Osteopontin promoter polymorphisms and risk of urolithiasis: a candidate gene association and meta-analysis study

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Research article

Keywords: Candidate Gene Association Study, Meta-analysis, Pakistan, Renal Calculi, SPP1, SNP

Posted Date: February 24th, 2020

DOI: https://doi.org/10.21203/rs.2.24295/v1

Version of Record: A version of this preprint was published on August 25th, 2020. See the published version at https://doi.org/10.1186/s12881-020-01101-2.

Abstract

Background Urolithiasis is a worldwide urological problem with significant contribution of genetic factors. Pakistan, which resides within the Afro-Asian stone belt, has a high reported prevalence (12%) of urolithiasis. Osteopontin (*SPP1*) is a urinary macromolecule with a suggested critical role in modulating renal stone formation, genetic polymorphisms of which may determine individual risk of developing urolithiasis. However, results of previous studies regarding SPP1 polymorphisms and susceptibility to urolithiasis have apparent inconsistencies with no data available for local population.

Methods 483 Pakistani subjects, including 235 urolithiasis patients and 248 healthy controls, were genotyped for 6 *SPP1* genetic polymorphisms to investigate association with urolithiasis in an indigenous candidate gene association study. Further, a comprehensive meta-analysis following a systematic literature search was also performed to provide evidence based account of any existent association between *SPP1* promoter polymorphisms and urolithiasis risk.

Results Three SPP1 promoter polymorphisms, rs2853744:G>T, rs11730582:T>C and rs11439060:delG>G, were found to be significantly associated with risk of urolithiasis in indigenous genetic association study (OR = 3.14; p = 0.006, OR = 1.78; p = 0.006 and OR = 1.60; p = 0.012, respectively). We also observed a 1.68-fold positive association of a tri-allelic haplotype of these SPP1 promoter polymorphisms (G-C-dG) with risk of urolithiasis (OR = 1.68; p = 0.0079). However, no association was evident when data were stratified according to gender, age at first presentation, stone recurrence, family history of urolithiasis, parental consanguinity and stone multiplicity. The overall results from meta-analysis, which included 4 studies, suggested a significant association of *SPP1* rs2853744:G>T polymorphism with susceptibility of urolithiasis (OR = 1.37; p = 0.004), but not for other SPP1 polymorphic variants analyzed.

Conclusions In conclusion, we report significant association of 3 *SPP1* polymorphisms with urolithiasis for the first time from South Asia, however, this association persisted only for *SPP1* rs2853744:G>T polymorphism after meta-analysis of pooled studies. Further studies with a larger sample size will be required to validate this association and assess any potential usefulness in diagnosis and prognosis of renal stone disease.

Background

Urolithiasis is a common urological problem (worldwide prevalence of 4–20%) (1) causing high patient morbidity and associated healthcare burden involving recurrence, frequent hospitalization and sometimes progression to chronic kidney disease (CKD) and renal failure (2, 3). Pakistan resides in the middle of Afro-Asian renal stone belt, characterized by relatively higher prevalence (12–15%) of urolithiasis, complicated by environmental determinants of urolithiasis risk such as chronic dehydration and nutrition (4).

The reported etiology of urolithiasis is multifactorial involving environmental and genetic risk factors with heritability of 50% (5). Only a few Genome Wide Association Studies (GWAS) are available regarding urolithiasis (predominantly from European and Japanese cohorts) that identified common genetic variants in various genetic loci regulating calcium and phosphate metabolism, urinary transporters and macromolecules among others, as urolithiasis associated risk factors (6, 7). The “common disease-common variant” paradigm stresses the small but significant risk contributed by common genetic variations in the development of multifactorial disorders like urolithiasis (8). Despite the current trend and availability of genome wide and global association options, candidate gene association studies, if performed appropriately, still provide a practical approach towards evaluation of genetic risk factors in complex diseases, especially in resource limited settings (9).

Evidence that macromolecular proteins, especially osteopontin, may play an important role in the modulation and development of urolithiasis, is growing (10). Osteopontin, also called as secreted phosphoprotein 1, is a macromolecular glycoprotein with pleotropic expression and function (11, 12). In kidneys, osteopontin is produced by renal epithelial cells and secreted into the urine as a normal macromolecular constituent of it (13). The hypothesis that osteopontin may play a critical role in modulating renal stone formation is supported by many observations such as; (1) SPP1 as organic component in the matrix of renal stones (14); (2) SPP1 as important regulator of renal calcification (15); (3) Changes in SPP1 expression and urinary SPP1 levels in hyperoxaluric rats and human subjects with urolithiasis, respectively (16); (4) In vitro cell culture based studies and in vivo SPP1 knockout animal models suggesting an important role of osteopontin in various phases of renal stone formation, including crystal nucleation, aggregation, retention, adhesion to renal epithelial cells and stone formation (17, 18); and (5) Candidate gene association studies demonstrating association of SPP1 polymorphisms and urolithiasis in different ethnic groups (19, 20).

