

ORIGINAL ARTICLE

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

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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).

SUBJECTS AND METHODS: Sex-combined and sex-stratified analyses were performed on each trait with (TRAIT–BMI) or without (TRAIT) adjustment for body mass index (BMI), and cohort-specific results were combined via a fixed effects meta-analysis. A total of 2513 subjects of European descent were available for the discovery phase. For replication, 2171 European Americans and 772 African Americans were available.

RESULTS: A total of 52 single-nucleotide polymorphisms (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.

International Journal of Obesity (2016) 40, 662–674; doi:10.1038/ijo.2015.217

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 1698 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 with 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 or computed tomography. Heritability of visceral adipose tissue (VAT) measured by computed tomography was first reported in QFS²⁴ and the HERITAGE Family Study.²⁵ After

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Received 11 June 2015; revised 5 October 2015; accepted 6 October 2015; accepted article preview online 20 October 2015; advance online publication, 24 November 2015

adjustment for total body fatness, significant genetic effects (48–56%) were found in both studies. Other family studies, which used dual-energy X-ray absorptiometry measurements to assess fat distribution, have reported heritability estimates in the range of 33–85% 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 fat³⁰ and changes in VAT³¹ in response to exercise training were influenced by genetic factors. Studies undertaken in pairs of male monozygotic twins submitted to a 100-day 1000 kcal per day caloric surplus³² or energy deficit induced by exercise³³ 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),^{41–47} 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 the Quebec family study (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.

PATIENTS AND 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 5115 subjects were recruited from the total community in Birmingham, AL, USA, from selected census tracts in Chicago, IL, USA and Minneapolis, MN, USA; and from the Kaiser Permanente health plan membership in Oakland, CA, USA. 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 1335 Whites.

The HERITAGE Family Study was designed to evaluate the role of genetic and nongenetic 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 ($BMI > 30 \text{ kg m}^{-2}$) 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 HERITAGE²⁵ 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, CA, USA). Genotyping was completed with a sample call rate $\geq 98\%$. A total of 578 568 SNPs passed quality control (minor allele frequency $\geq 2\%$, call rate $\geq 95\%$, 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 500 GX platform (San Diego, CA, USA). 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 500 GX 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 minor allele frequency $< 1\%$, 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 eight 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 2943 adult (18–84 years of age) subjects consisting of 2171 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 dual-energy X-ray absorptiometry (for 1707 subjects) or computed tomography (for 1236 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 paper by Fox et al.⁴⁸ showing evidence of association with abdominal fat. When multiple SNPs in strong linkage disequilibrium 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 (San Diego, CA, USA). Genotype calling was done using Illumina GenomeStudio software. All SNPs were in HWE (tested using the exact HWE test implemented in the PEDSTATS software package⁵⁹). 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’ (for example, phenotypes, diseases) to ‘targets’ (for example, 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 2513 subjects, including 1152 men and 1361 women, were available for the discovery phase plus 2943 (2171 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

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

Abbreviations: BMI, body mass index; CARDIA, coronary artery risk development in young adults; HERITAGE, health risk factors exercise training and genetics; QFS, Quebec Family Study; TAT, total abdominal adipose tissue; SAT, subcutaneous abdominal adipose tissue; VAT, visceral abdominal adipose tissue; VSR, VAT/SAT ratio. Values are mean ± s.d. or percentage.

