Effects of Obesity Related Genetic Variations on Visceral and Subcutaneous Fat Distribution in a Chinese Population

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Genome-wide association studies (GWAS) have uncovered numerous variants associated with body mass index (BMI), waist circumference, and waist-to-hip ratio. Our study aims to investigate how these variants are linked to fat distribution. We genotyped 56 validated variants of BMI, waist circumference, and waist-to-hip ratio in 2958 subjects from Chinese community-based populations and performed linear regression analyses to determine the association with visceral fat area (VFA) and subcutaneous fat area (SFA) imaged by magnetic resonance imaging (MRI). We found rs671 in ALDH2 exhibited the significant associations with VFA and the VFA-SFA ratio in all subjects ($P = 9.64 \times 10^{-5}$ and $6.54 \times 10^{-4}$). rs17782313 near MC4R for VFA and rs4846567 near LYPLAL1 for SFA were found in females only ($P = 2.93 \times 10^{-4}$ and 0.0015), whereas rs671 in ALDH2 for VFA and the VFA-SFA ratio was restricted to males ($P = 1.75 \times 10^{-8}$ and $4.43 \times 10^{-8}$). Given the robust association of rs671 with alcohol consumption, we next demonstrated the primary effects of rs671 on VFA and the VFA-SFA ratio were restricted to drinkers ($P = 1.45 \times 10^{-4}$ and $4.65 \times 10^{-3}$). Our data implied that variants of MC4R and LYPLAL1 modulated body fat distribution with sexual dimorphism and that alcohol consumption may mediate the impact of the ALDH2 locus on visceral fat in a Chinese population.

Obesity has become a major health concern in both developed and newly emerging economies1. The obesity epidemic is paralleled by an increased incidence of type 2 diabetes mellitus, metabolic syndrome, and cardiovascular diseases. There is abundant evidence that central obesity, particularly intra-abdominal fat accumulation, is more responsible for morbidity and mortality in obese patients with type 2 diabetes mellitus than overall adiposity2,3. Obesity is determined by both genetic and environmental factors. Although the “obesogenic environment” fuels the worldwide obesity epidemic, the notion that genetic variants could predispose individuals to common, polygenetic obesity seems to be an increasingly evident and persuasive argument. Certain SNPs that influence overall obesity (measured by BMI) and central adiposity (measured by the waist circumference or waist-to-hip ratio) have been identified in GWAS among European4–15 and other populations16–24. BMI, waist circumference and waist-to-hip ratio are regarded as commonly used but less precise measurements among a diverse group of obesity indices. The attempts to identify BMI loci have pointed toward the role of neuronal regulation of overall obesity8,9,25. Central obesity differs greatly from overall obesity in its pathogenesis, as well as in other areas; therefore, identifying the central obesity loci may aid in elucidating the signals shared with overall obesity or specific to central obesity. Fat distribution imaged by magnetic resonance imaging (MRI) is superior to waist circumference and waist-to-hip ratio in terms of distinguishing between visceral fat and subcutaneous fat. Additionally, there are few association studies of the genetic architecture of fat distribution, and the pathways determining how these variants influence the distribution of visceral and subcutaneous fat still remain unknown.

Different ethnicities have different genetic backgrounds. An indisputable fact is that large-scale obesity GWAS that include Asian and African populations are more likely to provide insight into different genetic architectures.

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Table 1. **Subject characteristics.** Data are shown as the mean ± SD, median (interquartile range), or N(%).

|                        | Overall  | Males        | Females       | P value |
|------------------------|----------|--------------|---------------|---------|
| N(%)                   | 2958     | 1352(45.71)  | 1606(54.29)   | —       |
| Age (years)            | 52.05 ± 6.93 | 52.11 ± 6.95 | 52.01 ± 6.92 | 0.7666  |
| BMI (kg/m²)            | 24.44 ± 3.36 | 24.90 ± 3.21 | 24.05 ± 3.43 | <0.001  |
| Waist circumference (cm)| 86(80.93) | 88.5(83.95) | 83.5(77.90.2) | <0.001  |
| Waist-to-hip ratio     | 0.913(0.8728,0.955) | 0.9295(0.8966,0.9645) | 0.8944(0.8563,0.9406) | <0.001  |
| VFA (cm³)              | 75.75(51.65,108.96) | 92.48(56.60,127.88) | 65.64(57.18,84.45) | <0.001  |
| SFA (cm³)              | 157.27(117.99,205.21) | 130.8(102.3,169.25) | 184.05(140.1,230.35) | <0.001  |
| VFA-SFA ratio          | 0.47(0.32,0.69) | 0.67(0.5,0.9) | 0.35(0.26,0.47) | <0.001  |

