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

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Keywords: uric acid; diabetic macrovascular disease; single nucleotide polymorphism; genetic risk score; Mendelian randomization analysis

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 3,207 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, 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 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 3,207 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/height². 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 $\pm$ 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.

3. Results

3.1 Association between Uric Acid and Diabetic Macrovascular Disease

The clinical characteristics of the patients are shown in Table 1. Among the total participants and the female patients, the prevalence of diabetic macrovascular disease was significantly higher in the hyperuricemia group compared with the normouricemic patients (66.49% vs 57.85%, $p=0.0001$; 72.12% vs 56.60%, $p<0.0001$), but no difference was observed in the male patients. A similar phenomenon was retained after diabetic macrovascular disease was divided into subgroups including cerebral atherosclerosis, carotid atherosclerosis, lower limb arteriosclerosis, and coronary atherosclerosis. In the total population and among the female patients, uric acid levels were significantly associated with diabetic macrovascular disease (OR 1.068 (1.005, 1.136), $p=0.0349$ and OR 1.122 (1.022, 1.231), $p=0.0158$, respectively) adjusted for sex, BMI, duration of diabetes, blood pressure, serum lipids and serum glucose, but this result was not observed in the male patients.

3.2 Instrumental Variable Construction

The associations between SNPs and uric acid are shown in Table 2. All SNPs satisfied the Hardy-Weinberg equilibrium. Nine SNPs were significantly associated with serum uric acid levels (adjusted for sex, age and BMI), including which, three SNPs (rs780094, rs1183201, rs1165205) were excluded for an association with LDL-c, three SNPs (rs11722228, rs16890979, rs3775948) were in the same gene region, and two of them (rs16890979, rs3775948) were excluded, 1 SNP (rs505802) was excluded for linkage disequilibrium with another SNP (rs506338). Finally, three SNPs (i.e., rs11722228, rs2231142, rs505802) which were not associated with any other biomarkers (except uric acid) were used for construction of a uric acid-related weighted GRS. The detailed selection procedures are shown in Fig. 1.

3.3 Three-Way Associations between Weighted GRS, Uric Acid and Diabetic Macrovascular Disease

For the females, we evaluated the uric acid level and the prevalence of diabetic macrovascular disease in layers of uric acid-related weighted GRS. As shown in Fig. 2, with the increased weighted GRS, a tendency towards higher uric acid level was observed ($p<0.0001$). The curve showed a J-shaped association between the prevalence of diabetic macrovascular disease and the weighted GRS, and there was a trend of increased levels from the second layer to the fifth layer ($p=0.0005$); however, there was no significant difference between the first two layers ($p=0.6085$). Next, we evaluated the association between the weighted GRS and uric acid. After adjustments for sex, age and BMI, there were significant correlations between the weighted GRS and uric acid in the total population, the male patients and the female patients ($\beta=0.203\pm0.025$, $p<0.0001$; $\beta=0.255\pm0.036$, $p<0.0001$; $\beta=0.142\pm0.034$, $p<0.0001$, respectively). When further tested for the relationship between uric acid-related weighted GRS and diabetic macrovascular disease susceptibility, the weighted GRS was significantly associated with diabetic macrovascular disease after adjusting for sex, BMI, duration of diabetes, blood pressure, serum lipids, serum glucose and eGFR (estimated glomerular filtration rate) in female patients (OR 1.184 [1.056, 1.327], $p=0.0039$); however, no such association was observed in the males.

The triangulation approach described in Fig. 3 showed the associations of diabetic macrovascular disease with uric acid and uric acid-related weighted GRS in females. The observed association between weighted GRS and diabetic macrovascular disease was larger than the predicted association (OR: 1.184 vs 1.016).

