Genome-Wide Linkage and Regional Association Study of Obesity-Related Phenotypes: The GenSalt Study

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Objective: To identify chromosomal regions harboring quantitative trait loci for waist circumference (WC) and body mass index (BMI).

Design and Methods: A genome-wide linkage scan and regional association study WC and BMI among 633 Chinese families was conducted.

Results: A significant linkage signal for WC was observed at 22q13.31-22q13.33 in the overall analysis (LOD = 3.13). Follow-up association study of 22q13.31-13.33 revealed an association between the TBC1D22A gene marker rs16996195 and WC (false discovery rate [FDR]-Q < 0.05). In gender-stratified analysis, suggestive linkage signals were attained for WC at 2p24.3-2q12.2 and 22q13.33 among females (LOD = 2.54 and 2.15, respectively). Among males, 6q12-6q13 was suggestively linked to BMI (LOD = 2.03). Single marker association analyses at these regions identified male-specific relationships of six single nucleotide polymorphisms (SNPs) at 2p24.3-2q12.2 (rs100955, rs13020676, rs13014034, rs12990515, rs17024325, and rs2192712) and five SNPs at 6q12-6q13 (rs7747318, rs7767301, rs12197115, rs12203049, and rs945847) with the obesity-related phenotypes (all FDR-Q < 0.05). At chromosome 6q12-6q13, markers rs7755450 and rs11758293 predicted BMI in females (both FDR-Q < 0.05).

Conclusions: Genomic regions on chromosomes 2, 6, and 22 which may harbor important obesity-susceptibility loci were described. Follow-up study of these regions revealed several novel variants associated with obesity related traits. Future work to confirm these promising findings is warranted.

Introduction

Obesity is a major risk factor for mortality globally because of its high prevalence and the concomitant increase in risk of type 2 diabetes, hypertension, certain cancers, and cardiovascular disease (1-3). Its development arises from a combination of environmental and genetic factors, along with their interactions. The genetic determinants of obesity-related phenotypes have been investigated extensively both in early heritability and linkage analyses (4,5) and more recently in candidate gene and genome-wide association studies (GWAS) (6,7). Still, our understanding of the genetic architecture of obesity remains limited, with currently identified variants explaining only a small proportion of its estimated heritability (4). Furthermore, while many GWAS have examined body mass index (BMI) (6-9) only a handful have examined waist circumference (WC) (10-13). Further nonhypothesis-driven research that takes advantage of dense single nucleotide polymorphism (SNP) data will be needed to
identify novel genetic variants and biological pathways with important influences on obesity susceptibility. The knowledge gained from this type of research may provide important insights into the biological mechanisms underlying obesity susceptibility, facilitate the discovery of novel drug targets for its treatment, and enable better prediction of individual obesity risks.

The objective of the current study was to identify chromosomal regions harboring quantitative trait loci (QTL) for WC and BMI by conducting a genome-wide linkage scan in a large, homogeneous sample of 3142 Han Chinese participants from 633 families included in the Genetic Epidemiology Network of Salt-Sensitivity (GenSalt) study. To localize signals for WC and BMI, the genome-wide scans were followed-up by fine-mapping promising linkage regions with a dense panel of SNPs and testing their association with the obesity-related traits.

Methods

Study population

The GenSalt study is a unique dietary feeding study designed to examine gene-dietary sodium and potassium interactions on blood pressure (BP). The GenSalt study includes 3142 Han Chinese participants from 633 families recruited from six field centers located in rural areas of northern China. A detailed description of the study design and participants has been presented elsewhere (14). In brief, probands and their families were identified through a community-based BP screening conducted among persons aged 18-60 years in the study villages. Probands with a mean systolic BP of 130-160 mm Hg and/or a mean diastolic BP between 85 and 100 mm Hg and no use of antihypertensive medications were recruited for the study, along with their siblings, spouses, parents, and offspring. After exclusion of parents from the GenSalt dietary feeding, a total of 1906 probands, siblings, spouses, and offspring participated in the 7-day low-sodium, 7-day high-sodium, and 7-day high-sodium plus potassium feeding study.

