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Original Article

Associations of gout with polymorphisms in *SLC2A9, WDR1, CLNK, PKD2,* and *ABCG2* in Chinese Han and Tibetan populations

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Abstract: Gout is a common inflammatory arthritis triggered by the presence of monosodium urate (MSU) crystals in joints and connective tissues. Current evidence suggests that heredity contributes to gout progression. It is increasingly assumed that gout occurs when serum uric acid (SUA) levels exceed the physiological saturation threshold for uric acid. This study aims to investigate whether variations in *SLC2A9*, *WDR1*, *CLNK*, *PKD2*, and *ABCG2* are associated with gout in Chinese Han and Tibetan populations. In this case-control study, 47 single nucleotide polymorphisms (SNPs) were analyzed in 438 cases (139 Han, 299 Tibetan) and 623 controls (309 Han, 314 Tibetan). The SNP-gout association analyses were performed with SPSS software, Sequenom MassARRAY RS1000, the Haploview software package, and the Chi-squared test. Using the Chi-squared test and analyses of genetic models, rs10034180 and rs16869430 in *CLNK*, rs717614 in *WDR1*, and rs12505410 in *ABCG2* showed associations with increased gout susceptibility in Han populations. In contrast, rs7663032 and rs10022499 in *SLC2A9* showed a reduced risk of gout in the recessive model. Additionally, in Tibetan populations, rs2108878 in *CLNK*, rs2725212, rs2725210, rs2725207, rs2728132, rs2725205, and rs2725203 in *PKD2* showed an increase in the risk of gout, whereas rs12505410 in *ABCG2* reduced the risk of gout. In summary, these data suggest significant associations between *SLC2A9*, *WDR1*, *CLNK*, *PKD2*, and *ABCG2* polymorphisms and the development of gout in Chinese Han and Tibetan populations.

Keywords: Gout, hyperuricemia, single nucleotide polymorphism, polymorphism, case-control studies

Introduction

Gout is a common inflammatory arthropathy that results from chronically elevated levels of uric acid, which can lead to tendonitis, bone damage, and tophus deposition [1]. The clinical manifestations of gout include severe pain, joint swelling, and skin erythema. The primary risk factor for gout is a SUA level that exceeds the physiological saturation threshold for uric acid [2]. In China, gout is commonly seen in men and postmenopausal women. Epidemiological studies have suggested that up to 2% of males over the age of 30 years and females over the age of 50 years will develop gout at some time. In 2001 [3], the prevalence of gout was only 0.33% in Shanghai. In 2009, Yu-Hong Jia et al. [4] reported an increased prevalence of gout of as high 1.21% in Tangshan. In recent years, the prevalence and incidence of gout are increasing due to changes in dietary habits, lifestyle, and environmental factors, such as excessive alcohol consumption, excessive calorie consumption, and high-protein diets [5], especially the environmental exposure and genetic factors play crucial important roles in the development of gout. Uric acid is the end-product of purine metabolism in humans, and

~75% of the uric acid produced daily is excreted by the kidney. In the majority of patients with gout, overproduction of uric acid or reduced renal excretion of uric acid may result in hyperuricemia [6]. Hyperuricemia has long been recognized as the most important predisposing factor for gout, but the great majority of persons with hyperuricemia never develop gout. Polymorphisms or genetic mutations are also associated with hyperuricemia and gout. Hypertension, insulin resistance, obesity, diabetes, and kidney disease are also associated with hyperuricemia and gout [7], thus genetic factors may play important roles in determining susceptibility to gout [8].

Previous genome-wide association studies (GWAS) identified many common single nucleotide polymorphisms (SNPs) in genes associated with elevated serum urate levels [9, 10], and SNPs at these loci explained 5-6% of the variance in serum urate concentrations [11]. These genes, *SLC2A9*, *WDR1*, *CLNK*, *PKD2*, and *ABCG2*, are located on chromosome 4, are associated with high SUA levels, and show the greatest variation and influence on SUA levels, especially in populations of European ancestry [10, 12, 13].

Xizang is located on the southwest China. This region is populated by a variety of ethnicities, but primarily by the Tibetan. The Tibetan has a unique genetic background, dietary and lifestyle habits. It is not known whether a difference in gout prevalence associated with *SLC2A9, WDR1, CLNK, PKD2,* and *ABCG2* gene is present between the Han and Tibetan populations. To investigate potential relationships between SNP polymorphisms, haplotypes, and locus-locus interactions in the etiology of gout, we performed a case-control association analysis in Chinese Han and Tibetan populations.

Study population

We recruited a total of 1061 subjects (438 cases, 623 controls) for this case-control study. All of the 438 gout patients came from the Second People's Hospital of Tibet Autonomous Region and the Affiliated Hospital of Xizang Minzu University in Xianyang City, Shanxi, China. Patients were diagnosed with gout according to the 1977 ARA preliminary classification criteria for acute gout between September 2011 and May 2013 [14]. All of the gout patients were recruited without age and gender restrictions.

Additionally, during the same period, 623 unrelated healthy individuals from the Affiliated Hospital of Xizang Minzu University in Xianyang City, Shanxi, China were chosen to serve as controls. These unrelated healthy individuals had no histories of nephropathy or other chronic diseases that can lead to high SUA concentrations. Subjects with blood disorders or other severe endocrinological diseases, as well as patients who had consumed alcohol before recruitment were also excluded. We obtained clinical information for the patients through consultations with their treating physicians or from reviews of their medical charts. The ethics Committee of the Affiliated Hospital of Xizang Minzu University approved our use of blood samples and our protocol. We also obtained signed informed consent from all of the subjects.

SNP selection and genotyping

We selected SNPs from previously published reports on polymorphisms associated with gout [10, 12, 15]. We identified 47 genome-wide SNPs with significance in *SLC2A9, WDR1, CLNK, PKD2*, and *ABCG2* with minor allele frequencies (MAFs) >5% in Chinese Han and Tibetan populations. Genomic DNA was extracted from the whole blood samples from the 438 cases and the 623 controls using a Blood DNA Extraction Kit (GoldMag Co. Ltd., Xi'an City, China), and DNA concentrations were measured using a NanoDrop 2000 (Thermo Scientific, Waltham, Massachusetts, USA). MassARRAY Assay Design 3.0 Software (Sequenom, San Diego, CA, USA) was used to design the Multiplexed SNP MassEXTEND assay [16], and SNP genotyping was performed using the Sequenom MassARRAY RS1000 (Sequenom Inc., San Diego, CA, USA) system according to the standard protocol. Sequenom Typer 4.0 Software (Sequenom Inc., San Diego, California, USA) was used to manage and analyze the data [16].