and provide evidence for fine mapping of causal genes. Thus, our aim was to replicate the impact of those validated loci on BMI, waist circumference and waist-to-hip ratio in Chinese populations, which were obtained from GWAS studies of European and non-European populations. More importantly, we tested the hypothesis that precise visceral and subcutaneous fat distribution indices could provide important information beyond BMI, waist circumference, and waist-to-hip ratio with respect to identify novel variants.

**Results**

**Validation of the impact of variants on BMI, waist circumference, and waist-to-hip ratio.** The subject characteristics are shown in Table 1. The associations of 19 loci among 56 validated SNPs of BMI, waist circumference, and waist-to-hip ratio were well replicated in our Chinese populations (Table 2). In general, most of 19 loci showed directionally consistent effect as previous studies except for the SNPs in ITIH4-AS1, MTF3 and ZNRF3. The SNP rs574367 in SEC16B showed the most significant association with BMI in nine loci for the lowest P (P = 1.33 × 10⁻⁴), and the result remained significant after multiple testing correction (empirical P = 0.0004). The most significant association with waist circumference was observed with rs671 in ALDH2, with or without BMI adjustments (P = 1.96 × 10⁻⁸ and 4.05 × 10⁻⁷, respectively, both empirical P = 1 × 10⁻⁸). Similarly, the analysis of the waist-to-hip ratio yielded nine SNPs with nominal associations; rs17782313 near MCAR was the maximum signal and the risk allele carriers showed a tendency toward elevating the waist-to-hip ratio after multiple testing correction (P = 0.0012; empirical P = 0.0641).

**The association of variants with visceral and subcutaneous fat distribution.** We also tested the genetic components of direct fat distribution imaged by MRI, namely, VFA, SFA, and the VFA-SFA ratio. The primary findings are presented in Table 3. Model 1 included the variables of sex and age for adjustment. Within five loci (SEC16B, ETV5, FTO, ALDH2, and MCAR) nominally associated with VFA, irrespective of BMI, the top locus was rs671 in ALDH2 (P = 1.94 × 10⁻⁸). Similarly, rs574367 in SEC16B was the top of ten loci (including SEC16B, LYPAL1, TMEM18, RBJ, GRB14-COBL1, NUDT3, ALDH2, MCC4, KCTD15, and ZNRF3) for SFA (P = 0.0017). Two SNPs in or near LYPAL1 and ALDH2 were associated with the VFA-SFA ratio, the metric describing the propensity to deposit visceral fat compared with subcutaneous fat (P = 0.0325 and 0.0001, respectively).

As BMI represents both fat and lean mass and correlates with regional fat depots, model 2 additionally adjusted for BMI. Although the majority of VFA signals were completely attenuated, rs671 in ALDH2 remained unchanged (P = 9.64 × 10⁻⁸). Similarly, the SNPs in or near RBJ and NUDT3 for SFA and SNPs near LYPAL1 and ALDH2 for the VFA-SFA ratio also showed nominal association after adjusting for BMI (P = 0.01 and 0.0318 for SFA; P = 0.0091 and 0.0007 for VFA-SFA ratio, respectively). We also noted that SNPs in or near POC5 and Cdkal1 showed nominal association only with VFA after adjusting for BMI (P = 0.024 and 0.0318, respectively). Similarly, the SNP in or near ITIH4-AS1 showed nominal association only with SFA after adjusting for BMI (P = 0.0225). Apart from the locus rs671 in ALDH2, none of the other loci survived the multiple comparisons (e.g., rs671 empirical P = 0.0043 for VFA, empirical P = 0.0345 for the VFA-SFA ratio).

