RESEARCH NOTE

Variation in the PTEN-induced putative kinase 1 gene associated with the increase risk of type 2 diabetes in northern Chinese

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Introduction

Type 2 diabetes mellitus (T2DM) is a multifactor disorder closely related to energy balance. Several susceptible genes that lead to mitochondrial dysfunction, such as mitochondrial D-loop gene, peroxisome proliferator activated receptor gamma coactivator 1 alpha (PGC1α) and uncoupling proteins, have been implicated in T2DM (Sun *et al.* 2006; Lee *et al.* 2008; Chen *et al.* 2009). PTEN-induced putative kinase 1 (*PINK1*) gene encodes a serine/threonine protein kinase that localizes to mitochondria and protects cells from stress-induced-mitochondrial dysfunction. Mutations in *PINK1* can cause autosomal recessive early-onset Parkinson's disease. Based on the products of *PINK1* and its role in mitochondria and energy metabolism, we hypothesized that in addition to Parkinson's disease, *PINK1* might also be involved in metabolic disorders such as T2DM.

PINK1 is mapped to human chromosome 1 (Valente *et al.* 2004) and functions as a mitochondrial $Na^+/Ca(2^+)$ exchanger regulator. PINK1 deficiency causes mitochondrial accumulation of calcium, and the resulting calcium overload stimulates reactive oxygen species (ROS) production via NADPH oxidase, ROS production inhibits the glucose transporter, which reduces substrate delivery and impairs mitochondrial respiration, ultimately inducing cell death (Gandhi *et al.* 2009). Two missense mutations at considerable frequency are found in the coding region of *PINK1* gene: a threonine/alanine substitution at residue 340 and an asparagine/threonine substitution at residue 521, which is located on exon 8 and indispensable for peptide substrate binding and phosphor-transfer. Results from gene mapping and genetic variation analysis have indicated the involvement of *PINK1* gene in the development of Parkinson's disease. However, there are still disagreements among the population association studies from different groups (Groen *et al.* 2004; Choi *et al.* 2008). Given its role in mitochondria and energy metabolism, we hypothesis that *PINK1* might also play a role in energy metabolic disorders such as T2DM. To test this hypothesis, we carried out a case–control study and our results indicate a significant association of Asn521Thr variation in *PINK1* with T2DM in a northern Chinese population.

Materials and methods

Two hundred and ninety two unrelated T2DM patients were recruited from Tianjin, People's Republic of China. Diagnoses were based on the fasting plasma criteria (American Diabetes Association 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 if they were receiving anti-diabetic treatment by oral hypoglycemic agents and insulin. Another 249 glucose tolerant normal subjects were recruited from routine health examinations in the same regions. The study was approved by ethics committee of Tianjin Medical University. Informed written consent was obtained from all subjects before participation.

All subjects were examined in the morning after overnight fasting. Seated blood pressure and regular anthropometric characteristics were obtained for all subjects. Anthropometric and clinical characteristics are outlined in table 1 of electronic supplementary material at http://www.ias.ac.in/jgenet/. Body mass index (BMI) was calculated as weight (kg) divided by height squared (m^2). All glucose-tolerant subjects and patients underwent a 75 g OGTT; fasting plasma glucose, and plasma glucose at 2 h after glucose intake were obtained by glucose oxidase

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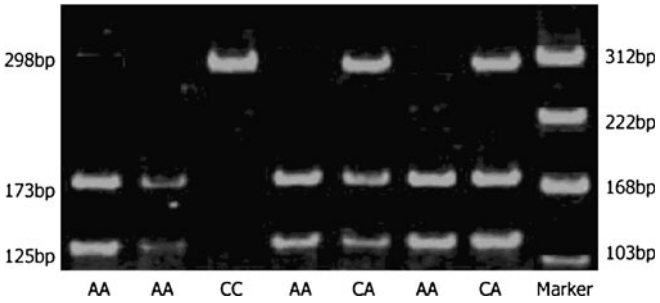

