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Original Article

ABCG2 and *PKD2* polymorphisms correlate with gout-related metabolic indices in Chinese Han and Tibetan populations

Yao Zhang[1,2*], Lifeng Ma[1,2*], Yiduo Zhao[1,2], Lijun Liu[1,2], Yuan Zhang[1,2], Zhiying Zhang[1,2], Jing Li[1,2], Yansong Li[1,2], Xingguang Luo[3], Xiaodian Sun[4], Wenling Chen[1,2], Peng Cai[1,2], Haijing He[1,2], Longli Kang[1,2]

[1]Key Laboratory for Molecular Genetic Mechanisms and Intervention Research on High Altitude Disease of Tibet Autonomous Region, School of Medicine, Xizang Minzu University, Xianyang, Shaanxi, China; [2]Key Laboratory of High Altitude Environment and Gene Related to Disease of Tibet Ministry of Education, School of Medicine, Xizang Minzu University, Xianyang, Shaanxi, China; [3]Division of Human Genetics, Department of Psychiatry, Yale University School of Medicine, USA; [4]Biostatistics and Bioinformatics Core, Sylvester Comprehensive Cancer Center, University of Miami, Miami, USA. [*]Co-first authors.

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Abstract: Gout is a complex inflammation disease resulting from an increase in serum uric acid (SUA), which is one of the most common forms of inflammatory arthritis worldwide. Identification of the genetic factors of gout could be paramount contribution to early prevention of gout. Recent evidence suggests that heredity and metabolic syndrome contribute to gout development. ATP-binding cassette, sub-family G (*ABCG2*) and *PKD2* may play a role in gout progression in Chinese and Han populations. A total of 455 patients (139 Han, 316 Tibetan) with gout and 627 healthy control subjects (309 Han, 318 Tibetan) were recruited for a case-control association study. Analysis of variance was used to evaluate the impact of polymorphism on gout based on metabolism indicators. Polymorphisms of the *ABCG2* and *PKD2* genes affected multiple risk factors for gout progression. Significant differences in alkaline phosphatase (AKP), high-density lipoproteins (HDL-C) and total bilirubin (TBIL) levels were detected between different genotypic groups with *ABCG2* polymorphisms rs4693924, rs2622621, rs17731799 and rs3114020. In addition, significant differences in AKP, HDL-C and glutamic oxalacetic transaminase (AST) levels were detected between different genotypic groups with the *PKD2* polymorphisms rs2728104, rs2728099 and rs2728121 in Chinese Han and Tibetan populations. Our results provided significant evidence for the potential relationships among *ABCG2*, *PKD2* polymorphisms correlate with gout related metabolic indices in Chinese Han and Tibetan populations.

Keywords: Gout, metabolic indices, single nucleotide polymorphisms, *ABCG2*, *PKD2*

Introduction

Gout is a complex metabolic disorder caused by hyperuricemia and characterized by joint inflammation and the presence of monosodium urate crystals in bones, joints, and soft tissues [1, 2]. Hyperuricemia is defined as Serum Uric Acid (SUA) level of ≥7 mg/dl (in males) or ≥6 mg/dl (in females). The prevalence of gout among USA adults in 2008 was 3.9% [3]. The prevalence of gout from 0.67% to 0.91% during the period from 2005 to 2009 in Italy [4]. In China, over the past few years, the prevalence and incidence of gout have risen, in part due to diet and lifestyle changes [5]. The primary risk factor for the development of gout is a SUA level exceeding the physiological saturation threshold of uric acid (UA) [6]. UA is a by product of certain normal chemical reactions in the body. In the bloodstream it acts as an antioxidant, protecting cells from the damaging effects of unstable molecules called free radicals. However, the excess of UA is reabsorbed into the bloodstream and can accumulate forming crystals in the joints, leading to a painful form arthritis and gout. UA has been associated with different components of metabolic syndrome, where multiple physiological pathways are compromised. To increase the understanding of the development to gout, it is crucial to study metabolism-related indicators in gout, such as globulin, urea nitrogen, alkaline phosphatase, glucose and UA levels and so forth [7].

In recent genome-wide association studies (GWAS) have identified an army of susceptibility

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

Variables	Han			Tibetan		
	Case (n=139)	Control (n=316)	*p*-valve	Case (n=309)	Control (n=318)	*p*-valve
Sex			<0.001[b]			<0.001[b]
Male	121	197		183	238	
Female	18	112		133	80	
Age			<0.001[a]			<0.001[a]
Mean ± SD	43.3±13.7	49.1±7.9		54.7±17.1	19.1±1.6	

p[a] values were calculated by Student t tests; *p*[b] values were calculated from two-sided Chi-square tests.

loci associated with elevated SUA levels, a sea of polymorphisms SUA loci have also been shown to be risk factors for gout [8]. Elevated SUA Concentrations is a pivotal "danger signal" for gout, and may be a risk factors for a variety of metabolic diseases, by triggering chronic interstitial nephritis and the formation of urinary tract stones composed of UA [9]. The most significant findings include single nucleotide polymorphisms (SNPs) located within the intergenic region between *ABCG2* and *PKD2* on chromosome 4. Woodward et al. [10] reported *ABCG2* is a hitherto unknown urate efflux transporter, and further demonstrated that native *ABCG2* is located in the brush border membrane of kidney proximal tubule cells, where it mediates renal urate secretion. *PKD2* gene product is expressed in the distal tubules, thick ascending limb in normal fetal, collecting duct and adult kidneys. It localizes to the endoplasmic reticulum but not the plasma membrane localized to the primary cilium. Chronic renal disease can influence the overproduction of UA or reduced renal excretion of UA may result in hyperuricemia or gout. Protein dysfunction caused by mutations in the gene *PKD2* is a paramount factor in the pathogenesis of chronic renal disease [11].

