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Original Article

Genetic polymorphisms in the *PDZK1* gene and susceptibility to gout in male Han Chinese: a case-control study

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Abstract: *PDZK1* acts as a scaffolding protein for a large variety of transporter and regulatory proteins, and has been identified in the kidney. The *PDZK1* locus has been determined to be associated with the serum urate concentration. However, the evidence supporting this protein's association with gout is equivocal. In the current study, we investigated the association between two single nucleotide polymorphisms (SNPs) (rs12129861 and rs1967017) in the *PDZK1* gene with gout in a male Chinese Han population. A total of 824 subjects were enrolled in this case-control study (400 gout cases and 424 controls). *PDZK1* genotyping was carried out by polymerase chain reaction (PCR) and ligase detection reaction (LDR) assays methods. The relationships were evaluated using the pooled odds ratios (ORs) and their 95 % confidence intervals (CI). The results of our case-control study demonstrated that the gout and control groups exhibited significant differences in the distribution of genotypes at rs12129861 (OR = 0.727, $P = 0.015$) and rs1967017 (OR = 0.705, $P = 0.016$), suggesting that *PDZK1* genetic polymorphisms were associated with increased risks of gout in male Han Chinese. However, there were no differences in the distribution of genotypes at rs12129861 (odds ratio (OR) = 0.744, $P > 0.05$) and rs1967017 (OR = 0.706, $P > 0.05$) in patients with gout with kidney stones and without kidney stones.

Keywords: Single nucleotide polymorphisms (SNPs), *PDZK1*, gout

Introduction

Familial clustering is often evident in common primary gout. The usual mechanism of hyperuricemia in primary gout is predominantly related to a relative inefficiency in excretion, rather than overproduction. It has been estimated that approximately 30% of the body's uric acid is excreted into the intestine by ill-defined mechanisms and is broken down by colonic bacteria (which possess uricase) to allantoin. The kidney excretes the majority (70%) of uric acid, and renal mechanisms appear to be crucial for understanding hyperuricemia. Multiple renal transporters contribute to the maintenance of the normal serum urate levels, but the identity and regulators of these transporters are incompletely understood. Therefore, recent interest has particularly focused on the genes that regulate renal urate transport [1].

PDZK1 encodes PDZ domain containing 1, a scaffolding protein that interacts with proteins that are believed to be associated with the handling of urate acid (e.g., URAT1 and NPT1) [2, 3]. In experimental setting, it has been shown the PDZK1 interacts directly with the protein products of SLC22A11 and SLC17A1, and this protein has been proposed as a regulator of urate transport activities [2]. Consequently, single nucleotide polymorphisms (SNPs) and some mutations of *PDZK1* will likely influence the serum urate concentrations. Previous studies have shown that genetic variants of *PDZK1* (rs12129861 and rs1967017) are associated with the serum urate concentration in European

[4, 5], Icelandic [6], and White populations [7]. However, Stark demonstrated that genetic variants of *PDZK1* (rs12129861) are not associated with gout in the German population [8]. This equivocal evidence for the association of *PDZK1* with gout was notable.

To clarify the global relevance of *PDZK1*, the association needs to be confirmed by independent studies in different ethnic groups. The objective of this study was to assess the genetic association of SNPs in the *PDZK1* gene with gout in a male Chinese Han population.

Materials and methods

Study population

A total of 407 male patients with gout and 438 gout-free males (controls) were recruited from the Fourth Affiliated Hospital of Harbin Medical University. We collected the clinical features (age, height, weight, and blood pressure) from all of the participants. The diagnosis of gout was based on the preliminary criteria for the classification of gout of the American Rheumatism Association for use in either clinical settings or population-based epidemiology studies [9]. Normal male controls with no personal or familial history of hyperuricemia, gout, or any other serious illness were recruited. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the Ethics Committee of the Fourth Affiliated Hospital of Harbin Medical University. All of the subjects who participated in the study provided informed consent.

Biochemical assays

We measured the blood glucose, urea nitrogen, creatinine, uric acid, total cholesterol, and triglycerides levels in the plasma of all of the participants using an automated multichannel chemistry analyzer (PPI; Roche, Germany). To assess obesity, the body mass index (BMI) was calculated as the weight in kilograms divided by the squared height in meters. Hyperuricemia in males was defined as uric acid levels greater than 420 μmol/L.

