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SHORT COMMUNICATION

Common variants in the *TCF7L2* gene are strongly associated with type 2 diabetes mellitus in the Indian population

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Abstract
Aims and hypothesis India has the greatest number of diabetic subjects in any one country, but the genetic basis of type 2 diabetes mellitus in India is poorly understood. Common non-coding variants in the transcription factor 7-like 2 gene (*TCF7L2*) have recently been strongly associated with increased risk of type 2 diabetes in European populations. We investigated whether *TCF7L2* variants are also associated with type 2 diabetes mellitus in the Indian population.
Materials and methods We genotyped type 2 diabetes patients (n=955) and ethnically matched control subjects (n=399) by sequencing three single nucleotide polymorphisms (SNPs) (rs7903146, rs12255372 and rs4506565) in *TCF7L2*.
Results We observed a strong association with all the polymorphisms, including rs12255372 (odds ratio [OR] 1.50 [95% CI=1.24–1.82], $p=4.0\times10^{-5}$), rs4506565 (OR 1.48 [95% CI=1.24–1.77], $p=2.0\times10^{-5}$) and rs7903146 (OR 1.46 [95% CI=1.22–1.75], $p=3.0\times10^{-5}$). All three variants showed increased relative risk when homozygous rather than heterozygous, with the strongest risk for rs12255372 (OR 2.28 [95% CI=1.40–3.72] vs OR 1.43 [95% CI=1.11–1.83]). We found no association of the *TCF7L2* genotypes with age at diagnosis, BMI or WHR, but the risk genotype at rs12255372 was associated with higher fasting plasma glucose (p=0.001), higher 2-h plasma glucose (p=0.0002) and higher homeostasis model assessment of insulin resistance (HOMA-R; p=0.012) in non-diabetic subjects.
Conclusions Our study in Indian subjects replicates the strong association of *TCF7L2* variants with type 2 diabetes in other populations. It also provides evidence that variations in *TCF7L2* may play a crucial role in the pathogenesis of type 2 diabetes by influencing both insulin secretion and insulin resistance. *TCF7L2* is an important gene for determining susceptibility to type 2 diabetes mellitus and it transgresses the boundaries of ethnicity.

Keywords Body mass index · Ethnicity · Polymorphisms · *TCF7L2* · Type 2 diabetes mellitus

Abbreviations

HOMA-R	homeostasis model assessment of insulin resistance
KCNJ11	potassium inwardly rectifying channel, subfamily J, member 11
OR	odds ratio
PMNS	Pune Maternal Nutrition Study
PPARγ	peroxisome proliferator-activated receptor gamma
RR	relative risk

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SNP single nucleotide polymorphism
TCF7L2 transcription factor 7-like 2

Introduction

The prevalence of type 2 diabetes mellitus in adult Indians living in cities is estimated to be between 12 and 16%, and India has the greatest number of diabetic subjects in any single country [1]. It is well established that Asian Indians are thin (low BMI) but have higher adiposity (percentage body fat), are more centrally obese [2] and more insulin-resistant than Europeans [3]. These factors are thought to increase their susceptibility to diabetes mellitus [4]. It has been reported that the genetic basis of some diseases in Indians may be different from that reported in Europeans [5], but only a few such studies have been performed.

There has been some progress in defining the genetic susceptibility to type 2 diabetes mellitus in European populations. The Pro12Ala polymorphism in *PPARG* (also known as *PPARγ*) and the E23K variant in *KCNJ11* predispose to type 2 diabetes with allelic odds ratios (OR) in the range of 1.15–1.3 [6, 7]. There are a limited number of studies on the genetic basis of type 2 diabetes mellitus in the Indian population, mostly on Southern Indians [8, 9]. Recently, variants in *TCF7L2* gene have been strongly associated with increased risk of type 2 diabetes mellitus in Icelandic individuals and replicated in Europid subjects from USA and Denmark, with a combined OR of 1.56 ($p=4.7\times10^{-18}$) [10]. Similar observations have been made in large case–control and familial-association studies from the UK [11]. However, all these studies are from European populations. We report for the first time the association of the variants in the *TCF7L2* gene with type 2 diabetes mellitus in a South Asian Indian population.

