Acta Diabetologica (2018) 55:901–908
https://doi.org/10.1007/s00592-018-1165-4

ORIGINAL ARTICLE

Association of branched chain amino acids related variant rs1440581 with risk of incident diabetes and longitudinal changes in insulin resistance in Chinese

Liping Xuan[1,2] · Yanan Hou[1,2] · Tiange Wang[1,2,3] · Mian Li[1,2,3] · Zhiyun Zhao[1,2,3] · Jieli Lu[1,2,3] · Yu Xu[1,2,3] · Yuhong Chen[1,2,3] · Lu Qi[4] · Weiqing Wang[1,2,3] · Yufang Bi[1,2,3] · Min Xu[1,2,3]

Received: 20 February 2018 / Accepted: 21 May 2018 / Published online: 31 May 2018

Abstract

Aims Previous genome-wide association studies reported rs1440581 was significantly associated with circulating branched chain amino acids (BCAAs) levels in Europeans. We aimed to investigate association of BCAAs related variant rs1440581 with incident T2D risk and longitudinal changes in glucose-related metabolic traits in a community-based prospective cohort of Chinese.

Methods 6043 non-diabetic participants aged ≥ 40 years from a community-based population at baseline were included and followed-up for 5 years. The BCAAs related variant rs1440581 was genotyped. Incident T2D was defined as fasting plasma glucose (FPG) ≥ 7.0 mmol/L or taking anti-diabetic therapy. Anthropometry and biochemical measurements were evaluated at both baseline and follow-up.

Results 576 (9.5%) participants developed T2D during the 5-year follow-up. Each C-allele was associated with a 20% higher risk of incident T2D (odds ratio = 1.20, 95% confidence interval [1.05, 1.36]) after adjustments for the confounders. We did not find a main effect of the variant on increase in fasting serum insulin (FSI) level or insulin resistance (IR). However, we found rs1440581 significantly modified effect of weight gain on increase in FSI and HOMA-IR. In the C-allele carriers, body mass index increase was associated with greater increase in Log_{10}_FSI ($\beta \pm$ SE 0.027 ± 0.002) and Log_{10}_HOMA-IR (0.030 ± 0.003), as compared to T-allele (both P for interaction = 0.003).

Conclusions BCAAs related genetic variant rs1440581 was associated with an increased risk of incident T2D in a Chinese population. This variant might modify effect of weight gain on development in IR.

Keywords Branched chain amino acids · Genetic variant · Longitudinal change · Interaction · Type 2 diabetes

Introduction

Compelling evidence has shown that circulating branched chain amino acids (BCAAs) were consistently associated with type 2 diabetes (T2D), insulin resistance, obesity and other metabolic disorders [1–3]. A recent genome-wide

Managed by Massimo Federici.

Liping Xuan and Yanan Hou contributed equally to this work.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00592-018-1165-4) contains supplementary material, which is available to authorized users.

✉ Min Xu
della.xumin@163.com

[1] 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 and Shanghai Clinical Center for Endocrine and Metabolic Diseases, Rui-Jin Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China

[2] Shanghai Institute of Endocrine and Metabolic Diseases, Rui-Jin Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China

[3] Department of Endocrine and Metabolic Diseases, Rui-Jin Hospital, Shanghai Jiao Tong University School of Medicine, 197 Rui-Jin 2nd Road, Shanghai 200025, China

[4] Department of Epidemiology, Tulane University School of Public Health and Tropical Medicine, New Orleans, LA, USA

association study (GWAS) performed in Caucasians identified a common variant rs1440581 near *PPM1K* (PP2C domain-containing protein phosphatase 1K) gene associated with higher ratio of circulating BCAAs to AAAs (aromatic amino acid) [4]. In addition, Mendelian randomization analysis in 16596 individuals indicated that the genetically predicted difference in leucine or valine by rs1440581 was causally related to T2D [5]. In an earlier study, we demonstrated that rs1440581 modified the effects of dietary intervention on long-term changes in weight loss and insulin sensitivity [6], and our findings were confirmed by an independent clinical trial [7].

Given the causal relation between BCAAs and diabetes observed in Caucasians, we assumed that such relation may persist in other populations such as Asians. Genetic marker could be better than biomarker in causality inference, because it is less likely to be affected by confounding and reverse causation. We used variant rs1440581 as a surrogate marker of circulating BCAAs, and tested the association between this BCAAs variant and risk of diabetes in a prospective cohort of Chinese.

In the present study, we investigated the relation of the BCAAs-associated variant with risk of incident T2D, and the longitudinal changes in glucose related metabolic traits. We further explored the potential gene–environment interactions in the T2D risk and changes in related metabolic traits. We selected rs1440581 as the genetic marker of BCAAs on considering that, it was found to be the strongest signal with circulating BCAAs and demonstrated previously having potential modifying effect on glucose metabolism and insulin resistance [6].