Phenotype measurement

All 3142 GenSalt participants underwent a 3-day baseline examination. During this period, a standardized questionnaire was administered by trained staff to gather information on demographic characteristics, family pedigree, personal and family medical history, and lifestyle risk factors. Height was measured twice on the second day of baseline observation with the participant standing on a firm level surface using a height board mounted at a 90° angle to a calibrated vertical height bar. Body weight was measured twice using a balance beam or digital scale on the second and third days of baseline observations in the morning before breakfast. WC measurements were taken twice on the second day of baseline observation using an anthropometric measuring tape, 1 cm above the navel. All measurements were obtained by trained GenSalt staff and with the study participants in light indoor clothing. If two measurements differed by ≥ 0.2 cm for height and WC or by ≥ 0.5 kg for weight, these measures were repeated. The means of the two height, four weight, and two WC measures were used for the analysis. BMI was calculated as weight in kilograms divided by height in meters squared (kg/m²).

Microsatellite marker and SNP genotyping

Lymphocyte DNA samples were collected from all 3142 GenSalt family members (proband, parents, spouses, siblings, and offspring) and used for genotyping microsatellite markers spaced at approximately 9 cm intervals across the genome (407 markers, Marshfield Screening Set 12). Fluorescently labeled PCR primers were used for marker amplification followed by capillary electrophoresis on an automated DNA sequencer (ABI 3730 DNA Analyser, Applied Biosystems, Foster City, USA). Quality control samples included blind duplicates, no DNA controls and CEPH DNA standards (mother, father, and offspring with known genotypes). Genotypes were assigned with the GeneMapper software (ABI). ASPEX and GRR were used to check for potential misreported relationships in the GenSalt pedigrees. MapMaker/Sibs and PedCheck were used to check for Mendelian inconsistencies within families for each marker.

SNPs located in promising linkage regions were genotyped among a subsample of 1906 GenSalt probands, siblings, spouses, and offspring who took part in the dietary intervention using chip-based hybridization assays (Affymetrix 6.0, In Santa Clara, CA). SNPs that had a call rate greater than 85%, were in Hardy Weinberg Equilibrium after adjustment for multiple comparisons (False Discovery Rate [FDR] Q-value < 0.05 [corresponding to a P < 2.75 × 10⁻⁴]), and had a minor allele frequency (MAF) greater than 1% were used for the statistical analysis. Data quality control revealed 59 SNPs with a low call rate, 154 SNPs, which deviated from Hardy Weinberg Equilibrium, and 5538 SNPs with low MAF. After the exclusion of these SNPs, 25,659 SNPs remained for the analysis.

Statistical analysis

The mean or percent of important covariables and obesity-related phenotypes was calculated for all study participants. Multipoint identity by descent estimates were calculated by Merlin software. Multipoint quantitative trait linkage-analyses of adjusted WC and BMI phenotypes were performed using SOLAR software. For the adjustment, each phenotype was regressed on covariates including, age, gender, and field center, in a stepwise fashion, and only significant terms (P < 0.05) were retained. The residual variance was examined by regressing the square residual from the first regression on the covariates (stepwise) and retaining significant terms. The final adjusted phenotype was computed as the residual from the first regression divided by the square root of the predicted score from the second regression. A final standardization step was taken to ensure a mean of 0 and a standard deviation of 1 for all the adjusted phenotypes.

Additive associations between single SNPs located in promising linkage regions (LOD > 3) and WC and BMI were assessed using a mixed linear regression model to account for familial correlations. The same covariates used in the linkage analysis, age, gender, and field center, were adjusted in the multivariable analysis. To adjust for multiple comparisons, the raw P-value was adjusted using the FDR method. For SNPs with an FDR Q-value < 0.05, we estimated the mean WC and BMI (95% confidence interval [CI]) for each genotype using a mixed linear regression model. The association analysis was conducted using SAS software (version 9.2; SAS Institute). Pairwise r² values between significant SNPs in each linkage region were assessed using Haplovview software (15). Finally, because there may be important gender differences in the genetic etiology of obesity-related phenotypes, we conducted additional linkage and association analyses stratified by gender. As a result of the limited power of gender-stratified analyses, promising linkage regions for follow-up association study were defined as those with LOD > 2. Additional linkage analysis conditional on significant SNPs were
performed to determine if there is a lowered LOD score for each respective region.

**Results**

The baseline characteristics of 3142 participants, including 676 probands, 69 spouses, 1236 parents, 956 siblings, and 205 offspring from 633 families are presented in Table 1. Age ranged from 23.5 years in offspring to 67.6 years in parents, and the percent male ranged from 33.3% among spouses to 60.4% among probands. GenSalt participants were relatively lean, with measures of WC ranging from 72.8 cm in the offspring to a high of 83.6 cm in probands. Similarly, BMI ranged from 21.5 kg/m² in the offspring to 24.2 kg/m² among probands.