Subjects and methods

We studied 955 type 2 diabetes mellitus patients and 399 ethnically matched control subjects (Indo-Europeans) from Pune, Maharashtra, Western India. The patients were from the Diabetology Research Centre, King Edward Memorial Hospital and the Research Centre, Pune, and were part of the Wellcome Genetic collection (WellGen) of young type 2 diabetes mellitus patients (diagnosed before age 45 years). The centre receives patients from Pune and adjoining areas for routine treatment, while some are referred for specific problems (secondary and tertiary referrals). We recruited consecutive young type 2 diabetic patients attending the outpatient department in the study. Diagnosis of type 2 diabetes mellitus was based on clinical criteria. Those clinically judged to be insulin-dependent (history of ketoacidosis, to be unresponsive to oral hypoglycaemic agents, to be on continuous insulin treatment since diagnosis) or to have exocrine pancreatic disease (fibrocalculous pancreatic diabetes) or to fulfil clinical criteria for monogenic forms of diabetes were excluded. We studied only one member from a family where more than one attended the clinic. Sixty-five per cent of the patients had osmotic symptoms or weight changes at diagnosis. Subsequent clinical course and response to treatment with oral hypoglycaemic agents supports the diagnosis of type 2 diabetes. The control subjects were parents of children studied in the Pune Maternal Nutrition Study (PMNS) in six villages near Pune [12], who were normal glucose tolerant on a 75 g OGTT. The clinical characteristics of the case and the control subjects are presented in Table 1. The study was approved by the Institutional Ethics Committee following the Indian Council of Medical Research guidelines for research on human subjects. All subjects gave written informed consent.

We genotyped the SNPs rs7903146 and rs12255372, which showed the strongest association in the study by Grant et al. [10], and rs4506565, as it was the best correlated proxy of these SNPs in HapMapII. SNP genotyping was carried out by direct sequencing (using ABI3730 Genetic Analyzer; Applied BioSystems, Foster City, CA, USA) of the purified PCR products, amplified using primers flanking the three variants (available on

Table 1 Clinical characteristics of the study population

	Patients	Control subjects
n	955	399
Sex (men/women)	513/442	186/213
Age at study (years)	47.2 (9.3)	30.9 (5.1)
Age at diagnosis (years)	37.1 (6.2)	NA
BMI (kg/m^2)		
Men	25.4 (3.6)	20.5 (3.1)
Women	27.1 (3.9)	19.1 (2.5)
WHR		
Men	0.98 (0.06)	0.90 (0.06)
Women	0.89 (0.06)	0.76 (0.05)
FPG (mmol/l)	NA	5.1 (4.7–5.5)
2-h PG (mmol/l)	NA	4.9 (4.3–5.8)
Fasting plasma insulin (pmol/l)	NA	28.13 (19.58–42.64)
2-h plasma insulin (pmol/l)	NA	136.68 (86.53–236.62)
HOMA-R	NA	1.15 (0.9)
Treatment (OHA/ins/OHA+ins/diet)	499/46/293/117	NA

FPG fasting plasma glucose; *2-h PG* 2-h plasma glucose; *ins* insulin; *OHA* oral hypoglycemic agents; *NA* not applicable
All values are mean±SD, except FPG, 2-h PG and insulin values, which are medians (interquartile ranges)

Table 2 Allelic and genotypic frequencies and estimates of relative risks for the *TCF7L2* variants in type 2 diabetes patients and control subjects

