Common Variants of *IL6*, *LEPR*, and *PBEF1* Are Associated With Obesity in Indian Children

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The increasing prevalence of obesity in urban Indian children is indicative of an impending crisis of metabolic disorders. Although perturbations in the secretion of adipokines and inflammatory molecules in childhood obesity are well documented, the contribution of common variants of genes encoding them is not well investigated. We assessed the association of 125 common variants from 21 genes, encoding adipocytokines and inflammatory markers in 1,325 urban Indian children (862 normal weight [NW group] and 463 overweight/obese [OW/OB group]) and replicated top loci in 1,843 Indian children (1,399 NW children and 444 OW/OB children). Variants of four genes (*PBEF1* [rs3801266] [$P = 4.5 \times 10^{-4}$], *IL6* [rs2069845] [$P = 8.7 \times 10^{-4}$], *LEPR* [rs1137100] [$P = 1.8 \times 10^{-3}$], and *IL6R* [rs7514452] [$P = 2.1 \times 10^{-3}$]) were top signals in the discovery sample. Associations of rs2069845, rs1137100, and rs3801266 were replicated ($P = 7.9 \times 10^{-4}$, 8.3×10^{-3}, and 0.036, respectively) and corroborated in meta-analysis ($P = 2.3 \times 10^{-6}$, 3.9×10^{-5}, and 4.3×10^{-4}, respectively) that remained significant after multiple testing corrections. These variants also were associated with quantitative measures of adiposity (weight, BMI, and waist and hip circumferences). Allele dosage analysis of rs2069845, rs1137100, and rs3801266 revealed that children with five to six risk alleles had an approximately four times increased risk of obesity than children with less than two risk alleles ($P = 1.2 \times 10^{-7}$). In conclusion, our results demonstrate the association of the common variants of *IL6*, *LEPR*, and *PBEF1* with obesity in Indian children. ***Diabetes* 61:626–631, 2012**

Manifestation of chronic metabolic disorders, including type 2 diabetes, cardiovascular diseases, and metabolic syndrome starts in early life, marked by a rapid increase in BMI during childhood (1). The increasing prevalence of obesity in Indian children (2) is indicative of an impending crisis of metabolic disorders that already has reached epidemic proportions in India. In this scenario, there is an urgent need to identify the underlying factors involved in the predisposition of childhood obesity and linking childhood obesity and adult chronic disorders.

Childhood obesity is a state of inflammation, as evident from the dysregulated secretions of adipokines and inflammatory molecules in the obese condition (3). In addition to inflammatory properties, these adipokines and inflammatory molecules also play an important role in energy homeostasis, metabolic processes, and regulation of body fat (3). Although perturbations in the secretion of adipokines and inflammatory molecules in childhood obesity are well documented, studies investigating the contribution of variants in genes encoding them to susceptibility of childhood obesity are few. Here, we examined 125 common variants from 21 genes encoding adipokines and inflammatory markers for association with overweight/obesity, markers of adiposity, and inflammation in urban Indian children.

RESEARCH DESIGN AND METHODS

The study involved the participation of 3,168 children (aged 11–17 years), including 2,261 normal-weight (NW group) and 907 overweight/obese (OW/OB group) children, recruited from school health surveys from four different zones of Delhi, as described previously (4). Subjects were classified as normal weight and overweight/obese according to the age- and sex-specific cutoffs provided by Cole et al. (5), which gives BMI cutoffs for overweight and obesity by sex for children between the age of 2 and 18 years corresponding to the cutoff points of 25 and 30 kg/m^2 for adults. Prior permission from school authorities, informed consent from parents/guardians, and verbal assent from the participants themselves was obtained. The study protocol was approved by ethics committees of the participating institutes, and the study was conducted according to principles of the Declaration of Helsinki.

