Meta-analysis of genome-wide association studies for body fat distribution in 694,649 individuals of European ancestry

Sara L. Pulit[1,2,3*], Charli Stoneman[4*], Andrew P. Morris[5,6], Andrew R. Wood[4], Craig A. Glastonbury[1], Jessica Tyrrell[4], Loïc Yengo[7], Teresa Ferreira[1], Eirini Marouli[8], Yingjie Ji[4], Jian Yang[7,9], Samuel Jones[4], Robin Beaumont[4], Damien C. Croteau-Chonka[10], Thomas W. Winkler[11], GIANT Consortium, Andrew. T. Hattersley[4], Ruth J. F. Loos[12], Joel N. Hirschhorn[13,14,15,16], Peter M. Visscher[7,9], Timothy M. Frayling[4#], Hanieh Yaghootkar[4#], Cecilia M. Lindgren[1,3,6#]

1. Big Data Institute, Li Ka Shing Center for Health for Health Information and Discovery, Oxford University, Oxford, UK
2. Department of Genetics, Center for Molecular Medicine, University Medical Center Utrecht, Utrecht, The Netherlands
3. Program in Medical and Population Genetics, Broad Institute, Boston, MA, USA
4. University of Exeter Medical School, University of Exeter, Royal Devon and Exeter NHS Trust, Exeter, UK
5. Biostatistics Department, University of Liverpool, Liverpool, UK
6. Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, UK
7. Institute for Molecular Bioscience, University of Queensland, Brisbane, Australia
8. William Harvey Research Institute, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, London, UK
9. Queensland Brain Institute, The University of Queensland, Brisbane, Queensland, Australia
10. Channing Division of Network Medicine, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA, USA
11. Department of Genetic Epidemiology, University of Regensburg, Regensburg, Germany
12. The Charles Bronfman Institute for Personalized Medicine, The Mindich Child Health and Development Institute, the Icahn School of Medicine at Mount Sinai, New York, USA
13. Broad Institute of MIT and Harvard, Cambridge, MA, USA
14. Department of Genetics, Harvard Medical School, Boston, MA, USA
15. Division of Endocrinology and Center for Basic and Translational Obesity Research, Boston Children's Hospital, Boston, MA, USA
16. Department of Pediatrics, Harvard Medical School, Boston, MA, USA

Corresponding authors:

Sara L Pulit
Department of Genetics, University Medical Center Utrecht
Heidelberglaan 100, 3584CX Utrecht
Utrecht, The Netherlands

Hanieh Yaghootkar
University of Exeter Medical School, RILD Building, RD&E Hospital
Wonford, Barrack Rd, Exeter, EX2 5DW, UK

Cecilia M Lindgren
The Big Data Institute, Li Ka Shing Centre for Health Information and Discovery
Old Rd, Oxford OX3 7FZ, UK
cecilia.lindgren@bdi.ox.ac.uk

We performed a genome-wide association study meta-analysis of body fat distribution, measured by waist-to-hip ratio adjusted for BMI (WHRadjBMI), and identified 463 signals in 346 loci. Heritability and variant effects were generally stronger in women than men, and the 5% of individuals carrying the most WHRadjBMI-increasing alleles were ~1.62 times more likely than the bottom 5% to have a WHR above the thresholds used for metabolic syndrome. These data, made publically available, will inform the biology of body fat distribution and its relationship with disease.

One in four adults worldwide are either overweight or obese[1,2] and are at increased risk of metabolic disease. While higher adiposity increases morbidity and mortality,[1,3] epidemiological studies indicate that the location and distribution of excess fat within particular depots is more informative than general adiposity for predicting disease risk. Independent of their overall body mass index (BMI), individuals with higher central adiposity have increased risk of cardiometabolic diseases, including type 2 diabetes (T2D) and stroke[4,5]; in contrast, individuals with higher gluteal adiposity have lower risk of such outcomes.[5] Previous studies indicate that fat distribution, as assessed by waist-to-hip ratio (WHR), is a trait with a strong heritable component, independent of overall adiposity (measured by BMI)[5,6], and recent Mendelian randomisation studies using known WHR-associated genetic variants showed putative causal effects of higher WHR on T2D and coronary artery disease independently of BMI.[7]