Genome-wide linkage results for WC and BMI in the entire sample are illustrated in Figure 1. Significant linkage (LOD > 3) was observed for WC from 22q13.31 to 22q13.33 (genetic distance [centimorgans]: 53-62, physical position [megabase pairs]: 44.6-48.1). A similar but smaller linkage peak, which did not attain statistical significance, was observed for the BMI phenotype. Maximum multipoint LOD scores of 3.13 and 1.89 were observed for WC and BMI, respectively, at 22q13.33. This region also attained a suggestive linkage signal for WC among women in gender-stratified analysis (maximum LOD of 2.15 at 22q13.33) but not in men (Figures 2a and b, respectively). In addition, suggestive signals (LOD > 2) were observed for BMI in females from 2p24.3 to 2q12.2 (genetic distance [centimorgans]: 32-49, physical position [megabase pairs]: 13.0-106.0) with a maximum LOD score of 2.54 at 2p22.3; and in males from 6q12-6q13 (genetic distance [centimorgans]: 82-85, physical position [megabase pairs]: 67.4-73.0) with a maximum LOD score of 2.03 at 6q13.

Figure 3 shows the association between 1234 SNPs at 22q13.31-22q13.33 and WC and BMI, respectively, among all GenSalt

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**TABLE 1** Baseline characteristics of 3142 GenSalt participants

| Variable          | Probands (n = 676) | Spouses (n = 69) | Parents (n = 1236) | Siblings (n = 956) | Offspring (n = 205) |
|-------------------|--------------------|-----------------|--------------------|--------------------|--------------------|
| Men (%)           | 60.4               | 33.3            | 48.6               | 51.1               | 44.4               |
| Age, years, mean (SD) | 41.0 (8.3)       | 48.9 (6.6)      | 67.6 (8.4)         | 39.6 (7.7)         | 23.5 (6.4)         |
| BP, mm Hg, mean (SD) |                  |                 |                    |                    |                    |
| Systolic BP       | 128.0 (11.4)      | 112.7 (15.0)    | 136.6 (23.9)       | 111.6 (11.5)       | 106.6 (10.3)       |
| Diastolic BP      | 80.3 (9.0)        | 72.7 (10.0)     | 75.0 (11.7)        | 71.0 (8.9)         | 65.3 (9.0)         |
| BMI, kg/m², mean (SD) | 24.2 (3.3)       | 23.4 (3.7)      | 22.8 (3.4)         | 23.1 (2.8)         | 21.5 (3.3)         |
| WC, cm, mean (SD) | 83.6 (5.6)        | 80.3 (9.9)      | 81.0 (10.7)        | 79.5 (8.9)         | 72.8 (10.1)        |

BP, Blood pressure; BMI, Body mass index; WC, waist circumference.
participants. One SNP, rs16996195 (MAF = 2.00%), which lays in an intronic region of the TBC1 domain family, member 22A (TBC1D22A) gene, was significantly associated with WC after FDR adjustment ($P = 3.86 \times 10^{-5}$; FDR-Q = 0.048). A similar association was observed for BMI, although it was not significant after adjustment for multiple comparisons ($P = 0.004$; FDR-Q = 0.98).

Compared to participants with the C/C genotype, WC was significantly decreased among T allele carriers, with similar trends for BMI (Table 2).

Figures 4a-e show the associations of SNPs in suggestive gender-stratified linkage regions on chromosomes 2, 4, and 6 with both WC and BMI in females and males, separately. On chromosome 2, the minor allele of intergenic marker rs13020676 (MAF = 1.30%) was significantly associated with decreased WC in males after FDR adjustment ($P = 2.6 \times 10^{-6}$; FDR-Q = 0.028; $P = 6.3 \times 10^{-6}$, FDR-Q = 0.044; $P = 9.6 \times 10^{-6}$, FDR-Q = 0.044; and $P = 1.0 \times 10^{-5}$, FDR-Q = 0.044, respectively). In addition, the minor allele of intergenic marker rs1099555 (MAF = 4.70%) significantly decreased both WC ($P = 3.2 \times 10^{-7}$, FDR-Q = 0.0035) and BMI ($P = 9.2 \times 10^{-7}$, FDR-Q = 0.020) in men.

