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Analysis of 32 common susceptibility genetic variants and their combined effect in predicting risk of Type 2 diabetes and related traits in Indians

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Abstract

Aims Recent genome-wide association studies have identified several Type 2 diabetes-related loci. We investigated the effect of susceptibility genetic variants, individually, together and in combination with conventional risk factors, on Type 2 diabetes and diabetes-related traits in Indians.

Methods We genotyped 33 variants in 1808 Indian patients and 1549 control subjects and performed association analyses with Type 2 diabetes and related traits using an additive model for individual variant and for genetic risk score based on 32 polymorphisms. The discriminatory value of genetic risk over conventional risk factors was analysed using receiver-operating characteristics curve analysis.

Results The allelic odds ratio ranged from 1.01 (95% CI 0.85–1.19) to 1.66 (95% CI 1.32–2.01) for single-variant analyses. Although, only 16 variants had significant odds ratios, the direction of association for others was similar to earlier reports. The odds ratio for Type 2 diabetes at each genetic risk score point was 1.11 (95% CI 1.09–1.14; $P = 5.6 \times 10^{-17}$) and individuals with extremes of genetic risk score (≥ 29.0 and ≤ 17.0) had a 7.5-fold difference in risk of Type 2 diabetes. The discrimination rate between control subjects and patients improved marginally on addition of genetic risk score to conventional risk factors (area under curve = 0.959 and 0.963, respectively; $P = 0.001$). Of all the quantitative traits analysed, *MC4R* variants showed strong association with BMI ($P = 4.1 \times 10^{-4}$), fat mass per cent ($P = 2.4 \times 10^{-4}$) and other obesity-related traits, including waist circumference and hip circumference ($P = 2.0 \times 10^{-3}$ for both), as well as insulin resistance ($P = 0.02$).

Conclusions We replicated the association of well-established common variants with Type 2 diabetes in Indians and observed a similar association as reported in Western populations. Combined analysis of 32 variants aids identification of subgroups at increased risk of Type 2 diabetes, but adds only a minor advantage over conventional risk factors.

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Keywords case–control, genetic risk score, genetic variants, predictive risk, Type 2 diabetes

Introduction

Type 2 diabetes is one of the leading health problems worldwide and its rapidly increasing prevalence is largely attributable to environmental factors acting on genetically susceptible individuals [1]. Several genome-wide association studies in the last 2 years have identified novel genetic risk variants, as well as confirmed the role of variants in various candidate genes [2–5]. However, most of these loci are identified in Europeans and hence are not necessarily generalizable to individuals of other ethnicities [6]. In addition, each of the risk variants is common in the general population (minor allele frequency > 5%), but has individually low penetrance [2–5]. Little is known about the exact mechanism through which these risk variants increase diabetes risk, although diverse pathways involving β-cell function, obesity and insulin sensitivity are likely to be implicated [7,8]. Indians are known to be centrally obese [9]

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and to develop Type 2 diabetes at a lower BMI and at least a decade earlier than Europeans [10]. Earlier onset of the disease means a longer burden of various complications and associated morbidity. In the face of the growing epidemic of Type 2 diabetes in Indians, the ability to predict and apply genotype-based early and individualized prevention or treatment strategies assumes importance. We have previously replicated the association of several Type 2 diabetes risk loci and showed the utility of combining eight of them in predicting risk of Type 2 diabetes in Indians. [11–13]. Here, we present an updated comprehensive case–control study of 3357 unrelated Indians, seeking to confirm association of 32 validated diabetes-susceptibility variants, to investigate their influence on diabetes-related intermediate traits and to examine their joint effect on risk of Type 2 diabetes. We also investigated the impact of conventional risk factors such as age, sex, BMI and waist–hip ratio on the genetic effects and their discriminatory ability in the presence of these conventional risk factors in Indians.

Patients and methods

Study participants

The study involved participation of 1808 patients with Type 2 diabetes of Indo-European ethnicity and 1549 control subjects from same ethnicity, recruited based on inclusion and exclusion criteria as described earlier [12,13]. The patients were diagnosed as having Type 2 diabetes according to the World Health Organization criteria [14] and the control group consisted of individuals recruited in different population cohorts. These included parents of the children in the village-based Pune Maternal Nutrition Study, which investigated the relationship between maternal nutrition, fetal growth and future risk of Type 2 diabetes [15], parents of children in the city-based Pune Children Study, which is a study of the relationship between a child's birth weight and his or her risk for Type 2 diabetes [16], and the Coronary Risk of Insulin Sensitivity in Indian Subjects study, which is a rural–urban comparison of adiposity and risk factors for Type 2 diabetes and coronary artery disease [17]. All participants gave informed consent and ethics committees of the participating institutions approved the study, in accordance with the principles of the Helsinki Declaration.