DNA extraction and genotyping

Blood samples were collected from the patients with gout and the healthy controls and stored at -20°C until analyzed. The genomic DNA from the peripheral blood leukocytes was extracted according to standard methods. The following two SNPs in the PDZK1 gene were selected: rs12129861 and rs1967017. The polymerase chain reaction (PCR) and ligase detection reaction (LDR) assays were employed to genotype the rs12129861 and rs1967017 SNPs. The PCR assay for rs12129861, which used the forward primer 5'-TGAATGAACTACAGCTACTC-3' and the reverse primer 5'-GTCT CTGGTTTATTTCATTC-3', was performed in a 10-μL reaction volume. The PCR assay for rs1967017 used the forward primer 5'-CACCCACACTGCTATAGAAC-3' and the reverse primer 5'-CTCTGCAT ACCTTTGGAGGA-3'. The probe for rs12129861_A was 5'-TTTTTTTTTTTTTTTTTTTTTTTTTT TTTTTTTTTTGATGTTTGTGAGGTTCACTCAT-3', and the probe for _G was 5'-TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTGATGTTTGTGAGGTTCACTCAC -3'. The probe for rs1967017_C was 5'-TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTGGAGG AATTTATTAGGCCAGG-3', and the probe for rs1967017_T was 5'-TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTGGAGGAATTTATTAGGCCAGA-3'. The PCR conditions consisted of a denaturation step at 95°C for 2 min, 35 cycles of 94°C for 30 s, 53°C for 1 min, and 65°C for 30 s, and a final extension step at 65°C for 10 min. The specific amplified fragments were used in an LDR assay to identify the mutations associated with rs12129861 and rs1967017. The LDR assay was performed in a reaction volume of 10 μL that contained 1 μL of 1× ligase reaction buffer, 1 μL of the probes (2 pmol/μL each), 0.05 μL (2 U) of thermostable Taq DNA ligase (Friendship Biotechnology Co., Ltd., China), and 4 μL of the PCR product. The ligation reaction was performed using a GeneAmp PCR System 9600 (Norwalk, CT, USA) with the following temperature program: 2 min at 95°C and 40 cycles of 15 s at 94°C and 25 s at 50°C. The products were separated by agarose gel electrophoresis and analyzed using an ABI PRISM 3730 DNA sequencer. The genotyping was performed using an independent external contractor (Biowing Applied Biotechnology Co., Ltd., China).

Statistical analysis

The statistical analyses were performed using SPSS version 13.0 (Stata, College Station, TX, USA). Student's t-test was used to assess the significance of the differences in the demo-

Table 1. Demographic and clinical characteristics (mean ± SD) of the study population

Parameter	Patients with gout (*n* = 400)	Controls (*n* = 424)	P
Age (year)	52.37 ± 13.32	54.33 ± 15.90	0.055
Body mass index (kg/m^2)	26.04 ± 2.99	25.33 ± 3.61	0.002
Waist to Hip ratio	0.92 ± 0.059	0.94 ± 0.077	< 0.001
Systolic pressure (mmHg)	134.28 ± 16.59	133.17 ± 17.14	0.346
Diastolic pressure (mmHg)	85.06 ± 10.45	80.81 ± 11.26	< 0.001
Blood glucose (mmol/L)	5.44 ± 1.43	5.60 ± 1.07	0.068
Uric acid (μmol/L)	477.52 ± 108.30	300.48 ± 62.62	< 0.001
Total cholesterol (mmol/L)	5.06 ± 1.03	4.65 ± 0.81	< 0.001
Triglycerides (mmol/L)	2.40 ± 1.97	1.44 ± 1.05	< 0.001
Creatinine (μmol/L)	92.82 ± 29.81	80.75 ± 12.27	< 0.001
Urea nitrogen (mmol/L)	5.63 ± 2.02	5.36 ± 1.24	0.018

The data are represented as the means ± standard deviation.

graphic and clinical characteristics between cases and controls. The differences between non-contiguous variables, genotype distribution, allele frequency, and haplotype analysis were analyzed by Chi-square analysis using SHEsis [10, 11]. The odds ratios and 95% confidence intervals (CIs) were calculated whenever possible. Significant differences between or among groups were indicated by a *P* value less than 0.05.