To identify genetic risk factors that significantly affect metabolism-related indicators in gout patients, we performed an association study between these 2 genes and metabolic traits, including SUA levels in Chinese Han and Tibetan populations. Tibet is a plateau region in Central Asia and the home to the indigenous Tibetan individuals. With an average elevation of 4,900 meters, it is the highest region on earth and is commonly referred to as the "Roof of the World". This region is populated by a variety of ethnicities, but primarily by the Tibetan. Tibetan has a unique genetic background, dietary and lifestyle habits. These results thus offer a new strategy for the potential relationships between *ABCG2* and *PKD2* gene variations that are significantly associates with gout arthritis in Chinese Han and Tibetan populations.

Materials and methods

Study population

After obtaining written informed consent, we recruited a total of 455 patients (139 Han, 316 Tibetan) with gout and 627 healthy control subjects (309 Han, 318 Tibetan) without history of major systemic diseases, histories of nephropathy, and medication for hypertension. The aim is to reduce the therapeutic factors and potential environmental impacting the variation of gout. All patients and controls were treated by the Second People's Hospital of Tibet Autonomous Region and the Affiliated Hospital of Xizang Minzu University between September 2011 and May 2013 in Xianyang City, Shanxi, China. All patients were recently diagnosed and histologically confirmed to have gout according to the 1977 ARA preliminary classification criteria for acute gout [12]. The ethics Committee of the Affiliated Hospital of Xizang Minzu University approved our use of blood samples and our protocol.

SNP selection and genotyping

Twelve SNPs from 2 genes were chosen for analysis in this study. A total of 6 SNPs in *ABCG2* and 6 SNPs in *PKD2* with minor allele frequencies greater than 5% in the Asian population HapMap were selected for further genotyping. Genomic DNA was extracted from 5 ml of peripheral blood using the Gold Mag-Mini Purification Kit (GoldMag Co. Ltd. Xian city, China), and DNA concentrations were measured using the NanoDrop2000 (Thermo Scientific, Waltham, Massachusetts, USA). Sequenom Mass ARRAY Assay Design3.0 software was used to design multiplexed SNP Mass EXTEND assay, and SNP genotyping was

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

SNP ID	Gene	Band	Alleles A/B	Han					Tibetan				
				MAF		HWE	OR (95% CI)	p^b-valve	MAF		HWE	OR (95% CI)	p^b-valve
				Case	Control	p^a-valve			Case	Control	p^a-valve		
rs2728109	PKD2	4q22.1	T/G	0.203	0.215	0.866	0.935 (0.663-1.320)	0.703	0.063	0.044	1.000	1.472 (0.896-2.417)	0.125
rs2728106	PKD2	4q22.1	T/C	0.479	0.444	0.421	1.155 (0.873-1.527)	0.313	0.428	0.365	0.279	1.302 (1.039-1.632)	0.022*
rs2728104	PKD2	4q22.1	G/A	0.241	0.229	0.872	1.072 (0.772-1.488)	0.678	0.134	0.102	0.349	1.37 (0.972-1.931)	0.072
rs2728099	PKD2	4q22.1	G/A	0.210	0.211	0.733	0.994 (0.706-1.400)	0.974	0.062	0.041	1.000	1.548 (0.931-2.575)	0.090
rs2728133	PKD2	4q22.1	G/A	0.479	0.437	0.646	1.185 (0.896-1.568)	0.233	0.418	0.357	0.329	1.29 (1.029-1.618)	0.027*
rs2728121	PKD2	4q22.1	A/G	0.448	0.423	1.000	1.110 (0.838-1.470)	0.466	0.321	0.295	0.590	1.132 (0.891-1.438)	0.309
rs4693924	ABCG2	4q22.1	A/G	0.231	0.227	0.198	1.021 (0733-1.422)	0.904	0.060	0.042	1.000	1.448 (0.873-2.401)	0.150
rs2622621	ABCG2	4q22.1	C/G	0.538	0.403	1.000	1.725 (1.302-2.286)	0.000*	0.396	0.346	0.387	1.24 (0.987-1.558)	0.065
rs2231142	ABCG2	4q22.1	A/C	0.293	0.295	0.586	0.990 (0.729-1.345)	0.949	0.089	0.061	0.617	1.498 (0.980-2.291)	0.060
rs3114018	ABCG2	4q22.1	A/C	0.476	0.389	0.721	1.428 (1.078-1.892)	0.013*	0.529	0.444	0.650	1.408 (1.128-1.757)	0.002*
rs17731799	ABCG2	4q22.1	G/T	0.390	0.352	0.619	1.177 (0.883-1.570)	0.266	0.432	0.464	0.911	0.877 (0.703-1.095)	0.246
rs3114020	ABCG2	4q22.1	T/C	0.376	0.350	0.901	1.118 (0.837-1.494)	0.448	0.430	0.455	0.910	0.905 (0.725-1.130)	0.378

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

Table 3. Metabolic indices of subjects based on different *ABCG2* genotypes in Tibetan and Han populations

