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Original Article

ORIGINAL ARTICLE

Predictive value of single-nucleotide polymorphism signature for nephrolithiasis recurrence: a 5-year prospective study

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ABSTRACT

Background. Genetic variations are linked to kidney stone formation. However, the association of single nucleotide polymorphism (SNPs) and stone recurrence has not been well studied. This study aims to identify genetic variants associated with kidney stone recurrences and to construct a predictive nomogram model using SNPs and clinical features to predict the recurrence risk of kidney stones.

Methods. We genotyped 49 SNPs in 1001 patients who received surgical stone removal between Jan 1 and Dec 31 of 2012. All patients were confirmed stone-free by CT scan and then received follow-up at least 5 years. SNP associations with stone recurrence were analyzed by Cox proportion hazard model. A predictive nomogram model using SNPs and clinical features to predict the recurrence risk of kidney stones was developed by use of LASSO Cox regression.

Results. The recurrence rate at 3, 5, 7 years were 46.8%, 71.2%, and 78.4%, respectively. 5 SNPs were identified that had association with kidney stone recurrence risk. We used computer-generated random numbers to assign 500 of these patients to the training cohort and 501 patients to the validation cohort. A nomogram that combined the 14-SNPs-based classifier with the clinical risk factors was constructed. The areas under the curve (AUCs) at 3, 5 and 7 years of this nomogram was 0.645, 0.723, and 0.75 in training cohort, and was 0.631, 0.708, and 0.727 in validation cohort, respectively. Results show that the nomogram presented a higher predictive accuracy than those of the SNP classifier or clinical factors alone.

Conclusion. SNPs are significantly associated with kidney stone recurrence and should add prognostic value to the traditional clinical risk factors used to assess the kidney stone recurrence. A nomogram using clinical and genetic variables to predict kidney stone recurrence has revealed its potential in the future as an assessment tool during the follow-up of kidney stone patients.

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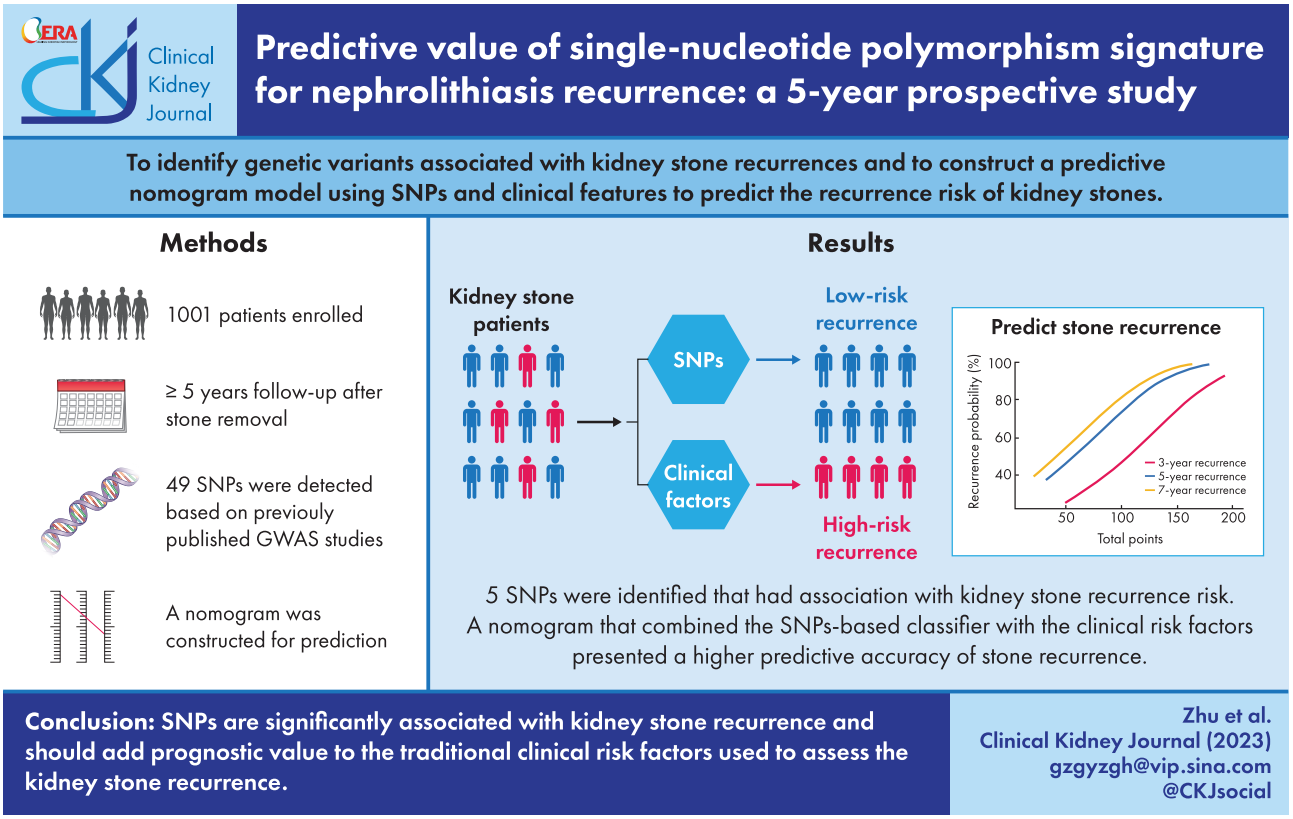
LAY SUMMARY

SNPs are significantly associated with kidney-stone recurrence and can add prognostic value to the traditional clinical risk factors used to assess the kidney-stone recurrence. A nomogram using clinical and genetic variables to predict kidney-stone recurrence has revealed its potential to be useful in the future as an assessment tool during the follow-up of kidney-stone patients.

GRAPHICAL ABSTRACT



Keywords: kidney stone, recurrence, single-nucleotide polymorphism signature

INTRODUCTION

Nephrolithiasis is a common condition that affects 5%–10% of individuals during their lifetime and is often associated with substantial healthcare and economic burdens [1, 2]. Despite the technological breakthroughs in the surgical control of stones which have significantly increased stone-free rates and reduced patient morbidity with shorter recovery time, stone recurrence remains an important health issue. After the initial episode, urolithiasis tends to tends to recur in up to 50% of cases within 5 years, with the percentage increasing to 90% within 10 years [3].

