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Genome-wide association studies suggest sex-specific loci associated with abdominal and visceral fat

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Abstract

Background—To identify loci associated with abdominal fat and replicate prior findings, we performed genome-wide association (GWA) studies of abdominal fat traits: subcutaneous adipose tissue (SAT), visceral adipose tissue (VAT), total adipose tissue (TAT) and visceral to subcutaneous adipose tissue ratio (VSR).

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Subjects and Methods—Sex-combined and sex-stratified analyses were performed on each trait with (TRAIT-BMI) or without (TRAIT) adjustment for BMI, and cohort-specific results were combined via a fixed effects meta-analysis. A total of 2,513 subjects of European descent were available for the discovery phase. For replication, 2,171 European Americans and 772 African Americans were available.

Results—A total of 52 SNPs encompassing 7 loci showed suggestive evidence of association ($p < 1.0 \times 10^{-6}$) with abdominal fat in the sex-combined analyses. The strongest evidence was found on chromosome 7p14.3 between a SNP near BBS9 gene and VAT (rs12374818; $p= 1.10 \times 10^{-7}$), an association that was replicated ($p = 0.02$). For the BMI-adjusted trait, the strongest evidence of association was found between a SNP near CYCSP30 and VAT-BMI (rs10506943; $p= 2.42 \times 10^{-7}$). Our sex-specific analyses identified one genome-wide significant ($p < 5.0 \times 10^{-8}$) locus for SAT in women with 11 SNPs encompassing the MLLT10, DNAJC1 and EBLN1 genes on chromosome 10p12.31 ($p = 3.97 \times 10^{-8}$ to 1.13×10^{-8}). The THNSL2 gene previously associated with VAT in women was also replicated ($p= 0.006$). The six gene/loci showing the strongest evidence of association with VAT or VAT-BMI were interrogated for their functional links with obesity and inflammation using the Biograph knowledge-mining software. Genes showing the closest functional links with obesity and inflammation were ADCY8 and KCNK9, respectively.

Conclusions—Our results provide evidence for new loci influencing abdominal visceral (BBS9, ADCY8, KCNK9) and subcutaneous (MLLT10/DNAJC1/EBLN1) fat, and confirmed a locus (THNSL2) previously reported to be associated with abdominal fat in women.

Introduction

Body fat distribution, particularly truncal abdominal fat, has long been recognized as a major determinant of the metabolic complications associated with an increased risk of type 2 diabetes and cardiovascular disease in obese individuals (1–7). A large number of studies, reviewed elsewhere (8–10), have clearly established that the pattern of fat distribution is influenced by genetic factors, generally to a larger extent than overall body fatness. The first evidence for a genetic component for body fat distribution was based on data from anthropometric measures obtained in 1,698 subjects from the Quebec Family Study (QFS). Truncal abdominal fat, assessed by computing the ratio of trunk skinfolds (sum of subscapular, suprailiac and abdominal skinfolds) to extremity skinfolds (sum of triceps, biceps and medial calf skinfolds), was found to be more influenced by genetic factors than total subcutaneous fat (sum of six skinfolds), with heritability estimates of 60% and 38%, respectively (11). Another study based on principal components analysis of the six skinfolds reported a heritability of 52% for a component contrasting trunk-to-extremity skinfolds (12). Waist circumference (WAIST) has also been widely used as an indicator of abdominal obesity, and a large number of twin (13–16) and family (17–22) studies have reported heritability estimates in the range of 40% to 75% for WAIST. It is noteworthy that in most studies, WAIST was not adjusted for body mass index (BMI) in order to obtain a heritability estimate of fat distribution independent of body mass. As observed in one study that reported a heritability estimate of 29% for BMI-adjusted WAIST, compared to 46% without adjustment for BMI (23), the heritability of WAIST is generally attenuated after adjustment for BMI.

Only a few studies have reported heritability estimates of fat distribution using imaging techniques such as dual-energy X-ray absorptiometry (DXA) or computed tomography. Heritability of visceral adipose tissue (VAT) measured by computed tomography was first reported in QFS (24) and the HERITAGE Family Study (25). After adjustment for total body fatness, significant genetic effects (48–56%) were found in both studies. Other family studies, which used DXA measurements to assess fat distribution, have reported heritability estimates in the range of 33% to 85% for the amount of fat in the trunk (26–29). Two reports based on data from the HERITAGE Family Study have shown that changes in the amount and distribution of subcutaneous fat (30) and changes in VAT (31) in response to exercise training were influenced by genetic factors. Studies undertaken in pairs of male MZ twins submitted to a 100-day 1,000 kcal/day caloric surplus (32) or energy deficit induced by exercise (33) showed significant within-pair resemblance, with intraclass coefficients reaching 0.72 and 0.84, for changes in abdominal visceral fat in response to overfeeding or negative energy balance protocol, respectively.

A large number of candidate gene studies have identified genes associated with various indices of body fat distribution (34–39) or changes in body fat distribution in response to diet (40). Multiple genome-wide association studies (GWAS) have identified several loci associated with anthropometric measures of fat distribution such as WAIST or waist-to-hip ratio (WHR) (41–43, 44 , 45–47), but few have been performed using direct measures of abdominal fat that can discriminate between abdominal visceral and subcutaneous fat deposition. Using measures of abdominal subcutaneous adipose tissue (SAT) and VAT obtained by computed tomography, Fox et al (48) performed a GWAS of SAT, VAT, VAT adjusted for BMI (VAT-BMI) and VAT/SAT ratio (VSR) in men and women from four population-based studies. They found genome-wide significant evidence of association for a single nucleotide polymorphism (SNP;rs11118316) at *LYPLAL1* gene for VAT/SAT ratio, in a region previously identified in a GWA study for WHR (41). A new locus for VAT was also identified on chromosome 2 in women (rs1659258 near *THNSL2* gene).

In the present study, we report results from GWA analysis of several measures for fat distribution obtained by computed tomography in the Coronary Artery Risk Development In young Adults (CARDIA) study, the HEalth RIsk factors exercise Training And GEnetics (HERITAGE) Family Study and QFS. We performed a GWA analysis of total abdominal (TAT), subcutaneous (SAT), visceral (VAT) adipose tissue and visceral to subcutaneous adipose tissue ratio (VSR), with and without adjustment for BMI. Given the importance of sexual dimorphism in the distribution of body fat and also as an attempt to replicate findings from the Fox et al., paper (48), we also performed sex-stratified GWA analysis of SAT, VAT, VAT-BMI and VSR.

Methods

Study Samples

Participants of European descent from CARDIA, HERITAGE and QFS were included in the GWA analysis. All three studies obtained informed consent from participants and approval from the appropriate institutional review boards.

The CARDIA study is a prospective multicenter study designed to investigate the development of cardiovascular disease risk factors and subclinical and clinical disease in young (18–30 years) Black and White men and women from four geographic locations in the United States. A total of 5,115 subjects were recruited from the total community in Birmingham, AL, from selected census tracts in Chicago, IL and Minneapolis, MN; and from the Kaiser Permanente health plan membership in Oakland, CA. The details of the study design for the CARDIA study have been published previously (49). Eight examinations have been completed since initiation of the study in 1985–1986, respectively in the years 0, 2, 5, 7, 10, 15, 20, and 25. For the present study, abdominal adipose tissue imaging data were available at year 25 in 1,335 whites.

The HERITAGE Family Study was designed to evaluate the role of genetic and non-genetic factors in cardiovascular, metabolic, and hormonal responses to aerobic exercise training (50). Extensive data, including body composition, cardiovascular risk factors, and lifestyle habits were gathered on almost 800 White and Black subjects in over 200 families, both before and after 20 weeks of supervised training. In the present study, analyses were performed using baseline data in Whites only (n= 496).