	SNP_ID	Genotype	AKP (Means ± SD)	*p*	HDL-C (Means ± SD)	*p*	AST (Means ± SD)	*p*	TBIL (Means ± SD)	*p*	TG (Means ± SD)	*p*
Tibietan	rs4693924	AG	103.58±38.944	0.874	1.33±0.381	0.043*	28.97±16.230	0.116	22.57±13.385	0.215	1.45±1.509	0.503
		GG	105.17±53.49		1.15±0.493		42.73±47.270		32.30±43.055		1.33±0.869	
	rs2622621	GG	112.33±50.453	0.004*	1.19±0.521	0.304	46.06±49.84	0.259	32.94±31.870	0.751	1.17±0.768	0.384
		GC	116.39±64.033		1.13±0.474		44.65±48.24		32.48±39.208		1.41±1.121	
		CC	91.33±31.715		1.23±0.475		34.73±37.76		28.28±44.634		1.35±0.883	
	rs3114018	AA	93.22±34.003	0.156	1.40±0.508	0.805	35.58±32.200	0.562	32.85±39.320	0.928	1.36±0.761	0.922
		CA	109.70±54.693		1.19±0.448		43.84±53.300		30.63±46.677		1.35±1.068	
		CC	108.92±58.300		1.19±0.524		40.32±35.090		29.90±26.892		1.30±0.965	
	rs17731799	TT	108.22±55.477	0.11	1.21±0.469	0.669	38.53±34.070	0.207	28.38±25.712	0.792	1.27±0.931	0.708
		GT	109.38±56.17		1.15±0.478		46.09±55.850		31.53±47.422		1.35±1.083	
		GG	90.62±30.504		1.19±0.521		32.38±23.810		33.65±40.451		1.41±0.735	
	rs3114020	TT	91.16±30.257	0.126	1.19±0.511	0.665	32.84±23.750	0.247	32.79±40.763	0.837	1.44±0.758	0.421
		TC	109.13±55.766		1.50±0.473		45.63±55.400		31.49±46.939		1.38±1.119	
		CC	108.27±55.451		1.21±0.480		38.52±34.050		28.40±25.691		1.23±0.862	
Han	rs4693924	AA	-	0.391	1.00±0.432	0.79	17.00±0.000	0.776	-	0.474	1.63±1.271	0.466
		AG	70.67±37.802		0.92±0.435		24.78±21.390		43.91±42.428		1.42±0.841	
		GG	87.00±32.945		0.96±0.368		28.71±18.410		27.71±25.587		1.77±1.765	
	rs2622621	GG	74.29±38.716	0.385	1.06±0.489	0.142	26.57±23.99	0.926	21.10±7.853	0.006*	1.50±1.003	0.294
		GC	72.30±26.495		0.882±0.317		28.80±18.87		57.01±41.886		1.51±0.916	
		CC	92.25±38.656		0.97±0.406		25.40±16.87		17.43±11.229		1.96±2.283	
	rs3114018	AA	105.83±45.644	0.07	0.93±0.345	0.775	20.17±5.913	0.561	20.50±13.176	0.194	2.08±2.411	0.105
		CA	67.71±16.717		0.93±0.368		30.29±19.990		43.81±41.039		1.58±1.005	
		CC	83.57±42.598		0.99±0.467		26.14±24.050		22.24±7.386		1.35±0.808	
	rs17731799	TT	80.78±38.376	0.001*	1.00±0.458	0.508	24.67±21.070	0.632	21.20±6.843	0.29	1.35±0.672	0.234
		GT	67.67±15.787		0.91±0.351		29.87±19.510		41.75±40.343		1.85±1.915	
		GG	142.00±32.047		0.95±0.373		19.33±4.933		25.00±18.976		1.59±0.901	
	rs3114020	TT	142.00±32.047	0.001*	1.01±0.317	0.492	19.33±4.933	0.74	25.00±18.976	0.339	1.67±0.868	0.267
		TC	66.75±15.687		0.91±0.365		28.75±19.370		40.60±39.247		1.83±1.937	
		CC	84.25±39.485		0.99±0.453		26.25±21.940		20.94±7.266		1.36±0.665	

AKP: Alkaline phosphatase; HDL-C: High-density lipoproteins; AST: Glutamic oxaloacetic aminopherase; TBIL: Total bilirubin; TG: Cholesterol. Data are reported as means ± standard deviation for continuous variables. $P<0.05$. *p* values were calculated for comparisons among the 3 genotype groups using ANOVA for continuous variables.

Table 4. Metabolic indices of subjects based on different *PKD2* genotypes in Tibetan and Han populations