With the goal of pinpointing genetic variants associated to body shape and fat distribution, and motivated by the recent release of genetic data from half a million individuals,[8] we performed a meta-analysis of WHR adjusted for BMI (WHRadjBMI). WHRadjBMI is an easily-measured fat distribution phenotype that correlates well with imaging-based fat distribution measures.[9] We performed genome-wide association studies (GWAS) of WHRadjBMI in the UK Biobank data set,[8] a collection of 484,563 individuals with densely-imputed genotype data, using a linear mixed model[10] to account for relatedness and ancestral heterogeneity. We then combined the results with publicly-available GWAS data generated by the GIANT consortium for the same phenotype (**Table 1** and **Methods**)[5], resulting in a meta-analysis of 694,649 samples (**Table 1**) and ~27.4M SNPs (**Methods**). As a sensitivity analysis and to evaluate the robustness of our results, we also performed a GWAS of WHR unadjusted for BMI (**Table 1**).

We identified 346 loci (300 novel) containing 463 independent signals associated with WHRadjBMI ($p < 5 \times 10^{-9}$, to account for the denser imputation data[11]; **Methods**, **Supplementary Table 1** and **Supplementary Fig 1**). The Linkage Disequilibrium (LD) Score Regression[12] intercept (1.0346) of the meta-analysis results indicated that the observed enrichment in genomic signal was primarily due to polygenicity and not confounding (**Supplementary Table 2**). Of the 300 novel signals, 234 (78%, $p_{binomial} < 1 \times 10^{-7}$) were directionally-consistent in an independent dataset with a relatively small sample size (N = 7,721) and signals were consistent in several sensitivity checks (**Supplementary Tables 3-5** and **Supplementary Fig 2-3**). These variants combined explained ~3.9% of the variance in WHRadjBMI in the independent study (**Methods** and **Table 1**). We constructed a weighted polygenic score using the 346 index SNPs discovered in the combined meta-analysis and tested this score in the same independent study. The 5% of individuals carrying the most WHRadjBMI-raising alleles were approximately 1.62 times more likely to meet the WHR threshold for metabolic syndrome[13] than the 5% carrying the fewest (**Methods**).

A number of analyses indicated that the majority of signals identified have genuine effects on body shape and that any bias caused by adjusting WHR for a correlated covariate (BMI)[14,15] was minimal. Of the 346 index variants, 311 were associated with stronger standard deviation effect sizes for WHR (unadjusted) than with standard deviation effect sizes for BMI (**Supplementary Table 3** and **Supplementary Fig 4**). This analysis also indicates that the WHR association is unlikely to be secondary to the known effect of

higher BMI leading to higher WHR. Furthermore, the common SNP associated with the largest known effect on BMI, that in the *FTO* gene, was not associated with WHRadjBMI (rs1421085, p = 0.40), despite a very strong association with WHR (4×10^{-118}). Finally, carrying each additional WHRadjBMI-raising allele (weighted) was associated with 0.0199 SD higher WHRadjBMI ($p = 6 \times 10^{-62}$; adjusted R^2 = 4%), 0.0111 SD higher WHR (unadjusted; $p = 3 \times 10^{-20}$; adjusted R^2 = 0.12%) and 0.0038 SD lower BMI (P-value = 0.0014; adjusted R^2 = 0.13%) in our independent dataset (consistent with the results obtained from an unweighted polygenic score; **Methods**).

Given the sex-dimorphism of fat distribution in humans, previously shown to have a genetic basis[5], we next performed meta-analyses of WHRadjBMI in women and men separately (**Table 1** and **Supplementary Fig 5**). We found SNP-based heritability (h_g^2) of WHRadjBMI, estimated using the restricted maximum likelihood method implemented in BOLT-REML[10] (**Methods**), to be stronger in women ($h_{\square}^2$= 25.6%) compared to men ($h_{\square}^2$= 16.7%, $p_{difference} = 9 \times 10^{-85}$; **Table 1**, **Supplementary Table 6**, and **Equation 2**). In addition to the heritability dimorphism, and in keeping with previous studies[5], we found signatures of sex-dimorphism amongst associated loci: a total of 266 loci associated with WHRadjBMI in women, compared to 91 loci in men ($p < 5 \times 10^{-9}$). The consistency between the effect size of 266 female index SNPs on WHRadjBMI in women and men (slope = 0.31; $p = 2 \times 10^{-33}$; adjusted R^2 = 51%) was greater than the consistency between the effect size of 91 male index SNPs on WHRadjBMI in men and women (slope = 0.20; p = 0.002; adjusted R^2 = 9%). Of all associated index SNPs ($p < 5 \times 10^{-9}$ in the combined or sex-specific analyses), 105 SNPs were sex-dimorphic (p_{diff}[16] $< 3.3 \times 10^{-5}$; **Methods**). Variants discovered in the combined sex analysis, will be enriched for those with similar effects in each sex, and variants discovered in sex-specific analyses will be enriched for those with different effects between sexes. In the absence of any sex-specific effects, we would only expect a slight shift towards stronger associations in women because the sample size for women was slightly larger. However, we observed that of the 105 sex-dimorphic signals, 97 (92.4%) showed stronger effects in women compared to men (**Figure 1**, **Supplementary Fig 6,** and **Methods**).