On chromosome 6, the minor alleles of intergenic variants rs7755450 (MAF = 1.60%) and rs11758293 (MAF = 4.60%) associated with significant increases in BMI in females ($P = 3.7 \times 10^{-7}$).
No SNPs on chromosome 22 reached statistical significance after adjusting for multiple testing in gender-stratified analyses (Figures 4d and e). Characteristics of significant SNPs from the overall and gender-stratified association analyses are shown in the Supporting Information Table.

**Discussion**

This study identified a significant QTL at chromosomal region 22q13 that may influence obesity susceptibility. Along with an independent signal at 2p24.3-2q12.2, 22q13 also demonstrated suggestive linkage to obesity-related traits among women in gender-stratified analysis. Chromosomal region 6q12-6q13 was linked to obesity-related phenotypes among men. Fine-mapping follow-up of the

| SNP          | Genotype | N   | WC (cm) Estimate (95% CI) | P-Value   | Q-Value | BMI (kg/m^2) Estimate (95% CI) | P-value | Q-value |
|--------------|----------|-----|--------------------------|-----------|---------|---------------------------------|---------|---------|
| rs16996195   | C/C      | 1791| 80.67 (80.09-81.25)      | 3.86 × 10^{-5} | 0.048   | 23.53 (23.33-23.72)             | 0.004   | 0.980   |
|              | C/T      | 65  | 77.55 (76.00-79.10)      |           |         | 22.71 (22.14-22.27)             |         |         |

HW, Hardy-Weinberg; WC, Waist circumference; BMI, Body mass index.
linkage regions revealed strong associations of novel genes and genetic markers for obesity susceptibility. Among all GenSalt participants, carriers of the low-frequency rs16996195 T allele of the chromosome 22 TBC1D22A gene had average WC measures approximately 3 cm smaller than those who were homozygous for the major C allele. In gender stratified analyses of 2p24.3-2q12.2

**FIGURE 4** Log $P$-values for the association of WC (black) and BMI (gray) with: 21,764 SNPs in suggestive linkage region 2q12.2 to 2p24.3 in (a) women and (b) men; 1336 SNPs in suggestive linkage region 6q12-6q13 in (c) women and (d) men; and 263 SNPs in suggestive linkage region 22q13.32 to 22q13.33 in (e) women and (f) men. Labeled SNPs had an adjusted $P < 0.05$. WC, Waist circumference; BMI, Body mass index.
### Table 3

Waist circumference and body mass index according to genotypes of significant SNPs in chromosomal regions 2p24.3-2q12.2 and 6q12-6q13 among GenSalt men and women.

