Association of TCF7L2 and ADIPOQ with Body Mass Index, Waist–Hip Ratio, and Systolic Blood Pressure in an Endogamous Ethnic Group of India

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Despite the increasing burden of type 2 diabetes (T2D) and its established association with anthropometric and physiological traits as a risk factor, genetic studies focusing on the association of T2D-related genes with quantitative traits like body mass index (BMI), waist–hip ratio (WHR), and systolic blood pressure (SBP) are only a few for western populations and rare for Indian populations. The present study tested the association of *TCF7L2*, *HHEX*, *KCNJ11*, and *ADIPOQ* with BMI, SBP, and WHR in men and women of the Aggarwal population of India and found a differential association of *TCF7L2* (rs7903146, rs4506565, and rs12256372) and *ADIPOQ* (rs2241766 and rs1501299) genes with increasing BMI, SBP, and WHR between the two sexes. We conclude that *TCF7L2* and *ADIPOQ* together might play an important role in explaining these traits and to understand the biological and genetic mechanisms underlying T2D, and the role of other T2D genes must also be evaluated with these continuous traits.

Introduction

OBESITY AND DIABETES in India have already achieved a *numero uno* status, and its epidemic is expected to rise further in the near future (International Diabetes Federation Report, 2009). Association of increase in various anthropometric (e.g., body mass index [BMI] and waist–hip ratio [WHR]) and physiological (e.g., systolic blood pressure [SBP]) measures with obesity and diabetes is well established (Vazquez *et al.*, 2007; Mullican *et al.*, 2009).

The advent of genome-wide association studies (GWAS) uncovered many genetic variants related to obesity and type 2 diabetes (T2D) and led to an ongoing series of efforts for their validation in different human populations (Horikoshi *et al.*, 2007). The lack of GWAS from India due to absence of large consortiums and only limited infrastructure along with small funding are forcing Indian genetic epidemiologists to produce at least validation studies of GWAS genes (e.g., Chauhan *et al.*, 2010). Despite of our knowledge that obesity is a risk factor for diabetes, very few genetic studies have tested the hypothesis that the genes related to diabetes are also associated with obesity-related continuous traits (e.g., BMI, WHR, and SBP) by significantly increasing their mean value (Helgason *et al.*, 2007; Pecioska *et al.*, 2010). Sanghera *et al.* (2008) studied the

impact of T2D genes on BMI, WHR, and lipid profile, but only found the association of *TCF7L2* with the cholesterol level in an Indian population. Therefore, in this article, we are presenting the results of testing the above hypothesis by evaluating the association of diabetes genes with anthropometric and physiological measures. We have found that T2D genes might play a role in explaining the genetics of anthropometric and physiological risk factors in India.

Materials and Methods

This article is a part of the case–control genetic association study for T2D among the Aggarwals of Delhi; the research design is given in Gupta *et al.* (2010). This endogamous population (intermarriage between clans) group was selected primarily to avoid false-positive association signals due to population stratification, secondly, due to its large population size (2–3 million in Delhi and surrounding area), and finally because this is a traders' community having a sedentary life style. Fortunately, this endogamous genetic structure of the Indian population has been comprehensively proved by the landmark study of Reich *et al.* (2009). For the purpose of analysis, we had first grouped the available case–control data (300 samples for which data were available from the parent

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study) for our target of continuous traits (BMI, WHR, and SBP), and then all data were segregated on the basis of the sex. The study was ethically cleared by the All India Institute of Medical Sciences, New Delhi.

All the genotyped 14 single-nucleotide polymorphisms (SNPs; *TCF7L2*—3 SNPs, *HHEX*—2 SNPs, *KCNJ11*—1 SNP, and *ADIPOQ*—8 SNPs) generated through sequencing (see Supplementary Data available online at www.liebertonline.com/gtmb) were used for the analysis. For testing the association of SNPs along with six variables (sex, age, WHR, physical activity, socioeconomic status, and SBP), the dataset was first cleaned by removing the observations with missing values >4 out of the 6 variables; therefore, several observations (111 for BMI, 94 for SBP, and 120 for WHR) were removed, and then the R-package—“Hmisc” (Harrell, 2010) was used for imputations of the missing values of variables. Multiple regression models were fitted for all the SNPs, and then based on a step-wise selection, using the Akaike’s information criterion, potential SNPs were identified separately for BMI, WHR, and SBP. Thereafter, multiple regression models were fitted for a single-SNP to detect association. All the analyses were done using R (R Development Core Team, 2008).

