Polymorphisms and Gene-Gene Interaction in AGER/IL6 Pathway are Associated with Diabetic Ischemic Heart Disease

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Abstract

Background: The aim of the present study is to demonstrate the association of *AGER* and *IL6R* gene polymorphisms with diabetic ischemic heart disease (IHD), and to investigate the effect of gene-gene interaction on disease risk.

Methods: Our study included 204 ischemic heart disease cases who have previously been diagnosed as diabetes before the diagnoses of IHD, and 882 health controls. Polygenic risk score (PRS) was calculated by summing the number of risk alleles of all the candidate single nucleotide polymorphisms (SNPs). Logistic regression was used to find the association of candidate SNPs and PRS with diabetic ischemic heart disease. Generalized multifactor dimensionality reduction (GMDR) was used to illustrate gene-gene interaction. Haplotypes were identified and analyzed via Haploview and Plink software.

Results: The rs184003 and rs2070600 in *AGER* gene were significantly associated with the risk of diabetic ischemic heart disease ($P_{additive}$=0.005; $P_{additive}$=0.025, respectively). For *IL6R* rs4845625, CT and TT genotype were associated with lower risk of the disease comparing with CC genotype (OR=0.692, *P*=0.045; OR=0.503, *P*=0.003, respectively). After adjustment for covariates, the association of rs4845625 with disease remained statistically significant. Haplotypes in *AGER* gene (rs184003-rs1035798-rs2070600-rs1800624) and *IL6R* gene (rs7529229-rs4845625-rs4129267-rs7514452-rs4072391) were both significantly associated with diabetic ischemic heart disease (*P*=0.008; *P*=0.007). PRS was associated with the disease (OR=1.106, *P*=0.020) after adjusting for covariates. The GMDR analysis suggested that rs184003 and rs4845625 was the best interaction model after permutation testing (*P*=0.001) with a cross-validation consistency of 10/10.

Conclusions: SNPs and haplotypes in *AGER* and *IL6R* gene and the interaction of rs184003 in *AGER* with rs4845625 in *IL6R* were significantly associated with diabetic ischemic heart disease.

Introduction

Cardiovascular disease (CVD) is the leading cause of mortality in people with type 2 diabetes mellitus (T2DM). About 68% of deaths in type 2 diabetic patients are caused by cardiac complications[1]. However, it is not clear how diabetes promotes cardiac dysfunction. The widely acceptable hypothesis is that many signaling cascades, ultimately resulting in pro-inflammatory reaction, oxidative stress or thrombotic pathways, and subsequently leading to vascular inflammation[2]. It has been demonstrated that advanced glycation end products (AGER) / interleukin-6 (IL-6) pathway plays an important role in the physiological mechanism of diabetic cardiovascular complication. High glucose level can trigger neutrophil to release S100 calcium-binding protein A8/A9 (S100A8/A9), which binds to AGER on Kupffer cells, and leads to IL-6 secretion[3]. IL-6 / IL-6R complex activate JAK2 / STAT3 pathway, which can mediate pro-inflammatory response and increase platelet thrombopoiesis by inducing thrombopoietin (TPO) production[4, 5].

Several single nucleotide polymorphisms (SNPs) in *AGER* gene have been reported to be associated with diabetes or its complications. The Atherosclerosis Risk in Communities Study showed an association between rs2070600 and an approximate 50% reduction in soluble AGER levels[6]. A meta-analysis has highlighted a significant association of rs2070600 with the risk of diabetic nephropathy development[7]. However, the association between rs2070600 and diabetic cardiovascular disease are under-reported. On *IL6R* gene, the rs1800624 and rs1800625 are in absolute or strong linkage disequilibrium, and were reported to be protective factors for cardiovascular disease[8, 9]. However, the effect of them on the vascular complications in T2DM remains inconsistent[10, 11], In addition, the gene-gene interactions on the increased risk of the disease requires further clarification .

The current study aimed at illustrating the association of *AGER* and *IL6R* gene polymorphisms with the risk of diabetic cardiovascular disease, and assess the modulatory effect of gene-gene interaction between these variants on disease risk. The result would provide evidence on the precise prevention of ischemic heart disease in diabetes.

Method

Study design and population

A total of 204 diabetic ischemic heart disease cases and 882 health controls were enrolled from communities in Beijing. All subjects gave written informed consent. This study was approved by the Ethics Committee of Capital Medical University (No:2016SY24).

Inclusion criteria for the cases were as follows: (1) T2DM patients diagnosed according to American Diabetes Association Criteria [12], or receiving pharmaceutical treatment on T2DM; (2) Ischemic heart disease defined by clinical history, including acute myocardium infarction, angina pectoris and/or ischemic electrocardiographic alterations; (3) T2DM was diagnosed earlier than ischemic heart disease. (4) The medical records or copies should be provided to verify the diagnose of diseases.

