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Original Article

The gene polymorphisms of insulin degrading enzyme (*IDE*) are associated with the risk of coronary heart disease in Chinese Han population

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Abstract: Objectives: Altered insulin degrading enzyme function leads to toxic amyloidand insulin aggregates, inflammation, and atherosclerosis. We hypothesized that gene polymorphisms of insulin degrading enzyme (IDE) are associated with the development of coronary heart disease (CHD). Methods: In this case-control study, 231 CHD patients and 200 non-CHD controls were selected according to the coronary angiography (CAG) results. Sequenom's MassARRAY system was used to genotype 5 IDE gene TagSNPs, namely rs1887922, rs2149632, rs6583817, rs4646954, and rs3758505. The frequencies of the alleles, genotypes, and haplotypes of these TagSNPs were compared between the CHD and control groups. Results: The rs1887922, rs2149632, and rs4646954 were significantly associated with the risk of developing CHD ($P<0.05$). Haplotype analysis of rs1887922-rs2149632-rs6583817-rs4646954-rs3758505 showed that the CTCGT haplotype increased the risk of developing CHD ($P<0.001$; OR=3.429, 95% CI: 1.816-6.475), while the TCCGT reduced the risk ($P=0.020$; OR=0.708, 95% CI: 0.530-0.947). In addition, the rs1887922 C allele was associated with reduced hepatic insulin clearance (HIC) ($P<0.001$), decreased glycosylated hemoglobin (HbA1c) ($P=0.032$), and increased plasma insulin concentration ($P=0.023$). Conclusions: IDE gene polymorphisms are closely associated with the risk of developing CHD in Chinese Han population.

Keywords: Insulin degrading enzyme, single nucleotide polymorphism, coronary heart disease, atherosclerosis, insulin degradation rate

Introduction

After establishing the association between insulin degrading enzyme (IDE) and Alzheimer's disease [1] and type 2 diabetes [2], several-studies investigated the functions of IDE. Two studies discussed the role of IDE inhibitors as a novel treatment for type 2 diabetes, in 2014 [3, 4]. Although IDE was closely associated with insulin metabolism, it was also closely related to the metabolism of amylin, amyloid β protein (Aβ), and α-synaptic protein [5], disputing the role of IDE inhibitor in reducing the vascular and neurotoxic effects of amylin and Aβ along with blood glucose regulation [6, 7]. Several studies showed that IDE dysfunction in male Ldlr-/- mice increased the levels of Aβ and advanced glycation endproducts (AGEs), and aggravated atherosclerotic lesions, suggesting that IDE dysfunction potentially induced vascular injuries [8]. However, to our knowledge, no genetic studies focused on the association between IDE and atherosclerotic diseases including coronary heart disease (CHD) have been reported to date.

IDE gene is located on the human chromosome 10q23-25. The association of *IDE* gene polymorphisms with Alzheimer's disease and diabetes is a research hotspot. A recent meta-analysis has shown that *IDE* gene polymorphisms are significantly associated with Alzheimer's disease [9]. However, the association with diabetes is still controversial [10]. To date, 45 *IDE*

Table 1. Selected *IDE* TagSNPs

SNP	MAF (CHB)	MAF (GLO)	Chromosome	Functional Consequence
rs1887922	0.089	0.1206	Chr10:94214145	intron variant
rs2149632	0.387	0.364	Chr10:94222227	intron variant
rs6583817	0.179	0.2031	Chr10:94237227	intron variant
rs4646954	0.135	0.2035	Chr10:94323807	utr variant 5 prime -51C>T
rs3758505	0.12	0.2027	Chr10:94324758	upstream variant 2KB -1002T>A

Abbreviations: MAF (CHB), minor allele frequency in Chinese Han population; MAF (GLO), minorallele frequency in global people.