Clinical, anthropometric and biochemical variables

All subjects were extensively characterized for different anthropometric and quantitative metabolic traits. Anthropometric variables were measured as per standardized protocols, and BMI and waist–hip ratio were calculated. Biochemical measurements, including levels of fasting plasma glucose, 2-h postprandial glucose, fasting plasma insulin, 2-h postprandial insulin, total cholesterol, HDL cholesterol and triglycerides, were performed using standard laboratory assays as described earlier [11–13]. Homeostasis model assessment of insulin resistance (HOMA-IR) and homeostasis model assessment of β-cell function (HOMA-B) values were computed using an online calculator (http://www.dtu.ox.ac.uk/homacalculator/index.php).

Selection of common diabetes-related variants and genotyping

We only selected variants that have been convincingly shown to associate with Type 2 diabetes and consistently replicated across various cohorts [18–20]. DNA samples were genotyped for 33 single nucleotide polymorphisms from 32 genes in a common multiplex pool using Sequenom-based MassARRAY technology [11–13]. The genotyping success rate was > 95% and duplicate samples (n = 384; ~10%) were genotyped with > 99% concordance, indicating high genotyping accuracy. The number of subjects available for analysis was marginally variable across the single nucleotide polymorphisms.

Computation of genetic risk score

As the effect size of each polymorphism was variable in the study, we constructed the weighted genetic risk score for each individual. Participants for whom data on seven or more genotypes were missing (n = 6) were excluded from the analyses. Genetic risk score was obtained by multiplying the allele dosage score (based on the number of risk alleles) with the number of single nucleotide polymorphisms and then summing the products.

Statistical analysis

The genotypes for all the single nucleotide polymorphisms were analysed for deviation from the Hardy–Weinberg equilibrium using χ^2 analysis. Chi-square tests and t-tests were used for comparing the proportions and means between patients and control subjects. We used logistic regression to determine the effect of each variant on risk of Type 2 diabetes after adjusting for sex, age, BMI and location, as appropriate. Odds ratios, 95% confidence intervals and P-values were calculated using an additive genetic model with reference to the risk allele as defined in earlier studies. We did not adjust for multiple comparison tests as the variants were chosen based on strong prior hypothesis; rather, we performed permutation analysis using 10^4 permutations. Power calculations were performed with the previously reported odds ratios using Quanto 1.2.3 (http://hydra.usc.edu/gxe). We used the Kruskal–Wallis test to analyse the association between genotypes at individual single nucleotide polymorphisms and other quantitative traits in the control subjects only. The genetic risk score was modelled as a continuous variable or categorized into quintiles and used for analysis. We plotted receiver-operating characteristic (ROC) curves and calculated corresponding areas under the curves (AUCs) for a logistic regression model including conventional risk factors and another including genetic risk score plus the conventional risk factors. The discriminatory power for Type 2

diabetes risk was assessed by comparing the area under the curve in both the models, using Analyze-it version 2.22 (http://www.analyze-it.com), which is based on the method of Hanley and McNeil for receiver-operating characteristic curve analyses [21]. All statistical analyses were performed using PLINK version 1.05 (http://pngu.mgh.harvard.edu/_purcell/plink) and SPSS version 17.0 (SPSS Inc., Chicago, IL, USA). The study sample had power varying from 27.8% (*MC4R*, rs12970134) to 99.9% (*TCF7L2*, rs7903146) to detect the association with Type 2 diabetes and both single nucleotide polymorphisms showed significant association.

Results

Patients with Type 2 diabetes had significantly higher BMI, waist–hip ratio and lipid levels compared with the control subjects (see also Supporting Information, Table S1). Nearly three quarters of the patients (n = 1339; 74.3%) had a history of diabetes in the family. Although the control subjects were younger than the patients, they were recruited from prospective cohorts that had been followed over the previous several years [11–13]. Only ~4% of these individuals developed diabetes in last 6 years and these individuals were excluded from the study.