Materials and methods

Study population

The current prospective analyses were based on a Chinese cohort study designed to explore the effects of genetic, lifestyle and diet on the development of diabetes, obesity and early cardiovascular diseases. The study design has been described elsewhere [8, 9]. Briefly, the participants were recruited from the Songnan community in Baoshan district, Shanghai, China, in 2008 as baseline. First, the permanent residents aged 40 or older were invited to participate a glucose survey. During this phase, anthropometric measurements were performed and standard questionnaires were used to collect information on lifestyle factors, history of diseases and medications, etc. Also, fasting blood samples were collected for biochemical measurements. The study protocol was approved by the Institutional Review Board of Rui-Jin Hospital affiliated to Shanghai Jiao Tong University School of Medicine. Every participant provided written informed consent. Totally, 10185 subjects agreed and joined the first stage of investigation. Among them, 6919 participants provided DNA samples. In 2013, all the eligible participants were invited to have follow-up examinations to determine their glucose metabolism status. The follow-up investigation included a standard questionnaire to collect information on lifestyle factors, history of diseases and medications, etc. Anthropometric measurements, blood and urine sampling were performed. We excluded those participants without genotype information for rs1440581 variant ($n = 19$), those with T2D at baseline ($n = 845$) and those missing information to define glucose metabolism status at the follow-up examination ($n = 12$). Then, 6043 participants were included for the current analyses.

Data collection and biochemical measurements

Questionnaires and anthropometric measurements were performed at baseline and follow-up by trained investigators according to standardized protocols [9]. A questionnaire was used to collect information about lifestyles, such as tobacco smoking and alcohol drinking habits, history of diseases, surgery and medications. If the subject smoked cigarettes or consumed alcohol in the past 6 months on a daily basis, the current smoking or drinking status was defined as 'yes'. The participants were divided into four groups according to their 5-year change status of smoking habits (categorical variable), as always-smokers, ex-smokers, never-smokers and new-smokers. Similarly, the change status of drinking habits was categorized into always-drinker, never-drinker, ex-drinker and new-drinker. The participants were asked whether their parents, siblings or children were ever diagnosed with T2D. If the answer was 'yes', we considered that they had a family history of diabetes. Body weight, height and waist circumferences were measured by trained physicians. Body mass index (BMI) was calculated as weight (in kilograms)/height (in square meters). Systolic and diastolic blood pressure (SBP and DBP) was measured on non-dominant arm three times using an automated electronic device (OMRON Model HEM-752, Omron Company, Dalian, China) with 1-min intervals after 10-min rest. The average value of the three measurements was used in analyses.

Venous blood samples were collected after a minimum 10-h overnight fast for biochemical analyses. Fasting plasma glucose (FPG) concentrations were measured using the glucose oxidase method through an autoanalyser (ADVIA-1650 Chemistry System, Bayer, Leverkusen, Germany). Fasting serum insulin (FSI) was measured using an electrochemiluminescence assay (Roche Diagnostics, Basel, Switzerland). Serum concentrations of triglycerides (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-c), high-density lipoprotein cholesterol (HDL-c) were measured by

an autoanalyser (ADVIA-1650 Chemistry System, Bayer, Leverkusen, Germany).

SNP selection and genotyping

We selected rs1440581 as the genetic marker of BCAAs, which was the strongest signal for plasma BCAAs in GWAS [5] and demonstrated to have potential modifying effect on glucose metabolism and insulin resistance in our previous study [6].

DNA was extracted from the buffy coat fraction of centrifuged blood using commercial blood genomic DNA extraction kit (OSR-M102-T1, TIANGEN BIOTECH CO, LTD, Beijing, China) through an automated nucleic acid extraction instrument (OSE-M48, TIANGEN BIOTECH CO, LTD, Beijing, China) according to the manufacturer's standard protocol. Specific assays were designed using the MassARRAY Assay Design software package (v3.1) (Agena Bioscience, http://agenabio.com/products/massarray-system/). Mass determination was carried out with the MALDI-TOF mass spectrometer and MassARRAY Type 4.0 software was used for data gathering (Sequenom, CapitalBio, Beijing, China) [9, 10]. The call rate for rs1440581 was 99.7% and the concordance rate was 100% based on genotyping 100 duplicates.

Incident cases ascertainment and definitions

T2D diagnosis evidence was included FPG level and glucose-lowering medication use. The known diabetes cases at baseline were excluded. Incident T2D was defined as according to the 1999 World Health Organization (WHO) criteria, as FPG $\geq$ 7.0 mmol/L or receiving anti-diabetic drugs or insulin injection at follow-up [9]. Incident impaired fasting glucose (IFG) was defined as 6.1 $\leq$ FPG < 7.0 mmol/L at follow-up, while < 6.1 mmol/L at baseline. The index of homeostasis model assessment of insulin resistance (HOMA-IR) was calculated as FSI (μIU/mL) × FPG (mmol/L)/22.5. The HOMA index of beta cell function (HOMA-β) was calculated as 20 × FSI (μIU/mL)/(FPG [mmol/L] − 3.5).