Statistical analysis

We used SPSS version 17.0 statistical software (SPSS Inc., Chicago, IL, United States) and Excel software (Microsoft Corp., Redmond, WA, United States) for statistical analyses. The Chi-

Table 1. Basic characteristics of the control individuals and patients with gout

Variables	Han		*p*-value	Tibet		*p*-value
	Case (n=139)	Control (n=309)		Case (n=299)	Control (n=314)	
Sex			< 0.001			< 0.001
Male	121	197		173	235	
Female	18	112		126	79	

p-values were calculated by the two-sided Chi-squared test.

squared test and the Student's t-test were used to compare genotype frequencies between patients and controls. The Hardy-Weinberg equilibrium (HWE) of each SNP was determined by the Chi-squared test, which compared the actual and expected frequencies of the genotypes in the controls. Genetic associations between SNPs and the risk of gout were tested with various genetic models, including codominant, dominant, recessive, and log-additive models, using SNP Stats Software (http://bioinfo.iconcologia.net). The effects of the polymorphisms on the risk of gout were expressed as odds ratios (ORs) with 95% confidence intervals (95% CIs) [17]. A linkage disequilibrium (LD) analysis was performed with genotype data from all of the subjects. All of the statistical tests were two-sided, and *p*-values < 0.05 were considered statistically significant.

Results

Our case-control analysis included 1061 subjects, including 438 cases (139 Han, mean age 43.3 ± 13.7 years; and 299 Tibetan, mean age 54.9 ± 16.9 years) and 623 controls (309 Han, mean age 49.1 ± 7.9 years; and 314 Tibetan). The basic characteristics of the patients and the control individuals are summarized (**Table 1**). The MAFs of the SNPs genotyped in the case and control groups are summarized (**Table 2**). Two SNPs, rs9291640 and rs2231164, deviated and were excluded when calculating the HWE in the Tibetan population. We compared the frequencies of the alleles in the different groups by the Chi-squared test, and we found that rs717614, rs10034180, rs16869430, and rs12505410 associated significantly with increased gout susceptibility in the Han population. We also found that rs2108878, rs2725212, rs2725210, rs2725207, rs2728132, rs2725205, rs2725203, and rs12505410 associated with an increased risk of gout in the Tibetan population ($P < 0.05$).

We used various genetic models with unconditional logistic regression adjusted for age and gender to investigate associations between these SNPs and gout in Chinese Han and Tibetan populations (**Table 3**). When we included the age and gender of the subjects, we found that the "T" allele of rs6823877 (OR = 0.67, $P = 0.036$) in *SLC2A9* correlated with a reduced risk of gout. Conversely, the "G" allele of rs717614 (OR = 1.45, $P = 0.024$) in *WDR1*, the "A" allele of rs10034180 (OR = 1.46, $P = 0.02$), the "T" allele of rs16869430 (OR = 1.76, $P = 0.0007$), the "C" allele of rs2041216 (OR = 1.44, $P = 0.025$), the "G" allele of rs997219 (OR = 1.46, $P = 0.02$) in *CLNK*, and the "G" allele of rs12505410 (OR = 1.59, $P = 0.0039$) in *ABCG2* associated with increased gout susceptibility in the log-additive model. In the codominant model, the "GG" genotype of rs717614 (OR = 2.41, $P = 0.026$), the "AG" genotype of rs10034180 (OR = 1.76, $P = 0.038$), the "CT" (OR = 2.39, $P = 0.024$) and "TT" (OR = 2.41, $P = 0.024$) genotypes of rs16869430, the "CT" genotype of rs2041216 (OR = 1.81, $P = 0.039$), the "AG" genotype of rs997219 (OR = 1.84, $P = 0.032$), and the "GG" genotype of rs12505410 (OR = 2.72, $P = 0.012$) associated with an increased risk of gout. We identified six significant SNP genotypes associated with increased gout susceptibility in the dominant model. They were genotype "A/G-A/A" of rs10034180 (OR = 1.79, $P = 0.011$), genotype "C/T-T/T" of rs16869430 (OR = 2.39, $P = 0.0001$), genotype "C/T-C/C" of rs2041216 (OR = 1.83, $P = 0.011$), genotype "A/G-G/G" of rs997219 (OR = 1.86, $P = 0.0087$), genotype "G/T-G/G" of rs12505410 (OR = 1.65, $P = 0.026$), and genotype "C/T-T/T" of rs2622624 (OR = 1.61, $P = 0.039$). In the recessive model, the genotype "GG" of rs717614 (OR = 2.28, $P = 0.0077$) and the genotype "GG" of rs12505410 (OR = 2.29, $P = 0.0091$) associated with an increased risk of gout.

The allele "C" (OR = 0.40, $P = 0.026$), the "TC" (OR = 0.21, $P = 0.037$) genotype, and the "T/C-C/C" (OR = 0.24, $P = 0.011$) genotype of rs2231164 in *ABCG2* correlated with reduced