SNPs have been reported in PubMed, and most of the studies focused on rs1887922. Karamohamed S *et al.* [11] first reported in the NHLBI Framingham Heart Study that the haplotype containing rs1887922 and rs2209772 was significantly associated with glycosylated hemoglobin (HbA1c), fasting plasma glucose (FPG), and the incidence of type 2 diabetes. In a clinical study with large sample size (n=3049) [12], Natalia Rudovich *et al.* further demonstrated that the rs1887922 C allele was associated with hepatic insulin clearance (HIC), reduced insulin sensitivity, and increased fasting insulin concentration. Multiple studies also showed that rs1887922CC+CT genotypes were closely associated with the incidence of Alzheimer's disease, mediated by IDE dysfunction attributed to *IDE* polymorphisms [13-16]. These pathogenic mechanisms are also closely associated with CHD and other atherosclerotic diseases. Basic research and clinical trials have demonstrated that abnormal insulin metabolism including insulin resistance, reduced insulin clearance, and hyperinsulinemia are important risk factors contributing to atherosclerotic disease [17-19]. In addition, the inflammatory effects of amyloid toxins including amylin and Aβ inducing vascular injuries have also attracted the attention of researchers in recent years [20, 21]. Therefore, we hypothesized that *IDE* gene polymorphisms induce changes in insulin metabolism and the clearance rate of amylin, Aβ, and α-synaptic protein, and thereby affect the development and progression of atherosclerotic disease including CHD. To verify this hypothesis, a case-control study was performed in Chinese Han population.

Subjects and methods

Subjects

A total of 231 CHD patients were included inthe Department of Cardiology, Daping Hospital, Third Military Medical University between May 2014 and March 2015. The CHD was confirmed by coronary angiography (CAG). The CHD was defined by the presence of stenosis of 50% or more in at least one of the following four blood vessels: left main coronary artery (LM), left anterior descending branch (LAD), circumflex artery, and right coronary artery (RCA) [22]. Another 200 non-CHD subjects with negative results on CAG were included in the control group. All the subjects included in the present study were unrelated Chinese Han population, and the age and BMI were not significantly different between the CHD and control groups. Subjects with tumor, hematonosis, hepatitis, liver dysfunction (ALT>2ULN; 1ULN=40 U/L), renal dysfunction (Cr≥133 umol/L), or autoimmune diseases were excluded. In addition, subjects requiring the use of insulin or other hypoglycemic drugs were also excluded as the present study was associated with the insulin signaling pathway. The present study was approved by the Ethics Committee of Daping Hospital, Third Military Medical University. Informed consent was obtained from all the subjects participating in this study.

Clinical data collection

Data including age, smoking, drinking, drug use, previous medical history, and family history were collected using a questionnaire. The subjects' height and weight were measured, and the body-mass index (BMI) was calculated. The sitting blood pressure at the upper arm was measured using a calibrated mercury sphygmomanometer. Fast blood was collected from the antecubital vein of the patients, and the serum levels of C-peptide (CP) and insulin (INS) were measured by electrochemiluminescence (cobase601, Roche, Basel, Switzerland). A BECKMAN DXC800 automatic biochemical analyzer (Brea, USA) was used to measure the

Table 2. Primers and PCR conditions of the five SNPs

SNPs	PCR primers	Tm (°C)	Extension primers	Direction
rs1887922	F:5'-ACGTTGGATGTGGTTCAGGAATGGATTATC-3'	45.9	5'-AGTTCCGCATATGAACA-3'	F
	R:5'-ACGTTGGATGACAGTCCTAGCTGTGTTTCC-3'			
rs2149632	F:5'-ACGTTGGATGTGTACCTGTTCTAGTAAGTG-3'	46.5	5'-CATTGGATAGGAGACTAGATT-3'	R
	R:5'-ACGTTGGATGGTTGATTGCCTCAGGCATTG-3'			
rs6583817	F:5'-ACGTTGGATGCTACCAAATCTATCGATGGG-3'	45.2	5'-AGTACTAGAAAGACTAACTCA-3'	R
	R:5'-ACGTTGGATGGATGTGGAGAGGAAGAGTAG-3'			
rs4646954	F:5'-ACGTTGGATGATCACCGCAAACGCTTCCTG-3'	61.7	5'-TGCGCAGGGCCGGCT-3'	R
	R:5'-ACGTTGGATGGGCTAGAGCATGCGCAGTG-3'			
rs3758505	F:5'-ACGTTGGATGAACCCTCCTACTGAATTCCC-3'	50.8	5'-AACGGTGTCCTTAGTCCA-3'	R
	R:5'-ACGTTGGATGACAGTCCTAGCTGTGTTTCC-3'			

