PDK2 and *ABCG2* genes polymorphisms are correlated with blood glucose levels and uric acid in Tibetan gout patients

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ABSTRACT. Previous studies have shown that the *PDK2* and *ABCG2* genes play important roles in many aspects of gout development in European populations. However, a detailed genotype-phenotype analysis was not performed. The aim of the present study was to investigate the potential association between variants in these two genes and metabolism-related quantitative phenotypes relevant to gout in a Chinese Tibetan

population. In total, 316 Chinese Tibetan gout patients were recruited from rheumatology outpatient clinics and 6 single nucleotide polymorphisms in *PDK2* and *ABCG2* were genotyped, which were possible etiologic variants as identified in the HapMap Chinese Han Beijing population. A significant difference in blood glucose levels was detected between different genotypes of rs2728109 (P = 0.005) in the *PDK2* gene. We also detected a significant difference in the mean serum uric levels between different genotypes of rs3114018 (P = 0.004) in the *ABCG2* gene. All P values remained significant after Bonferroni's correction for multiple testing. Our data demonstrate potential roles for *PDK2* and *ABCG2* polymorphisms in the metabolic phenotypes of Tibetan gout patients, which may provide new insights into the etiology of gout. Further studies are required to confirm these findings.

Key words: Gout; *PDK2*; *ABCG2*; Single nucleotide polymorphism; Metabolic phenotypes

INTRODUCTION

Gout, the most prevalent form of inflammatory arthritis, is a disease primarily triggered by urate overload with arthritis as a consequence of pathological accumulation (Pascual et al., 2013). Increased serum urate concentration or hyperuricemia results in the deposition of monosodium urate crystals in and around joints, which is the major underlying pathophysiological mechanism of gout. If left untreated, gout will result in progressive joint damage, chronic pain and disability, and clinically evident subcutaneous tophi (hard, impacted monosodium urate crystals) (Roddy et al., 2013; Roddy and Choi, 2014). With changes in lifestyle and society following World War II, the prevalence of gout in Asia increased, and moreover, has been steadily increasing over the past 20 years (Zhou et al., 2014). Patients with gout frequently have multiple comorbidities, including chronic kidney disease, cardiovascular disease, hypertension, diabetes, obesity, and hyperlipidemia, all of which have significant adverse impact on public health and quality of life (Robinson et al., 2013; Karis et al., 2014; Sakiyama et al., 2014). Importantly, the severity of gout is related to the number of comorbidities (Robinson et al., 2013).

Although the specific pathogenesis of gout is still unclear, major checkpoints in the regulation of urate metabolism in the pathogenesis of gout have been shown to be affected by different genetic and environmental factors that influence susceptibility. Observational studies have shown that dietary factors (i.e., animal purines, alcohol, and fructose), obesity, metabolic syndrome, hypertension, diuretic use, and chronic kidney disease may be clinically relevant risk factors for HUA and gout (Stark et al., 2008; Roddy and Choi, 2014). However, a large familial segregation study demonstrated that serum urate levels are highly impacted by hereditary factors (Kuo et al., 2015). Furthermore, the ATP-binding cassette, subfamily G, 2 (*ABCG2*) and *PDK2* gene loci, which are located on chromosome 4, have been identified to be associated with serum urate concentrations by recent genome-wide association studies of serum uric acid (UA) concentrations and gout (Kottgen et al., 2012).

Regardless of the underlying mechanism, we hypothesized that *PDK2* and *ABCG2* gene polymorphisms may lead to variations of the metabolism-related quantitative phenotypes in gout

patients. Therefore, in the current study, we investigated the potential relationships between *PDK2* and *ABCG2* gene variants and gout in a Chinese Tibetan population.

MATERIAL AND METHODS

Study participants

A total of 316 gout patients (183 males, 133 females) were recruited from the Affiliated Hospital of Tibet University for Nationalities and Xianyang Central Hospital from 2011 to 2013. All patients were native Tibet Chinese, and we certified that at least their three preceding generations had been living in the same locality. Gout patients were divided into an acute gouty arthritis (AGA) group and a non-AGA group based on the American College of Rheumatology classification criteria (1977). All cases recruited were verified to have no age, gender, or disease stage restrictions.