Table 2. Basic information on candidate SNPs analyzed in this study

SNP ID	Gene	Band	Alleles A/B	Han					Tibet				
				MAF		HWE	OR (95% CI)	p^b-value	MAF		HWE	OR (95% CI)	p^b-value
				Case	Control	p^a-value			Case	Control	p^a-value		
rs6823877	SLC2A9	4p16.1	T/C	0.213	0.275	1.000	0.714 (0.508-1.003)	0.052	0.256	0.283	0.889	0.872 (0.673-1.130)	0.300
rs16890979	SLC2A9	4p16.1	T/C	0.011	0.010	1.000	1.112 (0.276-4.481)	0.832	0.050	0.038	0.366	1.329 (0.768-2.301)	0.308
rs7675964	SLC2A9	4p16.1	T/C	0.457	0.469	0.647	0.952 (0.716-1.265)	0.735	0.448	0.428	0.207	1.084 (0.865-1.358)	0.485
rs4697698	SLC2A9	4p16.1	T/C	0.475	0.474	0.648	1.002 (0.755-1.331)	0.984	0.453	0.435	0.301	1.078 (0.860-1.350)	0.515
rs4235346	SLC2A9	4p16.1	C/T	0.471	0.472	0.733	0.994 (0.748-1.321)	0.969	0.453	0.438	0.357	1.066 (0.850-1.335)	0.581
rs4697701	SLC2A9	4p16.1	A/G	0.435	0.461	0.425	0.900 (0.677-1.197)	0.471	0.420	0.406	0.349	1.058 (0.843-1.328)	0.627
rs1122141	SLC2A9	4p16.1	T/C	0.475	0.468	0.648	1.029 (0.775-1.366)	0.842	0.451	0.435	0.302	1.070 (0.854-1.341)	0.558
rs998675	SLC2A9	4p16.1	C/T	0.478	0.468	0.648	1.044 (0.786-1.386)	0.765	0.441	0.435	0.302	1.028 (0.820-1.228)	0.812
rs7375599	SLC2A9	4p16.1	A/G	0.478	0.464	0.570	1.057 (0.796-1.404)	0.697	0.443	0.439	0.302	1.016 (0.810-1.273)	0.892
rs13103879	SLC2A9	4p16.1	T/C	0.478	0.460	0.493	1.078 (0.812-1.432)	0.600	0.440	0.439	0.252	1.001 (0.799-1.255)	0.991
rs11723970	SLC2A9	4p16.1	C/T	0.486	0.459	0.731	1.110 (0.835-1.475)	0.470	0.443	0.443	0.210	1.001 (0.799-1.255)	0.992
rs882223	SLC2A9	4p16.1	C/A	0.486	0.453	1.000	1.139 (0.858-1.513)	0.366	0.445	0.443	0.210	1.009 (0.805-1.264)	0.940
rs13125646	SLC2A9	4p16.1	A/G	0.007	0.011	1.000	0.632 (0.130-3.064)	0.832	0.050	0.037	0.342	1.389 (0.797-2.240)	0.244
rs7663032	SLC2A9	4p16.1	C/T	0.450	0.482	0.110	0.877 (0.660-1.166)	0.368	0.409	0.409	0.201	1.001 (0.797-1.257)	0.995
rs3775948	SLC2A9	4p16.1	G/C	0.356	0.405	0.637	0.814 (0.607-1.091)	0.169	0.308	0.293	1.000	1.072 (0.840-1.369)	0.575
rs3733588	SLC2A9	4p16.1	G/A	0.356	0.405	0.637	0.814 (0.607-1.091)	0.169	0.309	0.291	1.000	1.089 (0.853-1.391)	0.493
rs13113918	SLC2A9	4p16.1	A/G	0.004	0.016	1.000	0.219 (0.027-1.723)	0.210	0.030	0.029	1.000	1.052 (0.542-2.041)	0.882
rs6853437	SLC2A9	4p16.1	G/A	0.453	0.481	0.137	0.896 (0.674-1.190)	0.449	0.409	0.403	0.160	1.027 (0.818-1.291)	0.816
rs10022499	SLC2A9	4p16.1	C/A	0.460	0.482	0.088	0.916 (0.689-1.216)	0.546	0.425	0.406	0.199	1.081 (0.861-1.358)	0.500
rs9291640	SLC2A9	4p16.1	C/T	0.463	0.484	0.206	0.921 (0.692-1.227)	0.577	0.427	0.413	0.035	1.060 (0.844-1.332)	0.617
rs6826764	SLC2A9	4p16.1	G/C	0.450	0.471	0.254	0.918 (0.691-1.220)	0.557	0.373	0.376	0.400	0.988 (0.784-1.245)	0.917
rs717614	WDR1	4p16.1	G/C	0.434	0.359	0.109	1.372 (1.026-1.834)	0.032	0.480	0.492	0.573	0.951 (0.759-1.192)	0.664
rs9790491	CLNK	4p16.1	G/A	0.194	0.193	0.465	1.010 (0.706-1.446)	0.953	0.247	0.236	0.116	1.067 (0.821-1.386)	0.629
rs3749558	CLNK	4p16.1	T/C	0.193	0.194	0.465	0.997 (0.695-1.430)	0.989	0.241	0.238	0.087	1.019 (0.782-1.328)	0.887
rs12504795	CLNK	4p16.1	C/T	0.173	0.194	0.466	0.866 (0.598-1.253)	0.445	0.252	0.232	0.154	1.116 (0.858-1.451)	0.414
rs10034180	CLNK	4p16.1	A/G	0.424	0.341	0.614	1.422 (1.064-1.901)	0.017	0.371	0.341	1.000	1.142 (0.903-1.444)	0.268
rs16869430	CLNK	4p16.1	T/C	0.399	0.294	0.170	1.592 (1.184-2.140)	0.002	0.354	0.328	0.702	1.120 (0.884-1.420)	0.347
rs2041216	CLNK	4p16.1	C/T	0.453	0.390	1.000	1.296 (0.973-1.728)	0.076	0.396	0.377	0.810	1.080 (0.858-1.360)	0.513
rs997219	CLNK	4p16.1	G/A	0.453	0.386	1.000	1.316 (0.988-1.753)	0.060	0.395	0.373	0.716	1.097 (0.870-1.383)	0.433
rs2108878	CLNK	4p16.1	C/T	0.338	0.343	0.705	0.978 (0.725-1.319)	0.886	0.372	0.317	0.697	1.280 (1.010-1.621)	0.041
rs2725234	PKD2	4q22.1	T/C	0.230	0.228	0.872	1.011 (0.722-1.416)	0.946	0.087	0.065	1.000	1.364 (0.891-2.087)	0.152