| SNP       | Geno | Female | | Male | |  | Estimate (95% CI) | P-Value Q-Value | Estimate (95% CI) | P-Value Q-Value |
|-----------|------|--------|---|------|---|---|----------------|----------------|----------------|----------------|
|           |      | WC (cm) | BMI (kg/m²) | WC (cm) | BMI (kg/m²) | | | N | N | | |
| Chromosome 2 | | | | | | | | | | | |
| rs13020676 | G/G  | 857    | 78.54 | 0.62 | 0.98 | 23.63 | 0.25 | 0.94 | 962 | 82.44 | 2.3 \(\times\) 10^{-9} | 5.0 \(\times\) 10^{-5} |
| A/G  | 23   | 79.53 | (77.6-79.46) | 0.62 | 0.98 | 24.32 | 0.25 | 0.94 | 19 | 75.74 | (72.46-79.03) |
| A/A  | 0    | ... | (74.98-84.08) | 0.62 | 0.98 | 22.97-25.67 | 0.25 | 0.94 | 2 | 70.45 | (68.84-72.07) |
| rs17024325 | C/C  | 854    | 78.57 | 0.86 | 0.99 | 23.65 | 0.85 | 1.00 | 945 | 82.50 | 0.00010 | 0.24 |
| C/G  | 34   | 78.88 | (77.70-79.44) | 0.86 | 0.99 | 23.79 | 0.85 | 1.00 | 44 | 77.81 | (75.26-80.36) |
| rs2192712 | G/G  | 855    | 78.55 | 0.66 | 0.98 | 23.65 | 0.69 | 1.00 | 951 | 82.49 | 0.000050 | 0.269 |
| G/A  | 33   | 79.29 | (77.68-79.42) | 0.66 | 0.98 | 23.93 | 0.69 | 1.00 | 42 | 78.13 | (75.47-80.79) |
| A/A  | 5    | 75.94 | (75.55-83.04) | 0.66 | 0.98 | 22.42-25.44 | 0.69 | 1.00 | 2 | 78.38 | (71.32-85.45) |
| rs13014034 | G/G  | 778    | 78.50 | 0.68 | 0.98 | 23.62 | 0.40 | 0.97 | 872 | 82.68 | 0.00030 | 0.24 |
| G/A  | 105  | 79.17 | (77.63-79.38) | 0.68 | 0.98 | 23.92 | 0.40 | 0.97 | 119 | 79.58 | (77.87-81.29) |
| A/A  | 5    | 75.94 | (70.57-80.77) | 0.68 | 0.98 | 23.24 | 0.40 | 0.97 | 2 | 78.38 | (71.32-85.45) |
| rs12990515 | T/T  | 777    | 78.53 | 0.86 | 0.99 | 23.62 | 0.86 | 0.99 | 867 | 82.71 | 0.000012 | 0.24 |
| T/C  | 106  | 78.99 | (77.66-79.41) | 0.86 | 0.99 | 23.89 | 0.86 | 0.99 | 124 | 79.44 | (77.77-81.12) |
| C/C  | 5    | 75.92 | (77.36-80.62) | 0.86 | 0.99 | 23.24 | 0.86 | 0.99 | 2 | 78.40 | (71.33-85.45) |
| rs1009555 | C/C  | 800    | 78.75 | 0.03 | 0.77 | 23.71 | 0.08 | 0.81 | 909 | 82.69 | 3.2 \(\times\) 10^{-7} | 0.0035 |
| C/G  | 84   | 76.96 | (77.90-79.60) | 0.03 | 0.77 | 23.21 | 0.08 | 0.81 | 82 | 78.21 | (76.64-79.78) |
| G/G  | 4    | 75.45 | (75.11-78.81) | 0.03 | 0.77 | 22.30 | 0.08 | 0.81 | 2 | 75.73 | (55.33-96.13) |
|         |      |        | (71.62-79.28) | 0.03 | 0.77 | (21.11-23.49) | 0.08 | 0.81 |        | (13.02-27.47) |
| SNP             | Geno | Female | Male |
|-----------------|------|--------|------|
|                 | N    | WC (cm) | BMI (kg/m²) | WC (cm) | BMI (kg/m²) | N    | WC (cm) | BMI (kg/m²) |
|                 | N    | Estimate (95% CI) | P-Value | Q-Value | Estimate (95% CI) | P-Value | Q-Value | Estimate (95% CI) | P-value | Q-Value |
| Chromosome 6    |      |         |         |         |         |         |         |         |         |         |         |         |         |         |
| rs7755450      | C/C  | 829     | 78.44  | 0.0025 | 0.84   | 907    | 82.42  | 0.17   | 0.94   | 23.36   | 0.01   | 0.68   |          |
|                | C/T  | 25      | 82.80  | (77.53-79.36) | 25.47 | (24.62-26.31) | 26    | 80.03  | (76.83-83.22) | 3      | 79.18  | (62.75-95.60) |          |
|                | T/T  | 0       | ...    |         |         | ...    | ...    | ...    | ...    |         |         |         |          |
| rs11758293     | T/T  | 802     | 78.24  | 8.0 × 10⁻⁴ | 0.70   | 912    | 82.39  | 0.45   | 0.98   | 23.31   | 0.78   | 0.97   |          |
|                | T/G  | 80      | 81.19  | (77.40-79.08) | 24.82 | (24.16-25.48) | 74    | 81.44  | (79.00-83.88) | 4      | 81.00  | (68.22-93.78) |          |
|                | G/G  | 6       | 82.73  | (78.87-86.57) | 26.40 | (23.86-28.94) | 1     | 65.61  | (63.80-67.42) | 1      | 65.61  | (63.80-67.42) |          |
| rs7747318      | C/C  | 823     | 78.62  | 0.47   | 0.99   | 906    | 82.56  | 0.0010 | 0.84   | 23.42   | 1.1 × 10⁻⁴ | 0.035  |          |
|                | C/T  | 57      | 77.72  | (77.75-79.48) | 23.52 | (22.73-24.32) | 74    | 79.87  | (78.17-81.58) | 1      | 65.61  | (63.80-67.42) |          |
|                | T/T  | 0       | ...    |         |         | ...    | ...    | ...    | ...    |         |         |         |          |
| rs7767301      | A/A  | 833     | 78.65  | 0.37   | 0.99   | 919    | 82.54  | 0.0020 | 0.84   | 23.40   | 1.9 × 10⁻⁴ | 0.035  |          |
|                | A/G  | 54      | 77.73  | (77.75-79.52) | 23.39 | (22.69-24.35) | 71    | 79.53  | (77.74-81.31) | 3      | 79.45  | (65.49-93.42) |          |
|                | G/G  | 1       | 71.83  | (75.11-80.35) | 22.77 | (22.38-23.17) | 1     | 65.61  | (63.80-67.42) | 1      | 65.61  | (63.80-67.42) |          |
| rs12197115     | C/C  | 825     | 78.68  | 0.25   | 0.97   | 915    | 82.53  | 0.0043 | 0.84   | 23.40   | 1.9 × 10⁻⁴ | 0.035  |          |
|                | C/T  | 62      | 77.53  | (77.82-79.54) | 23.38 | (22.59-24.17) | 75    | 79.83  | (78.08-81.58) | 3      | 79.48  | (65.58-93.39) |          |
|                | T/T  | 1       | 71.76  | (75.20-79.86) | 22.73 | (22.34-23.11) | 1     | 65.61  | (63.80-67.42) | 1      | 65.61  | (63.80-67.42) |          |
| rs12203049     | C/C  | 814     | 78.63  | 0.38   | 0.99   | 882    | 82.51  | 0.0057 | 0.84   | 23.42   | 9.9 × 10⁻⁵ | 0.035  |          |
|                | C/T  | 63      | 77.83  | (77.74-79.52) | 23.46 | (22.65-24.28) | 83    | 79.93  | (78.20-81.66) | 1      | 65.61  | (63.80-67.42) |          |
|                |      |         |         |         |         |         |         |         |         |         |         |         |         |         |
## TABLE 3. (continued).