Table 3. Characteristics of the subjects in the CHD and control groups

Parameter	CHD group (n=231)	Control group (n=200)	*P* value
Age (y)	63.9 ± 9.6	62.2 ± 10.7	0.079
BMI (kg/m^2)	24.4 ± 3.3	24.1 ± 3.2	0.323
Hypertension (n, %)	150 (54.9%)	108 (54%)	0.021
Smokers (n, %)	81 (35.1%)	50 (25%)	0.023
Drinker (n, %)	54 (23.4%)	32 (16%)	0.056
Family history (n, %)	57 (24.7%)	31 (15.5%)	0.018
SBP (mmHg)	128.8 ± 18.4	126.2 ± 16.8	0.161
DBP (mmHg)	75.6 ± 10.7	74.7 ± 10.1	0.466
TC (mmol/L)	4.5 ± 1.1	4.2 ± 1.0	0.007
TG (mmol/L)	1.8 ± 1.2	1.5 ± 0.9	0.004
HDL-C (mmol/L)	1.1 ± 0.3	1.2 ± 0.3	<0.001
LDL-C (mmol/L)	2.9 ± 0.9	2.7 ± 0.8	0.004
ApoA1 (g/L)	1.2 ± 0.2	1.3 ± 0.2	0.002
ApoB (g/L)	0.9 ± 0.3	0.8 ± 0.3	0.008
HbA1c (%)	5.9 ± 0.6	5.8 ± 0.9	0.012
FPG (mmol/L)	5.5 ± 1.1	5.3 ± 1.3	<0.001
2hPBG (mmol/L)	9.7 ± 2.9	8.4 ± 2.8	<0.001
C-Peptide (ng/mol)	2.8 ± 1.8	2.4 ± 0.7	0.064
Plasma insulin (pmol/L)	76.0 ± 72.9	54.7 ± 28.7	<0.001
HIC	14.1 ± 4.5	16.6 ± 6.2	<0.001

Abbreviations: BMI, body-mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; TG, triglyceride; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; ApoA1, apolipoproteinA1; ApoB, apolipoproteinB; HbA1c, glycosylated hemoglobin; FPG, fasting blood-glucose; 2hPBG, 2-hour postprandial blood glucose; HIC, hepatic insulin clearance.

levels of total cholesterol (TC), triglyceride (TG), low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C), apolipoprotein (APO), glycosylated hemoglobin (HbA1c), fasting blood-glucose (FPG), and 2-hour postprandial blood glucose (2hPBG).

DNA extraction

Blood (2 mL) was collected from the antecubital vein in the early morning into EDTA-anticoagulant tubes, and stored at -20°C until use. Genome DNA was extracted using Blood Genome DNA Extraction Kit (DP332; Tiangen, Beijing, China), and stored at -70°C until use.

SNPs selection

The genotypes of *IDE* gene SNPs in Chinese Han population were first searched in the The International HapMap Project Web site (http://hapmap.ncbi.nlm.nih.gov/), and then checked in the SNP database system of the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/snp) and the Sanger Institute Biobank (http://www.ensembl.org/index.html). Then Haploview 4.2 software was used to select the TagSNPs with $r^2 \geq 0.8$ and a minor allele frequency (MAF) in Chinese Han population ≥0.08. Finally, the 5 TagSNPs of the *IDE* gene were selected (**Table 1**).

