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Association study of childhood obesity with eight genetic variants recently identified by genome-wide association studies

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BACKGROUND: Being overweight or obese is becoming increasingly common in low- and middle-income countries. The present study aimed to examine association of eight genetic variants with obesity and to estimate the cumulative effects of these variants in Chinese children.

METHODS: We conducted the case–control study in a total of 2,030 subjects. Genotyping of seven novel variants was performed with matrix-assisted laser desorption ionization time of flight mass spectrometry, while rs9939609 was genotyped with tetra-primer amplification refractory mutation system analysis.

RESULTS: The association of two fat mass and obesity-associated gene (*FTO*) single-nucleotide polymorphisms (SNPs; rs9939609 and rs62048402) with body mass index (BMI) or obesity reached nominal significance at $P < 0.05$. We found a cumulative effect of five SNPs on the risk of overweight and obesity (odds ratio (OR) = 1.197, 95% confidence interval (CI) = 1.068–1.342, $P = 0.002$). Subjects carrying 9 or more effect alleles had a 127% increased risk of overweight and obesity (OR = 2.270, 95% CI = 1.403–3.671, $P = 0.001$) compared with subjects who carry 6 or fewer effect alleles.

CONCLUSION: We confirmed two *FTO* SNPs (rs62048402 and rs9939609) had nominal significant effects on BMI or obesity. We identified the cumulative effect of five SNPs on risk of overweight and obesity. The results provided evidence for identifying genetic factors related to childhood obesity.

Being overweight or obese is a significant risk factor for the development of chronic diseases such as type II diabetes and cardiovascular diseases and is becoming increasingly common in low- and middle-income countries (1). Genetic factors contribute to the etiology of obesity and the heritability of body mass index (BMI) ranges 30–70% (2).

Genome-wide association studies (GWAS) have provided evidences for genetic risk loci for obesity. The largest GWAS of BMI was conducted by the Genetic Investigation of Anthropometric Traits (GIANT) Consortium in 2010 on a sample of 249,796 European individuals (3). The GIANT Consortium has confirmed 14 known obesity susceptibility loci, and identified 18 new loci associated with BMI ($P < 5 \times 10^{-8}$), bringing the total number of such loci to 32 (3). Subsequently, these 32 loci had been studied by many research groups and then replicated in some studies (4–6).

Besides the known 32 loci, we searched published literatures on electronic databases of Pubmed, EBSCO, Web of Science, etc. for novel obesity susceptibility loci, which were identified by GWAS studies. Three such publications were found during 2012–2013. Firstly, in 2012, Ng *et al.* (7) performed a meta-analysis of two GWAS and the replication in four cohorts of African Americans, revealing five SNPs at four loci (rs6794092 near transmembrane protein 212 (*TMEM212*), rs268972 near cadherin 12 (*CDH12*), rs2033195, and rs815611 between microfibrillar-associated protein 3 (*MFAP3*) and UDP-N-acetyl-alpha-D-galactosamine: polypeptide N-acetylgalactosaminyltransferase 10 (*GALNT10*), and rs6088887 near Fer-1-like 4 (*FER1L4*)) associated with BMI ($2.4 \times 10^{-6} < P < 2.5 \times 10^{-5}$). In the same year, Wang *et al.* (8) identified a novel locus that influences BMI based on meta-analysis of GWAS data of two Caucasian samples. The strongest associated marker was SNP rs2967951 in rhophilin-associated tail protein 1-like (*ROPN1L*) located at 5p15.2. Moreover, in 2013, Sällman *et al.* (9) identified SNP rs62048482 in intron 1 of fat mass and obesity-associated gene (*FTO*) in Swedish children, having stronger association with obesity than SNP rs9939609, although the association between *FTO* rs9939609 and obesity had been widely confirmed (10,11).