rs2728113	PKD2	4q22.1	G/A	0.230	0.230	0.750	1.002 (0.716-1.403)	0.988	0.087	0.067	1.000	1.329 (0.870-2.028)	0.187
rs2725215	PKD2	4q22.1	T/C	0.201	0.214	0.736	0.928 (0.654-1.318)	0.679	0.059	0.038	1.000	1.565 (0.919-2.663)	0.097
rs2725212	PKD2	4q22.1	G/A	0.471	0.443	0.489	1.119 (0.841-1.488)	0.437	0.426	0.358	0.463	1.333 (1.059-1.678)	0.014
rs2725211	PKD2	4q22.1	T/C	0.205	0.212	0.733	0.958 (0.676-1.359)	0.813	0.059	0.041	1.000	1.439 (0.855-2.422)	0.168
rs2725210	PKD2	4q22.1	G/A	0.475	0.443	0.421	1.135 (0.854-1.507)	0.382	0.425	0.356	0.459	1.334 (1.060-1.680)	0.014
rs2725207	PKD2	4q22.1	A/C	0.477	0.443	0.562	1.147 (0.857-1.534)	0.355	0.432	0.323	0.688	1.593 (1.252-2.028)	0.000
rs2728132	PKD2	4q22.1	C/A	0.474	0.437	0.729	1.161 (0.872-1.547)	0.305	0.418	0.343	0.451	1.376 (1.090-1.737)	0.007
rs2725205	PKD2	4q22.1	A/G	0.467	0.438	0.908	1.123 (0.843-1.496)	0.426	0.410	0.339	0.614	1.355 (1.073-1.711)	0.011
rs2725203	PKD2	4q22.1	C/T	0.478	0.453	0.565	1.107 (0.834-1.471)	0.479	0.441	0.382	0.339	1.277 (1.016-1.604)	0.036
rs2231164	ABCG2	4q22.1	C/T	0.529	0.471	0.731	1.261 (0.950-1.676)	0.108	0.359	0.390	0.024	0.875 (0.688-1.113)	0.277
rs4148157	ABCG2	4q22.1	A/G	0.230	0.228	0.199	1.011 (0.722-1.416)	0.946	0.057	0.045	1.000	1.292 (0.773-2.158)	0.327
rs2054576	ABCG2	4q22.1	G/A	0.230	0.225	0.143	1.027 (0.732-1.439)	0.876	0.051	0.045	1.000	1.144 (0.675-1.939)	0.617
rs12505410	ABCG2	4q22.1	G/T	0.417	0.314	0.895	1.564 (1.167-2.097)	0.003	0.329	0.406	0.294	0.717 (0.567-0.905)	0.005
rs2622626	ABCG2	4q22.1	C/A	0.388	0.348	0.802	1.190 (0.888-1.595)	0.242	0.429	0.462	1.000	0.877 (0.699-1.099)	0.253
rs2622624	ABCG2	4q22.1	T/C	0.392	0.351	0.708	1.191 (0.890-1.596)	0.239	0.433	0.465	1.000	0.879 (0.702-1.101)	0.262
rs2622605	ABCG2	4q22.1	T/C	0.380	0.351	1.000	1.129 (0.840-1.517)	0.418	0.428	0.457	1.000	0.889 (0.707-1.118)	0.314

SNP: Single nucleotide polymorphism; MAF: minor allele frequency; OR: odds ratio; 95% CI: 95% confidence interval; HWE: Hardy-Weinberg equilibrium; SNPs with HWE $P^a < 0.05$ were excluded; $P^b < 0.05$ indicates statistical significance for the allele model.

Table 3. Single loci associations with gout risk in Han populations (logistic regression adjusted for age and gender)

SNPs	Model	Genotype	control_Han	case_Han	OR (95% CI)	*p*-value
rs6823877	Codominant	C/C	162 (52.4%)	83 (61%)	1	0.110
		C/T	124 (40.1%)	48 (35.3%)	0.70 (0.44-1.10)	
		T/T	23 (7.4%)	5 (3.7%)	0.41 (0.14-1.21)	
	Dominant	C/C	162 (52.4%)	83 (61%)	1	0.061
		C/T-T/T	147 (47.6%)	53 (39%)	0.65 (0.42-1.02)	
	Recessive	C/C-C/T	286 (92.6%)	131 (96.3%)	1	0.150
		T/T	23 (7.4%)	5 (3.7%)	0.48 (0.16-1.38)	
	Log-additive	---	---	---	0.67 (0.46-0.98)	0.036
rs717614	Codominant	C/C	120 (39%)	45 (32.9%)	1	0.026
		G/C	155 (50.3%)	65 (47.5%)	1.10 (0.68-1.79)	
		G/G	33 (10.7%)	27 (19.7%)	2.41 (1.24-4.67)	
	Dominant	C/C	120 (39%)	45 (32.9%)	1	0.230
		G/C-G/G	188 (61%)	92 (67.2%)	1.32 (0.84-2.09)	
	Recessive	C/C-G/C	275 (89.3%)	110 (80.3%)	1	0.008
		G/G	33 (10.7%)	27 (19.7%)	2.28 (1.25-4.15)	
	Log-additive	---	---	---	1.45 (1.05-2.00)	0.024
rs10034180	Codominant	G/G	136 (44%)	44 (31.6%)	1	0.038
		A/G	135 (43.7%)	72 (51.8%)	1.76 (1.09-2.85)	
		A/A	38 (12.3%)	23 (16.6%)	1.90 (0.96-3.79)	
	Dominant	G/G	136 (44%)	44 (31.6%)	1	0.011
		A/G-A/A	173 (56%)	95 (68.3%)	1.79 (1.14-2.83)	
	Recessive	G/G-A/G	271 (87.7%)	116 (83.5%)	1	0.310
		A/A	38 (12.3%)	23 (16.6%)	1.39 (0.74-2.60)	
	Log-additive	---	---	---	1.46 (1.06-2.02)	0.020
rs16869430	Codominant	C/C	159 (51.5%)	48 (34.5%)	1	0.001
		C/T	118 (38.2%)	71 (51.1%)	2.39 (1.48-3.85)	
		T/T	32 (10.4%)	20 (14.4%)	2.41 (1.16-4.99)	
	Dominant	C/C	159 (51.5%)	48 (34.5%)	1	0.000
		C/T-T/T	150 (48.5%)	91 (65.5%)	2.39 (1.52-3.77)	
	Recessive	C/C-C/T	277 (89.6%)	119 (85.6%)	1	0.220
		T/T	32 (10.4%)	20 (14.4%)	1.53 (0.78-3.01)	
	Log-additive	---	---	---	1.76 (1.27-2.45)	0.001
rs2041216	Codominant	T/T	115 (37.3%)	39 (28.3%)	1	0.039
		C/T	146 (47.4%)	73 (52.9%)	1.81 (1.10-2.99)	
		C/C	47 (15.3%)	26 (18.8%)	1.89 (0.97-3.67)	
	Dominant	T/T	115 (37.3%)	39 (28.3%)	1	0.011
		C/T-C/C	193 (62.7%)	99 (71.7%)	1.83 (1.14-2.95)	
	Recessive	T/T-C/T	261 (84.7%)	112 (81.2%)	1	0.350
		C/C	47 (15.3%)	26 (18.8%)	1.32 (0.74-2.37)	
	Log-additive	---	---	---	1.44 (1.04-1.98)	0.025
rs997219	Codominant	A/A	116 (37.7%)	39 (28.1%)	1	0.032
		A/G	146 (47.4%)	74 (53.2%)	1.84 (1.12-3.03)	
		G/G	46 (14.9%)	26 (18.7%)	1.93 (0.99-3.76)	
	Dominant	A/A	116 (37.7%)	39 (28.1%)	1	0.009
		A/G-G/G	192 (62.3%)	100 (71.9%)	1.86 (1.16-2.99)	
	Recessive	A/A-A/G	262 (85.1%)	113 (81.3%)	1	0.330