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	s.e.	P-value
<i>No adjustment for BMI</i>										
TAT	rs9328211	6	3 989 654	<i>PRPF4B</i>	32	A	0.24	-33.12	6.708	7.93×10^{-7}
SAT	rs2679649	6	122 350 385	<i>HMGB3P18</i>	170	A	0.94	-48.92	9.730	4.97×10^{-7}
	rs2260078	6	122 350 567	<i>HMGB3P18</i>	171	A	0.06	46.36	9.423	8.67×10^{-7}
	rs2679647	6	122 351 503	<i>HMGB3P18</i>	172	T	0.06	47.28	9.486	6.23×10^{-7}
	rs2684270	6	122 352 450	<i>HMGB3P18</i>	173	A	0.06	47.25	9.486	6.33×10^{-7}
	rs2679643	6	122 353 149	<i>HMGB3P18</i>	173	T	0.94	-47.24	9.487	6.38×10^{-7}
	rs2684268	6	122 354 079	<i>HMGB3P18</i>	174	T	0.94	-47.27	9.499	6.46×10^{-7}
	rs2679641	6	122 355 637	<i>HMGB3P18</i>	176	T	0.94	-47.34	9.517	6.55×10^{-7}
	rs2816131	6	122 355 883	<i>HMGB3P18</i>	176	A	0.06	47.34	9.519	6.57×10^{-7}
	rs2816128	6	122 357 203	<i>HMGB3P18</i>	177	A	0.06	47.36	9.522	6.57×10^{-7}
	rs2679695	6	122 359 279	<i>HMGB3P18</i>	179	A	0.06	47.39	9.531	6.61×10^{-7}
	rs2816125	6	122 359 558	<i>HMGB3P18</i>	180	T	0.06	47.37	9.532	6.69×10^{-7}
	rs2679693	6	122 360 216	<i>HMGB3P18</i>	180	T	0.94	-47.37	9.533	6.72×10^{-7}
	rs2679692	6	122 361 247	<i>HMGB3P18</i>	181	T	0.06	47.22	9.500	6.69×10^{-7}
	rs2684266	6	122 361 317	<i>HMGB3P18</i>	181	T	0.94	-47.19	9.501	6.81×10^{-7}
	rs2684265	6	122 362 037	<i>HMGB3P18</i>	182	T	0.94	-47.26	9.538	7.25×10^{-7}
	rs1357056	6	122 363 734	<i>HMGB3P18</i>	184	A	0.94	-46.83	9.448	7.17×10^{-7}
	rs2684264	6	122 365 026	<i>HMGB3P18</i>	185	T	0.94	-46.86	9.461	7.33×10^{-7}
	rs9490391	6	122 368 622	<i>HMGB3P18</i>	189	T	0.94	-47.40	9.571	7.30×10^{-7}
	rs1521231	6	122 369 526	<i>HMGB3P18</i>	190	T	0.06	47.43	9.588	7.57×10^{-7}
VAT	rs12374818	7	33 694 108	<i>BBS9</i>	48	A	0.06	0.09	0.018	1.10×10^{-7}
	rs12374953	7	33 694 132	<i>BBS9</i>	48	A	0.08	0.08	0.015	4.81×10^{-7}
	rs4338001	7	33 701 080	<i>BBS9</i>	55	A	0.07	0.08	0.015	3.02×10^{-7}
<i>BMI-adjusted trait</i>										
TAT	rs17766701	2	215 115 236	<i>SPAG16</i>	0	A	0.90	-20.30	4.125	8.59×10^{-7}
	rs17817960	2	215 119 880	<i>SPAG16</i>	0	A	0.09	20.84	4.181	6.21×10^{-7}
	rs6038439	20	6 318 583	<i>FGFR3P3</i>	117	A	0.30	-12.94	2.564	4.48×10^{-7}
	rs2876017	20	6 321 944	<i>FGFR3P3</i>	121	T	0.30	-12.89	2.561	4.80×10^{-7}
	rs1305009	20	6 324 424	<i>FGFR3P3</i>	123	A	0.30	-12.90	2.561	4.74×10^{-7}
	rs6054136	20	6 326 709	<i>FGFR3P3</i>	125	A	0.70	12.91	2.560	4.64×10^{-7}
	rs8118802	20	6 334 683	<i>FGFR3P3</i>	133	A	0.30	-12.79	2.558	5.79×10^{-7}
	rs6076985	20	6 337 184	<i>FGFR3P3</i>	136	A	0.30	-12.64	2.555	7.46×10^{-7}
	rs6085518	20	6 345 709	<i>FGFR3P3</i>	144	T	0.30	-12.52	2.556	9.68×10^{-7}
	rs6133310	20	6 355 395	<i>FGFR3P3</i>	154	T	0.29	-12.68	2.562	7.41×10^{-7}
	rs6085522	20	6 357 707	<i>FGFR3P3</i>	156	A	0.70	12.71	2.561	6.90×10^{-7}
	rs6038468	20	6 360 394	<i>FGFR3P3</i>	159	T	0.71	12.68	2.563	7.45×10^{-7}
	rs959278	20	6 362 730	<i>FGFR3P3</i>	161	A	0.29	-12.67	2.564	7.69×10^{-7}
SAT	rs6866135	5	19 237 705	<i>HSPD1P15</i>	3	T	0.53	-9.53	1.877	3.87×10^{-7}
VAT	rs1588660	12	88 020 793	<i>CYCSP30</i>	120	A	0.97	0.07	0.014	8.89×10^{-7}
	rs1358302	12	88 021 930	<i>CYCSP30</i>	118	A	0.97	0.07	0.014	8.66×10^{-7}
	rs1398425	12	88 022 814	<i>CYCSP30</i>	118	A	0.97	0.07	0.014	8.33×10^{-7}
	rs2669107	12	88 022 848	<i>CYCSP30</i>	117	A	0.97	0.07	0.014	8.10×10^{-7}
	rs1398424	12	88 022 899	<i>CYCSP30</i>	117	T	0.97	0.07	0.014	7.68×10^{-7}
	rs2644752	12	88 023 316	<i>CYCSP30</i>	117	T	0.97	0.07	0.014	7.53×10^{-7}
	rs2137420	12	88 030 880	<i>CYCSP30</i>	109	A	0.97	0.07	0.014	7.23×10^{-7}
	rs2669106	12	88 031 123	<i>CYCSP30</i>	109	A	0.03	-0.07	0.014	6.95×10^{-7}
	rs17386404	12	88 031 977	<i>CYCSP30</i>	108	C	0.03	-0.07	0.014	6.79×10^{-7}
	rs2669105	12	88 032 132	<i>CYCSP30</i>	108	A	0.97	0.07	0.014	6.57×10^{-7}
	rs950414	12	88 033 419	<i>CYCSP30</i>	107	T	0.03	-0.07	0.014	6.56×10^{-7}
	rs704058	12	88 033 654	<i>CYCSP30</i>	107	A	0.03	-0.07	0.014	6.53×10^{-7}
	rs790448	12	88 033 786	<i>CYCSP30</i>	107	A	0.03	-0.07	0.014	6.53×10^{-7}
	rs12424284	12	88 107 791	<i>CYCSP30</i>	33	C	0.04	-0.06	0.012	2.44×10^{-7}
	rs10506943	12	88 114 376	<i>CYCSP30</i>	26	T	0.96	0.06	0.012	2.42×10^{-7}