| SNP      | Geno | N   | WC (cm) (95% CI) | BMI (kg/m²) (95% CI) | P-Value | Q-Value | Estimate (95% CI) | P-Value | Q-Value | Estimate (95% CI) | P-Value | Q-Value | N   | WC (cm) (95% CI) | BMI (kg/m²) (95% CI) | P-Value | Q-Value |
|----------|------|-----|------------------|----------------------|---------|---------|------------------|---------|---------|------------------|---------|---------|-----|------------------|----------------------|---------|---------|
| T/T      | 1    | 71.78 (70.60-72.95) | 22.74 (22.35-23.14) | 0.31 | 0.99 | 3 | 79.44 (65.55-93.34) | 0.0044 | 0.84 | 22.03 (18.48-25.58) | 0.036 | 23.40 (23.16-23.65) | 1.9 × 10⁻⁴ |
| rs9454847 | A/A  | 824 78.66 (77.80-79.52) | 23.68 (23.40-23.96) | 0.36 | 0.92 | 910 82.56 (81.83-83.30) | 0.0044 | 0.84 | 23.35 (21.80-22.90) | 0.036 | 23.87 (23.29-24.45) |
|          | A/G  | 62 77.69 (75.37-80.00) | 23.35 (22.57-24.13) | 77 | 79.64 (77.85-81.43) | 0.36 | 0.92 | 910 82.56 (81.83-83.30) | 0.0044 | 0.84 | 23.35 (21.80-22.90) | 0.036 | 23.87 (23.29-24.45) |
|          | G/G  | 1 71.80 (70.63-72.96) | 22.72 (22.33-23.10) | 2 | 86.77 (85.06-88.49) | 0.36 | 0.92 | 910 82.56 (81.83-83.30) | 0.0044 | 0.84 | 23.35 (21.80-22.90) | 0.036 | 23.87 (23.29-24.45) |
| rs12333199 | A/A  | 563 78.62 (77.70-79.54) | 23.70 (23.41-24.00) | 0.47 | 0.93 | 619 82.92 (82.06-83.78) | 0.0055 | 0.84 | 23.61 (23.33-23.88) | 0.035 | 23.35 (23.29-24.45) |
|          | A/C  | 289 78.60 (77.45-79.75) | 23.61 (23.21-24.01) | 333 | 81.45 (80.40-82.51) | 0.47 | 0.93 | 619 82.92 (82.06-83.78) | 0.0055 | 0.84 | 23.61 (23.33-23.88) | 0.035 | 23.35 (23.29-24.45) |
|          | C/C  | 36 77.83 (74.81-80.85) | 23.31 (22.05-24.57) | 41 | 80.00 (77.02-82.98) | 0.47 | 0.93 | 619 82.92 (82.06-83.78) | 0.0055 | 0.84 | 23.61 (23.33-23.88) | 0.035 | 23.35 (23.29-24.45) |
| rs4501394 | A/A  | 574 78.61 (77.69-79.54) | 23.72 (23.43-24.02) | 0.50 | 0.93 | 634 82.97 (82.12-83.82) | 0.0066 | 0.84 | 23.63 (23.36-23.91) | 0.035 | 23.35 (23.29-24.45) |
|          | A/G  | 277 78.75 (77.60-79.89) | 23.59 (23.19-23.99) | 306 | 80.93 (79.84-82.02) | 0.50 | 0.93 | 634 82.97 (82.12-83.82) | 0.0066 | 0.84 | 23.63 (23.36-23.91) | 0.035 | 23.35 (23.29-24.45) |
|          | G/G  | 26 77.64 (74.22-81.05) | 23.51 (21.92-25.09) | 37 | 81.54 (78.20-84.88) | 0.50 | 0.93 | 634 82.97 (82.12-83.82) | 0.0066 | 0.84 | 23.63 (23.36-23.91) | 0.035 | 23.35 (23.29-24.45) |