Currently, there is an unmet need to identify patients who are at a higher risk of recurrent kidney-stone events and who could benefit from more aggressive secondary prevention. The use of the 24-h urine test is guideline-supported for high-risk and interested patients [4, 5]. However, the 24-h urine test has various constraints, such as the complexity of interpretation, the need for repeated collections, the inability to foresee stone recurrence with individual parameters and supersaturation values, the ambiguous rationale of laboratory cutoff values and the difficulty of determining the adequacies of collected samples [6]. Therefore, identification of patients at the highest risk of recurrent kidney stone, including through the development of specific measures for early detection and prevention, remains a high priority.

Nephrolithiasis has a multifactorial etiology concerning factors from both genetic and environmental aspects. Prior studies have suggested that certain clinical and radiologic features of kidney stones are associated with disease recurrence [7–9]. In addition to traditional risk factors, genetics also play a key role in nephrolithiasis [10]. Single nucleotide polymorphism (SNP) is one of the most common types of genetic differences among humans. By adopting genome-wide association studies (GWASs), several SNPs associated with the genetic predisposition to kidney-stone formation have been identified [11, 12]. However, there is limited evidence that these SNPs are associated with stone recurrence and can be used to predict

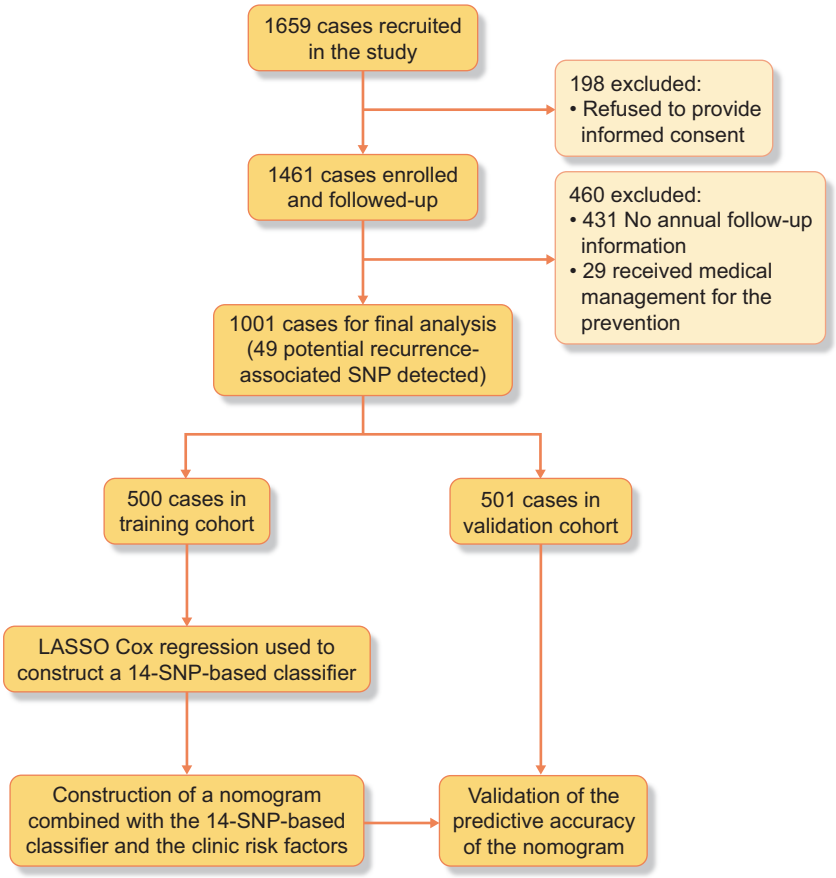


Figure 1: Enrollment and outcomes. Five to 10 years' follow-up for all participants was performed.

kidney-stone recurrence. No study with a sufficiently large sample size for statistical rigor has analyzed the role of individual genetic background in the risk of kidney-stones recurrence.

In this study, we performed a prospective study to identify genetic variants associated with kidney-stone recurrences and to identify a SNP signature to boost clinical features for predicting the recurrence risk of kidney stones. We also construct a predictive nomogram model using SNPs and clinical features to predict the recurrence risk of kidney stones.

MATERIALS AND METHODS

Study participants

We prospectively collected 1659 plasma samples from kidney-stone patients who underwent surgical stone removal, of which 1001 passed quality control for the final analysis (Fig. 1). All samples were obtained between 1 January and 31 December 2012 from patients treated at the First Affiliated Hospital of Guangzhou Medical University, a tertiary referral center of kidney-stone disease in southern China. Patients need to meet the following criteria in order to be considered in this study: (i) have received stone removal surgery and postoperative computed tomography (CT) scans confirmed total stone-free; (ii) have complete clinical and at least 5-year follow-up data, as well as common epidemiological data. Patients who refused to provide informed consent and did receive medical management for the prevention of recurrence stones were excluded. We used computer-generated random numbers to assign 500 of these patients to the training cohort and 501 patients to the validation cohort.

This study was approved by local ethics committee, and written informed consent was obtained from all individuals. The study was registered at http://clinicaltrials.gov/ (NCT04937192).

Demographic and clinical data

The demographic and clinical data on the patients' medical history, kidney-stones risk factors, current condition, laboratory results, medications at admission and discharge, and imaging information were collected. Stones were removed by percutaneous nephrolithotomy or flexible ureteroscopy. Stone analyses were performed by infrared spectroscopy. Post-operative stone-free status was assessed by low-dose CT scans obtained approximately 3 months after surgery. Stone-free status was defined as no stone of any size by CT scans and during endoscopy. The patients were followed for at least 5 years. The standard follow-up was updated at 6-month intervals through onsite interview or direct calling. The latest follow-up data in our analysis were obtained in January 2022. Recurrence was considered to be either silent or symptomatic. Silent recurrences were diagnosed on the basis of renal ultrasound, and kidney, ureter and bladder imaging (KUB) performed at 6-month intervals. If renal stones

were detected, a low-dose CT scan was also performed. A symptomatic recurrence was defined as typical renal colic, an episode of macroscopic hematuria or spontaneous stone passage. If a symptomatic recurrence was documented on the basis of renal colic or hematuria, the recurrence had to be confirmed by CT scans.