Anthropometric measurements including height, weight, waist circumference (WC), and hip circumference (HC) were taken using standard methods. BMI and waist-to-hip ratio (WHR) were calculated. Blood samples were drawn from the subjects after overnight fasting. Plasma levels of glucose and high-sensitivity C-reactive protein were measured using Cobas Integra 400 Plus (Roche Diagnostic, Mannheim, Germany). Levels of insulin and C-peptide were estimated using Elecsys 2010 (Roche Diagnostics). Plasma levels of leptin, resistin, and adiponectin were measured using commercial enzyme-linked immunosorbent assay kits (R&D Systems, Minneapolis, MN). Anthropometric and clinical characteristics of subjects are provided in Table 1.

Genotyping. In stage 1, we genotyped 1,325 children (862 NW and 463 OW/OB children) for 125 single nucleotide polymorphisms (SNPs) from 21 genes, encoding adipocytokines and inflammatory markers (Supplementary Table 1), using Illumina GoldenGate assay (Illumina, San Diego, CA). The SNPs were selected on the basis of their location in functionally important regions of genes, previous reports of association with metabolic disorders, minor allele frequency >0.05, and tag SNPs. Genotyping data were subjected to extensive quality control that included genotype confidence score >0.25, SNP call rate >0.9, GenTrans score >0.6, cluster separation score >0.4, Hardy-Weinberg equilibrium ($P > 0.01$ in NW, OW/OB, and all subjects], and minor allele frequency >0.05. Quality control passed SNPs ($n = 83$), had a call rate >98%, and a concordance rate of 99.97% based on 5% duplicate samples. Genotype frequencies for all the SNPs are provided in Supplementary Table 1.

In stage 2, genotyping for the replication of four loci (rs2069845 [*IL6*], rs1137100 [*LEPR*], rs3801266 [*PBEF1*], and rs7514452 [*IL6R*]) was performed in 1,843 children (1,399 NW and 444 OW/OB children) using iPLEX (Sequenom, San Deigo, CA). The average genotyping success rate was 93.5%, with >99.7% consistency in genotyping based on 5% duplicates.

Statistical analyses. Statistical analyses were performed using PLINK version 1.07 (http://pngu.mgh.harvard.edu/~purcell/plink) and SPSS version 17.0 (SPSS, Chicago, IL). Genotype frequencies were checked for Hardy-Weinberg equilibrium using the χ^2 test. Prior to analysis, continuous variables were inverse normal transformed to achieve normal distribution, and internal age- and

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TABLE 1
Anthropometric and clinical characteristics of study subjects