Previous studies have shown that in addition to redistributing body fat, some WHRadjBMI variants are also associated with total body fat percentage (BF%)[5,17-19]. Of relevance to the biology of adipose tissue storage capacity, these studies have shown that these pleiotropic associations can occur in both directions - some alleles associated with higher WHRadjBMI are associated with higher total BF%, whilst others are associated with lower BF%.[5,17-19] To test the hypothesis that alleles associated with higher WHRadjBMI could have pleiotropic effects on total BF%, and that these effects could occur in both directions, we next investigated whether the 346 index variants associated with WHRadjBMI also associated with BF%. Of the 59/346 variants associated with BF% in 443,001 European-ancestry UK Biobank individuals ($p < 0.05/346 = 1.44 \times 10^{-4}$), 25 alleles were associated with higher WHR and higher BF%, whilst 34 alleles were associated with higher WHR but lower BF% (**Supplementary Fig 7**). Additionally, a large proportion (29%) of WHRadjBMI index SNPs with a stronger effect in women had a BF% phenotype in men (28 of the 97 female-specific WHRadjBMI SNPs were associated with BF% in men and 25 were associated with BF% in women ($p < 0.05/105 = 4.8 \times 10^{-4}$)) (**Supplementary Fig 8)**. These variants appear to alter total BF% in men and women to a similar extent, but distribute body fat between the upper and lower body to a much greater extent in women (**Supplementary Table 7-9** and **Supplementary Fig 8**). Finally, we tested the index SNPs from each of the meta-analyses (combined and sex-specific) in a recent GWAS of CT and MRI image based measures of ectopic and subcutaneous fat depots.[20] Adjusting for the three sample groups and the 8 depots examined in the imaging-based GWAS ($p < 0.05/24 = 2.1 \times 10^{-3}$), the alleles associated with higher WHRadjBMI were collectively associated with lower measures of subcutaneous fat, and higher measures of visceral fat, including pericardial and visceral adipose tissue (**Supplementary Fig. 9).**

While we have performed the largest meta-analysis of a measure of body-fat distribution to date, a number of limitations remain. First, the substantially larger number of signals with a stronger effect in women compared to men may be influenced by the smaller sample size in the men-only analysis (**Table 1**), although we would not expect the difference in sample size to result in 92% of signals being stronger in women. Second, our replication sample was too small (~1% of the discovery) to formally replicate individual SNP associations, but the fact that 78% of the 300 previously unknown index associations showed consistent direction of effect suggests a low false positive rate. Finally, our meta-analysis focused only on European-ancestry samples. Given the very different body-fat distributions between people of European and non-European ancestry, and their very different risks of adiposity-related disease, studies in non-Europeans are urgently needed.[21,22]

