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Original Contribution

Evaluation of Genetic Susceptibility Loci for Obesity in Chinese Women

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Recent genome-wide association (GWA) studies have identified 18 genetic loci for obesity. Using directly observed and imputed GWA genotyping data on approximately 5,000 Chinese women (1996–2007), the authors evaluated 17 single nucleotide polymorphisms (SNPs) that represent 17 distinct obesity loci. Two SNPs near the *BAT2* and *MC4R* genes and 3 SNPs within the *FTO*, *SEC16B*, and *SH2B1* genes were significantly associated with body mass index (weight (kg)/height $(m)^2$), body weight, and the prevalence of obesity. The per-allele increase in body mass index ranged from 0.16 units (*BAT2*) to 0.38 units (*SH2B1*). Odds ratios for obesity ranged from 1.46 (95% confidence interval (CI): 1.12, 1.92) for *BAT2* to 2.16 (95% CI: 1.39, 3.37) for *MC4R*. A genetic risk score calculated by summing the number of risk-increasing alleles that each woman carried at these 5 loci was significantly associated with the prevalence of obesity. Women carrying 5 or more risk alleles had a 3.13-fold (95% CI: 2.06, 4.77) higher prevalence of obesity than women carrying 1 or no risk alleles. Results from this study extend some previous GWA findings to Chinese women and show the need for additional studies to identify susceptibility loci in Chinese and other Asian populations.

body mass index; genome-wide association study; linkage disequilibrium; obesity; polymorphism, genetic; women

Abbreviations: BAT2, HLA-B-associated transcript 2; BMI, body mass index; CI, confidence interval; FAIM2, Fas apoptotic inhibitory molecule 2; FTO, fat mass- and obesity-associated; GWA, genome-wide association; HLA-B, human leukocyte antigen B; MC4R, melanocortin 4 receptor; NEGR1, neuronal growth regulator 1; OR, odds ratio; PTER, phosphotriesterase-related; SEC16B, SEC16 homolog B (*Saccharomyces cerevisiae*); SH2B1, SH2B adaptor protein 1; SNP, single nucleotide polymorphism; TMEM18, transmembrane protein 18.

Obesity is a well-established risk factor for many common chronic diseases, including type 2 diabetes, cardiovascular disease, and certain forms of cancer (1). Although lifestyle and environmental factors are believed to contribute significantly to the etiology of obesity (2, 3), genetic predisposition has also been suggested to play an important role (4, 5). Candidate gene association studies have previously suggested several genetic variants that appear to be related to obesity. However, very few, if any, of these genetic variants have been validated in subsequent studies (6). Recently, genome-wide association (GWA) studies, through examination of hundreds of thousands of single nucleotide polymorphisms (SNPs) across the human genome, have identified at least 18 loci associated with obesity-related traits, including body mass index (BMI; weight (kg)/height $(m)^2$) and body weight (7–15). Several loci are also associated with waist circumference and/or hip circumference. These findings are summarized in Web Table 1, which is posted on the *Journal*'s Web site (http://aje.oxfordjournals.org/). Virtually all of these studies, however, were conducted among populations of European ancestry. Investigating these loci in other populations would be helpful to evaluate the generalizability of these findings and identify causal variants for obesity. Using GWA scan data from our ongoing studies, which include approximately 5,000 Chinese women, we conducted an in silico validation study for the obesity loci identified in published GWA studies (7–15).

MATERIALS AND METHODS

Study population

Samples used in the current analysis were donated by participants in GWA studies of breast cancer and type 2 diabetes. The breast cancer GWA study included 4,157 women who participated in the Shanghai Breast Cancer Study. Participant recruitment, sample collection, data cleaning, and laboratory protocols for the Shanghai Breast Cancer Study have been described in detail elsewhere (16). Briefly, participants were selected from 2 rounds of recruitment. The initial round was conducted between 1996 and 1998 and included women aged 25–64 years. Through a rapid case-ascertainment system and the population-based Shanghai Cancer Registry, 1,602 eligible breast cancer cases were identified, and 1,459 patients (91.1%) were interviewed in person. Controls were randomly selected from the general population and were frequency-matched to cases by age via the Shanghai Resident Registry, a population registry containing demographic information on all residents of urban Shanghai. The inclusion criteria for controls were identical to those for cases, except for a breast cancer diagnosis. Of the 1,724 eligible controls, 1,556 (90.3%) completed in-person interviews. Blood samples were obtained from 1,193 (82%) cases and 1,310 (84%) controls who completed interviews.

The second round of recruitment was conducted between 2002 and 2005 using a protocol similar to the one used in the initial round, except that the age range was expanded to include women up to 70 years of age. A total of 1,989 incident cases and 1,989 community controls were recruited, with response rates of 83.7% and 70.4%, respectively. Most newly recruited cases ($n = 1{,}932$; 97.1%) and controls ($n = 1{,}857$; 93.4%) provided a blood sample or an exfoliated buccal cell sample. Blood samples were genotyped initially using the Affymetrix GeneChip Human Mapping 500K Array Set (Affymetrix, Inc., Santa Clara, California) ($n = 300$) and subsequently using the Affymetrix Genome-Wide Human SNP Array 6.0 ($n = 3{,}918$), following the manufacturer's protocols. The gender of all participants was consistent with the gender determined from X chromosome genotyping data. An identity-by-descent analysis based on an identity-by-state analysis was performed using the corresponding function in *PLINK* (17) to detect first-degree cryptic relations in the study population. After exclusion of samples with a genotyping call rate less than 95%, sample duplication, or DNA contamination, GWA data on 4,157 persons (291 for the Affymetrix 500K Array Set and 3,866 for the Affymetrix SNP Array 6.0) were included in the breast cancer GWA study. We excluded an additional 11 persons from the present analysis of obesity-related phenotypes, because of missing data on BMI or body weight (4 breast cancer patients and 2 women from the control group) or a BMI greater than 4 standard deviations from the mean (5 women from the control group). One of the remaining women in the control group had type 2 diabetes, was included in another GWA study (see below), and was removed from the control group of the current study. Thus,

Table 1. Characteristics of Participants Included in a Validation Study of Obesity-Related Genetic Loci Identified in Genome-Wide Association Studies, Shanghai, China[a]

Variable	Community Controls ($n = 2{,}076$)		Breast Cancer Cases ($n = 2{,}069$)		Type 2 Diabetes Cases ($n = 885$)	
	Mean (SD)	%	Mean (SD)	%	Mean (SD)	%
Age, years	49.4 (8.5)		49.3 (8.3)		51.7 (6.3)	
Low educational level (less than middle school)		13.4		9.9		18.1
Energy intake, kcal/day	1,778 (444)		1,802 (451)		1,757 (425)	
No exercise		69.8		74.8		67.1
Postmenopausal		41.4		38.7		53.1
Weight at study enrollment, kg	58.5 (8.6)		59.9 (8.8)[b]		66.2 (9.7)	
Height at study enrollment, cm	158.4 (5.4)		158.7 (5.2)		157.3 (5.5)	
BMI[c] at age 20 years	19.4 (2.4)		19.3 (2.4)		19.6 (2.4)	
BMI at study enrollment	23.3 (3.3)		23.8 (3.3)[b]		26.7 (3.6)	
Waist circumference, cm	77.1 (8.9)		79.4 (8.78)		84.5 (8.5)	
Hip circumference, cm	95.3 (7.5)		95.5 (7.5)		100.5 (8.3)	
Waist:hip ratio	0.81 (0.06)		0.82 (0.06)		0.84 (0.05)	
Obesity[d] at study enrollment		11.0		12.8		38.1

Abbreviations: BMI, body mass index; SD, standard deviation.