Osteopontin gene on chromosome 4q21-25 exhibits many functional polymorphisms in the promoter/coding regions that may influence osteopontin expression/activity (21) and have been analyzed for potential association with urolithiasis in different ethnic groups (19, 20, 22, 23), but with varied results. Therefore, in this context, the present study was designed to investigate any potential association between common genetic variants in osteopontin gene and the susceptibility of urolithiasis in the indigenous sample set. Further, we also applied a systematic approach by collecting and analyzing the previously available data on the osteopontin polymorphisms in association with urolithiasis susceptibility in the form of a meta-analysis that evaluated the varying results of previous studies and provided a more comprehensive and accurate estimate of any existent association.

Material And Methods

Case control study cohort

Study participants

We recruited 235 urolithiasis patients, based on ultrasound finding of at least one renal stone [supplemented by other renal stone diagnostic procedures including X-ray imaging or non-contrast-enhanced computed tomography (NCCT) and urine analysis in most cases], presenting at 5 different tertiary care hospitals of Punjab, during a period of 29 months. All urolithiasis patients provided clinical and pedigree details, with confirmation provided by their urologist and/or relevant medical records, and EDTA blood samples for genetic analysis. In addition, 243 age and gender matched healthy subjects with same ethnic origin having no personal or familial antecedents of urolithiasis were also enrolled as control group. Details of patient recruitment process and study cohort have been described earlier (24).

Genotyping of SPP1 polymorphisms

Subjects were genotyped for six polymorphisms of SPP1 gene including five promoter polymorphisms (rs28357094:T > G, rs17524488, rs11730582:T > C, rs2853744:G > T and T-593A) by Sanger sequencing and one coding polymorphism (rs1126616) by PCR-RFLP based approach (assay details provided in Additional file 1). The genotypes of all SPP1 polymorphisms were scored by two independent researchers.

Statistical analysis

The analysis of coded study data, including allele and genotype frequencies expressed as numbers (percentage), was accomplished using the statistical package for social sciences (SPSS) version 20 for windows and online web tool SNPstats (25). Using a Chi-square test, Hardy-Weinberg equilibrium (HWE) was performed which served as a statistical control for systematic genotyping error and population stratification where SPP1 polymorphisms that violated HWE (p-value of < 0.05 for control group) were excluded from further data analysis. Odds ratios (ORs) with associated 95% confidence intervals (CI) were determined to assess strength of statistical association, if any, considering allelic, genotypic, recessive, dominant and log-additive models by the same SNPstats program. The pairwise linkage disequilibrium and haplotype analysis for SPP1 polymorphisms was conducted using the Haploview program (26). Bonferroni correction for multiple testing was performed in calculating the ORs and associated p-values for genotype and haplotype associations between SPP1 polymorphisms and urolithiasis. Also, the post-hoc power of the study estimates for SPP1 polymorphisms were performed using the Power and Sample Size Program (PS) version 3.0 available at http://biostat.mc.vanderbilt.edu/PowerSampleSize (27). A $p < 0.05$ in two-sided analysis was considered significant unless otherwise stated.

Meta-analysis

The meta-analysis of SPP1 polymorphisms and risk of urolithiasis was performed using a modified protocol described in a previous study (28). A brief description of meta-analysis protocol employed is as follows.

Systematic literature search

We did not pre-registered the review protocol of the present study. The meta-analysis investigation included published studies with a case control study design that explored genetic association of SPP1 polymorphisms and urolithiasis. Initial systematic literature search identified such published studies available before September, 2018 from the online databases of the Google Scholar, PubMed, ScienceDirect, Cochrane library and Embase. The literature search used relevant keywords related to the urolithiasis (urolithiasis, renal stones, kidney stones, nephrolithiasis) and osteopontin gene polymorphisms (osteopontin, SPP1, OPN). The search was restricted to studies with human subjects only and limited to publications in English language. Additional relevant articles were included by screening the references cited in the articles retrieved during the initial search. Three researchers, working independently, completed the literature search step and discrepancies were resolved through discussion.

Eligibility criteria for study selection and data extraction

The eligibility criteria used for inclusion of studies in the meta-analysis was; 1) original investigations with a case-control study design analyzing association of SPP1 polymorphisms and urolithiasis; 2) the SPP1 polymorphic sites should include at least one of the mentioned sites (rs2853744, rs11730582 and rs11439060); 3) Data presentation is appropriate enabling the calculations of Odds Ratios, confidence intervals and p-values. In accordance with this eligibility criteria, the relevancy and sufficiency of data was determined by obtaining and screening the full texts of the selected articles. To ensure the robustness of analysis performed, three researchers independently performed the screening process resolving any conflicting issues through discussion. The information extracted from each selected study included; reference, publication year, region, ethnicity, total number of study subjects including number of cases and controls, source of control samples, genotyping method, Hardy-Weinberg equilibrium status and genotype frequencies of the three SPP1 polymorphisms in cases and controls. All the data for studies included in the meta-analysis were obtained from the published articles only and no additional information was collected by approaching any corresponding author. The studies excluded from meta-analysis were; (1) studies with insufficient data presentation; (2) studies not pertaining to urolithiasis patients or SPP1 polymorphisms; (3) studies not following a case-control study design; (4) review articles; (5) meta-analysis studies;