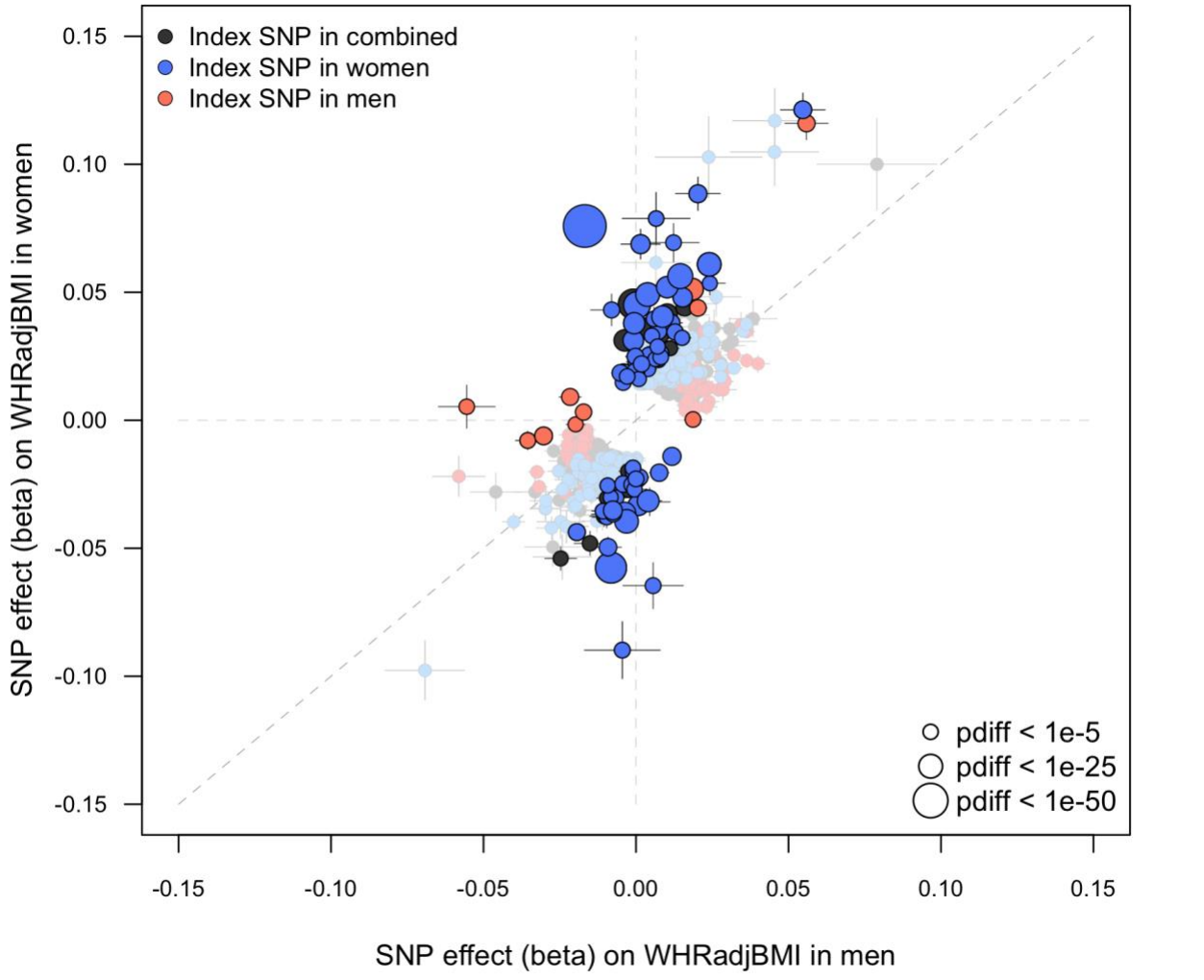
In summary, the genetic variants and loci identified by this meta-analysis will likely provide starting points for further understanding the biology of body fat distribution and its relationship with disease.

Tables and Figures

Table 1 | Large-scale meta-analysis in body fat distribution. We performed a meta-analysis of fat distribution as measured by WHRadjBMI in up to 694,649 individuals. We performed analyses of WHR as a sensitivity measure. Our analyses increase the number of WHRadjBMI-associated loci ($p < 5 \times 10^{-9}$, to account for SNP density in UK Biobank) to 346 loci. SNP-based heritability (h_g^2) results (estimated using the restricted maximum likelihood method implemented in BOLT-REML[10] (**Methods**)) and top-associated loci indicate patterns of sex-dimorphism. The top-associated index SNPs explain 3.9% of the overall phenotypic variance in fat distribution (calculated in an independent dataset, N = 7,721).

Phenotype	Sex	Sample sizes			Associated loci $p < 5 \times 10^{-9}$		Dimorphic index SNPs (% of total)	$h_□^2$(se)	Variance explained (adjusted R^2)
		UKBB	GIANT	Meta	Loci	Indep. signals			
WHRadjBMI	Combined	484,563	210,086	694,649	346	463	53 (15.3)	0.174 (0.002)	3.9%
	Women	262,759	116,742	379,501	266	363	77 (28.9)	0.256 (0.003)	3.6%
	Men	221,804	93,480	315,284	91	102	13 (14.3)	0.167 (0.003)	1.0%
WHR	Combined	485,486	212,248	697,734	316	382	37 (11.7)	0.194 (0.002)	3.0%
	Women	263,148	118,004	381,152	203	261	64 (31.5)	0.254 (0.003)	4.0%
	Men	222,338	94,434	316,772	79	82	10 (12.7)	0.208 (0.003)	0.3%