Since the associations between obesity and these novel SNPs recently identified by GWAS have not been validated in independent populations with different ethnicity, it is crucial to explore their effects in Chinese population. So we conducted the present study to examine association of eight gene variants (rs6794092 (*PP13439-TMEM212*), rs2967951 (*ROPN1L*), rs268972 (*CDH12*), rs2033195 (*MFAP3-GALNT10*), rs815611 (*MFAP3-GALNT10*), rs62048402 (*FTO*), rs9939609 (*FTO*), rs6088887 (*FER1L4*)) with obesity, and to estimate the cumulative effects of these variants in Chinese children. The study will provide evidences for these SNPs' associations with obesity in

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Table 1. General characteristics of study groups

	ALIR			CPOOA		
	Normal weight	Overweight and obese	*P* value	Normal weight	Overweight and obese	*P* value
N	151	786		456	637	
Female (%)	63 (19.1)	267 (80.9)	0.068	260 (53.9)	222 (46.1)	<0.001
Age (y)	14.8 ± 0.7	14.6 ± 0.6	0.002	11.8 ± 3.1	11.1 ± 2.6	<0.001
BMI (kg/m^2)	20.4 ± 1.8	27.5 ± 3.4	<0.001	18.2 ± 2.5	24.1 ± 3.5	<0.001

ALIR, adolescent lipids, insulin resistance and candidate genes; CPOOA, Comprehensive Prevention project for Overweight and Obese Adolescents.

Table 2. Genotyping information of eight SNPs in Chinese children

				Allele								Genotype (11/12/22)	
SNP	Chr	Position	Nearest gene	Effect (1)	Other (2)	EAF	EAF in literature	Call rate	Total sample (11/12/22)	HWE (*P* value)	F_{ST}	Normal weight	Overweight and obese
rs6794092	3	173041038	*PP13439-TMEM212*	G	A	1.00	0.90 (ref. 7)	99.3	0/0/2016	–	0.053	0/0/600	0/0/1416
rs2967951	5	10517107	*ROPN1L*	T	C	0.67	0.10 (ref. 8)	99.3	209/893/914	0.297	0.343	71/255/277	138/638/637
rs268972	5	22692284	*CDH12*	C	A	0.87	0.66 (ref. 7)	99.8	28/462/1534	0.625	0.061	10/146/447	18/316/1087
rs2033195	5	153489789	*MFAP3-GALNT10*	C	T	0.97	0.57 (ref. 7)	99.6	3/112/1906	0.706	0.226	1/39/564	2/73/1342
rs815611	5	153498959	*MFAP3-GALNT10*	G	A	0.97	0.58 (ref. 7)	99.6	3/107/1911	0.591	0.218	1/36/567	2/71/1344
rs62048402	16	52360724	*FTO*	A	G	0.13	0.44 (ref. 9)	99.5	38/458/1523	0.598	0.118	7/128/469	31/330/1054
rs9939609	16	52378028	*FTO*	A	T	0.13	0.44 (ref. 9)	100	41/462/1527	0.670	0.118	7/126/474	34/336/1053
rs6088887	20	33634738	*FER1L4*	G	A	0.91	0.79 (ref. 7)	99.5	28/323/1668	0.108	0.028	10/104/490	18/219/1178

Chr, chromosome; EAF, effect allele frequency; HWE, Hardy–Weinberg equilibrium; Position, NCBI build 36.3 (NCBI, Bethesda, MD); SNP, single-nucleotide polymorphism.

a population whose ethnicity is different from that in original studies.

RESULTS

The general characteristics of the study groups were shown in **Table 1**. The study on adolescent lipids, insulin resistance and candidate genes (ALIR) consisted of 151 normal weight children (63 females, mean age 14.8 ± 0.7 y, mean BMI 20.4 ± 1.8 kg/m^2) and 786 overweight and obese children (267 females, mean age 14.6 ± 0.6 y, mean BMI 27.5 ± 3.4 kg/m^2). The differences in age and BMI between two groups of ALIR were statistically significant ($P < 0.05$). The Comprehensive Prevention project for Overweight and Obese Adolescents (CPOOA) study group consisted of 456 normal-weight children (260 females mean age 11.8 ± 3.1 y, mean BMI 18.2 ± 2.5 kg/m^2) and 637 overweight and obese children (222 females, mean age 11.1 ± 2.6 y, mean BMI 24.1 ± 3.5 kg/m^2). The differences in sex, age, and BMI between two groups of CPOOA were statistically significant ($P < 0.05$).

