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Original Article

Association of the extracellular superoxide dismutase Arg213Gly and Ala40Thr polymorphisms with the susceptibility to type 2 diabetes mellitus in a Chinese population

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Abstract: We investigated the association between Extracellular superoxide dismutase (*EC-SOD*) Arg213Gly and Ala40Thr polymorphisms in the risk of Type 2 Diabetes Mellitus in a Chinese population. A total of 205 patients with type 2 diabetes mellitus and 226 control subjects were enrolled from the First Affiliated Hospital of Xinxiang Medical University. Genotyping of *EC-SOD* Arg213Gly and Ala40Thr was performed using polymerase chain reaction restriction fragment length polymorphism method. The Chi-square test revealed a statistically significant difference in the genotype frequencies of *EC-SOD* Ala40Thr ($\chi^2 = 8.27$, $P = 0.02$) between type 2 diabetes mellitus patients and control subjects. Using unconditional regression analysis, we observed that the AA genotype of *EC-SOD* Ala40Thr was correlated with a higher risk of developing type 2 diabetes mellitus compared to the GG genotype, and the adjusted OR (95% CI) was 2.20 (1.27-3.82). Moreover, the A allele of *EC-SOD* Ala40Thr had 1.47 fold risk of developing type 2 diabetes mellitus when compared with the G allele (adjusted OR = 1.53, 95% CI = 1.15-2.04). However, no significant association was found between *EC-SOD* Arg213Gly polymorphism and development of type 2 diabetes mellitus. In summary, our study suggests that individuals carrying the AA genotype and A allele of *EC-SOD* Ala40Thr expose higher risk to type 2 diabetes mellitus significantly with regardless of age and gender.

Keywords: Extracellular superoxide dismutase, Arg213Gly, Ala40Thr, polymorphism, type 2 diabetes mellitus

Introduction

Type 2 diabetes mellitus is the most common endocrine disease, which has the property of damaged insulin secretion and pancreatic beta cells to insulin resistance [1]. It is estimated that about 285 million people were diagnosed with type 2 diabetes worldwide by the end of 2010, and about 70% of these type 2 diabetes patients lived in low- and middle income countries. The pathological process of type 2 diabetes mellitus is involved in many environmental and lifestyle factors, including impaired glucose tolerance, hypertension, individuals over 40 years suffering from dyslipidemia, high fat dietary, high cholesterol dietary, and obesity or overweight as well as lack of physical activity practice [2, 3]. Hereditary or genetic factors have also been reported to play an important role in the development of this disease. For example, polymorphisms in genes coding for transcription factor 7-like 2 (*TCF7L2*), Melanocortin-4 receptor, mitochondrial DNA 5178 C/A, Vitamin D Receptor, ATP-Binding Cassette Transporter A1 and glutathionine S-transferases are associated with the development of type 2 diabetes mellitus [4-9].

Extracellular superoxide dismutase (*EC-SOD*) is the predominant superoxide dismutases in the extracellular matrix, and it is anchored to the extracellular matrix and cell surfaces through an interaction with heparansulfate proteoglycan and collagen [10]. *EC-SOD* is a homotetrameric copper- and zinc-containing glycoprotein, and is found in pancreas, skeletal muscle and particularly abundant in blood vessels. It is reported that the *EC-SOD* has an important role in protecting various tissues from oxidative stress through catalysis of dismutation of two

Table 1. Primers, restriction enzyme and product sizes for *EC-SOD* Arg213Gly and Ala40Thr

EC-SOD	Primers (5'-3')	Restriction enzyme	Product size, bp
Arg213Gly	Forward: CGGGCGACTTCGGCAACTT	*Eco*52I	A allele: 81, 23 and 104 bp
	Reverse: GAGAGGGCTGCGGGGAGAC		G allele: 104 bp
Ala40Thr	Forward: GGCTGGCCTGCTGCGTGGTGG	HhaI	A allele: 106, 213, 163, 2, 48 and 37 bp
	Reverse: CCTTGCACTCGCTCTCGCGCG		G allele: 213, 106, 163, 2, 48, 37 bp

superoxide radicals to hydrogenperoxide and oxygen [11]. Previous studies have reported that *EC-SOD* gene plays an important role in the development and complications of type 2 diabetes [10, 12]. Previous studies has analyzed the association of *EC-SOD* Arg213Gly genetic polymorphism with the development of type 2 diabetes and the risk of diabetic patients for ischemic cardiovascular and cerebrovascular diseases [10, 12]. In this hospital based case-control study, we firstly investigate the association between *EC-SOD* Arg213Gly and Ala40Thr polymorphisms and the risk of developing type 2 diabetes mellitus in a Chinese population.