(6) meeting abstracts with insufficient data; and (7) unpublished reports. The Newcastle-Ottawa scale (NOS) was the reference used to evaluate the quality of eligible studies where a quality score of 6 or better were considered for inclusion of studies in the meta-analysis.

Statistical analysis for meta-analysis part of study

Calculations of effect sizes and, other relevant meta-analysis measures and construction of graphical forest plots and Begg's funnel plots was done using Review Manager (RevMan) version 5 (29). Categorical/dichotomous data was analyzed using Mantel–Haenszel statistics. OR with associated CI was used as a measure to determine the strength of association between the SPP1 polymorphisms and urolithiasis risk. The chi-square ($\chi 2$) and the index of heterogeneity (I^2) tests were used for determination of overall heterogeneity among the studies included in meta-analysis. All the statistical tests performed were two tailed, with a statistical significance threshold of 0.05 except for the heterogeneity test where a p-value of < 0.10 reflected statistical significance as Chi-square test has limited statistical power for studies with a small sample size. The appropriate model for calculation of effect size was selected depending upon the value of overall study heterogeneity where fixed-effect model was used by default, however, for studies with I^2 values of > 50% (suggesting significant heterogeneity) effect size was determined using a random effect model. The estimation of between-study variance was done using tau-squared (τ^2) test based on a random-effect model. To assess the effect of an individual study on pooled results, sensitivity analysis was also performed by removing each study at a time. The assessment of any potential publication bias was performed using the Begg's rank correlation test (30) and Egger's linear regression test (31) using R version 3.5.2 (32). Stratified data analysis could not be performed due to limited number of studies available for meta-analysis part of the study.

Results

Case control study cohort

The basic characteristics of study cohort and primary information of SPP1 polymorphisms analyzed in this study are presented in Additional files 2 and 3, respectively. SPP1 -593 T/A polymorphism was found to be monomorphic in this study. The allelic and genotypic distribution for SPP1 rs28357094:T > G and rs1126616:C > T SNPs deviated from the HWE in control group, and therefore, were excluded from further data analysis. Sanger sequencing electropherograms for representative genotypes of each SPP1 polymorphism included in final analysis are presented in Additional file 4.

Data analysis for allelic and genotypic distribution suggested no significant association between the risk of urolithiasis and any of the SPP1 polymorphisms analyzed except for rs11439060:delG > G (OR = 0.40; p = 0.002 for G/dG genotype in co-dominant model) (Table 1). Additionally, SPP1 rs2853744:G > T polymorphism showed significant associated with increased risk of urolithiasis in a dominant model (OR = 3.14; p = 0.006). While, SPP1 rs11730582:T > C and rs11439060:delG > G polymorphisms were significantly associated with the risk of urolithiasis (OR = 1.78; p = 0.006 and OR = 1.60; p = 0.012, respectively) considering a recessive genetic model (Table 2).

Table 1

Allele and genotype distribution for SPP1 polymorphisms and their association with urolithiasis risk

SPP1 polymorphisms	Genotype/Allele	Patients n = 235, n (%)	Controls n = 243, n (%)	OR (95% CI)	p-value (corrected)†
rs2853744:G > T	T/T	07 (3.1%)	21 (9.1%)	Referent	0.024
	T/G	61 (27.2%)	62 (26.8%)	2.95 (1.17–7.45)	
	G/G	156 (69.6%)	148 (64.1%)	3.16 (1.31–7.66)	
	T	75 (17%)	104 (23%)	Referent	0.035
	G	373 (83%)	358 (77%)	1.44 (1.03–2.01)	
rs11730582:T > C	T/T	63 (28%)	69 (29.6%)	Referent	0.017
	T/C	87 (38.7%)	113 (48.5%)	0.84 (0.54–1.31)	
	C/C	75 (33.3%)	51 (21.9%)	1.61 (0.98–2.64)	
	T	213 (47%)	251 (54%)	Referent	0.056
	C	237 (53%)	215 (46%)	1.29 (1.00-1.68)	
rs11439060:delG > G	G/G	19 (8.3%)	12 (5%)	Referent	0.002
	G/dG	65 (28.5%)	103 (43.3%)	0.40 (0.18–0.88)	
	dG/dG	144 (63.2%)	123 (51.7%)	0.74 (0.35–1.58)	
	G	103 (23%)	127 (27%)	Referent	0.170
	dG	353 (77%)	349 (73%)	1.24 (0.92–1.68)	

†p-values shown reflect adjustment for age and gender. Bonferroni correction for multiple testing was applied (p-value threshold 0.016). Statistically significant p-values (< 0.016) and associated OR values are highlighted in bold.