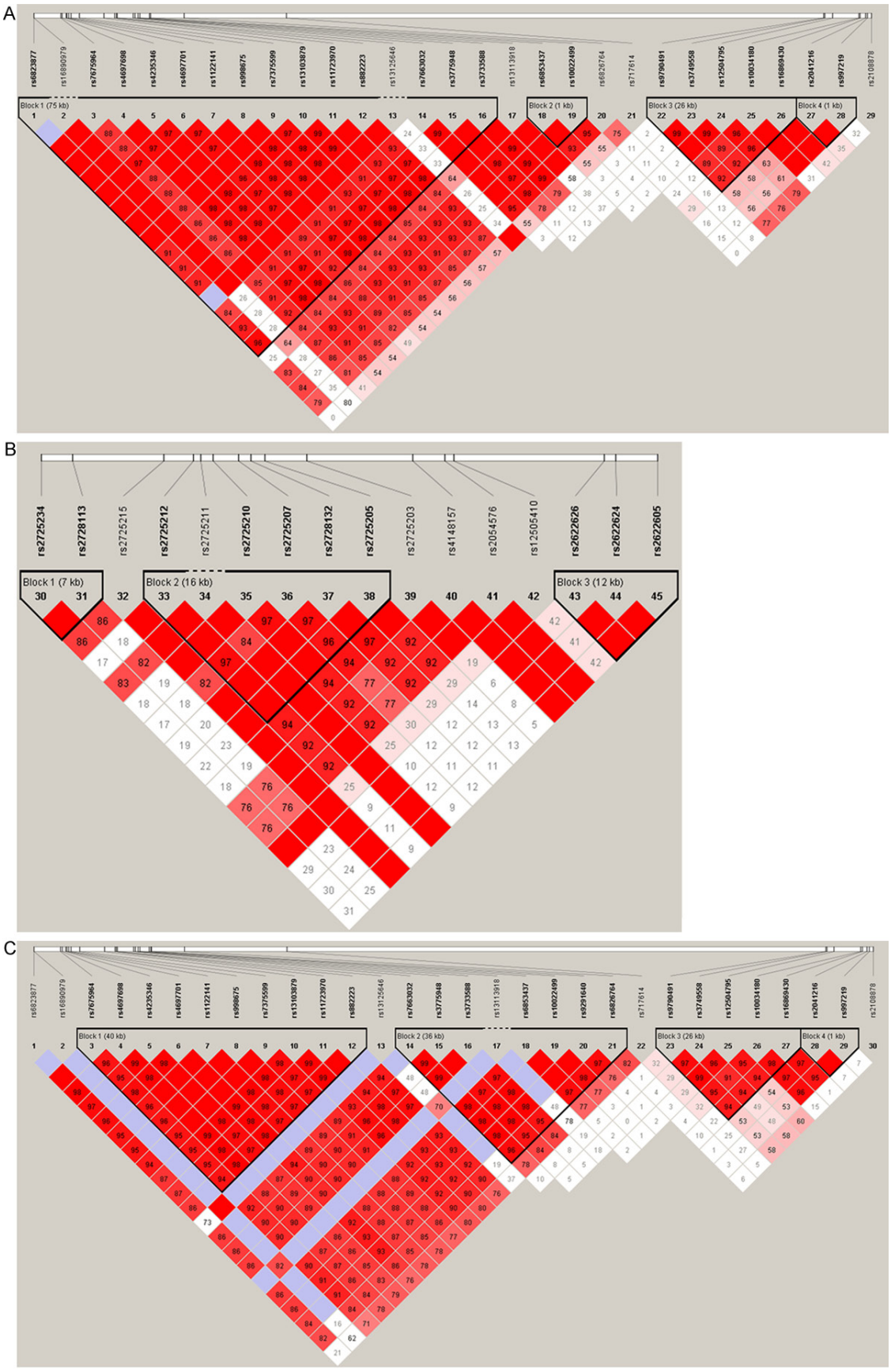
		G/G	46 (14.9%)	26 (18.7%)	1.34 (0.75-2.41)	
	Log-additive	---	---	---	1.46 (1.06-2.00)	0.020
rs12505410	Codominant	T/T	146 (47.2%)	50 (36%)	1	0.012
		G/T	132 (42.7%)	62 (44.6%)	1.41 (0.87-2.26)	
		G/G	31 (10%)	27 (19.4%)	2.72 (1.40-5.31)	
	Dominant	T/T	146 (47.2%)	50 (36%)	1	0.026
		G/T-G/G	163 (52.8%)	89 (64%)	1.65 (1.06-2.58)	
	Recessive	T/T-G/T	278 (90%)	112 (80.6%)	1	0.009
		G/G	31 (10%)	27 (19.4%)	2.29 (1.23-4.25)	
	Log-additive	---	---	---	1.59 (1.16-2.18)	0.004
rs2622624	Codominant	C/C	128 (41.4%)	47 (33.8%)	1	0.096
		C/T	145 (46.9%)	75 (54%)	1.68 (1.05-2.70)	
		T/T	36 (11.7%)	17 (12.2%)	1.33 (0.65-2.75)	
	Dominant	C/C	128 (41.4%)	47 (33.8%)	1	0.039
		C/T-T/T	181 (58.6%)	92 (66.2%)	1.61 (1.02-2.53)	
	Recessive	C/C-C/T	273 (88.3%)	122 (87.8%)	1	1.000
		T/T	36 (11.7%)	17 (12.2%)	1.00 (0.51-1.95)	
	Log-additive	---	---	---	1.28 (0.93-1.78)	0.130

P < 0.05 indicates statistical significance. SNPs: Single nucleotide polymorphisms; OR: odds ratio; CI: confidence interval.