Detailed exclusion criteria were used in this study. The exclusion criteria were as follows: 1) the use of any systemic inflammatory treatment including drug control treatment and elimination of UA; 2) the diagnosis of any blood diseases, which could lead to cell destruction disorders and higher nucleic acid metabolism, such as multiple myeloma, polycythemia, acute and chronic hemolytic anemia, leukemia, myelodysplastic syndrome, lymphoma, or other malignant tumor; 3) hyperuricemia caused by taking drugs (thiazide diuretics, aspirin, pyrazine amides, etc.) or alcohol intake prior to investigation; and 4) renal failure, non-gout calculi, renal dysfunction, or other renal diseases.

Clinical and demographic data

For all participants, serum albumin (ALB), ALB/globulin (A/G), uric acid (UA), blood glucose (GLU), total cholesterol, triglycerides, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, very low-density lipoprotein, urea nitrogen (UREA), and creatinine in plasma were measured. A standardized questionnaire was administered to collate the phenotypic characteristics from the gout patients, included demographic data (region, ethnicity, gender, age, education status, smoking status, alcohol use, occupational radiation exposure, family history of cancer, and other lifestyle factors) and clinical parameters (details of previous episodes of gout, tophi, and disease-related complications). Blood samples were obtained in the morning after the participants had been fasting for at least 12 h and after sitting for 15 min. Aliquots of serum were immediately obtained and stored at -80°C until further use. Written informed consent for each subject was obtained at recruitment, and the use of samples was approved by The Human Research Committee of the Affiliated Hospital of Tibet University for Nationalities for Approval of Research Involving Human Subjects.

Single nucleotide polymorphism (SNP) selection and genotyping

Candidate SNPs with minor allele frequencies (MAFs) >5% in the HapMap Chinese Han Beijing (CHB) population in the *PDK2* and *ABCG2* genes that had been previously published in association with gout were selected, which resulted in 6 relevant SNPs. The phenol-chloroform extraction method was performed to extract genomic DNA from whole blood as previously described (Köchl et al., 2005). DNA concentration was assessed by spectrophotometry (DU530UV/ VIS spectrophotometer, Beckman Instruments, Fullerton, CA, USA). Primers were designed by the Sequenom MassARRAY Assay Design 3.1 Software (Sequenom, San Diego, CA, USA).

The Sequenom MassARRAY Assay Design 3.0 Software was used to design a Multiplexed SNP MassEXTEND assay, and SNP genotyping was performed utilizing the Sequenom MassARRAY RS1000 as recommended by the manufacturer and as previously described (Gabriel et al., 2009). Genotyping quality was examined by a detailed quality control procedure consisting of over 95% successful call rate and duplicate calling of genotypes. The Sequenom Typer 4.0 Software was used to perform data management and analyses (Thomas et al., 2007; Gabriel et al., 2009).

Statistical analysis

Microsoft Excel (Microsoft, Redmond, WA, USA) and SPSS 16.0 statistical package (SPSS Inc., Chicago, IL, USA) were used to perform all statistical analyses. In the current study, reported P values are two-sided, and $P < 0.05$ was considered to be statistically significant. Comparison of clinical data between different genotype groups was carried out using one-way analysis of variance (ANOVA).

RESULTS

The primers for the 6 SNPs selected are shown in Table 1. The SNPs that were examined herein in the *PDK2* and *ABCG2* genes are shown in Table 2. The MAFs of the SNPs selected were each higher than 5% in the HapMap CHB population and Tibetan population.

Table 1. Primers for the 6 selected *PDK2* and *ABCG2* SNPs.

SNP_ID	Allele	1st-PCRP	2nd-PCRP	UEP_SEQ
rs2728109	T/G	ACGTTGGATGGGCATAGTCTTAGTACTTACC	ACGTTGGATGAGAACTTTGCCTGTGCCTTG	ccCACTGTGCTGAGTGTTT
rs2728106	T/C	ACGTTGGATGAGAGACCACTTCATTAGCCC	ACGTTGGATGCCAAGCTGGTCTTTGCTATG	atCTGGTCTTTGCTATGAGAATCA
rs2728133	G/A	ACGTTGGATGGTGGATTGTGTACTATGAGG	ACGTTGGATGCTTCGTAAGGGCCTAATCAG	CAGGCTGTTTTCCTTACCCC
rs3114018	A/C	ACGTTGGATGGGAAACCTCACAAAAGTGTC	ACGTTGGATGGGAAAAGACTTCTTAAGAGT	TATCAAATAACTTATTGCTTGAATTT
rs17731799	G/T	ACGTTGGATGCTGGTATATATTGCTAGAGAG	ACGTTGGATGGGAGCACAGGAGATCAGCTTG	aaAAGACCCTGTCTCAAAAAAAC
rs3114020	T/C	ACGTTGGATGGTTTCATATTTATAGGTCGG	ACGTTGGATGTTATTTGATTTACCACTGC	TTTACCACTGCCTCCCA

SNP = single nucleotide polymorphism; PCR = polymerase chain reaction; UEP = unextended mini-sequencing primer.

