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ORIGINAL ARTICLE

Association of E-Selectin Gene +A561C Polymorphism with Type 2 Diabetes in Chinese Population

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SUMMARY

Background: **Diabetes is associated with endothelial cell dysfunction. E-selectin is an endothelial cell adhesion molecule, which is bound for endothelial cell activation. E-selectin gene+A561C polymorphism is associated with many different disorders: essential hypertension, stroke, angina pectoris, coronary heart disease, etc. But the association with type 2 diabetes remains unclear. Therefore, we aimed to investigate the role of E-selectin gene+A561C polymorphism and soluble E-selectin in type 2 diabetes in a Chinese population.**
Methods: **This study involved 317 patients with type 2 diabetes and 285 normal healthy controls. Genotyping of E-selectin gene+A561C polymorphism was examined by polymerase chain reaction-restricted fragments length polymorphism (PCR-RFLP). Soluble E-selectin was examined by enzyme linked immunosorbent assay (ELISA). Biochemical markers were measured by Roche 7600 Automated Biochemical Analyzer.**
Results: **We found that C allele frequency in E-selectin A561 C polymorphism of Chinese T2DM group was higher than control group. The level of soluble E-selectin in T2DM group was higher than control group. TC, TG, LDL-C, ApoB, and sE-selectin (soluble E-selectin) in AC and CC genotypes were higher than AA genotype.**
Conclusions: **Our findings showed that E-selectin +A561C polymorphism was correlated in the Chinese population with type 2 diabetes. C allele and soluble E-selectin may be predisposing factors of Chinese population with type 2 diabetes.**
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INTRODUCTION

Type 2 diabetes (T2D) is a one of the most common chronic metabolic diseases throughout the world. It is characterized by high blood sugar, resulting in impaired insulin sensitivity and increased insulin resistance [1,2]. It was estimated that more than 300 million people worldwide were affected by T2DM [3]. In 2010, the prevalence of T2DM was reported to be 11.6% in Chinese population [4].

T2DM is a complex metabolic disorder resulting from multiple genetic components and environmental risk factors. The lipid metabolism and insulin signal transduction candidate genes may participate in the pathogenesis of T2DM. The variants of these candidate genes could change the expression of protein and also lead to abnormal signal transduction or metabolic disorder. Finally, these variants may influence the susceptibility of T2DM. Patients with diabetes have a high risk of developing long-term microvascular and macrovascular complications that contribute to considerable morbidity and mortality [5], which not only affect the quality of life of patients but additionally cause a large economic impact to China and the world [6]. However, the exact mechanism of T2DM is still unclear.

E-selectin is a cell-surface membrane glycoprotein expressed on endothelial cells after activation by cytokines such as interleukin-1, lipopolysaccharide, and tumor necrosis factor-alpha. It belongs to the selectin superfamily of adhesion molecules. E-selectin can adhere circulating leukocytes to endothelial cells [7]. E-selectin gene exon 2 and 4 mutations were first reported, and it was related with atherosclerosis (AS) [8,9]. E-selectin gene polymorphisms were found to be associated with inflammation related diseases, such as severe atherosclerosis and CHD. A number of studies explored the relationships between E-selectin gene polymorphisms and different diseases, but the results varied in different ethnic groups [10]. Several studies concluded that these polymorphisms might increase the susceptibility to essential hypertension (EH) [11]. However, no studies have been reported on E-selectin gene polymorphisms and diabetes mellitus.

In resting endothelial cells, the concentration of E-selectin is very low. When inflammatory factors stimulate the vascular endothelial cells, the expression of E-selectin is greatly increased. The level of E-selectin is significantly higher in EH/CAD group than controls [12-14]. In this study, the genotype of E-selectin was detected by PCR-RFLP. ELISA was used to detect soluble E-selectin, so as to explore and screen susceptible genes of Chinese population with T2DM.

MATERIALS AND METHODS

Study subjects

From Dec. 2018 to Apr. 2020, 317 patients with type 2 diabetes (n = 317, mean age 51.54 ± 10.32 years) and 285 controls (n = 285, mean age 49.93 ± 11.56 years) were selected randomly from Anhui Provincial Hospital. All DM diagnosis had been confirmed based on the World Health Organization (WTO) diagnostic criteria [15]. Patients presenting with symptoms of DM had a random plasma glucose (PG) > 11.1 mmol/L (200 mg/dL) and fasting plasma glucose (FPG) > 7.0 mmol/L (126 mg/dL) or 2-hour plasma glucose (2 h PG) > 11.1 mmol/L (200 mg/dL) during the oral glucose tolerance test (OGTT). The control group were healthy, without a family history of DM, and without liver, kidney, or other chronic diseases. The study was carried out with the approval of the local hospital's ethics committee. All participants signed informed consent forms.