	SNP_ID	Genotype	AKP (Means ± SD)	*p*	HDL-C (Means ± SD)	*p*	AST (Means ± SD)	*p*	TBIL (Means ± SD)	*p*	TG (Means ± SD)	*p*
Tibetan	rs2728109	TT	88.50±0.707	0.899	1.65±0.983	0.052	-	0.349	24.60±0.566	0.461	1.05±0.453	0.27
		GT	104.21±39.268		1.34±0.344		29.62±17.616		22.42±13.84		1.60±1.575	
		GG	105.24±53.588		1.15±0.491		42.55±47.249		32.30±43.05		1.31±0.864	
	rs2728106	TT	119.72±77.157	0.135	1.22±0.423	0.814	35.14±28.265	0.622	28.39±30.054	0.826	1.49±0.786	0.332
		TC	101.83±44.143		1.16±0.503		40.80±48.089		32.48±48.450		1.29±0.868	
		CC	100.55±41.105		1.17±0.483		44.29±46.415		29.45±28.129		1.27±0.851	
	rs2728104	AA	97.79±38.867	0.002*	1.18±0.475	0.997	39.32±41.228	0.446	29.95±32.030	0.606	1.30±0.812	0.199
		GA	122.61±71.391		1.18±0.504		44.63±52.257		33.20±56.748		1.47±1.305	
	rs2728099	AA	105.44±53.543	0.737	1.49±0.488	0.018*	42.26±47.250	0.139	32.35±43.053	0.2	1.35±1.007	0.87
		AG	102.06±38.417		1.36±0.411		29.63±17.141		22.31±13.212		1.32±0.732	
	rs2728133	AA	99.82±40.552	0.233	1.19±0.492	0.76	43.44±45.453	0.682	29.02±27.523	0.806	1.27±0.836	0.649
		AG	103.38±45.624		1.16±0.496		40.93±47.419		32.54±48.169		1.36±1.080	
		GG	118.26±78.093		1.22±0.414		35.06±29.907		28.55±29.662		1.45±0.803	
	rs2728121	AA	97.28±25.111	0.617	1.28±0.381	0.073	35.45±31.442	0.001*	25.88±17.111	0.068	1.47±0.773	0.582
		GA	104.25±60.425		1.22±0.453		28.82±17.02		24.53±25.809		1.28±0.782	
		GG	108.08±49.498		1.10±0.519		51.54±57.01		37.99±53.847		1.38±1.169	
Han	rs2728109	TT	-	0.495	0.94±0.368	0.997	-	0.951	-	0.203	1.70±1.213	0.799
		GT	72.43±39.429		0.95±0.429		26.57±23.985		19.66±7.966		1.50±0.856	
		GG	83.05±33.426		0.95±0.384		27.10±17.508		37.73±35.837		1.70±1.715	
	rs2728106	TT	88.50±50.205	0.856	0.84±0.422	0.26	17.00±0.000	0.073	17.85±11.243	0.792	1.64±0.986	0.965
		TC	77.84±38.127		0.99±0.390		23.26±15.169		34.62±32.181		1.68±1.848	
		CC	85.33±20.265		0.95±0.369		42.00±25.768		33.12±37.944		1.59±1.001	
	rs2728104	AA	90.87±33.228	0.193	0.95±0.380	0.973	29.73±19.429	0.669	26.009±26.415	0.408	1.77±1.772	0.416
		GA	68.36±34.200		0.95±0.416		24.09±19.201		43.17±38.472		1.39±0.794	
		GG	-		0.92±0.436		-		-		1.70±1.215	
	rs2728099	AA	89.18±32.049	0.211	0.93±0.374	0.841	28.88±18.354	0.751	33.58±34.936	0.974	1.73±1.719	0.687
		AG	66.56±36.95		0.97±0.434		24.44±21.413		32.83±29.343		1.47±0.849	
		GG	-		1.00±0.432		-		-		1.63±1.271	
	rs2728133	AA	85.33±20.265	0.856	0.95±0.369	0.296	42.00±25.768	0.073	33.12±37.944	0.792	1.59±1.001	0.865
		AG	77.84±38.127		0.99±0.388		23.26±15.169		34.62±32.181		1.71±1.857	
		GG	88.5±50.205		0.85±0.430		17.00±0.000		17.85±11.243		1.55±0.873	
	rs2728121	AA	-	0.734	0.82±0.368	0.131	17.00±0.000	0.143	-	0.254	1.48±0.865	0.677
		GA	80.71±41.489		1.00±0.398		22.24±15.786		23.70±5.748		1.76±1.860	
		GG	82.56±18.729		0.93±0.385		37.00±22.091		42.76±14.254		1.55±1.005	

AKP: Alkaline phosphatase; HDL-C: High-density lipoproteins; AST: Glutamic oxaloacetic aminopherase; TBIL: Total bilirubin; TG: Cholesterol. Data are reported as means ± standard deviation for continuous variables. $P<0.05$. *p* values were calculated for comparisons among the 3 genotype groups using ANOVA for continuous variables.

Table 5. Single loci association with gout risk in Han populations (logistic regression adjusted by age and gender)