SNP	Position (NCBI 35.1)[a]	Allele	Patients (n=955)	Control subjects (n=399)	Genotype	Patients (n=955)	Control subjects (n=399)	Allele OR (95% CI)	p value	Het OR (95% CI)	p value	Hom OR (95% CI)	p value
		C	0.63	0.71	CC	391 (40.9)	205 (51.4)	1.46 (1.22–1.75)	3.0×10^{-5}	1.39 (1.08–1.78)	9.8×10^{-3}	2.17 (1.44–3.28)	1.7×10^{-4}
rs7903146	114748339				CT	423 (44.3)	160 (40.1)						
		T	0.37	0.29	TT	141 (14.8)	34 (8.5)						
		A	0.63	0.71	AA	376 (39.4)	204 (51.1)	1.48 (1.24–1.77)	2.0×10^{-5}	1.50 (1.17–1.92)	1.4×10^{-3}	2.12 (1.41–3.20)	2.5×10^{-4}
rs4506565	114746031				AT	442 (46.3)	160 (40.1)						
		T	0.37	0.29	TT	137 (14.3)	35 (8.8)						
		G	0.70	0.78	GG	479 (50.1)	243 (60.9)	1.50 (1.24–1.82)	4.0×10^{-5}	1.43 (1.11–1.83)	5.3×10^{-3}	2.28 (1.40–3.72)	6.9×10^{-4}
rs12255372	114798892				GT	377 (39.5)	134 (33.6)						
		T	0.30	0.22	TT	99 (10.4)	22 (5.5)						

For the number of individuals n, values in parentheses indicate percentage
Genotype relative risk (GRR) was calculated compared with the baseline genotype (homozygote for the common allele)
Allele OR allelic odds ratio; *Het OR* GRR for heterozygotes; *Hom OR* GRR for minor allele homozygotes
[a] National Centre for Biotechnology Information, Build 35.1

Table 3 Correlation of *TCF7L2* rs12255372 genotype with measures of quantitative traits in non-diabetic control subjects

	GG	GT	TT	p value	p value corrected for BMI
n	243	134	22		
BMI (kg/m^2)	19.57 (19.19–19.96)	20.01 (19.52–20.51)	20.47 (19.67–21.26)	0.070	–
WHR (men)	0.90 (0.89–0.91)	0.90 (0.89–0.92)	0.90 (0.87–0.93)	0.91	0.92
WHR (women)	0.75 (0.74–0.76)	0.77 (0.75–0.78)	0.75 (0.72–0.78)	0.23	0.72
FPG (mmol/l)	4.95 (4.88–5.02)	5.14 (5.02–5.25)	5.24 (5.01–5.47)	0.001	0.002
2-h PG (mmol/l)	4.71 (4.57–4.85)	5.10 (4.91–5.30)	5.42 (4.74–6.19)	0.0002	0.0003
Fasting plasma insulin (pmol/l)	27.15 (25.21–29.24)	31.60 (28.27–35.42)	30.21 (24.93–36.60)	0.043	0.13
2-h plasma insulin (pmol/l)	129.80 (117.65–143.21)	144.32 (126.75–164.32)	154.46 (108.83–219.18)	0.14	0.31
HOMA-R	0.86 (0.80–0.93)	1.04 (0.92–1.17)	1.02 (0.83–1.25)	0.012	0.04

FPG fasting plasma glucose; *2-h PG* 2-h plasma glucose

request). Fifteen per cent of the randomly selected samples were re-genotyped and the discrepancy rate on duplicate genotyping was 1/752 (0.13%).

Allele and genotype frequencies were calculated and consistency of genotype frequencies at each SNP with Hardy–Weinberg equilibrium was tested on a contingency table of observed and expected genotype frequencies using the Markov simulation-based goodness of fit test [13]. The allele and genotype frequencies between the diabetic and the control subjects were compared using standard contingency table analysis. Continuous trait data are shown as mean±SD unless otherwise specified. Variables with skewed distributions were log transformed to satisfy assumptions of normality and back-transformed values are shown. We used regression analysis to examine the effects of *TCF7L2* genotypes on quantitative traits. Homeostasis model assessment of insulin resistance (HOMA-R) was calculated using the equation (fasting plasma insulin×fasting plasma glucose)/22.5, and all analyses were carried out with Stata (version 7; Stata, College Station, TX, USA).

