RESEARCH NOTE

The Ser311Cys variation in the *paraoxonase 2* gene increases the risk of type 2 diabetes in northern Chinese

YANCHUN QU[1,2,3], ZE YANG[3], FENG JIN[6], LIANG SUN[3], CHUANFANG ZHANG[1], LINONG JI[5], HONG SUN[4], BINYOU WANG[4] and LI WANG[1]*

[1]*Institute of Genetics and Developmental Biology, Graduate School of the Chinese Academy Sciences, Beijing 100101, People's Republic of China*
[2]*Tianjin Institute of Urology, The Second Hospital of Tianjin Medical University, Tianjin 300211, People's Republic of China*
[3]*National Institute of Geriatric Medicines, Beijing Hospital, Ministry of Health, Beijing 100730, People's Republic of China*
[4]*Public Health School, Harbin Medical University, Harbin 150081, People's Republic of China*
[5]*Endocrinology Department, The People's Hospital, Beijing University, Beijing 100044, People's Republic of China*
[6]*Institute of Psychology, Chinese Academy of Sciences, Beijing 100101, People's Republic of China*

Introduction

T2DM (type 2 diabetes mellitus) susceptibility genes associated with glucose absorption were mapped to chromosome 7q21-22 where paraoxonase gene family is located (Prochazka *et al*. 1995) by molecular marker linkage analyses in Pima Indians raising the possibility of paraoxonase genes as potential candidates for T2DM. *Paraoxonase 2* gene (*PON2*) is a new member of paraoxonase gene family which is similar in function to *PON1* (Primo-Parmo *et al*. 1996), that was widely concerned in oxidative damage and cardiovascular diseases. Previous studies suggested that *paraoxonase 1* gene (*PON1*) played a significant role in the protection of oxidative damage and detoxification of organophosphate pesticides, nerve agents and pharmaceutical drugs, such as glucocorticoids and statins (Jarvik *et al*. 2003; Ferretti *et al*. 2004). It is possible that the antioxidant feature of *PON1* rendered a protective function in slowing the progress of coronary artery disease, β cell dysfunction and metabolic syndrome including dyslipidemia and diabetes mellitus (Senti *et al*. 2003; Chiu *et al*. 2004; Oliveira *et al*. 2004).

Two polymorphisms were found in coding sequence of *PON2* gene: an alanine to glycine substitution at residue 148 and a serine to cysteine substitution at residue 311 (Mochizuki *et al*. 1998). In T2DM patients of Oji-Cree, the homozygosity for the *PON2* Gly148 allele had significantly higher mean fasting plasma glucose than that of the other two genotypes. However, no association of the *PON2* genotype with T2DM was found (Hegele *et al*. 1997). In Asian Indians, the polymorphism at codon 311 in *PON2* gene was found to be associated with coronary heart disease, with the frequency of *PON2* Ser allele being significantly higher in cases than the controls. *PON2* played a synergistic role in coronary disease in conjunction with Arg192 allele in *PON1* gene (Sanghera *et al*. 1998).

Although genome scanning provided some hints on the association between *PON* gene family and T2DM, and *PON2* Gly148 allele was associated with significantly higher mean fasting plasma glucose, there was still no association of the *PON2* genotype with T2DM was found. As a member of paraoxonase gene family, we speculated that perhaps Ser311Cys variation of *PON2* gene, which has a synergistic role in cardiovascular diseases interactively with *PON1* polymorphism, had a substantial impact on T2DM. In the present study, we have carried out a case–control study in the northern Chinese population, to reveal an association of Ser311Cys variation of *PON2* gene (rs17876171) with T2DM.

Materials and methods

Four hundred and thirty four unrelated patients of T2DM were recruited, from Beijing (228) and Harbin (206) regions (with an average age of 49.7 ± 13.2, 204 males and 230 females). Diabetes was diagnosed based on the American Diabetes Association (ADA) fasting plasma criteria (2005). Subjects were defined as diabetic, either through an oral glucose tolerance test (OGTT) using 75 g glucose load (dissolved in 250 ml water) or receiving anti-diabetic treatment by oral

*For correspondence. E-mail: lwang@genetics.ac.cn.