SNPs	Model	Genotype	Status=Control	Status=Case	OR (95% CI)	*p*-value
rs12498927	Codominant	G/G	120 (38.7%)	46 (34.1%)	1	0.042*
		A/G	158 (51%)	64 (47.4%)	0.99 (0.61-1.62)	
		A/A	32 (10.3%)	25 (18.5%)	2.22 (1.12-4.40)	
	Dominant	G/G	120 (38.7%)	46 (34.1%)	1	0.47
		A/G-A/A	190 (61.3%)	89 (65.9%)	1.19 (0.75-1.88)	
	Recessive	G/G-A/G	278 (89.7%)	110 (81.5%)	1	0.012*
		A/A	32 (10.3%)	25 (18.5%)	2.24 (1.20-4.17)	
rs10938799	Codominant	G/G	198 (63.9%)	85 (63%)	1	0.031*
		G/A	105 (33.9%)	40 (29.6%)	0.99 (0.61-1.59)	
		A/A	7 (2.3%)	10 (7.4%)	4.63 (1.41-15.20)	
	Dominant	G/G	198 (63.9%)	85 (63%)	1	0.52
		G/A-A/A	112 (36.1%)	50 (37%)	1.16 (0.73-1.83)	
	Recessive	G/G-G/A	303 (97.7%)	125 (92.6%)	1	0.0084*
		A/A	7 (2.3%)	10 (7.4%)	4.66 (1.44-15.09)	
rs10016022	Codominant	G/G	201 (64.8%)	88 (65.2%)	1	0.067
		G/A	103 (33.2%)	39 (28.9%)	0.98 (0.61-1.59)	
		A/A	6 (1.9%)	8 (5.9%)	4.51 (1.21-16.82)	
	Dominant	G/G	201 (64.8%)	88 (65.2%)	1	0.63
		G/A-A/A	109 (35.2%)	47 (34.8%)	1.12 (0.71-1.78)	
	Recessive	G/G-G/A	304 (98.1%)	127 (94.1%)	1	0.020*
		A/A	6 (1.9%)	8 (5.9%)	4.54 (1.23-16.76)	
rs2041215	Codominant	T/T	132 (42.9%)	44 (32.6%)	1	0.095
		G/T	138 (44.8%)	70 (51.9%)	1.64 (1.01-2.66)	
		G/G	38 (12.3%)	21 (15.6%)	1.73 (0.86-3.52)	
	Dominant	T/T	132 (42.9%)	44 (32.6%)	1	0.031*
		G/T-G/G	176 (57.1%)	91 (67.4%)	1.66 (1.04-2.63)	
	Recessive	T/T-G/T	270 (87.7%)	114 (84.4%)	1	0.41
		G/G	38 (12.3%)	21 (15.6%)	1.32 (0.69-2.52)	
rs2622621	Codominant	G/G	113 (36.6%)	27 (20%)	1	0.0002*
		G/C	143 (46.3%)	67 (49.6%)	2.32 (1.33-4.04)	
		C/C	53 (17.1%)	41 (30.4%)	3.61 (1.90-6.85)	
	Dominant	G/G	113 (36.6%)	27 (20%)	1	0.0001*
		G/C-C/C	196 (63.4%)	108 (80%)	2.68 (1.59-4.52)	
	Recessive	G/G-G/C	256 (82.8%)	94 (69.6%)	1	0.0047*
		C/C	53 (17.1%)	41 (30.4%)	2.13 (1.26-3.58)	
rs3114018	Codominant	C/C	114 (36.8%)	38 (28.1%)	1	0.021*
		C/A	151 (48.7%)	63 (46.7%)	1.47 (0.88-2.45)	
		A/A	45 (14.5%)	34 (25.2%)	2.45 (1.30-4.61)	
	Dominant	C/C	114 (36.8%)	38 (28.1%)	1	0.026*
		C/A-A/A	196 (63.2%)	97 (71.8%)	1.71 (1.06-2.76)	
	Recessive	C/C-C/A	265 (85.5%)	101 (74.8%)	1	0.018*
		A/A	45 (14.5%)	34 (25.2%)	1.96 (1.13-3.40)	
rs17731799	Codominant	T/T	128 (41.3%)	45 (33.3%)	1	0.077
		G/T	146 (47.1%)	73 (54.1%)	1.74 (1.07-2.83)	
		G/G	36 (11.6%)	17 (12.6%)	1.44 (0.69-2.99)	
	Dominant	T/T	128 (41.3%)	45 (33.3%)	1	0.028*
		G/T-G/G	182 (58.7%)	90 (66.7%)	1.67 (1.05-2.66)	

	Recessive	T/T-G/T	274 (88.4%)	118 (87.4%)	1	0.87
		G/G	36 (11.6%)	17 (12.6%)	1.06 (0.54-2.07)	
rs3114020	Codominant	C/C	130 (41.9%)	48 (35.6%)	1	0.1
		T/C	143 (46.1%)	71 (52.6%)	1.68 (1.04-2.73)	
		T/T	37 (11.9%)	16 (11.8%)	1.25 (0.60-2.60)	
	Dominant	C/C	130 (41.9%)	48 (35.6%)	1	0.048*
		T/C-T/T	180 (58.1%)	87 (64.4%)	1.58 (1.00-2.51)	
	Recessive	C/C-T/C	273 (88.1%)	119 (88.2%)	1	0.85
		T/T	37 (11.9%)	16 (11.8%)	0.94 (0.48-1.85)	

P<0.05 indicates statistical significance. SNPs: Single nucleotide polymorphisms. OR: Odds ratio. CI: Confidence interval.

performed utilizing the Sequenom Mass ARRAY RS1000 recommended by the manufacturer. The Sequenom Typer 4.0 Softwar was used to perform data management and analyses.

Statistical analysis

Data were analyzed using SPSS version 18.0 statistical software (SPSS Inc., Chicago, IL, United States) and Excel (Microsoft Corp., Redmond, WA, United States). Differences in demographic characteristics between the cases and controls were evaluated by the Chi-square test or the Student's t-test (The data considered were analyzed as continuous variables). Hardy-Weinberg equilibrium (HWE) was calculated using SHEs is online software for cases and controls, the odds ratios (ORs) and 95% confidence intervals (95% CIs) were tested using unconditional logistic regression analysis with adjustment for age and sex. Three genetic models (dominant, recessive, and additive) were performed using PLINK software to assess the association of SNPs with the risk of gout. Finally, the Haploview software package (version 4.2) was used for estimate the pairwise linkage disequilibrium (LD), haplotype construction, and genetic association at polymorphism loci.