Statistical analyses

Statistical analyses were performed with SAS version 9.3 (SAS Institute, Cary, NC). Deviation of Hardy–Weinberg equilibrium for BCAAs-associated variant rs1440581 genotype was assessed by the Chi square test. Participants were categorized into three groups according to rs1440581 genotypes. ANOVA for continuous variables and the Chi square tests for categorical variables were applied for the comparison of difference according to genotypes at baseline. As with skewed distribution variables, such as serum TG, FSI, HOMA-IR and HOMA-β, data were logarithmically transformed before analyses.

Multivariate logistic regression models were used to evaluate associations of rs1440581 with incident T2D and IFG risk. Three models were used. Model 1 was unadjusted. Model 2 was adjusted for sex, baseline age and BMI, changes in BMI and smoking and drinking habits over the follow-up period. Model 3 was further adjusted for family history of diabetes, baseline serum lipids and HOMA-IR based on Model 2.

We further examined the potential interactions between rs1440581 and traditional risk factors on the risk of incident T2D and the 5-year change in the glucose-related metabolic traits, followed by stratified analysis. Multivariate logistic regression models were fixed by introducing a multiplicative term of rs1440581 and the stratified variable, rs1440581 and the stratified variable in the models simultaneously, after adjustments for sex, baseline age and BMI, and family history of diabetes.

A two-sided *P* value of less than 0.05 was considered statistically significant. We performed a Bonferroni's correction to adjust for stratified analyses. A *P* value of 0.006 (0.05/8) was considered significant after adjustment of multiple comparisons.

Results

Baseline characteristics of the study participants and the changes in metabolic traits

The baseline characteristics of study participants according to rs1440581 genotypes were presented in Table 1. The mean age was 57.8 ± 9.3 years and 37.4% were males. The major allele (T) frequency of *PPM1K* variant rs1440581 was 50.1% and the genotype distribution was in Hardy–Weinberg equilibrium ($P = 0.81$). No significant difference was found in age, sex, BMI, blood pressure, FPG, FSI, HOMA-IR, or HOMA-β between the *PPM1K* rs1440581 genotypes.

The mean value of BMI over 5 years was increased by 0.61 (SD, 2.20) kg/m^2. The FPG and HOMA-IR levels were significantly increased; while the FSI and HOMA-β levels decreased (Supplemental Table 1).

Associations of BCAAs related variant rs1440581 with incident T2D and IFG

During the 5-year follow-up, 576 (9.5%) non-diabetic participants at baseline developed T2D, and 695 participants (12.0%) with normal glucose regulation developed IFG. The incidence of T2D was increased along with C-alleles, either in non-diabetic participants (*P* for trend = 0.01, Fig. 1a) or in those normal glucose regulation (*P* for trend = 0.0005,

Table 1 Baseline characteristics of the study participants according to BCAAs-associated variant rs1440581

	TT	CT	CC	*P* values
n	1522	3012	1509	–
Age (years)	57.8 ± 9.2	57.7 ± 9.3	58.0 ± 9.3	0.60
Male, *n* (%)	570 (37.5)	1114 (37.0)	577 (38.2)	0.66
Body mass index (kg/m^2)	24.9 ± 3.5	24.9 ± 3.5	24.9 ± 3.4	0.99
Family history of diabetes, *n* (%)	215 (14.1)	465 (15.4)	207 (13.7)	0.75
Systolic blood pressure (mmHg)	131 ± 21	131 ± 22	132 ± 21	0.90
Diastolic blood pressure (mmHg)	80 ± 11	80 ± 11	80 ± 10	0.88
Current smoker, *n* (%)	366 (24.1)	728 (24.2)	384 (25.5)	0.37
Current drinker, *n* (%)	285 (18.7)	534 (17.7)	277 (18.4)	0.79
Fasting plasma glucose (mmol/L)	4.94 ± 0.54	4.95 ± 0.54	4.94 ± 0.53	0.59
Fasting serum insulin (μIU/mL)	6.70 (5.00-9.30)	6.70 (4.90–8.95)	6.70 (4.90–9.20)	0.32
HOMA-IR	1.48 (1.05–2.12)	1.47 (1.06–1.99)	1.47 (1.04–2.08)	0.45
HOMA-β	98 (70–137)	96 (68–133)	98 (69–139)	0.36
Triglycerides (mmol/L)	1.38 (0.99–1.95)	1.37 (0.98–1.95)	1.38 (0.98–1.98)	0.92
Total cholesterol (mmol/L)	5.13 ± 0.91	5.10 ± 0.94	5.15 ± 0.89	0.20
LDL-c (mmol/L)	2.47 ± 0.66	2.45 ± 0.66	2.49 ± 0.68	0.09
HDL-c (mmol/L)	1.42 ± 0.31	1.41 ± 0.30	1.41 ± 0.30	0.23