[a] Community controls and breast cancer cases were compiled from the Shanghai Breast Cancer Study (1996–2005), and incident type 2 diabetes cases were compiled from the Shanghai Women's Health Study (1997–2007).

[b] Self-reported weight 1 year before diagnosis and BMI calculated using self-reported weight.

[c] Weight (kg)/height $(m)^2$.

[d] Obesity was defined as BMI $\geq$27.5.

Table 2. Associations of Obesity-Related Genetic Loci With Body Mass Index and Body Weight in Chinese Women, 1996–2007

Trait and SNP	Chromosome[a]	Base Position[a]	Nearest Gene	Effective Allele and Frequency (Proportion)[b]	Community Controls (n = 2,076)		Breast Cancer Cases (n = 2,069)		Type 2 Diabetes Cases (n = 885)		All Women (n = 5,030)	
					Per-Allele Effect[c]	*P* Value[d]	Per-Allele Effect	*P* Value	Per-Allele Effect	*P* Value	Per-Allele Effect	*P* Value
BMI[e]												
rs2815752[f]	1	72585028	*NEGR1*	A (0.92)	0.03	0.87	0.07	0.74	0.01	0.99	0.05	0.71
rs10913469	1	176180142	*SEC16B*[g]	C (0.21)	0.35	4.0×10^{-3}	0.26	0.03	0.23	0.26	0.28	4.1×10^{-4}
rs6548238[f]	2	624905	*TMEM18*	C (0.90)	0.16	0.34	0.01	0.93	0.30	0.32	0.12	0.29
rs7647305	3	187316984	*ETV5*	C (0.95)	−0.64	4.6×10^{-3}	0.57	0.01	0.74	0.07	0.10	0.49
rs10938397	4	44877284	*GNPDA2*	G (0.29)	−0.10	0.35	0.08	0.49	0.31	0.10	0.05	0.52
rs6235	5	95754654	*PCSK1*[g]	C (0.34)	0.09	0.37	−0.01	0.96	0.25	0.18	0.07	0.29
rs4712652	6	22186594	*PRL*	G (0.12)	0.06	0.70	−0.22	0.17	−0.34	0.21	−0.13	0.20
rs2844479	6	31680935	*BAT2*	T (0.56)	0.29	3.4×10^{-3}	0.05	0.62	0.13	0.46	0.16	0.01
rs4074134	11	27603861	*BDNF*	G (0.54)	0.13	0.19	0.18	0.08	−0.16	0.36	0.10	0.15
rs10838738	11	47619625	*MTCH2*[g]	G (0.33)	0.21	0.05	0.07	0.53	−0.04	0.83	0.10	0.13
rs7138803[f]	12	48533735	*FAIM2*	A (0.28)	−0.06	0.57	0.07	0.53	0.21	0.28	0.04	0.60
rs7498665	16	28790742	*SH2B1*[g]	G (0.11)	0.16	0.31	0.42	6.6×10^{-3}	0.70	7.6×10^{-3}	0.38	1.8×10^{-4}
rs9939609[f]	16	52378028	*FTO*[g]	A (0.12)	0.27	0.07	0.22	0.16	0.62	0.01	0.32	4.0×10^{-3}
rs1424233	16	78240252	*MAF*	A (0.67)	0.01	0.92	0.05	0.66	−0.18	0.31	−0.02	0.82
rs1805081	18	19394430	*NPC1*[g]	G (0.23)	−0.18	0.13	−0.19	0.12	0.18	0.39	−0.12	0.13
rs17782313[f]	18	56002077	*MC4R*	C (0.20)	0.41	1.1×10^{-4}	0.19	0.12	0.68	1.0×10^{-3}	0.37	4.6×10^{-6}
rs29941	19	39001372	*KCTD15*	C (0.23)	0.12	0.29	−0.04	0.75	−0.11	0.60	0.02	0.77
Body weight												
rs2815752[f]	1	72585028	*NEGR1*	A (0.92)	0.08	0.86	0.23	0.64	0.00	1.00	0.15	0.64
rs10913469	1	176180142	*SEC16B*[g]	C (0.21)	0.91	2.6×10^{-3}	0.64	0.04	0.60	0.24	0.71	3.5×10^{-4}
rs6548238[f]	2	624905	*TMEM18*	C (0.90)	0.44	0.28	0.00	1.00	0.75	0.32	0.29	0.29
rs7647305	3	187316984	*ETV5*	C (0.95)	−1.49	8.0×10^{-3}	1.50	8.6×10^{-3}	1.81	0.07	0.31	0.41
rs10938397	4	44877284	*GNPDA2*	G (0.29)	−0.25	0.36	0.17	0.52	0.77	0.09	0.11	0.53
rs6235	5	95754654	*PCSK1*[g]	C (0.34)	0.21	0.41	0.03	0.91	0.65	0.16	0.20	0.24
rs4712652	6	22186594	*PRL*	G (0.12)	0.15	0.70	−0.54	0.17	−0.78	0.24	−0.31	0.22
rs2844479	6	31680935	*BAT2*	T (0.56)	0.73	3.2×10^{-3}	0.10	0.69	0.35	0.42	0.40	0.01
rs4074134	11	27603861	*BDNF*	G (0.54)	0.31	0.22	0.40	0.12	−0.36	0.39	0.22	0.18
rs10838738	11	47619625	*MTCH2*[g]	G (0.33)	0.51	0.05	0.15	0.56	−0.05	0.92	0.26	0.13
rs7138803[f]	12	48533735	*FAIM2*	A (0.28)	−0.17	0.54	0.16	0.57	0.52	0.27	0.08	0.65
rs7498665	16	28790742	*SH2B1*[g]	G (0.11)	0.41	0.29	1.05	7.0×10^{-3}	1.75	6.7×10^{-3}	0.95	1.7×10^{-4}
rs9939609[f]	16	52378028	*FTO*[g]	A (0.12)	0.67	0.07	0.49	0.21	1.63	8.1×10^{-3}	0.79	1.4×10^{-3}
rs1424233	16	78240252	*MAF*	A (0.67)	0.03	0.91	0.11	0.68	−0.46	0.30	−0.05	0.79

rs1805081	18	19394430	*NPC1*[g]	G (0.23)	−0.49	0.09	−0.50	0.09	0.53	0.31	−0.31	0.11
rs17782313[f]	18	56002077	*MC4R*	C (0.20)	1.05	5.9×10^{-4}	0.51	0.09	1.67	1.1×10^{-3}	0.94	2.6×10^{-6}
rs29941	19	39001372	*KCTD15*	C (0.23)	0.33	0.26	−0.12	0.69	−0.21	0.68	0.07	0.71