4. Discussion

Our study revealed that among the total participants and female patients, the prevalence of diabetic macrovascular disease in the hyperuricemic group was significantly higher than that of the normouricemic group, and uric acid was significantly associated with diabetic macrovascular disease, while no similar results were witnessed in the males. With the increased weighted GRS, there was a tendency towards higher uric acid levels and a J-shaped trend in the diabetic macrovascular disease prevalence. The weighted GRS was positively correlated with uric acid in the total population, as well as in the female and male patients. A significant association between the weighted GRS and diabetic macrovascular disease was only observed in females. The predicted association, which was calculated using the weighted GRS as an instrumental variable and uric acid as the intermediate variable, was lower than the observed association between the weighted GRS and diabetic macrovascular disease.

Historically, uric acid has been considered to be a risk factor for macrovascular disease. Despite being studied in the general population [19], obese individuals [20], families [21], elderly populations [22] and individuals with diabetes [23], the association between uric acid and macrovascular disease is still uncertain. Moreover, cardiovascular disease is a multifactorial disease, and uric acid is associated with hypertension [24], type 2 diabetes mellitus [25], dyslipidemia [26], and chronic kidney disease [27]; thus, the association between uric acid and macrovascular disease tends to be confounded by other well-established risk factors [10]. Therefore, the association between uric acid and macrovascular disease has been heavily debated [8].

The validity of observational studies has been questioned regarding the influence of confounders, selection bias or reverse causation; thus, we chose Mendelian randomization analysis, which is based on the natural random allocation of alleles during meiosis and is independent of other confounders, to

verify the casual relationship between uric acid and diabetic macrovascular disease.

In our study, we selected SNPs based on previous studies, and we retested their associations with uric acid in this population. We only chose SNPs that are associated with uric acid to build the weighted GRS, thereby confirming the reliability and avoiding pleiotropy. Uric acid and weighted GRS were significantly associated with diabetic macrovascular disease in females. When we combined the weighted GRS-uric acid association and the uric acid-diabetic macrovascular disease association, we obtained the predicted association. The observed association of the weighted GRS with diabetic macrovascular disease and the predicted association were both over 1; thus, we determined that uric acid is a risk factor for diabetic macrovascular disease. Moreover, the observed association was greater than the predicted association, indicating that uric acid-related genes might also influence diabetic macrovascular disease through non-uric acid pathways, in which these uric acid-related genes might impact diabetic macrovascular disease through the regulation of glucose homeostasis, lipid metabolism or other unknown pathways. Our result is also in accordance with a previous Mendelian randomization study of uric acid levels and cardiovascular events [16], although this cohort study included patients with cardiovascular disease and had a greater focus on cardiovascular events.

After the weighted GRS was divided into five layers for the females, a J-shaped trend was found in the prevalence of diabetic macrovascular disease. Although the decrease in the prevalence of diabetic macrovascular disease from the first layer to the second layer was not significant, there was a trend of increases from the second layer to the fifth layer. Although uric acid is a risk factor for diabetic macrovascular disease, the J-shaped trend does not contradict our results or those of previous studies. Because the function of the uricase gene was lost in a mutation during primate evolution, the increased

uric acid level was the result of natural selection, and uric acid may have beneficial functions [28]. Therefore, there should be a normal range of uric acid levels; low as well as high levels of uric acid were associated with diabetic macrovascular disease [29]. A similar role of uric acid was also observed in a prospective cohort study of the association between uric acid and kidney function [30]. In addition, there was no significant difference in the diabetic macrovascular disease prevalence between the first two layers, likely because the uric acid level in type 2 diabetes patients is higher than that of normal glucose tolerance subjects [31]. The uric acid level of the first layer was not low enough to significantly increase the prevalence of diabetic macrovascular disease.