Data are unadjusted means ± standard deviation, or medians (interquartile ranges) for skewed variables, or *n* (proportions) for categorical variables

P values were calculated using ANOVA for continuous variables, and χ^2 tests for categorical variables

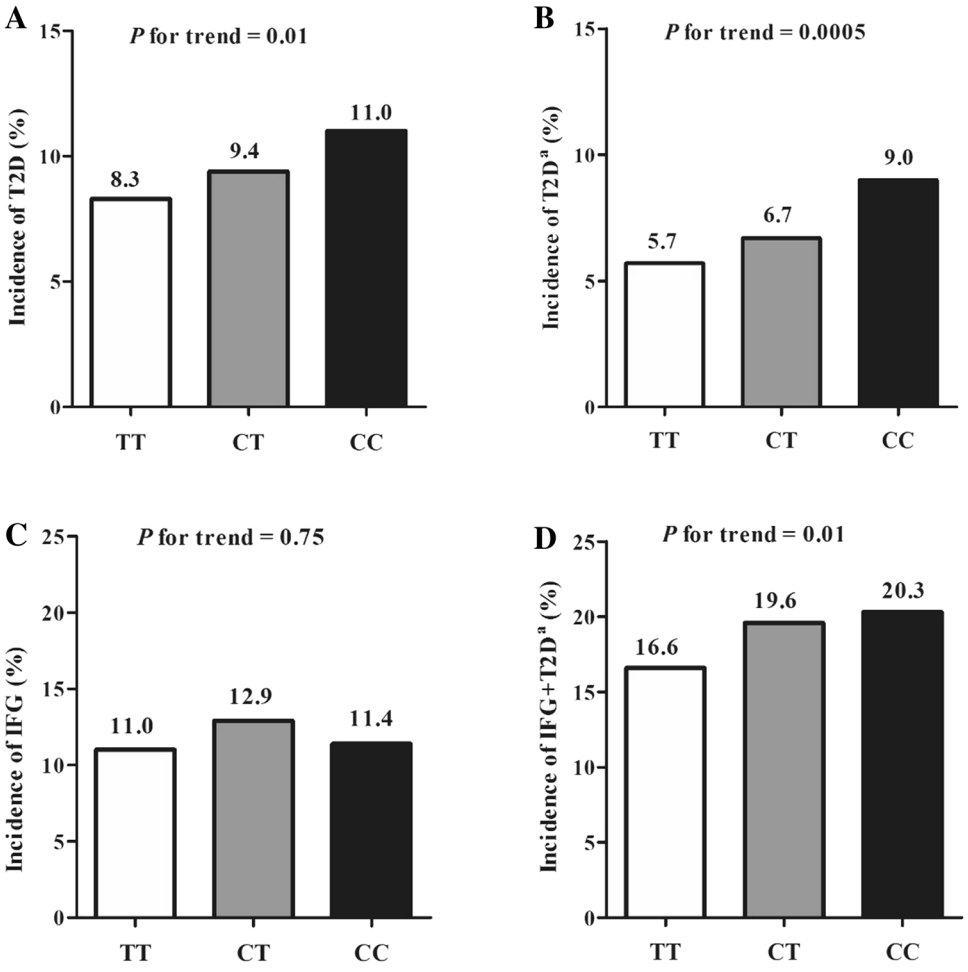

Fig. 1 **a** The incidence of T2D in non-diabetic participants at baseline by rs1440581 genotypes. **b** The incidence of T2D in participants with normal glucose regulation by rs1440581 genotypes. **c** The incidence of IFG in participants with normal glucose regulation by rs1440581 genotypes. **d** The incidence of IFG + T2D in participants with normal glucose regulation by rs1440581 genotypes. *P* for trend was calculated using Cochran–Armitage trend tests

Fig. 1b). However, no significant increasing trend for incidence of IFG with C-allele was found (Fig. 1c).

Logistic regression analyses showed that each C-allele in rs1440581 was associated with a 20% increased risk of diabetes (odds ratio [OR] = 1.20, 95% confidence interval [CI] [1.05–1.36]), after adjustments for sex, baseline age and BMI, changes in BMI and smoking and drinking habits over the follow-up period, family history of diabetes, serum lipids and HOMA-IR (Table 2). In participants with normal glucose regulation at baseline, rs1440581 C-allele was significantly associated with risk of incident T2D (OR = 1.29, 95% CI [1.11–1.50]) and composite outcome of combined T2D and IFG (OR = 1.15, 95% CI [1.04–1.27]), but not IFG ($P = 0.64$) (Table 2).