Figure 1. The genotyping of Asn521Thr variation in *PINK1* gene by PCR-RFLP. The PCR product was digested with *Tfi*I endonuclease, then detected by 1% agrose electrophoresis. The right lane is molecular weight reference marker, the fragment size of marker is denoted on the right side, while different fragment size of PCR-RFLP was denoted on the left side. In addition, the different genotypes are denoted at the bottom. There are two fragments of size 173 bp and 125 bp for AA genotype, a single fragment of size 298 bp for CC genotype, and three fragments of size 298 bp, 173 bp and 125 bp for CA heterozygotes.

method (Nakamura *et al.* 1996), while fasting plasma insulin was analysed using Dako kits (Dako, Glostreep, Denmark) for T2DM patients.

Genomic DNA was extracted from peripheral blood leucocytes by phenol–chloroform method. PCR primers spanning Asn521Thr variation were obtained by Oligo 6.0 software (Molecular Biology Insights, Inc., Cascade, CO, USA) according to GenBank genomic sequence (GI:193290126). Sense primer 5′-gttgggaccagagaagggaaga-3′ and antisense primer 5′-ccaggttagccagaaagagcat-3′ were used. PCR product was 298 bp in size. For genotyping of Asn521Thr variation, PCR-RFLP was employed by using *Tfi*I restriction endonuclease. There are two fragments in size of 173 bp and 125 bp for homozygote of Asn521 allele (AA genotype); and single fragment in size of 298 bp for homozygote of Thr521 allele (CC genotype) as indicated in figure 1. The genotyping results were confirmed by direct sequencing of 20 randomly chosen subjects.

Quantitative variables were expressed as mean ± S. D. and analysed with Student's *t*-test. A log transformation was conducted before further analysis for quantitative characteristics not in a normal distribution, such as fasting plasma insulin. Records with missing value were excluded during data analysis. Qualitative variables were analysed by a chi-squared test. The level of significance was set at $P \leq 0.05$. The risk of T2DM was expressed as odds ratios with 95% confidence intervals for genotype or allele. Statistical package for social science (SPSS) 11.5 software (International Business Machines Corp., New York, USA) was used for all statistical analyses.

Results and discussion

We tested the association between *PINK1* gene Asn521Thr variation and T2DM using a case–control study and reported that the variation was significantly associated with T2DM in a northern Chinese population. Three *PINK1* genotypes for Asn521Thr variation were identified through digestion of the 298-bp PCR products by *Tfi*I. As this is the first association study between *PINK1* gene variation and T2DM, we performed the Hardy–Weinberg equilibrium (HWE) test in controls to avoid false positive results from population stratification. The genotype frequency distribution in normal controls was consistent with HWE ($P = 0.175$). Genotypic and allelic frequencies of Asn521Thr variation are shown in table 1. The genotypic ($\chi^2 = 6.44$, $P = 0.040$) and C allele ($\chi^2 = 6.88$, $P = 0.009$) frequencies were both significantly different between T2DM patients and normal controls. The frequency of C allele in T2DM case was much higher than that of control, indicating that the allele C carriers associated with increased susceptibility of T2DM (odds ratio, OR = 1.40, 95% CI: 1.09–1.81, $P = 0.009$). One possible mechanism for this association was that the variation may affect the protein function by changing an amino acid from asparagine to threonine at the active site of PINK1, which often contains an active hydroxy as a target of phosphatation. The Asn521Thr variation is located at the exon 8 of PINK1, an important part of the C-terminal lobe which in charge of the binding of the peptide substrate and of phospho-transfer. Because *PINK1* gene is involved in mitochondrial function and energy metabolism, the coding change in *PINK1* gene could change the nature of amino acid at active site of PINK1 and impair its function in energy

Table 1. *PINK1* Asn521Thr variation in genotypic and allelic frequencies in patients with T2DM and in healthy control subjects.