Association analysis of common genetic variants with Type 2 diabetes

There were no significant departures from the Hardy–Weinberg equilibrium for all single nucleotide polymorphisms in the control subjects [d.f. = 2; $P > 0.0007$ (Bonferroni corrected P-value for 32 single nucleotide polymorphisms in patients with Type 2 diabetes and control subjects)]. In the Supporting Information (Table S2), for each single nucleotide polymorphism, we present the allele and genotype distributions, risk allele-specific odds ratios and P-values under an additive genetic model after correction for age, gender and BMI, as appropriate. Association analysis using other genetic models validated the observations made using the additive model. The minor allele frequencies of all single nucleotide polymorphisms are comparable with those in Gujarati Indians in Houston, but different at some variants from a Chinese Han population, Japanese and Europeans in HapMap [(http://www.hapmap.org/9); (see also Supporting Information, Table S3)].

In addition to confirming the earlier reported association of variants in *TCF7L2, FTO, PPARγ, HHEX/IDE, CDKN2A/B, KCNJ11, IGF2BP2, CDKAl1* and *SLC30A8* [11–13], we observed that the single nucleotide polymorphisms in *CDC123/CAMK1D, GCKR, LOC646279, MC4R, TCF2, THADA* and *WFS1* were significantly associated with the risk of Type 2 diabetes (see also Supporting Information, Table 2). As for the remaining loci, no association was observed, although the directions were consistent with previous reports [22–24]. The odds ratios for each individual variant ranged from 1.01 (95% CI 0.85–1.19; *BCL11A*; i.e. no association) to 1.66 (95% CI 1.32–2.01; *KCNJ11*; i.e. strong association) (see also Supporting Information, Table S2).

Genotype–phenotype correlation with Type 2 diabetes-related traits

We investigated the genotype–phenotype correlation for these variants in the control individuals, because treatment might distort the relationship in the patients (see also Supporting Information, Tables S4 and S5). The two single nucleotide polymorphisms near *MC4R* (rs12970134, rs17782313) showed significant association with BMI ($P = 4.1 \times 10^{-4}$ and 2.1×10^{-4}, respectively), fat mass per cent ($P = 2.4 \times 10^{-4}$ and 5.0×10^{-5}, respectively) and triglycerides ($P = 0.03$) (Table 1). We also observed significant association with waist circumference ($P = 0.002$), hip circumference ($P = 0.002$) and total weight ($P = 0.001$). The other variant, rs17782313, showed nominal association with fasting plasma insulin and HOMA-IR levels ($P = 0.02$), suggesting a link between regional fat deposition, central obesity and insulin resistance (see also Supporting Information, Table S5). The protective allele at *SGK1* variant, rs9402571, predicted higher HDL cholesterol levels ($P = 4.7 \times 10^{-3}$) and showed marginal association with fat mass per cent ($P = 0.04$) (see also Supporting Information, Tables S4 and S5). The risk allele at the *DCD* and *TCF2*

Table 1 Association of variants near *MC4R* with obesity-related traits in control subjects

	rs12970134		rs17782313	
Variants	Effect size, β (95% CI)*	*P*-value	Effect size, β (95% CI)*	*P*-value
Weight (kg)	1.18 (0.49–1.87)	1.0×10^{-3}	1.18 (0.50–1.87)	1.0×10^{-3}
Height (cm)	0.17 (−0.28 to 0.61)	0.461	0.09 (−0.35 to 0.53)	0.689
Body mass index (kg/m^2)	0.43 (0.19–0.66)	4.1×10^{-4}	0.45 (0.21–0.68)	2.1×10^{-4}
Waist circumference (cm)	1.06 (0.38–1.74)	2.0×10^{-3}	1.19 (0.52–1.87)	1.0×10^{-3}
Hip circumference (cm)	0.86 (0.31–1.41)	2.0×10^{-3}	0.89 (0.34–1.44)	1.0×10^{-3}
Waist–hip ratio	0.003 (−0.002 to 0.009)	0.225	0.004 (0.000–0.010)	0.103
Fat mass % by DEXA	1.28 (0.60–1.97)	2.4×10^{-4}	1.41 (0.73–2.09)	5.0×10^{-5}

* Effect sizes are shown as unit change per copy of high-risk allele as calculated by linear regression analysis assuming an additive genetic model with adjustment for age, gender and location.
DEXA, dual-emission X-ray absorptiometry.