Keywords. paraoxonase 2 gene; type 2 diabetes mellitus (T2DM); case control; northern Chinese.

hypoglycemic agents and insulin. The average duration of T2DM was 6.2 ± 5.4 years. And 533 glucose tolerant non-diabetic normal subjects were recruited at the routine health examination in the same regions (mean age 48.4 ± 8.2, 245 males and 288 females). The study was approved by ethics committee of Beijing Hospital, Ministry of Public Health, People's Republic of China. Informed written consent was obtained from all the subjects before participation.

All the subjects were examined in the morning after overnight fasting. Mean blood pressure (MBP) was calculated from systolic blood pressure and diastolic blood pressure as: MBP = SBP/3 + 2(DBP)/3 (Ferguson and Randall 1986). All glucose-tolerant subjects and patients underwent OGTT, fasting plasma glucose and plasma glucose were obtained by glucose oxidase method after 2 h of glucose intake (Nakamura *et al*. 1996), while fasting plasma insulin was analysed using Dako kits (Dako, Denmark) for T2DM patients.

Genomic DNA was extracted from peripheral blood leukocytes by phenol-chloroform method. Forward primer 5′-tggaaaacagggcttattgatga-3′ and reverse primer 5′-ctgggtcaatgttgctggttaaa-3′ were used for PCR amplification. PCR product was 331 bp in size. For genotyping of Ser311Cys variation, PCR-RFLP was conducted by using *Dde*I restriction endonuclease. The digested products were consisted of four fragments in size of 128 bp, 105 bp, 67 bp, and 31 bp for homozygote of Ser311 allele; and three fragments in size of 172 bp, 128 bp and 31 bp for homozygote of Cys311 allele. The successful rate of genotyping was 97% for this study and individuals that were not successfully genotyped were excluded. The genotyping results were confirmed directly by sequencing 20 subjects randomly chosen.

The quantitative variables were analysed using Student's *t*-test and one way ANOVA. A log transformation was conducted before further analysis for quantitative characteristics not in a normal distribution. The risk of T2DM was expressed as odds ratios (OR) with 95% confidence intervals (CI) for genotype or allele of *PON2* variants. Multiple logistic regression was conducted to find the factors that were associated with the susceptibility of T2DM independently. In the analysis stratified by blood pressure, subjects having blood pressure values ≥ 140/90 mm Hg at baseline were defined as hypertensives. Statistical package for social science (SPSS) 11.5 software was used for all statistical analyses.

Results

All phenotypic variables were compared by Student's *t*-test. The plasma glucose at 2 h after OGTT, SBP and DBP were significantly different between case and control ($P < 0.001$) and MBP was also significantly different between case and control ($P = 0.025$) whereas, age, sex and body mass index (BMI) revealed no significant differences (see table 1 in electronic supplementary material at http://www.ias.ac.in/jgenet/).

The three *PON2* genotypes for Ser311Cys variation were identified through digestion of the 331 bp PCR products by *Dde*I. The genotype and allele frequency of Ser311Cys variation in *PON2* gene for case and control subjects are shown in table 1. The genotype frequency distribution in normal controls was consistent with Hardy–Weinberg equilibrium ($P = 0.396$). Significant difference was revealed in genotype and allele distribution between case and control. And the frequency of G allele carriers in case was much higher than that of control. It was suggested that allele G and its carriers could have increase the susceptibility of T2DM. The OR for T2DM in northern Chinese who carried the G allele was 1.49 (95% CI, 1.13–1.96). Phenotypic variables of individuals with different genotypes were compared by one way ANOVA in case and control, respectively. No significant difference was revealed (data not shown).

A multivariate logistic regression analysis was performed to establish which were independently related with T2DM (table 2). Our analyses suggested that only G allele carrying

Table 1. *PON2* Ser311Cys genotype and allele frequencies in patients with T2DM and in healthy controls.

