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A causal relationship between uric acid and diabetic macrovascular disease in Chinese type 2 diabetes patients: A Mendelian randomization analysis

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ABSTRACT

Background: As the association between uric acid and macrovascular disease has been heavily debated, we aimed to confirm whether there is a causal relationship between uric acid and diabetic macrovascular disease through Mendelian randomization analysis.
Methods: In 3207 type 2 diabetes patients, seventeen SNPs (single nucleotide polymorphisms) related to uric acid were genotyped. A weighted GRS (genetic risk score) was calculated using selected SNPs and the strength of their effects on uric acid levels. Diabetic macrovascular disease was diagnosed through vascular ultrasound, magnetic resonance imaging or other clinical evidence. Associations of diabetic macrovascular disease with uric acid and weighted GRS were evaluated separately.
Results: In total participants and among females, the prevalence of diabetic macrovascular disease was significantly higher in hyperuricemic group than in normouricemic group, and uric acid was associated with diabetic macrovascular disease (OR = 1.068, $p = 0.0349$; OR = 1.122, $p = 0.0158$). The prevalence of diabetic macrovascular disease increased with the weighted GRS in a J-shaped manner for the females. The weighted GRS was positively correlated with uric acid in total population, male patients and female patients ($\beta = 0.203$, $p < 0.0001$; $\beta = 0.255$, $p < 0.0001$; $\beta = 0.142$, $p < 0.0001$, respectively). The weighted GRS was significantly associated with diabetic macrovascular disease in female patients (OR = 1.184, $p = 0.0039$). Among females, the observed association between weighted GRS and diabetic macrovascular disease was greater than predicted.
Conclusions: Using the uric acid-related weighted GRS as an instrumental variable for Mendelian randomization analysis, our study provided an evidence for causal relationship between uric acid and diabetic macrovascular disease in Chinese females with type 2 diabetes mellitus.

1. Introduction

As a highly prevalent complication of type 2 diabetes mellitus, diabetic macrovascular disease is the principal cause of disability and death [1,2], resulting in a heavy health burden. Therefore, an investigation of the risk factors for diabetic macrovascular disease may provide more clues about its treatment and prevention. As the final oxidation product of purine catabolism in humans and great apes, uric acid has been proven to be associated with endothelial dysfunction [3], vascular nitric oxide activity, smooth muscle cell proliferation and oxidative stress, which are the key mechanisms of macrovascular disease [4–6]. Epidemiological studies have identified an association between uric acid and macrovascular disease, but it is unclear whether uric acid is a coincidental, concomitant or causal factor of macrovascular disease [7]; in addition, whether uric acid is an independent risk factor for diabetic macrovascular disease is still debated [8]. This controversy may be attributed to the oxidant–antioxidant paradoxical function of uric acid [9], interference from other confounders (i.e., serum lipids, serum glucose, and blood pressure) [10], or the reverse causality in classical observational studies [11].

According to the laws of Mendelian genetics, at the time of gamete formation, alleles are randomly allocated; therefore, genetic variants should not be associated with other confounders in association

Abbreviations: SNPs, single nucleotide polymorphisms; GRS, genetic risk score; BMI, body mass index; LDL-c, low-density lipoprotein cholesterol; HDL-c, high-density lipoprotein cholesterol; eGFR, estimated glomerular filtration rate; OR, odds ratio; CI, confidence interval.

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analyses, and Mendelian randomization, which uses genetic variants as a surrogate marker for risk factors, can eliminate the influence of confounders and avoid reverse causality [12]. With the discovery of uric acid-related SNPs (single nucleotide polymorphisms) in recent studies [13–15], a Mendelian randomization study has verified the causal relationship between uric acid and cardiovascular events [16]. Thus, analyses using SNPs to construct a uric acid-related GRS (genetic risk score) may provide an unbiased approach for verifying the causal relationship between uric acid and diabetic macrovascular disease.

In this study, we used previous studies and information about our study population to select SNPs that were associated with serum uric acid. In addition, we created a weighted GRS, which was a composite GRS that accounted for a substantial amount of the variation in uric acid levels, and we investigated whether the uric acid level or the uric acid-related weighted GRS was related to diabetic macrovascular disease susceptibility.

2. Materials and methods

2.1. Ethics statement

Our study was performed in accordance with the second revision of the Declaration of Helsinki, and it was approved by the Institutional Review Board of Shanghai Jiao Tong University Affiliated Sixth People's Hospital. All participants provided informed consent.

2.2. Participants

A total of 3207 type 2 diabetes patients were recruited from the Shanghai Diabetes Institute Inpatient Database. All study participants were unrelated individuals, and they had the same predominant genetic background. The diagnosis of type 2 diabetes mellitus was based on the 1999 World Health Organization criteria. With clinical, immunological and genetic criteria, Type 1 diabetes and mitochondrial diabetes were excluded. Pregnant patients, as well as those with cancer, cardiac failure, or renal failure were excluded from the study. Patients using medications such as diuretics and urate-lowering therapies that might influence their uric acid levels were also excluded.