Inclusion criteria for the controls were as follows: (1) Subjects had not been diagnosed as T2DM before, and fasting blood glucose was less than 5.6 mmol/L in the current survey. (2) Subjects did not have cardiovascular disease, which included ischemic heart disease, ischemic stroke, or cerebral hemorrhage. (3) Subjects did not have chronic kidney disease. (4) Subjects were not in the acute phase of infection.

Measurements

Life style risk factors were obtained from structured questionnaire. Smoking status was categorized as: “currently smoking” and “past / never smoking”. Current smoking was defined as at least 1 cigarette per day, lasting for more than 1 year. Those who have never smoked before or have not smoked for at least 3 months were defined as past / never smoking. Alcohol drinking was categorized as “currently alcohol drinking” and “past / never alcohol drinking”. Currently drinking was defined as at least drink once per

week and still drank at that frequency in the previous month. Those who never drink alcohol or have not drank alcohol for at least one month were defined as never / past alcohol drinking.

Blood pressure (BP) was measured in the morning before participants use anti-hypertensive medication. Participants were asked to rest for at least 30 minutes before BP measurement if they had just smoked or had caffeinated products. BP (mmHg) was measured for three times at sitting positions by mercury sphygmomanometer. The average of the last two measurements was used for data analysis.

Serum markers

After an overnight fasting, all participants underwent fasting blood sampling. Fasting blood samples are collected and restored in 2% EDTA vacutainer for each participant. After centrifuging, plasma and blood cell samples are separated into two cryovials. Fasting plasma glucose (FPG), total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDLC), low-density lipoprotein cholesterol (LDLC) were tested using the Beckman coulter chemistry analyzer AU5800 in the clinical laboratory of Beijing Hepingli Hospital.

Venous blood samples were obtained and stored in 4^{o}C refrigerator. All the hematological analysis was done within 8 hours. Serum glucose and biochemical determinations were measured by an enzymatic method using a chemistry analyzer (Beckman LX20, Beckman, Brea, CA, USA) at the central laboratory of the hospital.

Genotyping

Important functional SNPs and previously reported susceptible SNPs were selected as candidate SNPs. Five SNPs (rs1035798, rs1800624, rs1800625, rs184003 and rs2070600) in *AGER* gene and seven SNPs (rs2228144, rs4072391, rs4129267, rs4537545, rs4845625, rs7514452 and rs7529229) in *IL6R* gene were selected in the current study.

Genomic DNA was extracted from 1 ml of peripheral blood cell using TIANGEN DNA kit (TIANGEN Biotech, China, DP319-01) according to the manufacturer's protocol. Primers were designed by the AssayDesigner3.1 software, and they were synthesized by Thermo Fisher Scientific Co., Ltd. Detailed information of the primers were shown in **supplementary table S1**. A Sequenom MassARRAY® matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS) platform (Sequenom Inc., San Diego, CA, USA) were used to genotype SNPs.

Definition of diseases and recommend level of their risk factors

T2DM was defined as FPG ≥ 7.0 mmol/L or self-reported physician-diagnosed diabetes and/or on use of antidiabetes agents, according to American Diabetes Association Criteria[12]. Ischemic heart disease (IHD) was defined as non-fatal ischemic heart disease, including acute myocardial infarction and angina pectoris. The incident of ischemic heart disease in T2DM patients were defined as diabetic ischemic heart disease. Hypertension was defined as systolic blood pressure (SBP) ≥ 140 mmHg and/or diastolic blood

pressure (DBP) ≥ 90 mmHg and/or on current antihypertensive medication. Participants with TG ≥ 2.3 mmol/L, or TC ≥ 6.2 mmol/L, or LDLC ≥ 4.1 mmol/L, or HDLC ≤ 1.0 mmol/L were defined as dyslipidemia according to the criteria of the 2016 Chinese guidelines for the management of dyslipidemia in adults[13].

Statistical analysis

Continuous variables with normal distribution are expressed as means ± standard deviations (SDs). Categorical variables were expressed as number (percentage). Student's *t* test was used to compare the difference of each continuous variables. Polygenic risk score (PRS) was calculated by summing the number of risk alleles of all the eleven candidate SNPs. Logistic regression was used to evaluate the association of the diabetic ischemic heart disease with candidate SNPs and PRS. SPSS25.0 software (SPSS Inc., Chicago, IL, USA) was used for all abovementioned statistical analysis. Generalized multifactor dimensionality reduction (GMDR) method was used to estimate the gene-gene interaction. For the adjustment for multiple testing, a permutation test with 1000 replications was performed. Haplotypes were identified and visualized by Haploview software. The association between haplotypes and diabetic cardiovascular disease were demonstrated by using Plink software. A two-sided $P \leq 0.05$ was considered statistically significant.