Abbreviations: BAT2, HLA-B-associated transcript 2; BDNF, brain-derived neurotrophic factor; BMI, body mass index; ETV5, ets variant 5; FAIM2, Fas apoptotic inhibitory molecule 2; FTO, fat mass- and obesity-associated; GNPDA2, glucosamine-6-phosphate deaminase 2; HLA-B, human leukocyte antigen B; KCTD15, potassium channel tetramerization domain containing 15; MAF, v-maf musculoaponeurotic fibrosarcoma oncogene homolog (avian); MC4R, melanocortin 4 receptor; MTCH2, mitochondrial carrier homolog 2 (*Caenorhabditis elegans*); NEGR1, neuronal growth regulator 1; NPC1, Niemann-Pick disease, type C1; PCSK1, proprotein convertase subtilisin/kexin type 1; PRL, prolactin; SEC16B, SEC16 homolog B (*Saccharomyces cerevisiae*); SH2B1, SH2B adaptor protein 1; SNP, single nucleotide polymorphism; TMEM18, transmembrane protein 18.

[a] Chromosome and base position were based on the March 2006 genome assembly (human genome version 18 (University of California, Santa Cruz), Build 36.1 (National Center for Biotechnology Information)).

[b] Frequency of the BMI- or weight-increasing allele (increasing effect defined by initially reported genome-wide association studies in populations of European ancestry; effective allele defined by nucleotide on forward strand in this study) in the Chinese community controls.

[c] Change in BMI or body weight (kg) for each copy of the effective allele.

[d] *P* values were not corrected for multiple tests.

[e] Weight (kg)/height (m)^2.

[f] This SNP was one of the 5 SNPs directly typed using Affymetrix SNP Array 6.0 (Affymetrix, Inc., Santa Clara, California). All other SNPs were imputed using MACH 1.0 (http://www.sph.umich.edu/csg/abecasis/MACH/).

[g] The tested SNPs are located on the corresponding genes: *SEC16B* rs10913469 (intron), *PCSK1* rs6235 (missense $^{Ser}690^{Thr}$ in exon 14), *MTCH2* rs10838738 (intron), *SH2B1* rs7498665 (missense $^{Ala}484^{Thr}$ in exon 5), *FTO* rs9939609 (intron), and *NPC1* rs1805081 (missense $^{His}215^{Arg}$ in exon 5).

a total of 4,145 women from the breast cancer GWA study (2,069 cases and 2,076 controls) were included in the present obesity study (Table 1).

Additional samples included in the current analysis were donated by 885 participants in the Shanghai Women's Health Study, a population-based prospective cohort study of 74,942 adult women aged 40–70 years at recruitment (between March 1997 and May 2000) who developed type 2 diabetes during the course of follow-up (18). At study enrollment, trained interviewers conducted in-person interviews (92% response rate) and collected anthropometric data and blood or buccal cell samples (87.6%). Follow-up of study participants (1997–2007) was conducted through biennial in-person interviews for collection of information on survival status and cancer, type 2 diabetes, and the occurrence of other chronic disease. We genotyped samples from a total of 901 women who met the following criteria: self-reported diagnosis of type 2 diabetes after study enrollment, age $\leq$65 years at baseline, use of diabetes medication or a fasting glucose level greater than 125 mg/dL on at least 2 occasions, and donation of a blood sample. Samples were genotyped using the Affymetrix SNP Array 6.0 immediately following the scan phase of the breast cancer GWA study, using an identical protocol. Quality checking was conducted using the same protocol as in the breast cancer GWA study, and genotyping information on 885 women was available for the obesity association analyses (Table 1).

Body weight and height were measured in the Shanghai Breast Cancer Study and the Shanghai Women's Health Study using identical protocols. All measurements were taken twice by trained medical professionals during the in-person interviews while participants were wearing light indoor clothes and no shoes. If the differences between 2 measurements were larger than predefined tolerance limits (0.5 kg for weight and 1 cm for height), a third measurement was taken. The average of the 2 closest measurements was used in the current analysis. For patients with breast cancer, self-reported weight 1 year before cancer diagnosis and BMI derived from this weight were included in the analysis to minimize the influence of cancer and its associated treatment on body weight. Exercise participation during the 10 years prior to study enrollment was assessed for all participants. Information on weight and height at age 20 years was self-reported.

SNP imputation and selection

There were 291 participants (5.8% of all Shanghai Breast Cancer Study samples) whose samples were genotyped using the Affymetrix 500K array and 4,771 subjects whose samples were genotyped using the Affymetrix 6.0 array. We imputed genotypes that were not on the Affymetrix 500K or 6.0 array and genotypes for samples that failed in laboratory assays by means of the program MACH (http://www.sph.umich.edu/csg/abecasis/MACH/), using Asian data from the International HapMap Project (http://hapmap.ncbi.nlm.nih.gov/), release 22, as the reference data set. The directly genotyped SNPs and the imputed SNPs (a total of 2.4 million) were then combined into a single data set. Among the 45 reported GWA SNPs, the minor allele frequency of rs10508503 near the phosphotriesterase-related (*PTER*)

gene was less than 0.05% in our study populations; thus, this locus was excluded from our validation study. Of the remaining SNPs, 14 were directly genotyped and 30 were imputed (Web Table 1). We selected 1 representative SNP for each of the 17 obesity loci identified by published GWA studies for our study, because the obesity-related SNPs in the same chromosome region that have been reported to date are all in high linkage disequilibrium. Representative SNPs were selected using the following criteria: 1) for SNPs on the Affymetrix 6.0 array, a genotyping call rate greater than or equal to 95% in our GWA scan and a consistency rate greater than or equal to 95% among the duplicated, quality control samples; 2) a minor allele frequency greater than or equal to 5% in our community controls; 3) high imputation quality for ungenotyped SNPs ($R^2 > 0.8$); 4) designation as the most significant SNP in previous reports; and/or 5) cross-validation in 2 or more published GWA studies. Among the 17 representative SNPs, 5 SNPs (neuronal growth regulator 1 (*NEGR1*) rs2815752, transmembrane protein 18 (*TMEM18*) rs6548238, Fas apoptotic inhibitory molecule 2 (*FAIM2*) rs7138803, fat mass- and obesity-associated (*FTO*) rs9939609, and melanocortin 4 receptor (*MC4R*) rs17782313) are on the Affymetrix SNP Array 6.0. The genotyping data for the other 12 SNPs were imputed (Table 2). For each woman, the directly observed (by chip scanning) or most likely (by imputation) genotype of each SNP was coded as 0, 1, or 2, respectively, for carrying 0, 1, or 2 obesity risk-increasing alleles that had been previously identified in reports from other GWA studies.