The genotype and allele frequencies of all the SNPs were shown in **Table 2**. Except for the monomorphic SNP rs6794092, the genotypes of the other seven SNPs were in Hardy–Weinberg equilibrium among normal-weight children ($P > 0.05$). *F*-statisics (F_{ST}) values between the population of original study and our sample were shown in **Table 2**, which varied from 0.028 (rs6088887) to 0.343 (rs2967951). Based on the F_{ST} values, we identified three SNPs having very great or great genetic differentiation, four SNPs moderate differentiation, and only one SNP little differentiation.

Table 3. Association of seven SNPs with BMI in Chinese children

		Our study		Discovery study	
SNP	Nearest gene	β (SE)	*P* value	β (SE)	*P* value
rs2967951	*ROPN1L*	0.187 (0.134)	0.162	0.76 (ref. 8)	3.05×10^{-4} (ref. 8)
rs268972	*CDH12*	0.218 (0.189)	0.248	0.091 (0.023) (ref. 7)[a]	5.00×10^{-5} (ref. 7)
rs2033195	*MFAP3-GALNT10*	0.084 (0.366)	0.819	0.094 (0.021) (ref. 7)[a]	5.57×10^{-6} (ref. 7)
rs815611	*MFAP3-GALNT10*	0.024 (0.373)	0.948	0.095 (0.021) (ref. 7)[a]	5.36×10^{-6} (ref. 7)
rs62048402	*FTO*	0.450 (0.183)	0.014	–[b]	–[b]
rs9939609	*FTO*	0.458 (0.181)	0.011	–[b]	–[b]
rs6088887	*FER1L4*	−0.189 (0.208)	0.364	0.106 (0.025) (ref. 7)[a]	2.53×10^{-5} (ref. 7)

SNP, single-nucleotide polymorphism.
[a]β and SE were reported for the changes in SD unit per copy of the effect allele. [b]There is no coefficient with BMI in the reference study.

As shown in **Table 3**, the association of two *FTO* SNPs (rs62048402 and rs9939609) with BMI reached nominal significance at $P < 0.05$. Each effect allele of rs62048402 was associated with increase of 0.450 kg/m^2 in BMI ($P = 0.014$). Each effect allele of rs9939609 was associated with increase of 0.458 kg/m^2 in BMI ($P = 0.011$). As shown in **Table 4**, the association of two *FTO* SNPs (rs62048402 and rs9939609) with obesity also reached nominal significance at $P < 0.05$. Each effect allele of rs62048402 was associated with a 25.9%

Table 4. Association of seven SNPs with risk of overweight and obesity in Chinese children

		Our study		Discovery study	
SNP	Nearest gene	OR (95% CI)	*P* value	OR (95%CI)	*P* value
rs2967951	*ROPN1L*	1.063 (0.912, 1.238)	0.435	–[a]	–[a]
rs268972	*CDH12*	1.098 (0.887, 1.358)	0.390	–[a]	–[a]
rs2033195	*MFAP3-GALNT10*	1.316 (0.875, 1.980)	0.188	–[a]	–[a]
rs815611	*MFAP3-GALNT10*	1.233 (0.810, 1.876)	0.329	–[a]	–[a]
rs62048402	*FTO*	1.259 (1.016, 1.560)	0.035	1.35 (1.13–1.60) (ref. 9)	<0.0007 (ref. 9)
rs9939609	*FTO*	1.322 (1.067, 1.637)	0.011	1.25 (1.05–1.48) (ref. 9)	<0.012 (ref. 9)
rs6088887	*FER1L4*	1.162 (0.919, 1.469)	0.210	–[a]	–[a]

OR, odds ratio; SNP, single-nucleotide polymorphism.
[a]There is no OR value with risk of overweight and obesity in the reference studies.

increased risk of overweight and obesity (OR = 1.259, 95% CI = 1.016–1.560, $P = 0.035$). Each effect allele of rs9939609 was associated with a 32.2% increased risk of overweight and obesity (OR = 1.322, 95% CI = 1.067–1.637, $P = 0.011$). After randomization tests (100,000 permutations), none of the seven SNPs was significantly associated with BMI or risk of overweight and obesity. We found the effects of the SNPs were in the same direction as those in the discovery studies except for rs6088887 (**Tables 3** and **4**).