Figure 1 | Sex-dimorphic association signals in fat distribution. For each associated locus from the combined or sex-specific meta-analyses, we tested the index SNP for sex-dimorphism. Whilst index SNPs identified in the combined (men and women) sample will be biased away from sex-dimorphism, and index SNPs identified in the sex-specific sample will be biased towards sex-dimorphism, due to winner's curse, when we compared all SNPs we observed stronger effects in women. For example, of the index SNPs from the men-only analysis (red points), 14% showed evidence of sex-dimorphism. In contrast, ~29% of the index SNPs from the women-only analysis show evidence of dimorphism. 92.4% sex-dimorphic SNPs show a stronger effect in women compared to men. Index SNPs that are not sex-dimorphic are plotted with faded colors. Points are sized by the $-\log_{10}(p_{diff})$ sex-dimorphism test. Horizontal bars indicate standard error in men; vertical bars indicate standard error in women.



Online Methods

Data and code availability

Code and data related to this project, including summary-level data from the meta-analyses, can be found on-line at https://github.com/lindgrengroup/fatdistnGWAS.

Phenotypes

To generate phenotypes for the waist-to-hip ratio (WHR) and waist-to-hip ratio adjusted for body mass index (WHRadjBMI) analyses in the UK Biobank data (**Supplementary Table 10**), we followed a phenotype conversion consistent with that performed in previous efforts investigating WHR and WHRadjBMI by the GIANT consortium.[5,23] Using phenotype information from UK Biobank, we divided waist circumference by hip circumference to calculate the WHR measure, and then regressed the WHR measure on age at assessment, age at assesment squared, and assessment centre. To generate the WHRadjBMI phenotype, we followed the same procedure and included body mass index (BMI) as an additional independent variable in the regression. We performed rank inverse normalization on the resulting residuals from the regression (**Supplementary Fig 10**), and used these normalized residuals as the tested phenotype in downstream genome-wide association testing. To generate phenotypes for the sex-specific analyses, we followed this same procedure but ran the regressions in sex-specific groups.

Genome-wide association analyses

The UK Biobank data

We conducted genome-wide association testing in the second release (June 2017) version of the UK Biobank data[8]; this release did not contain the corrected imputation at non-Haplotype Reference Consortium (HRC[24]) sites and we therefore subsetted all of the SNP data down to HRC SNPs only. The UK Biobank applied quality control to samples and genotypes, and imputed the resulting genotype data using sequencing-based imputation reference panels. We performed all of our genome-wide association testing and downstream analyses on the publicly-available imputation data (released in bgen format).

We excluded samples as suggested by the UK Biobank upon release of the data (**Supplementary Table 11**). Sample exclusions included samples with genotype but no imputation information, samples with missingness > 5%, samples with mismatching phenotypic and genotypic sex, and samples that have withdrawn consent since the initiation of the project.

LD scores and genetic relationship matrix for BOLT-LMM

We implemented all genome-wide association studies (GWAS) in BOLT-LMM[10], which performs association testing using a linear mixed model. To run, BOLT-LMM requires three primary components: the (imputed) genotypic data for association testing; a reference panel of Linkage Disequilibrium (LD) scores per SNP, calculated using LD Score Regression[12]; and genotype data used to approximate a genetic relationship matrix (GRM), which is the best method available in this sample size to account for all forms of relatedness, ancestral heterogeneity in the samples, and other (potentially hidden) structure in the data.

We performed sensitivity testing (**Supplementary Information**, **Supplementary Tables 12-13** and **Supplementary Fig 11**) using three LD Score reference datasets and four SNP-sets to construct the GRM. For our final GWAS, we used LD scores calculated from a randomly-selected, 9,748 unrelated UK Biobank samples (~2% of the full UK Biobank sample set; **Supplementary Information**) and a GRM constructed using: imputed SNPs with imputation info score > 0.8; MAF > 1%; Hardy Weinberg P-value > 1×10^{-8}; genotype missingness < 1%, after converting imputed dosages to best-guess genotypes; LD pruned at a threshold (r^2) of 0.2; and excluding the major histocompatibility complex, the lactase locus, and the inversions on chromosomes 8 and 17 (**Supplementary Information**).

Association testing

For genome-wide association testing, we used BOLT-LMM to run a linear mixed model (LMM). We tested SNPs with imputation quality (info) > 0.3, minor allele frequency (MAF) > 0.01%, and only those single-nucleotide variants (SNVs) and single-nucleotide polymorphisms (SNPs) represented in the Haplotype Reference Consortium[25] imputation reference panel. We used only the standard LMM implementation (i.e., infinitesimal model, using --lmm) in BOLT-LMM (**Supplementary Fig 12-13**); we did not run association testing using a non-infinitesimal model. The only covariate used in the LMM was the SNP array used to genotype sample; we included no other covariates.