Results

A total of 407 male gout patients and 424 male gout-free controls participated in this study. The genotypes from 400 of 407 gout patients and 424 of 438 gout-free controls were successfully sequenced. There were 159 cases of renal calculi patients, 241 patients without renal calculi in 400 patients with primary gout. The allele frequencies for each SNP were in Hardy-Weinberg equilibrium in both the patients and the controls (data not shown). The demographic data of the study population are shown in **Table 1**. The results showed that the gout patients had significantly higher abnormal body mass index, Waist to Hip ratio, diastolic pressure, levels of uric acid, creatinine, urea nitrogen, total cholesterol, and triglycerides, and rates of obesity, hypertension, and hyperuricemia than the gout-free controls ($P < 0.05$) (**Table 1**).

The allele frequencies of rs12129861 [OR 0.727, (95% CI = 0.562~0.940), $P = 0.015$] and rs1967017 [OR 0.705, (95% CI = 0.530~0.938), $P = 0.016$] SNPs of *PDZK1* were significantly associated with the development of gout (**Table 2**). Genotype frequencies of these two SNPs were found correlation with the development of gout ($P = 0.022$, 0.037) (**Table 2**). Because the haplotypes were multi-allelic, we analyzed the association between the haplotypes and gout to determine how the haplotype alterations affected the morbidity of gout, e.g., whether it is risky or protective and its association in the population. The analysis of two-marker haplotypes (rs12129861-rs1967017; **Table 3**) revealed consistent evidence for association for the G-T and A-C haplotypes [OR = 1.394 (95% CI = 1.078~1.803), $P = 0.011$; OR = 0.741 (95% CI= 0.550~0.997), $P = 0.047$], and the associates were significant in a male Chinese Han population.

Then, we investigated the association of the SNPs and clinical characteristics in gout and controls. Comparison between GG group and AA+GA group of rs12129861 genotype of *PDZK1* gene, no statistical differences were found in age, body mass index, waist to hip ratio, blood pressure, blood uric acid, total cholesterol, triglyceride, creatinine and urea nitrogen in two groups ($P > 0.05$), Blood glucose in GG group was higher than that in AA+GA group (5.61 ± 1.34 vs. 5.34 ± 1.05), the difference was statistically significant ($P < 0.01$) (**Table 4**). Comparison between TT group and CC+TT group of rs1967017 genotype of *PDZK1* gene, no statistical differences were found in age, body mass index, waist to hip ratio, blood pressure, blood uric acid, total cholesterol, triglyceride, creatinine and urea nitrogen in two groups ($P > 0.05$), Blood glucose in TT group was higher than that in CC+TC group (5.58 ±1.31 vs. 5.37 ± 1.09), the difference was statistically significant ($P < 0.05$) (**Table 5**).

Some medical factors such as obesity and dyslipidaemia have been indicated to be associated with serum uric acid levels. Therefore, we

Table 2. Genotype distribution and relative allele frequencies of the rs12129861 and rs1967017 polymorphisms of the *PDZK1* gene in Chinese patients with gout (*n* = 400) and controls (*n* = 424)

SNP		Distribution, n (%)			OR 95% CI	*P* value
rs12129861	Genotype	A/A	A/G	G/G		
	Gout	12 (3.0)	97 (24.2)	291 (72.8)		0.022
	Control	14 (3.3)	139 (32.8)	271 (63.9)		
	Allele	A	G	A vs. G		
	Gout	121 (15.1)	679 (84.9)	0.727	0.562~0.940	0.015
	Control	167 (19.7)	681 (80.3)			
rs1967017	Genotype	C/C	C/T	T/T		
	Gout	7 (1.8)	78 (19.5)	315 (78.7)		0.037
	Control	9 (2.1)	114 (26.9)	301 (71.0)		
	Allele	C	T	C vs. T		
	Gout	92 (11.5)	708 (88.5)	0.705	0.530~0.938	0.016
	Control	132 (15.6)	716 (84.4)			

OR, odds ratio; SNP, single nucleotide polymorphism; CI, confidence interval.