Table 2 Association of genetic risk score with risk of Type 2 diabetes

	Continuous genetic risk score		Quintile genetic risk score				
	Odds ratio (95% CI)	*P*-value	Q1	Q2	Q3	Q4	Q5
n			670	670	671	670	670
Genetic risk score, median (range)			15.5 (7.3–20.2)	21.9 (20.3–22.9)	24.1 (23.0–25.0)	26.2 (25.1–27.4)	29.2 (27.5–39.2)
Risk of Type 2 diabetes*	1.11 (1.09–1.14)	5.6×10^{-17}	1.00	1.65 (1.16–2.33)	2.55 (1.77–3.67)	3.20 (2.23–4.60)	4.99 (3.50–7.12)

*Adjusted for age, sex, BMI and location

polymorphisms showed nominal association with HOMA-B ($P = 0.04$). No other locus showed any significant association with any other diabetes-related quantitative traits. Correction for multiple testing nullified all the associations, except those of *MC4R* and *SGK1* variants.

Combined analysis of 32 variants with risk of Type 2 diabetes and related traits

The combined effect of the 32 variants was estimated by calculating the percentage of normal individuals and patients with Type 2 diabetes stratified according to genetic risk score. The genetic risk score followed a normal distribution in both patients with Type 2 diabetes and control subjects. On average, patients with Type 2 diabetes had a higher genetic risk score, indicating more risk alleles and thus shifting the curve to the right compared with that of normal individuals (see also Supporting Information, Fig. S1). There is an increase in odds ratios for Type 2 diabetes with the increasing genetic risk score against the baseline group of individuals with a genetic risk score of ≤ 17.0 (Fig. 1). For each unit increase in genetic risk score, risk of Type 2 diabetes increased by 1.11-fold ($P = 5.6 \times 10^{-17}$) (Table 2). Those with a genetic risk score ≥ 29.0 had a 7.5-fold higher risk of having Type 2 diabetes compared with the baseline reference group with a genetic risk score ≤ 17.0 (OR = 7.52; 95% CI 4.51–12.54, $P = 1.1 \times 10^{-14}$) (Fig. 1). On similar lines, compared with the persons in the lowest quintile of genetic risk score [15.5 (range 7.3–20.2)], subjects in the highest quintile [29.2 (range 27.5–39.2)] had an odds ratio of 4.99 (95% CI 3.50–7.12) (Table 2 and Supporting Information, Fig. S2). Thus, the risk of Type 2 diabetes significantly increased with increasing quintiles of genetic risk score.

Discriminatory power analysis of genetic variants and conventional risk factors by receiver-operating characteristic curve

Receiver-operating characteristic curves analysis showed that genetic risk score alone had a relatively low discriminatory ability for risk of Type 2 diabetes (area under the curve = 0.634; 95% CI 0.615–0.653) (Fig. 2). Removal of *FTO* and/or *MC4R* variants from analysis did not significantly influence the results (area under the curve = 0.633; $P > 0.05$). The area under the curve for the conventional risk factors, including age, sex, BMI and waist–hip ratio, was 0.959 (95% CI 0.953–0.966), which marginally increased to 0.963 (95% CI 0.957–0.969) with the addition of the genetic risk score ($P = 0.001$) (Fig. 2). Thus, the genetic risk score adds only marginally to the discriminatory power of the conventional risk variables in Indians, as has been observed in many of the populations [18–20].

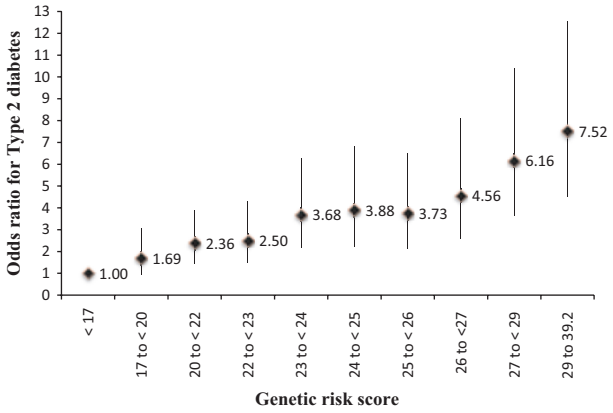

FIGURE 1 Relationship between genetic risk score and Type 2 diabetes mellitus. The plot shows increasing odds ratios with increasing genetic risk score vs. the baseline genetic risk score of ≤ 17. Odds ratios are calculated relative to the baseline genetic risk score of ≤ 17 and are represented as diamonds (◆); 95% confidence intervals are represented as vertical lines.