The simple count genetic risk score (GRS), calculated as the effect allele number of 5 SNPs carried by each individual, varied from 2 to 10. As shown in **Figure 1**, the risk of overweight and obesity increased in a linear model as the simple count GRS increased. On average, each additional effect allele was associated with a 19.7% increased risk of overweight and obesity (OR = 1.197, 95% CI = 1.068–1.342, $P = 0.002$). Subjects carrying 9 or more effect alleles had a 127% increased risk of overweight and obesity (OR = 2.270, 95% CI = 1.403–3.671, $P = 0.001$) compared with subjects that carry 6 or fewer effect alleles. Additionally, OR–weighted GRS value increased the risk of overweight and obesity (OR = 1.191, 95% CI = 1.075–1.319, $P = 0.001$).

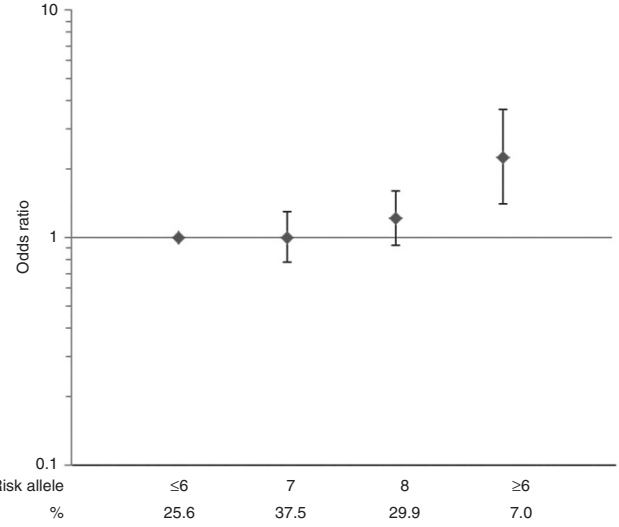

Figure 1. Odds ratios (ORs) and 95% confidence intervals (CIs) for the risk of overweight and obesity among children with different genetic risk scores. The score ≤6 was used as the reference in logistic regression analysis. On average, each additional effect allele was associated with increased risk of overweight and obesity (OR = 1.197, 95% CI = 1.068–1.342, $P = 0.002$).

DISCUSSION

Except for rs9939609, the other seven SNPs were identified in recent 2 y. To our knowledge, the present study was the first to validate these SNPs and examine their cumulative effect on BMI or overweight and obesity in other population with different ethnicity.

Recent GWAS studies found novel SNPs associated with BMI and obesity but the proportion of variance explained by individual SNPs is very limited. The aggregation of genetic information, obtained from many markers, into a single GRS variable permits to condense this information into a statistical metric of low dimensionality (12). Li *et al.* (13) assessed the cumulative effect of 12 SNPs identified by GWAS, and found each additional risk allele being associated with 10.8% ($P = 1.54 \times 10^{-22}$) and 5.5% ($P = 3.38 \times 10^{-10}$) increased risks of overweight and obesity. Ntalla *et al.* (5) assessed cumulative effect of 34 variants in Greek Adolescents, and found a significant association with overweight (OR = 1.09; 95% CI = 1.04–1.16; $P = 0.001$). Similar to these previous studies, we calculated simple count GRS for identifying cumulative effects of genetic variants on overweight or obesity. We found the cumulative effect of five SNPs on overweight and obesity in Chinese children (OR = 1.197, 95% CI = 1.068–1.342, $P = 0.002$). The OR–weighted GRS had association with overweight and obesity similar to that of simple count GRS (OR = 1.191, 95% CI = 1.075–1.319, $P = 0.001$).