Results

Our study included 455 patients (139 Han, 316 Tibetan) with gout and 627 healthy control subjects (309 Han, 318 Tibetan). The characteristics of cases and controls were listed in **Table 1**. The major allelic frequencies (MAFs) of the SNPs genotyped in the case and control groups were summarized in **Table 2**. We compared the frequencies of the alleles in the different groups by the Chi-squared test, and we found rs2622621 and rs3114018 associated significantly with increased gout susceptibility in the Han populations.

The genotype distributions of the *ABCG2* and *PKD2* gene SNPs from the gout patients were presented in **Tables 3** and **4**. In Tibetan populations, we found that the rs4693924 (*P*= 0.043) and rs2622621 (*P*=0.004) in *ABCG2* were significantly associated with HDL-C and AKP levels, respectively. Additionally, the rs27-28104 (*P*=0.004), rs2728099 (*P*=0.012) and rs2728121 (*P*=0.016) in *PKD2* were associated with AKP, HDL-C and AST levels, respectively. Similarly, in Han populations, the rs177-31799 (*P*=0.001) and rs3114020 (*P*=0.001) in *ABCG2* were associated with AKP levels, and the rs2622261 (*P*=0.006) in *ABCG2* were significantly associated with TBIL levels.

We also found that rs2728106, rs2728133 and rs3114018 associated with an increased risk of gout in the Tibetan populations (*P*< 0.05). We further analyzed the association between SNPs and gout risk by unconditional logistic regression analysis using three models in Han and Tibetan populations (**Tables 5**, **6**). In Han populations, we found the risk allele "A" of rs12498927 (*P*=0.042, *P*=0.012) and rs10938799 (*P*=0.031, *P*=0.0084) were associated with an increased risk of gout based on analysis using the codominant model and the recessive model. The minor allele "T" of rs2041215 (*P*=0.031), rs17731799 (*P*=0.028) and rs3114020 (*P*=0.048) were associated with an increased risk of gout based on the results of the dominant model. The minor allele "C" of rs2622621 (*P*=0.0002, *P*=0.0001, *P*= 0.0047) and "A" of rs3114018 (*P*=0.021, *P*= 0.026, *P*=0.018) were also associated with an increased risk of gout susceptibility in the codominant model, dominant model and recessive model. In addition, the minor allele "A" of rs10016022 (*P*=0.02) was associated with an increased risk of gout susceptibility in the recessive model. However, in Tibetan popula-

Table 6. Single loci association with gout risk in Tibetan populations (logistic regression adjusted by age and gender)

SNPs	Model	Genotype	Status=Control	Status=Case	OR (95% CI)	*p*-value
rs10033825	Codominant	C/C	161 (50.8%)	169 (54%)	1	0.14
		T/C	125 (39.4%)	134 (42.8%)	1.00 (0.36-2.74)	
		T/T	31 (9.8%)	10 (3.2%)	0.02 (0.00-20.62)	
	Dominant	C/C	161 (50.8%)	169 (54%)	1	0.62
		T/C-T/T	156 (49.2%)	144 (46%)	0.78 (0.29-2.09)	
	Recessive	C/C-T/C	286 (90.2%)	303 (96.8%)	1	0.046*
		T/T	31 (9.8%)	10 (3.2%)	0.02 (0.00-20.38)	
rs17467273	Codominant	T/T	153 (48.3%)	160 (50.6%)	1	0.048*
		T/C	129 (40.7%)	141 (44.6%)	1.05 (0.38-2.92)	
		C/C	35 (11%)	15 (4.8%)	0.02 (0.00-4.00)	
	Dominant	T/T	153 (48.3%)	160 (50.6%)	1	0.55
		T/C-C/C	164 (51.7%)	156 (49.4%)	0.74 (0.27-1.99)	
	Recessive	T/T-T/C	282 (89%)	301 (95.2%)	1	0.014*
		C/C	35 (11%)	15 (4.8%)	0.02 (0.00-3.85)	
rs2622621	Codominant	C/C	139 (43.7%)	120 (38.2%)	1	0.087
		G/C	137 (43.1%)	139 (44.3%)	3.58 (1.06-12.12)	
		G/G	42 (13.2%)	55 (17.5%)	2.81 (0.64-12.37)	
	Dominant	C/C	139 (43.7%)	120 (38.2%)	1	0.029*
		G/C-G/G	179 (56.3%)	194 (61.8%)	3.32 (1.05-10.48)	
	Recessive	C/C-G/C	276 (86.8%)	259 (82.5%)	1	0.62
		G/G	42 (13.2%)	55 (17.5%)	1.37 (0.40-4.74)	

P<0.05 indicates statistical significance. SNPs: Single nucleotide polymorphisms. OR: Odds ratio. CI: Confidence interval.

tions, we found the "C" allele of rs17467273 (*P*=0.048, *P*=0.014) was associated with an increased risk of gout in the codominant model and the recessive model, and the "G" allele of rs2622621 (*P*=0.029) was associated with an increased risk of gout susceptibility in the dominant model. Conversely, we found the "T" allele of rs10033825 (*P*=0.046) was associated with a reduced risk of gout in the recessive model. Furthermore, the candidate SNPs in the *PKD2* and *ABCG2* genes showed strong linkage (**Figures 1**, **2**). The results for the association between the *PKD2* and *ABCG2* haplotype and the risk of gout were listed in **Tables 7** and **8**. The *PKD2* haplotype "GTGAGG" was found to be associated with an increased risk of gout (*P*=0.012). Conversely, haplotypes "GGCTCTC" in *ABCG2* associated with a decreased risk of gout by the logistic regression adjusted by age and sex (*P*=0.0019).

