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SHORT COMMUNICATION

FTO gene variants are strongly associated with type 2 diabetes in South Asian Indians

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Abstract
Aims and hypothesis Variants of the *FTO* (fat mass and obesity associated) gene are associated with obesity and type 2 diabetes in white Europeans, but these associations are not consistent in Asians. A recent study in Asian Indian Sikhs showed an association with type 2 diabetes that did not seem to be mediated through BMI. We studied the association of *FTO* variants with type 2 dia-

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betes and measures of obesity in South Asian Indians in Pune.

Methods We genotyped, by sequencing, two single nucleotide polymorphisms, rs9939609 and rs7191344, in the *FTO* gene in 1,453 type 2 diabetes patients and 1,361 controls from Pune, Western India and a further 961 population-based individuals from Mysore, South India.

Results We observed a strong association of the minor allele A at rs9939609 with type 2 diabetes (OR per allele 1.26; 95% CI 1.13–1.40; $p=3\times10^{-5}$). The variant was also associated with BMI but this association appeared to be weaker (0.06 SDs; 95% CI 0.01–0.10) than the previously reported effect in Europeans (0.10 SDs; 95% CI 0.09–0.12; heterogeneity $p=0.06$). Unlike in the Europeans, the association with type 2 diabetes remained significant after adjusting for BMI (OR per allele for type 2 diabetes 1.21; 95% CI 1.06–1.37; $p=4.0\times10^{-3}$), and also for waist circumference and other anthropometric variables.

Conclusions Our study replicates the strong association of *FTO* variants with type 2 diabetes and similar to the study in North Indians Sikhs, shows that this association may not be entirely mediated through BMI. This could imply underlying differences between Indians and Europeans in the mechanisms linking body size with type 2 diabetes.

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

Abbreviation

SNP Single nucleotide polymorphism

Introduction

Common variants of the *FTO* (fat mass and obesity associated) gene have been found to be strongly associated with BMI, obesity and type 2 diabetes in white European adults and children [1, 2]; the association with type 2 diabetes was entirely explained by the association with BMI. The association of *FTO* variants with type 2 diabetes and BMI has been independently identified in several white European populations [3] but the findings are somewhat inconsistent in Asians, which may be the result of varying study designs, inadequate sample sizes or ethnic differences [4–6]. India is called the 'diabetes capital of the world'. Indians develop diabetes at a much lower BMI than that in white Europeans, and for any given BMI Indians have a higher prevalence of diabetes [7]. A recent study in North Indian Sikhs showed a strong association of *FTO* variants with type 2 diabetes, which did not seem to be mediated through BMI [8]. This raises the possibility that the association of *FTO* variants with BMI and type 2 diabetes might be different in Asian populations. We studied the association of *FTO* variants with type 2 diabetes and different measures of obesity in Pune, India.

Methods

Study participants—patients and controls The data reported in this study were obtained from 1,453 type 2 diabetes patients of Indo-European ethnicity, diagnosed before 45 years of age, as reported earlier [9]. The study also included two groups of controls comprising 1,361 and 961 non-diabetic participants of Indo-European and Dravidian ethnicity, respectively (see Electronic supplementary material [ESM]). Participants in both the cohorts have been extensively phenotyped for different biochemical–metabolic and anthropometric variables.

Genotyping and replication analysis Samples were genotyped for two single nucleotide polymorphisms (SNPs), rs9939609 and rs7191344, in the *FTO* gene by sequencing. Genotypes for ~10% samples ($n=516$) were validated by re-genotyping them using other genotyping platforms that did not detect any error (0/516).