	Number (frequency) of genotype/allele		
Genotype	T2DM (n = 434)	Controls (n = 533)	
C/C	276 (63.6%)	385 (72.2%)	P = 0.002
C/G	151 (34.8%)	133 (25.0%)	OR = 1.49 (1.13 – 1.96)*
G/G	7 (1.6%)	15 (2.8%)	
Allele	T2DM (n = 868)	Controls (n = 1066)	
C allele	703 (81.0%)	903 (84.7%)	P = 0.030
G allele	165 (19.0%)	163 (15.3%)	OR = 1.30 (1.02 – 1.65)

Number and frequency of each genotype and allele are given.
P value was obtained by comparison between T2DM patients and controls.
OR for genotype was calculated for CC and XG (CG or GG) genotype.
*Numbers in parantheses are 95% confidence intervals.

Table 2. Multivariate logistic regression analysis results of Ser311Cys variations in *PON2* gene in all the subjects.

Variables	B	SE	*P* value	OR	95%CI
Ser311Cys (1)	0.402	0.140	0.004	1.495	1.136–1.969
MBP (mm/Hg)	0.010	0.006	0.081	1.010	0.999–1.021
Age	0.008	0.006	0.204	1.008	0.996–1.021
Sex(1)	0.105	0.132	0.429	1.110	0.857–1.438
BMI	0.007	0.008	0.397	1.007	0.991–1.023

Logistic regression analysis: Risk (OR) of type 2 diabetes.
Dependent variable: normal glucose tolerant control (0) vs. T2DM (1).
Independent variable Ser311Cys (dummy variable): (1) G/G and C/G versus C/C (reference category: C/C). B, logistic regression coefficient; SE, standard error.

Table 3. *PON2* Ser311Cys genotypic and allelic frequencies after stratified by blood pressure.

Hypertensive group			
Number (frequency) of genotype/allele			
Genotype	T2DM (n = 161)	Controls (n = 209)	
C/C	95 (59.0%)	151 (72.2%)	P = 0.007
C/G & G/G	66 (41.0%)	58 (27.8%)	OR = 1.81(1.17–2.80)
Allele	T2DM (n = 322)	Controls (n = 418)	
C allele	252 (78.3%)	355 (82.3%)	0.019
G allele	70 (21.7%)	63 (17.7%)	1.56 (1.07–2.28)
Nonhypertensive group			
Number (frequency) of genotype/allele			
Genotype	T2DM (n = 273)	Controls (n = 324)	
C/C	181 (66.3%)	234 (72.2%)	P = 0.117
C/G & G/G	92 (33.7%)	90 (27.8%)	OR = 1.32 (0.93–1.87)
Allele	T2DM (n = 546)	Controls (n = 648)	
C allele	451 (82.6%)	548 (84.6%)	P = 0.360
G allele	95 (17.4%)	100 (15.4%)	OR = 1.15 (0.85–1.57)

G/G and C/G genotype was combined as G allele carriers because of the few individuals with G/G genotype. Genotypic and allelic frequencies distribution in hypertensive ones were significantly different between the case and control (χ^2 = 7.16, P = 0.007 and χ^2 = 5.48, P = 0.019, respectively) while, genotypic and allelic frequencies in subjects with normal blood pressure were not significantly different between case and control.

status of *PON2* gene polymorphism was independent factor for susceptibility of T2DM. The OR of G allele carriers for T2DM was 1.49.

Due to significant difference in blood pressure between case and control, we carried out additional analysis stratified by hypertension (no and yes). The results suggested that the association of Ser311Cys variation with T2DM was significant especially for hypertensive (P = 0.019), but not for normal blood pressure (P = 0.360) (table 3).

Discussion

Recent studies demonstrated that paraoxonases have antioxidant function and are involved in many age-related disorders. The results of meta analysis in 11,212 cases of coronary heart disease and 12,786 controls from 43 case control studies indicated a significant difference in allele frequencies of variations in *PON1* and *PON2* genes among different populations (Wheeler *et al*. 2004). In this study, we have explored the association of Ser311Cys variation in *PON2* gene with T2DM in the northern Chinese. Our analyses suggested that Ser311Cys (C→G) variation could significantly increase the risk for T2DM, which further validated the linkage results by Prochazka *et al*. (1995) and suggested *PON2* gene may be likely a candidate responsible for the linkage. Accordingly, Mackness *et al*. (2005) found that polymorphisms in *PON2* gene were associated with microvascular complications in diabetes.

