Journal of Human Genetics (2011) 56, 720–726
© 2011 The Japan Society of Human Genetics All rights reserved 1434-5161/11 $32.00
www.nature.com/jhg

ORIGINAL ARTICLE

Common variants of *FTO* and the risk of obesity and type 2 diabetes in Indians

Ganesh Chauhan[1,7], Rubina Tabassum[1,7], Anubha Mahajan[1], Om Prakash Dwivedi[1], Yuvaraj Mahendran[1], Ismeet Kaur[1], Shubhanchi Nigam[1], Himanshu Dubey[1], Binuja Varma[2], Sri Venkata Madhu[3], Sandeep K Mathur[4], Saurabh Ghosh[5], Nikhil Tandon[6] and Dwaipayan Bharadwaj[1]

Common variants of fat mass and obesity-associated gene (*FTO*, fat mass- and obesity-associated gene) have been shown to be associated with obesity and type 2 diabetes in population of European and non-European ethnicity. However, studies in Indian population have provided inconsistent results. Here, we examined association of eight *FTO* variants (rs1421085, rs8050136, rs9939609, rs9930506, rs1861867, rs9926180, rs2540769 and rs708277) with obesity and type 2 diabetes in 5364 North Indians (2474 type 2 diabetes patients and 2890 non-diabetic controls) in two stages. None of the variants including previously reported intron 1 variants (rs1421085, rs8050136, rs9939609 and rs9930506) showed body mass index (BMI)-dependent/independent association with type 2 diabetes. However, rs1421085, rs8050136 and rs9939609 were associated with obesity status and measures of obesity (BMI, waist circumference and waist-to-hip ratio) in stage 2 and combined study population. Meta-analysis of the two study population results also revealed that rs1421085, rs8050136 and rs9939609 were significantly associated with BMI both under the random- and fixed-effect models (*P* (random/fixed)=0.02/0.0001, 0.004/0.0006 and 0.01/0.01, respectively). In conclusion, common variants of *FTO* were associated with obesity, but not with type 2 diabetes in North Indian population.
Journal of Human Genetics (2011) **56**, 720–726; doi:10.1038/jhg.2011.87; published online 4 August 2011

Keywords: diabetes; *FTO*; genetic variants; Indians; obesity

INTRODUCTION

Type 2 diabetes and obesity have reached epidemic proportions worldwide and the prevalence is increasing at rising rates, especially in developing countries.[1,2] Both genetic and environmental factors with overlapping etiological mechanisms are believed to be involved in the development of obesity and type 2 diabetes. *FTO* (fat mass- and obesity-associated gene) is one of the best examples of diabetogenic gene mediating its effect through obesity. *FTO* was first identified as susceptibility gene for obesity[3] and later body mass index (BMI)-dependent association with type 2 diabetes was revealed in European population.[4] Among the variants known to influence obesity, *FTO* variants had maximal effect in Europeans.[5] Subsequently, a number of studies confirmed the association of common variants of *FTO* in population of European and non-European ethnicity.[6–10] Independent studies in Asian population[11–15] had yielded conflicting results, but a recent meta-analysis of common variants of *FTO* in East Asian population confirmed their association with obesity and type 2 diabetes.[16]

However, association of common variants of *FTO* in Indian population remains inconclusive. Two studies from Pune–Mysore[17] and North Indian Sikhs[18] demonstrated a BMI-independent association of rs9939609 with type 2 diabetes, but failed to replicate its association with obesity. A recent study on a South Indian population from Chennai[19] demonstrated BMI-dependent association of rs8050136 with type 2 diabetes and also with obesity. These contradictions in association results for variants in *FTO* with obesity and type 2 diabetes demand their further validation in Indian population.

In this study, we evaluated association of previously associated common variants of *FTO* in intron 1 (rs9939609, rs1421085, rs8050136 and rs9930506) and additional variants (rs1861867—intron 2, rs9926180—intron 7, rs2540769—intron 8 and rs708277—3′-UTR) of *FTO* with type 2 diabetes and obesity in North Indians of Indo-European ethnicity.

MATERIALS AND METHODS

Study participants

Study involved participation of 5364 subjects from North India belonging to Indo-European ethnicity residing in and around Delhi, in two stages. In stage 1, association analysis was performed in 2115 subjects comprising of 1073 type 2

[1]Genomics and Molecular Medicine Unit, Institute of Genomics and Integrative Biology (CSIR), Delhi, India; [2]The Centre of Genomics Applications, Okhla Industrial Area Phase III, New Delhi, India; [3]Division of Endocrinology, University College of Medical Sciences, Delhi, India; [4]Department of Endocrinology, SMS Medical College and Hospital, Rajasthan, India; [5]Human Genetics Unit, Indian Statistical Institute, Kolkata, India and [6]Department of Endocrinology, All India Institute of Medical Sciences, New Delhi, India
[7]These authors contributed equally to this work.
Correspondence: Professor N Tandon, Department of Endocrinology, All India Institute of Medical Sciences, Ansari Nagar, New Delhi 110 029, India.
E-mail: nikhil2811tandon@gmail.com
or Dr D Bharadwaj, Genomics and Molecular Medicine Unit, Institute of Genomics and Integrative Biology, CSIR, Mall Road, Delhi 110 007, India.
E-mail: db@igib.res.in
Received 20 February 2011; revised 18 June 2011; accepted 5 July 2011; published online 4 August 2011

diabetes patients and 1042 controls, who were recruited before September 2008. All type 2 diabetes patients of stage 1 were recruited from the Clinic (Endocrinology Clinic, All India Institute of Medical Sciences), whereas control subjects were collected by organizing 'Diabetes Awareness Camps'. In stage 2, further replication was assessed in an independent sample set of 3249 individuals recruited after September 2008 that included 1401 type 2 diabetes patients (recruited from Clinic and 'Diabetes Awareness Camps') and 1848 controls (enrolled from 'Diabetes Awareness Camps'). Type 2 diabetes was diagnosed as per the World Health Organization criteria.[20] Control subjects had age $\geqslant$40 years, no family history of diabetes with glycosylated hemoglobin level $\leqslant$6.0% and fasting glucose level $\leqslant$6.11 mmol l^{-1}. Detailed inclusion and exclusion criteria for type 2 diabetes patients and control subjects have been provided earlier.[21,22] Informed consent was obtained from all the participants and the study was approved by the Ethics committee of the participating institutions. The study was conducted in accordance with the principles of the Declaration of Helsinki.