Result

General characteristics of the studied participants

A total of 882 health controls and 204 diabetic cardiovascular disease cases were included in the current study. DBP, TG and FPG were significantly higher in cardiovascular disease cases than controls ($P < 0.001$). SBP, TC, LDLC and HDLC were significantly higher in controls compared with cases ($P < 0.001$). According to recommendation of "2017 Guidelines for the prevention and treatment of type 2 diabetes in China", the percentage of SBP, DBP, HDLC, LDLC, TG and TC in ideal range were significantly higher in control group compared with cases ($P < 0.001$). In people with diabetic cardiovascular disease, the proportion of current smoker or alcohol drinker was significantly lower than controls ($P < 0.001$). Details were show in Table1.

Table 1
Demographic and biochemical characteristics of the participants

	Controls	T2DM + CHD	*P* value
Age (years)	61.83 ± 10.55	63.97 ± 9.43	0.058
Male	488(55.3)	119(58.3)	0.436
SBP (mmHg)	136.79 ± 18.15	131.24 ± 11.78	< 0.001**
DBP (mmHg)	78.74 ± 10.73	81.02 ± 8.34	0.001**
BMI (kg/m^2)	25.89 ± 3.47	25.67 ± 3.18	0.428
TC (mmol/L)	5.16 ± 1.07	4.76 ± 1.26	< 0.001**
HDLC (mmol/L)	1.39 ± 0.38	1.26 ± 0.40	< 0.001**
LDLC (mmol/L)	3.06 ± 0.88	2.73 ± 0.86	< 0.001**
TG (mmol/L)	1.62 ± 1.01	2.54 ± 1.38	< 0.001**
FPG (mmol/L)	5.87 ± 2.43	7.82 ± 2.91	< 0.001**
Current smoking	194(22.0)	18(8.8)	< 0.001**
Current drinking	295(33.5)	32(15.7)	< 0.001**
AGEs (mmol/L)	35.31 ± 15.91	37.76 ± 17.13	0.151
IL-6 (mmol/L)	137.75 ± 41.25	135.94 ± 35.65	0.675

BMI body mass index, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *FPG* fasting plasma glucose, *TG* triglyceride, *TC* total cholesterol, *LDL-C* low density lipoprotein cholesterol, *HDL-C* high density lipoprotein cholesterol, *AGEs* advanced glycation end products, *IL-6* interleukin 6. ***P*< 0.01.

Association of AGER, IL6R **polymorphisms with diabetic cardiovascular disease**

All polymorphisms were in Hardy-Weinberg equilibrium (all *P*-values were more than 0.05). For *AGER* rs184003, participants with CA and AA genotype have significantly higher risk of diabetic cardiovascular disease compared with CC genotype (OR = 1.435, *P* = 0.039; OR = 2.525, *P* = 0.030, respectively). The A allele is associated with an increased risk of diabetic cardiovascular disease by 50% in additive and dominant models (*P* = 0.005; *P* = 0.013, respectively). For *AGER* rs2070600, the T allele is associated with a lower risk of diabetic cardiovascular disease by 30% in additive and dominant models (*P* = 0.025; *P* = 0.030, respectively). However, after adjusting for potential confounders, the association between the above two SNPs and disease were null.

For *IL6R* rs4845625, participants with CT and TT genotype have significantly lower risk of diabetic cardiovascular disease compared with CC genotype (OR = 0.692, *P* = 0.045; OR = 0.503, *P* = 0.003, respectively). The T allele can significantly decrease the risk of diabetic cardiovascular disease in additive and dominant models (OR = 0.707, *P* = 0.005; OR = 0.632, *P* = 0.013, respectively). The

association between rs4845625 and disease was still significant after adjusting for potential confounders. Details were shown in Table 2. Polygenic risk score is also associated with an increased risk of diabetic cardiovascular disease by 10% (OR = 1.098, 95%*CI*: 1.041 ~ 1.160, *P* = 0.001). After adjusting for dyslipidemia, hypertension, smoking, and drinking status, PRS was consistently associated with the disease (OR = 1.106, 95%*CI*: 1.016 ~ 1.205, *P* = 0.020).