OR, odds ratio; CI, confidence interval; n (%), frequency.

Table 2
Association of the studied SPP1 polymorphisms with urolithiasis under different genetic models

SPP1 polymorphisms	Model	Genotypes	Patients n = 235, n (%)	Controls n = 243, n (%)	OR (95% CI)	p-value (corrected)†
rs2853744:G > T	Dominant	T/T	07 (3.1%)	21 (9.1%)	1.00	0.006
		G/T-G/G	217 (96.9%)	210 (90.9%)	3.14 (1.29–7.45)	
	Recessive	T/T-G/T	68 (30.4%)	83 (35.1%)	1.00	0.210
		G/G	156 (69.4%)	148 (64.9%)	1.29 (0.87–1.90)	
	Log-additive	-	-	-	1.38 (1.01–1.89)S	0.040
rs11730582:T > C	Dominant	T/T	63 (28%)	69 (29.6%)	1.00	0.700
		T/C-C/C	162 (72%)	164 (70.4%)	1.08 (0.72–1.62)	
	Recessive	T/T-T/C	150 (66.7%)	182 (78.1%)	1.00	0.006
		C/C	75 (33.3%)	51 (21.9%)	1.78 (1.18–2.71)	
	Log-additive	-	-	-	1.26 (0.99–1.61)	0.062
rs11439060:delG > G	Dominant	G/G	19 (8.3%)	12 (5%)	1.00	0.150
		dG/G-dG/dG	209 (91.7%)	226 (95%)	0.58 (0.28–1.23)	
	Recessive	G/G-dG/G	84 (36.8%)	115 (48.3%)	1.00	0.012
		dG/dG	144 (63.2%)	123 (51.7%)	1.60 (1.11–2.60)	
	Log-additive	-	-	-	1.24 (0.92–1.67)	0.15

†p-values shown reflect adjustment for age and gender. Bonferroni correction for multiple testing was applied (p-value threshold 0.016). Statistically significant p-values (< 0.016) and associated OR values are highlighted in bold.

OR, odds ratio; CI, confidence interval; n (%), frequency.

Frequency of G-C-dG haplotype (SPP1 rs2853744-rs11730582-rs11439060 polymorphisms, respectively) was significantly higher in urolithiasis patients as compared to controls (OR = 1.68; p = 0.0079), suggesting an association with increased risk of urolithiasis in haplotype analysis (Table 3). However, pair wise linkage disequilibrium (LD) and haplotype plot structure analysis demonstrated no significant D' measures between each pair of SPP1 loci analyzed, suggesting that LD in this region is low Additional file 5.

Table 3
Distribution of SPP1 haplotype frequencies and their association with urolithiasis

SPP1 haplotypes (rs2853744:G > T- rs11730582:T > C- rs11439060:delG > G)	Haplotype frequency†	Case, control ratios	OR (95% CI)	p-value (corrected)‡
G-T-dG	0.323	0.313, 0.332	Referent	-
G-C-dG	0.312	0.374, 0.248	1.68 (1.15–2.46)	0.0079
G-C-G	0.090	0.086, 0.107	0.95 (0.57–1.58)	0.840
G-T-G	0.078	0.079, 0.068	1.25 (0.61–2.56)	0.550
T-T-dG	0.060	0.083, 0.053	1.43 (0.72–2.87)	0.310
T-C-dG	0.059	0.025, 0.081	0.53 (0.24–1.17)	0.120
OR – odds ratio; 95% CI – 95% confidence interval.				
†Haplotypes with a frequency > 5% were analyzed.				
‡p-values shown reflect adjustment for age and gender. Bonferroni correction for multiple testing was applied (p-value threshold 0.0083). Statistically significant p-values (< 0.0083) and associated OR values are highlighted in bold.				

The SPP1 polymorphisms data was also analyzed after dividing the urolithiasis patients into sub-groups based on gender, age at first presentation, stone recurrence, family history of urolithiasis, parental consanguinity and stone multiplicity. However, no significant associations were observed in any of the comparisons made (Additional file 6).

Meta-analysis

Qualitative synthesis for meta-analysis of SPP1 polymorphisms and urolithiasis

A flow diagram, reflecting the sequence of study selection for association of 3 SPP1 polymorphisms and susceptibility of urolithiasis, is described in Fig. 1. An initial online database search performed using different MeSH terms pertaining to urolithiasis and osteopontin gene, resulted in retrieval of a total of 217 articles. However, in the end a total of 4 studies were included in the present meta-analysis, comprising of 3 previously published reports obtained after rigorous screening according to the eligibility criteria, combined with the indigenous genetic epidemiology study. There were 2 studies exploring the association of SPP1 polymorphism rs2853744, 4 for rs11730582 and 3 for rs11439060.