Table 4. Single loci associations with gout risk in Tibetan populations (logistic regression adjusted for age and gender)

SNPs	Model	Genotype	control_Tibetan	case_Tibetan	OR (95% CI)	*p*-value
rs7663032	Codominant	T/T	115 (36.6%)	101 (33.9%)	1	0.077
		C/T	141 (44.9%)	150 (50.3%)	1.73 (0.54-5.59)	
		C/C	58 (18.5%)	47 (15.8%)	0.25 (0.03-1.87)	
	Dominant	T/T	115 (36.6%)	101 (33.9%)	1	0.890
		C/T-C/C	199 (63.4%)	197 (66.1%)	1.08 (0.36-3.27)	
	Recessive	T/T-C/T	256 (81.5%)	251 (84.2%)	1	0.039
		C/C	58 (18.5%)	47 (15.8%)	0.19 (0.03-1.21)	
	Log-additive	---	---	---	0.70 (0.34-1.45)	0.330
rs2231164	Codominant	T/T	114 (40.4%)	124 (43.2%)	1	0.037
		T/C	116 (41.1%)	120 (41.8%)	0.21 (0.06-0.83)	
		C/C	52 (18.4%)	43 (15%)	0.28 (0.05-1.57)	
	Dominant	T/T	114 (40.4%)	124 (43.2%)	1	0.011
		T/C-C/C	168 (59.6%)	163 (56.8%)	0.24 (0.07-0.76)	
	Recessive	T/T-T/C	230 (81.6%)	244 (85%)	1	0.390
		C/C	52 (18.4%)	43 (15%)	0.50 (0.10-2.60)	
	Log-additive	---	---	---	0.40 (0.17-0.96)	0.026
rs10022499	Codominant	A/A	116 (37.1%)	95 (31.8%)	1	0.076
		C/A	140 (44.7%)	154 (51.5%)	1.76 (0.54-5.68)	
		C/C	57 (18.2%)	50 (16.7%)	0.26 (0.04-1.89)	
	Dominant	A/A	116 (37.1%)	95 (31.8%)	1	0.870
		C/A-C/C	197 (62.9%)	204 (68.2%)	1.09 (0.36-3.32)	
	Recessive	A/A-C/A	256 (81.8%)	249 (83.3%)	1	0.040
		C/C	57 (18.2%)	50 (16.7%)	0.19 (0.03-1.22)	
	Log-additive	---	---	---	0.71 (0.34-1.46)	0.350

P < 0.05 indicates statistical significance. SNPs: Single nucleotide polymorphisms; OR: odds ratio; CI: confidence interval.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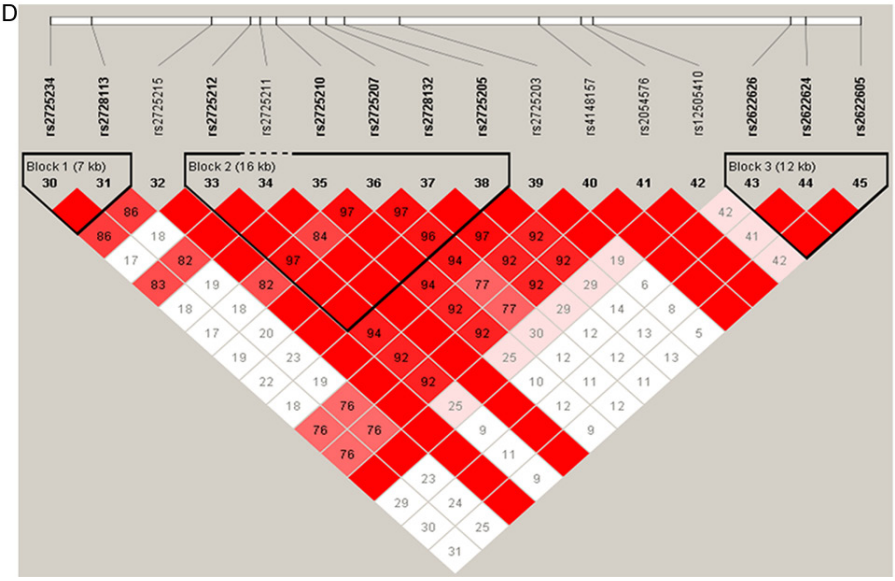

Figure 1. LD analysis of the associations between SNPs and gout. LD maps were shown by D, for 438 case and 623 control individuals. A. All of the SNPs in *SLC2A9, WDR1*, and *CLNK* that were analyzed in Tibetan populations. B. All of the SNPs in *PKD2* and *ABCG2* that were analyzed in Tibetan populations. C. All of the SNPs in *SLC2A9*, *WDR1*, and *CLNK* that were investigated in Han populations. D. All of the SNPs in *PKD2* and *ABCG2* that were analyzed in Han populations.

Table 5. *CLNK* haplotype frequencies and their associations with gout risk in Han subjects

Haplotype	Frequency		OR (95% CI)	p^a	OR (95% CI)	p^b
	Case	Control				
ACTGC	0.430	0.468	1	---	1	0
ACTAT	0.350	0.283	1.21 (0.86-1.71)	0.280	1.36 (0.93-1.99)	0.120
GTCGC	0.145	0.181	0.82 (0.52-1.28)	0.380	0.87 (0.53-1.42)	0.580
ACTAC	0.025	0.047	0.62 (0.27-1.44)	0.270	0.46 (0.18-1.14)	0.094
GTCAT	0.028	0.007	12.10 (1.48-99.10)	0.021	17.12 (1.42-206.34)	0.026

$P \leq 0.05$ indicates statistical significance. The SNPs are rs9790491, rs3749558, rs12504795. rs10034180, and rs16869430. P^a values were calculated using the two-sided Chi-squared test. p^b values were calculated by unconditional logistic regression adjusted for age and gender.

gout susceptibility in the log-additive, codominant, and in the dominant model, respectively (**Table 4**). The "CC" genotype of rs7663032 (OR = 0.19, P = 0.039) and rs10022499 (OR = 0.19, P = 0.04) in *SLC2A9* showed reduced risk in the recessive model. Furthermore, we performed an LD analysis, in which we analyzed all of the loci and the haplotypes of the 47 SNPs distributed in *SLC2A9, WDR1, CLNK, PKD2,* and *ABCG2* using Haploview software (**Figure 1**). The candidate SNPs in these genes showed strong linkages in Chinese Han and Tibetan populations. The *CLNK* and *PDK2* haplotypes associated with an increased risk of gout in Chinese Han and Tibetan populations, respectively. The haplotype "GTCAT" in *CLNK* (OR 17.12; 95% CI 1.42-206.34; P = 0.026) associated with an increased risk of gout in Han subjects (**Table 5**). The *PKD2* haplotypes "GGACA" (P = 0.0048) and "AAAAG" (P = 0.041) also associated with an increased risk of gout, but after adjusting for age and gender, these haplotypes were not associated with a risk of gout (**Table 6**).