Statistical analysis We used logistic regression to investigate the association between type 2 diabetes and the *FTO* genotype in Indo-European patients and non-diabetic controls, while linear regression was used to analyse the association between the *FTO* genotype and BMI or other anthropometric measures. Finally, meta-analysis was performed to combine the linear regression coefficients from within the three cohorts, using study- and sex-specific *Z*-scores for BMI (see ESM). All statistical analyses were carried out using STATA (version 9; Stata Corporation, College Station, TX, USA) and inter-study heterogeneity was estimated using Cochran's Q test and the I^2 statistic [10]. Power calculations were performed using QUANTO v.1. 2. (http://hydra.usc.edu/gxe). The Indo-European case–control study had >92% power to detect the association between *FTO* genotype and type 2 diabetes observed in the European populations (OR 1.27 [1]; log-additive model) at $\alpha=0.01$, given the minor allele frequency in Indians (0.30). Although the Indo-European cases ($n=1,448$) and controls ($n=1,355$) and Dravidian ($n=960$) studies individually had limited power to detect the 0.1 SD per allele association with BMI seen in Europeans at $\alpha=0.01$ (power 46%, 43% and 29%, respectively), the meta-analysis ($n=3,763$) gave >85% power.

Results

We compared the distribution of genotypes in 1,453 type 2 diabetes patients and 1,361 non-diabetic controls of Indo-

Table 1 Basic characteristics of patients and control groups

Characteristic	Patients	Controls	
		Pune	Parthenon
N	1,453	1,361	961
Sex			
Male (%)	818 (56.3)	729 (53.6)	446 (46.4)
Female (%)	635 (43.7)	632 (46.4)	515 (53.6)
Present age (years)	46.6 (9.3)	34.5 (6.1)	32.4 (5.9)
Age at diagnosis (years)	37.0 (16.4)	–	–
Ethnicity	Indo-European	Indo-European	Dravidian
Systolic blood pressure (mmHg)	128 (16)	116 (14)	112 (13)
Diastolic blood pressure (mmHg)	80 (9)	69 (10)	69 (10)
Height (cm)	160.9 (9.1)	159.7 (8.5)	160.4 (8.7)
Weight (kg)	67.8 (12.0)	55.0 (11.9)	61.4 (12.5)
BMI (kg/m^2)			
Male	25.4 (4.0)	21.9 (3.6)	23.9 (3.6)
Female	27.2 (4.3)	20.9 (4.1)	23.6 (4.5)
Waist circumference (cm)			
Male	95.4 (10.7)	83.2 (10.5)	86.3 (10.3)
Female	92.4 (10.3)	69.9 (10.4)	82.1 (11.9)
Hip circumference (cm)			
Male	97.3 (7.4)	91.6 (7.5)	92.8 (7.4)
Female	103.2 (10.2)	90.9 (9.3)	92.5 (8.7)
WHR			
Male	0.98 (0.06)	0.91 (0.06)	0.93 (0.06)
Female	0.89 (0.06)	0.77 (0.06)	0.88 (0.07)
Fat mass percentage by DEXA			
Male	–	17.7 (8.4)	–
Female	–	27.3 (8.7)	–
FPG (mmol/l)	8.50 (6.89–11.28)	5.06 (4.61–5.56)	5.49 (5.11–5.88)
2 h PG (mmol/l)	13.17 (10.72–16.39)	5.50 (4.56–6.72)	5.94 (5.09–6.74)
FPI (pmol/l)	–	32.8 (20.6–48.7)	44.8 (30.1–69.1)
2 h PI (pmol/l)	–	173.3 (97.9–319.9)	246.9 (160.6–378.3)
TC (mmol/l)	4.19 (0.98)	3.91 (0.86)	4.30 (0.91)
TG (mmol/l)	1.66 (1.12)	1.09 (0.81)	1.57 (1.17)
HDLC (mmol/l)	1.06 (0.25)	1.07 (0.32)	1.06 (0.22)
HOMA-R	–	1.85 (1.89)	2.17 (1.54)

Values are mean (SD) for all variables except for FPG, 2 h PG, FPI and 2 h PI, which are median (interquartile range)
DEXA, dual-energy X-ray absorptiometry; FPG, fasting plasma glucose; FPI, fasting plasma insulin; HOMA-R, homeostasis model assessment of insulin resistance; 2 h PG, 2 h plasma glucose; 2 h PI, 2 h plasma insulin; TC, plasma total cholesterol; TG, plasma triacylglycerol

