GMR Genetics and Molecular Research
Online Journal
ISSN:1676-5680

Case Report

SLC2A9 and *ZNF518B* polymorphisms correlate with gout-related metabolic indices in Chinese Tibetan populations

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Genet. Mol. Res. 14 (3): 9915-9921 (2015)
Received March 4, 2015
Accepted June 25, 2015
Published August 19, 2015
DOI http://dx.doi.org/10.4238/2015.August.19.26

ABSTRACT. Current evidence suggests that heredity and metabolic syndrome contribute to gout progression. *SLC2A9* and *ZNF518B* may play a role in gout progression in different populations, but no studies have focused on the Tibetan Chinese population. In this study, we

determined whether variations in these 2 genes were correlated with gout-related indices in Chinese-Tibetan gout patients. We detected 6 single nucleotide polymorphisms in *SLC2A9* and *ZNF518B* in 319 Chinese Tibetan gout patients. One-way analysis of variance was used to evaluate the polymorphisms' effects on gout based on mean serum levels of metabolism indicators. Polymorphisms in *SLC2A9* and *ZNF518B* affected multiple risk factors related to gout development. Significant differences in serum triglyceride levels and high-density lipoprotein-cholesterol level were detected between different genotypic groups with *SLC2A9* polymorphisms rs13129697 (P = 0.022), rs4447863 (P = 0.018), and rs1014290 (P = 0.045). Similarly in *ZNF518B*, rs3217 (P = 0.016) and rs10016022 (P = 0.046) were associated with high creatinine and glucose levels, respectively. This study is the first to investigate and identify positive correlations between *SLC2A9* and *ZNF518B* gene polymorphisms and metabolic indices in Tibetan gout patients. We found significant evidence indicating that genetic polymorphisms affect gout-related factors in Chinese Tibetan populations.

Key words: Gout; Metabolic indices; Single nucleotide polymorphism; *SLC2A9*; *ZNF518B*

INTRODUCTION

Gout is the most common inflammatory joint disease in men above 40 years of age (Luk and Simkin, 2005). Elevation of serum urate levels is an essential prerequisite for gout development (Riches et al., 2009). However, uric acid levels are closely associated with components of metabolic syndrome and other factors related to multiple physiological pathways. To increase the understanding of the predisposition to gout, it is important to study metabolism-related indicators in gout.

In addition to environmental components, there is evidence that strong genetic control influences the regulation of blood uric acid concentrations (Yang et al., 2005). Genome-wide association studies have identified over 30 common sequence variants influencing serum uric acid concentration and gout (Hindorff et al., 2010). Among these, the most significant findings include single nucleotide polymorphisms (SNPs) located within the *SLC2A9* gene and the intergenic region between *SLC2A9* and *ZNF518B* on chromosome 4 (Döring et al., 2008). *SLC2A9* encodes the GLUT9 renal molecule, which transports glucose and later transports uric acid (Caulfield et al., 2008; Witkowska et al., 2012). *ZNF518B* is a novel gene that has been identified to be associated with gout (Döring et al., 2008). It is possible that both *SLC2A9* and *ZNF518B* regulate uric acid levels in the human body.

To identify genetic risk factors that significantly affect metabolism-related indicators in gout patients, we conduct an association study between these 2 genes and metabolic traits, including serum uric acid concentrations, in an isolated population in China. Tibetans have a higher incidence of gout (Liu et al., 2011). Our data provided new evidence for the potential relationships between *SLC2A9* and *ZNF518B* gene variations and gout susceptibility in the Chinese Tibetan population.

MATERIAL AND METHODS

Study population

In our study population, all analyses were restricted to Tibetan Chinese subjects. A total of 316 patients with gout between September 2011 and May 2013 were recruited into an ongoing molecular epidemiological study at the Department of Rheumatology of the Affiliated Hospital of Tibet Institute For Nationalities and the Center's Hospital in Xianyang City, China. All participants were Tibetan Chinese living in the Tibet Autonomous Region of China, with at least 3 generations of paternal ancestry within this ethnic group. Subjects with any type of medical illness, organ transplant, or drug or alcohol addiction were excluded from the study. There were no gender, age, or disease stage restrictions for case recruitment. All patients were recently diagnosed and histologically confirmed to have gout according to the 1977 ARA preliminary classification criteria for acute gout (Malik et al., 2009).

Blood samples and signed informed consent forms were obtained from all enrolled participants. This protocol was approved by the Clinical Research Ethics Boards of Tibet Nationality College and Northwest University and was in compliance with Department of Health and Human Services regulations for the protection of human research subjects.