Character	Stage 1		Stage 2			
	NW children	OW/OB children	NW children	OW/OB children	*P**	*P*†
n (male/female)	830 (370/464)	453 (173/279)	1,399 (420/979)	444 (132/312)		
Age (years)	14.00 (12.50–15.00)	13.00 (12.00–15.00)	13.00 (12.00–14.50)	13.2 (12.0–15.0)	3.0×10^{-17}	0.43
Height (m)	1.54 (1.48–1.61)	1.56 (1.50–1.62)	1.52 (1.46–1.58)	1.56 (1.51–1.62)	4.8×10^{-8}	0.24
z Height	−0.11 ± 0.99	0.27 ± 0.89	−0.11 ± 1.0	0.35 ± 0.93	0.93	0.18
Weight (kg)	42.30 (37.00–48.85)	64.00 (55.45–73.90)	42.00 (36.00–48.40)	63.5 (56.0–71.0)	0.18	0.73
z Weight	−0.51 ± 0.75	1.20 ± 0.54	−0.40 ± 0.70	1.17 ± 0.55	4.4×10^{-4}	0.35
BMI (kg/m^2)	17.58 (15.86–19.44)	25.85 (23.97–28.97)	17.91 (16.26–19.78)	25.6 (24.0–28.0)	2.9×10^{-3}	0.14
z BMI	−0.56 ± 0.76	1.27 ± 0.49	−0.40 ± 0.65	1.18 ± 0.50	7.3×10^{-7}	0.01
WC (m)	0.66 (0.61–0.72)	0.84 (0.77–0.90)	0.65 (0.60–0.71)	0.85 (0.78–0.90)	4.2×10^{-5}	1.2×10^{-3}
z WC	−0.42 ± 0.77	1.11 ± 0.59	−0.40 ± 0.79	1.11 ± 0.63	1.49×10^{-4}	2.7×10^{-13}
HC (m)	0.81 (0.76–0.86)	0.97 (0.92–1.04)	0.80 (0.75–0.86)	0.97 (0.91–1.02)	8.3×10^{-3}	2.1×10^{-8}
z HC	−0.42 ± 0.76	1.23 ± 0.63	−0.41 ± 0.71	1.11 ± 0.62	0.11	1.4×10^{-14}
WHR	0.82 (0.78–0.87)	0.86 (0.81–0.90)	0.82 (0.78–0.86)	0.87 (0.83–0.92)	0.05	0.21
z WHR	−0.22 ± 0.89	0.41 ± 0.93	−0.20 ± 0.98	0.62 ± 0.96	0.14	1.3×10^{-9}
Glucose (mmol/L)	5.04 (4.72–5.33)	5.00 (4.72–5.33)	4.77 (4.44–5.11)	4.60 (4.22–4.90)	3.3×10^{-23}	6.3×10^{-27}
Insulin (pmol/L)	37.80 (23.40–54.60)	76.80 (52.20–108.00)	42.00 (28.80–58.80)	71.40 (47.40–111.60)	4.4×10^{-5}	1.8×10^{-3}
C-peptide (nmol/L)	0.46 (0.33–0.59)	0.61 (0.39–0.80)	0.48 (0.37–0.62)	0.70 (0.53–0.89)	0.86	0.19
Homeostasis model assessment of insulin resistance	1.41 (0.83–2.09)	2.83 (1.86–4.13)	1.44 (0.99–2.09)	2.41 (1.52–3.89)	8.1×10^{-3}	1.4×10^{-4}
High-sensitive C-reactive protein (mg/L)	0.25 (0.1–0.76)	1.26 (0.48–3.06)	0.34 (0.13–0.84)	1.11 (0.53–2.38)	3.0×10^{-3}	0.94
Adiponectin (ng/mL)	8.45 (5.95–12.50)	4.62 (2.81–7.18)	8.61 (4.80–13.59)	7.79 (4.76–10.83)	0.07	0.18
Leptin (ng/mL)	6.68 (4.30–11.51)	17.37 (10.72–26.81)	8.14 (5.10–12.75)	19.53 (13.43–30.34)	5.4×10^{-5}	3.0×10^{-3}
Resistin (ng/mL)	5.59 (4.39–7.82)	5.76 (4.33–7.25)	5.30 (4.25–6.80)	5.67 (4.60–7.25)	9.7×10^{-3}	7.6×10^{-8}

Data are median (interquartile range) and were compared using the Mann-Whitney *U* test or are mean *z* score ± SD and were compared by *t* test. **P* values for comparison between NW children from stage 1 and NW children from stage 2. †*P* values for comparison between OW/OB children from stage 1 and OW/OB children from stage 2.

sex-specific *z* scores (SDs from the study group mean) were generated by dividing the difference between the study group mean and the individual's value by SD for that age and sex. The association of SNPs with overweight/obesity was tested using logistic regression analysis under additive model adjusting for age and sex. Linear regression analysis was performed to assess the association of SNPs with quantitative traits adjusting for age and sex for obesity-related traits and age, sex, and *z* BMI for other traits. Meta-analysis was performed by combining summary estimates of stage 1 and stage 2. Bonferroni correction was applied in the meta-analysis stage to account for multiple testing, and a *P* value of $<7.6 \times 10^{-4}$ was considered significant after correcting for the number of independent SNPs ($r^2 < 0.80$; $n = 66$). For quantitative clinical traits, a *P* value of $<5.4 \times 10^{-5}$ was considered significant after accounting for multiple comparison (0.05/[66*14]). The statistical power of the study was determined using Quanto software (http://hydra.usc.edu/gxe/), assuming a log additive model of inheritance and 24% prevalence of overweight/obesity (1) at $\alpha = 0.05$. Sample size in stage 1 and combined analysis provided 44–76% and 76–97% power, respectively, to detect the association of the variant with an allele frequency of 0.20 and an effect size of 1.20–1.30.

