Genome-Wide and Abdominal MRI Data Provide Evidence That a Genetically Determined Favorable Adiposity Phenotype Is Characterized by Lower Ectopic Liver Fat and Lower Risk of Type 2 Diabetes, Heart Disease, and Hypertension

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Recent genetic studies have identified alleles associated with opposite effects on adiposity and risk of type 2 diabetes. We aimed to identify more of these variants and test the hypothesis that such favorable adiposity alleles are associated with higher subcutaneous fat and lower ectopic fat. We combined MRI data with genome-wide association studies of body fat percentage (%) and metabolic traits. We report 14 alleles, including 7 newly characterized alleles, associated with higher adiposity but a favorable metabolic profile. Consistent with previous studies, individuals carrying more favorable adiposity alleles had higher body fat % and higher BMI but lower risk of type 2 diabetes, heart disease, and hypertension. These individuals also had higher subcutaneous fat but lower liver fat and a lower visceral-to-subcutaneous adipose tissue ratio. Individual alleles associated with higher body fat % but lower liver fat and lower risk of type 2 diabetes included those in *PPARG*, *GRB14*, and *IRS1*, whereas the allele in *ANKRD55* was paradoxically associated with higher visceral fat but lower risk of type 2 diabetes. Most identified favorable adiposity alleles are associated with higher subcutaneous and lower liver fat, a mechanism consistent with the beneficial effects of storing excess triglycerides in metabolically low-risk depots.

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Many overweight or obese individuals do not carry the expected metabolic disease risks associated with a higher BMI (1,2), whereas some lean or normal weight individuals develop diseases like type 2 diabetes (3–5). We (6,7) and others (8–10) have shown that genetic variation is likely to contribute to these differences by increasing adiposity but lowering the risk of type 2 diabetes. We labeled these variants favorable adiposity because the alleles associated with higher BMI are associated with a favorable metabolic profile and lower risk of type 2 diabetes. The alternative alleles of the same variants could be characterized as unfavorable lack of adiposity or limited adipose tissue storage capacity. The identification of these variants differ by study. One study started with a genome-wide association study (GWAS) of body fat percentage (%) in 76,150 individuals and showed that a common allele near the *IRS1* gene was associated with higher adiposity but lower insulin resistance and risk of disease (8). The remaining studies were limited to genetic variants associated with fasting insulin levels at genome-wide levels of statistical confidence and used a combination of data and approaches to identify genetic scores of between 10 and 53 variants that collectively were associated with opposite effects on BMI and risk of type 2 diabetes (6,7,9,10).

More detailed characterization of these alleles revealed several insights. First, the alleles associated with higher BMI but lower risk of type 2 diabetes were associated with a lower risk of hypertension and heart disease as well as type 2 diabetes (6,7,9). Second, most of the alleles associated with higher insulin sensitivity, as identified by GWAS of fasting insulin levels, were associated with higher BMI or a redistribution of fat into the lower body, as estimated by waist-to-hip ratio (6,7,9,10). Third, these alleles were associated with more refined measures of adipose tissue distribution: The alleles associated with higher BMI but lower risk of disease also were associated with higher adiposity in the lower body (gynoid area and legs) as measured by DEXA (9).

A likely explanation for the association of favorable adiposity alleles with higher peripheral adiposity that these studies proposed is altered adipose tissue storage capacity (6,7,9,10) consistent with the adipose tissue expandability hypothesis (11). To have a clear understanding about the underlying mechanisms associated with favorable adiposity in the context of the adipose tissue expandability hypothesis, we need to study whether favorable adiposity alleles are specifically associated with lower levels of ectopic fat. Furthermore, because men and women have different body fat distribution regulated by sex steroids (12), separate study of underlying mechanisms in men and women may help to elucidate the biology of the cardiometabolic diseases.

The aim of the current study was to identify additional alleles associated with favorable adiposity and to combine genetic and MRI data to understand more about the underlying mechanisms. In contrast to most previous studies that focused on variants associated with surrogate measures of insulin resistance (fasting insulin), we started with variants associated with altered body fat %. We describe an approach that led to the characterization of 14 alleles collectively associated with higher body fat % but lower risk of type 2 diabetes, hypertension, and heart disease. We show that these alleles are associated with lower ectopic fat in the liver on the basis of MRI data.