Material and methods

Subjects

A total of 205 patients with type 2 diabetes mellitus were enrolled from the First Affiliated Hospital of Xinxiang Medical University, between December 2012 and January 2015. The type 2 diabetes mellitus were diagnosed according to the criteria established by the World Health Organization-International Diabetes Federation (Supplementary Table 1) [13]. The exclusion criteria of this study were those suffering from other endocrine diseases, acute or chronic infection diseases, and/or autoimmune diseases.

During the same period of time, a total of 226 control subjects were enrolled from the outpatient clinics and the health examination center at the First Affiliated Hospital of Xinxiang Medical University. All the control subjects received blood glucose examination, and they are free of type 2 diabetes mellitus and other endocrine disorders. Control subjects with a history of endocrine diseases, acute or chronic infection diseases and/or autoimmune diseases were excluded from the study.

The demographic variables, including age, gender, BMI, tobacco smoking, alcohol consumption and hypertension, were collected from an face-to-face interview with a structured questionnaire. The clinical variables of investigated subjects, including fasting glucose and fasting insulin, were collected from medical records.

At the time of recruitment, the mean age of type 2 diabetes mellitus patients and control subjects were 54.28 ± 10.36 and 54.13 ± 9.49 years, respectively. There were 91 (44.39%) females and 114 (55.61%) females in type 2 diabetes mellitus patients, respectively. There were 90 (39.82%) females and 136 (60.18%) males in control subjects, respectively. The mean Body Mass Index (BMI) for type 2 diabetes mellitus patients and control subjects were 27.07 ± 3.07 and 24.27 ± 2.84 kg/m^2, respectively. The mean fasting glucose levels were 8.93 ± 2.61 and 4.87 ± 1.67 mmol/L for type 2 diabetes mellitus patients and controls, respectively. The fasting insulin levels for type 2 diabetes mellitus patients and controls were 56.68 ± 15.33 and 47.82± 12.14 mmol/L, respectively. Peripheral blood samples were obtained from all type 2 diabetes mellitus patients and controls, and stored in ethylenediamine tetra-acetic acid (EDTA) coated tubes at -20°C until use. The Clinical Research Ethics Committee of the First Affiliated Hospital of Xinxiang Medical University agreed the performance of this study.

Genotyping analysis

DNA was isolated from peripheral blood samples, using QIAamp DNA Blood Mini Kit (QIAGEN, USA) following the instruction. The *EC-SOD* Arg213Gly and Ala40Thr polymorphisms was genotyped by polymerase chain reaction restriction fragment length polymorphism method. The primers, restriction enzymes and product sizes for *EC-SOD* Arg213Gly and Ala40Thr are provided in **Table 1**. PCR was carried out in a 25 μl reaction mixture, including 2.0 μL of DNA, 40 ng genomic DNA, l U Taq enzyme, 2.5

Table 2. Patients and controls' characteristics

Characteristics	Patients N = 205	%	Controls N = 226	%	*t* test or χ^2 test	*P* value
Age, years	54.28 ± 10.36		54.13 ± 9.49		0.16	0.87
Gender						
Female	91	44.39	90	39.82		
Male	114	55.61	136	60.18	0.92	0.34
BMI, kg/m^2	27.07 ± 3.07		24.27 ± 2.84		9.80	<0.001
Tobacco smoking						
No	124	60.49	144	63.72		
Yes	81	39.51	82	36.28	0.48	0.49
Alcohol consumption						
No	137	60.62	151	66.81		
Yes	68	30.09	75	33.19	1.11	0.29
Hypertension						
No	106	51.71	173	76.55		
Yes	99	48.29	53	23.45	29.06	< 0.001
Fasting glucose, mmol/L	8.93 ± 2.61		4.87 ± 1.67		19.40	< 0.001
Fasting insulin, mmol/L	56.68 ± 15.33		47.82± 12.14		6.68	<0.001

μL 10 × PCR mix (15 mmol/L $MgCl_2$), 2.0 μL 2.0 mol/L dNTP Mixture, 20 μmmol/L forward primer and reverse primer. The PCR program were performed with an initial denaturation at 94°C for 5 min, 35 cycles of denaturation at 94°C for 60 s, annealing at 58°C for 60 s, and extension at 72°C for 30 s, with a final extension at 72°C for 10 min. The enzyme-digested products were determined using a 1% agarose gel electrophoresis.