The GRS results of our study have also possible biological implications. Previous functional studies provided evidences that six out of eight SNPs in our study have potential effects in the central nervous system (CNS), which had multiple roles on body weight regulation, including appetite, energy expenditure, and other behavioral aspects. *GALNT10* with rs2033195 and rs815611 nearby and *CDH12* with rs268972 nearby are expressed in CNS (14). Besides, the SNP rs6794092 is located near the gene transmembrane protein 212 (*TMEM212*) (7), whose family member *TMEM18* was found to be associated

with BMI and expressed at high levels in hypothalamus (15). FTO is highly expressed in hypothalamus, which regulates the energy balance (15). Many studies showed that *FTO* regulates BMI by influencing energy-dense food intake (16,17). But the functions of two SNPs (*ROPN1L* and *FER1L4*) are unknown. The GRS results of our study and the functional evidences suggested further biological studies could study the multigenetic effect and interaction of these genes in a CNS pathway.

We found nominal associations between the two *FTO* SNPs (rs62048402 and rs9939609) and BMI or risk of overweight and obesity. All the two SNPs were within intron 1 of *FTO* (15). Sällman *et al.* (9) found that the intron 1 is the only region within *FTO* associated with obesity, and reported that rs62048402 (OR = 1.35, 95% CI = 1.13–1.60, $P < 0.0007$) had a stronger association with obesity than SNP rs9939609 (OR = 1.25, 95% CI = 1.05–1.48, $P < 0.012$). But our study showed that rs62048402 had a lower odds ratio (OR = 1.259, 95% CI = 1.016–1.560, $P = 0.035$) than rs9939609 (OR = 1.322, 95% CI = 1.067–1.637, $P = 0.011$). In Sällman Almén's study (9), rs9939609 and rs62048402 was in a linkage disequilibrium (LD) with r^2 value of 0.87 among Swedish children. The r^2 value was 0.96 in our Chinese population, indicating LD of the two FTO SNPs among Chinese population was stronger than that of European population. We considered the difference in association statistics of two SNPs was due to the LD difference.

Despite the fact that the variants had been previously identified to be associated with BMI, we observed that apart from two *FTO* SNPs, the other six SNPs had no individual effect on BMI or obesity. The unexpected finding might be due to the following reasons. Firstly, there is population differentiation between Chinese ancestry and other populations. The effect allele frequencies of the eight SNPs in the present study were similar to those reported in HapMap Han Chinese, however, different from the frequencies of the original findings in other populations (see **Table 2**). The effect allele frequency of rs2967951 varied at most, which was 0.67 in our study and 0.10 in Wang's study among Caucasians (8). The SNP rs6794092 was monomorphic in our study, but its effect allele frequency was 0.90 in Ng's study among African American (7). Secondly, the F_{ST} values of these SNPs varied from 0.028 to 0.343. We found seven out of eight SNPs having F_{ST} values over 0.05, indicating moderate to very great genetic differentiation (18). Moreover, heterogeneity of effect size may partly explain the inconsistency in replication. We considered that the effects of these SNPs in Chinese might be lower than that in original populations, which could not be determined with our limited sample size. Further studies should perform fine mapping of this region to clarify the LD difference between different ethnic populations and identify causal variations of obesity.

Different from the discovery studies in adults, our study population was consisted of children and adolescents. Compared with adults, children have higher BMI or obesity heritability and most obese children have simple obesity without complications. Distilling the genetic component of obesity should be easier in children, where environmental exposure and impact had occurred for a relatively short period in their lifetime (19). So our study in children could help to identify the effects of genetic variations on obesity. In addition, previous studies showed the importance of age-related aspects upon interpretation of GWAS signals. Although the currently known major common variants related to obesity overlap to a substantial degree between children and adults, Scherag *et al.* identified TNKS/MSRA locus had effects in children and adolescents but no effect in adults (20). We selected the SNPs identified in adults and provided evidences for their effects in children.