Results

All the 14 SNPs in four genes (*TCF7L2*, *HHEX*, *KCNJ11*, and *ADIPOQ*) were found to be in Hardy–Weinberg equilibrium (Supplementary Table S1). While testing for associations with continuous traits like BMI, SBP, and WHR, only two genes (*TCF7L2* and *ADIPOQ*) were found to be significant. The number of men and women tested for association is given in Supplementary Table S1. For BMI, we found two significant SNPs of *TCF7L2*, that is, rs4506565 (genotype-AT increasing BMI to 27.37, $p=0.009$) and rs7903146 (genotype-CT increasing BMI to 27.45, $p=0.019$) among men, and one SNP of *ADIPOQ*, that is, rs2241766 (genotype-AT increasing BMI to 34.24, $p=0.048$) among women (Table 1). Similarly, for SBP, we found same two significant SNPs of *TCF7L2*, that is, rs4506565 (genotype-TT increasing SBP to 141.35mmHg, $p=0.01$) and rs7903146 (genotype-TT increasing SBP to 141.12, $p=0.028$) among men, and one SNP of *ADIPOQ*, that is, rs2241766 (genotype-AT decreasing SBP to 124.78, $p=0.042$) among women (Table 1). For WHR, we found one SNP of *ADIPOQ*, that is, rs1501299 (genotype-GT increasing WHR to 1.026, $p=0.02$) among men, and one significant SNP of *TCF7L2*, that is, rs12256372 (genotype-GT increasing WHR to 0.921, $p=0.029$) among women (Table 1). When we treated one of the alleles having lower frequency as the risk allele, the number of categories reduced from three to two by combining it into two categories (based on the risk and nonrisk category). While testing in this two-category scenario, except for BMI in men, we were unable to detect significant association for other target quantitative variables. For BMI, we found *TCF7L2* SNP rs4506565 (AT + TT risk category) significantly increasing BMI to 27.15 among men.

Discussion

Despite of the discovery of loci such as *TCF7L2*, *HHEX*, *FTO*, and *CDKN2B*, through GWAS, where cases were not selected for BMI (Scott *et al.*, 2007), the role of T2D genes has not been much evaluated for risk factors like BMI, WHR,

TABLE 1. EFFECT OF SINGLE-NUCLEOTIDE POLYMORPHISMS OF TCF7L2 AND ADIPOQ ON ADJUSTED BODY MASS INDEX AND SYSTOLIC BLOOD PRESSURE AND WAIST–HIP RATIO

Gene	SNP	Genotype	Trait Value	Regression coefficient	p
BMI: men					
TCF7L2	rs4506565	AA	25.48		
		AT	27.37	0.0716	0.009
		TT	26.52	0.0402	0.285
TCF7L2	rs7903146	CC	25.77		
		CT	27.45	0.063	0.019
		TT	25.90	0.005	0.885
BMI: women					
ADIOPQ	rs2241766	TT	29.08		
		TG	30.02	0.031	0.452
		GG	34.24	0.163	0.048
SBP: men					
TCF7L2	rs4506565	AA	132.34		
		AT	130.62	−0.005	0.806
		TT	141.35	0.077	0.010
TCF7L2	rs7903146	CC	132.51		
		CT	130.56	−0.014	0.501
		TT	141.12	0.062	0.028
SBP: women					
ADIOPQ	rs2241766	TT	132.99		
		TG	124.78	−0.063	0.042
		GG	135.72	0.020	0.742
WHR: men					
ADIOPQ	rs1501299	GG	0.998		
		GT	1.026	0.027	0.020
		TT	1.012	0.014	0.558
WHR: women					
TCF7L2	rs12256372	GG	0.887		
		GT	0.921	0.037	0.029
		TT	0.887	0.0002	0.989

For BMI, association is adjusted for age, WHR, PAS, SES, and SBP. For SBP, association is adjusted for age, WHR, PAS, SES, and BMI. For WHR, association is adjusted for age, SBP, PAS, SES, and BMI.