E-selectin gene +A561C polymorphism determination

Genomic DNA was extracted from blood samples of the study subjects using a blood genome DNA extraction kit (GeneCore; BioTeke Corporation (wuxi) Co., Ltd., Beijing, China). Gene expression was assessed with the following primers: +A561C primer:
forwards:
5'-ATGGCACTCTGTAGGACTGCT-3';
backwards:
5'-GTCTCAGCTCACGATCACCAT-3'.

PCR was performed in a total volume of 25 µL, containing 3.0 µL DNA, 0.5 µL of each primer, 12.5 µL PCR mix (including dNTPs and $MgCl_2$; Shanghai Sangon Biological Engineering Technology and Services Co., Ltd.), and 8.5 µl distilled water. The Techne TC-512 gradient PCR instrument was used. Next, the reaction was performed: an initial denaturation at 94℃ for 5 minutes, followed by 35 cycles of denaturing at 94℃ for 30 seconds, annealing at 59℃ for 30 seconds, extension for 45 seconds at 72℃, and a final extension at 72℃ for 10 minutes. HphI restriction endonuclease (Fermentas, Vilnius, Lithuania) was used to digest PCR product (357 bp). The digested products were run on a 2.5% agarose gel and visualized under UV light by ethidium bromide staining.

Biochemical determination

Venous blood was drawn from the patient, serum and plasma were separated quickly and stored at -70℃. Serum blood glucose, cholesterol (TC), triglyceride (TG), LDL-cholesterol, and HDL-cholesterol were detected by automated enzymatic methods on a Hitachi 7600 automated analyzer (Tokyo, Japan). All procedures were performed by laboratory personnel.

ELISA for the detection of soluble E-selectin

Soluble E-selectin was detected by ELISA method (produced by Shenzhen Jingmei biotech Co. Ltd.). The operation followed the instructions, OD value was detected by a BIO-RAD550 microplate reader. Each level of samples was calculated.

Table 1. Comparison of clinical data between T2DM group and control group (X ± S).

Group	T2DM group (n = 317)	Control group (n = 285)	p-value
Gender (male/female)	124/193	115/170	0.822
Age (years)	59.43 ± 11.12	61.14 ± 10.64	0.231
BMI (kg/m^2)	26.74 ± 3.96	23.09 ± 3.22	< 0.001
Glucose (mmol/L)	8.12 ± 0.66	5.43 ± 0.37	0.007
ALT (IU/L)	34.27 ± 11.51	12.84 ± 10.95	< 0.001
AST (IU/L)	22.31 ± 9.56	21.46 ± 8.55	0.089
CRE (mmol/L)	76.54 ± 17.62	68.47 ± 15.49	< 0.001
TC (mmol/L)	5.49 ± 1.23	4.21 ± 1.19	0.023
TG (mmol/L)	1.88 ± 0.11	1.61 ± 0.07	0.039
HDL-C (mmol/L)	1.46 ± 0.32	1.62 ± 0.42	0.225
LDL-C (mmol/L)	3.08 ± 0.75	2.81 ± 0.63	0.046
ApoA1 (mmol/L)	1.55 ± 0.39	1.66 ± 0.27	0.132
ApoB (mmol/L)	0.92 ± 0.11	0.84 ± 0.18	0.485
sE-selectin (μg/L)	95.1 ± 19.7	84.9 ± 14.6	0.038

Table 2. Frequency and allele distribution of E-selectin gene +A561C polymorphism in T2DM group and control group [n (%)].

	T2DM group (n = 317)	Control group (n = 285)	p-value	OR value (95% CI)
A	418 (65.93)	430 (75.44)	0.000	1
C	216 (34.07)	140 (24.56)		1.289 (1.115 - 1.491)
AA	148 (46.69)	166 (58.25)	0.003	1
AC	122 (38.49)	98 (34.39)		1.187 (0.991 - 1.422)
CC	47 (14.83)	21 (7.36)		1.172 (1.182 - 2.480)
AA	148 (46.69)	166 (58.25)	0.005	1
AC + CC	169 (53.31)	119 (41.75)		1.279 (1.076 - 1.521)

Table 3. Association between E-selectin gene polymorphisms and T2DM populations in univariate and multivariate analyses.

	Univariate			Multivariate		
	Odds ratio	95% CI	p-value	Odds ratio	95% CI	p-value
Essential hypertension	3.133	2.226 - 4.864	0.021			
Alcoholism	2.513	1.194 - 2.996	0.017			
BMI	1.549	1.121 - 2.168	0.033			
Smoking	0.917	0.572 - 1.108	0.019			
+A561C	1.929	1.134 - 2.576	0.014	2.268	1.741 - 2.863	0.008

Table 4. Serum soluble E-selectin expression distribution in T2DM group and control group [X ± S, μg/L].