Table 2. SNPs examined in the *PDK2* and *ABCG2* genes.

Gene	SNP_ID	Chromosome	Alleles A[a]/B	Position	MAF	
					CHB	Tibetan gout patients
PDK2	rs2728109	4q22.1	T/G	88957723	0.268	0.063
	rs2728106	4q22.1	T/C	88972051	0.524	0.428
	rs2728133	4q22.1	G/A	88981690	0.500	0.418
ABCG2	rs3114018	4q22.1	C/A	89064581	0.634	0.471
	rs17731799	4q22.1	T/G	89068455	0.683	0.432
	rs3114020	4q22.1	C/T	89083666	0.683	0.43

[a]Minor allele.

In the subsequent genotype-phenotype analysis, the various quantitative phenotypes among the different genotypes in gout patients are shown in Table 3. A statistically significant association was found between the rs2728109 genotypes in the *PDK2* gene and the specific value of ALB and globulin (A/G) and mean serum glucose levels, where the TT genotype had a higher specific A/G value (1.86 ± 0.714, P = 0.001) and displayed higher serum glucose levels (9.26 ±

5.445 mM, P = 0.005) compared to those of the other genotypes. Statistically significant differences were also observed between rs3114020 genotypes in the *ABCG2* gene and the mean serum uric levels, where the CC genotype had higher serum uric levels (444.52 ± 164.176 μM, P = 0.004) compared to those of the other genotypes. After a strict Bonferroni correction, these results still remained significant (P < 0.05/6).

Table 3. Comparison of phenotypes among the different genotypes in the *PDK2* and *ABCG2* genes in gout patients.

		N	ALB (g)	P	A/G	P	GLU (mM)	P	UREA (mM)	P	UA (μM)	P
PDK2												
rs2728109	T/T	3	37.35 ± 10.112	0.009*	1.86 ± 0.714	0.001*	9.26 ± 5.445	0.005*	12.70 ± 10.394	0.319	314.00 ± 86.470	0.181
	G/T	34	44.31 ± 5.122		1.66 ± 0.363		5.60 ± 2.042		6.35 ± 3.392		441.39 ± 165.065	
	G/G	279	40.41 ± 6.637		1.39 ± 0.385		5.31 ± 1.612		6.27 ± 6.225		399.97 ± 145.838	
rs2728106	T/T	51	40.95 ± 5.964	0.881	1.42 ± 0.399	0.95	5.44 ± 1.788	0.517	7.09 ± 5.874	0.038*	425.29 ± 165.961	0.332
	T/C	167	41.04 ± 6.512		1.44 ± 0.400		5.47 ± 1.942		6.89 ± 7.154		405.68 ± 148.059	
	C/C	96	40.50 ± 7.113		1.44 ± 0.398		5.15 ± 1.181		4.77 ± 1.884		387.38 ± 139.012	
rs2728133	A/A	99	40.64 ± 6.995	0.919	1.45 ± 0.391	0.847	5.14 ± 1.167	0.405	4.72 ± 1.872	0.026*	388.02 ± 137.138	0.249
	A/G	170	41.00 ± 6.666		1.42 ± 0.395		5.52 ± 1.998		6.89 ± 7.077		404.83 ± 147.488	
	G/G	47	41.16 ± 5.706		1.45 ± 0.415		5.38 ± 1.750		7.24 ± 6.124		431.93 ± 169.514	
ABCG2												
rs3114018	A/A	76	40.47 ± 6.748	0.844	1.42 ± 0.402	0.99	5.38 ± 1.228	0.891	6.03 ± 3.152	0.786	402.15 ± 148.658	0.004*
	C/A	142	40.97 ± 6.784		1.43 ± 0.407		5.33 ± 1.706		6.61 ± 6.804		378.53 ± 131.026	
	C/C	94	41.22 ± 6.255		1.43 ± 0.366		5.48 ± 2.257		6.13 ± 6.512		444.52 ± 164.176	
rs17731799	T/T	107	41.59 ± 6.211	0.377	1.45 ± 0.376	0.524	5.38 ± 2.118	0.992	6.28 ± 6.149	0.812	436.00 ± 166.297	0.012*
	G/T	143	40.22 ± 6.733		1.40 ± 0.413		5.39 ± 1.723		6.56 ± 6.853		379.24 ± 127.589	
	G/G	64	41.35 ± 6.716		1.48 ± 0.392		5.42 ± 1.251		5.92 ± 3.211		402.75 ± 152.263	
rs3114020	T/T	64	41.42 ± 6.742	0.595	1.49 ± 0.394	0.275	5.47 ± 1.278	0.677	6.54 ± 4.192	0.861	409.95 ± 153.555	0.016*
	T/C	142	40.45 ± 6.698		1.39 ± 0.398		5.28 ± 1.518		6.11 ± 6.461		378.59 ± 126.018	
	C/C	108	41.36 ± 6.354		1.47 ± 0.391		5.51 ± 2.329		6.51 ± 6.235		432.71 ± 167.199	