Table 2
Associations of gene polymorphisms with the risk of diabetic cardiovascular disease

	Genotype	Crude OR£ (95%CI)	Crude *P* value	Adjusted OR¤ (95%CI)	Adjusted *P* value
rs1035798	GG	Ref	Ref	Ref	Ref
	AG	1.156 (0.817, 1.637)	0.413	1.200 (0.781, 1.845)	0.406
	AA	0.887 (0.360, 2.183)	0.794	0.767 (0.237, 2.481)	0.658
	additive	1.069 (0.805, 1.420)	0.645	1.072 (0.750, 1.531)	0.704
	dominant	1.124 (0.804, 1.570)	0.495	1.149 (0.758, 1.742)	0.514
	recessive	0.852 (0.348, 2.086)	0.725	0.726 (0.226, 2.333)	0.591
rs1800624	AA	Ref	Ref	Ref	Ref
	AT	1.105 (0.776, 1.572)	0.580	1.168 (0.756, 1.806)	0.484
	TT	0.987 (0.446, 2.183)	0.974	0.941 (0.347, 2.549)	0.905
	additive	1.054 (0.799, 1.389)	0.711	1.077 (0.764, 1.518)	0.673
	dominant	1.088 (0.778, 1.521)	0.622	1.136 (0.749, 1.721)	0.549
	recessive	0.961 (0.437, 2.114)	0.921	0.900 (0.335, 2.419)	0.835
rs1800625	AA	Ref	Ref	Ref	Ref
	AG	1.071 (0.746, 1.538)	0.709	1.387 (0.884, 2.177)	0.150
	GG	2.324 (0.672, 8.041)	0.183	1.992 (0.440, 9.022)	0.371
	additive	1.157 (0.835, 1.601)	0.381	1.393 (0.934, 2.077)	0.104

£ No variables were adjusted in logistic regression model

¤ Dyslipidemia, hypertension, smoking, and drinking were adjusted in the logistic regression model.

*P< 0.05, **P< 0.01.

	Genotype	Crude OR£ (95%CI)	Crude *P* value	Adjusted OR¤ (95%CI)	Adjusted *P* value
	dominant	1.117 (0.785, 1.589)	0.539	1.418 (0.914, 2.201)	0.119
	recessive	2.286 (0.663, 7.885)	0.191	1.842 (0.410, 8.286)	0.426
rs184003	CC	Ref	Ref	Ref	Ref
	CA	1.435 (1.019, 2.020)	0.039*	1.306 (0.840, 2.032)	0.236
	AA	2.525 (1.092, 5.837)	0.030*	1.399 (0.486, 4.027)	0.533
	additive	1.491 (1.125, 1.976)	0.005**	1.255 (0.880, 1.789)	0.210
	dominant	1.518 (1.093, 2.017)	0.013*	1.317 (0.864, 2.007)	0.200
	recessive	2.282 (0.993, 5.241)	0.052	1.312 (0.459, 3.752)	0.613
rs2070600	CC	Ref	Ref	Ref	Ref
	CT	0.713 (0.496, 1.024)	0.067	0.842 (0.541, 1.310)	0.445
	TT	0.536 (0.237, 1.211)	0.134	0.690 (0.230, 2.071)	0.508
	additive	0.721 (0.542, 0.960)	0.025*	0.838 (0.584, 1.202)	0.336
	dominant	0.684 (0.485, 0.964)	0.030*	0.823 (0.539, 1.256)	0.266
	recessive	0.587 (0.261, 1.320)	0.198	0.724 (0.243, 2.158)	0.562
rs2228144	GG	Ref	Ref	Ref	Ref
	AG	1.301 (0.889), 1.904	0.175	1.127 (0.685, 1.854)	0.637
	AA	1.679 (0.520, 5.425)	0.386	1.747 (0.429, 7.122)	0.437

£ No variables were adjusted in logistic regression model

¤ Dyslipidemia, hypertension, smoking, and drinking were adjusted in the logistic regression model.

*$P < 0.05$, **$P < 0.01$.

	Genotype	Crude OR£ (95%CI)	Crude *P* value	Adjusted OR¤ (95%CI)	Adjusted *P* value
	additive	1.300 (0.935, 1.806)	0.118	1.184 (0.776, 1.807)	0.433
	dominant	1.326 (0.917, 1.917)	0.134	1.171 (0.726, 1.889)	0.519
	recessive	1.594 (0.495, 5.135)	0.435	1.709 (0.421, 6.943)	0.454
rs4072391	CC	Ref	Ref	Ref	Ref
	CT	1.149 (0.777, 1.698)	0.487	1.258 (0.765, 2.069)	0.367
	TT	0.675 (0.081, 5.644)	0.717	1.172 (0.121, 10.178)	0.891
	additive	1.300 (0.935, 1.806)	0.118	1.142 (0.711, 1.835)	0.583
	dominant	1.129 (0.767, 1.662)	0.537	1.208 (0.736, 1.982)	0.455
	recessive	0.658 (0.079, 5.493)	0.658	1.128 (0.116, 10.918)	0.917
rs4129267	CC	Ref	Ref	Ref	Ref
	CT	1.327 (0.937, 1.877)	0.111	1.224 (0.791, 1.895)	0.364
	TT	1.430 (0.898, 2.279)	0.132	1.559 (0.863, 2.818)	0.141
	additive	1.217 (0.973, 1.521)	0.085	1.224 (0.993, 1.660)	0.137
	dominant	1.351 (0.970, 1.880)	0.075	1.294 (0.853, 1.963)	0.226
	recessive	1.208 (0.799, 1.825)	0.371	1.377 (0.814, 2.330)	0.232
rs4537545	CC	Ref	Ref	Ref	Ref
	CT	1.395 (0.984, 1.978)	0.062	1.439 (0.924, 2.239)	0.107