SNP selection and genotyping

The candidate SNPs were selected by manual searching PubMed using the keywords "kidney stone, nephrolithiasis, urolithiasis" for phenotypes and the keywords "polymorphism, SNP, mutation, variant" for polymorphisms. Only articles in English were considered. The potential functional SNPs were selected based on previously published GWAS studies, and their respective functional effect, such as those with a formerly known modification at transcription, translation, protein activity or an amino acid substitution–based hypothetic modification. We finally identified 49 SNPs in 37 genes selected from the literature related to kidney-stone formation [11–22] (Supplementary data, Table S1).

The genotyping in the study has been presented elsewhere in detail. Briefly, genomic DNA was extracted from the whole blood by using customized multiple targeted capture reagents. After amplifying with PCR, the products were genotyped by using the Illumina Nextseq CN500 platform and following its manufacturer's protocol. The quality control was performed using Bbduk software (v37.48). Contamination detection was performed on data that passed quality control using BLAST+ (v2.7.1).

Statistical methods

The numbers are presented as mean ± standard deviation or the number and percentage. Categorical variables were compared by χ^2 analysis or Fisher's exact test. Normally distributed scale variables were analyzed with Student's *t*-test for independent variables. Statistical significance for each SNP was assessed by Kaplan–Meier curves using the Cox model. The Cox regression model was used for multivariate survival analysis. Hazard ratios (HRs) and 95% confidence intervals (CIs) are reported. The LASSO Cox regression model was used to construct an SNP-based classifier. A simplified nomogram based on the final statistical model was developed to predict the probability of stone recurrences at 3, 5 and 7 years. Finally, calibration curves were the assessing method to evaluate whether the actual outcomes predicted outcomes for the nomogram, with prognostic accuracy assessed by time-dependent receiver operating characteristic (ROC) curves and areas under the curves (AUCs). All statistical tests were conducted using R software (version 3.5.0) and statistical significance was set at *P*-values <.05.

RESULTS

Characteristics of the study population

The distributions of 1001 patients' demographic and clinical characteristics are summarized in Table 1. Some 61.5% of patients had complex stones. Of these, 314 were multiple stones and 301 were staghorn stones. During the median follow-up time of 7 years (range 5–10 years), 787 patients (78.6%) developed recurrence. The recurrence rates at 3, 5 and 7 years were 46.8%, 71.2% and 78.4%, respectively.

Kidney-stone recurrence clinical predictor

Univariate analysis identified nine clinical variables nominally associated with the kidney-stone recurrence ($P < .05$). Significantly higher recurrence risks were observed in male patients, and patients with hyperparathyroidism, history of recurrent stone episode, bilateral stones, multiple stones, stone size >20 mm, urinary tract infection or infected stone. Patients with calcium oxalate stones were negatively associated with stone recurrence as compared with other stone compositions (Table 1). A multivariable Cox model in the training cohort including the 11 clinical variables with $P < .1$ was established to predict the stone recurrence. The AUC at 3, 5 and 7 years of the model was 0.596, 0.661 and 0.695, respectively (Fig. 2).

The associations between SNPs and kidney-stone recurrence

After excluding 1 SNP (rs184187143 in *SLC26A6*) as all cases were homozygous wild type, we eventually analyzed 48 SNPs in 36 genes (Table 2). We assessed the association of each individual SNP with kidney-stone recurrence by Cox regression. The characteristics and association results of the 48 variants are displayed in Table 3. There were three SNPs (rs1544935 in *KCNK5*, rs11746443 in *RGS14* and rs56235845 in *SLC34A1*) that exhibited significant association with increased kidney-stone recurrence risk. In addition, we found that two SNPs (rs10735810 in *VDR* and rs3798519 in *TFAP2B*) had negative association with kidney-stone recurrence risk.

The LASSO Cox regression model was used to select the most useful prognostic markers among the 48 SNPs in training cohort and to construct an SNP-based classifier for prediction of kidney-stone recurrence. The SNP-based classifier comprised 14 of the 48 SNPs in the regions of the following genes: rs10735810 (in *VDR*), rs11746443 (in *RGS14*), rs13003198 (in *DGKD*), rs13041834 (in *BCAS1*), rs1544935 (in *KCNK5*), rs2043211 (in *CARD8*), rs2286526 (in *LOC645722*), rs3798519 (in *TFAP2B*), rs4793434 (in *SOX9*), rs56235845 (in *SLC34A1*), rs6464214 (in *HIPK2*), rs7057398 (in *CLDN2*), rs755622 (in *MIF-AS*) and rs780093 (in *GCKR*). Using the LASSO Cox regression models, we calculated a risk score for each patient based on the 14-SNP status:

$$\begin{aligned}\text{Risk score} = {} & (0.0632 \times \text{rs11746443}) - (0.0611 \times \text{rs10735810}) \\ & + (0.0026 \times \text{rs13003198}) - (0.0384 \times \text{rs13041834}) + (0.1157 \times \text{rs1544935}) \\ & - (0.0563 \times \text{rs2043211}) - (0.0194 \times \text{rs2286526}) - (0.0065 \times \text{rs3798519}) \\ & - (0.0361 \times \text{rs4793434}) + (0.0160 \times \text{rs56235845}) - (0.0070 \times \text{rs6464214}) \\ & + (0.0104 \times \text{rs7057398}) + (0.0455 \times \text{rs755622}) - (0.0399 \times \text{rs780093})\end{aligned}$$

The risk scores of the 500 patients in the training cohort range from −0.4576 to 0.2414. We assessed the distribution of risk scores for the 14-SNP-based classifier and recurrence status in the training cohort. Patients in the training cohort were divided into high-risk ($n = 250$) and low-risk ($n = 250$) groups, with the median risk score (of −0.1567) as the cutoff. Compared with patients in the low-risk group, patients in the high-risk group had shorter recurrence-free survival [HR 1.38 (95% CI 1.13–1.69), $P = .0014$] (Fig. 2). The AUC at 3, 5 and 7 years of the classifier was 0.594, 0.632 and 0.636, respectively. The risk score for each patient in the validation cohort ($n = 501$) was calculated with the same formula as that used in the training cohort. The SNP classifier achieved similar predictive ability in the validation cohort (the AUC at 3, 5 and 7 years were 0.523, 0.572 and 0.588, respectively) (Fig. 4).