The gender-specific analysis revealed significant associations among uric acid, weighted GRS, and diabetic macrovascular disease in females but not in males, suggesting that the role of uric acid in macrovascular disease might differ between genders. Due to the influence of estrogen, females have higher serum uric acid concentrations during menopause than during the premenopausal period [32]. In our study, the mean age of the females was over 60 years, and the protective effect of estrogen had been attenuated. The percentage of hyperuricemia was higher in females than in males, indicating that females are more susceptible to hyperuricemia without estrogen protection. Moreover, although it was not demonstrated in this study, the high incidence of macrovascular disease risk factors, such as smoking, drinking and eating habits, in males might conceal the uric acid effect.. Additionally, although we have adjusted for age in the analysis, the mean age of females was higher than that of males, suggesting the possibility that higher age in females might allow more time for the manifestation of the macrovascular changes; prospective studies are needed for further confirmation.; thus, uric acid might have different effects on diabetic macrovascular disease in males and females [34, 35]. Therefore, further studies are needed to clarify the influence of gender on the effect of serum uric

acid in diabetic macrovascular disease. Moreover, considering the difference between females and males, the role of uric acid-related GRS in diabetic macrovascular disease should be separately considered in clinical practice.

5. Conclusion

In summary, our study using a uric acid-related weighted GRS as an instrumental variable supports the causal role of uric acid in diabetic macrovascular disease in female Chinese type 2 diabetes patients.

5.1 Limitations

This study has several limitations. First, we only retested the association between SNPs and uric acid in the type 2 diabetes patients in a cross-sectional study; therefore, we still cannot exclude population bias. Mendelian randomization analyses of different populations, multi-center investigations or prospective studies are needed to replicate this result [36]. Second, although this study included seventeen SNPs in thirteen genes based on previous studies, we could not completely avoid the pleiotropy of genes or exclude other interactions in the same pathway. Further functional genomic studies are urged. Third, although Mendelian randomization is an accepted method to confirm causal relationships, our analysis is based on an observational study, and further functional research is essential to verify the exact mechanism underlying the effect of uric acid on diabetic macrovascular disease. Fourth, although we could certify the role of uric acid in diabetic macrovascular disease through significant associations among uric acid, weighted GRS and diabetic macrovascular disease, more research involving the association of uric acid-related GRS with risk factors, such as endothelial dysfunction, vascular nitric oxide activity, smooth muscle cell proliferation or oxidative stress, can offer additional evidence for the

role of uric acid in diabetic macrovascular disease.

List of 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;

Authors' Contributions

Conceived and designed the experiments: WJ, CH. Performed the experiments: DY, JW, FJ, RZ. Analyzed the data: DY CH. Contributed reagents/materials/analysis tools: TW, SW, DP, ZH, HC, YB. Wrote the paper: DY, JW, FJ, RZ, TW, SW, DP, ZH, HC, YB, CH, WJ. Drafted the manuscript: DY, CH. Read and approved the final manuscript: DY, JW, FJ, RZ, TW, SW, DP, ZH, HC, YB, CH, WJ.

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Table 1. Clinical characteristics of the study participants.