The QFS was designed to investigate the contribution of genetic factors in obesity and its related metabolic complications in French-Canadian families (51). The cohort represents a mixture of random sampling and ascertainment through obese (BMI > 30 kg/m^2) probands. Measurements of abdominal fat by computed tomography were available on a total of 682 subjects.

Phenotype Data

In all three studies, the amounts of VAT, SAT and TAT were assessed by computed tomography with a scan performed at the abdominal level (L4 and L5 vertebrae) as described elsewhere for CARDIA (52), HERITAGE (25) and QFS (53). Participants were examined in the supine position with both arms stretched above head. TAT area was calculated by delineating the abdominal scan with a graph pen and then by computing the TAT with an attenuation range of −190 to −30 Hounsfield units. VAT was measured by drawing a line within the muscle wall surrounding the abdominal cavity and SAT was calculated by subtracting VAT from TAT. The VSR was also computed.

Genotype Data

For the CARDIA Study, genotyping was performed using the Affymetrix Genome-Wide Human SNP Array 6.0 (Santa Clara, California). Genotyping was completed with a sample call rate ≥98%. A total of 578,568 SNPs passed quality control (minor allele frequency (MAF) ≥2%, call rate ≥95%, Hardy-Weinberg equilibrium (HWE) $\geq 10^{-4}$) and were used for imputation.

For HERITAGE, genotyping was performed using the Illumina HumanCNV370-Quad v3.0 BeadChips on Illumina BeadStation 500GX platform. The genotype calls were done with the Illumina GenomeStudio software and all samples were called in the same batch to eliminate batch-to-batch variation. Monomorphic SNPs and SNPs with only one heterozygote, as well as SNPs with more than 30% missing data were filtered out with

GenomeStudio. Twelve samples were genotyped twice with 100% reproducibility across all SNPs. All GenomeStudio genotype calls with a GenTrain score less than 0.885 were checked and confirmed manually. Quality control of the GWAS SNP data confirmed all family relationships and found no evidence of DNA sample mix-ups.

For QFS, genotyping was performed using the Illumina 610-Quad chip containing 620.901 markers including 582,591 autosomal SNPs. The 610-Quad BeadChips were scanned on an Illumina BeadArray™ reader and the BeadStudio software package included with the Illumina® BeadStation 500GX system was used to extract genotyping data from images collected from the reader. The BeadStudio Genotyping Module software was used to call SNP genotypes. After exclusion of copy number variations, SNPs called in less than 95% of the subjects, SNPs not in HWE ($p < 10^{-4}$) and those with a MAF < 1%, a total of 543,714 SNPs were available for analysis.

For all three studies, imputation was performed using CEU reference panel consisting of 120 haplotypes from HapMap Phase II data (release 22, build 36) and the MACH software (54). A total of 2,473,256 directly typed or imputed SNPs were tested for association with the abdominal fat phenotypes.

Statistical Analyses

We performed meta-analyses for a total of 8 abdominal fat phenotypes: TAT, SAT, VAT, VSR, TAT-BMI, SAT-BMI, VAT-BMI and VSR-BMI. Log transformation was used to normalize the distribution of VAT and VSR. The primary analysis was performed in each cohort separately using regression models, additive genetic effects and accounting for phenotype correlation among family members when appropriate. For all phenotypes, age and sex were used as covariates. When a SNP was both genotyped and imputed, genotyped SNPs were used for analysis. These cohort- specific results were combined with fixed effects meta-analysis using the inverse-variance weighting method in METAL (55). In addition to the analyses performed in combined men and women and in an attempt to replicate the findings of Fox et al., (48), sex-stratified analyses were also performed in each cohort for the following phenotypes: SAT, VAT, VAT-BMI and VSR. These cohort-specific results were then combined through meta-analysis.

Replication cohort

To replicate findings from the meta-analysis, the Pennington Center Longitudinal Study (PCLS) cohort was used. The PCLS cohort is composed of individuals who participated in various clinical studies (diet interventions, weight loss and other metabolic studies) conducted at the Pennington Biomedical Research Center since 1992 (56). The total PCLS sample included 2,943 adult (18–84 years of age) subjects consisting of 2,171 European American men (n = 897) and women (n = 1,274) and 772 African American men (n = 185) and women (n= 587). All participants provided written, informed consent. In PCLS, abdominal fat was measured using either DXA (for 1,707 subjects) or computed tomography (for 1,236 subjects) as described elsewhere (57, 58).

PCLS replication genotyping

A total of 23 SNPs were selected for replication in the PCLS cohort, including 10 SNPs showing evidence ($p < 1 \times 10^{-6}$) of association with abdominal fat phenotypes in our sex-combined GWAS meta-analysis as well as 13 SNPs from the Fox et al. paper (48) showing evidence of association with abdominal fat. When multiple SNPs in strong linkage disequilibium were associated with abdominal fat traits on a given region, two SNPs were selected for genotyping to make sure that at least one SNP was available for data analysis if the other one failed the assay. DNA for the replication studies was extracted from buffy coats. The SNPs were genotyped using Illumina GoldenGate assay and Veracode technology on the BeadXpress platform. Genotype calling was done using Illumina GenomeStudio software. All SNPs were in HWE (tested using the exact HWE test implemented in the PEDSTATS software package (59)). In addition, five CEPH DNA samples included in the HapMap Phase II CEU panel (NA10851, NA10854, NA10857, NA10860. NA10861) were genotyped in triplicate. Concordance between the replicates as well as with the SNP genotypes from the HapMap database was 100%.

In silico generation of functional hypotheses

In order to prioritize gene/loci showing evidence of association and to explore the possible functional links among these loci and obesity-related traits, we used the Biograph knowledge-mining software (60). Biograph assembles and analyzes information from 22 heterogeneous biomedical databases via unsupervised data mining techniques and stochastic random walks to generate a map of relationships linking ‘source concepts’ (e.g. phenotypes, diseases) to ‘targets’ (e.g. candidate genes). This network of relationships is analyzed to score and rank the different ‘paths’ linking concepts to targets, resulting in an automated formulation of functional hypotheses. The relative strength of each hypothesis is computed to assess the ‘proximity’ of the association between a ‘concept’ and a ‘target’(61).

Results

Descriptive statistics of the phenotype data for the three cohorts are presented in Table 1. A total of 2,513 subjects, including 1,152 men and 1,361 women, were available for the discovery phase plus 2,943 (2,171 whites) for the replication component. Participants were mostly middle-aged, with a mean age of 35.9, 40.5 and 50.8 years in HERITAGE, QFS and CARDIA, respectively.

Sex-combined analyses

To assess population stratification, quantile-quantile (QQ)-plots were examined for all phenotypes in the sex-combined (Supplementary Figure S1) and the sex-specific (Supplementary Figure S2) meta-analyses. A genomic control lambda value of 1.0 indicates no stratification and values below 1.05 are generally considered as benign (62). As shown in Supplementary Figures S1 and S2, lambda values range from 0.999 to 1.021, suggesting little evidence for unaccounted population stratification.