Table 1: Baseline clinical variables for patients with recurrence of kidney stones.

Characteristic	Total	No recurrence	Recurrence	HR	95% CI		P-value
					Lower	Upper	
n (%)	1001	214 (21.4)	787 (78.6)				
Age (years)	48.93 ± 13.12	49.6 ± 13.28	48.75 ± 13.08	0.99	0.94	1.04	.655
Gender (male)	577 (57.6)	104 (48.5)	473 (60.2)	1.22	1.05	1.4	.008
BMI (kg/m^2)	23.44 ± 3.75	23.58 ± 3.73	23.4 ± 3.75	0.99	0.97	1.01	.453
Recurrent stone episode, *n* (%)	204 (20.4)	7 (3.3)	197 (25.1)	1.9	1.62	2.24	<.001
Urinary tract infection, *n* (%)	224 (22.4)	33 (15.4)	191 (24.3)	1.25	1.06	1.48	.007
Comorbidities, *n* (%)							
Hyperternsion	265 (27.5)	66 (30.8)	209 (26.6)	0.88	0.75	1.03	.111
Diabetes	141 (14.1)	36 (16.8)	105 (13.4)	0.84	0.68	1.03	.092
Gout	10 (0.9)	2 (0.9)	8 (1.1)	1.27	0.68	2.36	.458
Hyperparathyroidism	44 (4.4)	6 (2.8)	38 (4.8)	1.45	1.05	2.01	.025
Hyperlipidemia	21 (2.1)	2 (0.9)	19 (2.5)	1.14	0.72	1.79	.578
Imaging, *n* (%)							
Stone number							
Single	386 (38.5)	114 (53.2)	272 (34.5)				
Multiple or staghorn stone	615 (61.5)	100 (46.8)	515 (65.5)	1.36	1.18	1.58	<.001
Stone laterality, *n* (%)							
Unilateral	635 (63.5)	163 (76.2)	472 (59.9)				
Bilateral	366 (36.5)	51 (23.8)	315 (40.1)	1.44	1.25	1.66	<.001
Stone diameter, *n* (%)							
<20 mm	430 (42.9)	127 (59.3)	303 (38.5)				
≥20 mm	571 (57.1)	87 (40.6)	484 (61.5)	1.39	1.2	1.61	<.001
Grade of hydronephrosis, *n* (%)							
None or mild	650 (64.9)	144 (67.3)	506 (64.3)				
Moderate or severe	351 (35.1)	70 (32.7)	281 (35.7)	1.11	0.96	1.29	.157
Laboratory parameters, *n* (%)							
ALT >40 U/L	91 (9.1)	20 (9.4)	71 (9.1)	0.98	0.77	1.25	.867
ALB <30 g/L	239 (23.8)	49 (22.8)	190 (24.2)	1.09	0.93	1.28	.308
Serum Cr >133 μmol/L	114 (11.4)	19 (8.9)	95 (12.1)	1.22	0.99	1.51	.068
Stone composition, *n* (%)							
Calcium oxalate	438 (43.7)	111 (51.8)	327 (41.6)	0.75	0.65	0.87	<.001
Calcium phosphate	7 (0.7)	1 (0.5)	6 (0.7)	1.11	0.5	2.47	.807
Infection stone	220 (21.9)	33 (15.45)	187 (23.7)	1.35	1.14	1.59	<.001
Uric acid	72 (7.2)	16 (7.4)	56 (7.1)	1	0.76	1.31	1
Unknown	264 (26.4)	53 (24.8)	211 (26.8)				

Data are mean ± standard deviation or *n* (%).
BMI, body mass index; Cr, creatinine.

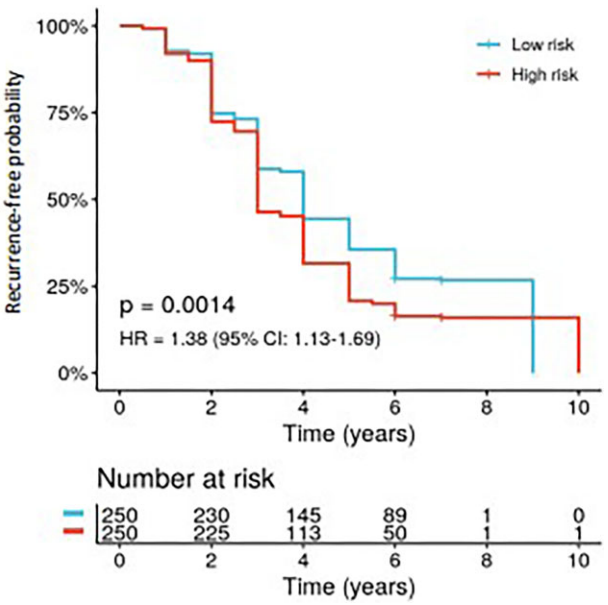


Figure 2: Kaplan–Meier curves for patients stratified into low-risk and high-risk groups according to the 14-SNP-based risk score.