Statistical analysis

The SAS statistical package, version 9.1 (SAS Institute Inc., Cary, North Carolina), was used for all analyses unless otherwise indicated. Means or proportions for demographic factors were estimated and are presented separately for each sample set, including community controls, breast cancer patients, and type 2 diabetes patients. Calculation of allele frequencies and testing for Hardy-Weinberg equilibrium in 2,076 controls without breast cancer from the Shanghai Breast Cancer Study were carried out using the corresponding functions in the *PLINK* software package (17).

The association of each SNP with quantitative traits (BMI or body weight) was evaluated separately for each sample set by means of linear regression analysis under an additive genetic model, with age, age squared, and menopausal status included as covariates. For body weight associations, we also adjusted for height. Analyses for obesity were carried out by comparing obese women (BMI $\geq$27.5, the World Health Organization's recommended cutpoint for Asians (19)) with women of normal weight (BMI 18.5–23) in logistic regression analyses with adjustment for age, age squared, menopausal status, and sample set. We also conducted logistic regression analyses to investigate the relation between reported obesity SNPs and type 2 diabetes.

We evaluated the joint effects of the 17 SNPs examined in this study, as well as the 5 significant, validated SNPs, using the counting genetic risk score approach (20). A simple summing of obesity risk-increasing alleles was used to calculate the genetic risk score. In logistic regression analyses of the association between obesity and the genetic risk score, the lower 20% of the genetic risk score in women with normal weight was chosen as the reference category—that is, a genetic risk score less than or equal to 11 for the 17 tested SNPs and a genetic risk score less than or equal to 1 for the 5 validated SNPs. We further examined the relation of the genetic risk score with BMI measured at age 20 years and at study enrollment. Controls were women with normal weight at both age 20 years (BMI 18–<23) and study enrollment (BMI 18.5–<23). Two case groups were created. Case group 1 had normal weight at age 20 years but were obese at study enrollment (BMI $\geq$27.5). Case group 2 were overweight or obese at age 20 years (BMI $\geq$23) and obese at study enrollment. We also evaluated whether genetic factors modified the associations of energy intake (quartiles) and physical activity (any exercise vs. no exercise) with obesity. Multiplicative interactions between the genetic risk score and daily calorie intake or exercise were examined using the log likelihood ratio test, which compared the model including only the main effects with the model that included both the main effects and the interactive terms. *P* values presented in this paper were not corrected for multiple tests.

RESULTS

Table 1 shows selected characteristics of the women included in the present study. Because of the late age of onset, cases with type 2 diabetes were significantly older and thus more likely to be postmenopausal than breast cancer patients and their matched controls. Energy intakes and the proportion of nonexercisers were higher in cancer cases than in controls or in type 2 diabetes cases. As expected, healthy controls had the lowest BMI at study enrollment, followed by breast cancer cases and diabetes cases. However, no significant differences in BMI at age 20 years were observed among these 3 groups. Compared with breast cancer cases and community controls from the Shanghai Breast Cancer Study, incident type 2 diabetes cases from the Shanghai Women's Health Study had a significantly higher waist circumference, hip circumference, and waist: hip ratio ($P < 0.05$, Student's *t* tests).

All SNPs were in Hardy-Weinberg equilibrium and had a *P* value greater than 0.10, except for rs7138803 ($P = 0.02$). SNPs at 5 loci—human leukocyte antigen B (HLA-B)-associated transcript 2 (*BAT2*) rs2844479, *FTO* rs9939609, *MC4R* rs17782313, SEC16 homolog B (*Saccharomyces cerevisiae*) (*SEC16B*) rs10913469, and SH2B adaptor protein 1 (*SH2B1*) rs7498665—were found to be associated with both BMI and body weight in the combined sample set at $P < 0.05$ (Table 2). The direction of the association was consistent across all 3 sample sets (i.e., breast cancer cases, controls, and type 2 diabetes cases), although not all individual risk estimates were statistically significant, perhaps because of small sample sizes. Similarly, these 5 SNPs were significantly associated with obesity (Table 3). The association direction for BMI, body weight, and obesity was consistent with initial GWA study findings from European-ancestry populations. The per-allele effect on BMI was generally comparable as well (European

Table 3. Odds Ratios for Associations Between Reportedly Obesity-Related Single Nucleotide Polymorphisms and Obesity in Chinese Women, 1996–2007

SNP	Normal Weight (n = 1,863)		Obesity (n = 830)		AB vs. AA		BB vs. AA		P Value[a]
	Effective Allele Frequency (Proportion)	AA/AB/BB[b]	Effective Allele Frequency (Proportion)	AA/AB/BB[b]	OR[c]	95% CI	OR[c]	95% CI	
rs2815752	0.92	9/261/1,593	0.92	4/123/703	0.90	0.25, 3.28	0.89	0.25, 3.17	0.87
rs10913469	0.19	1,240/547/72	0.23	480/311/38	1.40	1.14, 1.71	1.38	0.85, 2.24	1.6×10^{-3}
rs6548238	0.90	16/332/1,515	0.91	10/126/694	0.56	0.23, 1.37	0.59	0.25, 1.43	0.91
rs7647305	0.95	4/186/1,669	0.95	1/76/752	3.47	0.23, 53.0	3.21	0.21, 48.3	0.82
rs10938397	0.29	912/798/149	0.31	389/366/74	0.91	0.74, 1.11	1.04	0.73, 1.47	0.69
rs6235	0.33	811/858/190	0.34	349/390/90	1.15	0.94, 1.40	1.16	0.84, 1.61	0.18
rs4712652	0.12	1,428/409/22	0.12	641/178/10	0.91	0.72, 1.15	1.16	0.48, 2.81	0.56
rs2844479	0.54	379/934/546	0.58	154/392/283	1.11	0.86, 1.44	1.46	1.12, 1.92	3.3×10^{-3}
rs4074134	0.55	372/929/558	0.57	137/437/255	1.34	1.04, 1.74	1.21	0.91, 1.60	0.35
rs10838738	0.32	858/808/193	0.35	354/371/104	1.10	0.90, 1.35	1.29	0.95, 1.76	0.10
rs7138803	0.28	943/802/118	0.27	441/334/55	0.92	0.76, 1.12	1.13	0.76, 1.69	0.89
rs7498665	0.11	1,485/347/27	0.14	614/199/16	1.37	1.09, 1.72	1.70	0.84, 3.46	2.7×10^{-3}
rs9939609	0.11	1,478/360/25	0.14	621/186/23	1.23	0.97, 1.54	2.04	1.03, 4.04	0.01
rs1424233	0.67	200/836/823	0.67	90/373/366	1.04	0.76, 1.44	1.01	0.73, 1.40	0.95
rs1805081	0.24	1,064/693/102	0.22	498/294/37	0.91	0.75, 1.12	0.68	0.43, 1.06	0.10
rs17782313	0.20	1,193/607/63	0.25	464/317/49	1.22	1.00, 1.49	2.16	1.39, 3.37	7.1×10^{-4}
rs29941	0.23	1,099/661/101	0.22	496/295/38	1.06	0.87, 1.30	0.86	0.55, 1.33	0.98

Abbreviations: CI, confidence interval; OR, odds ratio; SNP, single nucleotide polymorphism.

