Gene 651 (2018) 62–69

Contents lists available at ScienceDirect

Gene

journal homepage: www.elsevier.com/locate/gene

The polymorphism rs671 at *ALDH2* associated with serum uric acid levels in Chinese Han males: A genome-wide association study

Dandan Zhang[a,b], Min Yang[c], Dan Zhou[a,b], Zhenli Li[a,b], Libin Cai[d], Yuqian Bao[e], Hong Li[f], Zhongyan Shan[g], Juan Liu[h], Duo Lv[i], Yi Liu[i], Chunxiao Xu[i], Jie Ling[i], Yuyang Xu[i], Shuai Zhang[a,b], Qiong Huang[a,b], Yongyong Shi[j], Yimin Zhu[i,*], Maode Lai[a,b,**]

[a] *Department of Pathology, School of Medicine, Zhejiang University, Hangzhou, Zhejiang 310058, PR China*
[b] *Key Laboratory of Disease Proteomics of Zhejiang Province, Hangzhou, Zhejiang 310058, PR China*
[c] *Department of Nutrition and Food Safety, School of Public Health, Zhejiang University, Hangzhou, Zhejiang 310058, PR China*
[d] *The People's No.3 Hospital of Xiaoshan, Hangzhou, Zhejiang 322251, PR China*
[e] *Department of Endocrinology and Metabolism, Shanghai Jiao Tong University Affiliated Sixth People's Hospital, Shanghai Diabetes Institute, Shanghai Key Laboratory of Diabetes Mellitus, Shanghai Clinical Center for Diabetes, 600 Yishan Road, Shanghai 200233, PR China*
[f] *Department of Endocrinology, Affiliated Sir Run Run Shaw Hospital, School of Medicine, Zhejiang University, Zhejiang 310058, PR China*
[g] *Department of Endocrinology and Metabolism, The Endocrine Institute and The Liaoning Provincial Key Laboratory of Endocrine Diseases, The First Hospital of China Medical University, Shenyang, Liaoning 110001, PR China*
[h] *Endocrinology and Diabetes Center, The First Affiliated Hospital of Sun Yat-sen University, Guangzhou, Guangdong 510080, PR China*
[i] *Department of Epidemiology & Biostatistics, School of Public Health, Zhejiang University, Hangzhou, Zhejiang 310058, PR China*
[j] *Bio-X Institutes, Key Laboratory for the Genetics of Developmental and Neuropsychiatric Disorders, Ministry of Education, Shanghai Jiao Tong University, Shanghai 200030, PR China*

ARTICLE INFO

Keywords:
Serum uric acid
Genome-wide association studies
Mendelian randomization
Alcohol consumption
ALDH2

ABSTRACT

Serum uric acid (SUA) levels are highly heritable and an increased SUA level is one of important risk factors for gout, diabetes, metabolic syndrome, and cardiovascular diseases. The genetic variants underlying SUA remains largely unexplored. The aim was to explore new genetic variants underlying SUA in Chinese Han. We performed a genome-wide association study of SUA levels in Han Chinese. The discovery set contained 1634 samples and subsequent replication was comprised of 1649 females and 1169 males. 2620 subjects were recruited in the detailed analysis of rs671, alcohol drinking and SUA. We found a genome-wide significant association between SUA level and the SNP rs671 at *ALDH2* ($P = 1.2 \times 10^{-10}$) in the merged data. In addition, we also replicated the signal from rs3733590 at *SLC2A9* ($P = 1.0 \times 10^{-10}$). In males, about 0.21% to 1.95% of the total variance for SUA can be explained by rs671 using linear regression models in four independent cohorts. Of those, 56.75% to 93.51% might be explained by altering alcohol consumption due to rs671. No statistical association of rs671 and SUA was observed in females ($P = 0.409$). Furthermore, we observed a causal relationship between alcohol consumption and SUA in males using rs671 as an instrumental variable ($P = 5.1 \times 10^{-4}$). We replicated the previous findings in *SLC2A9*. Our evidence supported that rs671 was associated with SUA by affecting alcohol consumption in males. This finding strongly suggests a role for alcohol consumption in the development of hyperuricaemia and uric acid related traits.