£ No variables were adjusted in logistic regression model

¤ Dyslipidemia, hypertension, smoking, and drinking were adjusted in the logistic regression model.

*$P < 0.05$, **$P < 0.01$.

	Genotype	Crude OR£ (95%CI)	Crude *P* value	Adjusted OR¤ (95%CI)	Adjusted *P* value
	TT	1.386 (0.866, 2.220)	0.174	1.603 (0.877, 2.929)	0.125
	additive	1.217 (0.973, 1.521)	0.085	1.294 (0.969, 1.727)	0.081
	dominant	1.393 (0.998, 1.944)	0.051	1.474 (0.964, 2.253)	0.073
	recessive	1.132 (0.747, 1.716)	0.558	1.276 (0.751, 2.168)	0.368
rs4845625	CC	Ref	Ref	Ref	Ref
	CT	0.692 (0.483, 0.991)	0.045*	0.572 (0.362, 0.904)	0.017*
	TT	0.503 (0.318, 0.795)	0.003**	0.541 (0.311, 0.940)	0.029*
	additive	0.707 (0.563, 0.888)	0.003**	0.723 (0.545, 0.960)	0.025*
	dominant	0.632 (0.448, 0.889)	0.009**	0.562 (0.365, 0.866)	0.009**
	recessive	0.644 (0.434, 0.955)	0.028	0.784 (0.491, 1.252)	0.308
rs7514452	TT	Ref	Ref	Ref	Ref
	CT	1.113 (0.751, 1.650)	0.594	1.193 (0.789, 1.805)	0.403
	CC	0.671 (0.080, 5.610)	0.712	1.166 (0.120, 11.317)	0.895
	additive	1.069 (0.739, 1.544)	0.724	1.108 (0.686, 1.789)	0.675
	dominant	1.095 (0.742, 1.616)	0.647	1.170 (0.710, 1.928)	0.539
	recessive	0.658 (0.079, 5.493)	0.699	1.128 (0.116, 10.918)	0.917
rs7529229	TT	Ref	Ref	Ref	Ref

£ No variables were adjusted in logistic regression model

¤ Dyslipidemia, hypertension, smoking, and drinking were adjusted in the logistic regression model.

*$P < 0.05$, **$P < 0.01$.

	Genotype	Crude OR£ (95%CI)	Crude *P* value	Adjusted OR¤ (95%CI)	Adjusted *P* value
	CT	1.289 (0.912, 1.822)	0.151	1.223 (0.790, 1.893)	0.366
	CC	1.403 (0.881, 2.234)	0.153	1.557 (0.861, 2.815)	0.143
	additive	1.069 (0.739, 1.544)	0.724	1.243 (0.932, 1.659)	0.138
	dominant	1.315 (0.946, 1.829)	0.103	1.292 (0.852, 1.960)	0.228
	recessive	1.206 (0.798, 1.822)	0.375	1.377 (0.814, 2.328)	0.233
£ No variables were adjusted in logistic regression model					
¤ Dyslipidemia, hypertension, smoking, and drinking were adjusted in the logistic regression model.					
*P < 0.05, **P < 0.01.					

Association between haplotypes and diabetic cardiovascular disease

Four out of five SNPs in *AGER* gene (Block1: rs184003-rs1035798-rs2070600-rs1800624) and five out of seven SNPs in *IL6R* gene (Block2: rs7529229-rs4845625-rs4129267-rs7514452-rs4072391) showed linkage disequilibrium, see Fig. 1. These two blocks were both significantly associated with diabetic cardiovascular disease (Block1: P = 0.008; Block2: P = 0.007). Four haplotypes were constructed in block 1, and two of them associated with diabetic ischemic heart disease (CGTA: P = 0.018; AGCA: P = 0.004). Four haplotypes were constructed in block 2, and two of them associated with diabetic cardiovascular disease (TCCTC: P = 0.033; TTCTC: P = 0.001). Details of haplotype analysis were shown in Table3.