Genotyping

Sequenom's MassARRAY system was used. The advantages include high sensitivity (accuracy >99.7%), low error, and multiplex analysis, simultaneously. The Beijing Liuhe Genomics Technology Co., Ltd. (Shenzhen, China) provided the genotyping service, using the primers designed by AssayDesigner 3.1 software (**Table 2**). The samples were added

Table 4. Distributions of the *IDE* genotypes (N=431)

SNPs	Alleles (1/2)	Groups	Genotypes (n, %)			P	Allele (n, %)		OR (95% CI)	P
			1/1	1/2	2/2		1	2		
rs1887922	C/T	CHD	1 (0.004)	48 (0.208)	182 (0.788)	<0.001	50 (0.108)	412 (0.892)	2.912 (1.160~5.201)	<0.001
		Control	1 (0.005)	14 (0.070)	185 (0.925)		16 (0.040)	384 (0.960)		
rs2149632	C/T	CHD	92 (0.398)	120 (0.519)	19 (0.082)	0.042	304 (0.658)	158 (0.342)	0.786 (0.589~1.049)	0.102
		Control	102 (0.510)	80 (0.400)	18 (0.090)		284 (0.710)	116 (0.290)		
rs6583817	C/T	CHD	161 (0.697)	66 (0.286)	4 (0.017)	0.270	388 (0.840)	74 (0.160)	0.871 (0.599~1.267)	0.471
		Control	149 (0.745)	45 (0.225)	6 (0.030)		343 (0.858)	57 (0.142)		
rs4646954	A/G	CHD	1 (0.004)	68 (0.294)	162 (0.701)	0.013	70 (0.152)	392 (0.848)	1.522 (1.012~2.290)	0.043
		Control	3 (0.015)	36 (0.180)	161 (0.805)		42 (0.105)	358 (0.895)		
rs3758505	G/T	CHD	2 (0.009)	50 (0.216)	179 (0.775)	0.108	54 (0.117)	408 (0.883)	1.298 (0.835~2.019)	0.245
		Control	4 (0.020)	29 (0.145)	167 (0.835)		37 (0.092)	363 (0.907)		