Table 3. Association of two-marker (rs12129861-rs1967017) haplotypes with gout

Haplotype	Frequency		OR [95% CI]	*P* Value
	Case	Control		
A-C	85.74 (0.107)	117.09 (0.138)	0.741 [0.550~0.997]	0.047
A-T	35.26 (0.044)	49.91 (0.059)	0.730 [0.469~1.136]	0.161
G-T	672.74 (0.841)	666.09 (0.785)	1.394 [1.078~1.803]	0.011

Table 4. Demographic and clinical characteristics (Mean ± SD) between GG and AA+GA in rs12129861 genotype in *PDZK1* gene

Parameter	GG (*n* = 562)	AA+GA (*n* = 262)	P
Age (year)	53.33 ± 14.55	53.48 ± 15.13	0.893
Body mass index (kg/m^2)	25.74 ± 3.16	25.62 ± 3.36	0.618
Waist to Hip ratio	0.93 ± 0.066	0.93 ± 0.076	0.536
Systolic pressure (mmHg)	133.89 ± 17.22	133.42 ± 16.22	0.707
Diastolic pressure (mmHg)	83.14 ± 11.05	82.31 ± 11.15	0.313
Blood glucose (mmol/l)	5.61 ± 1.34	5.34 ± 1.05	0.005
Uric acid (umol/l)	389.28 ± 124.45	380.30 ± 125.13	0.336
Total cholesterol (mmol/ll)	4.85 ± 0.94	4.84 ± 0.97	0.887
Triglycerides (mmol/l)	1.89 ± 1.68	1.94 ± 1.52	0.728
Creatinine (umol/l)	86.44 ± 22.90	86.99 ± 24.29	0.752
Urea nitrogen (mmol/l)	5.53 ± 1.71	5.41 ± 1.57	0.345

Data are represented as the mean-standard deviation.

conducted a multiple regression analysis to examine the interaction between serum uric acid levels and *PDZK1* polymorphisms together with age, BMI and serum triglyceride levels. Age, BMI, serum triglycerides were significantly associated with serum uric acid levels ($P < 0.001$). While the rs12129861 and rs1967017 genotypes of *PDZK1* gene had no correlation with serum uric acid levels ($P > 0.05$) (**Table 6**).

There were 159 cases of renal calculi patients, 241 patients without renal calculi in patients with primary gout. The age in group with kidney stone was greater than that in group without renal stone, the difference was statistically significant ($P < 0.05$), uric acid level in group with renal calculi was higher than that in group without renal calculi, but the difference was not statistically significant ($P > 0.05$), body mass index, waist to hip ratio, systolic blood pressure, diastolic blood pressure, fasting blood glucose, serum uric acid, total cholesterol, triglyceride, serum creatinine and urea nitrogen in two groups had no significant difference ($P > 0.05$) (Table S1). The allele frequencies and genotype frequencies of the rs12129861 and rs1967017 genotypes of *PDZK1* gene were not significantly associated with the development of renal stones in patients with primary gout ($P > 0.05$) (Table S2). Analysis of rs12129861-rs1967017 haplo-

Table 5. Demographic and clinical characteristics (Mean ± SD) between TT and CC+TC in rs1967017 genotype in *PDZK1* gene

Parameter	TT (*n* = 616)	CC+TC (*n* = 208)	P
Age (year)	53.39 ± 14.63	53.33 ±15.04	0.956
Body mass index (kg/m^2)	25.65 ± 3.18	25.85 ± 3.35	0.440
Waist to Hip ratio	0.93 ± 0.068	0.94 ± 0.074	0.124
Systolic pressure (mmHg)	133.72 ± 17.06	133.79 ± 16.46	0.985
Diastolic pressure (mmHg)	83.10 ± 10.91	82.22 ± 11.57	0.324
Blood glucose (mmol/l)	5.58 ±1.31	5.37 ± 1.09	0.042
Uric acid (umol/l)	390.99 ± 125.55	372.90 ± 121.27	0.070
Total cholesterol (mmol/ll)	4.84 ± 0.94	4.89 ± 0.97	0.501
Triglycerides (mmol/l)	1.88 ± 1.65	1.99 ± 1.57	0.422
Creatinine (umol/l)	87.00 ± 23.23	85.47 ± 23.67	0.415
Urea nitrogen (mmol/l)	5.51 ± 1.72	5.42 ± 1.50	0.495

Data are represented as the mean-standard deviation.