After association testing, we looked at known SNPs already reported in WHR, WHRadjBMI, and BMI.[5,23] At the previously-described loci, we checked correlation of frequency, beta, standard error, and $-\log_{10}$(P-value) between our UK Biobank GWAS and the previous GWAS results (**Supplementary Fig 14**). Additionally, we estimated genomic inflation (lambda) and the LD Score Intercept to check if the P-values were well calibrated (**Supplementary Table 2**); calculations were performed using the LD Score software (https://github.com/bulik/ldsc).[12]

Meta-analysis of results from UK Biobank and GIANT

Data preparation and quality control

We downloaded summary-level results from previous meta-analyses of WHR and WHRadjBMI (https://portals.broadinstitute.org/collaboration/giant/index.php/GIANT_consortium_data_files and **Supplementary Information**) performed by the GIANT consortium.[5] Marker names in both the GIANT data and UK Biobank were lifted over to their dbSNP151 identifier. We additionally renamed markers as "rsID:A1:A2" to avoid ambiguity at multiallelic SNPs in the UK Biobank data. As the GIANT data was imputed with HapMap 2[25,26] data (hg18), we additionally lifted chromosomal positions to hg19 for this data. SNPs with a frequency difference > 15% between GIANT and UK Biobank were removed from the data (**Supplementary Fig 15**).

Meta-analysis and downstream quality control

We performed inverse variance-weighted fixed effects meta-analysis in METAL.[28] To estimate LD score intercepts and genomic inflation (lambda) for the meta-analysis results, we first estimated LD scores from the same samples used to estimate the LD score reference for BOLT-LMM. LD scores were only estimated at high-quality SNPs (using the same criteria as used for SNPs included in the GRM in BOLT-LMM, but without applying a MAF threshold; **Supplementary Information**). We then calculated LD Score Regression intercepts and lambda with the LDSC software.[12]

As an additional quality control check, we reran all of our GWAS using two different subsets of the UK Biobank samples: (1) the unrelated samples only, and (2) the unrelated white British samples only. These subsamples were selected to test if our initial UK Biobank-wide GWAS was confounded by either relatedness or ancestral heterogeneity. After running these GWAS, we meta-analyzed the results with the existing GIANT summary-level data, and checked the concordance of our signals (**Supplementary Fig 2-3**).

Identification of index and secondary signals

Linkage disequilibrium clumping

To identify genomic loci (i.e., genomic windows) containing independent association signals, we first constructed a reference dataset of best-guess genotypes from 20,275 unrelated UK Biobank samples (equivalent to 5% of the unrelated sample). We converted imputed dosages of SNPs with info score > 0.3 and MAF > 0.001% to best-guess genotypes using PLINK (version 1.9),[29,30] and a conversion threshold (--hard-call-threshold) of 0.1 (**Supplementary Information**). SNPs with missingness > 5% after conversion or Hardy-Weinberg equilibrium $p < 1 \times 10^{-7}$ were removed.

We then used the PLINK 'clumping' algorithm to select top-associated SNPs ($p < 5 \times 10^{-9}$) and identify all SNPs in LD ($r^2 > 0.05$) with the top associated SNP and ±5Mb away. We determined the genomic span of each LD-based clump and added 1kb up- and downstream as buffer to the region. If any of these windows overlapped, we merged them together into a single (larger) locus. As a sensitivity analysis, we ran clumping also using a smaller genomic window to calculate LD (±2Mb); the results were effectively unchanged, as <5 loci appeared independent using the ±2Mb window but were found to correlate using ±5Mb windows. Therefore we report loci using the ±5Mb window.

Proximal conditional and joint testing

To identify index and secondary signals within each of the clumping-based loci, we ran proximal joint and conditional analysis as implemented in the Genome-wide Complex Trait Analysis (GCTA) software[30]. We ran this model (--cojo-slct) using the summary-level data within each locus, the LD reference panel constructed from UK Biobank data and also used for the locus 'clumping,' and setting genome-wide significance with $p < 5 \times 10^{-9}$.