Demographic and clinical data

We collected demographic and clinical data through face-to-face interviews using a standardized epidemiological questionnaire, including information on age, gender, ethnicity, residential region, alcohol use, smoking status, educational status, and family history of cancer. In fasting venous blood samples, serum albumin, fasting glucose (GLU), triglycerides (TG), total cholesterol, low-density lipoprotein-cholesterol, high-density lipoprotein-cholesterol (HDL-C), creatinine (CREA), urea nitrogen, and uric acid were measured (B-bridge, Tokyo, Japan).

SNP selection and genotyping

We selected SNPs form previously published polymorphisms associated with gout (Döring et al., 2008). A total of 4 SNPs in *SLC2A9* and 2 SNPs in *ZNF518B* with minor allele frequencies greater than 5% in the Asian population HapMap were selected for further genotyping because they were reported to be associated with gout. Genomic DNA was extracted from peripheral blood using phenol-chloroform, and its concentration was measured using a DU530 UV/VIS spectrophotometer (Beckman Instruments, Brea, CA, USA). A multiplexed SNP MassEXTEND assay was designed with the Sequenom MassARRAY Assay Design 3.0 Software (San Diego, CA, USA). Genotyping was performed using the Sequenom MassARRAY RS1000 with a standard protocol recommended by the manufacturer (Gabriel et al., 2009), and data were managed using the Sequenom Typer 4.0 Software (Thomas et al., 2007; Gabriel et al., 2009).

Statistical analysis

All statistical analyses were performed using SPSS 17.0 statistical package (SPSS, Inc., Chicago, IL, USA). The data elements considered were analyzed as continuous variables.

We used analysis of variance for comparisons of metabolism-related indicators among the subjects with 3 different genotypes. All P values in our study were 2-sided, and $P < 0.05$ was considered to indicate statistical significance.

RESULTS

A total of 316 Tibetan Chinese patients (183 males, 133 females; mean age 54.70 ± 17.140 years; SD) were included in our study. Basic characteristics of the patients such as gender, age, albumin, glucose, TG, cholesterol, high-density lipoproteins, carbamide, and uric acid level are listed in Table 1.

Table 1. Demographic and clinical characteristics of study participants.

Gender	N	Age (years)	ALB (g/L)	GLU (mM)	TC (mg/dL)	TG (mg/dL)	HDL-C (mg/dL)	CREA (μM)	UA (μM)
Male	183	56.74 ± 1.41	41.64 ± 1.84	5.59 ± 0.16	4.925 ± 0.91	1.49 ± 1.15	1.31 ± 1.08	77.50 ± 41.22	382.19 ± 137.89
Female	133	57.52 ± 15.40	41.05 ± 6.51	5.37 ± 1.97	4.29 ± 1.52	1.44 ± 1.30	1.26 ± 0.46	73.69 ± 26.25	384.98 ± 137.50

ALB: albumin; GLU: glucose; TC: triglyceride; TG: cholesterol; HDL-C: high-density lipoproteins; CREA: creatinine; UA: uric acid.

We genotyped 4 SNPs in *SLC2A9* using analysis of variance to compare different genotypes with clinical metabolism indices and found that the "TT" genotype of rs13129697 ($P = 0.022$) and rs4447863 ($P = 0.018$) were significantly associated with a high TG level, while the "CC" genotype of rs1014290 ($P = 0.045$) had a statistically significant association with low HDL-C level (Table 2). Similarly, in *ZNF518B*, the "TT" genotype of rs3217 ($P = 0.016$) and the "GA" genotype of rs10016022 ($P = 0.046$) were associated with high CREA and GLU levels, respectively (Table 2).

DISCUSSION

This study of *SLC2A9* and *ZNF518B* and their association with metabolic traits revealed 2 important findings. First, the SNPs examined (rs13129697, rs4447863, and rs1014290) were strongly associated with TG and HDL-C levels, respectively. This was surprising, as we expected the *SLC2A9* transporter gene variants to be strongly associated with gout in the Tibetan population. Second, we found an association between *ZNF518B* gene variants (rs3217 and rs10016022) and CREA and GLU levels, suggesting that *ZNF518B* is also associated with gout in the Tibetan population.

SLC2A9 is located on chromosome 4 and is the most extensively replicated genetic locus associated with serum uric acid levels. It is a causative gene for renal hypouricemia and plays a key role in urate reabsorption by renal proximal tubular cells (Preitner et al., 2009). Recently, studies have reported that *SLC2A9* plays a crucial role in the incidence of gout (Tin et al., 2011; Witkowska et al., 2012). Another study showed that rs13129697, located in intron 7 of *SLC2A9*, showed the strongest association with gout (Karns et al., 2012). Our results also indicate that rs13129697 is strongly associated with TG level. High TG levels cause some of free fatty acids to be largely re-esterified or stored in other tissues, resulting in accelerated degradation of ATP and higher serum uric acid levels (Shaw et al., 2014). In addition, we also found that rs1014290 was associated with low HDL-C level, which can cause the loss of the negative regulation of inflammation, possibly aggravating gout-related inflammatory reactions (Dong et al., 2014). Collectively, our results support an association between genetic polymorphisms in *SLC2A9* and gout susceptibility, suggesting that this gene may function in

Table 2. Metabolic indices of subjects based on different SLC2A9 and ZNF518B genotypes.

