EBioMedicine 6 (2016) 162–170

Contents lists available at ScienceDirect

EBioMedicine

journal homepage: www.ebiomedicine.com

Research Paper

Type 2 Diabetes, Diabetes Genetic Score and Risk of Decreased Renal Function and Albuminuria: A Mendelian Randomization Study

Min Xu [a,b,c,1], Yufang Bi [a,b,c,1], Ya Huang [a,b,c,1], Lan Xie [d,1], Mingli Hao [a,b,c], Zhiyun Zhao [a,b,c], Yu Xu [a,b,c], Jieli Lu [a,b,c], Yuhong Chen [a,b,c], Yimin Sun [d,e,*], Lu Qi [f], Weiqing Wang [a,b,c], Guang Ning [a,b,c,*]

[a] State Key Laboratory of Medical Genomics, Key Laboratory for Endocrine and Metabolic Diseases of Ministry of Health, National Clinical Research Center for Metabolic Diseases, Collaborative Innovation Center of Systems Biomedicine, Shanghai Clinical Center for Endocrine and Metabolic Diseases, Rui-Jin Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai 200025, China
[b] Shanghai Institute of Endocrine and Metabolic Diseases, Rui-Jin Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai 200025, China
[c] Department of Endocrine and Metabolic Diseases, Rui-Jin Hospital, Shanghai Jiao Tong University School of Medicine, 197 Rui-Jin 2nd Road, Shanghai 200025, China
[d] Department of Biomedical Engineering, Medical Systems Biology Research Center, Tsinghua University School of Medicine, 18 Life Science Park Road, Beijing 100084, China
[e] National Engineering Research Center for Beijing Biochip Technology, Beijing 100084, China
[f] Department of Epidemiology, Tulane University School of Public Health and Tropical Medicine, New Orleans, LA, USA

ARTICLE INFO

Article history:
Received 8 January 2016
Received in revised form 14 February 2016
Accepted 17 February 2016
Available online 20 February 2016

Keywords:
Type 2 diabetes
Genetic epidemiology
Albuminuria
Renal function
Causal modeling

ABSTRACT

Background: Type 2 diabetes (T2D) is a risk factor for dysregulation of glomerular filtration rate (GFR) and albuminuria. However, whether the association is causal remains unestablished.
Research Design and Methods: We performed a Mendelian Randomization (MR) analysis in 11,502 participants aged 40 and above, from a well-defined community in Shanghai during 2011–2013, to explore the causal association between T2D and decreased estimated GFR (eGFR) and increased urinary albumin-to-creatinine ratio (uACR). We genotyped 34 established T2D common variants in East Asians, and created a T2D-genetic risk score (GRS). We defined decreased eGFR as eGFR < 90 ml/min/1.73 m^2 and increased uACR as uACR ≥ 30 mg/g. We used the T2D_GRS as the instrumental variable (IV) to quantify the causal effect of T2D on decreased eGFR and increased uACR.
Results: Each 1-standard deviation (SD, 3.90 points) increment in T2D_GRS was associated with decreased eGFR: odds ratio (OR) = 1.18 (95% confidence interval [CI]: 1.01, 1.30). In the MR analysis, we demonstrated a causal relationship between genetically determined T2D and decreased eGFR (OR = 1.47, 95% CI: 1.15, 1.88, P = 0.0003). When grouping the genetic loci according to their relations with either insulin secretion (IS) or insulin resistance (IR), we found both IS_GRS and IR_GRS were significantly related to decreased eGFR (both P < 0.02). In addition, T2D_GRS and IS_GRS were significantly associated with Log-uACR (both P = 0.04).
Conclusion: Our results provide novel evidence for a causal association between T2D and decreased eGFR by using MR approach in a Chinese population.

1. Introduction

With a rapid rise in number of type 2 diabetes (T2D) patients, diabetic nephropathy has become the leading cause of chronic kidney disease (CKD). In recent decades, CKD has been an important public health problem in both developed and developing countries (Levey and Coresh, 2012; Zhang et al., 2012). The evaluation of diabetic nephropathy depends on assessment of two markers, albumin excretion rate and glomerular filtration rate (GFR) (Jerums et al., 2009). It is generally believed that microalbuminuria is an early clinical manifestation of diabetic nephropathy, and that decreased GFR occurs secondarily, mainly in individuals with longstanding diabetes (Reutens, 2013). Randomized controlled trials (RCTs) have also shown that intensive diabetes treatment was associated with better renal outcomes, including lowering risk of albuminuria and increasing estimated GFR (eGFR) (de Boer

Abbreviations: BMI, body mass index; CKD, chronic kidney disease; CI, confidence interval; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; FPG, fasting plasma glucose; 2 h PG, 2-hour post-loading plasma glucose; GRS, genetic risk score; HDL, high-density lipoprotein; HWE, Hardy–Weinberg equilibrium; HOMA, homeostasis model assessment; IR, insulin resistance; IS, insulin secretion; IV, instrumental variable; LDL, low-density lipoprotein; MR, Mendelian Randomization; OGTT, oral glucose tolerance test; OR, odds ratio; RCT, randomized controlled trial; SBP, systolic blood pressure; SNP, single nucleotide polymorphism; TC, total cholesterol; T2D, type 2 diabetes; TG, triglyceride; uACR, urinary albumin-to-creatinine ratio.