Clinical measurements

All subjects were extensively characterized for anthropometric and quantitative metabolic traits. Anthropometric measurements including height, weight, waist circumference (WC), hip circumference, systolic and diastolic blood pressure were taken as per standardized protocols. BMI and waist-to-hip ratio (WHR) were calculated. Biochemical measurements including levels of glycosylated hemoglobin, fasting plasma glucose, 2-h post-load plasma glucose, fasting plasma insulin, C-reactive protein, C-peptide, total cholesterol (TC), high-density lipoprotein-cholesterol, low-density lipoprotein-cholesterol, triglyceride, blood urea, creatinine and uric acid were performed using standard laboratory assays as described earlier.[21] Homeostasis model assessment for insulin resistance was calculated using the formula: (fasting plasma insulin (mU l^{-1})$\times$fasting plasma glucose (mmol l^{-1}))/22.5 as reported previously.[23] General characteristics of study population are presented in Table 1.

SNP selection

Four single-nucleotide polymorphisms (SNPs) from intron 1 (rs1421085, rs8050136, rs9939609, rs9930506) that were previously shown to be associated with obesity/type 2 diabetes were selected. In addition, four more SNPs (rs1861867—intron 2, rs9926180—intron 7, rs2540769—intron 8 and rs708277-3′—UTR) were selected from the database (NCBI dbSNP Build 129) to cover the gene at regular intervals. These SNPs were prioritized based on

Table 1 General characteristics of the study populations

	Stage 1		*Stage 2*			
Characteristics	*Type 2 diabetes patients*	*Controls*	*Type 2 diabetes patients*	*Controls*	P-*value*[a]	P-*value*[b]
N (men/women)	1019 (592/427)	1006 (606/400)	1401 (851/550)	1848 (1017/831)	—	—
Age (years)	53 (45–62)	50 (44–60)	55 (48–62)	52 (45–62)	0.03	1.9×10^{-6}
Age of diagnosis (years)	45 (39–52)	—	47 (40–55)		2.3×10^{-4}	
Individuals on medication for lipids (%)	4.00	—	7.35	0.87	—	—
BMI (kg m^{-2})						
Women	26.70 (24.20–29.20)	24.90 (21.10–28.60)	27.34 (24.46–31.00)	26.30 (23.11–29.23)	0.001	1.3×10^{-5}
Men	23.80 (22.00–26.00)	23.20 (20.20–25.70)	25.40 (22.94–28.36)	24.69 (22.15–27.35)	3.0×10^{-15}	3.7×10^{-13}
WC						
Women	91.44 (86.36–96.52)	85.00 (75.60–93.00)	86.68 (40.00–98.00)	88.00 (81.00–95.50)	3.8×10^{-34}	1.1×10^{-5}
Men	86.36 (86.36–91.44)	88.50 (80.64–95.00)	90.00 (36.00–98.00)	93.00 (86.00–100.00)	8.4×10^{-44}	1.9×10^{-16}
WHR						
Women	1.00 (0.97–1.03)	0.86 (0.82–0.92)	0.95 (0.89–0.98)	0.87 (0.82–0.91)	5.9×10^{-23}	0.57
Men	1.00 (0.97–1.03)	0.97 (0.92–1.00)	0.98 (0.96–1.03)	0.97 (0.92–1.00)	0.002	0.53
FPG (mmol l^{-1})	7.90 (6.40–10.30)	4.90 (4.50–5.30)	8.05 (6.39–10.60)	4.82 (4.41–5.20)	0.28	2.8×10^{-4}
2-h PPG (mmol l^{-1})	—	5.60 (5.80–6.30)	—	5.76 (4.95–6.55)		0.06
HbA1c (%)	7.80 (6.50–9.40)	5.20 (4.90–5.60)	8.19 (6.90–9.70)	5.70 (5.40–5.90)	2.6×10^{-5}	1.3×10^{-114}
FPI (pmol l^{-1})	—	32.20 (17.50–57.20)	—	43.68 (28.20–63.36)	—	4.1×10^{-16}
HOMA IR	—	1.16 (0.59–2.02)	—	1.56 (0.97–2.38)	—	9.2×10^{-15}
C-peptide (nmol l^{-1})	0.89 (0.56–1.36)	0.53 (0.36–0.73)	0.95 (0.60–1.45)	0.67 (0.51–0.86)	2.3×10^{-9}	3.6×10^{-26}
hsCRP (mg l^{-1})	2.20 (0.90–4.70)	1.30 (0.60–3.00)	1.93 (0.93–3.63)	1.68 (0.92–3.11)	3.4×10^{-5}	8.8×10^{-10}
TC (mmol l^{-1})	4.20 (3.50–5.00)	4.40 (3.70–5.10)	4.57 (3.73–5.39)	4.83 (4.16–5.46)	0.13	1.8×10^{-25}
HDL-C (mmol l^{-1})	1.03 (0.88–1.22)	1.06 (0.88–1.28)	1.08 (0.92–1.30)	1.20 (1.01–1.42)	2.0×10^{-7}	1.2×10^{-27}
LDL-C (mmol l^{-1})	2.57 (1.99–3.36)	2.79 (2.33–3.41)	2.70 (2.04–3.40)	2.97 (2.46–3.49)	0.009	2.5×10^{-6}
TG (mmol l^{-1})	1.60 (1.10–2.20)	1.30 (1.00–1.80)	1.39 (0.91–2.11)	1.16 (0.81–1.62)	0.84	1.2×10^{-14}
Urea (mmol l^{-1})	9.28 (7.13–11.83)	8.5 (6.78–10.31)	9.5 (7.57–12.50)	8.57 (6.96–10.42)	7.7×10^{-7}	0.33
Uric acid (μmol l^{-1})	291.45 (231.97–356.88)	291.45 (238.51–341.42)	289.37 (236.14–350.93)	303.35 (248.63–365.80)	7.6×10^{-10}	1.2×10^{-4}
Creatinine (μmol l^{-1})	74.26 (59.23–94.59)	66.3 (58.34–77.79)	67.18 (56.58–82.21)	64.53 (53.92–76.02)	3.8×10^{-5}	1.0×10^{-4}
Systolic BP (mm Hg)	130 (130–140)	120 (112–133)	130 (122–140)	128 (120–140)	7.6×10^{-10}	6.1×10^{-5}
Diastolic BP (mm Hg)	80 (78–90)	80 (70–88)	82 (80–90)	80 (80–90)	3.9×10^{-5}	5.7×10^{-17}

Abbreviations: BMI, body mass index; BP, blood pressure; FPG, fasting plasma glucose; FPI, fasting plasma insulin; HbA1c, glycosylated hemoglobin A1c; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment for insulin resistance; hsCRP, high-sensitivity C-reactive protein; LDL-C, low-density lipoprotein cholesterol; N, number of individuals; 2-h PPG, 2-h post-load plasma glucose; TC, total cholesterol; TG, triglycerides; WC, waist circumference; WHR, waist-to-hip ratio.
Data are presented as median (interquartile range).
[a]Difference in quantitative trait values of type 2 diabetes patients (Mann–Whitney *U*-test).
[b]Difference in quantitative trait values of controls (Mann–Whitney *U*-test).

their minor allele frequency (MAF) (>0.05), polymorphic state in at least two population and localization in functionally important region.