Results and discussion

The allele and genotype frequencies for all the variants in the patients and the control subjects are shown in Table 2. The risk allele frequency at rs12255372 in control subjects was lower in Indian subjects than Icelandic subjects (22 vs 29%, $p=0.001$), but that at rs7903146 was very similar to the cohorts reported by Grant et al. [10]. The genotype distribution at all the SNPs did not show any deviation from the Hardy–Weinberg equilibrium ($p>0.05$ in controls). All three SNPs showed strong linkage disequilibrium with $D'=0.86$–0.93 and $r^2=0.71$–0.88 (Electronic supplementary material [ESM] Table 1).

We found a strong association of *TCF7L2* variants with type 2 diabetes mellitus in this Indian population, replicating observations in European populations [10, 11]. All the SNPs showed similar association with the rare allele (rs12255372 [OR=1.50, 95% CI=1.24–1.82, $p=4.0\times10^{-5}$], rs7903146 [OR=1.46, 95% CI=1.22–1.75, $p=3.0\times10^{-5}$] and rs4506565 [OR=1.48, 95% CI=1.24–1.77, $p=2.0\times10^{-5}$]). For all variants, the risk of type 2 diabetes mellitus in homozygotes was higher than for the heterozygous carriers (Table 2). This was most marked for rs12255372, with heterozygotes OR=1.43 (95% CI=1.11–1.83, $p=5.3\times10^{-3}$) and homozygotes OR=2.28 (95% CI=1.40–3.72, $p=6.9\times10^{-4}$). These results support the multiplicative mode of inheritance proposed by Grant et al. [10].

As recent studies have suggested that the higher prevalence of type 2 diabetes mellitus in the South Asian Indians could be partially due to central obesity and altered distribution of fat and muscle mass [2], we investigated whether *TCF7L2* genotypes were associated with altered BMI and WHR. However, no significant association with BMI or WHR was observed in the patients or control subjects. Similarly, the *TCF7L2* genotypes were not associated with age at diagnosis, sex or family history of diabetes mellitus ($p>0.05$) (data not shown). However, in the non-diabetic subjects, we found that possessing the at-risk allele at rs12255372 predicted higher fasting and 2-h plasma glucose concentrations and higher HOMA-R, suggesting both a defect in insulin secretion from the beta cells and an increase in insulin resistance (Table 3).

Our results confirm that genetic variation in *TCF7L2* is as strongly associated with type 2 diabetes mellitus in India, as previously described in European populations [10, 11]. Variation in *TCF7L2* is the most significant genetic factor for diabetes mellitus described in the Indian population to date [8, 9]. The similar strength of association for *TCF7L2* in this Indian study contrasts with the results observed for the *PPARG* Pro12Ala SNP. The 12Ala allele is consistently associated with protection of type 2 diabetes in European populations, but not in South Asian Indians ($n=697$) in India and in Dallas, TX, USA [9]. However, in view of the genetic diversity of Indians, both these results need to be replicated in other groups of patients. Our results show that the strongest risk variant found for type 2 diabetes mellitus to date in European populations is a risk allele of similar effect size in Indians. This is consistent with genetic factors playing an important role in risk for type 2 diabetes mellitus, even in populations where environmental factors may have resulted in a dramatic recent increase in prevalence.

A potential limitation of our study is the relatively small number of patients and control subjects; thus, estimates of the association have relatively large confidence risks, especially for homozygous subjects. However, they are larger than the replication cohorts used in the initial study by Grant et al. [10]. Despite testing our control subjects with OGTT, there may be some potential diabetic patients in the control group, since they were younger and thinner than the patients and might develop diabetes in later life. However, the fact that some control subjects are at risk of diabetes would result in a slight reduction in the ORs, so our results may represent an underestimate of the strength of the association. Since we do not have data on the 30-min insulin levels in the control subjects, the dynamic measurements of insulin secretion, such as the insulinogenic and insulin disposition indices, are not available.