Abbreviations: Chr, chromosome; BMI, body mass index; EA, Effect allele; EAF, effect allele frequency; SAT, subcutaneous abdominal adipose tissue; SNP, single-nucleotide polymorphisms; TAT, total abdominal adipose tissue; VAT, visceral abdominal adipose tissue. *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.

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 Table 4). 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 Supplementary 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 seven 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', as VAT mass is highly correlated with total adiposity. We also considered 'inflammation' as a target phenotype, because VAT is considered a proinflammatory organ having 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 with all other genes linking to the same phenotype in Biograph's knowledge base. The phenotype-proximity ranks for the six genes are shown in Table 6. For each phenotype, the global rank represents the rank percent of a gene's proximity score compared with the proximity scores for all other Biograph entities (genes, compounds, metabolites and so on) for the same phenotype, whereas 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 Figures 2a and b. Connectivity diagrams for the other genes are shown in Supplementary Figure S5 for obesity and Supplementary Figure 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 has 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 signaling 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-BBS 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 (cytochrome *c*, somatic pseudogene 30; location

12q21.32), one of the numerous processed cytochrome *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

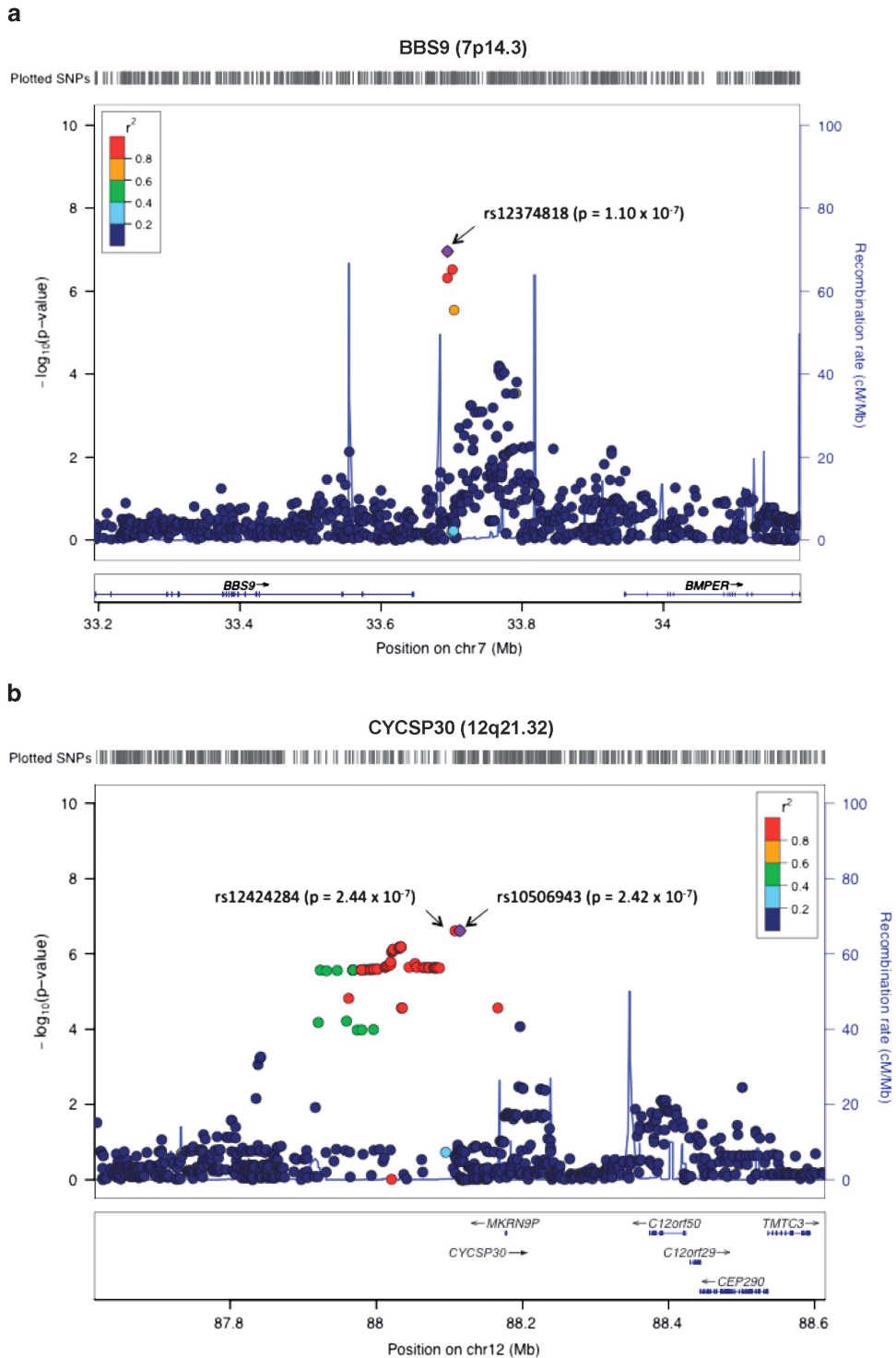


Figure 1. Regional plots for loci showing the strongest evidence of association with VAT (a), VAT-BMI (b), and SAT in women (c) SNPs are plotted by position on chromosome against association ($-\log_{10} P$ -value) and estimated recombination rate (from HapMap-CEU). SNPs surrounding the most significant 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/>).