SNP, single-nucleotide polymorphism; BMI, body mass index; SBP, systolic blood pressure (mmHg); WHR, waist–hip ratio; PAS, Physical Activity Score; SES, Socioeconomic Status Score.

and SBP. For instance, Pecoska *et al.* (2010) analyzed T2D-associated risk alleles (related to nine genes) with human measures of fatness like the BMI, the fat mass index, fat percentage, waist circumference, and WHR and found association only with the *FTO* gene, a well-established gene for obesity. However, not even a single study has evaluated the possibility for these associations in an Indian context. Therefore, we tried to detect associations between T2D SNPs and these diabetes-related continuous traits.

Since anthropometric traits, alone (height, weight, etc.) and in combination (like BMI and WHR), have different standards for men and women and the variation corresponding to these differences influences the risk for T2D for them, we therefore analyzed the effect of SNPs on these continuous traits separately. We found the significant association of *TCF7L2* SNPs like rs4506565 (AT) and rs7903146 (CT) among men and *ADIPOQ* SNP rs2241766 (GG) among women with an increase in the mean BMI of this population. One cross-sectional

study has shown that with increasing BMI and decreasing adiponectin levels in girls could contribute to their faster deterioration in lipid profiles in comparison with boys (Ong *et al.*, 2006). To some extent, this explains the significant association of *ADIPOQ* with BMI among women.

Despite a well-appreciated validation of *TCF7L2* SNPs in different world populations, the association of *TCF7L2* with obesity-related quantitative traits and body fat in humans is still unclear (Haupt *et al.*, 2010). For *TCF7L2*, we found a heterozygous genotype significant for BMI but not the homozygous genotype, possibly due to lack of enough power to capture its effect or maybe because *TCF7L2* has effect on increasing BMI in its heterozygous form. The role of *ADIPOQ* is known for obesity, but its differential role in men and women remains yet to be evaluated. Among women, homozygous rs2241766-GG (*ADIPOQ*) has the effect of increasing BMI, which may be due to a recessive effect on BMI.

Similarly, for SBP, we found a significant effect of *TCF7L2* SNPs (like rs4506565-TT and rs7903146-TT) for men and *ADIPOQ* SNP rs2241766-TG among women in increasing in the mean SBP. For SBP, this study has sufficient power to capture a homozygous effect of *TCF7L2* SNPs, which is likely to have a recessive effect on SBP.

Both *TCF7L2* and *ADIPOQ* are expressed in adipose tissue and involved in Wnt-dependent regulation of adipogenesis (Ross *et al.*, 2002; Sladek *et al.*, 2007; Cauchi *et al.*, 2008), which maintains pre-adipocytes in an undifferentiated state (Ross *et al.*, 2002). The few studies that analyzed the effect of *TCF7L2* SNPs on BMI (Schafer *et al.*, 2007; Grant *et al.*, 2006; Zhang *et al.*, 2006; Helgason *et al.*, 2007) hinted toward the possible association, but were not confirmed. Haupt *et al.* (2010) investigated the association of *TCF7L2* variants with body fat composition and ectopic lipid storage in a cross-sectional setting and found that the *TCF7L2* gene influenced lifestyle intervention-induced changes in BMI, total fat, and non-visceral and visceral fat, but these results must be replicated as suggested by the authors.

Interestingly, for WHR we found a significant effect of *ADIPOQ* SNP (rs1501299-GT) for men and *TCF7L2* SNP (rs12256372-GT) among women in increasing the mean WHR. Since both men and women have a different body composition, it is expected that the genes explaining the BMI and WHR might also differ, but the present study is not sufficient to support this hypothesis.

Thus, in our study also, both *TCF7L2* and *ADIPOQ* are found to be associated with BMI, SBP, and WHR and point in the same direction; this supports the growing evidence for the hypothesized role of *TCF7L2* in adipocyte differentiation (Haupt *et al.*, 2010). The detected differences in genetic association in the present study among men and women need replication with a larger sample size to confirm these findings. It is difficult to say that the nonassociation of other SNPs of *HHEX* and *KCNJ11* is because they do not have any effect on these continuous traits or due to low power.

We conclude that *TCF7L2* and *ADIPOQ* together might play an important role in regulating the human BMI, WHR, and SBP in the Aggarwal population; therefore, to confirm this, their role must be evaluated through larger genetic and epigenetic studies. In the end, we would like to emphasize that to understand the underlying, overlapping biological pathways and genetic spectrum of T2D, the role of T2D genes must be evaluated with these continuous traits.

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Author Disclosure Statement

No competing financial interests exist.

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