Table 3
Haplotype analysis for blocks in *AGER* and *IL6R* genes

	haplotypes	F_U £	F_A ¤	Chi-square	df	*P* value
Block1§	Omnibus test	-	-	11.750	3	0.008**
	CACT	0.162	0.170	0.162	1	0.687
	CGTA	0.202	0.150	5.575	1	0.018*
	AGCA	0.140	0.197	8.229	1	0.004**
	CGCA	0.497	0.482	0.247	1	0.620
Block2¢	Omnibus test	-	-	11.99	3	0.007**
	TTCCT	0.093	0.100	0.227	1	0.634
	CCTTC	0.387	0.431	2.639	1	0.104
	TCCTC	0.009	0.131	4.551	1	0.033
	TTCTC	0.426	0.338	10.32	1	0.001**

§ Block1: rs184003-rs1035798-rs2070600-rs1800624; ¢ Block2: rs7529229-rs4845625-rs4129267-rs7514452-rs4072391; £ F_U: minor allele frequency in controls; ¤ F_A: minor allele frequency in cases; **P* < 0.05; ***P* < 0.01

The effect of gene-gene interaction on diabetic cardiovascular disease

GMDR analysis were performed to assess the gene-gene interaction on diabetic cardiovascular disease risk, after adjustment for dyslipidemia, hypertension, smoking, and drinking. The GMDR analysis suggested that rs184003 in *AGER* gene and rs4845625 in *IL-6R* gene was the best model in terms of statistical significance after permutation testing (*P* = 0.001). The two-locus models had a cross-validation consistency of 10/10, and had a testing accuracy of 0.597. Logistic regression was subsequently used to obtain the odds ratios (ORs) and 95% confidence intervals (CI) for the interaction between rs184003 and rs4845625. In additive model, the joint effect

of rs184003 and rs4845625 is associated with an increased risk of diabetic cardiovascular disease by 38% (OR = 1.38, 95%*CI*: 1.13–1.69, *P* = 0.002).

Discussion

Individuals with T2DM are with an increased risk of CVD which cannot be fully explained by elevated glucose[14]. Genetic risk factors contribute a lot to the pathogenesis of diabetic macrovascular complications, but its role has not been fully illustrated yet. In the present community-based case-control

study, rs4845625 in *IL-6R* gene, and the interaction of rs184003 in *AGER* gene and rs4845625 in *IL-6R* were significantly associated with diabetic ischemic heart disease. Polygenic risk score calculated by summing the number of risk alleles of the SNPs located in the above two genes were also associated with the elevated risk of diabetic ischemic heart disease.

AGER is a multiligand cell surface receptor. Advanced glycation end products (AGEs) which is produced after high glucose exposure can bind to AGER. Their interaction has been implicated in the pathogenesis of atherosclerosis. In addition, HMGB1 (high-mobility group protein 1) and neutrophil-derived S100 calcium-binding family members (S100A8/A9/A11/A12, and S100B) were also ligands of AGER. After ligand binding, proinflammatory and procoagulant pathways will be activated. The rs2070600 was found to be significantly associated with diabetic ischemic heart disease in the current study. But after adjustments for covariates, the associations became null. The rs2070600 is located in ligand-binding V domain of the AGER gene, often referred to as Gly82Ser[15]. Genome-wide association studies (GWAS) showed that rs2070600 were strongly and dose-dependently correlated with sRAGE level in whites and blacks from Atherosclerosis Risk in Communities Study and Chinese population [6, 16]. Interestingly, although soluble-RAGE levels were found to be associated with diabetic complications in many researches, the association between rs2070600 and cardiovascular disease or other diabetic complications were not consistent. In Atherosclerosis Risk in Communities Study, the rs2070600 was not significantly associated with incident coronary heart disease or diabetes in both whites and blacks with a median follow-up of 20 years[6]. Gao et al. has found a significant association between rs2070600 and coronary arterial disease in 175 cases and 170 controls [17]. Meta-analysis found that the discrepancy may be attributable to ethnicity, subjects with rs2070600 risk allele were at higher risk of coronary arterial disease (CAD) in the Chinese population, rather than non-Chinese population. However, our study found the association between rs2070600 and diabetic ischemic heart disease was null. Another research also found rs2070600 was associated with the circulating levels of esRAGE but not with CAD in Chinese patients with T2DM [18]. These results might indicate that the association between rs2070600 and CAD may also be different in general population and T2DM patients.

