (AG+AA *vs.* GG) of rs564481 overall.

In the funnel plot, however, in the dominant model (AG + AA *vs.* GG) and heterozygote comparison (AG *vs.* GG) of rs1207568, study no 1.1.2, 1.1.3 and 1.3.1 extended beyond the diagonal line. The diagonal line indicated the pseudo-95% CI limit for the effect estimate. In allelic comparison (A *vs.* G), dominant model (AG + AA *vs.* GG), and heterozygote comparison (AG *vs.* GG) of rs3752472's Asian subgroup, studies no 2.1.1, 2.1.3 and 2.2.1 extended beyond the diagonal line. In the homozygote comparison (AA *vs.* GG) of rs3752472's Asian subgroup, study no 2.2.1 extended beyond the diagonal line. In allelic comparison (A *vs.* G) of rs3752472 overall, studies no 2.1.3 and 2.2.1 extended beyond the diagonal line.

DISCUSSION

Klotho rs1207568 adenine (A) was related to a decreased urolithiasis risk in the dominant model (AG + AA *vs.* GG), heterozygote comparison (AG *vs.* GG), and allelic comparison (A *vs.* G) in the Caucasian subgroup and overall; however, significant heterogeneity and unstable sensitivity analysis results were found for rs1207568 overall. In the Asian subgroup of rs1207568, unstable sensitivity analysis results were obtained by allelic comparisons (A *vs.* G). Publication bias and sensitivity analyses could not be made in the Han and Uyghur subgroups. The publication bias analysis results suggested differences between the subgroups. These results suggested that *Klotho* rs1207568 adenine (A) is related to a decreased urolithiasis risk in Caucasians. There were inadequate data to confirm the relation between urolithiasis susceptibility and *Klotho* rs1207568 in Asians, and the results should be interpreted with caution.

For *Klotho* rs3752472, heterogeneity was significant in each genetic model of the Han subgroup, and publication bias analyses could not be carried out in Han subgroup, but the results were stable in sensitivity analyses. Statistically significant changes were found in the recessive model (AA *vs.* GG + AG) and the homozygote comparison (AA *vs.* GG) of rs3752472 in the Uyghur subgroup; however, publication bias and sensitivity analyses could not be carried out. Only one study was included in the Caucasian subgroup of rs3752472. The publication bias analysis results suggested differences between the subgroups. These results showed that *Klotho* rs3752472 may not be related to the risk of urolithiasis in the Han subgroup of Asians. There were inadequate data to confirm the association between urolithiasis susceptibility and *Klotho* rs3752472 in Uyghur and Caucasians, and the results should be explained with caution.

No statistically significant change in urolithiasis risk was detected in any genetic model or subgroup for rs564481, rs650439, F352V, and rs145682430. In each study included in each SNP, no statistically significant changes were found. However, publication bias analysis could not be performed. Heterogeneity was not found in any genetic model or subgroup for rs564481, rs650439, or F352V. The results for rs564481 overall and the Asian subgroup of rs650439 showed stability in the sensitivity analyses. These results showed that *Klotho* rs564481 might not be related to urolithiasis risk in Asians or Caucasians, and that *Klotho* rs650439 might not be related to urolithiasis risk in Asians. There were inadequate data to confirm the relation between urolithiasis susceptibility and *Klotho* F352V in Caucasians or *Klotho* rs145682430 in Asians, and the results should be interpreted with caution.

Simultaneously, limitations of this meta-analysis should be addressed. To date, there had been few practical studies and their subgroups that could be absorbed by meta-analysis. In some groups or subgroups, sensitivity or publication bias analyses could not be operated. Studies no 1.1.1, 1.1.2, 1.1.3, 1.3.1 and 5.2 departed from HWE significantly. Unpublished studies or studies written by other languages were excluded. With imperfection, this meta-analysis and systematic review provided insights into the underlying relation between urolithiasis and *Klotho* gene polymorphisms.

CONCLUSION

Klotho rs1207568 adenine (A) may be related to a decreased urolithiasis risk in Caucasians. *Klotho* rs3752472 may not be related to urolithiasis risk in Han Asian subgroup. *Klotho* rs564481 may not be related to urolithiasis risk in Asians or Caucasians, and *Klotho* rs650439 may not be related to urolithiasis risk in Asians.

There were inadequate data to confirm the relation between urolithiasis susceptibility and *Klotho* rs1207568 in Asians, the relation between urolithiasis susceptibility and *Klotho* rs3752472 in Uyghur or Caucasians, and the relation between urolithiasis susceptibility and *Klotho* F352V in Caucasians or *Klotho* rs145682430 in Asians, and the results should be interpreted with caution. Elaborately designed studies with added subgroups and larger sample sizes will be needed to check the risk identified in systematic reviews and meta-analyses.

COMPETING INTEREST:
The authors declared that they have no competing interests.

AUTHORS' CONTRIBUTION:
JQ, BD: Designed the study and drafted the manuscript, accumulated the data, analysis and interpretation of the data, substantively revised the manuscript.
JX: Designed the study and drafted the manuscript, substantively revised the manuscript.
BC: Accumulated the data, conducted the analysis and interpretation of the data, substantively revised the manuscript.
TW: Accumulated the data, conducted the analysis and interpretation of the data.
All authors read and approved the final manuscript for publication.

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