Genotyping

Genotyping in stage 1 was performed by Golden Gate assay using Illumina platform (http://www.illumina.com/documents/products/workflows/workflow_goldengate_assay.pdf). Stringent quality control criteria were met to qualify an SNP for further analysis, including genotype confidence score >0.25, call frequency >0.9, GenTrans score >0.6, cluster separation score >0.4, MAF >0.05 and Hardy–Weinberg equilibrium $P>0.01$ in controls.[24] A total of 147 samples (7.2%) were also genotyped in duplicate and an error rate of <0.01 was estimated. The variant rs708277 did not qualify the quality control (GenTrans score <0.6) and was excluded from further analysis.

In stage 2, genotyping of rs9926180 (associated in stage 1) and rs9939609 (established obesity/type 2 diabetes variant in stage 2) was performed in 1047 cases and 1038 controls using iPLEX (Sequenom, San Diego, CA, USA). Approximately 5% (N=110) of the samples of stage 2 were genotyped in duplicates and a concordance of 99.99% was observed. In addition, 810 controls were genotyped for all the seven SNPs of stage 1 and 354 cases for rs9939609 and rs9926180, to increase power of the study. These samples were genotyped by single base extension using SNaPshot ddNTP Primer Extension Kit (Applied Biosystems, Foster City, CA, USA) and electrophoresis of extended probes on an ABI Prism 3730 Genetic Analyzer (Applied Biosystems). The genotype calls were determined using GeneMapper. Approximately 6% of the samples were genotyped in duplicates and a concordance of 99.99% was observed. SNaPshot assay for two SNPs rs1861867 and rs2540769 failed in this phase.

Statistical analysis

Statistical analyses were performed using PLINK v. 1.07 (http://pngu.mgh.harvard.edu/purcell/plink/[25]) and SPSS v. 17.0 (SPSS, Chicago, IL, USA), unless specified otherwise. Statistical power of the study was calculated under log-additive model, assuming 10% population risk using Quanto (http://hydra.usc.edu/gxe/) (Supplementary Tables 1 and 2, Supplementary Figure 1). Hardy–Weinberg equilibrium for genotypes was checked by χ^2 analysis. Linkage disequilibrium between SNPs was estimated using Haploview v. 4.1.[26] Single marker- and haplotype-based association with obesity and type 2 diabetes was assessed by logistic regression analysis assuming log-additive model. A P-value of <0.01 (α=0.05/5) was considered significant after Bonferroni correction for the number of independent SNPs investigated. All quantitative trait values were inverse normally transformed to achieve normal distribution and then analyzed using linear regression. Means and standard deviations are presented in inverse normal units of the parameters in the tables. For quantitative traits association, a P-value of <0.00053 (α=0.05/(5×19)) was considered significant (association for five independent loci with 19 traits). Association with obesity and quantitative traits was performed only in control subjects.

Meta-analysis of stage 1 and 2 results were performed by combining summary data of two study population both under fixed and random models. Similarly, the summary data of previous studies on Indians for association with type 2 diabetes[17–19] and this study were combined for meta-analysis. Association of variants with type 2 diabetes, obesity and quantitative traits were also performed by combining data for two study population and adjusting for study population in addition to other covariates. Non-parametric Mann–Whitney U-test was performed to compare the median values of clinical traits of two study population. Allele frequencies of cases and controls of two study population were compared by equality of proportions Z-test (Supplementary Table 3).

All analyses performed were adjusted for age, sex and BMI as appropriate. The odds ratios (ORs) and 95% confidence interval (CI) were calculated with respect to minor alleles and uncorrected P-values are presented in the manuscript.

RESULTS

Association with type 2 diabetes

In stage 1, rs9926180 (intron 7) was associated with type 2 diabetes that remained significant after adjusting for sex, age and BMI (OR_{adj}=1.24, 95% CI=1.07–1.43, P_{adj}=0.005) and even after correcting for multiple tests (Table 2). However, association of rs9926180