* Correspondence to: G. Ning, Shanghai Clinical Center for Endocrine and Metabolic Diseases, Rui-Jin Hospital, Shanghai Jiao Tong University School of Medicine, 197 Rui-Jin 2nd Road, Shanghai 200025, China or Y. Sun, Department of Biomedical Engineering, Medical Systems Biology Research Center, Tsinghua University School of Medicine, 18 Life Science Park Road, Beijing, 100084, China.
E-mail addresses: gning@sibs.ac.cn (G. Ning), ymsun@capitalbio.com (Y. Sun).
[1] These authors contributed equally to this study.

http://dx.doi.org/10.1016/j.ebiom.2016.02.032

et al., 2011; DCCT/EDIC research group, 2014; de Boer, 2014), suggesting a possible causal link between diabetes and renal outcomes. However, because conventional epidemiological studies are subject to a variety of bias such as confounding or reverse causation, and the RCTs are largely limited by short-term intervention, other novel approaches are needed to investigate the causal relation between T2D and renal dysfunction.

Recently, the Mendelian Randomization (MR) approach has been widely used for assessing causality in population studies (Jansen et al., 2014; Ding et al., 2009). Because the genetic alleles are allocated randomly during gamete formation and are inherited independent of potential confounding factors and represented as a life-long exposure, using the genetic variants as the instrumental variable (IV) have become a widely-used approach for causal inference (Lawlor et al., 2008).

In the present study, we performed a MR analysis to explore the causal relation between T2D and decreased eGFR and increased uACR, in a large community-based sample of Chinese participants. In order to reduce the statistical errors with multi-testing, we created a T2D genetic risk score (GRS) by using 34 T2D associated common variants that were identified in East Asians and represented the comprehensive genetic susceptibility of T2D, to be used as the IV (Palmer et al., 2012; Liu and Song, 2010). In addition, we also explored the genetic variations according to their roles in insulin secretion and insulin resistance.

2. Material and Methods

2.1. Study Participants

The present analysis was one part of an ongoing study of the Risk Evaluation of cAncers in Chinese diabeTic Individuals: a lONgitudinal (REACTION) study, which is a large, nationwide, prospective study involving 259,657 community-dwelling adults, aged 40 years or older. Details of the study rationale and profile have been published elsewhere (Ning, 2012; Bi et al., 2014). The study participants were recruited from two nearby communities at Baoshan district in Shanghai during 2011 and 2013. A standard questionnaire was used to collect information about lifestyle factors, disease and medical history. Anthropometric measurements, 75-g oral glucose tolerance tests (OGTT) were performed to determine the glucose metabolism status; blood and urine samples were collected for the measurements of interests.

11,935 participants (average age 63.5 years and 35.6% men) were recruited, in which genotype information was available in 11,837 ones (99.2%). Individuals with missing information on eGFR or urinary albumin-to-creatinine ratio (uACR) ($n = 95$) were excluded. We further excluded the participants who were missing more than two variants ($n = 240$). Thus, a total of 11,502 participants were included in the final analysis. The Institutional Review Board of Rui-Jin Hospital affiliated to Shanghai Jiao Tong University School of Medicine approved the study protocol. Written informed consent was obtained from each participant.

2.2. Anthropometric Information and Biochemical Measurements

A standard questionnaire was used to collect the social demographic information, the history of chronic diseases and medications, and lifestyle factors such as smoking and drinking status and physical activity. The current smoking or drinking was defined as 'yes' if the subject smoked at least one cigarette or consumed alcohol at least once a week in the past 6 month. Physical activity at leisure time was estimated by using the short form of the International Physical Activity Questionnaire (IPAQ) (Hagstromer et al., 2006). Trained investigators measured body height and body weight. Body mass index (BMI) was calculated as weight in kilograms divided by height squared in meters (kg/m^2). Systolic and diastolic blood pressures (SBP and DBP) were measured in triplicate on the same day after at least ten-min rest by using an automated electronic device (OMRON Model HEM-752 FUZZY, Omron Company, Dalian, China), and the average value of the three measurements was used for analysis. The SBP ≥ 140 mm Hg or DBP ≥ 90 mm Hg, or those who were taking anti-hypertension medications were diagnosed as hypertension.