European ethnicity (Table 1). The minor allele A at rs9939609 in the *FTO* gene had a frequency of 0.30 in controls and the genotypes were in Hardy–Weinberg equilibrium. The A allele was strongly associated with type 2 diabetes (OR per allele 1.26; 95% CI 1.13–1.40; $p=3\times10^{-5}$) (Table 2), but not with BMI either in patients ($p=0.29$) or in controls ($p=0.82$) and not with other anthropometric variables (Table 3). The association of the *FTO* genotype with type 2 diabetes remained significant after adjusting for BMI (OR per allele for type 2 diabetes 1.21;

Table 2 Allelic and genotypic frequency and estimate of relative risk for *FTO* variant rs9939609 in type 2 diabetes patients and control participants (Indo-Europeans)

SNP	Position (NCBI 36.3)[a]	Allele	Patients (n=1,453)	Controls (n=1,361)	Genotype	Patients (n=1,453)	Controls (n=1,361)	Allele OR (95% CI)	p value
rs9939609	52378028	T	0.646	0.700	TT	633 (43.6)	678 (49.8)		
					TA	613 (42.2)	550 (40.4)		
		A	0.354	0.300	AA	207 (14.2)	133 (9.8)	1.26 (1.13–1.40)	3.0×10^{-5}

Values in parentheses indicate percentage
[a] National Centre for Biotechnology Information, Build 36.3

Table 3 Relationship of obesity (body size characteristics) in patients and non-diabetic control participants according to *FTO* rs9939609 genotype

Variable	Patients					Controls									
	Indo-Europeans					Indo-Europeans					Dravidians				
	Genotype			*p* value	*p*1 value	Genotype			*p* value	*p*1 value	Genotype			*p* value	*p*1 value
	TT	TA	AA			TT	TA	AA			TT	TA	AA		
N	633	613	207			678	550	133			435	419	107		
Weight (kg)	67.6 (11.9)	67.8 (12.5)	68.8 (11.1)	0.262	0.381	55.2 (11.7)	54.7 (12.2)	55.6 (11.9)	0.88	0.98	60.2 (12.0)	62.4 (12.9)	61.8 (12.0)	0.04	0.05
Height (cm)	160.9 (9.2)	160.9 (9.1)	161.1 (8.6)	0.915	0.844	160.1 (8.6)	159.4 (8.5)	159.4 (8.6)	0.17	0.49	160.1 (8.5)	160.8 (9.1)	160.6 (7.9)	0.33	0.61
BMI (kg/m^2)	26.1 (4.0)	26.2 (4.6)	26.5 (3.8)	0.201	0.289	21.5 (3.8)	21.4 (3.9)	21.8 (4.0)	0.64	0.82	23.4 (3.9)	24.0 (4.3)	23.9 (3.9)	0.07	0.09
Waist circumference (cm)	93.9 (10.6)	94.1 (10.9)	95.2 (9.6)	0.106	0.266	77.2 (12.0)	76.7 (12.7)	77.7 (12.8)	0.94	0.95	83.4 (11.4)	84.7 (11.6)	84.1 (10.3)	0.21	0.25
Hip circumference (cm)	99.7 (8.9)	99.9 (9.6)	100.4 (9.2)	0.303	0.562	91.4 (8.1)	91.0 (8.6)	91.8 (9.0)	0.99	0.89	91.9 (7.8)	93.4 (8.5)	92.9 (7.5)	0.03	0.02
WHR	0.94 (0.07)	0.94 (0.07)	0.95 (0.08)	0.168	0.232	0.84 (0.09)	0.84 (0.09)	0.84 (0.09)	0.88	0.88	0.90 (0.07)	0.91 (0.07)	0.90 (0.07)	0.95	0.98
Fat mass % by DEXA	–	–	–	–	–	22.3 (9.7)	22.1 (9.9)	23.4 (10.0)	0.58	0.59	–	–	–	–	–