Table 2 Association of variants with type 2 diabetes

Study	*SNP*	N *(cases/controls)*	*Minor/major allele*	*MAF (cases/controls)*	*Genotype*		*Analysis not adjusted for covariates*		*Analysis adjusted for BMI*		*Analysis adjusted for sex, age and BMI*	
					Cases (11/12/22)[a]	*Controls (11/12/22)*[a]	*OR (95% CI)*	*P-value*	*OR (95% CI)*	*P-value*	*OR (95% CI)*	*P-value*
Stage 1	rs1421085	1012/1005	C/T	0.38/0.36	142/480/390 (0.14/0.47/0.39)	136/447/422 (0.14/0.44/0.42)	1.09 (0.96–1.24)	0.20	1.07 (0.93–1.21)	0.34	1.07 (0.94–1.22)	0.32
	rs8050136	1019/1005	A/C	0.35/0.34	132/455/432 (0.13/0.45/0.42)	122/437/446 (0.12/0.43/0.45)	1.06 (0.93–1.21)	0.36	1.05 (0.92–1.19)	0.51	1.05 (0.92–1.20)	0.48
	rs9939609	1019/1006	A/T	0.35/0.34	132/454/433 (0.13/0.45/0.42)	121/440/445 (0.12/0.44/0.44)	1.06 (0.93–1.20)	0.38	1.04 (0.91–1.19)	0.53	1.05 (0.92–1.19)	0.51
	rs9930506	946/956	G/A	0.44/0.43	196/449/301 (0.21/0.47/0.32)	196/431/329 (0.21/0.45/0.34)	1.06 (0.93–1.20)	0.40	1.06 (0.94–1.21)	0.34	1.07 (0.94–1.21)	0.31
	rs1861867	1018/1006	T/C	0.29/0.3	85/426/507 (0.08/0.42/0.50)	90/415/501 (0.09/0.41/0.50)	0.96 (0.86–1.13)	0.83	0.99 (0.86–1.14)	0.89	1.00 (0.87–1.15)	0.95
	rs9926180	1011/995	A/T	0.27/0.24	72/403/536 (0.07/0.40/0.53)	54/364/577 (0.05/0.37/0.58)	1.20 (1.04–1.38)	0.01	1.22 (1.05–1.41)	0.008	1.24 (1.07–1.43)	0.005
	rs2540769	1018/1006	G/T	0.32/0.31	98/447/473 (0.10/0.44/0.46)	101/425/480 (0.10/0.42/0.48)	1.02 (0.89–1.16)	0.77	1.04 (0.91–1.19)	0.60	1.03 (0.90–1.18)	0.67
Stage 2[b]	rs9939609	1342/1749	A/T	0.35/0.34	161/619/562 (0.12/0.46/0.42)	200/776/773 (0.11/0.44/0.44)	1.07 (0.96–1.19)	0.27	1.06 (0.95–1.18)	0.30	1.06 (0.95–1.19)	0.27
	rs9926180	1293/1610	A/T	0.25/0.26	71/493/729 (0.05/0.38/0.56)	114/611/885 (0.07/0.38/0.55)	0.92 (0.82–1.04)	0.19	0.96 (0.85–1.08)	0.49	0.96 (0.85–1.09)	0.53
Combined study	rs1421085	1012/1750	C/T	0.38/0.35	142/480/390 (0.14/0.47/0.39)	229/774/747 (0.13/0.44/0.43)	1.11 (1.00–1.25)	0.06	1.07 (0.93–1.21)	0.34	1.07 (0.94–1.22)	0.32
population[c]	rs8050136	1106/1800	A/C	0.35/0.34	140/502/464 (0.13/0.45/0.42)	209/792/799 (0.12/0.44/0.44)	1.08 (0.97–1.21)	0.18	1.05 (0.93–1.19)	0.41	1.06 (0.93–1.19)	0.39
	rs9939609	2361/2755	A/T	0.35/0.34	293/1073/995 (0.12/0.45/0.42)	321/1216/1218 (0.12/0.44/0.44)	1.06 (0.98–1.15)	0.13	1.05 (0.97–1.15)	0.22	1.06 (0.97–1.15)	0.19
	rs9930506	946/1743	G/A	0.44/0.43	196/449/301 (0.21/0.47/0.32)	332/848/563 (0.19/0.49/0.32)	1.04 (0.93–1.17)	0.45	1.06 (0.94–1.21)	0.34	1.07 (0.94–1.21)	0.31
	rs9926180	2304/2605	A/T	0.26/0.25	143/896/1265 (0.06/0.39/0.55)	168/975/1462 (0.06/0.37/0.56)	1.03 (0.94–1.12)	0.57	1.06 (0.96–1.16)	0.23	1.07 (0.97–1.17)	0.18

Abbreviations: BMI, body mass index; CI, confidence interval; MAF, minor allele frequency; N, number of individuals; OR, odds ratio; SNP, single-nucleotide polymorphism.
[a]The genotype distributions along with the genotype frequencies have been provided. 11—minor allele homozygous; 12—heterozygous; 22—major allele homozygous.
[b]In phase 2, the variants rs1421085, rs8050136 and rs993056 were genotyped only in control subjects; hence, association with type 2 diabetes was not performed.
[c]All the three analyses in the combined study population were adjusted for study.

could not be replicated in another independent study population in stage 2. Meta-analysis and combined analysis of two stages also did not show association of rs9926180 with type 2 diabetes (Table 4). None of the previously reported SNPs showed either BMI-dependent or -independent association with type 2 diabetes in our population. Interestingly, the most widely studied variant rs9939609 also failed to show any association with type 2 diabetes in our study population. Similar results were obtained when association analysis were performed by adjusting for sex, age and BMI as covariates and only BMI as a covariate (Table 2).

Association with obesity and its measures

For association analysis with obesity, control subjects were categorized as normal weight (BMI $<23\,kg\,m^{-2}$), overweight (BMI 23–$25\,kg\,m^{-2}$) and obese (BMI $\geqslant 25\,kg\,m^{-2}$) based on BMI cutoffs for Asians defined by World Health Organization expert committee[27] (Supplementary Table 4). None of the variants showed association with BMI-defined obesity in stage 1 of the study (Table 3). However, in stage 2, rs1421085 (OR=1.57, 95% CI=1.21–2.04, *P*=0.0007) and rs8050136 (OR=1.37, 95% CI=1.06–1.77, *P*=0.02) showed association with obesity when we compared obese/overweight with normal weight subjects. In the same stage, the association was also observed when we compared obese with normal weight subjects (rs1421085: OR=1.65, 95% CI=1.26–2.17, *P*=0.0003; rs8050136: OR=1.45, 95% CI=1.11–1.91, *P*=0.007) with increased risk. The associations were also observed after combined analysis and meta-analysis (Table 4). As WC has been shown to be a better predictor of obesity,[28] we also tested association by defining obesity using WC (>90 cm in men and >80 cm in women)[28] and WHR (>0.95 in men and >0.80 in women)[29] as measures of central obesity (Supplementary Table 5). The same variant rs1421085 was associated with WC-defined obesity in stage 2 (OR=1.43, 95% CI=1.11–1.85, *P*=0.006) and combined study population (OR=1.23, 95% CI=1.06–1.43, *P*=0.006). In WHR-defined obesity analysis, rs1421085 and rs9930506 showed nominal association only in stage 2 of the study. Association analysis with quantitative measures of obesity (BMI, WC and WHR) in stage 1 showed only nominal association of rs1421085 (*P*=0.04), rs8050136 (*P*=0.03) and rs9939609 (*P*=0.03) with WC (Supplementary Table 6). However in stage 2 (Supplementary Table 7), association with BMI and WC was observed for rs1421085 (BMI: *P*=0.0002; WC: *P*=0.001), rs8050136 (BMI: *P*=0.002; WC: *P*=0.05) and rs9930506 (BMI: *P*=0.04; WC: *P*=0.02). The variant rs9939609 also showed nominal association with WC (*P*=0.03). In the combined study population (Supplementary Table 8), all the above three variants were associated with BMI and WC in addition to nominal association of rs9939609 with WHR (*P*=0.03).