ALB = albumin; A/G = albumin/globulin; GLU = glucose; UREA = urea nitrogen; UA = uric acid. Data are reported as means ± standard deviation for continuous variables. Bonferroni's multiple adjustment was applied to the level of significance, which was set at P < 0.008 (0.05/6). P values were calculated using ANOVA for continuous variables. *P < 0.05 was considered statistically significant.

We also found that there was a statistically significant association between rs2728109 genotypes and the mean albumin levels, where the TT genotype displayed the lowest albumin levels (37.35 ± 10.112 g, P = 0.009) compared to those of the other genotypes. Additionally, a statistically significant association was discovered between the rs2728106 and rs2728133 SNP genotypes in the *PDK2* gene and blood urea nitrogen levels, as specific genotypes exhibited higher UREA levels than those of the other genotypes including the TT (rs2728106, 7.09 ± 5.874 mM, P = 0.038) and GG (rs2728133, 7.24 ± 6.124 mM, P = 0.026). Additionally, statistically significant differences were observed between the rs17731799 and rs3114020 SNP genotypes in the *ABCG2* gene and the mean serum uric levels, where specific genotypes had higher serum uric levels than those of the other genotypes including TT (rs17731799, 436.00 ± 166.297 μM, P = 0.012) and CC (rs3114020, 432.71±167.199 μM, P = 0.016). However, these results were not statistically significant after a strict Bonferroni's correction (P > 0.05/6).

DISCUSSION

In the current study, we found for the first time that polymorphisms of the *PDK2* and *ABCG2* genes played important roles in the pathogenesis and clinicopathological phenotypes of gout patients, which may provide novel insights into the pathogenesis of gout.

It has been unequivocally demonstrated that polymorphisms of rs2728109 in *PDK2* significantly affect the levels of GLU. GLU, as the sole nitrogen and carbon source, is in dynamic equilibrium in the human body and remains relatively constant under normal circumstances. The levels of GLU also have been shown to influence renal uric acid excretion during hyperglycemia and glycosuria (Christensen and Steenstrup, 1958; Cook et al., 1986). Interestingly, relationships

have previously been observed between serum UA and serum glucose in subjects with gout (Beckett and Lewis, 1960). Meanwhile, the polymorphisms of rs2728106 and rs2728133 in *PDK2* may have a strong influence on blood UREA levels. UREA is an important index of kidney function, which is intimately related to clinical and pathological findings in gout (Talbott and Terplan, 1960). Moreover, the polymorphisms rs3114018, rs17731799, and rs3114020 in *ABCG2* have been shown to be significantly associated with levels of serum uric, and genome-wide scans discovered that rs3114018 genotypes in *ABCG2* were associated with serum uric acid levels as a quantitative trait among different ethnic groups (Yang et al., 2014). These results were the first to suggest that *PDK2* and *ABCG2* variants have a significant modulating effect on metabolism-related quantitative phenotypes in gout patients.