	Total			Male			Female		
	Normouricemic	Hyperuricemic	<i>P</i> value	Normouricemic	Hyperuricemic	<i>P</i> value	Normouricemic	Hyperuricemic	<i>P</i> value
N (%)	2643 (82.4%)	564 (17.6%)	--	1424 (85.0%)	252 (15.0%)	--	1219 (80.0%)	312 (20.0%)	--
Age (year)	60.3±12.1	63.3±13.8	<0.0001	58.6±12.7	58.7±15.3	0.5278	62.3±11.2	67.0±11.2	<0.0001
BMI (kg/m ²)	23.9(21.7, 26.2)	25.5(23.0, 27.7)	<0.0001	24(22, 26.2)	25.5(23.2, 27.8)	<0.0001	23.7(21.5, 26.3)	25.5(22.8, 27.7)	<0.0001
SP (mmHg)	130(120, 145)	140(125, 150)	<0.0001	130(120, 140)	135(120, 150)	0.0028	135(120, 150)	140(130, 157.5)	<0.0001
DP (mmHg)	80(75, 90)	80(76, 90)	0.0044	80(75, 90)	80(80, 90)	0.0206	80(70, 85)	80(75, 90)	0.057
Uric acid (mg/dl)	4.8(4.0, 5.6)	7.3(6.7, 8.0)	<0.0001	5.2(4.4, 6.0)	7.7(7.3, 8.5)	<0.0001	4.4(3.7, 5.0)	6.8(6.3, 7.5)	<0.0001
LDL-c (mmol/L)	2.5(1.5, 3.3)	2.6(1.8, 3.4)	0.0028	2.4(1.5, 3.2)	2.5(1.6, 3.2)	0.1545	2.6(1.5, 3.4)	2.7(1.9, 3.4)	0.0183
LDL-c (mg/dl)	96.5(57.9, 127.4)	100.4(69.5, 131.3)	0.0028	92.7(57.9, 123.6)	96.5(61.8, 123.6)	0.1545	100.4(57.9, 131.3)	104.2(73.4, 131.3)	0.0183
HDL-c (mmol/L)	1.6(1.1, 4.3)	1.4(1.0, 4.4)	0.0591	1.4(1.0, 4.2)	1.4(0.9, 4.2)	0.3147	1.7(1.2, 4.5)	1.4(1.0, 4.6)	0.0128
HDL-c (mg/dl)	61.8(42.5, 166)	54.1(38.6, 169.9)	0.0591	54.1(38.6, 162.2)	54.1(34.7, 162.2)	0.3147	65.6(46.3, 173.7)	54.1(38.6, 177.6)	0.0128
TC (mmol/L)	3.7(1.2, 5.0)	3.8(1.1, 5.0)	0.5673	3.6(1.1, 4.7)	3.6(1.1, 4.8)	0.6511	3.9(1.3, 5.2)	4.0(1.2, 5.2)	0.3491
TC (mg/dl)	142.9(46.3, 193.1)	146.7(42.5, 193.1)	0.5673	139(42.5, 181.5)	139(42.5, 185.3)	0.6511	150.6(50.2, 200.8)	154.4(46.3, 200.8)	0.3491
Triglyceride (mmol/L)	2.1(1.3, 3.0)	2.3(1.7, 3.3)	<0.0001	2.1(1.1, 2.9)	2.3(1.5, 3.3)	0.0006	2.1(1.3, 3.0)	2.5(1.7, 3.3)	<0.0001
Triglyceride	185.8(115,	203.5(150,	<0.0001	185.8(97.3,	203.5(132.7, 292)	0.0006	185.8(115, 885)	221(150, 292)	<0.0001

(mg/dl)	265.5)	292)		256.6)					
eGFR (mL/min per 1.73 m ²)	125.5(105.7,149.1)	98.2(68.7,123.8)	<0.0001	125.5(105.7,148.5)	99.3(70.4,126.6)	<0.0001	125.4(106.0,150.9)	97.2(66.8,118.7)	<0.0001
HbA1c (%)	9.2(7.5, 10.8)	8.1(6.8, 10.0)	<0.0001	9.4(7.6, 11.1)	8.2(6.6, 10.6)	<0.0001	9.0(7.3, 10.4)	8.0(6.9, 9.7)	<0.0001
Diabetic macrovascular disease	1529(57.85%)	375(66.49%)	0.0001	839(58.91%)	150(59.52%)	0.8571	690(56.60%)	225(72.12%)	<0.0001
Cerebral atherosclerosis	317 (12.0%)	118 (20.9%)	<0.0001	171 (12.0%)	35 (13.9%)	0.4020	146 (12.0%)	83 (26.6%)	<0.0001
Carotid atherosclerosis	916 (34.7%)	229 (40.6%)	0.0075	500 (35.1%)	90 (35.7%)	0.8537	416 (34.1%)	139 (44.6%)	0.0006
Lower limb atherosclerosis	1176 (44.5%)	228 (45.2%)	0.7584	648 (45.5%)	115 (45.6%)	0.9697	528 (43.3%)	173 (55.4%)	0.0001
Coronary atherosclerosis	27 (1.1%)	6 (1.1%)	0.9333	19 (1.4%)	4 (1.7%)	0.7669	8 (0.7%)	2 (0.7%)	1

Data are shown as the mean±SD or as the median (interquartile range). BMI: Body mass index. SP: Systolic blood pressure. DP: Diastolic blood pressure. LDL-c: Low-density lipoprotein cholesterol. HDL-c: High-density lipoprotein cholesterol. TC: Total cholesterol. eGFR: Estimated glomerular filtration rate. The variables with a skewed distributed were compared using the Wilcoxon test. Proportions of the categorical variables were compared by the χ^2 test.