[a] P value from an additive logistic regression model adjusted for age, age squared, menopausal status, and sample set. P values were not corrected for multiple tests.

[b] AA indicates homozygosity for the non-risk allele A, AB indicates heterozygosity, and BB indicates homozygosity for the risk allele B (effective allele in Table 2). Four controls and 1 obese case were excluded from the analyses for SNPs not on the Affymetrix array, because they were among 8 samples not imputed for genotypes.

[c] Adjusted for age, age squared, menopausal status, and sample set.

ancestry: 0.15–0.56 units; Chinese: 0.16–0.38 units). We did not find the remaining 12 SNPs to be associated with BMI or body weight.

To estimate the cumulative effect of genetic risk factors on obesity, we constructed 2 genetic risk scores, one by summing the number of risk-increasing alleles each woman carried for all 17 SNPs and one for the 5 SNPs validated in the current study. The odds ratio per risk allele was 1.08 (95% confidence interval (CI): 1.04, 1.13; $P = 1.1 \times 10^{-4}$) for the genetic risk score comprised of 17 SNPs and 1.27 (95% CI: 1.18, 1.37; $P = 1.9 \times 10^{-10}$) for the genetic risk score comprised of the 5 confirmed SNPs in the combined sample set (Table 4). Women carrying 5 or more risk alleles had an odds ratio of 3.13 (95% CI: 2.06, 4.77) compared with women who carried 1 or none of the risk alleles. Similar gene-dose association patterns were seen in postmenopausal and premenopausal women (Table 4). Furthermore, the 5-SNP genetic risk score was also found to be associated with early onset of obesity. Compared with women who had normal weight at both age 20 years and study enrollment, women who had normal weight at age 20 years but were obese at study enrollment were 2.65 times more likely (95% CI: 1.48, 4.77) to carry 5 or more risk alleles, while women who were overweight or obese at age 20 years and were also obese at study enrollment were 11.0 times more likely (95% CI: 4.68, 25.9) to carry 5 or more risk alleles (Table 5).

We conducted additional analyses to evaluate the joint effects of genetic risk score, energy intake, and exercise on obesity. We found that the genetic risk score was associated with a higher prevalence of obesity among women with higher energy intake or who did not participate in exercise than in those with lower energy intake or who were exercisers. Similarly, the obesity-energy and diet-exercise associations were also stronger among women with a high genetic risk score than among women with a low genetic risk score. However, we did not observe any effect of the multiplicative interaction between genetic risk score and calorie intake or physical activity on obesity risk (data not shown).

DISCUSSION

In this study, we validated 5 obesity loci discovered in previous GWA studies. The effect of these obesity loci appears to follow an additive pattern. Women who carried 1 risk allele at any of these 5 loci had an approximately 27% increase in the prevalence of obesity. Women who carried 5 or more risk alleles had a 3.13-fold increased prevalence of

Table 4. Association Between Genetic Risk Score and Estimated Risk of Obesity in Chinese Women, 1996–2007

No. of SNPs Used for GRS Computation and GRS[a]	All Samples[b]						Premenopausal Women		Postmenopausal Women	
	Normal Weight		Obesity		OR	95% CI				
	No.	%	No.	%			OR	95% CI	OR	95% CI
17 tested SNPs										
Continuous variable	1,859	100	829	100	1.08	1.04, 1.13	1.10	1.03, 1.17	1.07	1.01, 1.13
P value[c]					1.1×10^{-4}		2.5×10^{-3}		0.02	
Categorical variable										
≤11	278	15.0	82	9.9	1.00	Reference	1.00	Reference	1.00	Reference
12	236	12.7	99	11.9	1.40	0.95, 2.06	1.26	0.68, 2.34	1.46	0.87, 2.43
13	271	14.6	124	15.0	1.53	1.05, 2.22	1.81	1.03, 3.19	1.30	0.79, 2.14
14	338	18.2	125	15.1	1.22	0.85, 1.76	1.48	0.86, 2.56	1.01	0.61, 1.66
15	278	15.0	137	16.5	1.62	1.13, 2.35	2.08	1.21, 3.58	1.25	0.75, 2.07
16	212	11.4	112	13.5	1.73	1.18, 2.56	1.78	1.00, 3.18	1.66	0.98, 2.83
17	129	6.9	80	9.7	2.08	1.35, 3.20	2.47	1.30, 4.70	1.81	1.01, 3.23
≥18	117	6.3	70	8.4	1.79	1.15, 2.80	1.78	0.92, 3.45	1.80	0.97, 3.34
5 validated SNPs										
Continuous variable	1,859	100	829	100	1.27	1.18, 1.37	1.28	1.15, 1.42	1.27	1.14, 1.41
P value[c]					1.9×10^{-10}		5.5×10^{-6}		7.1×10^{-6}	
Categorical variable										
≤1	501	27.0	156	18.8	1.00	Reference	1.00	Reference	1.00	Reference
2	596	32.1	235	28.4	1.20	0.92, 1.57	1.11	0.74, 1.66	1.26	0.88, 1.81
3	463	24.9	218	26.3	1.49	1.13, 1.97	1.55	1.03, 2.33	1.46	1.00, 2.13
4	218	11.7	147	17.7	2.07	1.50, 2.84	2.29	1.45, 3.60	1.87	1.20, 2.92
≥5	81	4.4	73	8.8	3.13	2.06, 4.77	2.43	1.34, 4.42	4.28	2.25, 8.14

Abbreviations: CI, confidence interval; GRS, genetic risk score; OR, odds ratio; SNP, single nucleotide polymorphism.

[a] Number of effective alleles. Controls had normal weight (body mass index (weight (kg)/height (m)^2) 18.5–<23) at study enrollment, while obesity was defined as a body mass index greater than or equal to 27.5 for participants without breast cancer at study enrollment and for cancer cases 1 year before diagnosis.