The cumulative effect of associated SNPs was determined through allele dosage analysis by calculating effective risk scores (ERSs), as provided earlier (6). Subjects were classified into different groups by ERS (<2, 2–3, 3–4, 4–5, and >5–6), and odds ratios (ORs) were calculated considering an ERS of <2 as the reference.

RESULTS

Stage 1 analysis revealed the association of 17 variants in *LEPR*, *IL6*, *IL6R*, *PBEF1*, *CES2*, and *TNFRSF1B* with overweight/obesity at $P < 0.05$ (Supplementary Table 1), with the strongest signal at rs3801266 of *PBEF1* ($P = 4.5 \times 10^{-4}$). The *IL6* variants (rs2069845 [$P = 8.7 \times 10^{-4}$] and rs2069849 [$P = 1.6 \times 10^{-3}$]) and the *LEPR* nonsynonymous variants (rs1137100 [K109R] [$P = 1.4 \times 10^{-3}$], rs1137101 [Q223R] [$P = 2.5 \times 10^{-3}$], and rs8179183 [K656N] [$P = 2.6 \times 10^{-3}$]) were associated with the risk of overweight/obesity. Among the *IL6R* variants, rs7514452 and rs10752641 were found to be associated ($P = 2.3 \times 10^{-3}$ and 0.03, respectively). Association analysis by considering BMI as a continuous trait provided similar results (Supplementary Table 1).

We performed a replication analysis for rs3801266 (*PBEF1*), rs2069845 (*IL6*), rs1137100 (*LEPR*), and rs7514452 (*IL6R*) in stage 2. Associations of rs2069845, rs1137100, and rs3801266 were replicated ($P = 7.9 \times 10^{-4}$, 8.3×10^{-3}, and 0.036, respectively) and corroborated in a subsequent meta-analysis ($P = 2.3 \times 10^{-6}$, 3.9×10^{-5}, and 4.3×10^{-4}, respectively) that remained significant after multiple testing correction (Table 2). Variants rs2069845, rs1137100, and rs3801266 also were significantly associated with BMI in stage 2 and meta-analysis and remained significant after multiple testing correction (Table 2). All three variants (rs2069845, rs1137100, and rs3801266) showed association with weight, WC, and HC (β range 0.05–0.14 *z* score units and *P* range 2.0×10^{-3} to 8.3×10^{-7}) (Fig. 1). The association of rs1137100, rs2069845, and rs3801266 with weight, and of rs3801266 with HC remained significant after correcting for multiple comparisons ($P < 5.4 \times 10^{-5}$).

Allele dosage analysis of rs2069845, rs1137100, and rs3801266 revealed a significant trend in the increase of risk for overweight/obesity by 1.32-fold, with an increase in each ERS ($P_{Trend} = 8.1 \times 10^{-11}$) (Fig. 1). The distribution of NW and OW/OB children in different ERS groups is provided in Supplementary Fig. 1. Children with ERSs 5–6 had an OR of ~4 for being overweight/obese compared with individuals with an ERS <2 ($P = 1.2 \times 10^{-7}$).

TABLE 2
SNPs showing the association with obesity and BMI in urban Indian children