How might these *ABCG2* and *PDK2* variants differentially affect gout and further affect clinicopathological phenotypes? The *ABCG2*, belongs to the ABC transporter superfamily, and encodes for a multispecific transporter that is expressed on the apical membrane in several tissues, including intestine, liver, and kidney (Matsuo et al., 2009). In humans, UA is the end product of purine metabolic oxidation. When lacking uricase, UA cannot be converted into its soluble and excretable form, and as a consequence, its concentration is mainly controlled by endogenous metabolism (synthesis and cell turnover), and excretion and reabsorption in the kidney (Dehghan et al., 2008). Increasing evidence suggests that ABCG2, a high-capacity urate transporter, regulates serum UA levels by mediating urate excretion, and studies have shown that a decrease in ABCG2 protein expression levels is directly related to a decrease in urate transport activity (Matsuo, et al., 2009; Matsuo et al., 2013; Woodward et al., 2013). *PDK2* is a gene that encodes for the kinase that phosphorylates serine 473 of Akt. Akt, also known as protein kinase B (PKB), is a member of the AGC protein kinase family and is a downstream target of PI 3-kinase in B cells (Gold et al., 1999; Chan and Tsichlis, 2001). This is important as PI-3Ks are likely to be involved in joint and tissue damage that occurs in gout patients (Popa-Nita et al., 2007). Thus, *PDK2* may play a pivotal role in inflammatory reactions caused by gout.

There were limitations inherent in our study worth mentioning. First, the race of all participants was limited to Tibet Chinese, which has a higher incidence of gout. Hence, substantial population admixture can be ignored in our study. Second, these subjects were not subdivided by age or gender, and gender-specific significant variants were not evaluated. Third, we performed Bonferroni's corrections in our statistical analysis and found no statistical significance in some comparisons. This may have been caused by the weakness of the Bonferroni's corrections. Once the Bonferroni's corrections were performed, the positive findings depend on the number of other tests performed. Thus, true important differences may be deemed non-significant since the likelihood of type II errors is also increased. However, Bonferroni's corrections are considered acceptable when performing associations without pre-established hypotheses.

Based on the results reported herein, it would be of interest to verify if the associated SNPs in the *PDK2* and *ABCG2* genes may contribute to gout development by regulating the variation of different clinicopathological phenotypes. Our study offers important insights into the etiology of gout. However, further functional investigations and analyses need to be performed to confirm our results.

Conflicts of interest

The authors declare no conflict of interest.

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REFERENCES

Beckett AG and Lewis J (1960). Gout and the serum uric acid in diabetes mellitus. *Q.J. Med.* 29: 443-458.

Chan TO and Tsichlis PN (2001). PDK2: a complex tail in one Akt. *Sci. STKE* 2001: pe1.

Christensen PJ and Steenstrup O (1958). Uric acid excretion with increasing plasma glucose concentration (pregnant and non-pregnant cases). *Scand. J. Clin. Lab. Invest.* 10: 182-185. http://dx.doi.org/10.3109/00365515809079942

Cook DG, Shaper AG, Thelle DS and Whitehead TP (1986). Serum uric acid, serum glucose and diabetes: relationships in a population study. *Postgrad. Med. J.* 62: 1001-1006. http://dx.doi.org/10.1136/pgmj.62.733.1001

Dehghan A, Kottgen A, Yang Q, Hwang SJ, et al. (2008). Association of three genetic loci with uric acid concentration and risk of gout: a genome-wide association study. *Lancet* 372: 1953-1961. http://dx.doi.org/10.1016/S0140-6736(08)61343-4

Gabriel S, Ziaugra L and Tabbaa D (2009). SNP genotyping using the Sequenom MassARRAY iPLEX platform. *Curr. Protoc. Hum. Genet.* Chapter 2: Unit 2 12.

Gold MR, Scheid MP, Santos L, Dang-Lawson M, et al. (1999). The B cell antigen receptor activates the Akt (protein kinase B)/glycogen synthase kinase-3 signaling pathway via phosphatidylinositol 3-kinase. *J. Immunol.* 163: 1894-1905.

Köchl S, Niederstatter H and Parson W (2005). DNA extraction and quantitation of forensic samples using the phenol-chloroform method and real-time PCR. *Methods Mol. Biol.* 297: 13-30.