[b] Four controls and 1 obese case with missing genotypes were excluded from GRS analyses. The GRS consisted of 5 validated SNPs. The frequencies of GRS = 0 in the normal-weight and obesity groups were 6.1% and 4.1%, respectively. These samples (GRS = 0) were combined with those with GRS = 1 as the reference group.

[c] *P* values were derived from logistic regression analyses under the additive model and were adjusted for age, age squared, and sample set for stratified samples (additionally adjusted for menopausal status for all samples).

obesity as compared with women who carried 1 or none of the risk alleles (Table 4).

Among the 5 validated obesity loci in our study, 2 are located in regions of the *MC4R* and *FTO* genes, the 2 genes most consistently reported as being related to obesity. The locus near *MC4R*, the gene that encodes the melanocortin 4 receptor, was first identified by combining data from 7 GWA studies ($n = 16{,}876$) and was replicated in 60,352 persons from 13 additional studies (11). This *MC4R* locus was subsequently confirmed in 4 other obesity GWA studies (12, 14, 15, 21), a GWA study on waist circumference and insulin resistance (8), a cohort study conducted in northern Sweden (22), and a Danish population-based analysis (23). The *FTO* gene has been reported to increase obesity risk in 7 GWA studies (9, 10, 12–15, 24) and has been validated in multiple candidate gene studies (22, 25–39), with 1 exception (40).

Of the 3 other validated obesity loci in our study, *BAT2*, *SEC16B*, and *SH2B1*, the *SH2B1* locus was independently identified in 2 GWA studies (14, 15). Identification of this locus was subsequently replicated in the northern Sweden cohort study (22) and in a recent GWA scan for extreme obesity loci (24). No validation of these 3 loci has been published to date.

It has been reported that the *MC4R* and *FTO* genes increase risk for childhood obesity (12, 29). Studies have also suggested that the A allele of rs9939609 in the first intron of the *FTO* gene and the C allele of rs17782313 near the *MC4R* gene are both associated with higher caloric intake (24, 41) and rs17782313 with fat intake (41). A recent report found that a boy carrying a null mutation of *MC4R* was hyperphagic and gained weight rapidly in the first months of life (42). These data suggested that the *FTO* and *MC4R* variants may predispose carriers to obesity at a young age, possibly by controlling food intake and/or energy expenditure, as demonstrated in transgenic mice (43, 44). In our study, we found that both loci were strongly associated with overweight at age 20 years among currently obese women (for

Table 5. Genetic Risk Scores for 5 Validated Loci and Estimated Risk of Obesity at Age 20 Years and at Study Enrollment in Chinese Women, 1996–2007[a]

Genetic Risk Score	Controls		Case Group 1		Case Group 1 vs. Controls		Case Group 2		Case Group 2 vs. Controls	
	No.	%	No.	%	OR	95% CI	No.	%	OR	95% CI
Continuous variable	1,004	100	521	100	1.25	1.14, 1.39	98	100	1.60	1.35, 1.89
P value[b]					6.7×10^{-6}				5.9×10^{-8}	
Categorical variable										
≤1	266	26.5	98	18.8	1.00	Reference	14	14.3	1.00	Reference
2	338	33.7	158	30.3	1.16	0.82, 1.64	29	29.6	1.70	0.84, 3.41
3	242	24.1	129	24.8	1.44	0.99, 2.08	19	19.4	1.89	0.89, 4.03
4	114	11.4	100	19.2	2.15	1.42, 3.25	18	18.4	3.54	1.61, 7.77
≥5	44	4.4	36	6.9	2.65	1.48, 4.77	18	18.4	11.0	4.68, 25.9

Abbreviations: BMI, body mass index; CI, confidence interval; OR, odds ratio.

[a] This analysis was restricted to women whose phenotypes were defined by BMI (weight (kg)/height $(m)^2$) at age 20 years and at study enrollment. The controls had normal weight at age 20 years (BMI 18–<23) and at study enrollment (BMI 18.5–<23); case group 1 had normal weight at age 20 years and were obese (BMI ≥27.5) at study enrollment; and case group 2 were overweight (BMI ≥23) or obese at age 20 years and obese at study enrollment.

[b] Adjusted for sample set.

the A allele of rs9939609 in *FTO*, odds ratio (OR) = 1.65, 95% CI: 1.07, 2.56 ($P = 0.02$); for the C allele of rs17782313 near *MC4R*, OR = 1.94, 95% CI: 1.35, 2.81 ($P = 4.0 \times 10^{-4}$)) (data not shown in tables). The *SH2B1* gene is located at another obesity-related locus, which was confirmed in our study. This gene is highly expressed in the hypothalamus (45). Recent observations suggest that the neuronal SH2B1 protein regulates energy balance and glucose homeostasis, possibly by enhancing hypothalamic leptin signaling (46, 47). In addition, *SH2B1*-knockout mice are hyperphagic and obese (48), and some severe, early-onset obese patients carry de novo 16p11.2 deletions that encompass the *SH2B1* gene (49). The biologic mechanism linking the *BAT2* and *SEC16B* genes to obesity is largely unknown, but *BAT2* is highly expressed in the hypothalamus (45). In our study, we found that the prevalence of obesity, particularly early-onset obesity, increased with the number of risk alleles women carried for these 5 loci, suggesting that these genes may each contribute to individual susceptibility, though not necessarily via the same mechanism.

Obesity is a disorder of imbalance between food intake and energy expenditure. Recent findings from genetic epidemiology studies suggest that genetic factors play a role in regulating energy balance, possibly by modifying appetite and food intake and/or lifestyle (50, 51). As described previously, the C allele of rs17782313 near the *MC4R* gene has been associated with higher intakes of total energy and dietary fat in women (41), as well as overeating in obese Europeans (52). Low physical activity has been found to strengthen the association of the *FTO* SNP rs1421085 (but not the *MC4R* SNP rs17782313) with obesity in French adults (53). In our study, we found suggestive signals that the genetic risk score was related to a higher prevalence of obesity among women who had high energy intake and did not participate in exercise compared with women who had lower energy intake or exercised. Similarly, energy intake and exercise were more strongly associated with obesity among women with a high genetic risk score than among women with a low genetic risk score. However, the tests for multiplicative interaction between the genetic risk score comprised of the 5 validated loci and energy intake or exercise did not reach statistical significance, nor did we detect significant interaction between single SNP markers and calorie intake or exercise (data not shown). Nevertheless, our study had limited power to evaluate moderate interactions.

The 12 other previously identified obesity loci were not validated in our population of Chinese women. None of these loci has been validated in populations other than the original GWA discovery study. There are several possible reasons why these 12 obesity loci were not validated in the current study. First, most of these SNPs are noncausative genetic variants. The distinct linkage disequilibrium structures and allele frequency differences for genetic variants between populations of European and Chinese ancestry may mask the underlying causal SNPs. Second, the sample sizes both in previous studies and in our replication study may not have been large enough to detect genetic variants with modest effects or interactions between genetic and environmental factors (54–56). Finally, the possibility of population-specific genetic association cannot be ruled out.