Fasting and 2-hour post-loading plasma glucose (FPG and 2 h PG) were measured by using hexokinase method on a clinical chemistry diagnostic system (C16000, Abbott Laboratories, Otawara-shi, Japan). Serum fasting insulin was measured by using the immunoassay diagnostic system (I2000, Abbott Laboratories, Dallas, USA). The homeostasis model assessment of insulin resistance index (HOMA-IR) was calculated as fasting insulin (μIU/ml) × fasting glucose (mmol/l) / 22.5 (Matthews et al., 1985). The homeostasis model assessment of β cell function or insulin secretion (HOMA-β) was calculated by using the formula: HOMA-β = [20 ∗ fasting insulin (uIU/ml)] / [fasting glucose (mmol/l) − 3.5] (Cersosimo et al., 2014). According to the 1999 World Health Organization diagnostic criteria (Alberti and Zimmet, 1998), type 2 diabetes was defined as FPG ≥ 7.0 mmol/l and/or 2 h-OGTT PG ≥ 11.1 mmol/l and/or treatment with anti-diabetic medication and/or previously diagnosed diabetes by physicians. Fasting serum total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-cholesterol) and low-density lipoprotein cholesterol (LDL-cholesterol) were measured by using the clinical chemistry diagnostic system (C16000, Abbott Laboratories, Otawara-shi, Japan).

2.3. Assessment of Decreased eGFR and Increased uACR

The abbreviated Modification of Diet in Renal Disease (MDRD) formula recalibrated for Chinese was used to estimate GFR (Ma et al., 2006). The formula was: $eGFR = 186 \times [\text{serum creatinine} \times 0.011]^{-1.154} \times [\text{age}]^{-0.203} \times [0.742 \text{ if female}] \times 1.233$, where 1.233 was the adjusting coefficient for Chinese and eGFR was expressed in $ml/min/1.73\ m^2$. Decreased eGFR was defined as eGFR < 90 $ml/min/1.73\ m^2$ (Inker et al., 2014), with mildly decreased eGFR defined as eGFR of 60–89 $ml/min/1.73\ m^2$, and moderately to severely decreased eGFR defined as eGFR < 60 $ml/min/1.73\ m^2$.

Urinary albumin and creatinine concentrations were determined in the first-void sterile urine samples in the early morning by rate nephelometry (Beckman Coulter, Fullerton, CA) and alkaline nitroxanthic acid method (Beckman LX/20, Brea, CA), respectively. The increased uACR was defined as a uACR ≥ 30 mg/g, with micro-albuminuria defined as a uACR of 30–299 mg/g, and macro-albuminuria as a uACR ≥ 300 mg/g (Reutens, 2013).

2.4. Selection of Genetic Loci, Genotyping, and Genetic Risk Score Construction

On considering the population specificity of genetic background, the selected variants or single nucleotide polymorphisms (SNPs) were either discovered in Europeans and replicated in East Asians (Cho et al., 2012a; Kato, 2013) or those identified and validated in meta-analysis including GWASs from East Asians (Cho et al., 2012b), including: *PROX1* rs213985913, *BCL11* rs60357484, *GCKR* rs27518370, *IRS1* rs226229029, *IGF2BP2* rs185793899, *PPARG* rs12351626, *PSMD6* rs64062621, *UBE2E2* rs23294959, *MAEA* rs1316113, *CDKAL1* rs20685255, *ZFAND3* rs38139068, *DGKB* rs15024684, *GCC1/PAX4* rs127524904, *JAZF1* rs28140937, *SLC30A8* rs117172544, *TP53INP1* rs94948283, *CDKN2A/B* rs22134095, *GLIS3* rs4287466, *PTPRD* rs8879118, *CDC123/CAMK1D* rs12272998, *CDC123/CAMK1D* rs12286011, *HHEX/IDE* rs92703125, *TCF7L2* rs112998590, *CENTD2* rs72722053, *KCNQ1* rs2670241, *KCNQ1* rs2818521, *KCNJ11* rs17387083, *SPRY2* rs80143021, *C2CD4A/C2CD4B* rs62104190, *FTO* rs53779455, *TCF2* (*HNF1B*) rs37738049, *SRR* rs2312964, *PEPD* rs33402102 and *FITM2/R3HT2DL/HNF4A* rs44318326 (Supplementary Table 1). They all reached a genome-wide significance level ($P < 5 \times 10^{-8}$) and no linkage disequilibrium relationship existed among the above loci ($r^2 = 0.000$, except it was 0.055 between rs10906115 and rs12779790 in *CDC123/CAMK1D*) according to the data of East Asian ancestries in the International HapMap release 21.