**Gender differences in variants influence on fat distribution of visceral and subcutaneous fat.** Taking into account the heterogeneity of fat distribution in both genders, we performed the male and female analyses separately, which yielded 27 SNPs associated with at least one of three traits in one gender. The association of rs671 in ALDH2 with fat distribution traits was restricted to males (P = 1.75 × 10⁻⁸ for VFA, P = 4.43 × 10⁻⁸ for the VFA-SFA ratio, Table 4), whereas rs17782313 near MCAR for VFA and rs4846567 near LYPAL1 for SFA were only observed in females (P = 2.93 × 10⁻⁸ and 0.0015, respectively, Supplemental Table 2). All associations described above remained significant or exhibited a tendency after correction for multiple testing (empirical P range 1 × 10⁻⁸ to 0.0778). Moreover, other loci, including CPEB, NRXN3, PPARG, and SPRY2, also displayed the marked sexual dimorphism. To reduce the basis of the power loss in the subgroup analysis, we performed further joint interaction analyses of the entire group. The results indicated that the gender interaction of ALDH2 for the VFA and VFA-SFA ratio, MCAR for VFA, and LYPAL1 for SFA remained significant (P for interaction range from 9.88 × 10⁻⁸ to 0.0398).

**Alcohol consumption mediated the effect of the ALDH2 locus on visceral fat accumulation.** As rs671 in ALDH2 previously demonstrated a robust association with alcohol consumption, we also confirmed the finding in our study (odds ratio 0.27, 95% confidence interval [CI] 0.09–0.23, P = 6.16 × 10⁻⁶ per copy of A allele) and then performed further analysis to evaluate the underlying effect of alcohol consumption on
| SNP       | Gene     | Alleles | MAF | Traits | Model 1 | Model 2 |
|-----------|----------|---------|-----|--------|---------|---------|
|           |          |         |     | BETA ± SE | P       | BETA ± SE | P       |
| rs984222  | TRX15-WARS2 | C/G    | 0.41 | BMI = −0.0378 ± 0.0879 | 0.6672  | BMI = −0.0021 ± 0.0012 | 0.0916  |
|           |          |         |     | WC = −0.0011 ± 0.0007 | 2011    | WC = −0.0016 ± 0.0007 | 0.0124  |
| rs574367  | SEC16B   | T/G    | 0.2  | BMI = 0.5353 ± 0.1105 | 1.33 × 10^−6a | BMI = 0.0056 ± 0.0006 | 0.6158  |
|           |          |         |     | WC = 0.0011 ± 0.0009 | 373     | WC = −0.0007 ± 0.0008 | 0.0681  |
| rs4846567 | LYPLA1   | T/G    | 0.3  | BMI = 0.192 ± 0.0939 | 0.0409  | BMI = 0.0021 ± 0.0013 | 0.1218  |
|           |          |         |     | WC = −0.0007 ± 0.0008 | 3893    | WC = 0.00002 ± 0.0008 | 0.9806  |
| rs6548238 | TMEM18   | T/C    | 0.09 | BMI = −0.4554 ± 0.1546 | 0.0033  | BMI = 0.0059 ± 0.0006 | 0.0066  |
|           |          |         |     | WC = 0.0011 ± 0.0009 | 3893    | WC = −0.0007 ± 0.0008 | 0.373   |
| rs1057001 | TRIB2    | T/A    | 0.15 | BMI = 0.0864 ± 0.1243 | 0.4873  | BMI = 0.0036 ± 0.0017 | 0.0413  |
|           |          |         |     | WC = 0.0020 ± 0.0010 | 0.0512  | WC = 0.0197 ± 0.0130 | 0.131   |