Associations of 5-year changes in BMI with changes in glucose-related metabolic traits according to rs1440581 genotypes

We observed that BMI increase was positively associated with 5-year increase in FPG, Log_{10}_FSI, Log_{10}_HOMA-IR and Log_{10}_HOMA-β (all $P < 0.0001$); whereas we did not find significant associations between BCAAs related variant rs1440581 and changes in these glucose-related metabolic traits (Supplemental Table 2). We further performed the gene–environment interaction analysis and found that rs1440581 significantly modified the effects of weight gain on increase in Log_{10}_FSI and Log_{10}_HOMA-IR (both P for interaction = 0.003), after adjustments for sex, baseline age, BMI, change in smoking and drinking habits over the follow-up period, and baseline values for the corresponding outcomes (Table 3). In the C-allele carriers, the association of BMI increases with increase in Log_{10}_FSI ($\beta \pm$ SE, 0.027 ± 0.002) and Log_{10}_HOMA-IR (0.030 ± 0.003) were more prominent than that in the

Table 2 Association of BCAAs-associated variant rs1440581 with incident T2D and IFG

Variable	Incident cases/participants	Model 1	Model 2	Model 3
Incident T2D	576/6043	1.17 (1.03–1.32)	1.18 (1.04–1.34)	1.20 (1.05–1.36)
Incident T2D[a]	405/5776	1.29 (1.12–1.49)	1.31 (1.13–1.51)	1.29 (1.11–1.50)
Incident IFG	695/5776	1.02 (0.91–1.14)	1.03 (0.92–1.15)	1.04 (0.93–1.17)
Incident IFG + T2D[a]	1100/5776	1.13 (1.03–1.24)	1.14 (1.04–1.25)	1.15 (1.04–1.27)

Data are odds ratios (ORs), 95% confidence interval (95% CI), calculated using multivariate logistic regression models. Model 1: unadjusted; model 2: adjusted for sex, baseline age and BMI, changes in BMI and smoking and drinking habits over the follow-up period; model 3: further adjusted for family history of diabetes, baseline serum lipids and HOMA-IR based on model 2

[a]Incident T2D and IFG were defined among participants whose fasting plasma glucose levels were less than 6.1 mmol/L at baseline

Table 3 Stratified analysis of associations of the 5-year changes in BMI with changes in glucose-related metabolic traits according to rs1440581 genotypes

	n	TT		CT		CC		P for interaction
		$\beta \pm$ SE	P value	$\beta \pm$ SE	P value	$\beta \pm$ SE	P value	
Δ FPG (mmol/L)	5654	0.019 ± 0.010	0.06	0.006 ± 0.009	0.46	0.032 ± 0.013	0.01	0.34
Δ Log_{10}_FSI (μIU/mL)	5508	0.019 ± 0.002	< 0.0001	0.024 ± 0.001	< 0.0001	0.027 ± 0.002	< 0.0001	0.003
Δ Log_{10}_HOMA-IR	5508	0.020 ± 0.002	< 0.0001	0.025 ± 0.002	< 0.0001	0.030 ± 0.003	< 0.0001	0.003
Δ Log_{10}_HOMA-β	5508	0.014 ± 0.002	< 0.0001	0.022 ± 0.002	< 0.0001	0.019 ± 0.003	< 0.0001	0.16

Data are regression coefficients (β) ± standard error (SE). Analysis was performed in participants excluding known diabetes at baseline, and with two measurements of those metabolic traits available

P values were calculated from generalized linear regression models, with the 5-year changes in FPG, Log_{10}_FSI, Log_{10}_HOMA-IR and Log_{10}_HOMA-β as dependent variables, after adjustments for sex, baseline age and BMI, changes in smoking and drinking habits over the follow-up period, and baseline values for the corresponding outcomes; P values for interaction were calculated from generalized linear regression models, with the 5-year changes in FPG, Log_{10}_FSI, Log_{10}_HOMA-IR and Log_{10}_HOMA-β as dependent variables, and the BMI changes, the rs1440581 genotype, and the multiplicative term of BMI changes and rs1440581 genotype simultaneously in the model, after adjustments for sex, baseline age and BMI, changes in smoking and drinking habits over the follow-up period, and baseline values for the corresponding outcomes

T-allele carriers (0.019 ± 0.002 and 0.020 ± 0.002, respectively). We did not detect a significant interaction between BMI change and the genetic variant on changes in FPG and Log_{10}_HOMA-β (both $P \geq 0.16$, Table 3).

Interactions between genetic variant and traditional risk factors

We found a nominal significant interaction of rs1440581 variant with baseline SBP and DBP levels on risk of incident T2D, after adjustments for the confounders (*P* for interaction = 0.01 and 0.02, respectively). The C-allele was associated with a stronger risk of T2D with SBP ≥ 120 (OR = 1.28, 95% CI [1.11–1.47]) and DBP ≥ 80 mmHg (OR = 1.36, 95% CI [1.15–1.60]), as compared to the lower blood pressure (the corresponding OR = 0.85 and 0.99). No significant interactions were observed between the genetic variant and sex, baseline age, BMI, smoking and drinking status, and FPG (Fig. 2).