The Manhattan plots for the abdominal fat phenotypes with (right panel) and without (left panel) adjustment for BMI are shown in Supplementary Figure S3. The horizontal lines in

the plots correspond to p-values of 1.0×10^{-6} and 5.0×10^{-8}, respectively. No SNP reached genome-wide significance (p value $< 5 \times 10^{-8}$). However, a total of 52 SNPs showed suggestive evidence (p values $< 1.0 \times 10^{-6}$) of association with the various abdominal fat phenotypes (results shown in Table 2). For the phenotypes not adjusted for BMI, our most significant finding was with rs12374818 on chromosome 7p14.3 for VAT ($p = 1.10 \times 10^{-7}$; Table 2 and Figure 1A). This SNP is located near *BBS9.* For the other abdominal fat phenotypes not adjusted for BMI, the top hits were with rs9328211 on chromosome 6 for TAT near the *PRPF4B* locus ($p = 7.93 \times 10^{-7}$) and rs2679649 on chromosome 6 for SAT near the *HMGB3P18* locus ($p = 4.97 \times 10^{-7}$). For the BMI-adjusted phenotypes, the most significant finding was with rs10506943 on chromosome 12q21.32 for VAT-BMI near the *CYCSP30* locus ($p= 2.42 \times 10^{-7}$; Table 2 and Figure 1B). For the other BMI-adjusted traits the most significant findings were with rs6038439 on chromosome 20 for TAT-BMI near the *FGFR3P3* locus ($p= 4.48 \times 10^{-7}$) and rs6866135 on chromosome 5 for SAT-BMI near the *HSPD1P15* locus ($p=3.87 \times 10^{-7}$). For VSR, no suggestive evidence of association was found, whether adjusted for BMI or not; the best evidence of association (results not shown) was found with two SNPs on chromosomes 4: rs2292298 for VSR ($p = 1.00 \times 10^{-6}$) and rs11946679 ($p = 1.25 \times 10^{-6}$) for VSR-BMI.

Sex-specific analyses

The Manhattan plots for the sex-specific analyses are presented in Supplementary Figure S4. Table 3 presents the results of the sex-specific analyses for the SNPs achieving suggestive evidence of association ($p < 1.0 \times 10^{-6}$). Except for VAT, the top SNPs for each abdominal fat phenotype analyzed showed stronger evidence of association in women than in men. In men, the best evidence of association was found with rs170053 on chromosome 13 for SAT near the *PCDH17* locus ($p = 5.99 \times 10^{-7}$); rs10505574 on chromosome 8 for VAT near the *ADCY8* locus ($p = 2.62 \times 10^{-7}$) and rs2930176 on chromosome 3 for VSR near the *CACNA1D* locus ($p = 6.06 \times 10^{-7}$). In women, 11 SNPs on chromosome 10 reached genome-wide significant evidence of association ($p < 5.0 \times 10^{-8}$) with SAT, the strongest evidence of association being found with SNP rs7919823 on chromosome 10p12.21 near the *MLLT10* locus ($p = 1.13 \times 10^{-8}$; Table 3 and Figure 1C). Two other SNPs on chromosome 13 (rs12866352 near *EFNB2*, $p = 8.16 \times 10^{-7}$) and chromosome 14 (rs4384548 near *BDKRB2*, $p = 5.27 \times 10^{-8}$) reached suggestive evidence of association with SAT. The key hits for the other abdominal fat phenotypes in women were on chromosome 8 (rs16910486 near *KCNK9*, $p = 5.82 \times 10^{-7}$), chromosome 11 (rs7927727 near *FAR1*, $p = 1.86 \times 10^{-7}$) and chromosome 8 (rs10095849 near *ADAM18*, $p = 2.42 \times 10^{-7}$) for VAT, VAT-BMI and VSR, respectively.

Replication

Table 4 presents the results of replication analyses in the PCLS cohort for the SNPs showing evidence of suggestive association in the sex-combined analyses. Among the 14 SNPs that were tested for association in European Americans and African Americans, separately, three showed evidence of replication (indicated in bold in the table). The association found on chromosome 6 with SAT and two SNPs in perfect linkage disequilibrium (rs2260078 and rs2679647) was replicated in African Americans (p = 0.0013), while the association found

on chromosome 7 with VAT and rs4338001 was replicated in European Americans (p = 0.024).

Replication results for the top SNPs of Fox et al. (48) are presented in Table 5. Since the beta value was not provided in the Fox et al. paper, we cannot say for sure if we really replicated the original finding, because the direction of the association is not known. Nevertheless, the main finding of Fox et al. of an association between rs1659258 near *THNSL2* gene and VAT in women was replicated in PCLS White women (p = 0.0056) and was borderline significant in our meta-analysis (p= 0.059). Another SNP on chromosome 6 showing evidence of association with VAT-BMI was replicated in PCLS Whites (p = 0.0165). Their most significant finding of an association between rs11118316 and VSR near the *LYPLAL1* gene ($p= 3.13 \times 10^{-9}$) was not replicated in our meta-analysis nor in the PCLS cohort, but we found an association between this SNP and SAT in our sex-combined meta-analysis (p= 0.048).

In an attempt to further replicate the findings from Fox et al. we also verified whether the SNPs from their Table S2, which showed evidence of association with p-values $< 1.0 \times 10^{-4}$, were associated ($p < 0.05$) with abdominal fat in our sex-combined or sex-specific GWAS meta-analysis. The results presented in Supplementary Table S1 reveal that several SNPs were associated with abdominal fat in both studies, but not necessarily with the same trait. Replications (associations with the same trait) were found for 7 different loci: chromosome 3 for VAT-BMI in women (rs7638389 near *ADAMTS9*, p = 0.006); chromosome 6 for VSR (rs12204127 near *BACH2*, p = 0.03); chromosome 7 for VAT-BMI in men (rs1299548 near *C1GALT1*, p = 0.039); chromosome 14 for VAT in women (rs3783938 near *TSHR*, p= 0.026); chromosome 15 for VSR in men (rs8036080 near *VPS18*, p= 0.035); chromosome 19 for VAT-BMI in men (rs8106493 near *SLC7A10*, p= 0.046) and chromosome 20 for VAT-BMI in men (rs13043330 near *HSPA12B*, p= 0.011).

Exploratory analysis of functional associations

The Biograph tool was utilized to explore the possible functional links among VAT-associated loci, and obesity-related phenotypes. Six VAT-associated genes (*BBS9, ROBO1, ADCY8, FAR1, KCNK9 and EFR3*) were individually queried for association to target phenotypes. With the exception of *CYCSP30*, which is a pseudogene, these genes were those showing the strongest evidence of association in our sex-combined (Table 2) or sex-stratified (Table 3) analyses. An obvious target phenotype was “obesity”, since VAT mass is highly correlated with total adiposity. We also considered ‘inflammation’ as a target phenotype, because VAT is considered a pro-inflammatory organ playing an important role in the etiology of obesity-related cardiometabolic complications (63–65), and also because of previous evidence of genetic pleiotropy between inflammation and abdominal obesity (21). It is therefore conceivable that, at least for a subset of genes, the observed association to VAT reflects association to VAT-related inflammation. For each target phenotype, the proximity of a gene to the phenotype was quantified as a relative rank of the gene compared to all other genes linking to the same phenotype in Biograph’s knowledge base. The phenotype-proximity ranks for the 6 genes are shown in Table 6. For each phenotype, the global rank represents the rank percent of a gene’s proximity score compared to the

proximity scores for all other Biograph entities (genes, compounds, metabolites, etc) for the same phenotype, while for gene rank the comparison is restricted only to genes. A lower rank percentage indicates higher proximity between the gene and the phenotype. Based on the scores, *ADCY8* (5.36%) and *ROBO1* (11.68%) were ranked in the top 20% of all genes linked to 'obesity', whereas *ADCY8* (9.59%), *ROBO1* (8.48%) and *KCNK9* (0.54%) scored in the top 20% for their global strength of connection to 'inflammation'. The remaining genes had poorer ranks for both targets. The Biography-derived connectivity graphs between *ADCY8* and obesity and *KCNK9* and inflammation are shown in Figure 2a–b. Connectivity diagrams for the other genes are shown in Supplementary Figure S5 for obesity and S6 for inflammation.