RESEARCH DESIGN AND METHODS

UK Biobank Study

UK Biobank recruited >500,000 individuals aged 37–73 years (99.5% were between 40 and 69 years of age) between 2006 and 2010 from across the U.K. (Supplementary Table 1). The study has been described in detail elsewhere (13).

UK Biobank Genetic Data

Single nucleotide polymorphism (SNP) genotypes underwent extensive central quality control (http://biobank.ctsu.ox.ac.uk). We based our study on 451,099 individuals of white European descent as defined by principal component analysis. Briefly, principal components were generated in the 1000 Genomes cohort using high-confidence SNPs to obtain their individual loadings. These loadings then were used to project all the UK Biobank samples into the same principal component space, and individuals were clustered using principal components 1–4. We removed seven participants who withdrew from the study and 348 individuals whose self-reported sex did not match their genetic sex on the basis of relative intensities of X and Y chromosome SNP probe intensity.

Measures of Disease and Disease-Related Traits in UK Biobank

We used three cardiometabolic diseases—type 2 diabetes, hypertension (also represented by continuous measures of systolic and diastolic blood pressure), and heart disease—according to baseline data and while following similar definitions to those used in previous GWAS

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(Supplementary Table 1). We defined type 2 diabetes using baseline data if three criteria were present: *1*) reports of diabetes at the interview, *2*) at least a 1-year gap from diagnosis without requiring insulin, and *3*) reported age at diagnosis >35 years to limit the numbers of individuals with slow-progressing autoimmune diabetes or monogenic forms. Individuals not reporting an age at diagnosis were excluded. We also excluded individuals diagnosed with diabetes within the year before the baseline study visit because we were unable to determine whether they were using insulin within the 1st year. Control subjects were individuals not fulfilling these criteria. We defined subjects as hypertensive if systolic blood pressure was >140 mmHg, diastolic blood pressure was >90 mmHg, or blood pressure medication was reported. Control subjects were individuals who did not fulfill these criteria. For the analysis of systolic and diastolic blood pressure, we corrected blood pressure measures in those on antihypertensive drugs by adding 15 mmHg to systolic and 10 mmHg to diastolic blood pressure. We defined subjects as having heart disease if they reported angina and/or a heart attack at the interview stage. We defined control subjects as individuals without these conditions.

Identification of Genetic Variants Associated With Favorable Adiposity

The study design included three steps to identify genetic variants associated with favorable adiposity (Supplementary Fig. 1).

Step 1: Genetic Variants Associated With Adiposity

To measure adiposity, we used bioimpedance measures of body fat % taken by the Tanita BC-418MA body composition analyzer (n = 442,278 individuals from UK Biobank). We used a linear mixed model implemented in BOLT-LMM to account for population structure and relatedness (14). We used age, sex, genotyping platform, study center, and the first five principal components as covariates in the model.

Step 2: Genetic Variants Associated With a Multivariate Metabolic Outcome

We used summary statistics from published GWAS (not including UK Biobank) of metabolic biomarkers, including body fat % (n = 120,000) (15), HDL cholesterol (HDL-C) (n = 99,900) (16), adiponectin (n = 29,400) (17), sex hormone–binding globulin (SHBG) (n = 21,800) (18), triglycerides (n = 96,600) (16), fasting insulin (n = 51,800) (19), and alanine transaminase (n = 55,500) (20). We used these biomarkers to be consistent with our previous approach (7). These biomarkers are used to discriminate monogenic disorders of fat storage (lipodystrophy) from other monogenic conditions where insulin sensitivity and adiposity are affected (7,21,22).