Table 6. Multiple regression analysis of the association rs12129861 and rs1967017 polymorphism and otherfactors with sUA levels in gout and controls

Variable	Regression coefficient (beta)	95% CI	*P* Value
(Intercept)	256.932	177.104-336.761	
rs12129861	0.028		0.397
rs1967017	0.060		0.065
Age	-0.141	-1.741-0.650	< 0.001
BMI	0.114	1.822-6.679	< 0.001
Triglycerides	0.266	15.267-25.253	< 0.001

types in *PDZK1* gene showed that A-C, A-T and G-T haplotypes (OR = 0.666 (95% CI = 0.412~1.076); OR = 1.017 (95% CI = 0.511~2.026) and OR = 1.340 (95% CI = 0.892~2.012) in Chinese Han population were not associated with the pathogenesis of kidney stones in patients with primary gout (*P* > 0.05) (Table S3).

Discussion

PDZK1, which encodes PDZ domain containing 1, acts as a scaffolding protein for a large variety of transporter and regulatory proteins and has been identified in the kidney, liver, small intestine, and adrenal cortex [12]. Within the kidney, *PDZK1* is localized in the apical membrane of the proximal tubule. *PDZK1* is a 519-amino-acid protein that contains four PDZ-binding domains (PSD-95, DglA, and ZO-1) ranging from 80 to 90 amino acids in length and typically binds to proteins that contain the tripeptide motif (S/T)-X-Ø (X = any amino acid and Ø = a hydrophobic residue) at their C terminus [3, 12]. Each of the four PDZ-binding domains of *PDZK1* independently binds to a sequence-specific PDZ motif at the carboxy-terminal end of transporter proteins. PDZ domains are thought to play important roles in the targeting of proteins to specific cell membranes, the assembly of proteins into signaling complexes for efficient transduction, and the regulation of the function of transporters. PDZ-binding domains have been identified in various proteins and are known to be modular protein-protein recognition domains that play a role in protein targeting and protein complex assembly [13]. These multidomain molecules not only target and provide scaffolds for protein-protein interactions but also modulate the function of receptors and ion channels with which they associate. Several PDZ proteins, such as NHERF1, NHERF2, and PDZK1, were recently identified in the proximal tubules, and these are hypothesized to be important for the generation and maintenance of epithelial polarity and the formation of large protein complexes [14-16]. The disruption of the association between PDZ proteins and their targets contributes to the pathogenesis of a number of human diseases likely due to the failure of PDZ proteins to appropriately target and modulate the actions of their associated proteins. Kolz et al. and van der Harst et al. demonstrated that one of the five new loci that influence uric acid concentrations is near *PDZK1* [4, 5].

In this study, we provide further evidence supporting a role of the *PDZK1* locus {rs12129861 [OR = 0.727 (95% CI = 0.562~0.940), *P* = 0.015] and rs1967017 [OR = 0.705 (95% CI = 0.530~0.938), *P* = 0.016]} in gout in a male Han Chinese population. The G-T and A-C haplotypes [OR = 1.394 (95% CI = 1.078~1.803), *P* = 0.011; OR = 0.741 (95% CI = 0.550~0.997), *P* = 0.047] were significantly associated with the development of gout. This study provides

the first indication of an association between two SNPs in the *PDZK1* gene and the development of gout in a male Han Chinese population.

We found blood glucose in GG group was higher than that in AA+GA group of rs12129861 genotype of *PDZK1* gene (5.61 ± 1.34 vs 5.34 ± 1.05, $P < 0.05$) and that in TT group was higher than that in CC+TC group of rs1967017 genotype of *PDZK1* gene (5.58 ± 1.31 vs. 5.37 ± 1.09, $P < 0.05$). But the specific mechanism is still not clear and need to be further studied. We also found no association between two SNPs in the *PDZK1* gene and the development of kidney stones in Chinese patients with primary gout.