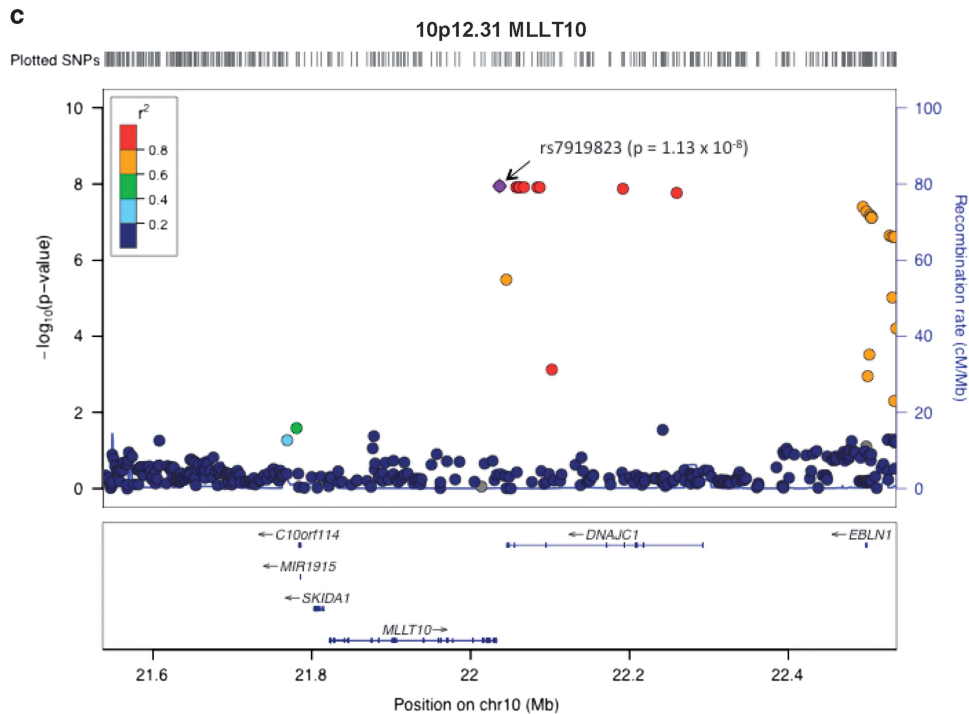


Figure 1. Continued

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 with 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 with 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 seven new loci for waist circumference adjusted for BMI and three 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.*⁴⁷ to be associated with anthropometric measures of abdominal, 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 chromosome 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 *CACNA1D* on chromosome 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 5560 women and 4997 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