| rs887912  | FANCL    | A/G    | 0.002 | BMI = 0.8386 ± 0.9268 | 0.3656  | BMI = 0.0035 ± 0.0013 | 0.0054  |
|           |          |         |     | WC = 0.0016 ± 0.0006 | 2821    | WC = 0.0006 ± 0.0007 | 0.2694  |
| rs2535633 | ITIH4-AS1 | C/G    | 0.41 | BMI = 0.2331 ± 0.0889 | 0.0098  | BMI = 0.0027 ± 0.0016 | 0.0943  |
|           |          |         |     | WC = 0.0810 ± 0.1148 | 4807    | WC = 0.0010 ± 0.0009 | 0.0387  |
| rs987237  | TFAP2B   | G/A    | 0.17 | BMI = 0.0504 ± 0.1082 | 0.6416  | BMI = 0.0009 ± 0.0015 | 0.5359  |
|           |          |         |     | WC = 0.0007 ± 0.0015 | 6553    | WC = −0.0021 ± 0.0009 | 0.016   |
| rs10968576| LRRN6C-LINGO2 | G/A  | 0.21 | BMI = −0.2932 ± 0.1052 | 0.0053  | BMI = −0.0075 ± 0.0015 | 4.05 × 10^−6c |
|           |          |         |     | WC = −0.0030 ± 0.0012 | 0.1516  | WC = −0.0011 ± 0.0007 | 0.1369  |
| rs671     | ALDH2    | A/G    | 0.22 | BMI = 0.3047 ± 0.1361 | 0.0253  | BMI = 0.0037 ± 0.0019 | 0.563   |
|           |          |         |     | WC = 0.0018 ± 0.0011 | 1168    | WC = 0.0018 ± 0.0011 | 0.168   |
| rs4771122 | MTIF3    | G/A    | 0.18 | BMI = −0.293 ± 0.1140 | 0.7969  | BMI = −0.0018 ± 0.0016 | 0.2532  |
|           |          |         |     | WC = −0.0022 ± 0.0009 | 0.0173  | WC = −0.0022 ± 0.0009 | 0.0173  |
| rs939609  | FTO      | A/T    | 0.12 | BMI = 0.3047 ± 0.1361 | 0.0253  | BMI = 0.0037 ± 0.0019 | 0.563   |
|           |          |         |     | WC = 0.0037 ± 0.0019 | 1168    | WC = 0.0018 ± 0.0011 | 0.168   |
| rs17782313| MC4R     | C/T    | 0.22 | BMI = 0.3600 ± 0.1051 | 0.0006d | BMI = 0.0073 ± 0.0015 | 9.73 × 10^−6c |
|           |          |         |     | WC = 0.0028 ± 0.0009 | 0.0012  | WC = 0.0028 ± 0.0009 | 0.0012  |
| rs29941   | KCTD15   | C/T    | 0.24 | BMI = 0.2437 ± 0.1017 | 0.0166  | BMI = 0.0030 ± 0.0014 | 0.0344  |
|           |          |         |     | WC = 0.0010 ± 0.0008 | 0.2458  | WC = 0.0010 ± 0.0008 | 0.2458  |
| rs3810291 | TMEM160  | A/G    | 0.29 | BMI = 0.1594 ± 0.0984 | 0.1053  | BMI = 0.0025 ± 0.0014 | 0.0662  |
|           |          |         |     | WC = 0.0020 ± 0.0008 | 0.014   | WC = 0.0020 ± 0.0008 | 0.014   |
| rs4823006 | ZNRF3    | A/G    | 0.46 | BMI = −0.1732 ± 0.0884 | 0.0502  | BMI = −0.0020 ± 0.0012 | 0.1013  |
|           |          |         |     | WC = −0.0016 ± 0.0007 | 0.0268  | WC = −0.0016 ± 0.0007 | 0.0268  |
Table 2. The impact of variants on BMI, waist circumference, and the waist-to-hip ratio. SNP, single nucleotide polymorphism; Alleles, minor/major alleles; MAF minor allele frequency; SE, standard error; WC, waist circumference; WHR, waist-to-hip ratio. Only SNPs that showed significant associations with traits are shown in Table 2. P values < 0.05 are shown in bold. Traits were adjusted for age and sex in the additive genetic model 1 and adjusted for age, sex, and BMI in model 2. *Empirical P = 0.0004. †Empirical P = 0.0187. ‡Empirical P = 1 × 10−4. §Empirical P = 0.0326. ¶Empirical P = 0.0029; Empirical P values were based on 10000 permutations within each trait.