**Geno, Genotype; WC, Waist circumference; BMI, Body mass index.**
SNPs, the minor allele of intergenic marker rs13020676 was related to decreased WC in men, whereas men carrying the minor alleles of correlated THUMPD2 markers rs17024325 and rs2192712 and intergenic markers rs13014034 and rs12990515 had decreased BMI. The minor allele of intergenic marker rs1009555 significantly decreased both WC and BMI in men. Gender-stratified analyses of chromosome 6 revealed an inverse dose-allele relationship between intergenic variants rs7755450 and rs11758293 and BMI in females, whereas the minor alleles of intergenic markers rs7747318, rs7767301, rs12197115, rs12203049, and rs9454847, and correlated markers rs12333199 and rs4501394 were associated with decreased BMI among men. In aggregate, these results contribute promising information towards elucidating the genomic mechanisms underlying obesity susceptibility.

We identified a significant linkage signal for obesity at chromosomal region 22q13.31-22q13.33 in the overall analysis, which also achieved suggestive linkage among women in our gender-stratified analysis. Both animal and human linkage studies have implicated this region in obesity susceptibility. In mouse models, Marsh and colleagues found evidence for linkage at chromosome 15 in a region homologous to human chromosome 22q13.3, whereas both Miyazaki et al. and Finck et al. identified significant linkage at chromosome 15 band 48.8, corresponding to human chromosome 22q13.31 (16-18). In humans, Wilson and colleagues found evidence of linkage at 22q13.31 for body weight in a study of at least 168 Caucasian sib-pairs (19). Despite strong evidence that this region may harbor important QTLs for obesity1 susceptibility, no fine-mapping studies were previously conducted to comprehensively follow-up on this promising region.

In addition to the signal on 22q13, gender-stratified linkage analyses revealed suggestive linkage to obesity susceptibility at chromosomal region 2p24.3-2q12.2 in females and at 6q12-6q13 in males. Few previous studies have conducted gender-stratified linkage analyses for this phenotype (20-23). However, similar to our finding, a previous report from HyperGEN showed suggestive linkage of 2p24.2 to percent body fat in women, which also did not replicate in the overall sample (21). Further, 6p12.3 showed suggestive linkage to hip circumference in a previous study of Han Chinese men (22). These findings suggest a potential gender-specific role for variants in this region on obesity-related phenotypes. In previous analyses unrestricted to gender, the 2p22.3 locus has been linked to various obesity-related traits, including leptin, adiponectin, BMI, and energy intake (24-26). Within the 6q12-6q13 region, a scan of 2188 individuals in a European twin cohort identified suggestive linkage to total fat percentage (27). Our findings combined with evidence from previous studies suggest that follow-up of these signals could provide important information on the genetic etiology of obesity-related traits.