Discussion

It is well known that polymorphisms have an effect on the regulation of gene expression, which could contribute to the differences between individuals in the susceptibility to a disease and its severity. Gout is a polygenic

Table 6. *PKD2* haplotype frequencies and their associations with gout risk in Tibetan subjects

Haplotype	Frequency		OR (95% CI)	p^a	OR (95% CI)	p^b
	Case	Control				
AACAG	0.555	0.638	1	---	1	---
GGACA	0.405	0.331	1.41 (1.11-1.79)	0.0048	0.78 (0.34-1.78)	0.550
AAAAG	0.017	0.005	3.92 (1.06-14.49)	0.041	0.83 (0.00-760.79)	0.960

$P \leq 0.05$ indicates statistical significance. The SNPs are rs2725212, rs2725210, rs2725207, rs2728132, and rs2725205. P^a values were calculated using the two-sided Chi-squared test. P^b values were calculated by unconditional logistic regression adjusted for age and sex.

heterogeneity disease, and the pathogenesis of gout showed individualization in human. The distributions of SNP loci in different races showed genetic heterogeneity. Overall, our studies established correlations between 47 SNPs and the risk of gout and confirmed the associations between *SLC2A9*, *WDR1*, *CLNK*, *PKD2*, and *ABCG2* and gout susceptibility. Our results demonstrated differences in the SNPs associated with gout in Chinese Han and Tibetan populations.

ATP-binding cassette transporter, sub-family G, member 2 (*ABCG2*) is identified as a high-capacity urate exporter, and its dysfunction has an association with serum uric acid levels and gout/hyperuricemia risk. A previous GWAS found a strong correlation with another *ABCG2* SNP (rs2622605) and uric acid levels in European-American obesity cases and controls [18]. It has also been shown that the associations between *ABCG2* SNPs and SUA concentrations are affected by obesity. Many studies reported that genotyping of only two dysfunctional variants, rs72552713 and rs2231142 is sufficient to estimate the severity of *ABCG2* dysfunction. This dysfunction increases gout risk markedly, conferring an OR of more than 3.0 [19]. Amanda J et al. [20] suggest that rs2231142 relate to serum urate levels with gout susceptibility in Caucasian and Japanese. Polymorphic forms of *ABCG2* are associated with gout because these forms are more active as transporters and reabsorb more uric acid from the tubules leading to a lowered renal clearance of urate and hyperuricemia. *ABCG2*, also known as the drug exporter BCRP, is expressed on the epithelial cells of the small intestine and the renal tubules and encodes an ATP-dependent transporter that functions in urate excretion from the gut and the kidney [21]. Molecular functional studies revealed that *ABCG2* dysfunction elevates SUA levels, and genetic studies have demonstrated a strong association between SUA levels and hyperuricemia and gout. In our study, we found that only one SNP (rs12505410) in *ABCG2* gene strongly correlated with gout in both Chinese Han and Tibetan populations.

The significant associations emerged from multiple SNPs in and around *SLC2A9* on chromosome 4, a widely replicated SUA-associated region. Previous studies have demonstrated that genetic variations in solute carrier family 2, member 9 (*SLC2A9*) might increase the risk of gout. *SLC2A9* is the most extensively replicated genetic locus associated with renal hyperuricemia, and it plays a significant role in urate reabsorption in renal proximal tubular cells [22]. Twenty-one SNPs in *SLC2A9* associated with gout in our study, and these SNPs are primarily found in the exons and introns of *SLC2A9*. Voruganti et al. reported there was non-additive interaction between sex and *SLC2A9* in determining urate levels [23]. Given the urate transporter activity of *SLC2A9* and the strong correlations between genetic variants and SUA levels, *SLC2A9* appears to be an important modulator of uric acid levels. In particular, variants in *SLC2A9* (rs11942223) have a very large effect on urate levels and gout, explaining 2-3% of the variance in serum urate in European individuals and a substantially stronger effect in women than in men [24]. We have found a statistically significant difference in genotype distribution between the normo- and the hyperuricemic groups. However, there was no effect of the mutated allele on the SUA concentrations and gout. Previously reported associated studies in different ethnic groups are thus inconsistent in the impact of genetic variants affecting SUA and gout. This discrepancy is probably caused by linkage disequilibrium with the more strongly associated variants that include rs16890979. The rs16890979 SNP has been

found to be associated with SUA concentrations and gout, with a stronger association in women in the Framingham and Rotterdam cohorts and in the island population of the Adriatic coast of Croatia [25]. Several recent studies have found that the rs16890979 in the *SLC2A9* is highly associated with gout [26-28]. Kolz M et al. [29] reported rs3775948 and rs7663032 were among the twenty-one most frequently SNPs in *SLC2A9* associated with SUA in European-ancestry populations. Voruganti et al. [23] reported there was non-additive interaction between sex and *SLC2A9* in determining urate levels. Amanda J et al. [20] reported that *SLC2A9* is a strong risk factor for gout in both Māori and Pacific Island people. However, in our studies, we found no differences in the distribution of the rs7663032 and rs16890979 genetic variants between the gout and control groups and no association with gout in Chinese Han and Tibetan populations. These differences may be due to ethnic and regional differences, environmental factors, and dietary habits.