The interaction of ALDH2 and visceral fat accumulation. While adjusting for alcohol consumption, the associations of ALDH2 with VFA and the VFA-SFA ratio were substantially attenuated in the overall group (P = 0.0043 and 0.0149, respectively), as well as in males (P = 5.72 × 10−5 and 7.22 × 10−4). Next, we performed a subgroup analysis stratified by alcohol consumption. Data from 1211 drinkers (938 males and 273 females) and 1726 non-drinkers (407 males and 1319 females) were available, and the results are depicted in Fig. 1. Note that nominal associations between the ALDH2 variant and visceral fat accumulation were restricted to drinkers overall (P = 1.45 × 10−4 for VFA, P = 4.65 × 10−5 for the VFA-SFA ratio) and to male drinkers specifically (P = 4.22 × 10−5 for VFA, P = 0.0031 for the VFA-SFA ratio). The interaction analysis of SNP × drinking revealed significant in overall individuals for VFA (P for interaction = 0.0055). Additionally, we also performed SNP × environment (gender × drinking) interaction analyses for rs671 in ALDH2 and found that the SNP × environment interaction of ALDH2 for the VFA and VFA-SFA ratio remained significant (P for interaction = 0.0007 and 0.0058, respectively).

In order to strengthen our finding, we performed subgroup analysis which divided subjects into three groups (i.e. non-drinkers, chance drinkers and regular drinkers). We found that the nominal associations between the ALDH2 variant and VFA-SFA ratio were restricted to overall regular drinkers and to male regular drinkers specifically (P = 0.0453 and 0.0429, respectively) and that a tendency toward elevated VFA were restricted to overall regular drinkers and to male regular drinkers specifically (P = 0.0503 and 0.0634, respectively), but did not observe associations in chance drinkers.

Discussion
We replicated 19 of 56 loci, such as FTO, MC4R and KCTD15, were nominally associated with BMI, waist circumference, and waist-to-hip ratio, but SNPs in MC4R, ALDH2 and SEC16B were shown significant association after multiple testing correction. More importantly, in search for fat distribution variants in a Chinese population, our study revealed 15 of 56 loci nominally associated with at least one trait within three fat distribution indices, and the SNPs in or near MC4R, LYPLAL1, and ALDH2 were significantly associated with fat distribution after multiple testing correction.

To our knowledge, this report is the first to focus on fat distribution variants in a Chinese population. Previous efforts have focused on this issue in European and other Asian populations. The results indicated that several loci, such as LYPLAL1, FTO, THNSL2, GCCR, TRIB2, and IRS1, substantially impacted fat distribution indices. The reported signals for fat distribution of visceral fat and subcutaneous fat from previous GWAS by the GIANT consortium such as LYPLAL1, TMEM18, GB14-COBB1 and ETV5 were directionally consistent with our results. Besides, their finding highlighted the associations of rs11118316 in LYPLAL1 with the ratio of visceral fat area to subcutaneous fat area and rs1558902 in FTO with subcutaneous fat area. The former locus failed to be analysed for departure from Hardy-Weinberg equilibrium and the proxy of latter locus was not replicated in our study as well as in that GWAS. With the current sample size, the statistical power was 45%~95% to detect the effect size ranging 0.003 to 0.006 for waist-to-hip ratio (minor allele frequency = 0.2, two-sided type one error rate = 0.05) in our study. One of the probability for negative association is thus the differences in genetic architecture among varied populations. Some variants with the modest effect size or low minor allele frequency need to be replicated in large-scale meta-analyses of GWAS across varied populations.

Table 2. The impact of variants on BMI, waist circumference, and the waist-to-hip ratio. SNP, single nucleotide polymorphism; Alleles, minor/major alleles; MAF minor allele frequency; SE, standard error; WC, waist circumference; WHR, waist-to-hip ratio. Only SNPs that showed significant associations with traits are shown in Table 2. P values < 0.05 are shown in bold. Traits were adjusted for age and sex in the additive genetic model 1 and adjusted for age, sex, and BMI in model 2. *Empirical P = 0.0004. †Empirical P = 0.0187. ‡Empirical P = 1 × 10−4. §Empirical P = 0.0326. ¶Empirical P = 0.0029; Empirical P values were based on 10000 permutations within each trait.