The cases and the controls were derived from northern China and all subjects were Han people, who were ready to

detect a risk allele to T2DM with enough power. Genotype frequency in controls was consistent with HWE in our population, excluding the possibility of false positive association result from the sampling bias. Together these data suggested that Ser311Cys variation was associated with T2DM. One possible mechanisms for this association is that this variation may affect the protein function by changing its coding, from a serine, that often locates at active site of protein and contains an active hydroxy group as a target of phosphatation, to a cysteine which often binds metal ion and impacts dimensional structure of protein. Because *PON2* gene is involved in anti-oxidation, the possible coding change in *PON2* gene could change the nature of amino acid at active site of *PON2* and impair its anti-oxidation function and consequently change the metabolic potency of the cell.

Since no significant differences in clinical characteristics are found in individuals with different genotypes and the functional role and metabolic pathway of *PON2* gene are to be defined, it is difficult at this stage to explain why *PON2* gene can act as a critical candidate of T2DM. However, some other studies suggested that *PON2* may play a similar role as *PON1* in protection of oxidative damage and detoxification in liver, and *PON2* gene was expressed more widely than *PON1* gene in many tissues or organs including spleen. Several studies demonstrated the association between *PON1* gene polymorphism and disorders in oxidative damage complications such as coronary heart disease and metabolic syndrome (Ikeda *et al*. 2003; Robertson *et al*. 2003). In addition, it was demonstrated that *PON2* played a synergistic role with *PON1* and the *PON1* activity in *PON2* C/C genotype T2DM patients of Ser311Cys variation was significantly higher than that in patients with other genotypes (Mackness *et al*. 2000). This may imply a possible mechanism that the Ser311Cys variation in *PON2* gene is implicated in the susceptibility to T2DM.

Further, analysis with subjects stratified by hypertension suggested significant association only with hypertension. Our results suggested that *PON2* Ser311Cys variation was an independent risk factor and could increase the susceptibility of T2DM, especially in those with hypertension. However, the detection power of our stratified analysis was low because of the small sample size of T2DM complicated with hypertension. In addition, our data suggested that *PON2* Ser311Cys variation was associated with hypertensive phenotypes (data not shown), a few studies demonstrated *PON1* activity and its gene polymorphism were associated with hypertension. Studies of Fortunato *et al*. (2003) suggested that *PON1* Met55Leu polymorphism is an independent parameter with systolic blood pressure, while Aynacioglu and Kepekci (2000) suggested that both systolic and diastolic blood pressure levels were slightly higher in patients with the Q/Q genotype of Gln192Arg polymorphism in *PON1* gene. These suggested the role of *PON1* in blood pressure regulation. Since the genotype of *PON2* Ser311Cys variation had an effect on *PON1* activity and the function of *PON2* is similar to *PON1* (Primo-Parmo *et al*. 1996), these may explain that the *PON2* Ser311Cys variation played a role in blood pressure regulation of T2DM patients.

In summary, our association study revealed a significant association between Ser311Cys variation in *PON2* gene and T2DM in the northern Chinese, especially one with hypertension, providing an evidence for the role of *PON2* gene in T2DM. Further, multi-loci analyses and studies on the function of the Ser311Cys variation in *PON2* gene are still necessary to help us understand the mechanism underlying the involvement of *PON2* gene in T2DM for Chinese.

Acknowledgements

We thank Professor M. S. Qiu from University of Louisville for review of the manuscript. We thank the National High Technology Research Development Project (No.2002AA223031) and the National Natural Science Foundation (No. 30225019, 30471926) for their financial support.

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Received 12 December 2007, in revised form 24 December 2007; accepted 03 February 2008
Published on the Web: 4 June 2008