	Obesity												BMI					
	Stage 1				Stage 2		Meta-analysis			Stage 1			Stage 2			Meta-analysis		
SNP (gene)	Risk allele frequency OW/OB NW	OR (95% CI)	P	Risk allele frequency OW/OB NW	OR (95% CI)	P	OR^r; OR^f	P^r; P^f	Q	β (SE)	P	β (SE)	P	β^r; β^f	P^r; P^f	Q
rs2069845																		
(A/G); (*IL6*)	0.28; 0.22	1.39 (1.14–1.68)	8.7×10^{-4}	0.29; 0.23	1.35 (1.14–1.62)	7.9×10^{-4}	1.37; 1.37	2.3×10^{-6}; 2.3×10^{-6}	0.85	0.17 (0.05)	1.1×10^{-3}	0.09 (0.04)	8.8×10^{-3}	0.12; 0.12	6.9×10^{-4}; 5.7×10^{-5}	0.24
rs1137100																		
(A/G); (*LEPR*)	0.84; 0.79	1.45 (1.15–1.82)	1.4×10^{-3}	0.84; 0.80	1.33 (1.07–1.67)	8.3×10^{-3}	1.39; 1.39	3.9×10^{-5}; 3.9×10^{-5}	0.65	0.26 (0.06)	2.2×10^{-6}	0.09 (0.04)	0.02	0.17; 0.15	0.04; 3.2×10^{-6}	0.01
rs3801266																		
(T/C); (*PBEF1*)	0.84; 0.78	1.47 (1.19–1.82)	4.5×10^{-4}	0.83; 0.80	1.23 (1.01–1.51)	0.04	1.35; 1.35	4.3×10^{-4}; 8.5×10^{-5}	0.26	0.13 (0.05)	0.01	0.11 (0.04)	4.4×10^{-3}	0.17; 0.17	1.7×10^{-4}; 1.7×10^{-4}	0.70
rs7514452																		
(T/C); (*IL6R*)	0.25; 0.20	1.36 (1.12–1.67)	2.3×10^{-3}	0.23; 0.22	1.06 (0.88–1.27)	0.52	1.20; 1.19	0.146; 0.011	0.07	0.13 (0.05)	0.01	0.009 (0.04)	0.81	0.06; 0.05	0.30; 0.12	0.06

Meta-analysis was performed by combining the summary estimates of stage 1 and stage 2 using PLINK. The ORs and βs are presented with respect to the risk alleles as observed in the current study, which are underlined. β, change in z score of BMI per increase in risk allele; β^f, β for fixed-effects meta-analysis; β^r, β for random-effects meta-analysis; OR^f, OR for fixed-effects meta-analysis; OR^r, OR for random-effects meta-analysis; P^f, P value for fixed-effects meta-analysis; P^r, P value for random-effects meta-analysis; Q, P value for the Cochrane Q statistic.

A significant increase in the measures of adiposity with the increase in ERS was observed, with the highest influence on weight and BMI, which both increased by 0.13 z score units per increase in ERS ($P = 1.2 \times 10^{-12}$ and 5.0×10^{-12}) (Fig. 2).

Variants rs2069845, rs1137100, and rs3801266 did not show a significant association with the plasma levels of adipokines and markers of glucose homeostasis. In addition, we performed exploratory analysis to investigate the association of variants of these adipokines and inflammatory marker genes with quantitative clinical traits using stage 1 data (Supplementary Table 2). Variants in *RETN* (rs3745367 and rs1862513) and *ADIPOQ* (rs822395 and rs1063538) showed an association with the plasma levels of resistin ($P = 2.7 \times 10^{-5}$ and 2.0×10^{-3}) and adiponectin ($P = 2.0 \times 10^{-3}$ for both), respectively. Only a nominal association of SNPs with markers of glucose homeostasis was observed.

DISCUSSION

Our study demonstrates the association of rs2069845 (*IL6*), rs1137100 (*LEPR*), and rs3801266 (*PBEF1*) with the risk of overweight/obesity, BMI, body weight, WC, and HC in urban Indian children. Allele dosage analysis revealed a cumulative effect of these variants on the risk of overweight/obesity and the effect size on measures of adiposity, which greatly increased with the increase in each risk allele.

Studies investigating the association of *IL6* variants with adult and childhood obesity have generated contradictory results (7–9). A study in Greek school children had shown an association of the *IL6* variant rs1800795 with parameters related to obesity (10). We did not detect an association of this variant with overweight/obesity; rather, we found an association of rs2069845 in intron 4 of *IL6*, which is in moderate linkage disequilibrium with rs1800795 (D' = 1.0; $r^2 = 0.63$), with overweight/obesity and measures of adiposity in Indian children.

