RESEARCH NOTE

Lack of association of *PTPN1* gene polymorphisms with type 2 diabetes in south Indians

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[Bodhini D., Radha V., Ghosh S., Majumder P. P. and Mohan V. 2011 Lack of association of *PTPN1* gene polymorphisms with type 2 diabetes in south Indians. *J. Genet.* **90**, 323–326]

Introduction

The protein tyrosine phosphatase N1 (*PTPN1)* gene is located on chromosome 20q13 and encodes PTP-1B, an enzyme that negatively regulates the insulin receptor. A total of eight SNPs in the *PTPN1* gene have been reported to be associated with type 2 diabetes, insulin sensitivity and other type 2 diabetes related traits either individually or in combination (Echwald *et al*. 2002; Mok *et al*. 2002; Bento *et al*. 2004; Palmer *et al*. 2004). Subsequent replication studies provided weaker or negative results for association with type 2 diabetes (Florez *et al*. 2005; Traurig *et al*. 2007; Wanic *et al*. 2007). None of these SNPs have been studied in Asian Indians who have greater insulin resistance, increased susceptibility to type 2 diabetes, and a strong genetic background (Radha and Mohan 2007). Hence, the present study was designed to investigate the association of these eight SNPs: rs941798, rs3787345, rs2230604 (Pro303Pro), rs2282147, rs718049, rs718050, rs16995309 (Pro387Leu) and rs16989673 (1484insG), in the *PTPN1* gene with type 2 diabetes in an Asian Indian population from south India.

Materials and methods

Study subjects

A total of 511 subjects, 249 normal glucose tolerant (NGT) and 262 type 2 diabetic subjects were selected from the Chennai Urban Rural Epidemiology Study (CURES), an ongoing epidemiological study, conducted on a representative sample of Chennai city in southern India. The methodology of CURES has been published elsewhere (Deepa *et al*. 2003). NGT was defined as fasting plasma glucose <5.6 mmol/L and 2 h postglucose value ≤ 7.8 mmol/L. Diabetes was diagnosed if the fasting plasma glucose was ≥ 7 mmol/L or 2 h postglucose value ≥ 11.1 mmol/L (Alberti and Zimmet 1998). Informed consent was obtained from all subjects who participated in this study and this study was approved by the institutional ethical committee. Biochemical analyses were carried out on a Hitachi-912 Autoanalyzer (Hitachi, Mannheim, Germany) using commercial kits (Roche Diagnostics, Mannheim, Germany). Details of methods used for estimating fasting plasma glucose, serum insulin, serum cholesterol, serum triglycerides, HDL cholesterol and LDL cholesterol are all mentioned elsewhere (Deepa *et al*. 2003). Insulin resistance was calculated using the homeostasis model assessment-insulin resistance (HOMA-IR), using the formula: fasting insulin (μIU/mL) $\times$ fasting glucose (mmol/L)/22.5 (Matthews *et al*. 1985).

SNP genotyping

DNA was isolated from whole blood using the phenol–chloroform method. All SNPs were genotyped by PCR-RFLP followed by agarose gel electrophoresis. The restriction enzymes that were used for RFLP analysis include *Ale*I for rs941798, *Taa*I for rs3787345, *Bso*BI for rs2230604, *Bsl*I for rs2282147, *Hha*I for rs718049, HpyCH4III for rs718050, *Bfa*I for rs16995309, and *Sac*II for rs16989673. To assure that the genotyping was of adequate quality, we performed random duplicates in 20% of the samples. The assays were performed by a technician who was

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Keywords. *PTPN1* gene; type 2 diabetes; *PTPN1* polymorphisms; Asian Indians.

masked to the phenotype and there was 99% concordance in genotyping.

Statistical analysis

SPSS Windows, version 15.0, was used for statistical analysis. Data for continuous variables were expressed as mean±SD. Chi-square test was used to compare the proportions of genotypes or alleles. P values < 0.05 were considered statistically significant. Linkage disequilibrium (LD) pattern between the SNPs was analysed using the software Haploview (Barrett *et al*. 2005). HAPLOPOP program (Majumdar and Majumder 1999) was used to determine the frequencies of the haplotypes. Power was calculated using PS-Power and Sample Size Calculation, version 3.0.14 (http://ps-power-and-sample-size-calculation.software.informer.com/).