Discussion

The results of this GWA study of abdominal visceral fat measured by computed tomography in three cohort studies revealed the presence of several loci associated ($p < 1.0 \times 10^{-6}$) with measures of abdominal fat adjusted (*SPAG16*, *FGFR3P3*, *HSPD1P15*, *CYCSP30*) or not adjusted (*PRPF4B*, *HMGB3P18*, *BBS9*) for BMI. Our sex-combined analysis provided no genome-wide significant loci, but the evidence of association observed for VAT at *BBS9* and for SAT at *HMGB3P18* was replicated in an independent cohort. Our sex-stratified analysis provided one genome-wide significant locus ($p < 5 \times 10^{-8}$) for SAT in women with a block of 11 SNPs near the *MLLT10*, *DNAJC1* and *EBLN1* genes on chromosome 10. We also confirmed in an independent cohort a previous association observed between a SNP near the *THNSL2* gene and visceral fat in women (48).

In the sex-combined analyses, the strongest evidence of association was found for VAT with SNP rs12374818 near the *BBS9* gene on chromosome 7p14.3. The association between VAT and *BBS9* was replicated in the PCLS cohort (with SNP rs4338001, $r^2 = 1.0$). *BBS9* is one of the 15 genes/loci that have been associated with Bardet-Biedl syndrome (BBS), a genetically heterogeneous disorder characterized by several clinical features, including polydactyly, retinopathy, renal abnormalities, mental retardation and truncal obesity. Studies have shown that BBS proteins are involved in cilia-associated functions (66). The cilium is a specialized organelle projecting from plasma membrane of almost every vertebrate cell and plays a role in the transduction of extracellular signals. Using homozygosity mapping of small consanguineous BBS families followed by comparative genomics and gene expression studies of a BBS-knockout mouse model, Nishumira et al., (67) identified parathyroid hormone-responsive B1 (*PTHB1*) gene as the *BBS9* gene. Knockdown of *BBS9/PTHB1* gene in zebra fish was found to lead to developmental abnormalities in the retina and brain that were consistent with the core phenotypes observed in syndromic ciliopathies and human BBS9 mRNA rescued the bbs9 knockdown phenotype (68). The exact mechanism leading to obesity in BBS patients is not known, but a study using BBS knockout mouse models showed that $Bbs2^{-/-}$, $Bbs4^{-/-}$, $Bbs6^{-/-}$ mice were resistant to the action of leptin to reduce body weight and food intake regardless of serum leptin levels and obesity, suggesting that altered leptin receptor signalling is the major cause of obesity in BBS (69). Interestingly, variants in the *BBS2*, *BBS4* and *BBS6* genes were previously reported to be associated with obesity in non-BSS individuals (70).

Suggestive evidence of association was also found for SAT near the *HMGB3P18* gene (high mobility group box 3 pseudogene 18), a finding that was replicated in the PCLS cohort. This pseudogene is located on chromosome 6 near the *NKAIN2* locus (also known as *TCBA1* gene), which was also previously found to be associated with SAT (48). For the BMI-adjusted abdominal fat phenotypes, the strongest evidence of association was found for VAT-BMI near the *CYCSP30* gene (cyctochrome c, somatic pseudogene 30; location 12q21.32), one of the numerous processed cyctochrome c pseudogenes found throughout the human genome. Several SNPs in that region of chromosome 12 showed suggestive evidence of association with VAT-BMI. In a previous large GWA study of more than 10,000 Korean subjects (44), strong evidence of association was found in that region of chromosome 12 with systolic blood pressure (rs17249754, $p = 1.3 \times 10^{-7}$) and WHR (rs2074356, $p = 7.8 \times 10^{-12}$).

Given the importance of sexual dimorphism in the distribution of body fat, we performed sex-stratified meta-analyses. The analyses revealed that 12 loci were associated with abdominal fat in women compared to 6 loci in men. The strongest evidence of association, and the only one reaching genome-wide significant level ($p < 5.0 \times 10^{-8}$), was found for SAT in women with 11 SNPs encompassing three different loci on chromosome 10p12.31: *MLLT10*, *DNAJC1* and *EBLN1*. No evidence of association with obesity-related traits has been reported with these loci, but a SNP located in that region of chromosome 10 (rs16923476 at *OTUD1/KIAA1217* locus; $p = 3.69 \times 10^{-8}$) was previously found to be associated with severe early-onset obesity (71). In men, the strongest evidence of association was found for VAT with SNP rs10505574 on chromosome 8 between the *ADCY8* and *EFR3* genes ($p = 2.62 \times 10^{-7}$).

Recent data suggest that site-specific expression of developmental genes direct adipose tissue development, while providing a mechanistic basis to explain functional differences between upper-body and lower-body adipose tissue (72–74). These developmental genes include members of the homeobox (HOX) family, HOX-domain encoding genes and T-box genes, which are transcriptional factors involved in early embryonic development, body patterning and cell specification. One such gene, *TBX15*, was previously reported to be associated with fat distribution in GWA studies (41). Interestingly, *TBX15* was first identified by its higher expression in VAT compared to SAT in both rodents and humans (75). In our meta-analysis, a SNP located in *TBX15* (rs1779437) was associated with VAT-BMI ($p = 0.0006$) and VSR-BMI ($p = 0.02$). A second member of the T-box family of genes showing differences in expression level between abdominal fat and lower-body fat is *TBX5*, and results of our meta-analysis also revealed that SNPs in *TBX5* were associated with VAT-BMI in women (rs2236017, $p = 8.7 \times 10^{-5}$), TAT-BMI (rs2555025, $p= 0.007$) and VSR-BMI (rs10850336, $p= 0.009$).

Multiple GWA studies have identified several loci associated with anthropometric measures of fat distribution (41–45, 76). The most recent GWA meta-analysis of traits related to fat distribution in up to 224,450 individuals identified 49 loci associated with waist-to-hip ratio adjusted for BMI (WHRadjBMI), 33 of which were new and 16 previously described (47). The study also identified 7 new loci for waist circumference adjusted for BMI and 3 new loci for waist-to-hip ratio. None of the loci found to be associated with abdominal fat in the

present study were in the list of the 59 loci reported by Shungin et al., to be associated with anthropometric measures of abdominal (47), but two of the loci reported in the present study fall in the same genomic region as two WHRadjBMI loci. One is the *FGFR3P3* locus on chr. 20 (Table 2) associated with TAT in our sex-combined analyses (rs6038439. $p = 4.48 \times 10^{-7}$), which is in the same genomic region as *BMP2* (rs979012, $p = 3.3 \times 10^{-14}$), and the other is *CACNAD1* on chr. 3 (Table 3) associated with VSR in men (rs2930176, $p = 6.06 \times 10^{-7}$) that is close to the PBRM1 locus (rs2276824, $p = 3.2 \times 10^{-11}$). In total, 25 of these 59 loci associated with anthropometric measures of abdominal fat showed significant sexual dimorphism, the majority of them (21 out of 25) displaying stronger effects in women (47), which is consistent with the findings from our sex-specific analyses. Other GWA studies found significant sex-differences for loci associated with anthropometric measures of fat distribution (41, [43], [76]), which emphasize the need for considering sex-differences in association studies when searching for genes influencing the fat distribution profile.