Association with quantitative trait

We also examined association of *FTO* variants with other quantitative traits related to obesity and type 2 diabetes to see if they affect risk of obesity/type 2 diabetes by influencing them. In stage 1, rs9926180 was associated with high-density lipoprotein-cholesterol (*P*=0.002), and in stage 2, rs9930506 was associated with triglycerides (*P*=0.002). Nominal associations of *FTO* variants with other traits were also observed (Supplementary Table 6).

Haplotype-based association

Intron 1 variants—rs1421085, rs8050136 and rs9939609 formed a haplotype block encompassing a region of 19 kb (Supplementary Figure 2). Association analysis of this haplotype block with type 2

Table 3 Association of variants with BMI-defined obesity (controls only)

			(Obese+overweight) vs normal weight				*Obese vs normal weight*				*Overweight vs normal weight*			
Study	*SNP*	*Allele (minor/major)*	*N (OB+OW/NW)*	*MAF (OB+OW/NW)*	*OR (95% CI)*	*P-value*	*N (OB/NW)*	*MAF (OB/NW)*	*OR (95% CI)*	*P-value*	*N (OW/NW)*	*MAF (OW/NW)*	*OR (95% CI)*	*P-value*
Stage 1[a]	rs1421085	C/T	561/436	0.36/0.35	1.07 (0.89–1.28)	0.50	387/436	0.36/0.35	1.05 (0.86–1.29)	0.63	174/436	0.37/0.35	1.07 (0.84–1.38)	0.58
	rs8050136	A/C	561/436	0.35/0.33	1.07 (0.89–1.29)	0.49	387/436	0.35/0.33	1.05 (0.86–1.29)	0.63	174/436	0.35/0.33	1.08 (0.84–1.39)	0.57
	rs9939609	A/T	562/436	0.35/0.33	1.06 (0.88–1.28)	0.54	388/436	0.34/0.33	1.04 (0.85–1.28)	0.69	174/436	0.35/0.33	1.07 (0.83–1.38)	0.59
	rs9930506	G/A	532/416	0.43/0.44	0.95 (0.80–1.14)	0.60	369/416	0.42/0.44	0.93 (0.77–1.13)	0.49	163/416	0.43/0.44	0.98 (0.77–1.25)	0.87
	rs1861867	T/C	562/436	0.29/0.31	0.89 (0.73–1.08)	0.23	388/436	0.29/0.31	0.89 (0.72–1.10)	0.27	174/436	0.28/0.31	0.88 (0.67–1.16)	0.36
	rs9926180	A/T	557/430	0.23/0.25	0.91 (0.74–1.12)	0.38	384/430	0.24/0.25	0.98 (0.78–1.23)	0.86	173/430	0.21/0.25	0.78 (0.57–1.07)	0.12
	rs2540769	G/T	562/436	0.30/0.32	0.94 (0.77–1.13)	0.50	388/436	0.31/0.32	0.96 (0.78–1.18)	0.69	174/436	0.30/0.32	0.88 (0.67–1.16)	0.37
Stage 2[a]	rs1421085	C/T	433/197	0.38/0.28	1.57 (1.21–2.04)	0.0007	330/197	0.39/0.28	1.65 (1.26–2.17)	0.0003	103/197	0.34/0.28	1.34 (0.93–1.93)	0.12
	rs8050136	A/C	465/209	0.35/0.28	1.37 (1.06–1.77)	0.02	352/209	0.36/0.28	1.45 (1.11–1.91)	0.007	113/209	0.31/0.28	1.18 (0.84–1.68)	0.34
	rs9939609	A/T	1151/479	0.34/0.33	1.04 (0.89–1.22)	0.62	868/479	0.34/0.33	1.06 (0.90–1.25)	0.50	283/479	0.33/0.33	0.99 (0.80–1.24)	0.95
	rs9930506	G/A	462/206	0.44/0.40	1.21 (0.94–1.55)	0.13	351/206	0.45/0.40	1.25 (0.97–1.63)	0.09	111/206	0.41/0.40	1.06 (0.75–1.51)	0.73
	rs9926180	A/T	1054/439	0.25/0.27	0.92 (0.77–1.09)	0.33	795/439	0.25/0.27	0.89 (0.74–1.07)	0.22	259/439	0.27/0.27	0.99 (0.77–1.27)	0.93
Combined study population[b]	rs1421085	C/T	994/633	0.37/0.33	1.22 (1.05–1.41)	0.009	717/633	0.37/0.33	1.24 (1.06–1.46)	0.008	277/633	0.36/0.33	1.16 (0.95–1.43)	0.15
	rs8050136	A/C	1026/645	0.35/0.31	1.17 (1.01–1.36)	0.04	739/645	0.35/0.31	1.19 (1.01–1.40)	0.04	287/645	0.33/0.31	1.12 (0.91–1.38)	0.27
	rs9939609	A/T	1713/915	0.34/0.33	1.05 (0.93–1.19)	0.41	1256/915	0.34/0.33	1.06 (0.93–1.20)	0.41	457/915	0.33/0.33	1.03 (0.87–1.22)	0.72
	rs9930506	G/A	994/622	0.43/0.42	1.04 (0.90–1.20)	0.61	720/622	0.44/0.42	1.04 (0.89–1.22)	0.60	274/622	0.43/0.42	1.02 (0.83–1.24)	0.86
	rs9926180	A/T	1611/869	0.24/0.26	0.91 (0.80–1.05)	0.19	1179/869	0.24/0.26	0.92 (0.80–1.07)	0.28	432/869	0.24/0.26	0.90 (0.74–1.09)	0.27

Abbreviations: BMI, body mass index; CI, confidence interval; MAF, minor allele frequency; N, number of individuals; NW, normal weight; OB, obese; OR, odds ratio; OW, overweight; SNP, single-nucleotide polymorphism.
[a]Analysis adjusted for sex and age.
[b]Analysis adjusted for sex, age and study.