Mutations in *LEPR* are associated with a severe form of obesity (11); however, there have been conflicting reports regarding the association of its common variants with a complex form of obesity. *LEPR* coding variants (rs1137101 [Q223R] and rs8179183 [K656N]) have been inconsistently shown to be associated with adiposity in previous studies as a result of ethnic differences in allele frequencies (12). Here, we obtained a significant association of rs1137101, rs8179183, and rs1137100 (K109R) with childhood obesity. To the best of our knowledge, this is the first report demonstrating an association of K109R with obesity and adiposity measures in children. Although the functional significance of K109R is unknown, it causes a conservative change in the membrane-distal part of the leptin receptor extracellular domain (13) and thus might have a possible role in modulating the binding affinity of the receptor.

Visfatin, encoded by *PBEF1*, is a recently identified adipokine with a role in inflammation and insulin resistance (3). Only a rare variant in *PBEF1* has been reported to be associated with severe obesity in French subjects (14). However, no common variants influencing the risk of obesity or its measures has been identified to date. In our study, the *PBEF1* variant rs3801266 provided strong evidence for the association with overweight/obesity and anthropometric parameters in children.

Leptin, adiponectin, and resistin are the major adipocyte-secreted hormones with pleiotropic effects on metabolism, inflammation, and insulin resistance (3), the central factors