Only one GWA meta-analysis of abdominal adipose tissue assessed by computed tomography has been reported so far (48). In that meta-analysis of four GWA studies including 5,560 women and 4,997 men, the strongest association was observed between *LYPLAL1* rs11118316 and VSR ($p= 3.1 \times 10^{-9}$), a SNP in linkage disequilibrium with rs4846567 that was previously found to be associated with VSR in Japanese subjects (p = 0.002) (77) and with WHR adjusted for BMI ($p= 6.89 \times 10^{-21}$)(41). This result was not replicated in our study as we found only marginal evidence of association between rs11118316 and SAT in all subjects (p = 0.048). For SAT, the most significant finding of Fox et al., (48) was with SNP rs9922619 in the *FTO* gene ($p= 5.87 \times 10^{-8}$), a SNP that we also found to be associated with SAT, but in men only (p= 0.002) (see Table S1). For VAT-BMI, the most significant finding of Fox et al. (48) was with SNP rs1641895 in an intron of the sorting nexin 29 (*SNX29*) gene on chromosome 16, a variant that we found to be associated with SAT (p= 0.003) and VAT (p= 0.01) in women (see Table S1). Seven loci, which showed significant evidence of association with abdominal fat in the Fox et al. study (48), were replicated in our meta-analyses (see Table S1). A series of studies undertaken in overweight Japanese subjects have tested whether SNPs associated with increased susceptibility to obesity and obesity-related complications were associated with VAT and SAT measured by computed tomography. Overall, these studies tested associations between 83 SNPs in 66 genes/loci and found associations for *FTO* with SAT and VAT (78, [79]), *SH2B1* with VAT (80), *CYP17A1* and *NT5C2* with both SAT and VAT in women (81), *LYPLAL1* with VSR, *NISCH* with VAT and VSR (77) and *NUDT3* rs206936 with SAT in women (82).

As a way of exploring the potential mechanisms by which genetically associated loci may relate to biological function, we utilized the Biograph knowledge mining tool and derived exploratory functional links connecting the VAT-associated genes to the phenotypes of ‘obesity’ and ‘inflammation’. The graph linking the *ADCY8* gene to ‘obesity’ displays multiple routes traversing via the *GNB3* gene, an essential component of G-protein coupled receptor signalling. Notably, the *GNB3* 825C>T polymorphism has previously been associated with obesity in specific populations (83–[85]). Similarly, Biograph identified a very strong connection between the potassium-channel *KCNK9* gene and ‘inflammation’ (gene rank 0.7%). Among the many possible routes linking *KCNK9* to inflammation, one involved local anesthetic bupivacaine. Bupivacaine is a KCNK9 inhibitor (86), and is known to

display complex, context-dependent pro- and anti-inflammatory effects (87–89). In addition to the hypotheses from Biograph, the *KCNK9* channel activity appears to be directly enhanced by the pro-inflammatory cytokine TNF-alpha, eventually leading to cellular apoptosis (90). If regulation of *KCNK9* activity is upstream to the generation of inflammatory signals, then one might speculate how altered *KCNK9* activity could influence inflammatory signaling from VAT.

The present study has focused on the identification of genetic associations between individual loci and abdominal adipose tissue depots, with or without adjustment for total adiposity. The majority of the loci fall below the statistical threshold for genome-wide significance, suggestive of weaker effects when these loci are considered in isolation. Effect estimates of variants associated with the traits adjusted for BMI should be interpreted with caution, as suggested by a recent study which showed that estimates of variants identified in GWAS for traits adjusted for a covariate that is heritable can be biased, relative to the true direct effect on the trait (91). To illustrate this bias, the authors conducted a GWAS of WHR, BMI and WHRadjBMI and found that half of the reported associations with WHRadjBMI were likely influenced by a direct genetic association with BMI. The authors recommended avoiding such adjustment unless we know for certainty that the tested variant does not influence the covariate (91). Given the evidence of abundant pleiotropy among genes associated with complex traits (92), it is unlikely that a covariate such as BMI can fulfill that condition. In addition, it is important to remember that the genetic architecture underlying complex traits is often the result of joint interactions among multiple, weakly associated loci. Identification of these interactions can, therefore, provide additional insights into the bases of genetic susceptibilities. Among several methods, set-based techniques such as biological pathway analysis and interactome analysis (93–95) have proven successful in identifying joint interactions that contribute significantly to diverse traits, including multiple sclerosis, cardiorespiratory fitness, cholesterol metabolism and lung cancer (96–99). We have not examined such methods in the present study but plan on doing so in the future.

In conclusion, our study identified new loci influencing abdominal visceral (*BBS9, ADCY8, KCNK9*) and subcutaneous (*MLLT10*, *DNAJC1*, *EBLN1*) fat depots. We also confirmed in an independent cohort a previous association observed between the *THNSL2* gene and visceral fat in women and replicated in our meta-analysis seven loci that were previously found to be associated with various measures of abdominal fat obtained by imaging as in the present study. Our results also highlight the importance of sex-differences in the genetic architecture of body fat distribution.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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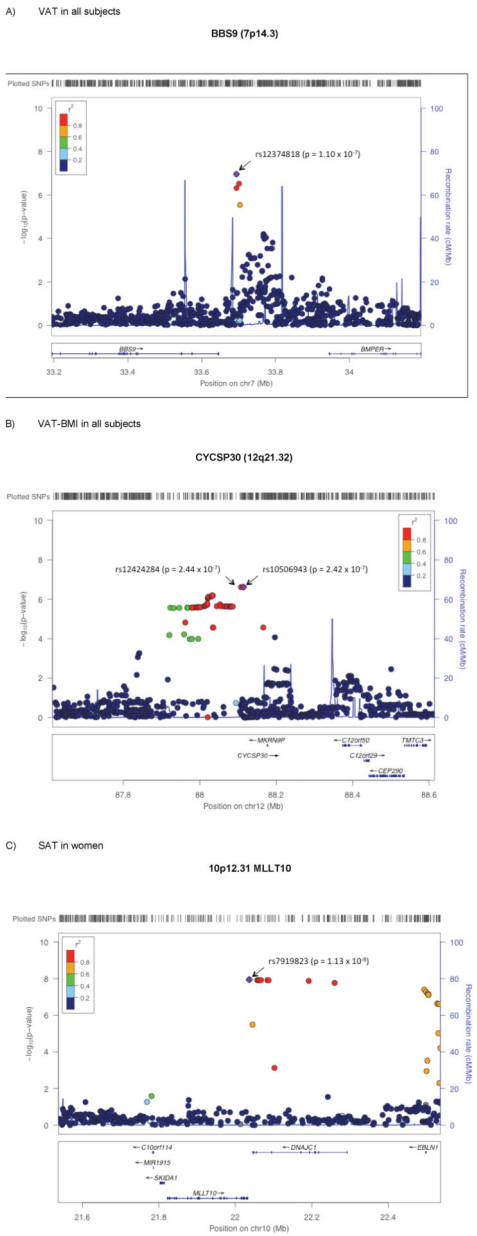

Figure 1. Regional plots for loci showing the strongest evidence of association

Regional plots for loci showing the strongest evidence of association with VAT (panel A), VAT-BMI (panel B), and SAT in women (panel C)

SNPs are plotted by position on chromosome against association (−log10 p-value) and estimated recombination rate (from HapMap-CEU). SNPs surrounding the most significant SNP (purple diamond) are color-coded to reflect their LD with this SNP. Genes and the positions of exons as well as the direction of transcription are shown below the plots. These regional plots were generated using LocusZoom (http://csg.sph.umich.edu/locuszoom/)