Collider bias analysis

Given that we had conditioned WHR on the BMI phenotype for analysis (and BMI and WHR are correlated; r = 0.433 in the UK Biobank data; **Supplementary Fig 16**), we tested all index signals found in the WHRadjBMI analysis for evidence of collider bias.[15,31] To do this, we ran meta-analyses of BMI and WHR using the UK Biobank samples and pre-existing summary-level data from GIANT[5,23] (**Supplementary Methods**). We performed these meta-analyses using identical methods to the meta-analysis of WHRadjBMI.

Then, for each index SNP from the WHRadjBMI meta-analyses (combined as well as sex-specific) we extracted the association results from the BMI and WHR meta-analyses (**Supplementary Fig 4**). WHRadjBMI-associated SNPs with a stronger association for BMI than WHR show evidence of collider bias or pleiotropy. We additionally looked at the effect size and direction of effect in BMI and WHR, but whether the effects are from collider bias or pleiotropy cannot be determined from this data.

Identification of sex-dimorphic signals

We performed sex-specific GWAS in UK Biobank and meta-analyzed the results with publicly-available sex-specific data from the GIANT consortium. We identified the primary and secondary signals from these meta-analyses using methods identical to those performed in the combined analysis. We tested each primary and secondary signal for a sex-dimorphic effect by estimating the t-statistic:

$$t = \frac{\beta_{females} - \beta_{males}}{\sqrt{SE_{females} + SE_{males} + (2r \times SE_{females} \times SE_{males})}} \quad (1)$$

where r is the genome-wide correlation between SNP effects in females and males. We estimated the t-statistic and the resulting so-called p_{diff}[16] (P-value from a t-distribution with one degree of freedom) as implemented in the EasyStrata software.[32] We tested a total of 2,162 different index SNPs for sex-dimorphism; we tested all of the secondary signals as well, but these signals are by definition in linkage disequilibrium with the index SNPs (and therefore not independent). Given that we tested for sex-dimorphism at index SNPs in not only WHRadjBMI but WHR and BMI as well, we performed a test at 1,502 distinct genomic loci. Therefore, we set significance for sex-dimorphism at a Bonferroni-corrected p = 0.05/1,502 = 3.3×10^{-5}.

SNPs were determined to have a stronger effect in women if they fell into one of the following categories (abs: absolute value):

(a) $beta_{females} \leq 0$ and $beta_{males} \leq 0$ and $abs(beta_{females}) > abs(beta_{males})$

(b) $beta_{females} \geq 0$ and $beta_{males} \geq 0$ and $abs(beta_{females}) > abs(beta_{males})$

(c) $beta_{females} \leq 0$ and $beta_{males} \geq 0$ and $p_{females} < p_{males}$ and $abs(beta_{females}) > abs(beta_{males})$, or

(d) $beta_{females} \geq 0$ and $beta_{males} \leq 0$ and $p_{females} < p_{males}$ and $abs(beta_{females}) > abs(beta_{males})$

Heritability calculations

SNP-based heritability calculations

We implemented all heritability calculations in BOLT-LMM.[10] We used the same genetic relationship matrix (GRM) to estimate SNP-based heritability as we did to run our GWAS (see *Genome-wide association analyses*). This GRM included 790,000 SNPs. Heritability was estimated using only the UK Biobank samples, for which we had individual level data; these estimates are likely more accurate than those resulting from only summary-level data. We used Restricted Maximum Likelihood Estimation, implemented as --reml in BOLT.

To test the impact of including lower-frequency SNPs in the heritability estimates, we constructed an additional GRM identically as we had for association testing, but including no minor allele frequency threshold. This GRM included ~1.7M SNPs. Heritability analyses were calculated identically using this GRM and --reml in BOLT.

To calculate whether heritability estimates in men and women were sex-dimorphic, we used the following equation to generate a z-score:

$$z = \frac{h^2_{females} - h^2_{males}}{\sqrt{variance_{females} + variance_{males}}} \quad (2)$$

We then converted the z-scores to P-values using the following formula in the statistical programming language and software suite R (version 3.4):

$$P = 2 * pnorm(-abs(z)) \quad (3)$$

Validation in an independent dataset

We used an independent dataset EXTEND (7,721 individuals of white European descent collected from South West England, **Supplementary Table 14**) to validate our findings. We extracted the index SNPs from the HRC imputed genotypes. To generate the WHRadjBMI variable, we regressed WHR on BMI, age, age-squared, sex and principal components 1-5. We then performed rank based inverse normalization on the resulting residuals. We validated the findings in 3 steps:

(1) Directional consistency. We checked for directional consistency between the effect of index SNPs on WHRadjBMI from the main meta-analysis and EXTEND. We performed linear regression of WHRadjBMI on each individual SNP. We ensured all alleles were aligned to the WHRadjBMI increasing allele in the original meta-analysis. We compared directions between all 346 index SNPs and then split these into novel and known signals to determine the number of novel signals showing consistent directionality.