Nomogram construction

As the clinical factors–based model and SNP-based classifier both showed poor predictive ability for stone recurrence, we constructed a nomogram that combined the 14-SNP-based classifier with the clinical risk factors to ascertain how the 14-SNP-based classifier added prognostic value to the clinical risk factors and to provide clinicians with a quantitative method to predict the probability of recurrence in a patient with kidney stones. The nomogram was developed with data from 500 cases in the training cohort (Fig. 3). The AUC at 3, 5 and 7 years of this nomogram in the training cohort was 0.645, 0.723 and 0.75, respectively. The AUC at 3, 5 and 7 years of the classifier or clinical risk factors ranged from 0.579 to 0.688 in the training cohort. The predictive accuracy of the nomogram was significantly higher than that of the classifier or clinical risk factors alone in the training cohort (Fig. 4). A total of 501 cases in the validation cohort were used to validate the predictive accuracy of the nomogram; the AUC at 3, 5 and 7 years was 0.631, 0.708 and 0.727, respectively, in the validation cohort (Fig. 4).

Table 2: The percentage of 1001 patients with wild-type homozygous/heterozygous/variant-type homozygous genotypes.

dbSNP rs ID	Chr	Physical position	Associated gene	SNP status (%)		
				WT (0)	HT (1)	VT-HO (2)
rs1010269	17	59 448 945	*BCAS*	14.49	48.15	37.36
rs1037271	13	42 779 410	*DGKH*	20.58	52.25	27.17
rs10735810	12	48 272 895	*VDR*	20.08	52.65	27.27
rs10917002	1	21 836 340	*ALPL*	50.45	39.16	10.39
rs1155347	6	39 146 230	*KCNK5*	70.13	27.57	2.30
rs11746443	5	176 798 306	*RGS14*	72.63	24.08	3.30
rs12539707	7	27 626 165	*HIBADH*	0.30	9.99	89.71
rs1260326	2	27 730 940	*GCKR*	23.78	52.35	23.88
rs12626330	21	37 835 982	*CLDN14*	27.47	51.75	20.78
rs12666466	7	30 916 430	*AQP1*	79.72	19.38	0.90
rs12837024	X	106 911 003	*CLDN2*	49.95	22.48	27.57
rs13003198	2	234 257 105	*DGKD*	39.56	48.55	11.89
rs13041834	20	52 703 284	*BCAS1*	56.84	38.36	4.80
rs13054904	22	23 410 918	*BCR*	88.31	10.99	0.70
rs1481012	4	89 039 082	*ABCG2*	47.85	42.46	9.69
rs1544935	6	39 124 448	*KCNK5*	70.83	26.77	2.40
rs17216707	20	52 732 362	*CYP24A1*	94.21	5.49	0.30
rs184187143	3	48 666 132	*SLC26A6*	100	0	0
rs2043211	19	48 737 706	*CARD8*	23.78	49.75	26.47
rs2058265	7	139 462 249	*HIPK2*	58.14	35.16	6.69
rs2231142	4	89 052 323	*ABCG2*	46.65	43.06	10.29
rs2286526	17	59 472 057	*LOC645722*	38.56	48.85	12.59
rs35747824	16	20 393 308	*PDILT*	70.73	27.17	2.10
rs3752472	13	33 629 393	*Klotho*	85.21	13.89	0.90
rs3760702	19	14 588 237	*GIPC1*	56.14	39.76	4.10
rs3798519	6	50 788 778	*TFAP2B*	58.24	35.46	6.29
rs4529910	11	111 243 102	*POU2AF*	33.97	48.95	17.08
rs4793434	17	70 352 537	*SOX9*	10.99	44.36	44.66
rs56235845	5	176 798 040	*SLC34A1*	65.23	24.08	10.69
rs578595	15	53 997 089	*WDR72*	70.33	26.87	2.80
rs6123359	20	52 714 706	*BCAS1*	18.78	49.65	31.57
rs6464214	7	139 454 165	*HIPK2*	57.64	35.36	6.99
rs6667242	1	21 826 566	*ALPL*	51.75	37.86	10.39
rs6928986	6	131 323 992	*EPB41L2*	15.48	49.45	35.06
rs6975977	7	30 917 831	*INMT-FAM188B*	75.42	23.18	1.40
rs7057398	X	106 901 299	*CLDN2*	44.96	23.28	31.77
rs7206790	16	53 797 908	*FTO*	71.33	26.47	2.20
rs7277076	21	37 836 973	*CLDN14*	21.28	52.05	26.67
rs731236	12	48 238 757	*VDR*	91.41	8.39	0.20
rs73247968	X	106 911 865	*CLDN2*	70.83	15.18	13.99
rs7456421	7	139 415 775	*HIPK2*	57.34	35.46	7.19
rs74956940	19	14 571 966	*PKN1*	59.74	36.36	3.90
rs755622	22	24 236 392	*MIF-AS*	63.14	34.57	2.30
rs7652589	3	121 889 088	*CaSR*	47.75	42.86	9.39
rs77648599	6	160 624 115	*SLC22A2*	89.31	10.19	0.50
rs77924615	16	20 392 332	*UMOD*	70.43	27.47	2.10
rs780093	2	27 742 603	*GCKR*	22.48	51.05	26.47
rs7975232	12	48 238 837	*VDR*	44.36	47.25	8.39
rs889299	16	23 381 914	*SCNN1B*	43.46	46.45	10.09

dbSNP, Database for SNPs; Chr, chromosome; WT, wild-type homozygous; HT, heterozygous; VT-HO, variant-type homozygous.

DISCUSSION

In this study, we evaluated the effect of SNPs on the recurrence prediction of 1001 patients. Five SNPs were identified as being closely related to the recurrence of kidney stones. We further developed a 14-SNP-based classified to complement the clinical factors for prediction of kidney-stone recurrence, which can enable physicians to make more informed treatment decision about recurrence prevention. To the best of our knowledge, this study was the first to comprehensively assess the association between genetic background and kidney-stone recurrence with a large number of cases.

Personalized medicine has widely accepted the role of investigating the genetic makeup of an individual patient in achieving optimized medical care. In turn, the clarification of gene polymorphism contribution to kidney-stone recurrence will be advantageous in clinics to improve diagnosis of at-risk patients as well as to provide treatment with maximum

Table 3: Association between SNPs and stone recurrence.