Values are mean (SD)
p by ANOVA; *p*1, adjusted for age and sex
DEXA, dual-energy x-ray absorptiometry

95% CI 1.06–1.37; $p=4\times10^{-3}$), for waist circumference (OR per allele 1.20; 95% CI 1.04–1.37; $p=0.01$), for hip circumference (OR per allele 1.24; 95% CI 1.10–1.40; $p=6\times10^{-4}$) or for WHR (OR per allele 1.22; 95% CI 1.08–1.39; $p=2\times10^{-3}$]. This suggests that the increased risk of developing type 2 diabetes in those participants with *FTO* variants was not entirely mediated through its effect on BMI or central obesity in our study. Analysis of non-diabetic Dravidian controls showed a similar lack of association with BMI and other anthropometric variables (Table 3). Another SNP rs7193144, which is highly correlated with rs9939609 ($r^2=1.0$) in Europeans [1, 2] and in our study ($r^2=0.99$), showed a similar association with type 2 diabetes (OR 1.31; 95% CI 1.12–1.54; $p=8.2\times10^{-4}$) but was not associated with BMI or other anthropometric variables (data not presented). We performed a meta-analysis combining Indo-European cases, Indo-European controls and Dravidian controls ($n=3,763$) and observed a borderline association between *FTO* genotype and BMI (per allele SD change in $\log_{10}$ BMI 0.06; 95% CI 0.01–0.10; $p=0.017$; $I^2=0\%$; Fig. 1), waist circumference (per allele SD change in $\log_{10}$ waist circumference 0.05; 95% CI 0.01–0.10; $p=0.030$; $I^2=0\%$) and hip circumference (per allele SD change in hip circumference 0.05; 95% CI 0.01–0.10; $p=0.027$; $I^2=0\%$), but not WHR ($p=0.42$). The *FTO* genotype at rs9939609 was not associated with traits associated with obesity including glycaemia, insulin concentrations, insulin resistance (by homeostasis model assessment of insulin resistance) or lipids in Indo-Europeans but was associated with fasting plasma insulin levels in Dravidians (see ESM Table 1).

Discussion

Our results demonstrate that in Asian Indians, variants in the *FTO* gene predispose to type 2 diabetes; however, unlike in Europeans, they do not appear to do this entirely through their influence on BMI, central obesity and adiposity [1, 2]. Sanghera et al. [8] recently reported a significant association of *FTO* gene variants with type 2 diabetes in North Indian Sikhs that was unaffected by controlling for BMI. They suggested that ethnic differences were responsible for the variable results. A recent study in East Asians also found borderline evidence that the association of the *FTO* gene with BMI was weaker compared with that in studies of Europeans, but the association with type 2 diabetes was similar [6]. The finding that the *FTO* variants are associated with type 2 diabetes argues against a difference in linkage disequilibrium with a putative functional variant in these two populations. Although population stratification may be a possible explanation that may confound the results, recent evidence suggests that despite the geographic and linguistic diversity, Indians as a whole display a low level of genetic heterogeneity [11]. Recent studies in Chinese and Japanese populations did not find an association of *FTO* variants with type 2 diabetes, but a weak association with BMI was found in Japanese [4, 5]. Another study reported that *FTO* variants