1. Introduction

Uric acid is the end product of purine metabolism in humans and unbalanced serum uric acid (SUA) levels have been observed to cause a variety of common disorders. Elevated SUA is associated with increased risk of gout, metabolic syndrome (MetS), diabetes, and cardiovascular diseases.(Fang and Alderman 2000; Dehghan et al. 2008b; Feig et al. 2008; Roddy and Doherty 2010) Low SUA levels are a risk factor for death of incidence hemodialysis patients and multiple sclerosis.(Spitsin and Koprowski 2008; Lee et al. 2009) Thus, having a normal SUA level is crucial for the optimal functioning of the human body.

SUA levels are influenced not only by environmental but also by

Abbreviations: Abbreviation, Description; SUA, serum uric acid; MetS, metabolic syndrome; GWASs, genome-wide association studies; CSIMS2010, Cross-Sectional Investigation on Metabolic Syndrome in 2010; SSGH, samples recruited from Shanghai, Shenyang, Guangzhou and Hangzhou; XS cohort, Xiaoshan Cohort

* Correspondence author at: Department of Epidemiology & Biostatistics, School of Public Health, Zhejiang University, 866 Yuhangtang Road, Hangzhou, Zhejiang 310058, PR China.
** Corresponding author at: Department of Pathology, School of Medicine, Zhejiang University, 866 Yuhangtang Road, Hangzhou, Zhejiang 310058, PR China.
E-mail addresses: zhuym@zju.edu.cn (Y. Zhu), lmp@zju.edu.cn (M. Lai).

https://doi.org/10.1016/j.gene.2018.01.064
Received 19 April 2017; Received in revised form 8 January 2018; Accepted 17 January 2018

genetic factors.(Whitfield and Martin 1983) Recently, several genome-wide association studies (GWASs) identified and replicated common variants in the *SLC2A9* gene, a glucose transporter, and these variants are strongly associated with SUA across different human populations. (Li et al. 2007; Dehghan et al. 2008a; Vitart et al. 2008; Wallace et al. 2008; Kamatani et al. 2010; Charles et al. 2011; Li et al. 2012; Sakiyama et al. 2016) *SLC2A9* was first identified as a SUA-associated gene by Li et al. in a genome-wide scan of 4305 Sardinian individuals. (Li et al. 2007) SNP rs6855911 showed the strongest association and reached genome-wide significance after Bonferroni correction (*P*-value = 1.84×10^{-16}).(Li et al. 2007) In Japanese population, a different SNP in *SLC2A9, namely* rs11722228, was found to be significantly associated with SUA (*P*-value = 7.09×10^{-24}) and was replicated by an independent study.(Kamatani et al. 2010; Hamajima et al. 2011) SNPs rs3775948, rs7663032, rs6856396, and rs6449213 were markedly associated with SUA concentrations in a GWA study of 1017 African Americans (*P*-value $< 1 \times 10^{-8}$).(Charles et al. 2011).

Besides the *SLC2A9* gene, *ABCG2* encoding for a urate transporter has been reported to reach genome-wide significance in association studies of people of European ancestry (EA) and Japanese.(Dehghan et al. 2008a; Kamatani et al. 2010) *SLC22A12* has been implicated in the GWASs of SUA concentrations in African Americans and Japanese. (Kamatani et al. 2010; Tin et al. 2011) Moreover, a meta-analysis of 28,141 individuals found five additional loci that affect SUA levels: rs17300741, rs780094, rs12129861, rs742132, and rs12356193.(Kolz et al. 2009) Recently, a GWAS with a large EA sample had identified 18 new loci associated with SUA levels.(Kottgen et al. 2013) *SLC2A9* and *ABCG2* were validated to be associated with SUA levels in a Chinese population.(Yang et al. 2014) GWASs provide a powerful strategy and have successfully identified genetic loci contributing to the regulation of SUA levels. Previous studies mainly focused on Caucasian and African and limited attention was paid to Chinese population. However, due to the ethnic difference on genetic variations, novel common variants underlying SUA levels remain to be investigated in Chinese. In the present study, we conducted a two-stage GWAS to identify common genetic loci associated with SUA in a Chinese population. We further genotyped additional 2620 subjects for detailed rs671 analysis.

2. Materials

2.1. Participants

XiaoShan Cohort (XS cohort) was a population-based study conducted among residents aged from 18 to 95 years in Xiaoshan (a suburban area of Hangzhou). It was launched in 2010 and designed to explore the effects of environmental and genetic factors on chronic diseases such as MetS. A comprehensive demographic and health survey was carried out among individuals who participated in a large-scale physical examination in the medical center of the Third People's Hospital in Xiaoshan from July 2010 to July 2011. Each subject provided written informed consent before enrollment in this study. The study protocols were approved by the Research Ethics Committees of School of Public Health, College of Medicine, Zhejiang University. All participants were of Han Chinese origin. The original GWAS discovery stage genotyped 996 controls and 998 subjects with MetS from XS cohort, which was part of two GWASs data as described elsewhere.(Zhu et al., 2017).