Results

The diabetic subjects were older (49 ± 10 years) compared to the NGT subjects (46 ± 11 years, $P = 0.001$). BMI (NGT: 23.7 ± 4.6; diabetes: 25.1 ± 4.3 kg/m^2, $P < 0.001$), total cholesterol (NGT: 4.78 ± 1.0; diabetes: 5.24 ± 1.0 mmol/L, $P < 0.001$) and serum triglycerides (NGT: 1.35 ± 0.73; diabetes: 2.02 ± 1.42 mmol/L, $P < 0.001$) were all significantly higher in type 2 diabetic subjects.

Genotype and allele frequencies of PTPN1 SNPs

The Pro387Leu polymorphism was not found in the 249 NGT and 262 type 2 diabetic subjects genotyped in the present study. Table 1 shows the genotype and allele frequency of rest of the seven SNPs genotyped in this study. The genotypic distributions of all the SNPs were in Hardy–Weinberg equilibrium. There were no significant difference

Table 1. Genotype and allele frequencies of the SNPs studied in *PTPN1* gene.

Genotype	Type 2 diabetic subjects ($n = 262$)	NGT subjects ($n = 249$)	HOMA-IR (NGT subjects)	P value**
rs941798				
GG	67 (25.6%)	60 (24.1%)	1.95 ± 1.57	0.45
GA	141 (53.8%)	126 (50.6%)	2.01 ± 1.35	
AA	54 (20.6%)	63 (25.3%)	1.72 ± 1.16	
MAF (A allele)	249 (48%)	252 (51%)		0.35
rs3787345				
TT	131 (50%)	117 (47%)	2.05 ± 1.49	0.79
TC	106 (40.5%)	107 (43%)	1.81 ± 1.26	
CC	25 (9.5%)	25 (10%)	1.81 ± 1.16	
MAF (C allele)	156 (30%)	157 (32%)		0.58
rs2230604 (Pro303Pro)				
CC	164 (62.6%)	144 (57.8%)	2.06 ± 1.49	0.45
CT	91 (34.7%)	95 (38.2%)	1.77 ± 1.17	
TT	7 (2.7%)	10 (4.0%)	1.27 ± 0.67	
MAF (T allele)	105 (20%)	115 (23%)		0.26
rs2282147				
CC	121 (46.2%)	110 (44.2%)	2.05 ± 1.52	0.60
CT	123 (46.9%)	116 (46.6%)	1.80 ± 1.22	
TT	18 (6.9%)	23 (9.2%)	1.93 ± 1.24	
MAF (T allele)	159 (30%)	162 (33%)		0.49
rs718049				
TT	128 (48.9%)	108 (43.4%)	2.05 ± 1.52	0.44
TC	113 (43.1%)	117 (47.0%)	1.83 ± 1.24	
CC	21 (8.0%)	24 (9.6%)	1.80 ± 1.20	
MAF (C allele)	155 (30%)	165 (33%)		0.24
rs718050				
GG	125 (47.8%)	106 (42.6%)	2.03 ± 1.57	0.51
GA	104 (39.6%)	112 (45.0%)	1.81 ± 1.22	
AA	33 (12.6%)	31 (12.4%)	2.17 ± 1.33	
MAF (A allele)	170 (32%)	174 (35%)		0.48
rs16989673 (1484insG)				
0/0*	257 (98.1%)	246 (98.8%)	–	0.52
0/G	5 (1.9%)	3 (1.2%)	–	
G/G	–	–		
MAF (G allele)	5 (1%)	3 (0.7%)		0.77

MAF, minor allele frequency, HOMA-IR, homeostasis assessment model for insulin resistance. *Refers to the deletion allele of the 1484insG polymorphism. **Chi square P value for genotypic and allelic frequency between NGT and type 2 diabetic subjects. None of the SNPs showed any significant difference when the HOMA-IR levels between the genotypes were compared.

Table 2. Comparison of haplotype frequencies of the *PTPN1* SNPs in NGT and type 2 diabetic subjects.