Only few studies had demonstrated the association between rs184003 and cardiovascular disease. A hospital-based case-control study found rs184003 can significantly increase the risk of coronary artery disease (OR = 1.23, $P = 0.008$), and haplotypes C-T-G-G and T-A-G-T in *AGER* gene (rs1800625-rs1800624-rs2070600-rs184003) were associated with significant increases in risk for CAD[19]. In the current study, we also haplotypes C-G-T-A and A-G-C-A in *AGER* gene (rs184003-rs1035798-rs2070600-rs1800624) were significantly associated with diabetic ischemic heart disease. Although the rs184003 was significantly associated with diabetic ischemic heart disease in the current study, the associations became null after adjustments for covariates. To our knowledge, few studies illustrated the relationship between rs184003 and diabetic macrovascular complications. More researches are still need to validate our results. Given the fact sRAGE level were found to be significantly associated with CAD [20, 21] in many researches, the null association between *AGER* polymorphisms and diabetic ischemic heart disease in the current study

indicated that sRAGE level could be served a marker of CAD, but not the a potential intervention targeting of reducing the burden of CAD.

Mendelian randomization analysis illustrated that IL6R signaling might have a causal role in development of coronary heart disease[22]. Previous meta-analysis demonstrated that rs2228145 and rs7529229 in *IL6R* could significantly reduce the risk of coronary heart disease[22, 23]. Although the meta-analysis constituting a large sample size, the data from Asian is insufficient. Chen et al. did not find an association of rs2228145 with coronary stenosis or acute myocardial infarction in the Chinese Han population[24]. Likewise, our current study, showed no association between rs2228145 and diabetic ischemic heart disease in Chinese population. The haplotype T-T-C-T-C (rs7529229-rs4845625-rs4129267-rs7514452-rs4072391) in *IL6R* gene and the rs4845625 was associated with diabetic cardiovascular disease in our study, and the association held after adjusting for potential confounders. The rs4845625 was found to be significantly associated with hypertriglyceridemia in Japanese population[25], and the T allele was associated with lower serum concentration of creatinine and increased eGFR[26]. Hypertriglyceridemia and chronic kidney disease (CKD) have common pathway leading to metabolic cardiovascular disease, like endothelial dysfunction, dyslipidemia, and inflammation[27]. Although there was seldom any study focus on the association between rs4845625 and diabetic heart disease, its association with triglyceride and kidney function might indicate the potential mechanisms of rs4645625 on diabetic ischemic heart disease.

It has been found that, in response to hyperglycemia, AGER will be activated by S100A8/A9 on hepatic Kupffer cells, leading to the secretion of IL-6. IL-6 would subsequently bind to its receptor (IL6R) on hepatocytes to enhance the production of thrombopoietin, thereby regulating platelet production and resulting diabetes-induced thrombocytosis[28]. In the current study, we found that gene-gene interaction between *AGER* and *IL6R* would increase the risk of diabetic ischemic heart disease. We subsequently used GeneMANIA to construct gene network and predict gene function. IL6R and AGER have physical interactions with each other, and several pathways including NF-kB /RelA and JAK/STAT are involved in these interactions. Details were shown in **Supplementary Figure S1**. These interactions illustrated that the interaction of SNPs in *IL6R* and *AGER* was not only a statistical interaction, but also a biological interaction. To our knowledge, this is the first study aimed to identify interaction of *AGER* and *IL6R* gene, and our results provided a genetic evidence on the physiological mechanism of diabetic macrovascular complications. Whether the main effect and gene-gene interaction in these two genes could be used to predict the risk of diabetic macrovascular complications are still need to be validated by cohort study in the future. Although we found the significant interaction of *AGER* gene and *IL6R* gene, the association between circulating IL-6 and diabetic ischemic heart disease was null. This result indicates that the role of circulating IL-6 in the pathogenesis and development of T2DM cardiovascular complications is complex. The most common hypothesis is that local IL-6 production and dynamics of sIL-6R which indicated the activation of IL-6 trans-signaling pathway were more likely to affect the TPO production and macrovascular complications[28, 29].

In the current study, SBP, TC, LDLC level and the proportion of people with smoking and drinking habits were significantly lower in cases than in controls, which is not consistent with other researches. According to “2017 Guidelines for the prevention and treatment of type 2 diabetes in China”, diabetes patients have more stringent standards on blood pressure (BP) and blood lipid compared with health population, and diabetes patients with ischemic heart disease should quit smoking and drinking[30]. Diabetes patients might change their lifestyles and medication to maintain their BP or blood lipid at a lower level. Due to the case-control study design of the current study, we were not able to collect the lifestyle risk factors and blood sample before the incident of diabetic ischemic heart disease. However, the percentage of SBP, DBP, HDLC, LDLC, TG and TC in ideal range were significantly higher in control group compared with cases ($P < 0.001$, **supplementary table S2**). Due to the above limitation of our study, more longitudinal researches are still needed to demonstrate whether genetic variants will increase the incident of diabetic macrovascular complications. What is more, medication information was not included in the investigation. Given the fact that some antidiabetic medication, like SGLT-2 inhibitor[31], will reduce the risk of cardiovascular disease in diabetes patients, future researches considering antidiabetic medication are still needed to validate the genetic effect on diabetic macrovascular complications.