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	58 514 665	<i>PCDH17</i>	211	T	0.02	383.17	5.99×10^{-7}
SAT women	rs6727879	2	231 026 592	<i>SP110</i>	7	A	0.99	-645.32	1.51×10^{-7}
	rs2679649	6	122 350 385	<i>HMGB3P18</i>	370	A	0.94	-77.55	2.16×10^{-7}
	rs2260078	6	122 350 567	<i>HMGB3P18</i>	370	A	0.06	72.13	5.51×10^{-7}
	rs2679647	6	122 351 503	<i>HMGB3P18</i>	369	T	0.05	74.15	3.11×10^{-7}
	rs2684272	6	122 351 593	<i>HMGB3P18</i>	369	A	0.06	71.30	8.83×10^{-7}
	rs2684270	6	122 352 450	<i>HMGB3P18</i>	368	A	0.05	74.10	3.17×10^{-7}
	rs2679643	6	122 353 149	<i>HMGB3P18</i>	367	T	0.95	-74.08	3.23×10^{-7}
	rs2684268	6	122 354 079	<i>HMGB3P18</i>	366	T	0.95	-73.85	3.61×10^{-7}
	rs2679641	6	122 355 637	<i>HMGB3P18</i>	365	T	0.95	-73.53	4.29×10^{-7}
	rs2816131	6	122 355 883	<i>HMGB3P18</i>	364	A	0.05	73.46	4.41×10^{-7}
	rs2816128	6	122 357 203	<i>HMGB3P18</i>	363	A	0.05	73.47	4.46×10^{-7}
	rs2679695	6	122 359 279	<i>HMGB3P18</i>	361	A	0.05	73.38	4.75×10^{-7}
	rs2816125	6	122 359 558	<i>HMGB3P18</i>	361	t	0.05	73.22	5.05×10^{-7}
	rs2679693	6	122 360 216	<i>HMGB3P18</i>	360	t	0.95	-73.18	5.14×10^{-7}
	rs2679692	6	122 361 247	<i>HMGB3P18</i>	359	T	0.06	72.92	5.29×10^{-7}
	rs2684266	6	122 361 317	<i>HMGB3P18</i>	359	t	0.94	-72.76	5.64×10^{-7}
	rs2684265	6	122 362 037	<i>HMGB3P18</i>	358	t	0.95	-72.73	6.17×10^{-7}
	rs1357056	6	122 363 734	<i>HMGB3P18</i>	357	a	0.94	-71.24	7.43×10^{-7}
	rs2684264	6	122 365 026	<i>HMGB3P18</i>	355	T	0.94	-71.27	7.69×10^{-7}
	rs9490391	6	122 368 622	<i>HMGB3P18</i>	352	T	0.95	-72.99	6.29×10^{-7}
	rs1521231	6	122 369 526	<i>HMGB3P18</i>	351	T	0.05	72.98	6.71×10^{-7}
	rs17241164	9	9 857 448	<i>PTPRD</i>	0	T	0.99	-407.73	6.55×10^{-7}
	rs7919823	10	22 036 491	<i>MLLT10</i>	4	A	0.98	-252.13	1.13×10^{-8}
	rs7903144	10	22 057 405	<i>DNAJC1</i>	0	A	0.98	-249.29	1.21×10^{-8}
	rs7899191	10	22 060 277	<i>DNAJC1</i>	0	A	0.02	249.21	1.21×10^{-8}
	rs7924175	10	22 061 450	<i>DNAJC1</i>	0	A	0.02	249.17	1.21×10^{-8}
	rs7924290	10	22 061 494	<i>DNAJC1</i>	0	T	0.98	-249.13	1.21×10^{-8}
	rs7895753	10	22 067 207	<i>DNAJC1</i>	0	T	0.98	-249.11	1.21×10^{-8}
	rs7071519	10	22 084 152	<i>DNAJC1</i>	0	T	0.02	248.89	1.22×10^{-8}
	rs7093279	10	22 087 030	<i>DNAJC1</i>	0	T	0.02	248.87	1.23×10^{-8}