SNP_ID		N	ALB (Means ± SD)	P	A/G (Means ± SD)	P	GLU (Means ± SD)	P	TC (Means ± SD)	P	TG (Means ± SD)	P
SLC2A9												
rs13129697	T/T	88	40.95 ± 7.099	0.363	1.40 ± 0.398	0.399	5.11 ± 1.342	0.197	4.44 ± 1.771	0.393	1.47 ± 1.005	0.022
	G/T	169	40.39 ± 6.645		1.42 ± 0.393		5.59 ± 2.063		4.35 ± 1.533		1.38 ± 1.045	
	G/G	57	42.10 ± 5.629		1.51 ± 0.402		5.24 ± 1.309		4.06 ± 1.354		1.01 ± 0.531	
rs4447863	T/T	75	41.01 ± 6.893	0.408	1.41 ± 0.404	0.199	5.24 ± 1.394	0.437	4.48 ± 1.813	0.502	1.52 ± 1.029	0.018
	T/C	171	40.35 ± 6.768		1.40 ± 0.391		5.30 ± 1.800		4.32 ± 1.526		1.31 ± 0.825	
	C/C	69	41.84 ± 5.548		1.52 ± 0.396		5.64 ± 1.877		4.16 ± 1.395		1.31 ± 0.825	
rs7686538	T/T	1	39.8 ± 0.000	0.58	2.41 ± 0.000	0.046	7.06 ± 0.000	0.611	2.19 ± 0.000	0.242	0.15 ± 0.000	0.434
	C/T	53	39.95 ± 6.451		1.42 ± 0.391		5.31 ± 1.861		4.14 ± 1.683		1.30 ± 0.718	
	C/C	262	41.16 ± 6.641		1.43 ± 0.392		5.40 ± 1.733		4.39 ± 1.556		1.36 ± 1.023	
rs1014290	T/T	139	40.52 ± 7.236	0.688	1.39 ± 0.403	0.243	5.40 ± 1.801	0.679	4.32 ± 1.715	0.99	1.34 ± 0.912	0.775
	C/T	143	41.13 ± 6.218		1.45 ± 0.388		5.33 ± 1.62		4.32 ± 1.477		1.37 ± 1.097	
	C/C	30	41.69 ± 5.603		1.53 ± 0.402		5.68 ± 2.070		4.37 ± 1.480		1.21 ± 0.648	
ZNF518B												
rs3217	T/T	13	39.09 ± 10.315	0.485	1.38 ± 0.432	0.601	4.60 ± 0.901	0.348	3.88 ± 1.219	0.634	1.43 ± 0.539	0.798
	C/T	135	41.45 ± 6.200		1.46 ± 0.405		5.49 ± 1.913		4.39 ± 1.615		1.30 ± 1.101	
	C/C	167	40.65 ± 6.602		1.41 ± 0.388		5.38 ± 1.661		4.32 ± 1.585		1.38 ± 0.884	
rs10016022	A/A	26	39.95 ± 7.572	0.809	1.31 ± 0.389	0.278	5.21 ± 1.434	0.046	4.21 ± 1.257	0.812	1.27 ± 0.768	0.334
	G/A	118	40.88 ± 6.322		1.48 ± 0.394		5.77 ± 2.241		4.41 ± 1.752		1.24 ± 0.798	
	G/G	172	41.09 ± 6.680		1.43 ± 0.397		5.15 ± 1.321		4.30 ± 1.503		1.43 ± 1.100	
		N	HDL-C (Means ± SD)	P	LDL-C (Means ± SD)	P	CREA (Means ± SD)	P	UREA (Means ± SD)	P	UA (Means ± SD)	P
SLC2A9												
rs13129697	T/T	88	1.17 ± 0.557	0.656	2.35 ± 1.131	0.704	72.92 ± 31.588	0.585	7.14 ± 7.390	0.272	411.67 ± 141.228	0.784
	G/T	169	1.15 ± 0.448		2.38 ± 0.997		71.20 ± 23.510		6.21 ± 5.994		401.63 ± 151.631	
	G/G	57	1.23 ± 0.450		2.23 ± 0.860		76.29 ± 25.841		5.37 ± 2.387		394.77 ± 151.826	
rs4447863	T/T	75	1.16 ± 0.580	0.365	2.38 ± 1.152	0.857	71.66 ± 30.752	0.343	7.21 ± 7.766	0.364	411.23 ± 138.516	0.821
	T/C	171	1.15 ± 0.440		2.36 ± 0.993		71.15 ± 24.245		6.17 ± 5.965		402.99 ± 152.747	
	C/C	69	1.25 ± 0.453		2.28 ± 0.915		77.73 ± 26.588		5.74 ± 3.015		395.71 ± 148.195	
rs7686538	T/T	1	1.29 ± 0.000	0.919	0.63 ± 0.000	0.18	89.2 ± 0.000	0.268	11.05 ± 0.000	0.666	313.00 ± 0.000	0.824
	C/T	53	1.15 ± 0.478		2.25 ± 11.086		78.58 ± 25.560		5.96 ± 3.710		401.70 ± 167.277	
	C/C	262	1.18 ± 0.486		2.38 ± 0.996		71.42 ± 26.577		6.39 ± 6.393		404.27 ± 144.234	
rs1014290	T/T	139	1.15 ± 0.528	0.045	2.33 ± 1.007	0.943	71.74 ± 26.172	0.408	6.68 ± 6.175	0.707	409.07 ± 141.524	0.845
	C/T	143	1.15 ± 0.437		2.35 ± 0.913		72.02 ± 26.645		6.11 ± 6.292		398.84 ± 155.203	
	C/C	30	1.40 ± 0.393		2.40 ± 1.011		79.61 ± 27.474		5.84 ± 3.435		401.52 ± 145.358	
ZNF518B												
rs3217	T/T	13	1.18 ± 0.391	0.954	2.07 ± 0.765	0.628	94.86 ± 40.778	0.016	7.03 ± 7.418	0.688	391.92 ± 165.72	0.403
	C/T	135	1.19 ± 0.501		2.39 ± 1.028		69.06 ± 22.417		5.98 ± 5.278		391.49 ± 150.27	
	C/C	167	1.17 ± 0.477		2.33 ± 1.022		73.92 ± 27.332		6.59 ± 6.469		414.31 ± 145.248	
rs10016022	A/A	26	1.24 ± 0.446	0.738	2.14 ± 0.867	0.552	80.14 ± 30.665	0.345	9.21 ± 13.990	0.086	387.19 ± 124.038	0.514
	G/A	118	1.19 ± 0.479		2.41 ± 1.083		74.65 ± 29.367		6.26 ± 3.870		415.49 ± 147.670	
	G/G	172	1.16 ± 0.492		2.33 ± 0.988		70.72 ± 23.614		5.97 ± 5.305		397.75 ± 151.753	