The salient characteristics of the studies comprising the present meta-analysis are described in Table 4. The publication period for the selected studies ranged from 2010 to 2018. All four studies included in the meta-analysis were case-control studies, based on Asian population, and most (3/4) studies used control groups collected from general population. Also, all studies had their control group in HWE. Among these, three studies also analyzed the association of other polymorphisms in SPP1 gene with urolithiasis. However, data pertaining to additional polymorphisms were not included in the present meta-analysis. All studies showed positive association of at least one SPP1 polymorphism with the risk of urolithiasis.

Table 4
Main characteristics and findings of the eligible studies included in this meta-analysis.

Reference (first author, year)	Region	Ethnic group	Controls source	Samples	Cases	Controls	Polymorphic sites	HWE status†	Genotyping method	Findings
Liu, 2010	Taiwan	Asian	Hospital based	496	249	247	rs11730582, rs11439060	Yes	TaqMan genotyping assay	rs11439060 of SPP1 promoter associated with risk of UL in allelic and genotypic models
Safarinejad, 2013	Iran	Asian	Population based	1026	342	684	rs2853744, rs11730582	Yes	PCR-FRET	SPP1 SNP rs2853744 showed significant association with UL
Xiao, 2016	China	Asian	Population based	480	230	250	rs11730582, rs11439060	Yes	TaqMan genotyping assay	rs11439060 in SPP1 promoter significantly associated with risk of UL as well as clinical characteristics in UL
Present study, 2018	Pakistan	Asian	Population based	478	235	243	rs2853744, rs11730582 and rs11439060	Yes	Sanger sequencing	All 3 SPP1 promoter SNPs associated with UL under different genetic models

†Yes indicates consistence with HWE.

FRET, fluorescence resonance energy transfer; HWE, Hardy-Weinberg equilibrium; SPP1, osteopontin; SNP, single-nucleotide polymorphism; UL, urolithiasis.

Quantitative synthesis for meta-analysis of SPP1 polymorphisms and urolithiasis

For the association of SPP1 rs2853744 polymorphism, only 2 studies were available including 577 cases and 927 controls where overall results from recessive model showed significant association with the risk of urolithiasis (OR = 1.37; p = 0.004, Fig. 2b). However, no association was detected under dominant model after considering correction for multiple testing (Fig. 2a). Heterogeneity in this group was not significant (I^2 = 0%, p = 0.61 for recessive model) therefore, fixed effect model was employed to determine the pooled results (Fig. 2c and 2d).

Meta-analysis of SPP1 rs11730582 and rs11439060 polymorphisms included 4 (1056 cases and 1424 controls) and 3 (714 cases and 740 controls) studies, respectively. The summarization of all studies indicated no significant associations between rs11730582, and rs11439060 polymorphisms and urolithiasis using either a dominant or recessive model (Additional files 7 and 8, parts a and b) after correction for multiple testing. Heterogeneity analysis for SPP1 rs11730582 polymorphism was insignificant, but not for SPP1 rs11439060 polymorphism, therefor fixed and random effect models, respectively, were applied in calculation of pooled results (Additional files 7 and 8, parts c and d).

Assessment of the publication bias was made on the basis of shape of funnel plots and Egger's test as depicted in Fig. 2, and Additional files 7 and 8. No publication bias was evident except for the analysis of SPP1 rs11439060 polymorphism and urolithiasis (Additional file 8, parts c and d); however, the Egger's test was not significant (p = 0.19 for dominant model and p = 0.15 for recessive model). Sensitivity analyses, performed by removing each study at a time, suggested no significant influence of an individual study on the overall pooled OR, indicating the reliability of the results.

Discussion

The role of genetic variations with low penetrance has earned special concern in urolithiasis research, which, in association with other risk factors, may determine the critical threshold necessary for the formation of renal stones. A number of genetic markers in different urolithiasis genes

including SPP1, VDR, CaSR, urokinase, prothrombin, interleukins and others have been investigated in this regard (6). Osteopontin has earned a particular prominence among these genetic risk factors as an importance determinant and regulator of renal calcification and stone formation (11, 13, 33). However, the results of genetic association studies in urolithiasis are still to achieve diagnostic and translational significance. The gap in existing knowledge and inconsistent results for potential genetic associations in urolithiasis indicate a need of genetic epidemiology studies performed in diverse populations.

For the first time, we present a genetic association study investigating the role of SPP1 polymorphisms in a cohort of Pakistani urolithiasis patients where we demonstrate significant association of 3 SPP1 promoter polymorphisms (rs2853744:G > T, rs11730582:T > C and rs11439060:delG > G) and their tri-allelic haplotype with urolithiasis. Moreover, estimates of post-hoc study power showed that the levels of power associated with SPP1 rs2853744:G > T, rs11730582:T > C and rs11439060:delG > G polymorphisms were 78.4%, 79.8% and 99.7%. These results reflect that the sample size of 235 provided fairly adequate power (almost 80%) in determining genetic associations between these polymorphic variants of SPP1 gene and urolithiasis in the indigenous population.