ID (SNP status)	Associated gene	HR	95% CI		P-value	[a]Adjusted P-value
			Lower	Upper		
rs1010269 (2 vs 1 vs 0)	*BCAS*	1.05	0.947	1.16	.358	.818
rs1037271 (2 vs 1 vs 0)	*DGKH*	1.02	0.925	1.14	.637	.983
rs10735810 (2 vs 1 vs 0)	**VDR**	**0.868**	**0.784**	**0.96**	**.006**	**.265**
rs10917002 (2 vs 1 vs 0)	*ALPL*	1.03	0.929	1.15	.558	.983
rs1155347 (2 vs 1 vs 0)	*KCNK5*	1.05	0.92	1.21	.451	.934
rs11746443 (2 vs 1 vs 0)	**RGS14**	**1.17**	**1.03**	**1.32**	**.018**	**.265**
rs12539707 (2 vs 1 vs 0)	*HIBADH*	0.972	0.778	1.22	.805	.983
rs1260326 (2 vs 1 vs 0)	*GCKR*	0.947	0.856	1.05	.285	.818
rs12626330 (2 vs 1 vs 0)	*CLDN14*	1.08	0.974	1.19	.149	.587
rs12666466 (2 vs 1 vs 0)	*AQP1*	1.02	0.864	1.19	.849	.983
rs12837024 (2 vs 1 vs 0)	*CLDN2*	1.01	0.926	1.09	.883	.983
rs13003198 (2 vs 1 vs 0)	*DGKD*	1.08	0.97	1.2	.159	.587
rs13041834 (2 vs 1 vs 0)	*BCAS1*	0.898	0.796	1.01	.079	.473
rs13054904 (2 vs 1 vs 0)	*BCR*	1.02	0.841	1.25	.809	.983
rs1481012 (2 vs 1 vs 0)	*ABCG2*	0.96	0.861	1.07	.467	.934
rs1544935 (2 vs 1 vs 0)	**KCNK5**	**1.19**	**1.04**	**1.36**	**.013**	**.265**
rs17216707 (2 vs 1 vs 0)	*CYP24A1*	0.937	0.71	1.24	.647	.983
rs2043211 (2 vs 1 vs 0)	*CARD8*	0.917	0.832	1.01	.079	.473
rs2058265 (2 vs 1 vs 0)	*HIPK2*	1	0.896	1.12	.947	.983
rs2231142 (2 vs 1 vs 0)	*ABCG2*	0.94	0.844	1.05	.265	.818
rs2286526 (2 vs 1 vs 0)	*LOC645722*	0.969	0.874	1.07	.544	.983
rs35747824 (2 vs 1 vs 0)	*PDILT*	1.12	0.974	1.28	.113	.493
rs3752472 (2 vs 1 vs 0)	*Klotho*	0.975	0.817	1.16	.782	.983
rs3760702 (2 vs 1 vs 0)	*GIPC1*	1.06	0.939	1.2	.338	.818
rs3798519 (2 vs 1 vs 0)	**TFAP2B**	**0.873**	**0.777**	**0.981**	**.022**	**.265**
rs4529910 (2 vs 1 vs 0)	*POU2AF*	1.04	0.944	1.15	.409	.892
rs4793434 (2 vs 1 vs 0)	*SOX9*	0.908	0.818	1.01	.072	.473
rs56235845 (2 vs 1 vs 0)	**SLC34A1**	**1.12**	**1.01**	**1.23**	**.030**	**.283**
rs578595 (2 vs 1 vs 0)	*WDR72*	1.03	0.908	1.18	.616	.983
rs6123359 (2 vs 1 vs 0)	*BCAS1*	1.01	0.911	1.11	.890	.983
rs6464214 (2 vs 1 vs 0)	*HIPK2*	0.999	0.892	1.12	.988	.988
rs6667242 (2 vs 1 vs 0)	*ALPL*	1.03	0.924	1.14	.625	.983
rs6928986 (2 vs 1 vs 0)	*EPB41L2*	1	0.906	1.11	.963	.983
rs6975977 (2 vs 1 vs 0)	*INMT-FAM188B*	1.01	0.874	1.18	.847	.983
rs7057398 (2 vs 1 vs 0)	*CLDN2*	1.01	0.93	1.09	.833	.983
rs7206790 (2 vs 1 vs 0)	*FTO*	0.934	0.814	1.07	.337	.818
rs7277076 (2 vs 1 vs 0)	*CLDN14*	1.06	0.954	1.17	.295	.818
rs731236 (2 vs 1 vs 0)	*VDR*	0.848	0.657	1.09	.206	.706
rs73247968 (2 vs 1 vs 0)	*CLDN2*	0.997	0.905	1.1	.958	.983
rs7456421 (2 vs 1 vs 0)	*HIPK2*	1.01	0.904	1.13	.849	.983
rs74956940 (2 vs 1 vs 0)	*PKN1*	1.04	0.919	1.18	.538	.983
rs755622 (2 vs 1 vs 0)	*MIF-AS*	1.11	0.977	1.27	.108	.493
rs7652589 (2 vs 1 vs 0)	*CaSR*	0.994	0.894	1.11	.918	.983
rs77648599 (2 vs 1 vs 0)	*SLC22A2*	1.05	0.849	1.3	.655	.983
rs77924615 (2 vs 1 vs 0)	*UMOD*	1.12	0.975	1.28	.109	.493
rs780093 (2 vs 1 vs 0)	*GCKR*	0.953	0.863	1.05	.344	.818
rs7975232 (2 vs 1 vs 0)	*VDR*	1.02	0.912	1.14	.746	.983
rs889299 (2 vs 1 vs 0)	*SCNN1B*	1.02	0.92	1.14	.677	.983

[a]Benjamini–Hochberg procedure.

efficacy. Accumulating evidence has suggested that genetic backgrounds affect the risk of kidney-stone formation. Previous GWAS analyses with large size samples have reported that SNPs in metabolism-related genes are linked to stone formation [11, 12]. However, there is limited evidence to show that these SNPs are associated with stone recurrence. On the other hand, these genetic variations likely will contribute to the recurrence of kidney stones as the remaining underlying metabolic basis of kidney-stone formation after surgical removal of prior mineral accumulations. Therefore, our choice of SNPs for this study can identify those genetic variations that persist and influence kidney-stone development even after likely lifestyle changes. Our effort thus represents an effective means to determine critical genetic contributions to kidney-stone recurrence without resort to *de novo* identification of recurrence SNPs with a required much larger patient population.