Our study included 3 sets of women: women with breast cancer, women who were healthy at study enrollment but developed type 2 diabetes during follow-up, and healthy control women. Since obesity is highly prevalent among type 2 diabetes patients and a GWA study has identified *FTO* rs9939609 as a type 2 diabetes locus (57), there is a concern as to whether the associations found in the current study were caused by type 2 diabetes. We evaluated the association of the 5 confirmed obesity-related SNPs with type 2 diabetes in our study using controls from the Shanghai Breast Cancer Study as the comparison group (none had diabetes). In models adjusted for age and menopausal status, *MC4R* rs17782313 was significantly associated with type 2 diabetes risk (OR = 1.20, 95% CI: 1.05, 1.37; $P = 9.3 \times 10^{-3}$). When the results were additionally adjusted for BMI, the association between type 2 diabetes and *MC4R* rs17782313

disappeared ($P = 0.58$), but *BAT2* rs2844479 (OR = 0.85, 95% CI: 0.75, 0.96; $P = 8.1 \times 10^{-3}$) and *SH2B1* rs7498665 (OR = 0.81, 95% CI: 0.67, 0.98; $P = 0.03$) were inversely related to type 2 diabetes risk. Taken together, these data suggest that not all observed SNP-obesity associations were caused by the SNP-type 2 diabetes association.

Our study had several strengths. First, anthropometric measurements were taken by trained interviewers using a standard protocol, which minimized measurement errors. Second, our study population was relatively homogeneous. Over 97% were Han Chinese, which reduced confounding from genetic admixture. Third, dietary intake and physical activity information, collected using validated questionnaires, was available for evaluating the joint effect of genes and energy balance. Last, our study's power to detect common SNPs with a moderate effect on obesity risk was reasonably good. For example, under an additive genetic model and at a significance level of $P < 0.05$, our study had 80% power to detect odds ratios greater than 1.27, 1.18, and 1.16 for SNPs with allele frequencies of 0.1, 0.3, and 0.5, respectively. However, our study had limited statistical power to evaluate SNPs with low allele frequency or modest gene-gene and gene-environment interactions. In addition, our genetic risk score was generated from the same study population in which the individual SNPs were evaluated, which may have resulted in an overly optimistic estimate. Our findings for the genetic risk score should be validated in an independent study setting.

In summary, we validated 5 loci (*BAT2*, *FTO*, *MC4R*, *SEC16B*, and *SH2B1*) for obesity-related phenotypes in adult Chinese women. Results from this study confirmed that women of European and Chinese ancestry share some genetic predispositions to obesity. We found that a summarized genetic risk score predicts obesity prevalence, particularly for early onset of obesity. The genetic risk score also appears to be related to individual susceptibility to high energy intake and low physical activity. If it is proven to be valid, this genetic risk score may serve as a potential marker for identifying populations at high risk of obesity for targeted disease intervention.

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REFERENCES

1. Haslam DW, James WP. Obesity. *Lancet*. 2005;366(9492):1197–1209.
2. James PT. Obesity: the worldwide epidemic. *Clin Dermatol*. 2004;22(4):276–280.
3. Wyatt SB, Winters KP, Dubbert PM. Overweight and obesity: prevalence, consequences, and causes of a growing public health problem. *Am J Med Sci.* 2006;331(4):166–174.
4. Barsh GS, Farooqi IS, O'Rahilly S. Genetics of body-weight regulation. *Nature*. 2000;404(6778):644–651.
5. Maes HH, Neale MC, Eaves LJ. Genetic and environmental factors in relative body weight and human adiposity. *Behav Genet.* 1997;27(4):325–351.
6. Rankinen T, Zuberi A, Chagnon YC, et al. The human obesity gene map: the 2005 update. *Obesity (Silver Spring)*. 2006;14(4):529–644.
7. Benzinou M, Creemers JW, Choquet H, et al. Common nonsynonymous variants in *PCSK1* confer risk of obesity. *Nat Genet.* 2008;40(8):943–945.
8. Chambers JC, Elliott P, Zabaneh D, et al. Common genetic variation near *MC4R* is associated with waist circumference and insulin resistance. *Nat Genet.* 2008;40(6):716–718.
9. Frayling TM, Timpson NJ, Weedon MN, et al. A common variant in the *FTO* gene is associated with body mass index and predisposes to childhood and adult obesity. *Science*. 2007;316(5826):889–894.
10. Hinney A, Nguyen TT, Scherag A, et al. Genome wide association (GWA) study for early onset extreme obesity supports the role of fat mass and obesity associated gene (*FTO*) variants. *PLoS One*. 2007;2(12):e1361.
11. Loos RJ, Lindgren CM, Li S, et al. Common variants near *MC4R* are associated with fat mass, weight and risk of obesity. *Nat Genet.* 2008;40(6):768–775.
12. Meyre D, Delplanque J, Chèvre JC, et al. Genome-wide association study for early-onset and morbid adult obesity identifies three new risk loci in European populations. *Nat Genet.* 2009;41(2):157–159.