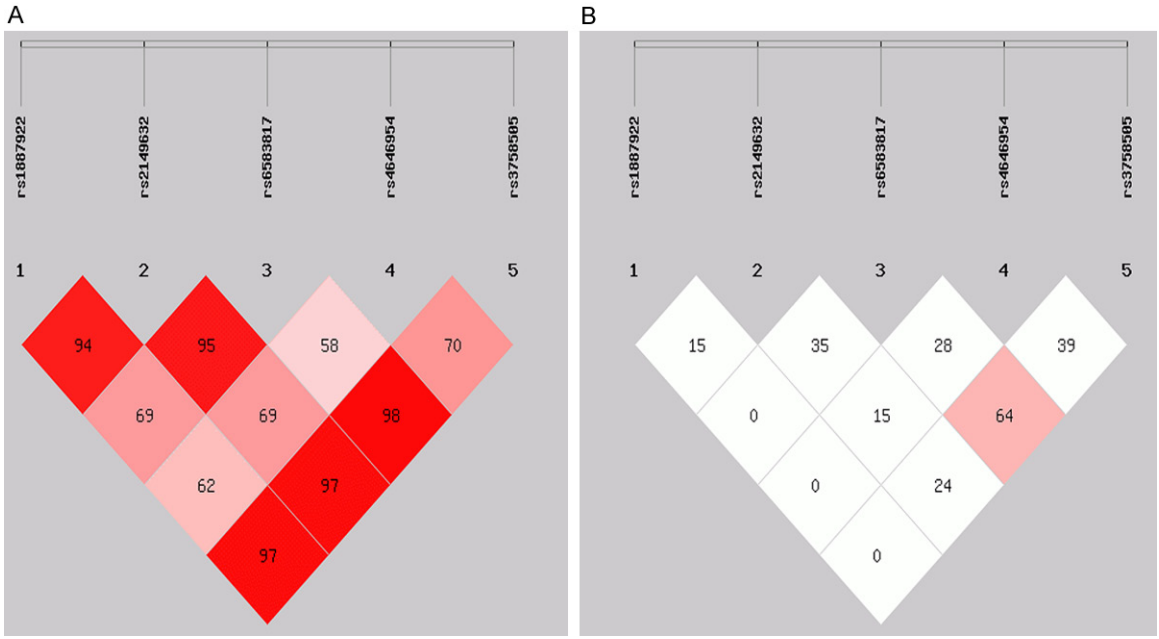

Figure 1. Genetic variation in human IDE gene. We calculated the linkage disequilibrium (LD) between each SNP. A: LD value shown: |D'|. B: LD value shown: r^2.

into a 384-well plate for PCR amplification, shrimp alkaline phosphatase (SAP) reaction, and desalting after extension. The sample data were fed into the Typer 4.0 software and the analysis plate was established. The samples were re-fed and the MassARRAY system was used for the last CHIP assay, followed by the genotyping analysis using the Typer 4.0 software.

Statistical analysis

Chi-square (χ^2) test or Fisher's exact test was used for the Hardy-Weinberg equilibrium test in each SNP for patients in both the CHD and control groups. Independent t-test, χ^2 test, or non-parametric test was performed using the SPSS 18.0 software for the comparison of age, sex, blood lipid, blood glucose, and insulin between the EH and control groups. The online SHEsis software (http://analysis.bio-x.cn/myAnalysis.php) was used for the analysis of genotypes, alleles, and haplotypes between the EH and control groups, as well as the linkage disequilibrium (LD) analysis. P<0.05 was considered statistically significant. 95% confidential interval (CI) was obtained in the analyses performed with SPSS 18.0 and SHEsis softwares. The haplotypes with frequencies less than 0.03 were excluded from the analysis using the SHEsis software.

Table 5. Distribution of the haplotypes in the CHD and control groups

Haplotypes	Frequency		*P* value	OR	95% CI
	CHD	Control			
C T C G T	0.101	0.032	<0.001	3.429	1.816~6.475
T C C A T	0.036	0.022	0.203	1.705	0.743~3.911
T C C G T	0.612	0.688	0.020	0.708	0.530~0.947
T T C G T	0.071	0.099	0.150	0.702	0.433~1.138
T T T A G	0.094	0.059	0.055	1.658	0.984~2.791
T T T G G	0.021	0.031	0.336	0.659	0.280~1.550
T T T G T	0.034	0.042	0.570	0.816	0.404~1.647

Results

Characteristics of subjects

The statistical results showed no significant differences in age, BMI, alcoholic intake, systolic blood pressure (SBP), diastolic blood pressure (DBP), and fasting CP between the CHD and case groups (*P*>0.05). However, significant differences in the history of hypertension, smoking, family history of cardiovascular diseases, TC, TG, HDL-C, LDL-C, ApoA1, ApoB, HbA1c, FPG, 2hPBG, INS, and HIC were found between the CHD and control groups (P<0.05) (**Table 3**). As the insulin and metabolic rate of CP in the liver were significantly different between the two groups, the fasting CP and molar ratio of insulin were used to reflect the HIC consistent with previous studies [23, 24].

Hardy-Weinberg equilibrium

The results of the χ^2 test and Fisher's exact test showed that the distributions of the genotypes of all the 5 SNPs investigated in the present study were in Hard-Weinberg equilibrium with the control group (P>0.05).