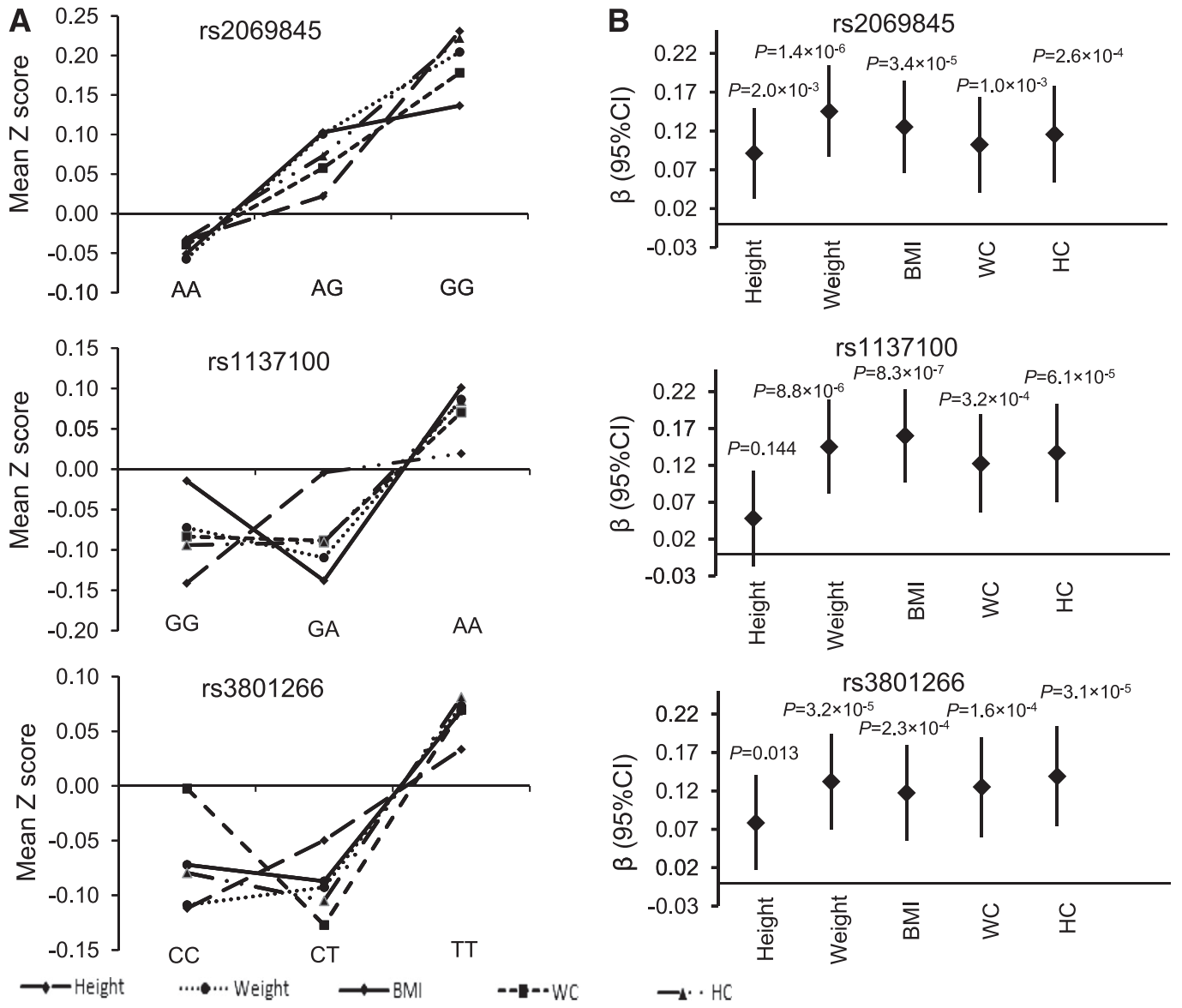

FIG. 1. Effect of overweight/obesity-associated SNPs on anthropometric measures (height, weight, BMI, WC, and HC). *A*: Effect of genotypes of associated SNPs on measures of adiposity. Mean *z* scores adjusted for age and sex are plotted on the *y*-axis, for the corresponding genotypes on the *x*-axis. *B*: *z* Score change per risk allele of associated SNPs (95% CI). Change in *z* scores was determined using regression analysis adjusted for age and sex.

underlying metabolic disorders. Genetic variants in genes encoding these adipokines are known to contribute to variations in their plasma levels. Consistent with the previous report of an association of rs3745367 of *RETN* with plasma resistin levels (15), we found strong evidence of an association of this polymorphism with plasma resistin levels. Our study also supports previous observations of an association of *ADIPOQ* variants with plasma adiponectin levels (16–18). The widely studied P12A polymorphism of *PPARG* has been shown to be associated with various metabolic disorders, including obesity and type 2 diabetes in adults. The attempts to assess the role of P12A on the susceptibility to obesity in children have provided inconsistent reports (19,20). Our study did not detect any association of *PPARG* variants with childhood obesity. On a similar note, variants from *LEP* and *RETN*, which have been suggested to be associated with adult/childhood obesity (21–24), were not found to be associated in our study.

Although the sample size of our study is considerable, there is a likelihood of false-negative observations for variants with small effect sizes, because the current study is sufficiently powered to capture only large effects (OR >1.3) of the common variants with frequencies >0.20. In addition, population stratification also can lead to spurious association results. To minimize the effect of population stratification, we have collected samples from a small geographical region that forms a homogenous cluster, as reported by the Indian Genome Variation Consortium (25). Moreover, the multidimensional scaling analysis using the genotype data for 595 unlinked markers ($r^2 < 0.20$) for stage 1 samples shows that the study population belongs to one cluster (Supplementary Fig. 2).

In conclusion, we demonstrate that common variants of *IL6*, *LEPR*, and *PBEF1* are associated with increased susceptibility to obesity and measures of adiposity in Indian school children. Although the case-control study design