In our study, five SNPs in five genes were shown to be associated with the recurrence of kidney stones. Consistent with previous studies, the genetic polymorphisms of *RGS14* and *SLC34A1* were associated with the risk of kidney-stone for-

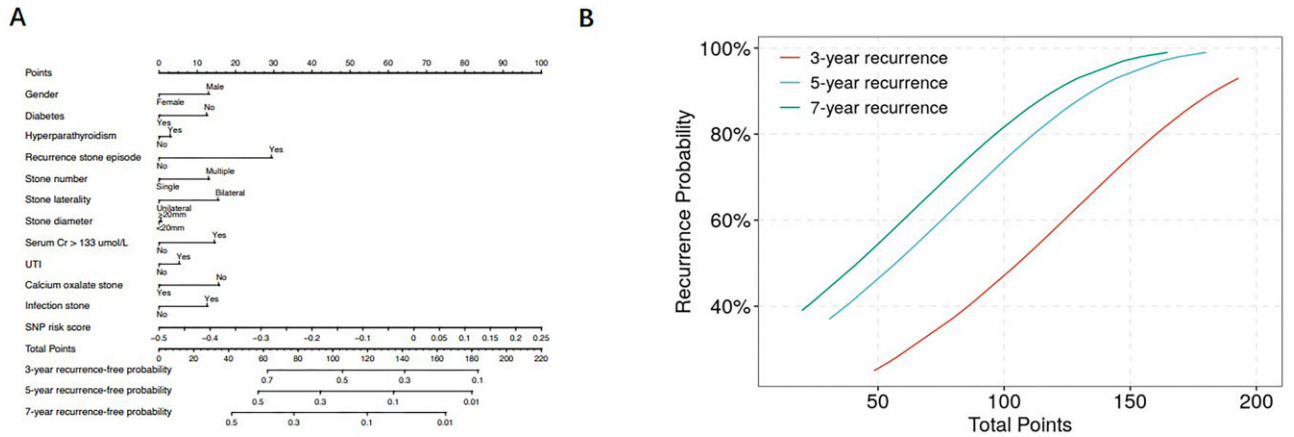


Figure 3: Nomogram predicting stone recurrence probability. (**A**) Determination of the total points based on the sum of 12 predicts. (**B**) Estimate of recurrence risk at 3, 5 and 7 years based on the total points.

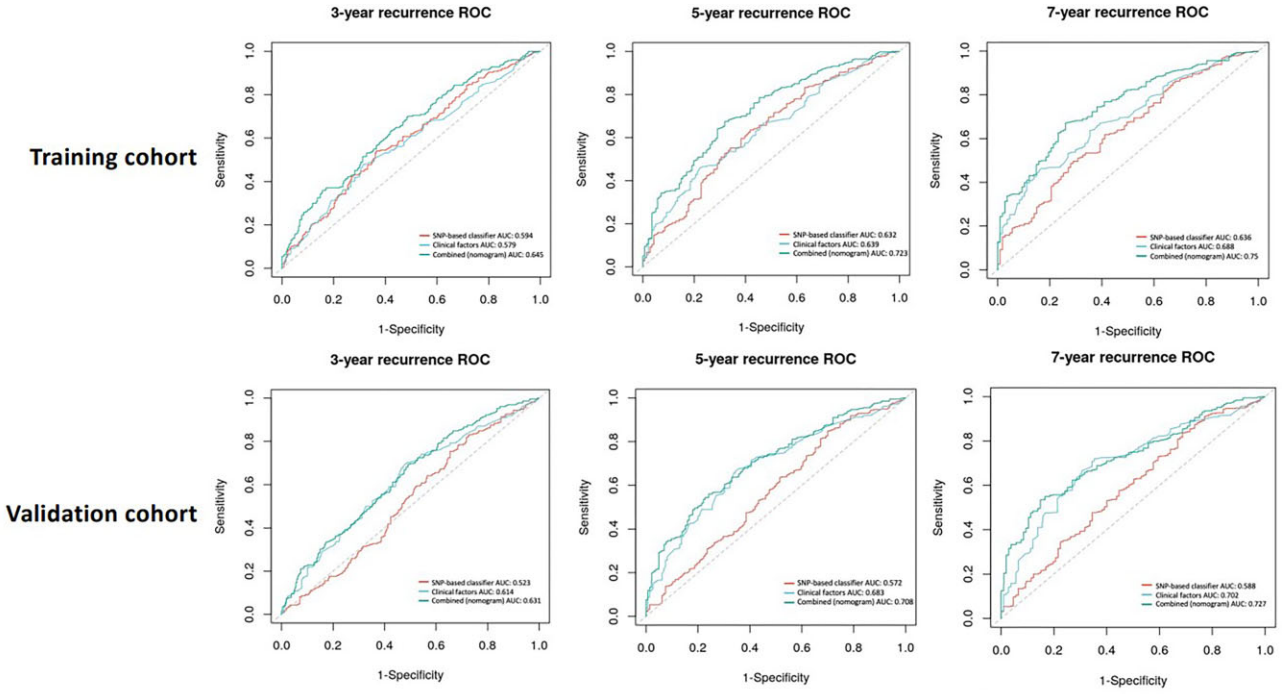


Figure 4: Better nomograms for predicting stone recurrence. Time-dependent ROC curves at 3, 5 and 7 years were used to assess the prognostic accuracy of the nomogram.