Genotype	T2DM group (n = 317)	Control group (n = 285)	p-value
AA	71.3 ± 9.64	69.7 ± 8.72	0.514
AC	92.6 ± 10.3 [a]	71.3 ± 9.81 [b]	0.029
CC	102.5 ± 9.78 [c]	91.4 ± 5.65 [d]	0.018
AA	71.3 ± 9.64	69.7 ± 8.72	0.514
AC + CC	98.6 ± 9.81 [e]	89.4 ± 12.5 [f]	0.023
Total	95.1 ± 19.7	84.9 ± 14.6	0.038

[a] AC - Compared with AA in T2DM group: p = 0.016;
[b] AC - Compared with AA in control group: p = 0.682.
[c] CC - Compared with AA in T2DM group: p = 0.021;
[d] CC - Compared with AA in control group: p = 0.011;
[e] AC + CC - Compared with AA in T2DM group: p = 0.033;
[f] AC + CC - Compared with AA in control group: p = 0.027.

Table 5. Demographic characteristics and biochemical features of study subjects by genotypes for E-selectin gene polymorphism in all subjects (X ± S).

Group	AA	AC	CC	AC + CC	[a] p-value	[b] p-value	[c] p-value
Number of subjects	314	220	68	288			
Age (years)	58.67 ± 12.41	60.52 ± 11.75	59.42 ±10.26	60.34 ± 9.87	0.476	0.568	0.611
BMI (kg/m^2)	24.33 ± 3.46	23.39 ± 3.19	24.61 ± 3.02	24.26 ± 2.92	0.712	0.623	0.394
Glucose (mmol/L)	6.81 ± 1.74	6.59 ± 1.07	6.62 ± 1.51	6.48 ± 0.82	0.416	0.532	0.541
ALT (IU/L)	15.54 ± 7.92	15.97 ± 7.86	14.93 ± 8.62	15.31 ± 9.68	0.294	0.336	0.382
AST (IU/L)	21.33 ± 8.94	20.64 ± 7.25	21.53 ± 7.76	21.37 ± 8.22	0.190	0.213	0.264
CRE (mmol/L)	71.03 ± 15.75	72.69 ± 17.16	71.46 ± 16.52	71.82 ± 16.30	0.716	0.673	0.578
TC (mmol/L)	5.02 ± 1.41	5.46 ± 1.16	5.58 ± 1.27	5.52 ± 1.06	0.032	0.031	0.028
TG (mmol/L)	1.89 ± 0.13	1.56 ± 0.12	1.81 ± 0.09	1.71 ± 0.10	0.028	0.033	0.044
HDL-C (mmol/L)	1.51 ± 0.24	1.29 ± 0.41	1.33 ± 0.37	1.72 ± 0.42	0.087	0.544	0.536
LDL-C (mmol/L)	2.84 ± 0.75	3.05 ± 0.72	3.11 ± 0.43	3.09 ± 0.61	0.022	0.019	0.037
ApoA1 (mmol/L)	1.45 ± 0.39	1.36 ± 0.27	1.33 ± 0.21	1.29 ± 0.18	0.544	0.692	0.368
ApoB (mmol/L)	0.62 ± 0.11	0.84 ± 0.18	0.75 ± 0.18	0.79 ± 0.18	0.020	0.017	0.015
sE-selectin (μg/L)	77.5 ± 12.4	85.4 ± 13.2	99.3 ± 11.9	94.6 ± 13.7	0.017	0.005	0.008

[a] AC - Compared with AA in all subjects.
[b] CC - Compared with AA in all subjects.
[c] AC + CC - Compared with AA in all subjects.

Statistical analysis

SPSS software 17.0 version was used. Each result was calculated as the mean ± SD. The Hardy-Weinberg equilibrium was used to check the sample with group representation. Allelic and genotypic frequencies were obtained by direct counting. Differences in genotypic and allelic frequency between ethnic groups were evaluated by χ^2 test. The odds ratio (OR) and its 95% confidence interval (CI) were calculated at the same time. When $p < 0.05$, it was considered significant different.

RESULTS

The research object of this study is Chinese T2DM patients, and 602 cases were selected in this research. General data showed that the age and gender between the two groups showed no significant difference ($p > 0.05$). HDL-C and ApoA1 levels of T2DM group were lower than the control group ($p < 0.05$), but BMI, glucose, ALT, CRE, TC, TG, sE-selectin were higher than the control group ($p < 0.05$), AST and ApoB showed no

significant difference (Table 1).