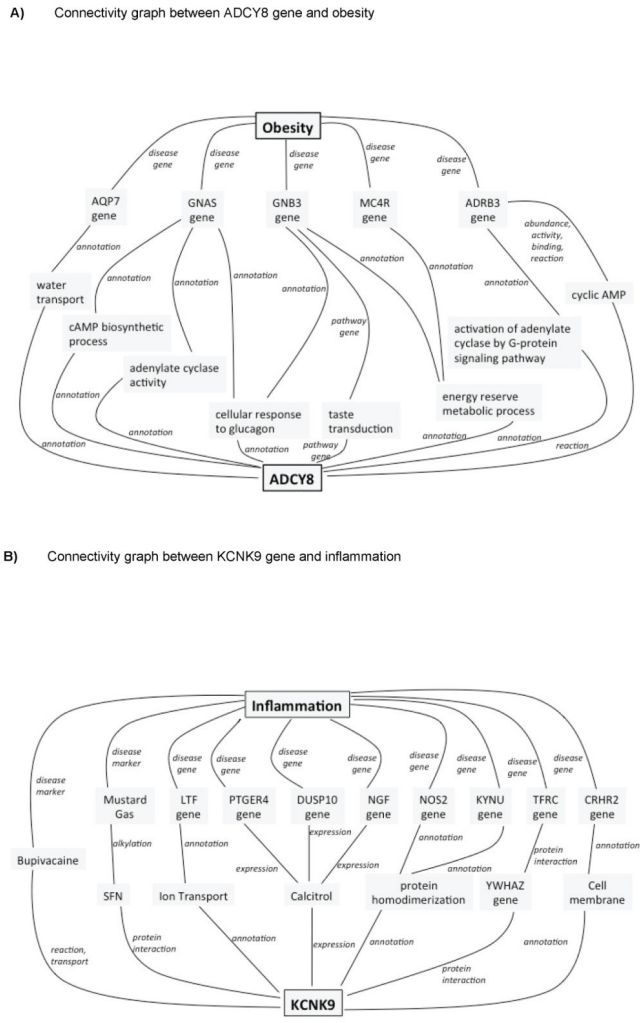

Figure 2. Biograph analysis of VAT-BMI associated genes to ‘obesity’ and ‘inflammation’ phenotypes Biograph analysis of VAT-BMI associated genes to ‘obesity’ and ‘inflammation’ phenotypes

A) Biograph generated connectivity graph between *ADCY8* gene and obesity. **B)** Biograph generated connectivity graph between *KCNK9* gene and inflammation. The intermediate linking the genes to the phenotype are indicated in a gray background along with the type of interaction.

Table 1

Descriptive statistics for the three studies used in the analysis

	N	Age (years)	BMI (kg/m^2)	TAT (cm^2)	SAT (cm^2)	VAT (cm^2)	VSR
CARDIA All	1335	50.8 ± 3.3	28.3 ± 6.1	452.2 ± 202.0	292.5 ± 143.8	139.95 ± 78.79	0.53 ± 0.30
CARDIA Men	618	50.8 ± 3.3	28.7 ± 4.9	450.7 ± 184.3	258.4 ± 118.2	172.4 ± 79.9	0.72 ± 0.32
CARDIA Women	717	50.8 ± 3.4	27.9 ± 7.0	453.4 ± 216.2	321.9 ± 156.9	112.0 ± 66.1	0.36 ± 0.15
HERITAGE All	496	35.9 ± 14.6	25.8 ± 5.0	355.2 ± 186.0	261.7 ± 144.8	93.4 ± 61.6	0.39 ± 0.22
HERITAGE Men	244	36.6 ± 15.0	26.7 ± 4.9	341.6 ± 185.3	231.1 ± 136.4	110.4 ± 64.9	0.52 ± 0.22
HERITAGE Women	252	35.2 ± 14.2	25.0 ± 4.9	368.3 ± 186.1	291.3 ± 136.4	77.0 ± 53.5	0.27 ± 0.12
QFS All	682	40.5 ± 15.5	27.3 ± 6.9	402.5 ± 226.2	287.5 ± 173.2	114.9 ± 80.5	0.46 ± 0.29
QFS Men	290	41.0 ± 16.1	27.0 ± 5.5	351.4 ± 203.2	220.7 ± 137.7	130.7 ± 87.3	0.65 ± 0.32
QFS Women	392	40.2 ± 15.0	27.6 ± 7.7	440.3 ± 234.9	336.9 ± 180.2	103.3 ± 73.1	0.31 ± 0.15

Values are mean ± the standard deviation or percentage. BMI = body mass index; TAT = total abdominal adipose tissue; SAT = subcutaneous abdominal adipose tissue; VAT = visceral abdominal adipose tissue; VSR = VAT/SAT ratio.

Table 2

Abdominal fat loci achieving suggestive evidence of association ($p < 1.0 \times 10^{-6}$) in sex-combined meta-analyses

Trait	SNP	Chr	Position (bp)	Locus	Distance	EA	EAF	Beta	StdErr	P-value
No adjustment for BMI										
TAT	**rs9328211**	**6**	**3 989 654**	**PRPF4B**	**32**	**A**	**0.24**	**−33.12**	**6.708**	**7.93×10^{-7}**
SAT	**rs2679649**	**6**	**122 350 385**	**HMGB3P18**	**170**	**A**	**0.94**	**−48.92**	**9.730**	**4.97×10^{-7}**
	rs2260078	6	122 350 567	HMGB3P18	171	A	0.06	46.36	9.423	8.67×10^{-7}
	rs2679647	6	122 351 503	HMGB3P18	172	T	0.06	47.28	9.486	6.23×10^{-7}
	rs2684270	6	122 352 450	HMGB3P18	173	A	0.06	47.25	9.486	6.33×10^{-7}
	rs2679643	6	122 353 149	HMGB3P18	173	T	0.94	−47.24	9.487	6.38×10^{-7}
	rs2684268	6	122 354 079	HMGB3P18	174	T	0.94	−47.27	9.499	6.46×10^{-7}
	rs2679641	6	122 355 637	HMGB3P18	176	T	0.94	−47.34	9.517	6.55×10^{-7}
	rs2816131	6	122 355 883	HMGB3P18	176	A	0.06	47.34	9.519	6.57×10^{-7}
	rs2816128	6	122 357 203	HMGB3P18	177	A	0.06	47.36	9.522	6.57×10^{-7}
	rs2679695	6	122 359 279	HMGB3P18	179	A	0.06	47.39	9.531	6.61×10^{-7}
	rs2816125	6	122 359 558	HMGB3P18	180	T	0.06	47.37	9.532	6.69×10^{-7}
	rs2679693	6	122 360 216	HMGB3P18	180	T	0.94	−47.37	9.533	6.72×10^{-7}
	rs2679692	6	122 361 247	HMGB3P18	181	T	0.06	47.22	9.500	6.69×10^{-7}
	rs2684266	6	122 361 317	HMGB3P18	181	T	0.94	−47.19	9.501	6.81×10^{-7}
	rs2684265	6	122 362 037	HMGB3P18	182	T	0.94	−47.26	9.538	7.25×10^{-7}
	rs1357056	6	122 363 734	HMGB3P18	184	A	0.94	−46.83	9.448	7.17×10^{-7}
	rs2684264	6	122 365 026	HMGB3P18	185	T	0.94	−46.86	9.461	7.33×10^{-7}
	rs9490391	6	122 368 622	HMGB3P18	189	T	0.94	−47.40	9.571	7.30×10^{-7}
	rs1521231	6	1223 69 526	HMGB3P18	190	T	0.06	47.43	9.588	7.57×10^{-7}
VAT	**rs12374818**	**7**	**33 694 108**	**BBS9**	**48**	**A**	**0.06**	**0.09**	**0.018**	**1.10×10^{-7}**
	rs12374953	7	33 694 132	BBS9	48	A	0.08	0.08	0.015	4.81×10^{-7}
	rs4338001	7	33 701 080	BBS9	55	A	0.07	0.08	0.015	3.02×10^{-7}
BMI-adjusted trait										