Genotypic frequencies	T2DM ($n = 292$)	Controls ($n = 249$)	P OR (95% CI)
A/A	119 (40.8%)	127 (51.0%)	0.040
C/A	127 (43.5%)	95 (38.2%)	1.51 (1.08–2.13)
C/C	46 (15.7%)	27 (10.8%)	
Allelic frequencies	T2DM ($n = 584$)	Controls ($n = 498$)	
A allele	365 (62.5%)	349 (70.1%)	0.009
C allele	219 (37.5%)	149 (29.9%)	1.40 (1.09–1.81)

Number and frequency of each genotype and allele were outlined and *P* value was obtained by comparison between T2DM patients and normal controls. Odd ratio for C allele carriers was given as a risk assessment for different genotype of *PINK1* Asn521Thr variation.

Table 2. Independent risk factor identification by logistic regression analysis.

Variables	B	Wald	*P*	OR	95% CI
Age	−0.012	1.474	0.225	0.99	0.97–1.01
Sex (1)	−0.065	0.128	0.720	0.94	0.66–1.34
BMI	0.115	17.553	<0.001	1.12	1.06–1.18
PINK1 (1)	0.700	6.006	0.014	2.01	1.15–3.53
Constant	−1.620	3.527	0.060	0.198	

Age, sex and BMI were considered as covariates, Sex (1) is a representative of male, while PINK1 (1) stands for the genotype CC of Asn521Thr variation.

balance regulation and consequently change the metabolic potency of cell.

Most of the previous studies on *PINK1* gene variations focussed on their association with Parkinson's disease (Toft *et al.* 2007; Kumazawa *et al.* 2008; Brooks *et al.* 2009). Due to few previous studies on the association of genetic variation in *PINK1* gene with T2DM, only a few other groups provided indirect evidences on the role of *PINK1* gene in the development of T2DM. Scheele *et al.* (2007) found a link between neurodegeneration and T2DM. They found that regulation of the *PINK1* locus, previously linked to neurodegenerative disease, is altered in obesity, T2DM and inactivity. Their studies partially support the view that the missense mutation of *PINK1* gene that results in protein dysfunction may spoil its role in cell energetics and lead to the development of metabolic disorders, including T2DM. It is also possible that the Asn521Thr variation can increase risks for both T2DM and Parkinson's disease.

All the other clinical characters tested in our model were significantly different between cases and controls with age and sex as two exceptions (see table 1 in electronic supplementary material). Further, to exclude the false positive association resulted from confounding effects, we adopted a logistic regression analysis which included age, sex and BMI as covariates to confirm the association. The results suggested that both Asn521Thr variation in *PINK1* gene and BMI can be taken as independent risk factors for T2DM ($P = 0.014$ and $P < 0.001$, respectively), as shown in table 2. We found that CC genotype was positively correlated with T2DM susceptibility (OR = 2.01, 95% CI: 1.15–3.53). BMI was also positively correlated with T2DM susceptibility with OR of 1.12 (95% CI: 1.06–1.18). Our results suggest that Asn521Thr variation in *PINK1* gene is significantly associated with increased susceptibility to T2DM as an independent risk factor in northern Chinese population.

If a population consists of subpopulations with heterogenous genetic background, it can result in confounding effects in association studies, so we confined our study subjects to northern Chinese Han people to keep the genetic background of our study population homogeneous. In this study, we focussed on the 521 coding variation based on its possible protein function, while other variations such as the Thr340Ala variation and haplotypes of two coding variations might also be causal variants for T2DM and should be evaluated in future studies. Moreover, studies in larger and multiple populations are still needed to confirm these results because the sample size of this study is not large enough to obtain a powerful conclusion ($P = 0.040$ for genotype frequency distribution). In summary, our association study revealed a significant association between Asn521Thr variation in *PINK1* gene and T2DM in a northern Chinese population, suggesting that *PINK1* gene Thr521 allele carriers have significantly increased susceptibility to T2DM, providing preliminary evidence for the role of *PINK1* gene in T2DM.

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