2.3. Clinical measurements

The history, anthropometric and biochemical traits related to diabetes were recorded in detail for every participant. Height (m) and weight (kg) were measured, and BMI (body mass index) was calculated as weight/height^2. Blood pressure (mmHg) was measured on the right arm with the patient in a seated position. The measurement was performed by an experienced medical staff member, and all measurements were repeated three times; the average values were calculated for further analysis. Serum uric acid, serum triglyceride, total cholesterol, LDL-c (low-density lipoprotein cholesterol), HDL-c (high-density lipoprotein cholesterol) and creatinine levels were measured with a type 7600–020 Automated Analyzer (Hitachi, Tokyo, Japan). HbA1c levels were measured using high performance liquid chromatography (HPLC) with a Bio-Rad Variant II hemoglobin testing system (Bio-Rad Laboratories, Hercules, CA, USA). Patients with serum uric acid levels over 7 mg/dl for males and serum uric acid levels over 6 mg/dl for females received a diagnosis of hyperuricemia [17].

2.4. Diagnosis of diabetic macrovascular disease

Bilateral carotid and bilateral lower limb arteries were examined with vascular ultrasound (Esaote MyLab Twice, Italy), and cerebrovascular disease was examined through brain magnetic resonance imaging (Achieva; Philips, Best, The Netherlands) or computed tomography (Definition AS; Siemens Medical Solutions, Forchheim, Germany). Myocardial infarction was diagnosed through either a combination of electrocardiography and clinical syndromes or prior coronary angioplasty. Patients with clinical evidence of carotid atherosclerosis, lower limb arteriosclerosis, coronary atherosclerosis, myocardial infarction or stroke were diagnosed with diabetic macrovascular disease.

2.5. Genotyping

We selected seventeen SNPs from thirteen loci that were identified as being associated with the serum uric acid level based on previous genome-wide association (GWA) studies and meta-analysis of GWA studies [13,14], and used them as representations of serum uric acid level genetically. These SNPs were genotyped using multiplex primer extension with matrix-assisted laser desorption ionization time-of-flight mass spectroscopy using the MassARRAY Compact Analyzer (Sequenom, San Diego, CA, USA). All SNPs passed the quality control with genotyping call rates over 90%. All participants had cleaned genotype information, and individuals with over 15% missing genotype data were excluded.

2.6. Statistical analysis

Analyses of the clinical characteristics were performed using SAS 9.2. Normality testing was performed to test the distribution of the data, and quantitative traits with a skewed distributed were logarithmically transformed. Continuous variables were summarized as the mean ± standard deviation or median (interquartile range). Between-group differences in the skewed distributed quantitative traits were analyzed using the Wilcoxon test, and between-group differences in the categorical variables were analyzed using the χ^2 test. Multivariable logistic regression analysis was used to analyze the association between the quantitative traits and diabetic macrovascular disease, and OR (odds ratio) with 95% CI (confidence interval) were obtained. Correlations between quantitative traits were analyzed through stepwise regression.

To further verify the association between uric acid and diabetic macrovascular disease, we selected the SNPs significantly associated with uric acid and then created the weighted GRS as an instrumental variable for further Mendelian randomization analysis. Associations between the SNPs and quantitative traits of diabetic macrovascular disease were analyzed through linear regression in PLINK [18] (v1.07; http://pngu.mgh.harvard.edu/~purcell/plink/), using an additive genetic model. To construct a valid instrumental variable, the selected SNPs should be reliably associated with the exposure and should be independent of other factors that affect the outcome; thus, we should exclude SNPs that are associated with blood glucose, blood pressure, serum lipids and BMI. Because a weighted GRS should account for the effect sizes of the SNPs, we coded each individual variant as 0, 1 or 2 based on the number of the serum uric acid-increasing alleles. We multiplied the risk alleles from each SNP by the predicted effect size of uric acid on that SNP (according to linear regression analysis), and then the total score was divided by the average effect size of these SNPs.

The weighted GRS was then divided into five layers. The uric acid level in each layer was calculated and compared using the Kruskal–Wallis Test, and the prevalence of diabetic macrovascular disease in each layer was compared using the χ^2 test. The association between the weighted GRS and the uric acid level was determined through stepwise regression, and multivariable logistic regression analysis was used to determine the association between the weighted GRS and the risk of diabetic macrovascular disease. The predicted association between the weighted GRS and diabetic macrovascular disease was calculated using the associations between the weighted GRS, uric acid levels and diabetic macrovascular disease. A two-tailed p value of <0.05 was considered statistically significant.