	rs7082546	10	22 191 842	<i>DNAJC1</i>	0	T	0.02	243.93	1.33×10^{-8}
	rs7923514	10	22 259 547	<i>DNAJC1</i>	0	T	0.02	188.38	1.71×10^{-8}
	rs12257323	10	22 494 143	<i>EBLN1</i>	4	T	0.02	237.97	3.97×10^{-8}
	rs10466081	10	22 498 450	<i>EBLN1</i>	0	A	0.98	-237.27	5.24×10^{-8}
	rs16922112	10	22 502 778	<i>EBLN1</i>	4	C	0.02	236.58	6.52×10^{-8}
	rs11012890	10	22 504 782	<i>EBLN1</i>	6	A	0.02	236.20	7.32×10^{-8}
	rs11012892	10	22 504 881	<i>EBLN1</i>	6	T	0.98	-236.15	7.42×10^{-8}
	rs11012893	10	22 504 966	<i>EBLN1</i>	6	A	0.98	-236.07	7.59×10^{-8}
	rs11012894	10	22 505 064	<i>EBLN1</i>	6	A	0.02	236.04	7.69×10^{-8}
	rs11012902	10	22 527 817	<i>EBLN1</i>	29	A	0.98	-237.85	2.27×10^{-7}
	rs12250660	10	22 531 317	<i>EBLN1</i>	32	T	0.02	238.02	2.43×10^{-7}
	rs11812422	10	22 533 935	<i>EBLN1</i>	35	A	0.02	238.07	2.47×10^{-7}
	rs12866352	13	107 045 074	<i>EFNB2</i>	97	A	0.02	219.72	8.16×10^{-7}
	rs4384548	14	96 645 143	<i>BDKRB2</i>	26	A	0.02	232.19	5.27×10^{-8}
VAT men	rs17377726	3	78 896 235	<i>ROBO1</i>	0	A	0.06	0.11	7.86×10^{-7}
	rs7017641	8	132 470 314	<i>ADCY8</i>	417	A	0.10	-0.09	2.74×10^{-7}
	rs10505574	8	132 472 102	<i>ADCY8</i>	419	A	0.90	0.09	2.62×10^{-7}
	rs715969	8	132 473 863	<i>ADCY8</i>	421	A	0.90	0.08	4.91×10^{-7}
	rs1507456	8	132 476 129	<i>ADCY8</i>	423	T	0.90	0.08	9.07×10^{-7}
	rs1118349	8	132 502 614	<i>EFR3A</i>	414	T	0.88	0.07	9.86×10^{-7}
	rs1395804	8	132 510 680	<i>EFR3A</i>	406	T	0.12	-0.08	5.70×10^{-7}
VAT women	rs16910486	8	140 352 359	<i>KCNK9</i>	261	A	0.86	0.07	5.82×10^{-7}
	rs13252823	8	140 352 050	<i>KCNK9</i>	261	T	0.14	-0.07	6.22×10^{-7}
VAT-BMI women	rs7927727	11	13 656 086	<i>FAR1</i>	34	A	0.37	-0.03	1.86×10^{-7}
VSR men	rs2930176	3	53 487 279	<i>CACNA1D</i>	42	A	0.52	-0.04	6.06×10^{-7}
VSR women	rs4947599	7	51 538 997	<i>COBL</i>	154	C	0.80	-0.04	5.17×10^{-7}
	rs7819481	8	39 405 882	<i>ADAM18</i>	36	A	0.17	0.04	3.45×10^{-7}
	rs10095849	8	39 408 586	<i>ADAM18</i>	34	T	0.83	-0.04	2.42×10^{-7}

Abbreviations: Chr, chromosome; BMI, body mass index; EA, effect allele; EAF, effect allele frequency; TAT, total abdominal adipose tissue; SAT, subcutaneous abdominal adipose tissue; VAT, visceral abdominal adipose tissue; VSR, VAT/SAT ratio. 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.