Our follow-up analysis of SNPs within the q13.31-13.33 region of chromosome 22 among all GenSalt participants provides the first report of an intronic variant (rs16996195) of the TBC1D22A gene strongly associated with WC. TBC1D22A helps to comprise a class of genes encoding the Rab-GTPase-activating proteins, which are essential in cellular membrane trafficking (28) and have been associated with smoking cessation in previous GWAS (29,30). A link between drug addiction, including nicotine, and food addiction has long been established(31), with both phenotypes shown to activate the same dopamine containing link in the brain reward pathway (32). These data suggest that genes involved in smoking cessation may have pleiotropic effects on traits related to food addiction, like obesity. Our novel finding of association with rs16996195 may in part be attributed to the unique population examined by this study. While past GWAS of WC have also explored this region, these studies have been conducted only in populations of European, Southeast Asian, and Indian Asian descent (33-35). Marker rs16996195 is monomorphic in European populations and has an unknown frequency among Southeast and Indian Asian populations (36). With a MAF of 2% among the Han Chinese participants of the GenSalt study, we were able to detect the relatively large influence of this low frequency variant on WC. Although future replication work is needed to confirm these findings, our results underscore the importance of trans-ethnic approaches to genetic discovery. Validation of these findings in populations of African ancestry, where the MAF of this variant ranges from 5% to 15%, could be of particular importance. In addition, as an intronic SNP not in linkage disequilibrium with any known functional variants, future sequencing and experimental study will be needed to delineate the true nature of the observed association.

Despite suggestive linkage of 2p24.3-2q12.2 to obesity susceptibility in women, follow-up association study of this region yielded six novel SNPs from four independent loci associated with obesity-related traits in men only. Among these variants, rs13020676 has not previously been associated with obesity-related traits and is located over 964kbp away from its closest gene neighbor (APOB). However, bioinformatics tools suggest that this variant is highly conserved, indicating that it could have trans-acting effects on distant obesity-related genes (37). In contrast, marker rs17024325 represents an intronic variant of the THUMPD2 gene and is in high LD with marker rs2192712, located just upstream of the THUMPD2 gene. While the exact function of THUMPD2 is unknown, (38) its variants have been associated with obesity related traits, including erectile dysfunction (39) and triglycerides (40). The remaining three variants identified in the chromosome 2 region, including correlated markers rs13014034 and rs12990515 along with rs1009555, have not been associated with obesity susceptibility in previous studies, and based on bioinformatics tools, have no known functional relevance. Future studies aimed at replicating these promising findings are needed.

At chromosome 6q12-6q13 we identified two uncorrelated SNPs at one locus associated with obesity susceptibility in women and seven SNPs from two independent loci associated with obesity traits in men. Markers rs7755450 and rs11758293, strongly associated with BMI in women, represent intergenic variants with no known functional importance. Similarly, correlated markers rs7747318, rs7767301, rs12197115, rs12203049, and rs9454847 as well as rs12333199 and rs4501394, which strongly associated with BMI in men, are also intergenic markers with no known function. While the associations identified here provide promising evidence of gender-specific genetic associations for obesity, replication evidence will be necessary to confirm our findings as well as sequencing studies to pinpoint the potentially causal variants. In addition, given the paucity of knowledge regarding the
functional relevance of these markers, functional studies will be necessary to elucidate the biological mechanisms underlying these associations.

Our study has several important strengths. The large sample size and homogeneity of the population with respect to lifestyle and environmental factors should have increased our power to detect both linkage and association signals. In addition, study attributes, including the recruitment of only Han Chinese individuals, should make the association analysis robust to population stratification. Furthermore, stringent quality control procedures were employed during phenotype measurement, genotyping, and data cleaning. Finally, based on the 1000 Genomes samples of East Asian Ancestry (CHB + JPT), coverage of common genetic variants was high within this population, with 83.6% coverage for the linkage regions. Still, some limitations should be addressed. Adjustment for associated SNPs could only explain a negligible portion of the linkage signals in their corresponding regions (data not shown). In addition, among women no SNPs were found to associate with obesity susceptibility in 2p24.3 to 2q12.2, which was suggestive for this group in gender-stratified linkage analysis. These results imply that additional genetic variants in these regions could be important for obesity susceptibility. Further examination of any untagged common variants, structural variation, and low-frequency or rare variants may be warranted to explain the remaining linkage signals.

This study described genetic regions on chromosomes 2, 6, and 22 that may harbor important susceptibility loci for obesity. Follow-up fine mapping using dense SNP panels identified variants in the TBC1D22A and THUMP2D2 genes as well many novel intergenic variants associated with obesity-related traits. The association of intronic TBC1D22A variant rs16996195 highlights the potential importance of low-frequency variants with relatively large effect sizes, whereas our gender-specific results provide some of the first evidence of sex differences in the genetic etiology of obesity. Future studies aimed at replicating these novel findings and pinpointing the causative variants are warranted.

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