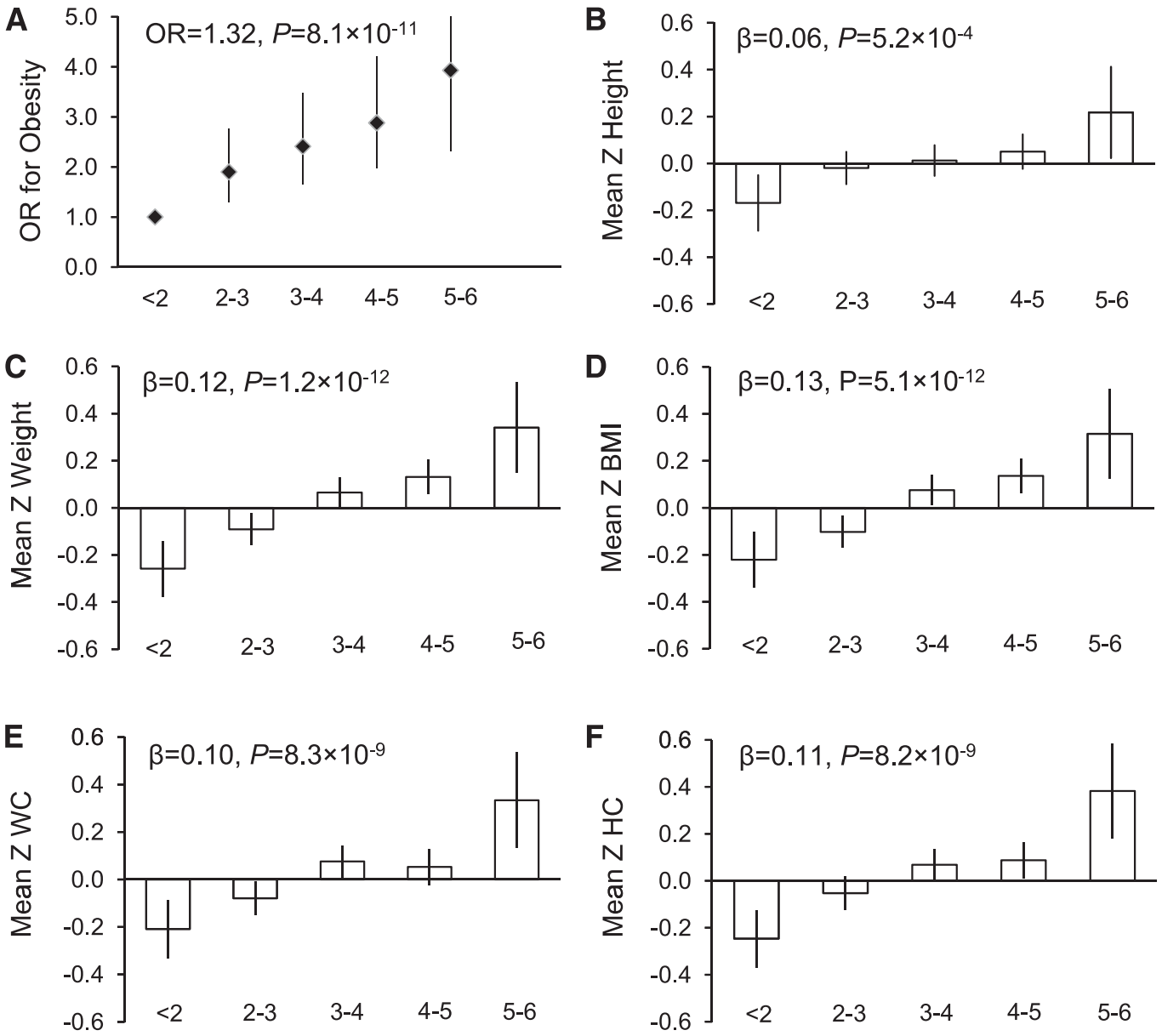

FIG. 2. Allele dosage analysis showing the association of combined risk alleles on childhood obesity and anthropometric measures. *A*: Obesity. *B*: Height. *C*: Weight. *D*: BMI. *E*: Waist circumference. *F*: Hip circumference. ORs or mean *z* scores with 95% CIs for combined effect are plotted on the *y*-axis, for the corresponding effective risk score on the *x*-axis.

limits the potential to identify the causal relationships, our study provides a lead for future investigations toward understanding the contribution of inflammatory genes in genetic predisposition to obesity in childhood. This would help in understanding the molecular mechanisms and exploring of therapeutic options toward prevention of childhood obesity.

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R.T. researched data, contributed to the discussion, and wrote the manuscript. Y.M. researched data and contributed to the discussion. O.P.D., G.C., and D.B. researched data, contributed to the discussion, and reviewed and edited the manuscript. S.G. researched data. R.K.M. reviewed and edited the manuscript. N.T. contributed to the discussion and reviewed and edited the manuscript. D.B. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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