Discussion

Our study supports a role for many common variants identified from genome-wide association studies in the aetiology of Type 2 diabetes in Indians and demonstrates that a genetic risk score based on 32 genetic variants could help in identifying individuals with a substantially increased risk of Type 2 diabetes.

Out of 23 new Type 2 diabetes susceptibility loci that we investigated, significant evidence of association was observed for several variants [11–13]. The observations are consistent with recent cross-sectional studies assessing individual impact of

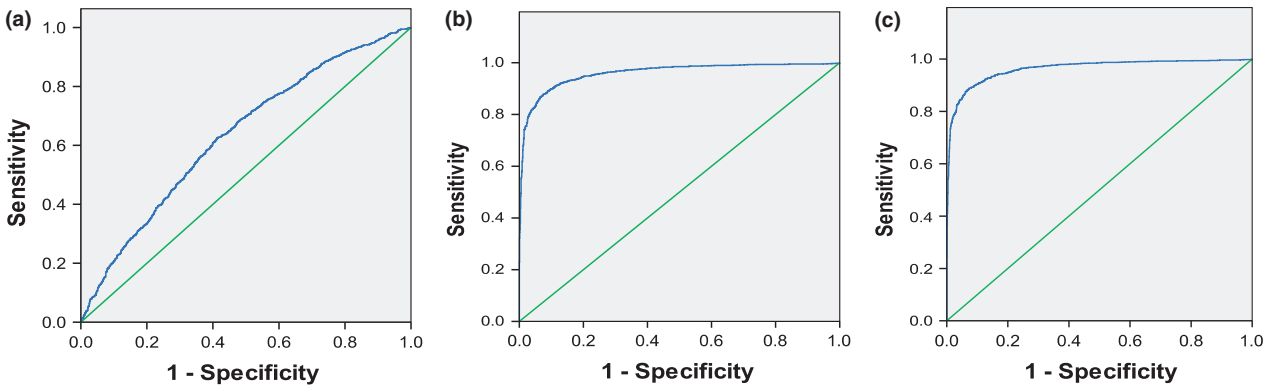

FIGURE 2 Receiver operating characteristic curves for discrimination between patients with Type 2 diabetes and control subjects. Graphs show the plots based on (a) genetic risk score (area under the curve = 0.634), (b) conventional risk factors, including age, sex, BMI, waist–hip ratio (area under the curve = 0.959), and (c) genetic risk score and conventional risk factors combined (area under the curve = 0.963).

several risk alleles of Type 2 diabetes in Europeans [22–24]. However, in a recent study of six genome-wide association studies, identified variants in Asian Sikhs failed to replicate their association with Type 2 diabetes, except the likely influence of *CDC123* on β-cell function [25]. We observed a significant association of *CDC123* with Type 2 diabetes, but not with any quantitative phenotype measuring insulin resistance or secretion. We also found a significant association of the single nucleotide polymorphisms near the *MC4R* gene with increased diabetes risk.

Consistent with earlier studies, *MC4R* variants were strongly associated with BMI, fat mass per cent and other obesity-related quantitative traits [23,24,26]. Individuals homozygous for the risk allele for rs12970134 had a 2.0-cm greater waist circumference and 2.3-cm higher hip circumference compared with the wild type. We could not confirm the previously reported association of rs12970314 with insulin resistance [26], but another *MC4R* variant, rs17782313, predicted ∼25% higher HOMA-IR between individuals with and without the risk allele. This variant has shown strong association with obesity and fat mass per cent in a recent meta-analysis of multiple genome-wide association studies in white Europeans [24]. However, both variants are in strong linkage disequilibrium (r^2 = 0.895 in the Gujarati Indians in Houston population in HapMap; http://www.hapmap.org/9), hence, the association of *MC4R* variants with obesity transgresses ethnic boundaries. Despite having a similar frequency to the risk allele, the strength of association was less strong than reported for Asians in the London Life Sciences Prospective Population (LOLIPOP) study [23]. This may be attributable to the smaller sample size, but could be because of the different genetic structure of the study population. Individuals in our study represent an ethnically homogeneous cluster, while the LOLIPOP cohort is a heterogeneous collection of Asian subjects from different geographical regions who migrated to the UK at different time points. The melanocortin-4 receptor is expressed in the brain and is part of the pathway controlling food intake and energy homeostasis [23,24] and thus may play a more important role in the regulation of body weight and regional fat deposition. We have previously demonstrated that the increased risk of developing Type 2 diabetes in Indian individuals with *FTO* variants was not entirely mediated through its effect on BMI or central obesity and we speculated on the underlying differences in the possible mechanism of association of Type 2 diabetes and related intermediate traits from those in Europeans [12]. Thus, in contrast to *FTO*, *MC4R* seems to play an equally important role in predicting obesity both in Indians and in Europeans.