Genotype and allele frequencies

The frequency of the C allele of rs1887922 (P<0.01) and a allele of rs4646954 was significantly higher in the CHD group than in the control group (P=0.043) (**Table 4**). In addition, the genotype frequencies were also significantly different between the two groups (P<0.001 and P=0.013, respectively) (**Table 4**). No significant differences in the frequencies of alleles of rs2149632 were found between the CHD and control groups (P=0.102). However, the frequency of the CT genotype was significantly higher in the CHD group than in the control group (P=0.042) (**Table 4**).

Haplotype analyses

Further haplotype analyses were performed using the online SHEsis software. According to the LD analysis results shown in **Figure 1**, *IDE* gene haplotypes consisting rs1887922-rs2149632-rs6583817-rs4646954-rs3758505 was selected for the analyses. The results showed that the risk of developing CHD in the subjects with CTCGT haplotype was 3.429-fold of the risk in those not carrying CTCGT haplotype, and the frequency of this haplotype was significantly higher in the CHD group than in the control group (P<0.001; OR=3.429, 95% CI: 1.816-6.475). In addition, the frequency of TCCGT in the CHD group was significantly lower than in the control group (P=0.020; OR=0.708, 95% CI: 0.530-0.947), suggesting that this haplotype was a cardioprotective factor (**Table 5**).

Blood-glucose and insulin metabolism

The association of *IDE* gene polymorphisms with the blood-glucose level and insulin metabolism were further investigated. The results showed no significant differences in the age, BMI, FPG, 2-hBPG, and CP between the subjects carrying only rs1887922T allele (TT) and those carrying rs1887922 C allele (CC+CT). However, the subjects in the CC+CT subgroup showed significantly higher insulin (P=0.023) and significantly lower HIC (P<0.001) and HbA1c (P=0.032) levels when compared with the TT subgroup (**Table 6**; **Figure 2**).

Discussion

IDE is closely associated with the metabolism of multiple physiologically active molecules including insulin, glucagon, amylin, and Aβ. First, studies reported that *IDE* gene polymorphisms were associated with the development of Alzheimer's disease, and further demonstrated that the pathogenic mechanism involved aggregates of amyloid toxins including Aβ in subjects with the *IDE* gene polymorphisms that reduce IDE function, which was confirmed in multiple clinical studies [25, 26]. The association of IDE with the metabolites of insulin and amylin in CHD is also a research hotspot. In 2013, Caravaggio JW *et al.* investigated Ldlr-/- mice and found that the reduced IDE levels in the mice increased the levels of Aβ and AGEs, and thus induced inflammation and atheroscle-

Table 6. Profile of rs1887922CC+CT vs. TT subgroups

Group	N	Age (y)	BMI (kg/m^2)	HbA1c (%)	FPG (mmol/L)	2hPBG (mmol/L)	CP (ng/mol)	INS (pmol/L)	HIC
rs1887922 (CC+CT)	64	63.3 ± 10.2	24.5 ± 3.1	5.7 ± 0.6	5.3 ± 0.9	8.8 ± 2.6	2.5 ± 1.0	70.4 ± 37.7	13.3 ± 3.8
rs1887922 (TT)	367	63.0 ± 10.1	24.3 ± 3.3	5.9 ± 0.8	5.4 ± 1.2	9.2 ± 3.0	2.6 ± 1.5	65.3 ± 60.6	15.6 ± 5.7
P value		0.864	0.414	0.032	0.567	0.370	0.608	0.023	<0.001