(2) Variance explained. We evaluated the proportion of variance explained by including all the index SNPs into a linear regression model and calculated the adjusted R^2. We performed these analyses using the lm() function in R.

(3) Polygenic scores. We created a weighted polygenic score based on the 346 index SNPs associated with WHRadjBMI. The weighted polygenic score was calculated by summing the dosage of the WHRadjBMI-increasing alleles (weighted by the effect size on WHRadjBMI from the meta-analysis). We then performed linear regression to test the association between WHRadjBMI and the GRS in our independent dataset.

To determine how likely 5% of individuals carrying the most number of WHRadjBMI increasing alleles were to meet the World Health Organization (WHO) WHR threshold for metabolic syndrome[13] comparing to 5% carrying the least, we used the WHR reference levels of > 0.9 in men and > 0.85 in women to define cases and WHR < 0.9 in men and < 0.85 in women to define controls. We excluded all individuals with missing data leaving a sample size of 7,513. We took 5% of individuals (7,513 x 0.05 = 376) from the two ends of weighted GRS and coded them as 1 or 2 respectively. We tested for the likelihood of the top 5% meeting the WHR threshold for metabolic syndrome (WHO criteria) compared to the bottom 5% using a binomial logistic regression model adjusting for age, age-squared, sex and principal components 1-5.

Comparison of WHRadjBMI-associated SNPs in other fat distribution phenotypes

Comparison with body fat percentage

Similarly to Shungin et al[5], we carried out analysis on the 346 index SNPs and their association with BF%. We obtained association statistics for the 346 SNPs with BF% from a GWAS of 443,001 unrelated, European-ancestry UK Biobank individuals. We aligned all results to the WHRadjBMI increasing allele and used a Bonferroni-corrected P-value (0.05/346 = 1..44 X 10^{-4}) to determine if a SNP was also associated with BF%. To determine whether sex-specific WHRadjBMI index SNPs have an adiposity phenotype, we took the 97 (female-specific) and 8 (male-specific) SNPs and independently compared their effects on WHR and BF% in men and women. To identify which sex-dimorphic SNPs were strongly associated with BF% in men and women separately, we used a Bonferroni-corrected P-value of 0.05/105 (4.8 x 10^{-4}) (**Supplementary Fig 8 and Supplementary Table 9**). We obtained Pearson's *r* correlations using the cor() function in R for each comparison.

Comparison with genome-wide analysis of depot-specific traits

Recently, Chu et al[20] performed a genome-wide association study of subcutaneous and ectopic fat depots, as measured by CT and MRI imaging, in a multi-ancestry sample. Since the meta-analysis results are publicly-available (https://grasp.nhlbi.nih.gov/FullResults.aspx and **Supplementary Information** for further details), we took the index SNPs from our WHRadjBMI meta-analyses (combined sample as well as sex-specific), checked for allele consistency, aligned effects to the reference allele, and tested for associations with the imaging based measures of subcutaneous and ectopic fat. We repeated these analyses in men and women separately. The depots investigated in the imaging-based GWAS were: pericardial tissue (PAT), PAT adjusted for height and weight (PATadjHtWt), subcutaneous adipose tissue (SAT) ,SAT Hounsfield units as measured by MRI (SATHU), visceral adipose tissue (VAT), VAT Hounsfield units (VATHU), ratio of VAT to SAT (VAT/SAT), and VAT adjusted for BMI (VATadjBMI).

We calculated Pearson's r correlations between z-scores in WHRadjBMI (calculated by dividing the SNP beta by the standard error) and SNP z-scores reported in Chu et al.[20] We evaluated significance of the correlation by performing a t-test (implemented as cor.test() in R). Correlations were considered significant if P-value < 0.05/3 sample groups/9 phenotypes = 1.9 x 10^{-3}.

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Authorship contributions

Data collection and analysis: S.L.P., C.S., A.P.M., A.R.W.,
Data collection: ATH
Data interpretation: S.L.P., C.S., C.M.L., T.M.F., H.Y., A.P.M., C.G.
Study supervision: H.Y., T.F., S.L.P, C.M.L
First draft of the manuscript: S.L.P, C.M.L.
Critical revisions of the manuscript: all co-authors

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