Table 4 Meta-analysis for the two study populations

Trait	*SNP*	P (F)	P (R)	*OR*/β (F)	*OR*/β (R)	Q	I
Type 2 diabetes	rs9939609	0.22	0.22	1.054	1.054	0.870	0
	rs9926180	0.19	0.52	1.065	1.086	0.010	85.1
BMI	rs1421085	**0.0001**	**0.02**	0.638	0.668	0.094	64.34
	rs8050136	**0.0006**	**0.004**	0.582	0.591	0.226	31.71
	rs9939609	**0.01**	**0.01**	0.324	0.324	0.668	0
	rs9930506	0.22	0.39	0.205	0.236	0.104	62.14
	rs9926180	0.09	0.09	−0.256	−0.256	0.760	0
Obese+overweight vs normal weight	rs1421085	**0.01**	0.20	1.215	1.280	0.015	83.02
	rs8050136	0.05	0.16	1.164	1.190	0.121	58.33
	rs9939609	0.44	0.44	1.048	1.048	0.878	0
	rs9930506	0.69	0.65	1.030	1.055	0.132	55.84
	rs9926180	0.20	0.20	0.916	0.916	0.941	0
Obese vs normal weight	rs1421085	**0.01**	0.24	1.232	1.304	0.010	85.11
	rs8050136	0.05	0.22	1.181	1.219	0.067	70.3
	rs9939609	0.46	0.46	1.052	1.052	0.902	0
	rs9930506	0.62	0.66	1.040	1.067	0.074	68.66
	rs9926180	0.32	0.32	0.926	0.926	0.536	0
Overweight vs normal weight	rs1421085	0.19	0.19	1.151	1.151	0.334	0
	rs8050136	0.32	0.32	1.111	1.111	0.681	0
	rs9939609	0.78	0.78	1.024	1.024	0.640	0
	rs9930506	0.96	0.96	1.005	1.005	0.720	0
	rs9926180	0.30	0.34	0.900	0.895	0.250	24.39

Abbreviations: P (F), fixed-effect meta-analysis *P*-value; P (R), random-effect meta-analysis *P*-value; OR (F), fixed-effect OR estimate; OR (R), random-effect OR estimate; Q, *P*-value for Cochrane's *Q* statistic; I, *I*^2 heterogeneity index (0–100).
P-value in bold are significant.

diabetes, obesity and BMI failed to show any association (Supplementary Table 9).

Meta-analysis with other studies

Meta-analysis of the four studies on Indians (including this study) for association with type 2 diabetes (Figure 1) showed association under the random-effect (OR_{random}=1.16, $P\text{-value}_{random}$=0.02) and fixed-effect models (OR_{random}=1.21, $P\text{-value}_{random}$=0.001).

DISCUSSION

Here we explored association of *FTO* variants with type 2 diabetes, obesity and related quantitative traits in North Indians. We did not find *FTO* variants to influence the risk of type 2 diabetes in our study population. Our preliminary study suggested a BMI-independent association of a new variant rs9926180 in intron 7 with type 2 diabetes, but that could not be replicated in an independent study population. However, variants of intron 1 (rs1421085, rs8050136 and rs9939609) were associated with obesity status and/or measures of obesity (BMI, WC and WHR). This is in accordance with previous reports of these variants for association with obesity.

It is pertinent to mention that consistent with results of the studies in other population, our previous study demonstrated association of top eight genome-wide association studies confirmed loci with type 2 diabetes in North Indian population.[30] However, in this study, we failed to observe association for *FTO* in the same study population. Phenotypic differences between Indians and Europeans might be one of the possible reasons for observed difference in association in two populations. Indians have higher body fat and lower muscle mass for a given BMI compared with Europeans, have different fat distribution (high truncal, subcutaneous and intra-abdominal fat) and are centrally obese.[31] Studies in different ethnic groups of Indian population have provided contradictory reports for association of *FTO* variants with type 2 diabetes and obesity. A study that recruited subjects from Western and Southern India (Pune–Mysore study),[17] representing Indo-European and Dravidian population, found rs9939609 to be associated with type 2 diabetes independent of BMI (MAF in controls 0.30). Similar results were obtained in another study of Indo-European population of North Indian Sikhs[18] (MAF in controls 0.31). However, these studies failed to replicate association of rs9939609 with obesity in the control subjects. A recent study in Dravidian population from India[19] demonstrated *FTO* variant association with type 2 diabetes independent of BMI and also with obesity in the control subjects (MAF in controls 0.11). However, they did not study rs9939609; instead, they studied rs8050136 (a proxy SNP for rs9939609). We investigated both these variants (rs9939609 and rs8050136) in this study and observed them to be in strong linkage disequilibrium ($r^2 > 0.99$, $D' > 0.99$) with each other (MAF in controls 0.34). By far this is the largest study on any Indian population, investigating the role of *FTO* variants with obesity and type 2 diabetes. Despite being sufficiently powered, our study did not detect association of common variants of *FTO* with type 2 diabetes (dependent or independent of BMI). We also meta-analyzed the data of all the four studies on the Indian population (including this study) and found variants of *FTO* to be associated with type 2 diabetes, but with a lower effect size (Figure 1). This may be indicative of a lower effect size of *FTO* variants in this study population.

The contradictory findings of the studies in diverse ethnic groups from different geographical regions of India might indicate ethnic-specific effect of *FTO* variants in Indians. However, study design, study population and sample size can significantly affect the outcome of association studies. There was considerable heterogeneity in subject recruitment criteria, statistical analyses, sample sizes and most importantly sample collection areas among these studies on Indian population. The study in North Indian Sikhs involved participation of