rs4846567 at LYPLAL1 has been previously reported by the GIANT consortium to be associated with the waist-to-hip ratio in subjects based on GWAS, but the association was restricted to females (P = 2.6 × 10−6). Our study did not replicate this finding in the entire group or in females, but there was an association with SFA and the VFA-SFA ratio, which are consistent with the other findings in European and Japanese populations. The former study also revealed the association of another independent SNP, rs11118316 near LYPLAL1 (r2 = 0, D' = 0.004 in HCB; r2 = 0.285, D' = 0.935 in CEU with rs4846567) with the VFA-SFA ratio in both males and females. This SNP was not analysed in our study, but we speculated that there were heterogeneous sex-related signals associated with the VFA-SFA ratio. LYPLAL1 encodes lysophospholipase-like protein 1, which plays a role in the consecutive steps of triglyceride degradation. This region showed an association with fasting serum triglycerides, insulin resistance, and non-alcoholic fatty liver disease, suggesting some involvement in hepatic lipid metabolism and insulin responsiveness. The molecular mechanism responsible for the link between LYPLAL1 and the pathogenesis of fat distribution according to gender remains to be elucidated in functional studies.

The locus rs671 in ALDH2 was previously reported to be associated with BMI in East Asians. Our novel findings were for visceral fat accumulation in overall subjects and restricted to males. However, we did note that the male to female ratio was not balanced between drinkers and non-drinkers, and the analysis of the associations of ALDH2 with VFA and SFA revealed a borderline sex-related significance among overall drinkers (P for interaction = 0.0473 and 0.0406, respectively). We cannot exclude the possibility that alcohol consumption does not affect visceral fat accumulation in a sex-dependent manner. ALDH2 encodes aldehyde dehydrogenase-2, a mitochondrial enzyme that metabolises acetaldehyde to acetic acid and ultimately removes it. Many analyses of
Table 3. Influence of variants on fat distribution (i.e., visceral fat and subcutaneous fat). SNP, single nucleotide polymorphism; Alleles, minor/major alleles; MAF minor allele frequency; SE, standard error; VFA, visceral fat area; SFA, subcutaneous fat area; VFA-SFA, the ratio of visceral fat to subcutaneous fat. Only SNPs that showed nominal significant associations with traits are shown in Table 3. P values < 0.05 are shown in bold. Traits were adjusted for age and sex in the additive genetic model 1 and adjusted for age, sex, and BMI in model 2. 1Empirical P = 0.0003. 2Empirical P = 0.0057. 3Empirical P = 0.0043. 4Empirical P = 0.0345; Empirical P values were based on 10000 permutations within each trait.

GWAS have demonstrated the robust association of rs671 in ALDH2 with alcohol consumption in Asian populations; however, this SNP does not appear to be polymorphic in Europeans34–36. The A allele of rs671, designated as the ALDH2*2 allele, encodes in an inactive form, resulting in a nearly complete loss of catalytic activity, which
Table 4. Gender differences in how the variants influence fat distribution. SNP, single nucleotide polymorphism; Alleles, minor/major alleles; MAF minor allele frequency; SE, standard error; VFA, visceral fat area; SFA, subcutaneous fat area; VFA/SFA, the ratio of visceral fat to subcutaneous fat. Only SNPs that showed nominal significant associations with traits are shown in Table 4. P values < 0.05 are shown in bold. Traits were adjusted for age and BMI in the additive genetic model. *Empirical P = 1 × 10⁻⁴; Empirical P values were based on 10000 permutations within each trait.
BMI was calculated as weight (kilograms) divided by height² (meters). Waist circumference was measured at the level of the umbilicus, and hip circumference was measured around the buttocks. The waist-to-hip ratio was calculated as the ratio between the waist and hip circumferences in centimetres. Each subject underwent abdominal MRI (Archive, Philips Medical System, Amsterdam, Netherlands) at the level of the umbilicus between L4 and L5 in the supine position for quantification of body fat distribution. Two trained observers used SLICE-O-MATIC image analysis software (version 4.2; Tom Vision Inc., Montreal, QC, Canada) to generate graphical displays of the imaging data and to calculate the visceral fat area (cm²) and subcutaneous fat area (cm²). If the results differed by more than 10%, a third observer warranted future molecular and biology investigations.