($P=0.048$). 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 ($P=0.002$; Supplementary 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 (Supplementary Table S1). Seven loci,

Table 4. Replication of top SNPs of the sex-combined GWA meta-analysis in the PCLS cohort

SNP	Chr	Position	Trait	Meta-analysis			PCLS European Americans			PCLS African Americans		
				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	TAT-BMI	A	12.91	4.64×10^{-7}	G	1.52	0.5671	G	0.38	0.9423

Abbreviations: BMI, body mass index; EA, Effect allele; PCLS, Pennington Center Longitudinal Study; SNP, single-nucleotide polymorphism; SAT, subcutaneous abdominal adipose tissue; TAT, total abdominal adipose tissue; VAT, visceral abdominal adipose tissue. 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

SNP	Chr	Position	Trait	Fox et al. 2012			Meta-analysis			PCLS European Americans			PCLS African Americans		
				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 ^a	0.0056 ^a	G	-0.004^a	0.822^a
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

Abbreviations: Chr, chromosome; BMI, body mass index; EA, Effect allele; NA, not applicable; PCLS, Pennington Center Longitudinal Study; SNP, single-nucleotide polymorphism; SAT, subcutaneous abdominal adipose tissue; TAT, total abdominal adipose tissue; VAT, visceral abdominal adipose tissue. 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. ^aResults for sex-stratified analysis with women.

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

Abbreviations: BMI, body mass index; VAT, visceral abdominal adipose tissue. Values are rank percent of a gene's proximity score compared to the proximity scores for all other Biograph entities (genes, compounds, metabolites and so on) to the same phenotype (Global Rank) or compared with the proximity scores for genes only (Gene Rank). A lower rank percentage indicates higher proximity between the gene and the phenotype.

which showed significant evidence of association with abdominal fat in the study by Fox et al.⁴⁸ were replicated in our meta-analyses (Supplementary 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 *NTSC2* with both SAT and VAT in women,⁸¹ *LYPLAL1* with VSR, *NISCH* with VAT and VSR⁷⁷ 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 signaling. Notably, the *GNB3* 825C>T polymorphism has previously been associated with obesity in specific