Discussion

In this prospective investigation performed in a large size of Chinese cohort, we reported that the most strong and leading genetic signal of blood BCAAs levels from the GWASs, rs1440581 near *PPM1K* gene, was significantly associated with incident T2D risk. Furthermore, rs1440581 significantly modified effects of BMI changes on changes in IR, as indicated in FSI and HOMA-IR level. The association of BMI increase with increases in FSI and HOMA-IR was more prominent in C-allele carriers than that in T-allele.

A limited number of studies have examined effects of genetic determinants of serum BCAAs levels on the risk of incident T2D and longitudinal changes in glucose-related traits; and particularly the potential interactions with traditional risk factors in prospective cohort studies. Previous studies demonstrated a positive association of circulating BCAAs and the risk of T2D in different ethnicity groups [11–13]. A recent systematic review and meta-analysis reported BCAAs (leucine, isoleucine, and valine) prospectively associated with pre-diabetes and/or incident

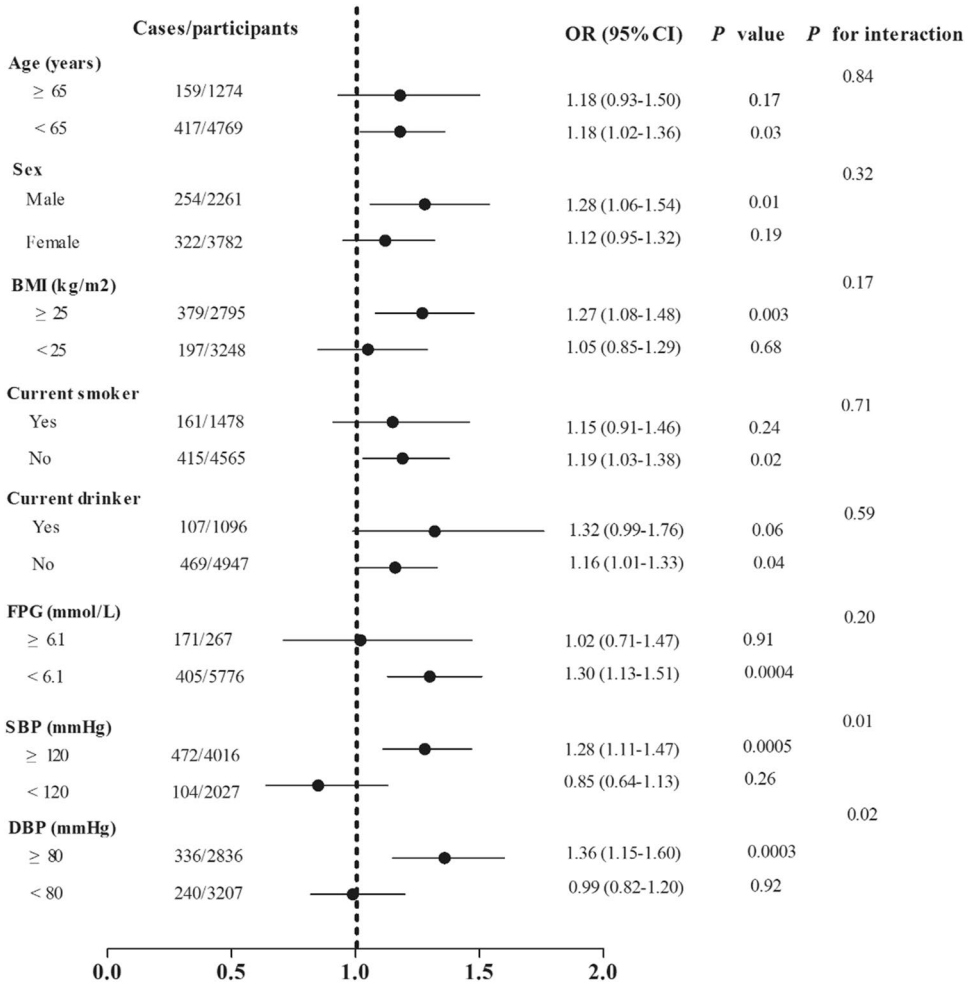

Fig. 2 Stratified analysis of BCAAs-associated variant rs1440581 on the risk of incident T2D. *P* values were calculated using logistic regression model after adjustments for sex, baseline age and BMI, and family history of diabetes. *P* for interaction was tested by logistic regression analysis, by introducing a multiplicative term of rs1440581 and the categorized stratified factor, rs1440581 and the categorized stratified factor in the model simultaneously, after same adjustments as above

T2D using comprehensive high-throughput metabolomics techniques [14]. In a very recent Mendelian randomization analysis performed in a combined large sample size of Caucasians, a genetically predicted difference of one standard deviation in amino acid level was associated with an odds ratio of T2D of 1.44 for isoleucine, 1.85 for leucine, and 1.54 for valine [5]. In our present study, we demonstrated a BCAAs related genetic variant rs1440581 was significantly associated with higher risk of incident T2D. The results were also in line with our previous findings that rs1440581 affected changes in insulin sensitivity. Our data provided supporting evidence for a causal relationship between serum BCAAs and an increased risk of T2D. According to the Mendel's Second Law, the random assortment of alleles/genotypes transferred from parent to offspring at the time of gamete formation [15, 16]. The independent distribution of alleles/genotypes allows a study relating a health outcome to a genetic variant would not be affected by confounders that often distort the interpretation of findings. Besides, because the random assignment to genotype occurs at conception, the association between a genetic variant and a disease is less affected by reverse causation [17].