In combination with these findings, previous researchers have also identified a genome-wide significant association of the SNPs in and upstream of *PDZK1*, which encodes for the scaffolding protein PDZ domain containing 1 that interacts with OAT4, URAT1, and NTP1 (SLC17A1) via their C-terminal PDZ motifs [2, 3]. Because URAT1 has a PDZ motif at its C terminus, researchers have speculated that the urate transport function of URAT1 may be regulated by its protein-protein interactions via the PDZ motif and employed the yeast two-hybrid method to screen a human kidney cDNA library using the C terminus of URAT1 as bait. As a result, it has been discovered that *PDZK1* is a binding partner for the urate transporter URAT1 [3]. Subsequently, the interactions of PDZK1 with the renal apical organic anion transporter OAT4 (SLC22A11) [17] as one of the five new loci that influence uric acid concentrations by Kolz et al. [4], the renal apical peptide transporter PEPT2 [18], NPT1 [19], and NPT4 [20] were discovered. The urate transporters URAT1, OAT4, and NPT1 interact with PDZK1 via a class I PDZ motif (-S/T-X-Φ, where X is any amino acid and Φ is a hydrophobic amino acid) [3, 12, 17, 19]. It has been proposed that the PDZ scaffold may form a bidirectional transport system by linking URAT1 (reabsorption) and NPT1 (secretion), leading to a functional complex that is responsible for the regulation of urate transport at the apical membrane of renal proximal tubules [2, 21]. These studies also detected interactions with other apical transporters, such as *NPT1* with *PDZK1*, and found a similar enhancement of the transport activities after *PDZK1* coexpression [19]. In addition to *PDZK1*, whose expression has been detected on the luminal side of the proximal tubules, where it binds to some transporters, researchers have speculated that membrane transport proteins are tethered by intracellular scaffolding proteins, such as PDZK1, and constitute a molecular complex at the plasma membrane that acts as a functional unit of the 'membrane transportome'. A previous study proposed the urate-transporting molecular complex (urate transportome) as a model of urate transport in the luminal membrane of renal proximal tubules [2]. According to this model, renal urate transport should be evaluated not only from the viewpoint of a single transporter, such as URAT1, but also from the viewpoint of a functional unit composed of urate transporters and other molecules supported by protein-protein interactions mediated by PDZK1. We believe that this concept is appropriate for the consideration of the physiological function of urate transport in the kidney.

In conclusion, our study provides further evidence that *PDZK1* polymorphisms are associated with the development of gout in a male Han Chinese population. A more in-depth investigation of these loci and functional experiments are needed in the future to unravel the complex mechanism by which this region is pathologically involved in gout.

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Disclosure of conflict of interest

None.

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References

[1] So A and Thorens B. Uric acid transport and disease. J Clin Invest 2010; 120: 1791-1799.

[2] Anzai N, Kanai Y and Endou H. New insights into renal transport of urate. Curr Opin Rheumatol 2007; 19: 151-157.

[3] Anzai N, Miyazaki H, Noshiro R, Khamdang S, Chairoungdua A, Shin HJ, Enomoto A, Sakamoto S, Hirata T, Tomita K, Kanai Y and Endou H. The multivalent PDZ domain-containing protein PDZK1 regulates transport activity of renal urate-anion exchanger URAT1 via its C terminus. J Biol Chem 2004; 279: 45942-45950.

[4] Kolz M, Johnson T, Sanna S, Teumer A, Vitart V, Perola M, Mangino M, Albrecht E, Wallace C, Farrall M, Johansson A, Nyholt DR, Aulchenko Y, Beckmann JS, Bergmann S, Bochud M, Brown M, Campbell H, Connell J, Dominiczak A, Homuth G, Lamina C, McCarthy MI, Meitinger T, Mooser V, Munroe P, Nauck M, Peden J, Prokisch H, Salo P, Salomaa V, Samani NJ, Schlessinger D, Uda M, Volker U, Waeber G, Waterworth D, Wang-Sattler R, Wright AF, Adamski J, Whitfield JB, Gyllensten U, Wilson JF, Rudan I, Pramstaller P, Watkins H, Doering A, Wichmann HE, Spector TD, Peltonen L, Volzke H, Nagaraja R, Vollenweider P, Caulfield M, Illig T and Gieger C. Meta-analysis of 28,141 individuals identifies common variants within five new loci that influence uric acid concentrations. PLoS Genet 2009; 5: e1000504.