ALB: albumin; A/G: albumin/globulin; GLU: glucose; TC: triglyceride; TG: cholesterol. HDL-C: high-density lipoproteins; LDL-C: low-density lipoproteins; CREA: creatinine; UREA: urea nitrogen; UA: uric acid. 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.

the same disease mechanism in the Tibetan populations as it does in others.

For *ZNF518B*, which was also identified by genome-wide association studies to be associated with gout, rs3217 and rs10016022 have been found to influence CREA and GLU levels in our study. However, there have been no previous studies showing a relationship between these 2 SNPs and gout onset; in fact, studies on this gene are rare. GLU is highly associated with the development of gout and hyperuticemia, indirectly increasing the level of serum uric acid and the risk of gout by enhancing insulin resistance and circulating insulin levels (Han et al., 2008), suggesting that *ZNF518B* is also associated with the incidence of gout in Tibetan populations.

In this study, a very specific population was examined. Additionally, novel genes were included. Although *SLC2A9* has been identified to be associated with gout by several previous studies, few studies have examined *ZNF518B*. Our study focused on the association between genes and the metabolic traits of disease, which differs from the approach used by general association studies. There are also several limitations to our study. Our sample size was limited and no controls were included because of the difficulty of recruiting Tibetan subjects. Thus, the novel associations we identified should be confirmed in further studies.

In conclusion, we analyzed SNPs in the *SLC2A9* and *ZNF518B* genes and identified a relationship between genetic polymorphisms and gout susceptibility in the Tibetan Chinese population. We examined the associations between *SLC2A9* and *ZNF518B* gene variations and metabolic traits in gout. Our study offers important insights into the etiology of gout.

Conflicts of interest

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

Research supported by the National Natural Science Foundation of China (#31260252), the Natural Science Foundation of Xizang (Tibet) Autonomous Region (#2014), the Social Science Foundation of the Chinese Ministry of Education (#12YJA850011) and the State Project for Essential Drug Research and Development (Grant #2012ZX09506001-007).

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