Furthermore, we found the rs1440581 variation significantly modified effects of BMI increase on increase in FSI and HOMA-IR level during 5 years of follow-up. These findings were consistent with and confirmed what we have identified in the previous study [6]. In the gene–diet interaction analysis, we reported that individuals carrying the C allele of the BCAAs-associated variant rs1440581 might benefit less in weight loss and improvement of insulin sensitivity than those without this allele when undertaking an energy-restricted high-fat diet [7]. These findings suggested the rs1440581 might play an important role in modifying weight regulation and related metabolic benefits.

PPM1K was identified as a T2D gene by a systems genetics approach and rs1440581 is at upstream of this gene [18]. The expression of *PPM1K* influenced insulin secretion in human islets. The *PPM1K* is a kind of branched chain α-ketoacid (BCKA) dehydrogenase phosphatase and acts importantly in the metabolism of BCAAs [19, 20]. A recent study in mice demonstrated that the expression of *PPM1K* in muscle biopsies during an oral glucose challenge failed to increase in patients with T2D, suggesting that impaired α-keto acid dehydrogenase (BCKD) activation might partly mediated the association between IR and higher BCAA levels [21]. Menni et al. reported that BCKA 3-methyl-2-oxovalerate was the strongest predictor of IFG and the genetic variant rs1440581 had the strongest associations with all BCAAs and all BCKAs in their study [22]. Further studies are needed to determine whether the rs1440581 polymorphism affects *PPM1K* expression, and further influences the risk of developing diabetes.

The strengths of this study included the large well-defined community-based prospective cohort study design and a relatively large number of participants. Moreover, it is the first time to assess a GWAS-identified and validated BCAAs-associated genetic variant rs1440581 with risk of incident T2D and its effect on longitudinal changes in glucose-related metabolic traits. Several limitations should also be acknowledged. First, the 75-g oral glucose tolerance test was not performed at baseline examinations. Absence of the 2 h post-loading glucose might misclassify participants with T2D into non-diabetic group at baseline and underestimate new cases during the follow-up period. Second, we did not measure circulating BCAAs levels in the participants, which prevented the potential analysis of the association between the genetic variant and circulating BCAAs levels. Although, a genetic marker could be a surrogate for a biomarker in revealing causal relationship according to Mendelian randomization theory [16, 23], we acknowledged that the present study is a genetic association analysis, not a standard Mendelian randomization analysis. Testing circulating levels of BCAAs and AAAs would have provided more evidence and strengthened the conclusion. Third, we studied a single one genetic variant associated with BCAAs levels. Rs1440581 near *PPM1K* gene was first identified in large-scale GWAS and consistently validated as strongest and leading signal of genetic predisposition to diabetes risk in relation to circulating BCAAs [4, 5]. Whereas, a genetic risk score consisting more established loci related circulating BCAAs levels as the instrument variable are warranted. Lastly, our population is limited to middle-aged and elderly Chinese subjects. Further studies from other demographic groups or ethnicities are expected.

In conclusion, we found that the BCAAs related variant rs1440581 was significantly associated with the risk of incident T2D in Chinese. This genetic variant might modify the longitudinal changes in IR in relation to body weight changes. It would provide supportive evidence for high-risk stratification of population and personalized prevention to prevent diabetes. Replication of the findings in other populations and potential mechanism exploration are warranted.

Acknowledgements We thank all the study participants for their participation and contribution. We are also grateful for research team who contributed to data collection and laboratory measurement.

Author contributions LX and YH performed/supervised genetic analyses, contributed to data interpretation, and wrote the manuscript; LX, YH, TW, ML, ZZ, JL, YX, and YC contributed to acquisition of clinical and genetic data and reviewed the manuscript; LX, YH, MX, YB, LQ and WW contributed to genetic analyses and data interpretation, and reviewed the manuscript; MX designed study, contributed to data interpretation, wrote the manuscript and takes full responsibility for the work as a whole.

Funding This work was supported by grants from the National Natural Science Foundation of China (81471062, 81471059, 81500660 and 81500610), the Ministry of Science and Technology of China (2016YFC1305600 and 2016YFC1304904), Shanghai Jiao Tong University SMC-Chen Xing Project (2016), the Gaofeng Clinical Medicine Grant Support from the Shanghai Municipal Education Commission (20152508 and 20161301) and the Shanghai Pujiang Program (18PJ1409600).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval The study protocol was approved by the Institutional Review Board of Rui-Jin Hospital Affiliated to Shanghai Jiao Tong University School of Medicine.