The participants with MetS included in the GWA study were randomly selected based on the Chinese Diabetes Society definition in which individuals are diagnosed as having MetS if they have three or more of the following components: (1) body mass index (BMI) ≥ 25 kg/m^2, (2) fasting glucose ≥ 6.1 mmol/L and/or plasma glucose ≥ 7.8 mmol/L at 2 horal glucose and/or previously diagnosed with type 2 diabetes mellitus and treatment, (3) blood pressure $\geq$ 140/90 mmHg and/or previously diagnosed with hypertension and medication, and (4)triglycerides ≥ 1.7 mmol/L and/or HDL-C $<$ 0.9 mmol/L for males and 1.0 mmol/L for females. The healthy controls were randomly selected according to the following exclusion criteria: (1) diabetes mellitus, (2) hypertension, (3) abnormal lipid levels,(4) obesity or other chronic diseases.

Since SUA was a quantitative trait, samples were randomly selected in the replication phase. The replication stage consisted of 1649 females and 1169 males randomly selected from XS corhot after removing samples included in GWAS discovery stage.

The subjects were recruited from our cross-sectional investigation on MetS launched in 2010 in China (Cross-Sectional Investigation on Metabolic Syndrome, CSIMS2010).(Zheng et al. 2015) The subjects were randomly sampled from seven geographically representative areas in China (Shanghai, Hangzhou) for eastern China, Beijing for northern China, Shenyang for northeast China, and Taiyuan for central China, Chengdu for southwest China and Guangzhou for southern China). 2620 individuals (1206 males and 1414 females) were included from Shenyang, Shanghai, Guangzhou and Hangzhou (SSGH) in CSIMS2010 for detailed rs671 analysis. A standardized questionnaire was administrated to each individual through face-to-face interviews. The questionnaires were about the demographic and lifestyle data of each subject, including smoking habits, alcohol intake, dietary preference. Subjects who had smoked at least one cigarette per day for more than half a year either currently or formerly were defined as smokers, otherwise they were considered as non-smokers. Each participant was asked whether he was a non-drinker or an occasional drinker or a frequent drinker. Non-drinkers were defined as those who had never drunk. An occasional drinker was defined as who consumed alcoholic beverages less than three times per week. A frequent drinker was referred to people who drink three times or more per week. Food diet was evaluated based on the salt intake (light, moderate and heavy).

2.2. Serum uric acid measurements

SUA analysis was performed on fresh serum samples in all cases. SUA concentrations were measured with enzymatic colorimetric test using a Hitachi 747Automatic Analyzer.

2.3. SNP genotyping

Genomic DNA was extracted from peripheral blood lymphocytes by using a TACO Nucleic Acid Automatic Extraction System (GeneReach Biotechnology Corp., Taiwan, China). The Illumina Omni-Express platform (Illumina Inc., San Diego, CA, USA) was used for the genome-wide assay of samples in the discovery stage. The Omni-Express BeadChip is designed to genotype 731,442 markers per sample. All the BeadChips were processed in the Bio-X Institute, Shanghai Jiao Tong University. Genotyping procedures were performed according to the manufacturer's standard protocol. In the replication stage, MassARRAY iPLEX of the Sequenom platform was used for genotyping (Sequenom, Inc., San Diego, CA, USA). The PCR and extension primers were designed using MassARRAY Assay Design software (Sequenom, Inc). All the primer and probe sequences are available upon request. The genotyping procedures were carried out according to the manufacturer's standard protocol. Double-blind duplicates and negative controls were included in each 384-well plate in the replication stage. In Mendelian randomization stage, Taqman (Applied Biosystems, Life Technologies Inc., CA, USA) was applied for genotyping.

2.4. Genotyping quality control (QC) and sample QC

SNPs with call rates $\geq$95%, Hardy-Weinberg equilibrium (HWE) *P*-values $\geq 10^{-6}$, and minor allele frequencies $\geq$5% were included in the association tests. Samples with missing SUA levels and genotyping call rate $<$ 95% were removed. Principal component analysis was used to identify population stratification by running EIGENSTRAT (Price et al. 2006). To assess the cryptic relatedness between samples, pair-wise