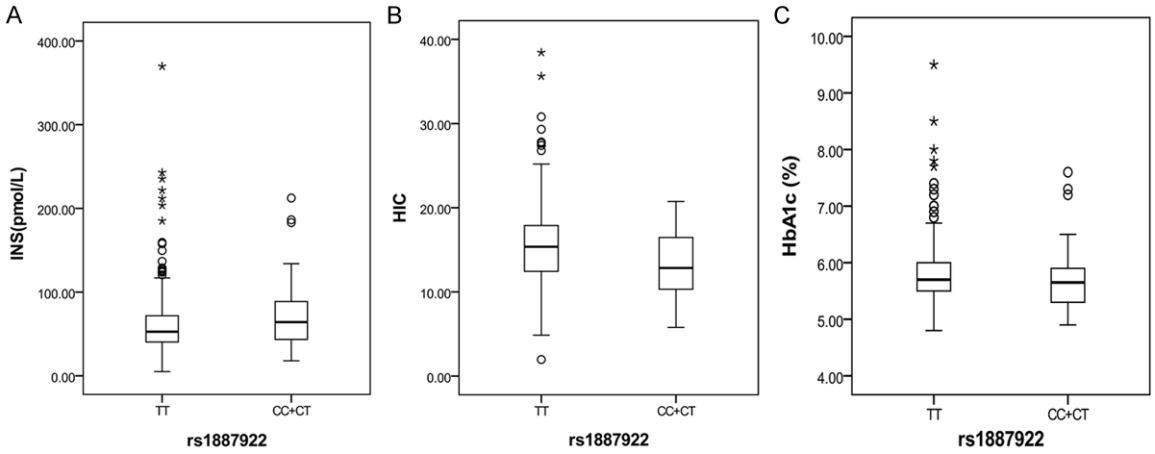

Figure 2. rs1887922 TT vs. CT+CC subgroups. A: INS level; B: HIC; C: HbA1c level.

rosis [8]. Therefore, we hypothesized that *IDE* gene polymorphisms were also associated with the development of atherosclerotic lesions including CHD, which was verified in this clinical study. The findings in the present case-control study showed that 3 *IDE*TagSNPs, namely rs-1887922, rs2149632, and rs4646954 polymorphisms, were associated with the development of CHD. Further investigation of the haplotypes consisting of rs1887922-rs21496-32-rs6583817-rs4646954-rs3758505 showed that CTCGT haplotype carriers manifested increased risk of developing CHD, while TCCGT haplotype carrierswere protected against CHD risk. To our knowledge, this is the first clinical study investigating the association between *IDE* gene polymorphisms and CHD. As susceptibility genes for Alzheimer's disease, diabetes, and CHD, more attention should be paid to *IDE* gene SNPs.

The association of each *IDE* SNP with insulin concentration and blood-glucose metabolism was investigated in the present study. Although no association was found between the rs-1887922 C alleles and the FPG and 2hBPG, this allele reduced the HbA1c and HIC levels, and increased the plasmainsulin levels. HIC and plasma insulin levels are two important indices reflecting altered IDE activity [27]. The present study showed that the rs1887922 C allele of the *IDE* gene was associated with reduced metabolic activities of IDE. Reduction in the IDE activity slowed down the metabolism of the amyloid toxins including Aβ and amylin, thereby elevating the vascular toxicity and inducing the development and progression of atherosclerotic diseases [28]. The present study also showed that the rs1887922 C allele was closely associated with reduced HIC and increased fasting insulin concentration, and is also a susceptible gene for CHD. In multiple studies involving Alzheimer's disease, the rs1887922 C allele closely associated with the development of Alzheimer's disease reduced the IDE activities [29], consistent with our findings. In 2013, Maria A *et al.* [30] used hyperinsulinemic euglycemic clamp technique in a clinical study and demonstrated that the decreased insulin clearance was closely associated with the severity of carotid atherosclerosis, which provided a reasonable explanation for our findings. We speculated that the decrease in insulin clearanceand increase in insulin concentration caused by IDE dysfunction was also one of the pathogenic mechanisms of *IDE* gene polymorphisms. Our findings and past experimental evidence demonstrate

that IDE dysfunction caused atherosclerosis [8]. If IDE inhibitor was recommended as a new approach for the treatment of type 2 diabetes, the treatment-related adverse effects for the development and progression of CHD remain to be investigated.

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Disclosure of conflict of interest

None.

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