It is not yet clear how *TCF7L2* contributes to the pathogenesis of type 2 diabetes mellitus [10]. Association of the at-risk alleles in non-diabetic control subjects with higher glycaemia and higher HOMA-R suggests defects in

both insulin secretion and insulin sensitivity mechanisms for its possible effects. A causal variant or functional defect in this gene is yet to be identified, so further study of the gene is required.

To conclude, we have replicated the strong association of variants in *TCF7L2* gene and shown it to be a susceptibility gene for type 2 diabetes mellitus in the South Asian Indians. In view of a similar strength of association in Indians as in European populations, *TCF7L2* is an important susceptibility marker for risk of type 2 diabetes mellitus in different ethnic groups.

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Duality of interest None of the authors had any conflicting interests in connection with this study.

References

1. Ramachandran A, Snehalatha C, Kapur A et al (2001) High prevalence of diabetes and impaired glucose tolerance in India: National Urban Diabetes Survey. Diabetologia 44:1094–1101
2. Shelgikar KM, Hockaday TDR, Yajnik CS (1991) Central rather than generalized obesity is associated with hyperglycemia in Asian subjects. Diabet Med 8:712–717
3. Ramachandran A, Snehalatha C, Viswanathan V, Viswanathan M, Haffner SM (1997) Risk of non-insulin-dependent diabetes mellitus conferred by obesity and central obesity in different ethnic groups. A comparative analysis between Asian Indians, Mexican Americans and Whites. Diabetes Res Clin Pract 36:121–125
4. McKeigue PM, Pierpoint T, Ferrie JE, Marmot MG (1992) Relationship of glucose intolerance and hyperinsulinaemia to body fat pattern in south Asians and Europeans. Diabetologia 35:785–791
5. Chandak GR, Idris MM, Reddy DN et al (2002) Mutations in pancreatic secretory trypsin inhibitor gene (*PSTI/SPINK1*) rather than cationic trypsinogen gene (*PRSS1*) are significantly associated with tropical calcific pancreatitis. J Med Genet 39:347–351
6. Altshuler D, Hirschhorn JN, Klannemark M et al (2000) The common PPARgamma Pro12Ala polymorphism is associated with decreased risk of type 2 diabetes. Nat Genet 26:76–80
7. Gloyn AL, Weedon MN, Owen KR et al (2003) Large-scale association studies of variants in genes encoding the pancreatic beta-cell K-ATP channel subunits Kir6.2 (KCNJ11) and SUR1 (ABCC8) confirm that the KCNJ11 E23K variant is associated with type 2 diabetes. Diabetes 52:568–572
8. Vimaleswaran KS, Radha V, Ghosh S et al (2005) Peroxisome proliferator-activated receptor-γ co-activator-1α (PGC-1α) gene polymorphisms and their relationship to type 2 diabetes in Asian Indians. Diabet Med 22:1516–1521
9. Radha V, Vimaleswaran KS, Babu HN et al (2006) Role of genetic polymorphism peroxisome proliferator-activated receptor-gamma2 Pro12Ala on ethnic susceptibility to diabetes in South-Asian and Caucasian subjects. Diabetes Care 29:1046–1051
10. Grant SF, Thorleifsson G, Reynisdottir I et al (2006) Variant of transcription factor 7-like 2 (*TCF7L2*) gene confers risk of type 2 diabetes. Nat Genet 38:320–323
11. Groves CJ, Zeggini E, Minton J et al (2006) Association analysis of 6,736 U.K. subjects provides replication and confirms *TCF7L2* as a type 2 diabetes susceptibility gene with a substantial effect on individual risk. Diabetes 55:2640–2644
12. Kinare AS, Natekar AS, Chinchwadkar MC et al (2000) Low mid pregnancy placental volume in rural Indian women: a cause for low birth weight? Am J Obstet Gynecol 182:443–448
13. Schaid DJ, Rowland CM, Tines DE, Jacobson RM, Poland GA (2002) Score tests for association between traits and haplotypes when linkage phase is ambiguous. Am J Hum Genet 70:425–434