Statistical analysis

Significant differences of demographic and clinical variables between the investigated groups were analyzed by performing either the Chi-square test or Student's *t*-test. Comparison of genetic frequencies of *EC-SOD* Arg213Gly and Ala40Thr between patients and controls was determined by Chi-square test. Confirmation of the genotype frequencies of the in patients and controls with the Hardy-Weinberg equilibrium was estimated using the Pearson's Chi-square test. Logistic regression analysis was taken to analyze the association between *EC-SOD* Arg213Gly and Ala40Thr genetic polymorphisms and risk of type 2 diabetes mellitus. Adjusted odds ratios (ORs), 95% confidence intervals (95% CI), and their corresponding *P*-values were used to calculate the results. Statistical analysis was conducted using the SPSS 20.0 package (IBM Corp., Armonk, NK, USA). A *P*-value of less than 0.05 indicated a statistically significance.

Results

Patients with type 2 diabetes mellitus patients and control subjects were comparable in terms of age (*t* value = 0.16, P =0.87), gender (chi-square value = 0.92, P = 0.34), tobacco smoking (chi-square value = 0.48, P =0.49) and alcohol consumption (chi-square value = 1.11, P = 0.29) (**Table 2**). We identified a statistical significant difference between type 2 diabetes mellitus patients and control subjects in BMI (*t* = 9.80, P < 0.001), hypertension (chi-square value = 29.06, P < 0.001), fasting glucose (*t* value = 19.40, P < 0.001) and fasting insulin (*t* value = 6.68, *P* < 0.001).

The genotype frequencies of *EC-SOD* Arg213Gly and Ala40Thr are shown in **Table 3**. For the Arg213Gly gene, 84 (40.98%), 91 (44.39%) and 30 (14.63%) type 2 diabetes mellitus patients carried the AA, AG and GG genotypes, respectively; 83 (36.72%), 108 (47.79%) and 35 (15.49%) control subjects carried the AA, AG and GG genotypes, respectively. For the *EC-SOD* Ala40Thr gene, 71 (34.63%), 89 (43.42%) and 45 (21.95%) type 2 diabetes mellitus patients carried the GG, GA and AA genotypes, respectively; 104 (46.02%), 92 (40.71%) and 30 (13.27%) control subjects carried the GG, GA

Table 3. Genotype frequencies of *EC-SOD* Arg213Gly and Ala40Thr in the study groups

EC-SOD	Patients N = 205	%	Controls N = 226	%	χ^2 test	*P* value	P for Hardy-Weinberg equilibrium	
							Patients	Controls
Arg213Gly								
AA	84	40.98	83	36.72				
AG	91	44.39	108	47.79				
GG	30	14.63	35	15.49	0.82	0.66	0.51	0.99
Ala40Thr								
GG	71	34.63	104	46.02				
GA	89	43.42	92	40.71				
AA	45	21.95	30	13.27	8.27	0.02	0.09	0.19

Table 4. Relationship between *EC-SOD* Arg213Gly (rs 1799895) and Ala40Thr (rs 2536512) genotype polymorphisms and development of type 2 Diabetes Mellitus

EC-SOD	Patients N = 205	%	Controls N = 226	%	OR (95% CI)[1]	*P* value
Arg213Gly						
AA	84	40.98	83	36.72	1.0 (Ref.)	-
AG	91	44.39	108	47.79	0.85 (0.48-1.50)	0.57
GG	30	14.63	35	15.49	1.02 (0.58-1.78)	0.95
Allele						
A	259	63.17	274	60.62	1.0 (Ref.)	-
G	151	36.83	178	39.38	0.90 (0.67-1.419)	0.44
Ala40Thr						
GG	71	34.63	104	46.02	1.0 (Ref.)	-
GA	89	43.42	92	40.71	1.55 (0.90-2.68)	0.12
AA	45	21.95	30	13.27	2.20 (1.27-3.82)	0.01
Allele						
G	231	56.34	300	66.37	1.0 (Ref.)	-
A	179	43.66	152	33.63	1.53 (1.15-2.04)	0.03

[1]Adjusted for gender and age.

and AA genotypes, respectively. Chi-square test revealed a significant difference in the genotype distribution of *EC-SOD* Ala40Thr (chi-square value = 8.27, P = 0.02) between type 2 diabetes mellitus patients and control subjects, whereas the genotype frequencies of *EC-SOD* Arg213Gly were comparable between the two study groups (chi-square value = 8.27, P = 0.02). The *P* values of *EC-SOD* Arg213Gly and Ala40Thr genetic frequencies confirmed with the Hardy-Weinberg equilibrium in type 2 diabetes mellitus patients and controls (All *P* value > 0.05).