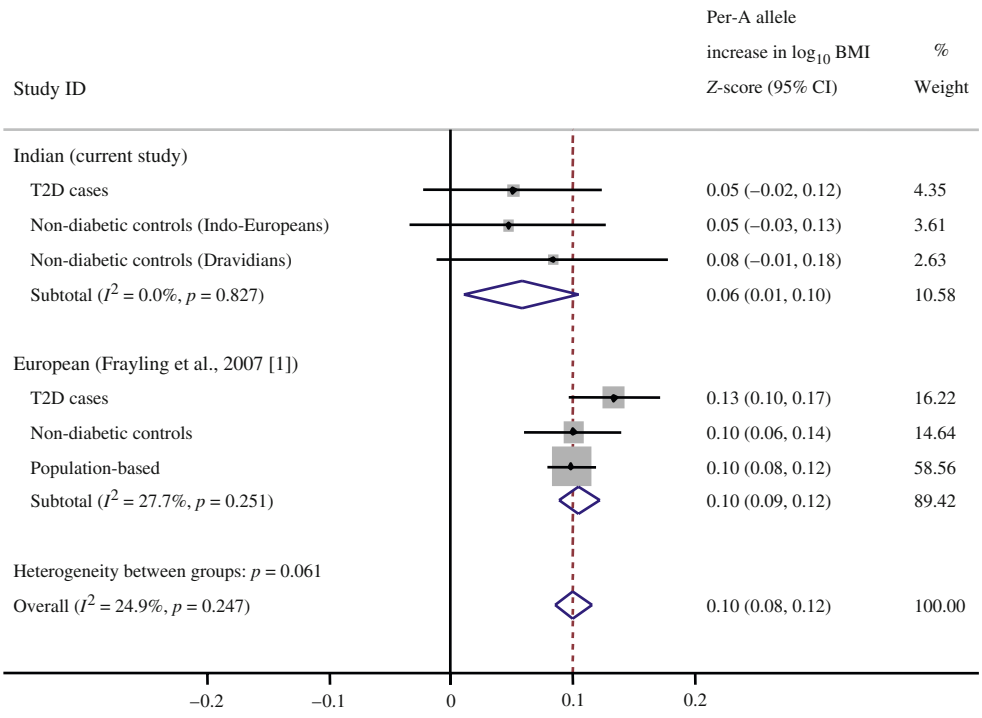

Fig. 1 Meta-analysis plot for *FTO* variant rs9939609 and BMI association for the present study and comparison with the European study of Frayling et al. [1]

might influence the risk of severe obesity in the Japanese [6]. The frequencies of the A allele at the rs9939609 variant were lower in Chinese (0.12) and Japanese (0.18) populations compared with those in Europeans (0.45) and Indians (0.30), meaning that the studies in East Asians have less power [4–6]. It is well known that Indians (and Asians in general) have a lower BMI than Europeans, and the relationship between obesity measures and risk of type 2 diabetes is steeper [7]. This has been ascribed to relatively higher central obesity (WHR) and higher adiposity (body fat percentage) in Asians for a given BMI compared with that in Europeans [7]. The association between *FTO* variants and type 2 diabetes in this study, independent of measures of obesity, could indicate that BMI is a poor measure of adiposity in South Asian Indians. However, the possibility remains that there could be underlying differences in the way the *FTO* gene works to influence the risk of type 2 diabetes in Asian Indians and in Europeans.

The functional role of the *FTO* gene is not yet understood, nor is it clear how the variants affect body size and predict the risk of type 2 diabetes. Based on sequence homology, the *FTO* gene is predicted to code for a 2-oxoglutarate-dependent demethylase enzyme [12], which influences nucleic acid demethylation and so may be important in epigenetic regulation. It is intriguing that maternal vitamin B_{12} and folate nutrition influenced adiposity and insulin resistance in Indian children [13], suggesting that 1-carbon (methyl) metabolism may have an important role in predicting risk for type 2 diabetes. It would be interesting to see if the micronutrients mentioned above influence the effect of the *FTO* gene on adiposity.

In conclusion, our results demonstrate that in Indians, variants of the *FTO* gene predispose to type 2 diabetes, but not entirely through their effect on BMI. Our results are consistent with those recorded in North Indian Sikhs but an independent replication of the weaker effect of *FTO* variants on BMI is required [8]. These results suggest a difference in the possible mechanism of the association from that in Europeans and reinforce our previous suggestion that the relationship between BMI and type 2 diabetes may be different in Indians. Comparative studies of *FTO* in South Asians and Europeans, including functional genomic and epigenetic analyses, may help to understand critical mechanisms in the pathogenesis of obesity and type 2 diabetes.

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Duality of interest The authors declare that there is no duality of interest associated with this manuscript.

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