Haplotypes*	Type 2 diabetes	NGT	Observed value of test statistics (z)	*P* values
GTCTG0**	0.483	0.465	0.280	0.779
ACTCA0	0.162	0.193	−0.632	0.528
ATCTG0	0.145	0.143	0.044	0.968
ACCCA0	0.101	0.095	0.157	0.872
GTCTA0	0.02	0.024		
ATTCA0	0	0.018		
ATCTA0	0	0.013		
GTTTG0	0.013	0		

*Haplotypes for SNPs rs941798, rs3787345, rs2230604, rs718049, rs718050 and 1484insG; **refers to the deletion allele of the 1484insG polymorphism (rs16989673).

either in the allele frequency or in the genotypic frequency among the NGT and type 2 diabetic subjects in any of the SNPs.

Analysis of the effect of PTPN1 SNPs on clinical and biochemical parameters

Since PTPN1 polymorphisms are known to influence insulin sensitivity and that Indians are known to be highly insulin resistant, we compared the HOMA IR levels of the NGT subjects stratified based on the PTPN1 SNPs and found no significant association (table 1). HOMA-IR values for rs16989673 were not compared because of very low frequency of the heterozygous genotype. When the other biochemical parameters were compared, NGT subjects with TT genotype of the Pro303Pro (rs2230604) had significantly lower total serum cholesterol levels (mean ± SD: 4.16 ± 0.9 mmol/L) when compared to CT (4.99 ± 1.0 mmol/L) and CC genotype (4.96 ± 0.8 mmol/L), the age and sex adjusted *P* values were 0.025 for CT vs TT and 0.015 for CC vs TT. The 95% CI for the difference between the means of cholesterol levels among CC and CT individuals is (−0.31, 0.25), that between CT and TT is (0.09, 1.57) and that between CC and TT is (0.32, 1.28). After multiple testing using Bonferroni, the nominal *P* value for the association of the TT genotype with lower total cholesterol levels as compared to CT ($P = 0.025$) and CC ($P = 0.015$) genotype is not lower than the Bonferroni threshold of 0.05/8, that is, 0.0062. Hence, this association finding might not be significant. No significant difference in the other biochemical parameters such as fasting plasma glucose, glycated haemoglobin, HDL cholesterol, LDL cholesterol and serum triglycerides was observed when the NGT and type 2 diabetic subjects were stratified according to the other SNPs.

LD estimation between PTPN1 SNPs and haplotype analysis

LD estimation between the *PTPN1* polymorphisms revealed that the rs2282147 and rs718049 polymorphisms exhibited strong LD ($r^2 = 0.90$ in NGT and 0.85 in type 2 diabetic group). Pairwise LD between the other SNPs was not high. Six locus haplotypes involving SNPs rs941798, rs3787345, rs2230604, rs718049, rs718050 and 1484insG were constructed and analysis was restricted to those haplotypes which have frequency of at least 0.05 in either cases or controls (table 2). Among the rs2282147 and rs718049 polymorphisms which were in strong LD, rs718049 polymorphism was included for the haplotype analysis. GTCTG0, ACTCA0, ATCTG0 and ACCCA0 of SNPs, rs941798, rs3787345, rs2230604, rs718049, rs718050 and 1484insG (rs16989673) were the only four haplotypes which were found with a frequency >5%. None of the haplotypes showed any significant difference in the frequency between the NGT and type 2 diabetic subjects.

Discussion

The failure of the present study to replicate the association between the *PTPN1* variants and type 2 diabetes can have several explanations. In the first place, this study was underpowered with the power ranging from 0.17 to 0.34 to detect an association at significance of 5% for the various SNPs studied. Secondly, as type 2 diabetes is polygenic, genetic variants at multiple loci will contribute in small effect towards its development and it is possible that this effect went undetected in the present study because of the small sample size. Another possibility is that the initial association signal reported by Bento *et al*. (2004) is valid, but there is heterogeneity among the various populations examined either in the phenotypic characteristics of patients studied or in the frequencies of genetic or environmental modifiers.

In the present study, *PTPN1* variants do not seem to have a major role in the aetiology of type 2 diabetes in this population. Since this study is underpowered, elucidation of these results with much larger sample size may help in better understanding of the role of *PTPN1* variants in type 2 diabetes.

Acknowledgements

This study was supported by a grant from the Department of Biotechnology (DBT), Government of India. This is the 98th publication from the CURES study (CURES 98).

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Received 3 May 2010, in final revised form 27 December 2010; accepted 25 January 2011
Published on the Web: 19 August 2011