Despite their association with Type 2 diabetes, each locus had a modest risk, thus limiting their clinical utility when considered in isolation. The utility of the genetic risk score as a proxy of an individual's genetic predisposition to Type 2 diabetes is well established [18–20,27,28]. We found that each additional risk allele increased the risk of Type 2 diabetes by approximately 11%. Thus, by comparing individuals with the lowest genetic risk score with those carrying the most risk alleles, we could identify the ∼11% of the population with a genetic risk score ≥ 29 that had a 7.5 times greater risk of diabetes compared with the ∼12% with a genetic risk score ≤ 17. This risk was similar in both obese and non-obese subjects, which is an easily measurable major risk factor for Type 2 diabetes. This assumes importance as the prevalence of obesity and Type 2 diabetes in India has escalated significantly over the last few years. Thus, obese people carrying a large number of risk alleles may specifically be targeted for potential intervention strategies, including lifestyle.

As observed earlier, information about genetic risk had a minor additional effect on case–control discrimination of Type 2 diabetes risk by conventional risk factors in this study [18–20,27,28]. This might be partly explained by the fact that several genetic variants may predict susceptibility to Type 2 diabetes through conventional risk factors such as BMI, waist–hip ratio, etc. [23,24]. Close to three quarters of the patients in this study had a positive family history and already contained some genetic information contributed by genetic risk score. We cannot rule out overestimation of the discriminatory value of the receiver-operating curve analysis, as case–control design for association studies necessitates including an equal number of patients and control subjects, while the population prevalence of

Type 2 diabetes is only 15–20% in India. Thus, despite great progress in identifying Type 2 diabetes susceptibility loci, their discriminatory value is still too limited to be of clinical utility.

Strengths and limitations of the study

Ours is the largest study in terms of the number of common susceptibility variants analysed, investigating their joint effects and the number of well-phenotyped patients and control subjects from India. One of the main limitations of this study has been the limited power to detect the association of several variants and the caveat that the majority of them were not causal in nature. This means that the predictive power of these susceptibility loci is likely to be an underestimate. We cannot rule out the possibility that some of the control participants may have undiagnosed Type 2 diabetes or may develop the disease in later life, but this would result in false negatives and not false positives. It may be worth mentioning that the control subjects are recruited in prospective cohorts and the conversion rate of these subjects to overt Type 2 diabetes on follow-up over several years has been extremely low (4%; C. S. Yajnik, unpubl. data) [15–17]. In addition, population stratification may also influence the observed association [29], but the possibility is low because both patients and the control subjects belong to well-characterized cohorts within a defined geographical region and the analysis was restricted to only Indo-European ethnicity. However, India is a huge country and replication of our association results in other ethnic groups, such as Dravidians, is warranted.

To conclude, we could replicate the association of many of the risk variants identified in Europeans and observed the association to be on similar lines as reported earlier in Western populations. The failure of association of individual variants could be attributable to the limited statistical power of the study or because of the different ethnic origins or the different social and environmental circumstances. However, we observed a strong association of the *MC4R* variants on obesity and related quantitative traits, stressing its global role in predicting obesity and future risk of Type 2 diabetes. Using the combined effects of the currently known susceptibility variants allows us to identify subgroups of the population at increased risk of developing Type 2 diabetes, but, on a population level, they may be of limited use in discriminating between individuals who will and will not develop Type 2 diabetes.

Competing interests

Nothing to declare.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Distribution of genetic risk scores in patients with Type 2 diabetes and control subjects.

Figure S2. Relationship between quintiles of genetic risk score and Type 2 diabetes risk.

Table S1. Basic anthropometric and clinical characteristics of patients with Type 2 diabetes and control subjects.

Table S2. Association analysis of 32 common variants with Type 2 diabetes.

Table S3. Comparison of minor allele frequency at Type 2 diabetes-associated variants between HapMap data and our study.

Table S4. Association analysis of 23 common susceptibility genetic variants with anthropometric and various lipid variables.

Table S5. Association analysis of 23 common susceptibility genetic variants with diabetes-related intermediate traits.

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