13. Scuteri A, Sanna S, Chen WM, et al. Genome-wide association scan shows genetic variants in the *FTO* gene are associated with obesity-related traits. *PLoS Genet.* 2007;3(7):e115.
14. Thorleifsson G, Walters GB, Gudbjartsson DF, et al. Genome-wide association yields new sequence variants at seven loci that associate with measures of obesity. *Nat Genet.* 2009; 41(1):18–24.
15. Willer CJ, Speliotes EK, Loos RJ, et al. Six new loci associated with body mass index highlight a neuronal influence on body weight regulation. *Nat Genet.* 2009;41(1):25–34.
16. Zheng W, Long J, Gao YT, et al. Genome-wide association study identifies a new breast cancer susceptibility locus at 6q25.1. *Nat Genet.* 2009;41(3):324–328.
17. Purcell S, Neale B, Todd-Brown K, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet.* 2007;81(3):559–575.
18. Zheng W, Chow WH, Yang G, et al. The Shanghai Women's Health Study: rationale, study design, and baseline characteristics. *Am J Epidemiol*. 2005;162(11):1123–1131.
19. WHO Expert Consultation. Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies. *Lancet*. 2004;363(9403):157–163.
20. Morrison AC, Bare LA, Chambless LE, et al. Prediction of coronary heart disease risk using a genetic risk score: the Atherosclerosis Risk in Communities Study. *Am J Epidemiol*. 2007;166(1):28–35.
21. Cho YS, Go MJ, Kim YJ, et al. A large-scale genome-wide association study of Asian populations uncovers genetic factors influencing eight quantitative traits. *Nat Genet.* 2009; 41(5):527–534.
22. Renström F, Payne F, Nordström A, et al. Replication and extension of genome-wide association study results for obesity in 4923 adults from northern Sweden. *Hum Mol Genet.* 2009;18(8):1489–1496.
23. Zobel DP, Andreasen CH, Grarup N, et al. Variants near *MC4R* are associated with obesity and influence obesity-related quantitative traits in a population of middle-aged people: studies of 14,940 Danes. *Diabetes*. 2009;58(3): 757–764.
24. Cotsapas C, Speliotes EK, Hatoum IJ, et al. Common body mass index-associated variants confer risk of extreme obesity. *Hum Mol Genet.* 2009;18(18):3502–3507.
25. Chu X, Erdman R, Susek M, et al. Association of morbid obesity with *FTO* and *INSIG2* allelic variants. *Arch Surg*. 2008;143(3):235–240.
26. Cecil JE, Tavendale R, Watt P, et al. An obesity-associated *FTO* gene variant and increased energy intake in children. *N Engl J Med*. 2008;359(24):2558–2566.
27. Cha SW, Choi SM, Kim KS, et al. Replication of genetic effects of *FTO* polymorphisms on BMI in a Korean population. *Obesity (Silver Spring)*. 2008;16(9):2187–2189.
28. Chang YC, Liu PH, Lee WJ, 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*. 2008;57(8):2245–2252.
29. Dina C, Meyre D, Gallina S, et al. Variation in *FTO* contributes to childhood obesity and severe adult obesity. *Nat Genet.* 2007;39(6):724–726.
30. Grant SF, Li M, Bradfield JP, et al. Association analysis of the *FTO* gene with obesity in children of Caucasian and African ancestry reveals a common tagging SNP. *PLoS One*. 2008; 3(3):e1746.
31. Hotta K, Nakata Y, Matsuo T, et al. Variations in the *FTO* gene are associated with severe obesity in the Japanese. *J Hum Genet.* 2008;53(6):546–553.
32. Marvelle AF, Lange LA, Qin L, et al. Association of *FTO* with obesity-related traits in the Cebu Longitudinal Health and Nutrition Survey (CLHNS) Cohort. *Diabetes*. 2008;57(7): 1987–1991.
33. Ng MC, Park KS, Oh B, et al. Implication of genetic variants near *TCF7L2, SLC30A8, HHEX, CDKAL1, CDKN2A/B, IGF2BP2*, and *FTO* in type 2 diabetes and obesity in 6,719 Asians. *Diabetes*. 2008;57(8):2226–2233.
34. Ohashi J, Naka I, Kimura R, et al. *FTO* polymorphisms in oceanic populations. *J Hum Genet.* 2007;52(12):1031–1035.
35. Sanghera DK, Ortega L, Han 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.* 2008;9:59.
36. Song Y, You NC, Hsu YH, et al. *FTO* polymorphisms are associated with obesity but not diabetes risk in postmenopausal women. *Obesity (Silver Spring)*. 2008;16(11):2472–2480.
37. Tan JT, Dorajoo R, Seielstad M, et al. *FTO* variants are associated with obesity in the Chinese and Malay populations in Singapore. *Diabetes*. 2008;57(10):2851–2857.
38. Villalobos-Comparán M, Teresa Flores-Dorantes M, Teresa Villarreal-Molina M, et al. The *FTO* gene is associated with adulthood obesity in the Mexican population. *Obesity (Silver Spring)*. 2008;16(10):2296–2301.
39. Zhang F, Xu L, Jin L, et al. A common variant in the *FTO* gene is associated with obesity in the Uyghur population. *J Endocrinol Invest*. 2008;31(11):1043.
40. Li H, Wu Y, Loos RJ, et al. Variants in the fat mass- and obesity-associated (*FTO*) gene are not associated with obesity in a Chinese Han population. *Diabetes*. 2008;57(1):264–268.
41. Qi L, Kraft P, Hunter DJ, et al. The common obesity variant near *MC4R* gene is associated with higher intakes of total energy and dietary fat, weight change and diabetes risk in women. *Hum Mol Genet.* 2008;17(22):3502–3508.
42. Dubern B, Bisbis S, Talbaoui H, et al. Homozygous null mutation of the melanocortin-4 receptor and severe early-onset obesity. *J Pediatr.* 2007;150(6):613–617, 617.e1.
43. Balthasar N, Dalgaard LT, Lee CE, et al. Divergence of melanocortin pathways in the control of food intake and energy expenditure. *Cell.* 2005;123(3):493–505.
44. Fischer J, Koch L, Emmerling C, et al. Inactivation of the *Fto* gene protects from obesity. *Nature*. 2009;458(7240):894–898.
45. Hofker M, Wijmenga C. A supersized list of obesity genes. *Nat Genet.* 2009;41(2):139–140.
46. Ren D, Zhou Y, Morris D, et al. Neuronal *SH2B1* is essential for controlling energy and glucose homeostasis. *J Clin Invest*. 2007;117(2):397–406.
47. Li Z, Zhou Y, Carter-Su C, et al. *SH2B1* enhances leptin signaling by both Janus kinase 2 Tyr813 phosphorylation-dependent and -independent mechanisms. *Mol Endocrinol*. 2007;21(9):2270–2281.
48. Ren D, Li M, Duan C, et al. Identification of *SH2-B* as a key regulator of leptin sensitivity, energy balance, and body weight in mice. *Cell Metab.* 2005;2(2):95–104.
49. Bochukova EG, Huang N, Keogh J, et al. Large, rare chromosomal deletions associated with severe early-onset obesity. *Nature*. 2010;463(7281):666–670.
50. Blakemore AI, Froguel P. Is obesity our genetic legacy? *J Clin Endocrinol Metab.* 2008;93(11 suppl 1):S51–S56.
51. Lam DD, Farooqi IS, Heisler LK. Melanocortin receptors as targets in the treatment of obesity. *Curr Top Med Chem.* 2007; 7(11):1085–1097.
52. Stutzmann F, Cauchi S, Durand E, et al. Common genetic variation near *MC4R* is associated with eating behaviour

patterns in European populations. *Int J Obes (Lond)*. 2009; 33(3):373–378.
53. Cauchi S, Stutzmann F, Cavalcanti-Proença C, et al. Combined effects of *MC4R* and *FTO* common genetic variants on obesity in European general populations. *J Mol Med*. 2009;87(5):537–546.
54. Chanock SJ, Manolio T, Boehnke M, et al. Replicating genotype-phenotype associations. NCI-NHGRI Working Group on Replication in Association Studies. *Nature*. 2007; 447(7145):655–660.
55. Ioannidis JP. Non-replication and inconsistency in the genome-wide association setting. *Hum Hered*. 2007;64(4): 203–213.
56. Kraft P. Curses—winner's and otherwise—in genetic epidemiology. *Epidemiology*. 2008;19(5):649–651.
57. Wellcome Trust Case Control Consortium. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. *Nature*. 2007;447(7145): 661–678.