Discussion

This study of *ABCG2* and *PKD2* polymorphisms and their association with metabolic traits revealed several crucial findings. The SNPs examined (rs2622621, rs17731799 and rs3114020 in *ABCG2* and the rs2728104 in *PKD2*) were strongly associated with AKP levels. We found the rs2728099 SNP of *PKD2* and the rs4693924 SNP of *ABCG2* could significantly affect HDL-C levels. These associations remained significant after Bonferroni correction. We also found the rs2728121 SNP of *PKD2* gene, and the rs2622621 of *ABCG2* gene affect the AST and TBIL levels, respectively. Taken together, these SNPs showed significant associations with multiple metabolic indices of gout, which is consistent with previous research that *ABCG2* and *PKD2* polymorphisms related to gout susceptibility.

HDL-C is one of the five major lipoproteins found in the body, HDL-C is about 20% to 30% of total cholesterol in human body, and may also reduce the risk of coronary heart disease. HDL-C consists of apolipoproteins, phospholipids, cholesterol, and a small amount of fatty acids. Yu et al. [13] reported the association between SUA and idiopathic venous thrombo-

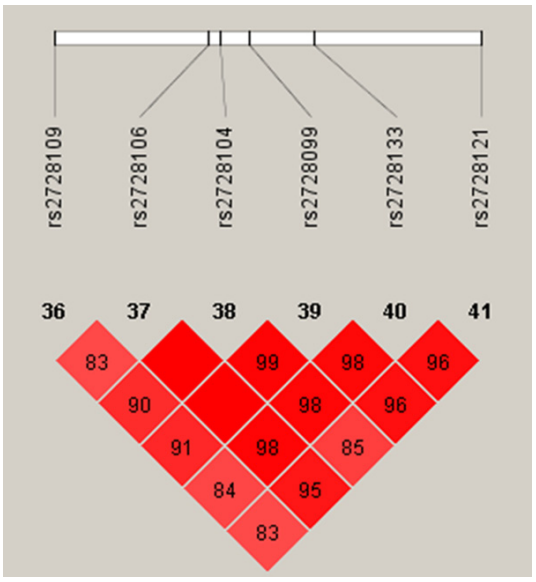

Figure 1. Haplotype block map for all the SNPs of the *PKD2* gene in Han populations.

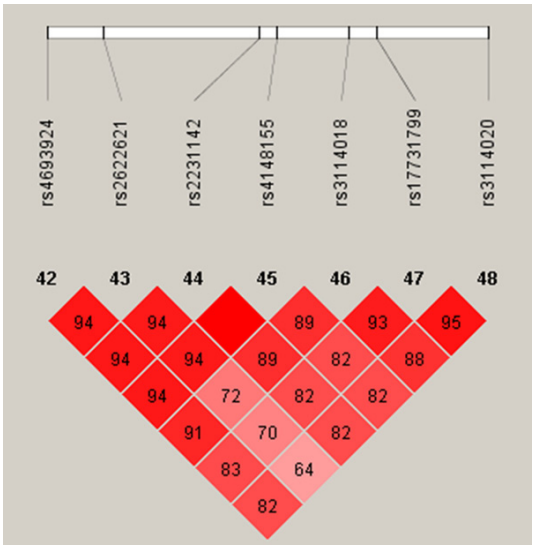

Figure 2. Haplotype block map for all the SNPs of the *ABCG2* gene in Han populations.

embolism varied with the HDL-C levels, in which SUA was significantly associated with idiopathic venous thromboembolism at a high HDL-C levels, while the correlation was no longer present when the HDL-C levels was decreased. SUA may also act as a crucial connection between atherosclerosis and idiopathicvenous thromboembolism by influencing the functional HDL-C and it should be considered a critical loop in the progress of pro-inflammatory and pro-thrombotic state in the disease. SUA is also highly associated with the development of gout. Taken together, these results indicate SUA, HDL-C are associated with susceptibility to gout. Further, Karimi et al. [14] reported SUA levels had significant correlation with AKP. Our findings are consistent with this conclusion. Gout involves an increase in UA levels, which is the end product of purine metabolism. Because of the lack of UA enzyme, degradation of UA allantoin and UA salt for eventual excretion by the kidneys. SUA levels reflect the formation and excretion of UA [15, 16]. The complex process of renal excretion of UA has been extensively examined in an ocean of studies. The excretion of UA occurs include glomerular, renal proximal tubular reabsorption, secretion and resorption.

ABCG2 is identified as a high-capacity urate exporter, and its dysfunction has an association with SUA levels and gout susceptibility. Moreover, *ABCG2* SNPs have been shown to be more specifically associated with gout among patients classified as “renal overload” [17]. *ABCG2* encodes for a multispecific transporter that is expressed on the apical membrane in several tissues, including intestine, kidney and liver [18]. *ABCG2* is also a major drug transporter, and a multitude of these drug transporters seem to be substrate inducible. Hence, it may lead to induction of urate transporters in the intestine and other non-renal tissues. A previous study demonstrated that SUA levels has a 63% heritability [19]. Previous GWASs have reported *ABCG2* loci to be positively associated with SUA levels and gout. It transports purine nucleoside analogues, which resemble the molecular structure of UA and mediates urate excretion in the kidney [20]. Matsuo and Ichida et al. [17, 21] indicated that a slice of characters with hyperuricaemia would be erroneously labelled “overproducers”, not because of abnormally high synthesis of UA rather than a risk allele for *ABCG2*. These individuals have a significantly reduced total body clearance of urate compared with those without the risk allele and a greater risk of hyperuricaemia and gout.