Trait	SNP	Chr	Position (bp)	Locus	Distance	EA	EAF	Beta	StdErr	P-value
TAT	rs17766701	2	215 115 236	SPAG16	0	A	0.90	−20.30	4.125	8.59×10^{-7}
	rs17817960	2	215 119 880	SPAG16	0	A	0.09	20.84	4.181	6.21×10^{-7}
	rs6038439	**20**	**6 318 583**	**FGFR3P3**	**117**	**A**	**0.30**	**−12.94**	**2.564**	**4.48×10^{-7}**
	rs2876017	20	6 321 944	FGFR3P3	121	T	0.30	−12.89	2.561	4.80×10^{-7}
	rs1305009	20	6 324 424	FGFR3P3	123	A	0.30	−12.90	2.561	4.74×10^{-7}
	rs6054136	20	6 326 709	FGFR3P3	125	A	0.70	12.91	2.560	4.64×10^{-7}
	rs8118802	20	6 334 683	FGFR3P3	133	A	0.30	−12.79	2.558	5.79×10^{-7}
	rs6076985	20	6 337 184	FGFR3P3	136	A	0.30	−12.64	2.555	7.46×10^{-7}
	rs6085518	20	6 345 709	FGFR3P3	144	T	0.30	−12.52	2.556	9.68×10^{-7}
	rs6133310	20	6 355 395	FGFR3P3	154	T	0.29	−12.68	2.562	7.41×10^{-7}
	rs6085522	20	6 357 707	FGFR3P3	156	A	0.70	12.71	2.561	6.90×10^{-7}
	rs6038468	20	6 360 394	FGFR3P3	159	T	0.71	12.68	2.563	7.45×10^{-7}
	rs959278	20	6 362 730	FGFR3P3	161	A	0.29	−12.67	2.564	7.69×10^{-7}
SAT	**rs6866135**	**5**	**19 237 705**	**HSPD1P15**	**3**	**T**	**0.53**	**−9.53**	**1.877**	**3.87×10^{-7}**
VAT	rs1588660	12	88 020 793	CYCSP30	120	A	0.97	0.07	0.014	8.89×10^{-7}
	rs1358302	12	88 021 930	CYCSP30	118	A	0.97	0.07	0.014	8.66×10^{-7}
	rs1398425	12	88 022 814	CYCSP30	118	A	0.97	0.07	0.014	8.33×10^{-7}
	rs2669107	12	88 022 848	CYCSP30	117	A	0.97	0.07	0.014	8.10×10^{-7}
	rs1398424	12	88 022 899	CYCSP30	117	T	0.97	0.07	0.014	7.68×10^{-7}
	rs2644752	12	88 023 316	CYCSP30	117	T	0.97	0.07	0.014	7.53×10^{-7}
	rs2137420	12	88 030 880	CYCSP30	109	A	0.97	0.07	0.014	7.23×10^{-7}
	rs2669106	12	88 031 123	CYCSP30	109	A	0.03	−0.07	0.014	6.95×10^{-7}
	rs17386404	12	88 031 977	CYCSP30	108	C	0.03	−0.07	0.014	6.79×10^{-7}
	rs2669105	12	88 032 132	CYCSP30	108	A	0.97	0.07	0.014	6.57×10^{-7}
	rs950414	12	88 033 419	CYCSP30	107	T	0.03	−0.07	0.014	6.56×10^{-7}
	rs704058	12	88 033 654	CYCSP30	107	A	0.03	−0.07	0.014	6.53×10^{-7}
	rs790448	12	88 033 786	CYCSP30	107	A	0.03	−0.07	0.014	6.53×10^{-7}
	rs12424284	12	88 107 791	CYCSP30	33	C	0.04	−0.06	0.012	2.44×10^{-7}

Trait	SNP	Chr	Position (bp)	Locus	Distance	EA	EAF	Beta	StdErr	P-value
	rs10506943	**12**	**88 114 376**	**CYCSP30**	**26**	**T**	**0.96**	**0.06**	**0.012**	**2.42×10^{-7}**

EA = Effect allele; EAF = effect allele frequency. *P* values and β coefficients (per change of the effect allele) for the association with abdominal fat phenotypes. Positions are reported in base pairs (NCBI Build 37). Distance (in kbp) represents the distance between the SNP and the locus; a value of zero means that the SNP is within the gene. Entries in bold indicate the top SNP for a given phenotype.

Table 3

Abdominal fat loci achieving suggestive evidence of association in sex-specific analyses

Trait	SNP	Chr	Position (bp)	Locus	Distance kbp	EA	EAF	Beta	P-value
SAT Men	**rs1370053**	**13**	**58514665**	**PCDH17**	**211**	**T**	**0.02**	**383.17**	**5.99×10^{-7}**
SAT Women	rs6727879	2	231 026 592	SP110	7	A	0.99	−645.32	1.51×10^{-7}
	rs2679649	6	122 350 385	HSF2	370	A	0.94	−77.55	2.16×10^{-7}
	rs2260078	6	122 350 567	HSF2	370	A	0.06	72.13	5.51×10^{-7}
	rs2679647	6	122 351 503	HSF2	369	T	0.05	74.15	3.11×10^{-7}
	rs2684272	6	122 351 593	HSF2	369	A	0.06	71.30	8.83×10^{-7}
	rs2684270	6	122 352 450	HSF2	368	A	0.05	74.10	3.17×10^{-7}
	rs2679643	6	122 353 149	HSF2	367	T	0.95	−74.08	3.23×10^{-7}
	rs2684268	6	122 354 079	HSF2	366	T	0.95	−73.85	3.61×10^{-7}
	rs2679641	6	122 355 637	HSF2	365	T	0.95	−73.53	4.29×10^{-7}
	rs2816131	6	122 355 883	HSF2	364	A	0.05	73.46	4.41×10^{-7}
	rs2816128	6	122 357 203	HSF2	363	A	0.05	73.47	4.46×10^{-7}
	rs2679695	6	122 359 279	HSF2	361	A	0.05	73.38	4.75×10^{-7}
	rs2816125	6	122 359 558	HSF2	361	t	0.05	73.22	5.05×10^{-7}
	rs2679693	6	122 360 216	HSF2	360	t	0.95	−73.18	5.14×10^{-7}
	rs2679692	6	122 361 247	HSF2	359	T	0.06	72.92	5.29×10^{-7}
	rs2684266	6	122 361 317	HSF2	359	t	0.94	−72.76	5.64×10^{-7}
	rs2684265	6	122 362 037	HSF2	358	t	0.95	−72.73	6.17×10^{-7}
	rs1357056	6	122 363 734	HSF2	357	a	0.94	−71.24	7.43×10^{-7}
	rs2684264	6	122 365 026	HSF2	355	T	0.94	−71.27	7.69×10^{-7}
	rs9490391	6	122 368 622	HSF2	352	T	0.95	−72.99	6.29×10^{-7}
	rs1521231	6	122369 526	HSF2	351	T	0.05	72.98	6.71×10^{-7}
	rs17241164	9	9 857 448	PTPRD	0	T	0.99	−407.73	6.55×10^{-7}
	rs7919823	**10**	**22 036 491**	**MLLT10**	**4**	**A**	**0.98**	**−252.13**	**1.13×10^{-8}**
	rs7903144	10	22 057 405	DNAJC1	0	A	0.98	−249.29	1.21×10^{-8}
	rs7899191	10	22 060 277	DNAJC1	0	A	0.02	249.21	1.21×10^{-8}