Logistic regression analysis indicated that individuals carried the AA genotype of *EC-SOD* Ala40Thr were associated with an increased risk of type 2 diabetes mellitus (OR =2.20, 95% CI = 1.27-3.82), when compared with the GG genotype (**Table 4**). Moreover, individuals expressing the A allele of *EC-SOD* Ala40Thr were associated with an increased risk of type 2 diabetes mellitus when compared to those carrying with the G allele (OR = 1.53, 95% CI = 1.15-2.04). We did not observe a significant relationship between *EC-SOD* Arg213Gly gene polymorphism and type 2 diabetes mellitus risk in a Chinese population.

Discussion

Recent advances in early detection have decreased the complications of Type 2 Diabetes Mellitus, but prevention of type 2 diabetes mellitus remains an important public health concern worldwide. In this study, we investigated the association between the *EC-SOD* gene polymorphism and the susceptibility to Type 2 Diabetes Mellitus in a Chinese population. We identified that individuals carrying the AA genotype and A allele of *EC-SOD* Ala40Thr had an increased risk of type 2 diabetes mellitus in comparison to the wide-type genotype.

It is reported that a C to G substitution at position 213 of *EC-SOD,* resulting in an amino acid changes from Arginine to Glycine, impairs the affinity of *EC-SOD* for heparin and endothelial surface; and thus this genetic variation increase

the susceptibility to insulin resistance and the risk of developing ischemic cardiovascular and cerebrovascular diseases, in patients with type 2 diabetes [10, 12, 14-16]. A transition mutation of G to A at position 172 of *EC-SOD,* resulting in an alanine to threonine substitution (Ala40Thr) in the SOD3 amino-terminal domain, disrupts the tetramerization of SOD3 enzyme and prevents the secretion of the protein into the extracellular matrix [16, 17]. Therefore, polymorphisms in *EC-SOD* Arg213Gly and Ala40Thr may be involved in the pathogenesis of type 2 diabetes mellitus.

Several studies have investigated the possible relationship between *EC-SOD* Ala40Thr and Arg213Gly polymorphisms and the risk of diabetes and its complications, but the results are not consistent [10, 12, 14, 18-22]. Samolia et al. reported that *EC-SOD* Ala40Thr genetic variation could determine the susceptibility to diabetes and hypertension in a Romanian population [19]. Tamai et al. carried out a study on 205 patients with type 2 diabetes and 220 controls, and reported that *EC-SOD* Ala40Thr genetic variant could affect the insulin resistance and the risk of developing type 2 diabetes in a Japanese population [12]. Strokov et al. discovered that the genes encoding the *EC-SOD* was significantly correlation with the pathogenesis of diabetic polyneuropathy in Russian type 1 diabetes mellitus patients [20]. Zotova et al. performed a study with 180 patients with diabetes, and reported that the *EC-SOD* Arg213Gly genetic polymorphism was significantly associated with the development of diabetic polyneuropathy in type 1 diabetes mellitus patients in a Russian population [21]. However, some studies reported conflicting results. Ukkola et al. reported that *EC-SOD* genetic polymorphism could not influence the development of type 2 diabetes mellitus [22]. In our study, only *EC-SOD* Ala40Thr polymorphism was found to be associated with the susceptibility to type 2 diabetes mellitus, but no association between *EC-SOD* Arg213Gly genetic variant and this disease. Discrepancies in these studies could be caused by differences in ethnicities, study design, subjects selection and sample size.

This study indicates that individuals carrying the AA genotype and A allele of *EC-SOD* Ala40Thr expose higher risk to type 2 diabetes mellitus significantly with regardless of age and gender. The results of this preliminary study mandate further investigations with much more larger sample size to confirm the possible role of the *EC-SOD* gene polymorphisms in the risk of type 2 diabetes mellitus.

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Disclosure of conflict of interest

None.

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