Considerable heterogeneity in correlation of these SPP1 polymorphisms and risk of urolithiasis have been reported by studies conducted in different ethnic groups. An Iranian study (19) reported a positive association of G allele/GG genotype of rs2853744:G > T with risk of developing urolithiasis. In contrast to our study results, no significant association of rs11730582:T > C with urolithiasis risk was reported in 3 independent studies from Taiwan, Iran and China (19, 20, 34). For rs11439060:delG > G, significant association with urolithiasis phenotype was described in two studies, however, in consistence with our study, first study of Taiwanese origin reported dG/dG genotype as the risk genotype associated with increased susceptibilty of urolithiasis (34), while the second study found insertion allele or genotype (G allele or G/G genotype) to be more prevalent in Chinese urolithiasis patients as compared to controls (20).

The observed overall heterogeneity in the results of different studies regarding association of SPP1 polymorphisms with urolithiasis may be attributable to many factors that include differences in the prevalence of urolithiasis among different ethnic groups (for example 12–15% in Pakistan vs 5.7% and 9.6% in Iran and Taiwan, respectively) (35–37), differences in genetic architecture of studied populations (variations in allele/genotype frequencies and linkage disequilibrium of SPP1 polymorphisms), differences in the context and contribution of environmental risk factors (including chronic dehydration, diet and lifestyle) (4) and differences in study methodologies (selection bias, control source, genotyping method used, statistical analysis approach including conformance to HWE and correction for multiple testing).

Meta-analysis is a powerful tool that provides evidence based comprehensive and reliable results compared to a single study when investigating association of potential risk factors and disease phenotype. Therefore, in addition to presenting results of indigenous study, we also conducted a meta-analysis to clarify the possible association between SPP1 polymorphisms and risk of urolithiasis. To the best of our knowledge, no meta-analysis has been carried out previously regarding association of SPP1 promoter polymorphisms with urolithiasis risk. The results of present meta-analysis reveal that GG genotype of SPP1 rs2853744:G > T significantly increased the risk of urolithiasis by 1.37 fold in a recessive model. However, no significant association between other SPP1 polymorphisms analyzed (rs11730582:T > C and rs11439060:delG > G) was observed after correction for multiple testing. All the studies included in the meta-analysis were in HWE, no publication bias or heterogeneity was observed except for rs11439060:delG > G, sensitivity analysis did not alter the overall pooled results, correction for multiple testing was applied, all of which, indicate the robustness of results generated. Inclusion of Pakistani samples in the overall analysis also strengthens the results of this study. However, the results should still be interpreted with caution because of the limited number of primary studies available for present meta-analysis.

Currently, there is only one meta-analysis available on the subject which revealed positive association of SPP1 coding region rs1126616:C > T polymorphism and lower serum and urine osteopontin levels with increased risk of developing urolithiasis (38), however, they did not include any other SPP1 polymorphism (including SPP1 promoter polymorphisms investigated in this study) in the analysis, which limits the usefulness and broader applicability of that study.

Despite the efforts made to generate evidence based and robust statistical results through current case control and meta-analysis study, a number of limitations should be acknowledged. First, a comprehensive investigation and correlation of biochemical parameters of renal stone disease (including stone analysis and serum/urine osteopontin levels) could not be done due to limited resources available. Second, all the eligible studies, including our own data, could not address all the known risk factors involved. Keeping in view the multifactorial nature of the urolithiasis phenotype, a more comprehensive and precise analysis should be based on adjusted estimates considering covariates such as age, gender, dietary habits, lifestyle and other genetic factors, thus also investigating gene-gene and gene-environment interactions. Third, sub-group analysis based on ethnicity, source of control samples and other factors, could not be performed due to limited number of published studies available for current meta-analysis. Further, we did not include other SPP1 polymorphisms because we could find only a couple of studies with limited sample size.

Conclusion

In conclusion, the current study provides first account of a modest but statistically significant association of three SPP1 promoter polymorphisms and their tri-allelic haplotype with increased risk of urolithiasis from South-Asian region under the indicated genetic model. In addition, evidence

from meta-analysis part of the study supports the positive association of rs2853744:G > T SPP1 SNP and susceptibility of urolithiasis. Further studies with larger sample sizes and analysis of gene-gene and gene-environment factors in diverse populations are suggested to validate and determine usefulness and broader relevance of SPP1 and other genetic polymorphisms in accessing risk and prognosis of urolithiasis.