The present study had limitations. Firstly, we only selected several novel variants which were recently identified by GWAS to be associated with obesity. We considered the other variants reported before the year 2012 had been studied by many research groups and then replicated in some studies (4–6), some of which in Chinese population (10). But the effects of these novel variants on obesity have not been validated in different populations. So we selected only these variants in this study. However, the cumulative effects or interaction of these SNPs and other known SNPs on pathogenesis of obesity await further studies. The second is the limited sample size and statistic power. Based on the effect estimates from the discovery papers and allele frequencies in the current study population, we calculated the statistic power of our sample for the SNPs. The power for three SNPs was higher than 70%, including *FTO* rs62048402 (92%), rs9939609 (73%) and *ROPN1L* rs2967951 (99%). The powers for *CDH12* rs268972 and *FER1L4* rs6088887 were 62% and 61%, respectively. But the power was lower for *MFAP3-GALNT10* rs2033195 (26%) and rs815611 (27%), for minor allele frequency (0.03) is low in the Chinese population. Even in the absence of statistically significant association, the consistency in effect estimates between our study and discovery studies may at least hint at a true association of the SNPs with BMI and obesity in Chinese population, which need further validation studies with large sample size and better power. Thirdly, the case–control study design did not permit us to make conclusions about causality.

In conclusion, we confirmed two SNPs (rs62048402 and rs9939609) in intron 1 of *FTO* had nominal significant effects on BMI or risk of overweight and obesity. We identified the cumulative effect of five SNPs (rs2967951, rs268972, rs2033195, rs9939609, and rs6088887) on risk of overweight and obesity. The results provided evidences for identifying genetic factors related to childhood obesity.

MATERIALS AND METHODS

Subjects

We conducted the case–control study in a total of 2,030 subjects of two independent study groups, including 1,423 overweight or obese cases and 607 normal-weight controls recruited from the urban regions of Beijing, China. The first group came from the study on ALIR. The second study group was from the CPOOA with physical exercise and healthy nutrition as instruments. The ascertainment strategies were very similar in the two studies, except for the age difference (21,22). All the obese individuals in the selected schools were recruited with their voluntary participation. The method of cluster sampling was adopted to recruit non-obese subjects from some classes of each grade in the same schools. The ALIR subjects were ascertained from adolescents aged 14–17 y in 9 middle schools of Dongcheng District of Beijing, including 786 overweight or obese adolescents and 151

normal-weight adolescents. The CPOOA subjects were recruited from children and adolescents aged 7–18 y in 5 elementary and middle schools of Haidian District of Beijing, comprising 637 overweight or obese children and adolescents and 456 normal-weight children and adolescents. The ascertainment strategies for the two study groups have been previously described in detail previously (23,24). We used the uniform BMI percentile criteria for the participants, which were determined in a representative Chinese population (25). According to criteria, the children and adolescents with an age- and gender-specific BMI ≥95th percentile are defined as obese; those with an age- and gender-specific BMI between 85th and 95th percentile are defined as overweight. The BMIs of normal-weight students were between the 15th and 85th percentiles. The individuals with any cardiovascular or metabolic disease were excluded. Two studies were approved by the Ethic committee of Peking University Health Science Center. Written informed consent was provided by all participants and, in the case of minors, their parents.

SNPs Selection and Genotyping

Firstly, we selected six SNPs associated with BMI or obesity from two new GWAS in 2012 (7,8). Secondly, the SNP rs62048402 in *FTO* recently identified by Sällman *et al.* (9) was included in the present study. Finally, to compare the effects of rs62048402 and rs9939609 (general confirmed to be associated with obesity), rs9939609 was included in the present study.