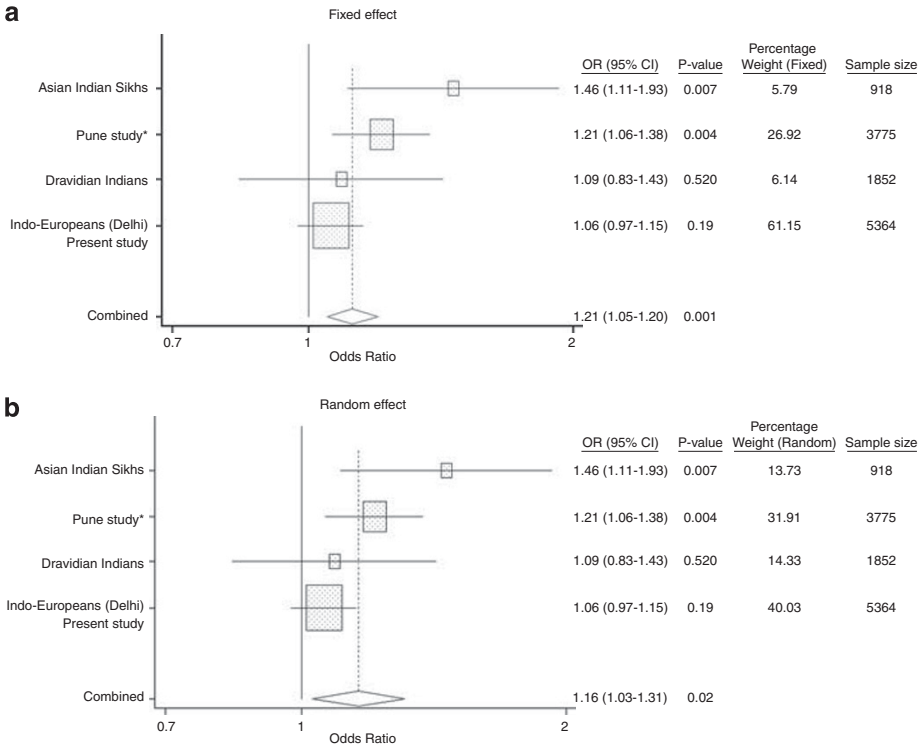

Figure 1 Forest plot of meta-analysis (fixed and random effects) for association of rs9939609 with type 2 diabetes in Indians. (**a**) Fixed effect and (**b**) random effect. *Pune study[17] involved participation of both Indo-European (2814) and Dravidian Indians (961). In all studies, analysis was adjusted for sex, age and body mass index (BMI), except for Indo-Europeans (Pune), where analysis was adjusted only for BMI. For Dravidian Indians,[19] rs8050136 was considered as proxy single-nucleotide polymorphism (SNP) for rs9939609 for this analysis as this SNP was not studied in Dravidian Indians. Test for heterogeneity: Q=6.571 on 3 d.f. (P=0.09). Moment-based estimate between studies variance=0.008.

only 920 subjects. While the Pune study involved participation of 3775 subjects (inclusive of individuals of two different ethnicities: Indo-European and Dravidians) and for reasons unknown analysis was not adjusted for sex and age. Diet and physical activity are shown to have considerable effect in modulating the susceptibility to obesity by *FTO* variants.[32,33] There is tremendous diversity in diet and physical activity not only among Europeans and Indians, but also within different ethnic groups of Indian population. Hence, differences in genetic susceptibility of obesity and type 2 diabetes observed in different population might be due to modulation by population-specific environmental factors and lifestyle. However, absence of data on diet and physical activity in this study population does not allow affirmative conclusion in this regard.

Although we observed differences in various anthropometric and biochemical measures of subjects in stage 1 and stage 2, there is no genetic heterogeneity between the subjects of the two stages (unpublished data). We performed a multidimensional scaling (MDS) analysis based on 608 unlinked markers genotyped in the initial study population of the present study that clearly demonstrates that our study population belongs to one cluster (Supplementary Figure 3). In stage 1, all type 2 diabetes patients were recruited from the Clinic, and thus having longer duration of diabetes, under hypoglycemic and lipid-lowering medication that can influence quantitative measurements. Although stage 2 comprised of a combination of patients from the Clinic and 'Diabetes Awareness Camps' that included a proportion of patients who were newly detected with type 2 diabetes and without any kind of medication, this might result in the observed differences in glycemic measures, lipid profile and creatinine levels of patients in two stages. 'Diabetes Awareness Camps' in stage 1 were held in areas of low socio-economic localities, whereas stage 2 predominantly comprised of subjects recruited from camps organized in more affluent localities. We believe that difference in socio-economic localities of the subjects could explain the differences in the anthropometric and biochemical measures.

In conclusion, common variants of *FTO* were associated with obesity, but not with type 2 diabetes in our study population. However, it is also possible that the studied variants had small effects on type 2 diabetes, and that variants of *FTO* other than the ones studied are involved in conferring genetic susceptibility of type 2 diabetes and modulations of their effect through environment (diet and physical activity). Hence, further large-scale studies on Indians of different ethnicities with data for diet and physical activity and dense SNP coverage (end-to-end tag SNPs) spanning the entire gene is required to elucidate the role of *FTO* on the predisposition to type 2 diabetes in Indians.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

We thank all the participants who volunteered for the study. We also thank Mr AK Sharma for his help in sample collection. This study was supported by 'Diabetes mellitus-New drug discovery R&D, molecular mechanisms and genetic and epidemiological factors' (NWP0032-11) funded by Council of Scientific and Industrial Research (CSIR), Government of India. We thank Dr Mitali Mukerji (IGIB) and Dr Anurag Agrawal (IGIB) for critical evaluation of the manuscript.