The genotypic and allelic distributions of E-selectin gene polymorphism were shown in Table 2. SNP genotypes were tested for departures from HWE. In this study, all polymorphisms were in HWE. In this study, three genotypes of E-selectin gene were found: AA, AC, and CC. In T2DM group, the frequencies of genotypes were 46.69%, 38.49%, and 14.83%, respectively. In the control group, the frequencies of genotypes were 58.25%, 34.39%, and 7.36%, respectively. Compared to AA genotype distribution, the risk factor of AC and CC genotype were 1.187 and 1.172 times larger than the AA genotype. Compared to the allele frequency distribution of the control group, C allele frequency of T2DM group was higher than control group, which showed a significant difference ($p = 0.000$). The risk factor of the C allele is 1.289 times larger than the A allele (Table 2).

Logistic regression analysis was performed for variables independently associated with essential hypertension. The variable odds ratio (95% CI) for essential hypertension with alcoholism was 3.133 (2.226 - 4.864), for BMI (≥ 30) it was 1.549 (1.121 - 2.568), and for smokers (≥ one pack per month) it was 0.917 (0.572 - 1.108). If we adjusted for potential confounding variables (age, gender, smoking, EH, etc.), the results showed that E-selectin +A561C polymorphism was independently associated with T2DM (OR = 2.268, 95% CI = 1.741 - 2.863, $p = 0.008$) (Table 3).

Soluble E-selectin was detected by ELISA. The results showed that soluble E-selectin in T2DM group was higher than the control group. AC and CC and AC + CC genotypes were higher than AA genotype (Table 4). In order to investigate the E-selectin gene polymorphism in all subjects, we studied the demographic characteristics and biochemical features of E-selectin +A561C gene polymorphism. Results showed that TC, TG, LDL-C, ApoB and sE-selectin in AC and CC genotypes were higher than AA genotype (Table 5).

DISCUSSION

E-selectin (E-selectin) is a major member of the selectin family, with a molecular weight of 115 kD and composed of 589 amino acids. It is expressed only on the surface of endothelial cells activated by cytokines and is important in immune responses, inflammatory responses, and the formation of atherosclerosis. The E-selectin gene is located on the long arm of chromosome 1 and is 13 kb long. Its DNA sequence contains 14 exons and 13 introns. Studies have shown that G/T and A/C are the second and fourth exons of E-selectin gene polymorphism. They may affect gene expression and function, and may be closely related to cardiovascular diseases [16,17]. In 1994, Wenzel had first confirmed the +A561C conversion, which leads to a change in its 128 amino acids by serine (Ser) into arginine (Arg) (S128R). It thus affects the biological function of E-selectin [8]. Study also found that the level of C alleles was higher than A alleles. E-selectin gene polymorphism may affect its expression in endothelial cells. Alterations in gene structure may result in changes in protein function [18,19].

Recent studies have confirmed the inflammatory cytokine E-selectin may affect hypertension. It is currently found that +A561C gene polymorphism is correlated with non-insulin dependent diabetes mellitus [19]. Moreover, this polymorphism has been confirmed in Europe and the United States in human CAD [20]. Our research is the first report on the E-selectin gene +A561C polymorphism and Chinese T2DM patients. We found that in the T2DM group, the C allele of the E-selectin +A561C genotype was higher in the control group. The risk of developing hypertension is 1.289 times that of the A allele.

A number of previous studies have reported the implication of E-selectin gene in the pathogenesis of diseases [21,22]. A study carried out by Shaker et al. in Egyptian patients with peripheral arterial occlusive disease also reported that S128R polymorphism is associated with increased risk of the disease [23]. In addition to this, E-selectin plasma levels in patients bearing AC + CC genotypes have been reported to be higher than in those carrying the AA genotype [24]. The 128Arg allele shows decreased binding specificity and increased affinity for additional ligands and the range of lymphocytes recruited by E-selectin is extended [25]. These effects have been suggested to provide a mechanistic link between this polymorphism and vascular inflammatory diseases [26]. We also evaluated the association between soluble E-selectin and Chinese T2DM patients. Results had indicated that soluble E-selectin level of the T2DM group was higher than that of the control group. Soluble E-selectin may be a predisposing factor of Chinese T2DM patients.

We showed the association of E-selectin gene +A561C polymorphism and Chinese T2DM patients. It will also help to better understand the genetic mechanism of diabetes. We have only just started the study of genetic polymorphisms of T2DM. It remains to be studied extensively.

CONCLUSION

Our findings showed that E-selectin +A561C polymorphism was correlated with T2DM in Chinese patients. C Allele and soluble E-selectin may be factors predisposing Chinese patients to develop T2DM.

Declaration of Interest:
None.

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