Abbreviations

CaSR Calcium Sensing Receptor

CI Confidence Interval

CKD Chronic Kidney Disease

EDTA Ethylenediaminetetraacetic Acid

GWAS Genome Wide Association Studies

HWE Hardy-Weinberg equilibrium

I^2 Index of Heterogeneity test

LD Linkage Disequilibrium

MeSH Medical Subject Headings

NCCT Non-Contrast-enhanced Computed Tomography

NOS Newcastle-Ottawa scale

OPN Osteopontin

OR Odds Ratio

PCR-RFLP Polymerase Chain Reaction-Restriction Fragment Length Polymorphism

PS Power and Sample Size Program

SPP1 Secreted Phosphoprotein 1

VDR Vitamin D Receptor

τ^2 Tau-squared test

$\chi 2$ Chi-square test

Declarations

Ethics approval and consent to participate

The Ethical Review Committee for Medical and Biomedical Research, University of Health Sciences Lahore, which adheres to the latest guidelines provided in Declaration of Helsinki, approved the study protocol. All the study participants (urolithiasis patients and healthy controls) provided written informed consent before enrollment in the study. In case of minor subjects (<16 years old), written informed consent was provided by their parents or legal guardians.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analyzed during this study are included in this published article and its supplementary information files.

Competing interests

The authors declare that they have no competing interests.

Funding

This work was supported by Higher Education Commission of Pakistan (grant number NRPU-1987 to Shagufta Khaliq). The funding body had no role in the design of the study and collection, analysis, and interpretation of data.

Authors' contributions

AAm performed data curation, genetic and statistical analysis, wrote the original manuscript and contributed in review/editing of final manuscript. **AAf** contributed towards data curation, genetic analysis and manuscript review/editing. **AH** and **MA** contributed in patient recruitment, clinical data acquisition and analysis and manuscript review/editing. **HN** contributed towards genetic analysis. **ARK** and **AAb** performed the meta-analysis and were significant contributors towards manuscript review/editing. **SK** designed and supervised the study, acquired funding and contributed significantly towards manuscript review/editing. All authors read and approved the final manuscript.

Acknowledgements

We thank patients who participated in the study and hospital staff who facilitated the research team in data and sample collection. We also acknowledge the facilities and support provided by university staff.

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Additional Files

Additional file 1 (.docx): Oligonucleotide sequences, PCR conditions and restriction enzyme used for the genotyping of *SPP1* gene polymorphisms.

Additional file 2 (.docx): Basic characteristics of the study participants in the case-control part of the study.

Additional file 3 (.docx): Basic information and HWE analysis for *SPP1* gene polymorphisms analyzed in this study.

Additional file 4 (.docx): Representative electropherograms for each genotype of three *SPP1* polymorphisms. (A) rs11730582:T>C, **(B)** rs2853744:G>T and **(C)** rs11439060:delG>G**.**

Additional file 5 (.docx): Pairwise linkage disequilibrium (LD) map, based on D´ values, of *SPP1* polymorphic markers analyzed in the present study. No significant LD was apparent in any of the *SPP1* polymorphic pairs analyzed.

Additional file 6A (.docx): Association of *SPP1* rs2853744:G>T with different clinical characteristics of urolithiasis.

Additional file 6B (.docx): Association of *SPP1* rs11730582:T>C with different clinical characteristics of urolithiasis.

Additional file 6C (.docx): Association of *SPP1* rs11439060:delG>G with different clinical characteristics of urolithiasis.

Additional file 7 (.docx): Meta-analysis of *SPP1* rs11730582:T>C polymorphism with risk of urolithiasis. a) and **b)** Forest plots of urolithiasis association with rs11730582 polymorphism using dominant and recessive model, respectively. **c)** and **d)** Funnel plots of rs11730582 polymorphism assuming dominant and recessive inheritance, respectively, using fixed effect model.

Additional file 8 (.docx): Meta-analysis of *SPP1* rs11439060:delG>G polymorphism with susceptibility of urolithiasis. a) and **b)** Forest plots of urolithiasis association with rs11439060 polymorphism following dominant and recessive model, respectively. **c)** and **d)** Funnel plots of rs11439060 polymorphism using dominant and recessive inheritance, respectively, by random effect model.

Figures

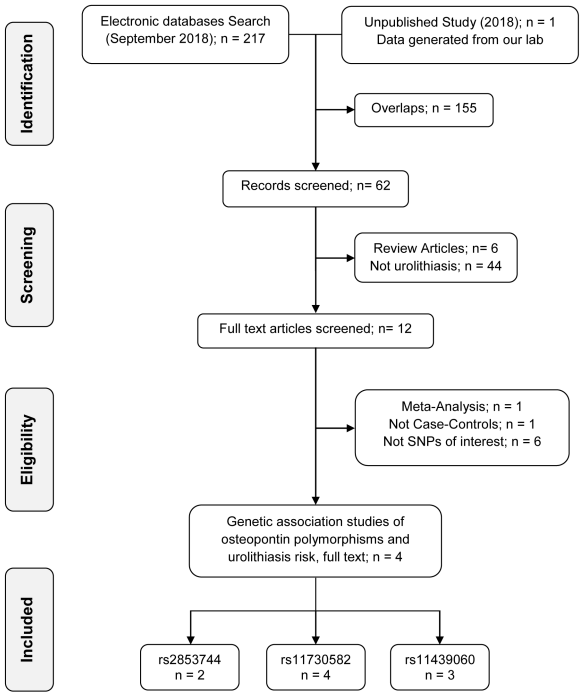

Figure 1

Flow diagram reflecting selection process of eligible studies included in meta-analysis.