Recently, several separate GWAS determined that common SNPs of the *WDR1* gene are associated with increased SUA levels and gout [30]. In some studies, *WDR1* is the vertebrate homolog of actininteracting protein 1, *WDR1* expression correlated with aging and inflammatory responses. *WDR1* may impact phagocytosis by macrophages and gout-related inflammatory reactions by regulating the degree of actin flip-flop. In addition, *WDR1* has been shown to regulate the MAPK signaling pathway based on its interaction with other proteins. In chondrocytes, MSU crystals have been shown to activate a signaling kinase via downstream p38 activation and upstream Src and FAK family activation [31]. *WDR1* gene variants and the disease associations have been reported in European populations [30]. Benjamin T. Kile et al. [32] reported *WDR1* disrupt megakaryocyte maturation and platelet shedding, provoke neutrophilic autoinflammatory disease. Our study also suggests that the rs717614 SNP in *WDR1* associates with an increase in gout susceptibility. Based on this evidence, these results suggest an association between *WDR1* and gout via the MAPK signaling pathway.

Our study suggested the rs10034180 and rs16869430 SNPs of *CLNK* could significantly affect the development of gout in Han population. We also found rs2108878 of *CLNK* associates with an increase in gout susceptibility in Tibetan populations. *CLNK* is a multi-function protein (also named MIST), which is expressed in several cell types, including T cells, natural killer cells, and mast cells, and its expression seems to be strictly dependent on sustained exposure to cytokines such as interleukin (IL)-2 and IL-3. The *CLNK* positively regulates immune reactions and immunoreceptor signaling, and is necessary for B cell development and activation. *CLNK* is a novel member of the SLP-76 family selectively expressed in cytokine-stimulated hemopoietic cells. Xu M et al. [33] reported that *CLNK* can mediating immunoreceptor signaling as an adaptor protein, may be is required for TNF induced cell death. Strong support for the notion that *CLNK* is involved in immunoreceptor signaling was provided by the observation that it inducibly associated with at least one tyrosine-phosphorylated polypeptide (p92) in response to immunoreceptor stimulation [34]. *CLNK* is abundantly expressed in a variety of hemopoietic cell types after sustained exposure to cytokines, that *CLNK* may also directly or indirectly impact the occurrence of gout by modulating the STAT signal transduction pathway and the concentration of circulating immune complexes. Thus, *CLNK* may modulate immune receptor signaling in response to cytokine receptor stimulation resulting in increased occurrences of gout arthritis symptoms.

PKD2 gene product is expressed in the distal tubules, collecting duct, and thick ascending limb in normal fetal and adult kidneys. It localizes to the endoplasmic reticulum and not the plasma membrane localized to the primary cilium. Autosomal dominant polycystic kidney disease (ADPKD) is a common genetic disorder characterized by the development of fluid-filled cysts in both kidneys and progressive chronic kidney disease. The disease shows significant phenotypic variability in terms of severity of renal disease and the occurrence of extrarenal manifestations. ADPKD is also a serious genetic disorder that can lead to chronic renal disease. Chronic renal disease can influence the overproduction of uric acid or reduced renal excretion of uric acid and may result in hyperuricemia. Protein dysfunction caused by mutations in the gene *PKD2* is an important factor in

the pathogenesis of ADPKD. Therefore, hyperuricemia and gout are closely correlated with *PKD2*. *PKD2* encodes a member of the polycystin protein family. Its protein product may be an integral membrane protein involved in cell-cell/matrix interactions, function in renal tubular development, function, and morphology, and may modulate intracellular calcium homoeostasis and other signal transduction pathways. *PKD2* is a calcium-permeable cation channel that spans the cell membranes of the distal tubules and collecting ducts of kidney cells, thus mutations in *PKD2* may cause a genetic predisposition to metabolic abnormalities. Wu-Chou et al. [35] performed a comprehensive clinical and mutational analysis in a cohort of ADPKD patients. This is the first study to describe the characteristics of *PKD2* mutations in Taiwan. They found in the Taiwanese population would expand the spectrum of *PKD2* mutations and contribute to the global references for genetic counseling of ADPKD patients. Besides, ADPKD patients who seek for the genetic diagnosis need to undergo a complete mutation analysis of *PKD2*. Kurashige M et al. [36] found that *PKD2* mutations were more frequent in Japanese ADPKD than that in European or American ADPKD. In populations of European origin, mutations within *PKD2* have shown to produce a more progressive renal phenotype. Their findings have highlighted various aspects of disease progression, and suggest that genetic factors are a powerful indicator of renal prognosis in patients with ADPKD. Lena Obeidova et al. [37] reported their study is the first detailed analysis a correlation between genetic polymorphisms in *PKD2* and ADPKD in Czech patients. In the majority of patients with ADPKD, it is similar to gout patients, overproduction of uric acid also may result in hyperuricemia. A recent meta-analysis of 14 GWAS also suggested that genetic variability in the *PKD2* locus contributes to SUA concentrations in European populations [13]. We identified for rs2725212, rs2725210, rs2725207, rs2728132, 2725205 and rs2725203 in the *PKD2* gene associated with an increased risk of gout in Tibetan populations. Other SNPs were not found associations in PKD2 gene. Collectively, these results indicate a correlation between genetic polymorphisms in *PKD2* and gout.

It has been suggested that several genetic polymorphisms are associated with susceptibility to gout, whereas each polymorphism may contribute to only a small relative risk of gout involves a complex interplay between exposure to multiple environmental stimuli and genetic background. This study is the first to investigate correlations between polymorphisms in *SLC2A9*, *WDR1*, *CLNK*, *PKD2*, and *ABCG2* genes and gout-related indices in Chinese Han and Tibetan populations. As a separate area, Tibetan has a unique genetic background, dietary and lifestyle habits. This could also be the main reason for the Tibetans and Han Chinese genetic background in terms of the disease. Despite the important discoveries revealed by these studies, there are some intrinsic limitations. First, the sample size (438 cases and 623 controls) is relatively small for correlation studies. Second, correlations between polymorphisms and histological subtypes were not evaluated in this study. Finally, the functions of the genetic variants and their mechanisms have not been evaluated in this study. Further research should focus on delineating the relationship between these gene polymorphisms and gout development.

Conclusion

In conclusion, this study provides significant evidence that polymorphisms in *SLC2A9*, *WDR1*, *CLNK*, *PKD2*, and *ABCG2* associate with gout in Chinese Han and Tibetan populations. To better understand the relationships between gene polymorphisms and gout progression, additional genetic risk factors and new candidate genes should be identified and analyzed.

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Disclosure of conflict of interest

None.

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