[5] van der Harst P, Bakker SJ, de Boer RA, Wolffenbuttel BH, Johnson T, Caulfield MJ and Navis G. Replication of the five novel loci for uric acid concentrations and potential mediating mechanisms. Hum Mol Genet 2010; 19: 387-395.

[6] Sulem P, Gudbjartsson DF, Walters GB, Helgadottir HT, Helgason A, Gudjonsson SA, Zanon C, Besenbacher S, Bjornsdottir G, Magnusson OT, Magnusson G, Hjartarson E, Saemundsdottir J, Gylfason A, Jonasdottir A, Holm H, Karason A, Rafnar T, Stefansson H, Andreassen OA, Pedersen JH, Pack AI, de Visser MC, Kiemeney LA, Geirsson AJ, Eyjolfsson GI, Olafsson I, Kong A, Masson G, Jonsson H, Thorsteinsdottir U, Jonsdottir I and Stefansson K. Identification of low-frequency variants associated with gout and serum uric acid levels. Nat Genet 2011; 43: 1127-1130.

[7] Yang Q, Kottgen A, Dehghan A, Smith AV, Glazer NL, Chen MH, Chasman DI, Aspelund T, Eiriksdottir G, Harris TB, Launer L, Nalls M, Hernandez D, Arking DE, Boerwinkle E, Grove ML, Li M, Linda Kao WH, Chonchol M, Haritunians T, Li G, Lumley T, Psaty BM, Shlipak M, Hwang SJ, Larson MG, O'Donnell CJ, Upadhyay A, van Duijn CM, Hofman A, Rivadeneira F, Stricker B, Uitterlinden AG, Pare G, Parker AN, Ridker PM, Siscovick DS, Gudnason V, Witteman JC, Fox CS and Coresh J. Multiple genetic loci influence serum urate levels and their relationship with gout and cardiovascular disease risk factors. Circ Cardiovasc Genet 2010; 3: 523-530.

[8] Stark K, Reinhard W, Grassl M, Erdmann J, Schunkert H, Illig T and Hengstenberg C. Common polymorphisms influencing serum uric acid levels contribute to susceptibility to gout, but not to coronary artery disease. PLoS One 2009; 4: e7729.

[9] Wallace SL, Robinson H, Masi AT, Decker JL, McCarty DJ and Yu TF. Preliminary criteria for the classification of the acute arthritis of primary gout. Arthritis Rheum 1977; 20: 895-900.

[10] Li Z, Zhang Z, He Z, Tang W, Li T, Zeng Z, He L and Shi Y. A partition-ligation-combination-subdivision EM algorithm for haplotype inference with multiallelic markers: update of the SHEsis (http://analysis.bio-x.cn). Cell Res 2009; 19: 519-523.

[11] Shi YY and He L. SHEsis, a powerful software platform for analyses of linkage disequilibrium, haplotype construction, and genetic association at polymorphism loci. Cell Res 2005; 15: 97-98.

[12] Kocher O, Comella N, Tognazzi K and Brown LF. Identification and partial characterization of PDZK1: a novel protein containing PDZ interaction domains. Lab Invest 1998; 78: 117-125.

[13] Hung AY and Sheng M. PDZ domains: structural modules for protein complex assembly. J Biol Chem 2002; 277: 5699-5702.

[14] Biber J, Gisler SM, Hernando N, Wagner CA and Murer H. PDZ interactions and proximal tubular phosphate reabsorption. Am J Physiol Renal Physiol 2004; 287: F871-875.

[15] Brone B and Eggermont J. PDZ proteins retain and regulate membrane transporters in polarized epithelial cell membranes. Am J Physiol Cell Physiol 2005; 288: C20-29.

[16] Hernando N, Wagner CA, Gisler SM, Biber J and Murer H. PDZ proteins and proximal ion transport. Curr Opin Nephrol Hypertens 2004; 13: 569-574.

[17] Miyazaki H, Anzai N, Ekaratanawong S, Sakata T, Shin HJ, Jutabha P, Hirata T, He X, Nonoguchi H, Tomita K, Kanai Y and Endou H. Modulation of renal apical organic anion transporter 4 function by two PDZ domain-containing proteins. J Am Soc Nephrol 2005; 16: 3498-3506.