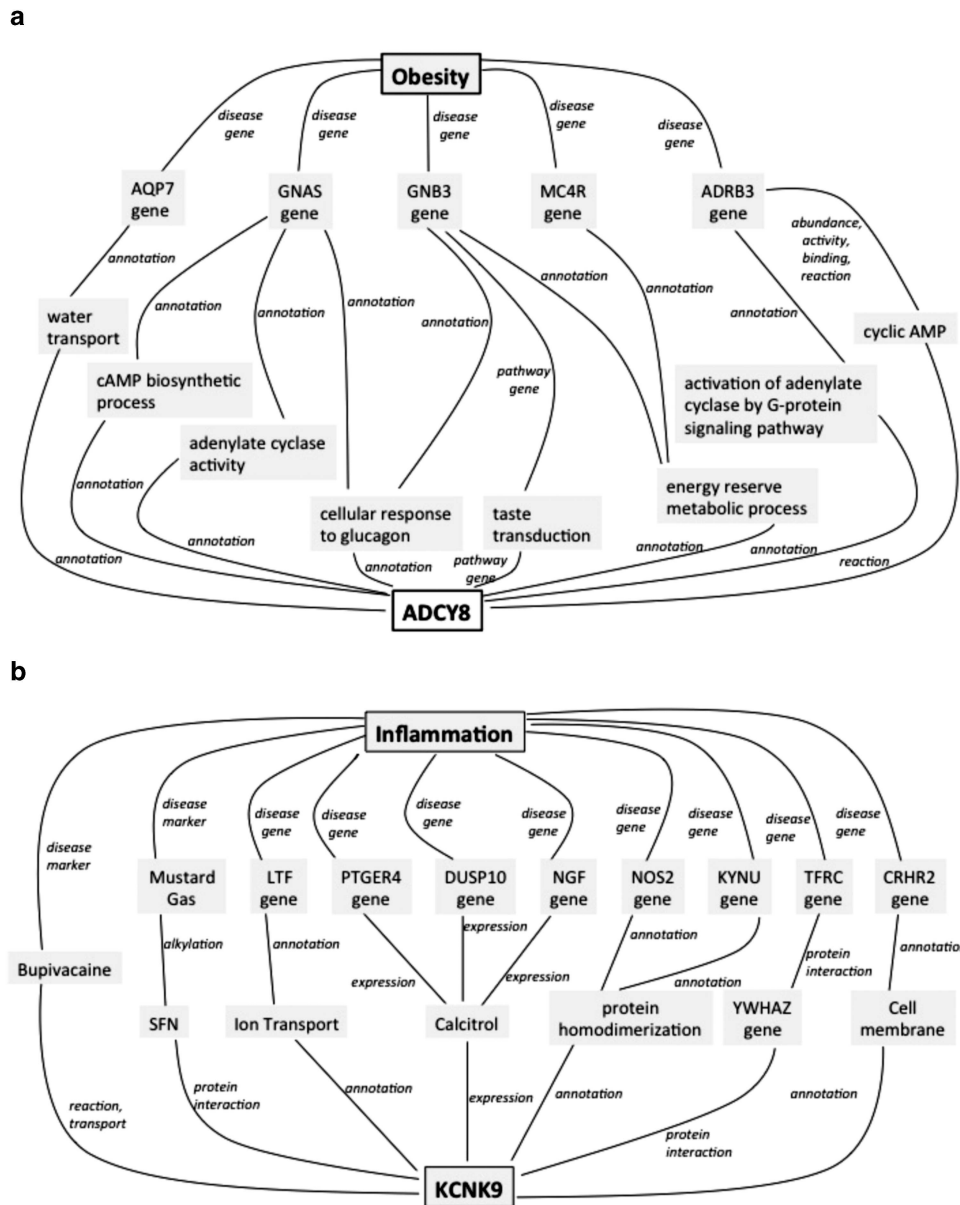


Figure 2. Biograph analysis of VAT-BMI-associated genes to 'obesity' and 'inflammation' phenotypes. **(a)** Biograph generated connectivity graph between ADCY8 gene and obesity. **(b)** Biograph generated connectivity graph between KCNK9 gene and inflammation. The intermediate linking the genes to the phenotype are indicated in a gray background along with the type of interaction.

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* inhibitor⁸⁶ 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 proinflammatory cytokine TNF- α , 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.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

We thank Mrs Allison Templet and Mrs Robin Post for their support in the development and submission of this manuscript. The Quebec Family Study (QFS) was supported for three decades by multiple grants from the Medical Research Council of Canada and the Canadian Institutes for Health Research. This project was supported by a team grant from the Canadian Institute for Health Research (FRCN-CCT-83028). We thank all PIs who contributed to the HERITAGE Family Study in the past via support from the National Heart, Lung, and Blood Institute (NHLBI) through the following grants: C Bouchard (HL-45670); AS Leon (HL-47323); DC Rao (HL-47317); JS Skinner (HL-47327); JH Wilmore (HL-47321). We also acknowledge grant 8P20 GM-1033528 (COBRE center grant to Pennington Biomedical Research Center, PI: Thomas Gettys, supporting M.A. Sarzynski). The Pennington Center Longitudinal Study is partially supported by a Nutrition Obesity Research Center (NIH 2P30DK072476) grant from the National Institutes of Health and by 1 U54 GM104940 from the National Institute of General Medical Sciences of the National Institutes of Health which funds the Louisiana Clinical and Translational Science Center. The Coronary Artery Risk Development in Young Adults Study (CARDIA) is conducted and supported by the NHLBI in collaboration with the University of Alabama at Birmingham (HHSN268201300025C and HHSN268201300026C), Northwestern University (HHSN268201300027C), University of Minnesota (HHSN268201300028C), Kaiser Foundation Research Institute (HHSN268201300029C), and Johns Hopkins University School of Medicine (HHSN268200900041C). CARDIA is also partially supported by the Intramural Research Program of the National Institute on Aging (NIA) and an intra-agency agreement between NIA and NHLBI (AG0005). This manuscript has been reviewed by CARDIA for scientific content. The CARDIA CT Scan year 25 data were obtained with the support of NHLBI (R01-HL-098445). C. Bouchard is partially funded by the John W. Barton Sr. Chair in Genetics and Nutrition.

DISCLAIMER

The funders had no role in the study design, data collection, decision to publish, or preparation of the manuscript. The funding agencies had no role in the design of the present study, collection and analysis of the data and the decision to publish. None of the authors are employed by institutions that stand to gain or lose financially as a result of this publication.

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