Table 2. Association between the SNPs and serum uric acid levels.

Chr*	Gene	SNP	Minor allele	MAF	β for minor allele	SE	<i>P</i> value	Empirical <i>p</i> value
1	<i>PZDK1</i>	rs12129861	A	0.1909	0.0398	0.0501	0.4280	0.9998
2	<i>GCKR</i>	rs780094	G	0.4913	-0.1345	0.0423	0.0015	0.0232
2	<i>LRP2</i>	rs2544390	T	0.4457	0.0695	0.0401	0.0830	0.7222
4	<i>SLC2A9</i>	rs11722228	T	0.2953	0.1446	0.0439	0.0010	0.0159
4	<i>SLC2A9</i>	rs16890979	T	0.0126	-0.3655	0.1734	0.0351	0.4164
4	<i>SLC2A9</i>	rs734553	C	0.0139	-0.2890	0.1666	0.0829	0.7217
4	<i>SLC2A9</i>	rs3775948	C	0.4166	-0.1535	0.0400	0.0001	0.0013
4	<i>SLC2A9-WDR1</i>	rs10489070	C	0.1340	-0.0788	0.0578	0.1725	0.9359
4	<i>ABCG2</i>	rs2231142	A	0.2985	0.2411	0.0433	2.79E-08	1.00E-04
6	<i>LRRC16A</i>	rs742132	C	0.2512	-0.0137	0.0454	0.7629	1
6	<i>SLC17A1</i>	rs1183201	T	0.1579	-0.1267	0.0538	0.0185	0.2423
6	<i>SLC17A3</i>	rs1165205	A	0.1631	-0.1452	0.0530	0.0062	0.0887
9	<i>NA</i>	rs1333049	C	0.4771	-0.0167	0.0395	0.6730	1
11	<i>SLC22A11</i>	rs17300741	G	0.0566	0.0737	0.0864	0.3939	0.9995
11	<i>SLC22A12</i>	rs505802	A	0.2581	-0.1875	0.0454	3.77E-05	0.0005
11	<i>NRXN2</i>	rs506338	C	0.2623	-0.1893	0.0452	2.89E-05	0.0004
11	<i>SF1</i>	rs606458	G	0.3707	-0.0458	0.0417	0.2727	0.9906

Chr*: Chromosome. *P* values were adjusted for sex, age and BMI. MAF: minor allele frequency.

Figure Legends

Fig. 1 Detailed procedures for selecting the SNPs used to construct the weighted GRS.

Fig. 2 Association between the weighted GRS and uric acid levels and the prevalence of diabetic macrovascular disease. The weighted GRS was divided into 5 layers (all individuals with a weighted GRS ≤ 1 were included in the first category, individuals with a weighted GRS >1 but ≤ 2 were included in the second category, etc. All individuals with a weighted GRS >4 were included in the fifth category), and the uric acid level, the prevalence of diabetic macrovascular disease were calculated separately in each layer. The uric acid levels are shown in box plots; for each box, the median is indicated by the band near the middle of the box. The lower and upper quartiles are shown by the bottom and top of the box, respectively, and the 5% and 95% percentiles are shown by the solid dots outside the box. The prevalence of diabetic macrovascular disease is marked by the simple straight line and the black plus sign. As the weighted GRS increased, the uric acid level tended to increase ($p < 0.0001$). The association between the weighted GRS and the prevalence of diabetic macrovascular disease was J-shaped.

Fig. 3 A triangulation approach was used to compare the observed association between the weighted GRS and diabetic macrovascular disease with that expected among the female type 2 diabetic patients. The associations determined through the observed regression or logistic regression analyses are depicted by the solid line. The predicted associations are depicted by the dashed line. The observed association between the weighted GRS and diabetic macrovascular disease was larger than the predicted association.