Karis E, Crittenden DB and Pillinger MH (2014). Hyperuricemia, Gout, and Related Comorbidities: Cause and Effect on a Two-Way Street. *South Med. J.* 107: 235-241. http://dx.doi.org/10.1097/SMJ.0000000000000082

Kottgen A, Albrecht E, Teumer A, Vitart V, et al. (2012). Genome-wide association analyses identify 18 new loci associated with serum urate concentrations. *Nat. Genet.* 45: 145-154. http://dx.doi.org/10.1038/ng.2500

Kuo CF, Grainge MJ, See LC, Yu KH, et al. (2015). Familial aggregation of gout and relative genetic and environmental contributions: a nationwide population study in Taiwan. *Ann. Rheum. Dis.* 74: 369-374. http://dx.doi.org/10.1136/annrheumdis-2013-204067

Matsuo H, Ichida K, Takada T, Nakayama A, et al. (2013). Common dysfunctional variants in ABCG2 are a major cause of early-onset gout. *Sci. Rep.* 3: 2014. http://dx.doi.org/10.1038/srep02014

Matsuo H, Takada T, Ichida K, Nakamura T, et al. (2009). Common defects of ABCG2, a high-capacity urate exporter, cause gout: a function-based genetic analysis in a Japanese population. *Sci. Transl. Med.* 1: 5ra11. http://dx.doi.org/10.1126/scitranslmed.3000237

Pascual E, Andres M and Vela P (2013). Gout treatment: should we aim for rapid crystal dissolution? *Ann. Rheum. Dis.* 72: 635-637. http://dx.doi.org/10.1136/annrheumdis-2012-202594

Popa-Nita O, Rollet-Labelle E, Thibault N and Gilbert C (2007). Crystal-induced neutrophil activation. IX. Syk-dependent activation of class Ia phosphatidylinositol 3-kinase. *J. Leukoc. Biol.* 82: 763-773. http://dx.doi.org/10.1189/jlb.0307174

Robinson PC, Merriman TR, Herbison P and Highton J (2013). Hospital admissions associated with gout and their comorbidities in New Zealand and England 1999-2009. *Rheumatology* 52: 118-126. http://dx.doi.org/10.1093/rheumatology/kes253

Roddy E and Choi HK (2014). Epidemiology of gout. *Rheum. Dis. Clin. North Am.* 40: 155-175. http://dx.doi.org/10.1016/j.rdc.2014.01.001

Roddy E, Mallen CD and Doherty M (2013). Gout. *BMJ* 347: f5648. http://dx.doi.org/10.1136/bmj.f5648

Sakiyama M, Matsuo H, Shimizu S, Nakashima H, et al. (2014). A common variant of organic anion transporter 4 (OAT4/SLC22A11) gene is associated with renal underexcretion type gout. *Drug Metab. Pharmacokinet.* 29: 208-210. http://dx.doi.org/10.2133/dmpk.DMPK-13-NT-070

Stark K, Reinhard W, Neureuther K, Wiedmann S, et al. (2008). Association of common polymorphisms in GLUT9 gene with gout but not with coronary artery disease in a large case-control study. *PloS One* 3: e1948.

Talbott JH and Terplan KL (1960). The kidney in gout. *Medicine* 39: 405-467. http://dx.doi.org/10.1097/00005792-196012000-00001

Thomas RK, Baker AC, Debiasi RM, Winckler W, et al. (2007). High-throughput oncogene mutation profiling in human cancer. *Nat. Genet.* 39: 567. http://dx.doi.org/10.1038/ng1975

Woodward OM, Tukaye DN, Cui J, Greenwell P, et al. (2013). Gout-causing Q141K mutation in ABCG2 leads to instability of the nucleotide-binding domain and can be corrected with small molecules. *Proc. Natl. Acad. Sci. U. S. A.* 110: 5223-5228. http://dx.doi.org/10.1073/pnas.1214530110

Yang B, Mo Z, Wu C, Yang H, et al. (2014). A genome-wide association study identifies common variants influencing serum uric acid concentrations in a Chinese population. *BMC Med. Genomics* 7: 10. http://dx.doi.org/10.1186/1755-8794-7-10

Zhou L, Liu L, Liu X, Chen P, et al. (2014). Systematic Review and Meta-Analysis of the Clinical Efficacy and Adverse Effects of Chinese Herbal Decoction for the Treatment of Gout. *PloS One* 9: e85008. http://dx.doi.org/10.1371/journal.pone.0085008