mation. Mutation in *SLC34A1*, encoding the proximal tubular sodium-phosphate transporter NaPi-lla, may cause infantile hypercalcemia, hypophosphatemic kidney stone and osteoporosis [23]. *RGS14* encodes a complex scaffolding protein, known as regulator of G protein signaling 14, which is enriched in hippocampal area CA2 dendritic spines. Several studies report that the rs11746443 of *RGS14* was associated with calcium-containing renal stones due to its position in the upstream of the *SLC34A1* and *AQP1* genes, which may be crucial for urine concentration [24, 25]. Vitamin D is a hormone that plays a critical role in the metabolism of calcium through binding to the VDR. Genetic variations in the *VDR* gene have been shown to influence the interactions of the vitamin D/VDR, modulating the susceptibility risk for several pathologic conditions. A series of studies investigated the association between these polymorphisms of the *VDR* gene and the risk of urolithiasis, but the findings were conflicting [26–29]. In our study, we found that rs10735810 was associated with stone recurrence while two other SNPs in *VDR* (rs7312366 and rs7975232) were not associated with stone recurrence. It has been reported that *VDR* variants have different effects on the receptor activity. In particular, the *FokI* isoform is synthesized from different start sites in the *VDR* gene and therefore it is involved in transcriptional activation [30]. Along this line, a potential start site of the *FokI* polymorphism (rs10735810) produces an altered VDR protein by generating an additional start codon, which results in proteins containing fewer amino acids,

which likely are more active in terms of their transactivation capacity [31].

Improving the prediction of kidney-stone recurrence following initial surgical interventions would undoubtedly contribute to more effective symptom management and patient care. Several studies have reported some clinical factors can be used for evaluating the risk of kidney-stone recurrence. Andrew D Rule *et al*. [7, 9] found a series of clinical risk factors for stone recurrence, such as younger age, male sex, family history of stones and uric acid stone compositions. Based on these clinical risk factors, they constructed a nomogram (called the ROKS nomogram) to predict stone recurrence, which could be helpful for better centralized management and early intervention. In our study, we identified 11 clinical factors associated with stone recurrence. Most of them were similar to those reported in the literature [7]. Such a constructed a nomogram based on the clinical risk factors did not perform well in predicting stone recurrence risk, with AUC values <0.7. Further improvement of this kind of nomogram will make it a practical tool in the routine clinical use to benefit kidney-stone patients.

One of the aspects to consider for improvements could be to include genetic information in the nomogram model. Indeed, genetic risk factors alone or in combination with clinical factors can be used for risk stratification and to guide strategies for treatment in various type of diseases [32, 33]. Kidney stone is generally acknowledged as a disorder caused by the interaction of multiple genetic and environmental factors. Therefore genetic signature alone is unlikely to predict kidney-stone recurrence risk. On the other hand, inclusion of genetic information likely will improve the accuracy of prediction based on clinical factors. Indeed, we built a nomogram using genetic factors combined with clinical factors to predict the stone recurrence. We found that the addition of SNP information to the nomogram improved the predictive accuracy, reflecting the contribution of inherent genetic predisposition in stone recurrence. Thus, our nomogram provides a simple, accurate and improved method for predicting kidney-stone recurrence risk.

In our study we found that the addition of SNPs to the clinical model did not significantly improve the clinical model AUC in the validation cohort. The possible reasons are many: first, the sample size of the validation set is relatively small. Increasing the sample size may reveal that SNPs can improve the clinical model AUC. Second, the clinical variations may partially reflect genetic variations, and the predictive value of genetic variation may overlap with clinical variations. Therefore, addition of SNPs did not significantly improve the accuracy of clinical model for predicting stone recurrence. This indirectly confirms the effectiveness of constructing stone recurrence prediction models using clinical variations, such as the ROKS nomogram [7], as previously discovered by other researchers. Finally, this study only tested 49 SNP loci based on previous GWAS results. If more genetic information is detected through whole-genome survey, it might identify novel genetic loci to significantly improve the predictive value of the clinical model.

Our study has several limitations. First, the cohort was of Chinese origin, therefore the generalizability to other ethnic background needs confirmation. Second, the AUC of 0.631–0.645 in 3-year recurrence for the nomogram was relatively low for a prediction tool, even though it was an improvement over the 0.579–0.614 for the nomogram based on clinical factors. Future studies may be needed to better identify predictors. Third, although no stone formers received stone prevention medications, most of them did receive dietary recommendations that might have influenced natural stone recurrence. Fourth, surgical interventions to remove renal stones were performed in all patients in our study, which might affect the natural history of renal stone recurrence. Thus, the recurrence findings seen in this study are most applicable to those with moderate to large sized stones who required surgical intervention at baseline. Fifth, silent recurrence was determined by KUB and renal ultrasound, which might miss smaller stones. It was reported that the sensitivity of KUB and ultrasound combined was 78% for renal calculi [34]. Finally, this nomogram needs to be evaluated externally in other community-based settings with a larger proportion of residents.

CONCLUSION

Our study indicates that genetic variations are significantly associated with kidney-stone recurrence, and should add prognostic value to the traditional clinical risk factors used to assess kidney-stone recurrence. The generation of a nomogram using clinical and genetic variables to predict kidney-stone recurrence has revealed its potential in the future as an assessment tool during the follow-up of kidney-stone patients.

SUPPLEMENTARY DATA

Supplementary data are available at *ckj* online.

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AUTHORS' CONTRIBUTIONS

G.Zeng had full access to all the data in the study and takes responsibility for the data and the accuracy of the data analysis. G.Zeng and Y.S. contributed to the conception and design of the study. W.Zhu, X.Z., Z.Zhou, G.Zhang, X.D., Y.L., Z.Zhao, W.Zhong, Z.H. and G.A. contributed to acquisition of data. W.Zhu, X.Z. and Z.Zhou contributed to the analysis and interpretation of the data. W.Zhu and G.Zeng drafted the manuscript. Y.S. participated in the critical revision of the manuscript for important intellectual content. W.Zhu, X.Z. and Z.Zhou carried out the statistical analysis. G.Zeng and W.Zhu contributed to the acquisition of funding. X.D. and W.Zhong provided administrative, technical or material support. W.Zhu, X.Z. and Z.Zhou contributed equally to the work. All authors contributed to reading the manuscript and approved the submitted version.

DATA AVAILABILITY STATEMENT

All data used to support the findings of this study are available from the corresponding author (Guohua Zeng, gzgyzgh@vip.sina.com) upon request.

CONFLICT OF INTEREST STATEMENT

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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