Trait	SNP	Chr	Position (bp)	Locus	Distance kbp	EA	EAF	Beta	P-value
VAT-BMI Women	**rs7927727**	**11**	**13 656 086**	**FAR1**	**34**	**A**	**0.37**	**−0.03**	**1.86×10^{-7}**
VSR Men	**rs2930176**	**3**	**53 487 279**	**CACNA1D**	**42**	**A**	**0.52**	**−0.04**	**6.06×10^{-7}**
VSR Women	rs4947599	7	51 538 997	COBL	154	C	0.80	−0.04	5.17×10^{-7}
	rs7819481	8	39 405 882	ADAM18	36	A	0.17	0.04	3.45×10^{-7}
	rs10095849	**8**	**39 408 586**	**ADAM18**	**34**	**T**	**0.83**	**−0.04**	**2.42×10^{-7}**

EA = Effect allele; EAF = effect allele frequency. *P* values and β coefficients (per change of the effect allele) for the association with abdominal fat phenotypes. Positions are reported in base pairs (NCBI Build 37). Distance (in kbp) represents the distance between the SNP and the locus; a value of zero means that the SNP is within the gene. Entries in bold indicate the top SNP for a given phenotype.

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Table 4

Replication of top SNPs of the sex-combined GWA meta-analysis in the PCLS cohort.

				Meta-analysis			PCLS European Americans			PCLS African Americans		
SNP	**Chr**	**Position**	**trait**	**EA**	**Beta**	**P-value**	**EA**	**Beta**	**P-value**	**EA**	**Beta**	**P-value**
rs17817960	2	215 119 880	TAT-BMI	A	20.84	6.21×10^{-7}	A	−3.15	0.4626	A	3.34	0.7347
rs6866135	5	19 237 705	SAT-BMI	T	−9.53	3.87×10^{-7}	C	2.64	0.1946	T	5.33	0.1721
rs2260078	**6**	**122 350 567**	**SAT**	A	46.36	8.67×10^{-7}	A	−4.47	0.6445	**A**	**25.67**	**0.001342**
rs2679647	**6**	**122 351 503**	**SAT**	T	47.28	6.23×10^{-7}	A	−5.05	0.6041	**A**	**25.67**	**0.001342**
rs4338001	**7**	**33 701 080**	**VAT**	A	0.08	3.02×10^{-7}	**A**	**0.036**	**0.02426**	A	−0.037	0.1047
rs704058	12	88 033 654	VAT-BMI	A	−0.07	6.53×10^{-7}	T	−0.001	0.9065	T	−0.011	0.2202
rs790448	12	88 033 786	VAT-BMI	A	−0.07	6.53×10^{-7}	T	−0.001	0.906	T	−0.010	0.3021
rs10506943	12	88 114 376	VAT-BMI	T	0.06	2.42×10^{-7}	C	0.008	0.4547	C	0.031	0.4837
rs1305009	20	6 324 424	VAT-BMI	A	−12.90	4.74×10^{-7}	A	0.96	0.7166	A	0.24	0.9644
rs6054136	20	6 326 709	ATF-BMI	A	12.91	4.64×10^{-7}	G	1.52	0.5671	G	0.38	0.9423

EA = Effect allele. *P* values and β coefficients (per change of the effect allele) for the association with abdominal fat phenotypes. Positions are reported using NCBI Build 37. Entries in bold indicate replication in the PCLS cohort.

Table 5

Replication of top SNPs of Fox et al. in the PCLS cohort

				Fox et al. 2012			Meta-analysis			PCLS European Americans			PCLS African Americans		
SNP	**Chr**	**Position**	**Trait**	**EA**	**Beta**	**P-value**	**EA**	**Beta**	**P-value**	**EA**	**Beta**	**P-value**	**EA**	**Beta**	**P-value**
rs10914967	1	34 761 824	VAT-BMI All	A	NA	6.33×10^{-6}	A	0.002	0.683	A	0.004	0.5043	G	0.002	0.7974
rs4657015	1	159 539 065	VAT-BMI All	G	NA	7.95×10^{-6}	A	−0.001	0.825	A	0.000	0.9704	A	−0.012	0.1551
rs11118316	1	217 723 786	VSR All	A	NA	3.13×10^{-9}	A	0.002	0.697	A	−0.002	0.6063	A	0.016	0.2907
rs1659258	**2**	**88 440 703**	**VAT women**	A	NA	1.58×10^{-8}	A	−0.037	0.059	G	**−0.056** *	**0.0056** *	G	−0.004 *	0.822 *
rs6781182	3	34 187 170	VAT-BMI All	T	NA	3.31×10^{-6}	T	−0.006	0.270	T	0.004	0.4053	T	0.007	0.3795
rs2554152	3	141 089 484	VAT-BMI All	G	NA	7.04×10^{-6}	T	−0.007	0.340	T	0.001	0.8663	G	0.003	0.6722
rs10516635	4	118 299 355	VAT-BMI All	A	NA	8.38×10^{-6}	A	−0.005	0.571	A	−0.006	0.3744	A	0.013	0.2419
rs11930273	4	151 121 207	VAT-BMI All	G	NA	1.14×10^{-6}	A	−0.003	0.775	A	−0.009	0.2955	A	−0.009	0.4626
rs2842895	**6**	**7 051 315**	**VAT-BMI All**	C	NA	4.32×10^{-6}	C	0.002	0.611	G	**−0.011**	**0.0165**	C	0.011	0.3684
rs4304868	12	116 654 292	VAT-BMI All	A	NA	7.57×10^{-6}	A	0.009	0.567	T	0.003	0.6631	T	−0.001	0.9123
rs1316952	12	122 965 503	VAT-BMI All	T	NA	4.60×10^{-6}	T	0.008	0.261	C	0.007	0.2793	C	−0.004	0.6268
rs1048497	12	123 065 495	VAT-BMI All	G	NA	8.51×10^{-6}	A	−0.009	0.346	A	0.006	0.3202	A	−0.013	0.547
rs746080	16	82 831 906	VAT-BMI All	T	NA	6.21×10^{-6}	T	0.007	0.381	A	0.001	0.9123	A	0.001	0.9175

*Results for sex-stratified analysis with women. EA = Effect allele. *P* values and β coefficients (per change of the effect allele) for the association with abdominal fat phenotypes. Positions are reported using NCBI Build 37. Entries in bold indicate replication in the PCLS cohort.

Table 6

Rank percent proximity scores for the 6 VAT-BMI associated genes to obesity and inflammation derived from Biograph analysis

Gene	Obesity		Inflammation	
	Global Rank (%)	Gene Rank (%)	Global Rank (%)	Gene Rank (%)
ADCY8	3.64	5.36	9.59	16.5
ROBO1	6.32	11.68	8.48	13.93
KCNK9	12.40	27.67	0.54	0.70
EFR3A	29.79	68.72	12.46	22.93
FAR1	18.00	42.27	25.97	52.89
BBS9	15.53	36.09	24.98	50.79

Values are rank percent of a gene's proximity score compared to the proximity scores for all other Biograph entities (genes, compounds, metabolites, etc) to the same phenotype (Global Rank) or compared to the proximity scores for genes only (Gene Rank). A lower rank percentage indicates higher proximity between the gene and the phenotype.