List of abbreviations

CVD, Cardiovascular disease

T2DM, Type 2 diabetes mellitus

AGER, Advanced glycation end products receptors

AGEs, Advanced glycation end products

IL-6, Interleukin-6

S100A8/A9, S100 calcium-binding protein A8/A9

FPG, Fasting plasma glucose

TC, Total cholesterol

TG, Triglycerides

HDLC, High-density lipoprotein cholesterol

LDLC, Low-density lipoprotein cholesterol

IHD, Ischemic heart disease

SBP, Systolic blood pressure

DBP, Diastolic blood pressure

PRS, Polygenic risk score

GMDR, Generalized multifactor dimensionality reduction

GWAS, Genome-wide association studies

Declarations

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Declarations

Ethical Approval and consent to participate: This study was approved by the Ethics Committee of Capital Medical University (No:2016SY24). All participants enrolled in this study have signed informed consent.

Consent for publication: Not applicable

Availability of data and materials: The datasets generated and/or analyzed during the current study are not publicly available due to the regulations of the people's Republic of China on the administration of human genetic resources, but part of the dataset is available from the corresponding author on reasonable request.

Competing interests: The authors declare that they have no competing interests.

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Author contributions: LK designed the study and wrote the manuscript, XY analyzed data and visualized the interaction diagram. ZQ provided the statistical plan and helped to revise the manuscript. PW contributed to the verification of diabetic ischemic heart diseases in case group. GC and ZJ contributed to the management of blood sample and DNA extraction. ZL contributed to the collection of controls and participated in the study design.

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Figures

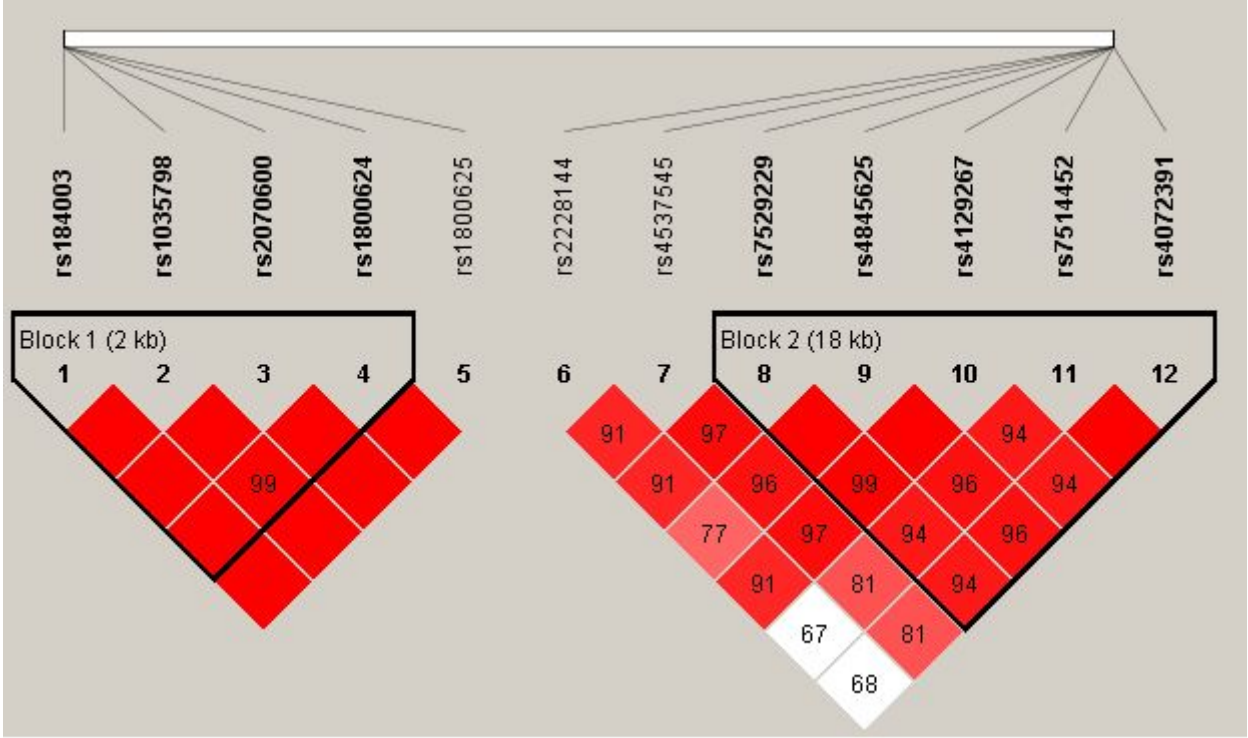

Figure 1

Haplotypes in AGER and IL6R gene

Supplementary Files

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