PKD2, which encodes a member of the polycystin protein family located on chromosome 4. Its protein product may be an integral membrane protein involved in cell and matrix interactions, function in renal tubular development, morphology, and may modulate intracellular calcium

Table 7. *PKD2* haplotype frequencies and the association with gout risk in Han populations

Haplotype	Frequency[a]	OR (95% CI)	p^a	Frequency[b]	OR (95% CI)	p^b
GCAAAG	0.519	1	---	0.520	1	---
GTAAGA	0.210	1.20 (0.83-1.72)	0.33	0.213	1.34 (0.89-2.01)	0.16
TTGGGA	0.193	1.09 (0.75-1.58)	0.66	0.188	0.96 (0.63-1.46)	0.84
GTGAGG	0.021	2.15 (0.84-5.53)	0.11	0.021	3.83 (1.36-10.79)	0.012
GTGGGA	0.015	1.52 (0.48-4.82)	0.48	0.015	1.52 (0.44-5.33)	0.51
TCAAAG	0.013	0.78 (0.21-2.97)	0.72	0.014	0.66 (0.15-2.87)	0.58

$P \leq 0.05$ indicates statistical significance SNPs: rs2728109, rs2728106, rs2728104, rs2728099 and rs2728133. Frequency[a] and p^a value from were calculated from two-sided Chi-squared test; Frequency[b] and p^b values were calculated by unconditional logistic regression adjusted for age and sex.

Table 8. *ABCG2* haplotype frequencies and the association with gout risk in Han populations

Haplotype	Frequency[a]	OR (95% CI)	p^a	Frequency[b]	OR (95% CI)	p^b
GCCTAGT	0.285	1	---	0.287	1	---
GGCTCTC	0.207	0.49 (0.31-0.77)	0.0019	0.209	0.46 (0.28-0.75)	0.0019
AGACCTC	0.206	0.89 (0.59-1.32)	0.55	0.201	0.71 (0.45-1.12)	0.14
GCCTCTC	0.073	1.21 (0.67-2.20)	0.53	0.074	1.10 (0.57-2.13)	0.78
GGACCTC	0.068	0.77 (0.41-1.45)	0.42	0.067	0.57 (0.27-1.18)	0.13
GCCTATC	0.058	1.88 (0.98-3.58)	0.057	0.058	1.53 (0.75-3.14)	0.24
GGCTAGT	0.038	0.44 (0.16-1.22)	0.12	0.038	0.37 (0.12-1.16)	0.09
GCCTAGC	0.012	0.91 (0.25-3.33)	0.88	0.012	0.80 (0.19-3.46)	0.77

$P \leq 0.05$ indicates statistical significance SNPs: rs4693924, rs2622621, rs2231142, rs4148155, rs3114018, rs17731799 and rs3114020. Frequency[a] and p^a value from were calculated from two-sided Chi-squared test; Frequency[b] and p^b values were calculated by unconditional logistic regression adjusted for age and sex.

homoeostasis and signal transduction pathways. *PKD2* gene product is expressed in cell membranes of the distal tubules and collecting ducts of kidney cells, thus mutations in *PKD2* may cause a genetic predisposition to metabolic abnormalities. Interestingly, a recent meta-analysis of 14 GWAS have identified that the genetic variability around the *PKD2* gene could contribute to SUA concentrations in European populations [22]. Surveys such as that conducted by Lena et al. [23] showed that genetic polymorphisms in *PKD2* and adult dominant polycystic kidney disease (ADPKD) in Czech patients. They found the majority of patients with ADPKD, which is similar to gout patients, overproduction of UA levels also may result in hyperuricemia and gout. We identified for rs2728106 and rs2728133 in the *PKD2* gene associated with an increased risk of gout in Tibetan populations. Other SNPs were not found associations in *PKD2* gene. Thus, these results indicated 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. As a unique geological condition in Central Asia, because of geographical reasons, Tibet's eating habits is a unique ethnic characteristic. Tibetan meal is a representative sample of burning sheep, beef, pigs, chickens and other meat. The main methods of cooking Tibetan meal there is roasted, fried, boiled, and other laws. This is a high fat and high protein dietary habit. The diets rich in purines or fructose can increase SUA levels. Due to the difference between the area and the dietary habit, Han population has another lifestyle. This is probably the main reason for differences between Tibetan and Han populations in hereditary diseases. Although there are important discoveries revealed by the studies, there are also limitations. On the one hand, due to practical constraints, this paper cannot provide enough sample size for correlation studies. On the other hand, the functions of the genetic variants and their mechanisms have not been evaluated in this study.

The conclusion gives a brief summary of the findings. We analyzed SNPs in the *ABCG2* and *PKD2* genes and identified a relationship between genetic polymorphisms and gout in Chinese Han and Tibetan populations. We examined the associations between *ABCG2* and *PKD2* variations and gout-relates metabolic indices. This study set out to determine paramount insights into the etiology of gout. However, additional genetic risk factors and functional investigations should be identified to confirm our results. Finally, areas for further research are identified.

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Disclosure of conflict of interest

None.

Address correspondence to: Dr. Longli Kang, Key Laboratory of High Altitude Environment and Gene Related to Disease of Tibet Ministry of Education, School of Medicine, Xizang Minzu University, 6 East Wenhui Road, Xianyang 712082, Shaanxi, China. Tel: +86-29-33755247; E-mail: longli_kang@163.com

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