This study has several limitations. First, we did not perform further analyses after adjusting for lifestyle (e.g., alcohol consumption and smoking), except for the analysis of the ALDH2 locus, which demonstrated a robust association with alcohol consumption. It is unknown whether there is an interaction between lifestyle and other variants on fat distribution. Second, we tested the one SNP of each locus obtained from the top signals of GWAS with alcohol consumption. It is unknown whether there is an interaction between lifestyle and other factors. The remaining subjects provided informed consent and completed a questionnaire on their medical histories; they also underwent anthropometric measurements and laboratory examinations. The study complied with the Declaration of Helsinki and was approved by the Institutional Review Board of Shanghai Jiao Tong University Affiliated Sixth People's Hospital.

Materials and Methods

Subjects. From 2009–2012, we recruited up to 2958 subjects from a community-based population with Chinese Han ancestry and excluded the subjects with cancer, severe disability, or severe psychiatric disturbances. The remaining subjects provided informed consent and completed a questionnaire on their medical histories; they also underwent anthropometric measurements and laboratory examinations. The study complied with the Declaration of Helsinki and was approved by the Institutional Review Board of Shanghai Jiao Tong University Affiliated Sixth People’s Hospital.

Phenotypes and assessment of alcohol consumption. BMI was calculated as weight (kilograms) divided by height² (meters). Waist circumference was measured at the level of the umbilicus, and hip circumference was measured around the buttocks. The waist-to-hip ratio was calculated as the ratio between the waist and hip circumferences in centimetres. Each subject underwent abdominal MRI (Archive, Philips Medical System, Amsterdam, Netherlands) at the level of the umbilicus between L4 and L5 in the supine position for quantification of body fat distribution. Two trained observers used SLICE-O-MATIC image analysis software (version 4.2; Tom Vision Inc., Montreal, QC, Canada) to generate graphical displays of the imaging data and to calculate the visceral fat area (cm²) and subcutaneous fat area (cm²). If the results differed by more than 10%, a third observer...
who was blinded to the results reanalysed the images. As for alcohol consumption, briefly, each subject was asked whether they had ever consumed alcohol in their lifetime (chance drunk less than three times in every week and regularly drunk equal or more than three times in every week) and individuals who gave a positive answer were defined as drinkers, whereas those who gave a negative answer were non-drinkers.

Genotyping and quality control analysis. Genomic DNA was extracted from blood samples collected from each subject. A total of 57 SNPs associated with BMI, waist circumference, and waist-to-hip ratio from previous literature (as shown in Supplementary Table 1 and Supplementary Figure 1) were selected to be genotyped using the MassARRAY Compact Analyzer (Sequenom, San Diego, CA, USA). None of the 57 SNPs failed quality control analyses, with call rates >95% and concordant rates >99%. Fifty-three subjects were excluded due to sample call rate <90%. The Hardy-Weinberg equilibrium test was performed prior to the analysis. Among the 57 SNPs, 56 SNPs were in accordance with Hardy-Weinberg equilibrium (P > 0.05), except for rs11118316.

Statistical analysis. Haploview (version 4.2; www.broad.mit.edu/mpg/haploview/) was used to determine the pairwise linkage disequilibrium. Using PLINK (http://pngu.mgh.harvard.edu/~purcell/plink/)41, logistic regression analysis was used to examine the associations between SNPs and dichotomous variables, and linear regression analysis was used to test for the effects of SNPs on quantitative traits under the additive genetic model. All analyses were adjusted for covariates, such as age, sex, and other variables, if appropriate. Waist circumference, waist-to-hip ratio, VFA, SFA, and VFA:SFA ratio were log10-transformed. Since no accurate data on type and amount of alcohol consumption, alcohol consumption was converted into a dichotomous variable that includes drinkers and non-drinkers. Multiple testing based on 10000 permutations was performed with PLINK. The statistical analyses were performed using SAS software (version 8.0; SAS Institute, Cary, NC, USA), unless otherwise specified. A two-tailed P value of <0.05 was considered to be significant. The power calculations were performed using Quanto software (http://biostats.usc.edu/Quanto.html, version 1.2.4, May 2009).

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Author Contributions
C.H. and W.J. conceived and designed the research. T.W., D.P., X.S., F.J. and R.Z. performed the experiments. T.W. and C.H. analysed the data. C.H., X.M., M.C., J.Y., Z.H., D.Y. and S.W. contributed reagents/materials/analysis tools. T.W. and C.H. drafted the manuscript. All authors contributed to the writing of this manuscript, and read and approved the final version.

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