Informed consent Informed consent was obtained from all individual participants included in this study.

References

1. Newgard CB, An J, Bain JR et al (2009) A branched-chain amino acid-related metabolic signature that differentiates obese and lean humans and contributes to insulin resistance. Cell Metab 9:311–326
2. Stancakova A, Civelek M, Saleem NK et al (2012) Hyperglycemia and a common variant of GCKR are associated with the levels of eight amino acids in 9,369 Finnish men. Diabetes 61:1895–1902
3. Suhre K, Meisinger C, Doring A et al (2010) Metabolic footprint of diabetes: a multiplatform metabolomics study in an epidemiological setting. PloS One 5:e13953
4. Kettunen J, Tukiainen T, Sarin AP et al (2012) Genome-wide association study identifies multiple loci influencing human serum metabolite levels. Nat Genet 44:269–276
5. Lotta LA, Scott RA, Sharp SJ et al (2016) Genetic predisposition to an impaired metabolism of the branched-chain amino acids and risk of type 2 diabetes: a mendelian randomisation analysis. PLoS Med 13:e1002179
6. Xu M, Qi Q, Liang J et al (2013) Genetic determinant for amino acid metabolites and changes in body weight and insulin resistance in response to weight-loss diets: the Preventing Overweight Using Novel Dietary Strategies (POUNDS LOST) trial. Circulation 127:1283–1289
7. Goni L, Qi L, Cuervo M et al (2017) Effect of the interaction between diet composition and the *PPM1K* genetic variant on insulin resistance and β cell function markers during weight loss: results from the Nutrient Gene Interactions in Human Obesity: implications for dietary guidelines (NUGENOB) randomized trial. Am J Clin Nutr 106(3):902–908
8. Bi Y, Wang W, Xu M et al (2016) Diabetes genetic risk score modifies effect of bisphenol A exposure on deterioration in glucose metabolism. J Clin Endocrinol Metab 101:143–150
9. Xu M, Lv X, Xie L et al (2016) Discrete associations of the GCKR variant with metabolic risk in a Chinese population: longitudinal change analysis. Diabetologia 59:307–315
10. Xu M, Huang Y, Xie L et al (2016) Diabetes and risk of arterial stiffness: a mendelian randomization analysis. Diabetes 65:1731–1740
11. Tillin T, Hughes AD, Wang Q et al (2015) Diabetes risk and amino acid profiles: cross-sectional and prospective analyses of ethnicity, amino acids and diabetes in a South Asian and European cohort from the SABRE (Southall And Brent REvisited) Study. Diabetologia 58:968–979
12. Wang TJ, Larson MG, Vasan RS et al (2011) Metabolite profiles and the risk of developing diabetes. Nat Med 17:448–453
13. Palmer ND, Stevens RD, Antinozzi PA et al (2015) Metabolomic profile associated with insulin resistance and conversion to diabetes in the Insulin Resistance Atherosclerosis Study. J Clin Endocrinol Metab 100:E463–E468
14. Guasch-Ferre M, Hruby A, Toledo E et al (2016) Metabolomics in prediabetes and diabetes: a systematic review and meta-analysis. Diabetes Care 39:833–846
15. Davey Smith G, Ebrahim S (2005) What can mendelian randomisation tell us about modifiable behavioural and environmental exposures? BMJ (Clin Res Ed) 330:1076–1079
16. Didelez V, Sheehan N (2007) Mendelian randomization as an instrumental variable approach to causal inference. Stat Methods Med Res 16:309–330
17. Qi L (2009) Mendelian randomization in nutritional epidemiology. Nutr Rev 67:439–450
18. Taneera J, Lang S, Sharma A et al (2012) A systems genetics approach identifies genes and pathways for type 2 diabetes in human islets. Cell Metab 16:122–134
19. Lu G, Sun H, She P et al (2009) Protein phosphatase 2Cm is a critical regulator of branched-chain amino acid catabolism in mice and cultured cells. J Clin Investig 119:1678–1687
20. Joshi M, Jeoung NH, Popov KM, Harris RA (2007) Identification of a novel PP2C-type mitochondrial phosphatase. Biochem Biophys Res Commun 356:38–44
21. Lian K, Du C, Liu Y et al (2015) Impaired adiponectin signaling contributes to disturbed catabolism of branched-chain amino acids in diabetic mice. Diabetes 64:49–59
22. Menni C, Fauman E, Erte I et al (2013) Biomarkers for type 2 diabetes and impaired fasting glucose using a nontargeted metabolomics approach. Diabetes 62:4270–4276
23. Interleukin-6 Receptor Mendelian Randomisation Analysis (IL6R MR) Consortium, Swerdlow DI, Holmes MV et al (2012) The interleukin-6 receptor as a target for prevention of coronary heart disease: a mendelian randomisation analysis. Lancet 379(9822):1214–1224