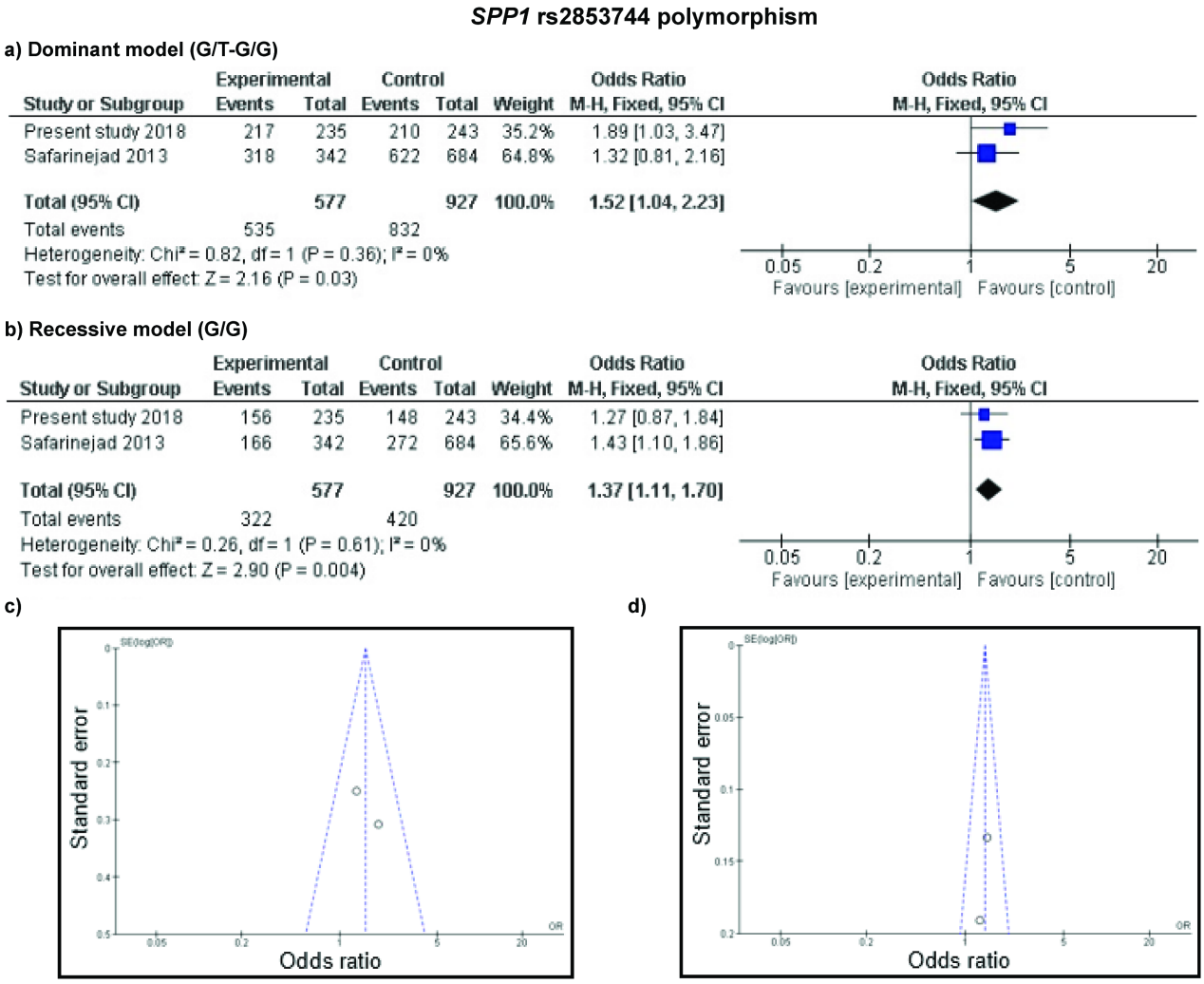

Figure 2

Meta-analysis of SPP1 rs2853744:G>T polymorphism with risk of urolithiasis. a) and b) Forest plots of urolithiasis association with rs2853744 polymorphism assuming dominant and recessive model, respectively. c) and d) Funnel plots of rs2853744 polymorphism in dominant and recessive inheritance, respectively, using fixed effect model.

Supplementary Files

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