[18] Noshiro R, Anzai N, Sakata T, Miyazaki H, Terada T, Shin HJ, He X, Miura D, Inui K, Kanai Y and Endou H. The PDZ domain protein PDZK1 interacts with human peptide trans-

porter PEPT2 and enhances its transport activity. Kidney Int 2006; 70: 275-282.

[19] Jutabha P, Anzai N and Endou H. Interaction of the multivalent PDZ domain protein PDZK1 with type I sodium-phosphate cotransporter (NPT1). J Am Soc Nephrol 2005; 16: 350A.

[20] Fukutomi T, Anzai N, Jutabha P, Kanai Y and Sakurai H. Interaction of the multivalent PDZ proteins with sodium-phosphate transporter 4 (NPT4). J Pharmacol Sci 2011; 115: 68P.

[21] Taniguchi A and Kamatani N. Control of renal uric acid excretion and gout. Curr Opin Rheumatol 2008; 20: 192-197.

Table S1. Demographic and clinical characteristics (Mean ± SD) in gout group with kidney stones (S) (*n* = 241) and gout group without kidney stones (G) (*n* = 159)

Parameter	G (*n* = 241)	S (*n* = 159)	P
Age (year)	51.15 ± 13.68	54.21 ± 12.56	0.022
Body mass index (kg/m^2)	25.92 ± 3.06	26.23 ± 2.88	0.299
Waist to Hip ratio	0.92 ± 0.06	0.92 ± 0.05	0.516
Systolic pressure (mmHg)	134.03 ± 16.92	134.67 ± 16.16	0.702
Diastolic pressure (mmHg)	84.52 ± 10.50	85.92 ± 10.37	0.189
Blood glucose (mmol/l)	5.47 ± 1.50	5.40 ± 1.32	0.650
Uric acid (umol/l)	471.47 ± 113.70	486.69 ± 99.21	0.158
Total cholesterol (mmol/ll)	5.03 ± 1.05	5.11 ± 1.02	0.443
Triglycerides (mmol/l)	2.46 ± 2.13	2.29 ± 1.70	0.376
Creatinine (umol/l)	91.77 ± 31.31	94.42 ± 27.41	0.372
Urea nitrogen (mmol/l)	5.52 ± 1.99	5.81 ± 2.05	0.156

Data are represented as the mean-standard deviation.

Table S2. Genotype distribution and relative allele frequencies of rs12129861 and rs1967017 polymorphismof *PDZK1* gene in Chinese in gout group with kidney stones (S) (*n* = 241) and gout group without kidneystones (G) (*n* = 159)

SNP		Distribution, n (%)			OR 95% CI	*P* value
rs12129861	Genotype	A/A	A/G	G/G		
	S	4(2.5)	33 (20.8)	122 (76.7)		0.348
	G	8 (3.3)	64 (26.6)	169 (70.1)		
	Allele	A	G	A vs G		
	S	41 (12.9)	277 (87.1)	0.744	0.495~1.117	0.152
	G	80 (16.6)	402 (83.4)			
rs1967017	Genotype	C/C	C/T	T/T		
	S	2 (1.3)	26 (16.4)	131 (82.4)		0.341
	G	5 (2.1)	52 (21.6)	184 (76.3)		
	Allele	C	T	C vs T		
	S	30 (9.4)	288 (90.6)	0.706	0.445~1.119	0.137
	G	62 (12.9)	420 (87.1)			

OR, odds ratio; SNP, single nucleotide polymorphism; CI, confidence interval.

Table S3. Association of two-marker rs12129861-rs1967017 haplotypes with gout group with kidney stones (S) and gout group without kidney stones (G)

Haplotype	Frequency		OR (95% CI)	*P* Value
	S	G		
A-C	26.87 (8.5)	58.87 (12.2)	0.666 [0.412~1.076]	0.095
A-T	14.13 (4.4)	21.13 (4.4)	1.017 [0.511~2.026]	0.961
G-T	273.87 (86.1)	398.87 (82.8)	1.340 [0.892~2.012]	0.158