1 Wild, S., Roglic, G., Green, A., Sicree, R. & King, H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. *Diabet. Care* **27,** 1047–1053 (2004).
2 Misra, A. & Khurana, L. Obesity and the metabolic syndrome in developing countries. *J. Clin. Endocrinol. Metab.* **93,** S9–S30 (2008).
3 Frayling, T. M., Timpson, N. J., Weedon, M. N., Zeggini, E., Freathy, R. M., Lindgren, C. M. *et al.* A common variant in the *FTO* gene is associated with body mass index and predisposes to childhood and adult obesity. *Science* **316,** 889–894 (2007).
4 Scott, L. J., Mohlke, K. L., Bonnycastle, L. L., Willer, C. J., Li, Y., Duren, W. L. *et al.* Genome-wide association study of type 2 diabetes in Finns detects multiple susceptibility variants. *Science* **316,** 1341–1345 (2007).
5 Loos, R. J. & Bouchard, C. *FTO*: the first gene contributing to common forms of human obesity. *Obes. Rev.* **9,** 246–250 (2008).
6 Scuteri, A., Sanna, S., Chen, W. M., Uda, M., Albai, G., Strait, J. *et al.* Genome-wide association scan shows genetic variants in the FTO gene are associated with obesity-related traits. *PLoS Genet.* **3,** e115 (2007).
7 Legry, V., Cottel, D., Ferrières, J., Arveiler, D., Andrieux, N., Bingham, A. *et al.* Effect of an *FTO* polymorphism on fat mass, obesity, and type 2 diabetes mellitus in the French MONICA Study. *Metabolism* **58,** 971–975 (2009).
8 Adeyemo, A., Chen, G., Zhou, J., Shriner, D., Doumatey, A., Huang, H. *et al.* FTO genetic variation and association with obesity in West Africans and African Americans. *Diabetes* **59,** 1549–1554 (2010).
9 Tönjes, A., Zeggini, E., Kovacs, P., Böttcher, Y., Schleinitz, D., Dietrich, K. *et al.* Association of FTO variants with BMI and fat mass in the self-contained population of Sorbs in Germany. *Eur. J. Hum. Genet.* **18,** 104–110 (2010).
10 Zhang, G., Karns, R., Narancic, N. S., Sun, G., Cheng, H., Missoni, S. *et al.* Common SNPs in FTO gene are associated with obesity related anthropometric traits in an island population from the eastern Adriatic coast of Croatia. *PLoS One* **28,** e10375 (2010).
11 Hotta, K., Nakata, Y., Matsuo, T., Kamohara, S., Kotani, K., Komatsu, R. *et al.* Variations in the *FTO* gene are associated with severe obesity in the Japanese. *J. Hum. Genet.* **53,** 546–553 (2008).
12 Horikoshi, M., Hara, K., Ito, C., Shojima, N., Nagai, R., Ueki, K. *et al.* Variations in the *HHEX* gene are associated with increased risk of type 2 diabetes in the Japanese population. *Diabetologia* **50,** 2461–2466 (2007).
13 Omori, S., Tanaka, Y., Takahashi, A., Hirose, H., Kashiwagi, A., Kaku, K. *et al.* Association of *CDKAL1*, *IGF2BP2*, *CDKN2A/B*, *HHEX*, *SLC30A8*, and *KCNJ11* with susceptibility to type 2 diabetes in a Japanese population. *Diabetes* **57,** 791–795 (2008).
14 Chang, Y. C., Liu, P. H., Lee, W. J., Chang, T. J., Jiang, Y. D., Li, H. Y. *et al.* Common variation in the fat mass and obesity-associated (*FTO*) gene confers risk of obesity and modulates BMI in the Chinese population. *Diabetes* **57,** 2245–2252 (2008).
15 Li, H., Wu, Y., Loos, R. J., Hu, F. B., Liu, Y., Wang, J. *et al.* Variants in the fat mass- and obesity-associated (*FTO*) gene are not associated with obesity in a Chinese Han population. *Diabetes* **57,** 264–268 (2008).
16 Liu, Y., Liu, Z., Song, Y., Zhou, D., Zhang, D., Zhao, T. *et al.* Meta-analysis added power to identify variants in FTO associated with type 2 diabetes and obesity in the Asian population. *Obesity* **18,** 1619–1624 (2010).
17 Yajnik, C. S., Janipalli, C. S., Bhaskar, S., Kulkarni, S. R., Freathy, R. M., Prakash, S. *et al.* FTO gene variants are strongly associated with type 2 diabetes in South Asian Indians. *Diabetologia* **52,** 247–252 (2009).
18 Sanghera, D. K., Ortega, L., Han, S., Singh, J., Ralhan, S. K., Wander, G. S. *et al.* Impact of nine common type 2 diabetes risk polymorphisms in Asian Indian Sikhs: PPARG2 (Pro12Ala), IGF2BP2, TCF7L2 and FTO variants confer a significant risk. *BMC Med. Genet.* **9,** 59 (2008).
19 Ramya, K., Radha, V., Ghosh, S., Majumder, P. P. & Mohan, V. Genetic variations in the FTO gene are associated with type 2 diabetes and obesity in south Indians (CURES-79). *Diabetes Technol. Ther.* **13,** 33–42 (2011).
20 Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Report of the expert committee on the diagnosis and classification of diabetes mellitus. *Diabetes Care* **26,** S5–S20 (2003).
21 Tabassum, R., Chavali, S., Dwivedi, O. P., Tandon, N. & Bharadwaj, D. Genetic variants of FOXA2: risk of type 2 diabetes and effect on metabolic traits in North Indians. *J. Hum. Genet.* **53,** 957–965 (2008).
22 Mahajan, A., Tabassum, R., Chavali, S., Dwivedi, O. P., Bharadwaj, M., Tandon, N. *et al.* High-sensitivity C-reactive protein levels and type 2 diabetes in urban North Indians. *J. Clin. Endocrinol. Metab.* **94,** 2123–2127 (2009).
23 Matthews, R. D., Hosker, J. P., Rudenski, A. S., Naylor, B. A., Treacher, D. F. & Turner, R. C. Homeostasis model assessment: insulin resistance and β cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* **28,** 412–419 (1985).
24 Mahajan, A., Tabassum, R., Chavali, S., Dwivedi, O. P., Chauhan, G., Tandon, N. *et al.* Obesity-dependent association of *TNF-LTA* locus with type 2 diabetes in North Indians. *J. Mol. Med.* **88,** 515–522 (2010).
25 Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M. A., Bender, D. *et al.* PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am. J. Hum. Genet.* **81,** 559–575 (2007).
26 Barrett, J. C., Fry, B., Maller, J. & Daly, M. J. Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics* **21,** 263–265 (2005).
27 WHO Expert Consultation. Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies. *Lancet* **363,** 157–163 (2004).
28 Misra, A., Wasir, J. S. & Pandey, R. M. An evaluation of candidate definitions of the metabolic syndrome in adult Asian Indians. *Diabet. Care* **28,** 398–403 (2005).
29 Willett, W. C., Dietz, W. H. & Colditz, G. A. Guidelines for healthy weight. *N. Engl. J. Med.* **341,** 427–434 (1999).
30 Chauhan, G., Spurgeon, C. J., Tabassum, R., Bhaskar, S., Kulkarni, S. R., Mahajan, A. *et al.* Impact of common variants of PPARG, KCNJ11, TCF7L2, SLC30A8, HHEX, CDKN2A, IGF2BP2 and CDKAL1 on the risk of type 2 diabetes in 5164 Indians. *Diabetes* **59,** 2068–2074 (2010).
31 Misra, A. & Khurana, L. Obesity-related non-communicable diseases: South Asians vs White Caucasians. *Int. J. Obes.* **35,** 167–187 (2011).
32 Sonestedt, E., Roos, C., Gullberg, B., Ericson, U., Wirfält, E. & Orho-Melander, M. Fat and carbohydrate intake modify the association between genetic variation in the FTO genotype and obesity. *Am. J. Clin. Nutr.* **90,** 1418–1425 (2009).
33 Vimaleswaran, K. S., Li, S., Zhao, J. H., Luan, J., Bingham, S. A., Khaw, K. T. *et al.* Physical activity attenuates the body mass index–increasing influence of genetic variation in the FTO gene. *Am. J. Clin. Nutr.* **90,** 425–428 (2009).

Supplementary Information accompanies the paper on Journal of Human Genetics website (http://www.nature.com/jhg)