Genomic DNAs of subjects were extracted from blood leukocytes by the phenol–chloroform extraction method. Genotyping of seven SNPs except for the SNP rs9939609 was conducted on MassARRAY System (Sequenom, San Diego, CA). Primers, including a pair of amplification primers and an extension primer for each SNP, were designed with SpectroDESIGNER software (Sequenom). A multiplex polymerase chain reaction was performed, and unincorporated double-stranded nucleotide triphosphate bases were dephosphorylated with shrimp alkaline phosphatase followed by primer extension. The purified primer extension reaction was spotted on to a 384-element silicon chip (SpectroCHIP, Sequenom) and analyzed in the matrix-assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF MS, Sequenom). The resulting spectra were processed with MassArray Typer (Sequenom).The call rates of the seven SNPs were more than 99.0%.

Genotyping of rs9939609 was carried out with tetra-primer amplification refractory mutation system analysis (tetra-primer ARMS-PCR) (26). The sequences of primers were: F_{out}: 5′– TGG CTC TTG AAT GAA ATA GGA TTC AGA A–3′; R_{out}: 5′– AGC CTC TCT ACC ATC TTA TGT CCA AAC A–3′; F_{in}: 5′–TAG GTT CCT TGC GAC TGC TGT GAA TAT A–3′; R_{in}: 5′–GAG TAA CAG AGA CTA TCC AAG TGC ATC TCA–3′ (product size: T-allele (321bp, 178bp), A-allele (321bp, 201bp)). Different PCR products were clearly distinguished on 2.5% agarose gels stained with ethidium bromide. For reference ARMS-PCR of individuals with genotypes identified by sequencing were included in every run. For validity of genotypes, allele assignments were made by at least two experienced individuals independently. Discrepancies were solved unambiguously either by reaching consensus or by repeating. We genotyped 5% of samples twice for quality control and the genotyping concordance rate was 100%. The call rate of rs9939609 was 100%.

Statistical Analyses

Statistical analyses were performed using SPSS 18.0 (SPSS, Chicago, IL) and PLINK (Massachusetts General Hospital, Boston, MA). The genotype data of normal-weight group was tested for deviation from Hardy–Weinberg equilibrium. *F*-statistics (F_{ST}), a metric representation of the effect of population subdivision, was calculated according to the following formula, $F_{ST} = (P_1 - P_2)^2/((P_1 + P_2)*(2 - (P_1 + P_2)))$, where P_1 = allele frequency in the population of original study and P_2 = allele frequency in the population of our study (27,28). A F_{ST} value between 0 and 0.05 indicates little genetic differentiation; a value between 0.05 and 0.15, moderate differentiation; a value between 0.15 and 0.25, great differentiation; and values above 0.25, very great differentiation (18).

The genotypes of each SNP were coded as 0, 1, and 2 according to the number of effect alleles. General linear model was performed to examine the independent effect of each SNP on BMI under additive model with adjustment for age, sex, and study population. Logistic regression model was performed to examine the independent effect of each SNP on risk of overweight and obesity.

To identify cumulative effects of the eight SNPs, we calculated two kinds of GRS, including simple count GRS and OR-weighted GRS. We used Haploview 4.2 (29) to estimate LD between the SNPs. Because rs62048402 was in strong LD with the SNP rs9939609 (r^2 = 0.958), and rs2033195 was in strong LD with rs815611 (r^2 = 0.956), rs62048402 and rs815611 were excluded from calculating the GRS. The SNP rs6794092 was also excluded for it was monomorphic in our samples. At first, the simple count GRS was calculated for each individual by adding the number of effect alleles of the remaining five SNPs (rs2967951, rs268972, rs2033195, rs9939609, and rs6088887). Secondly, taking into account the fact that effect sizes among SNPs vary, we calculated an OR-weighted GRS by using weights derived from logging per-allele OR from our study (30). Logistic regression model was used to calculate OR of simple count GRS and OR-weighted GRS for the risk of overweight and obesity.

A two-side *P* value of <0.05 was considered as nominal significance. Empirical significance levels were computed by randomization tests (100,000 permutations) of PLINK (Massachusetts General Hospital). Power calculation was performed using Quanto software (University of Southern California, Los Angeles, CA).

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