Within each GWAS, we standardized the effect sizes to correct for the differences in sample size and the various trait measurement units across different GWAS (Eq. 1):

$$\beta_{standardized} = \frac{\beta}{SE \times \sqrt{n}} \quad \text{Eq. 1}$$

We used metaCCA software (23) to run a multivariate GWAS. The phenotype-phenotype correlation matrix [ΣYY = cov(Y, Y)] was built according to the Pearson correlation between any pairs of traits across genome-wide genetic variants. The genotype-genotype correlation matrix [ΣXX = cov(X, X)] was computed using a reference database from 1000 Genomes. The canonical correlation analysis in metaCCA finds the maximal correlation coefficient R_metaCCA between genetic variants and linear combination of phenotypes on the basis of the phenotype-phenotype correlation matrix. We defined genetic variants associated with a multivariate metabolic outcome if metaCCA $P < 5 \times 10^{-8}$.

Step 3: Genetic Variants Associated With Favorable Adiposity

We selected genetic variants associated with both adiposity (step 1) and a multivariate metabolic outcome (step 2) at $P < 5 \times 10^{-8}$ and used a hierarchical clustering approach to narrow the list to ones showing a pattern of favorable adiposity. We calculated the frequency of times the variants were in the same cluster to identify the favorable adiposity cluster using the pvclust package in R (7).

Genetic Score Analysis

We constructed the genetic score of favorable adiposity variants as the number of favorable adiposity alleles carried by each individual (unweighted). We used age, sex, genotyping platform, study center, and the first five ancestry principal components as covariates in the model.

Additional Studies for Replication of the Nonimaging Findings

To provide additional evidence for the role of favorable adiposity alleles, we used five cohorts that were not part of the published GWAS used in our discovery stage (Supplementary Table 1): The Netherlands Epidemiology of Obesity (NEO) study (6,671 individuals of white European descent from the greater area of Leiden in the west of the Netherlands [24]), Exeter 10,000 (EXTEND) (7,340 individuals of white European descent from southwest England), Generation Scotland: Scottish Family Health Study (GS:SFHS) (20,000 individuals of white European descent from Scotland [25]), Tübingen Family Study for Type 2 Diabetes (TÜF) (2,679 individuals of white European descent from southern Germany [26]), and Innovative Medicines Initiative (IMI) Diabetes Research on Patient Stratification (DIRECT) (3,029 Caucasian subjects with prediabetes and type 2 diabetes recruited by clinical centers located across Europe [27]). To also provide evidence for the role of favorable adiposity alleles in risk of cardiometabolic diseases, we used published GWAS of type 2 diabetes (28), heart disease (29), and blood pressure (30).

Studies That Contributed to Imaging Findings (Liver Fat, Visceral Fat, and Subcutaneous Fat)

UK Biobank

We used 5,045 individuals who had available data obtained through UK Biobank Access Application number 6569. Subjects underwent MRI as previously described (31). Briefly, a single transverse slice located at the liver was acquired from each subject using multiecho spoiled gradient echo acquisition and analyzed as previously described (32). Assessment of abdominal subcutaneous and visceral fat was described previously (33).

NEO

Abdominal subcutaneous and visceral fat was assessed in 2,236 participants using MRI and were quantified by a turbo spin echo imaging protocol. At the level of the fifth lumbar vertebra, three transverse images, each with a slice thickness of 10 mm, were obtained during a breathhold. Proton ^{1}H magnetic resonance spectroscopy of the liver was used to assess hepatic triglyceride content (n = 1,821) (24).

TÜF

The TÜF study contributed subcutaneous and visceral adipose tissue measurements from 833 and 906 genotyped individuals, respectively, who underwent whole-body magnetic resonance tomography. The two fat depots were quantified by an axial T1-weighed fast spin echo technique with a 1.5-T whole-body imager (MAGNETOM Sonata; Siemens Healthcare), as previously described (26). Liver fat measurements were available from 911 genotyped individuals who underwent localized ^{1}H magnetic resonance spectrometry, as previously described (26).

IMI-DIRECT

The IMI-DIRECT consortium is a collaboration among investigators from a range of European academic institutions and pharmaceutical companies. Liver fat was assessed in 1,457 subjects using a multiecho acquisition as previously described (34). Briefly, the liver was identified from a scout abdominal image, and axial images were taken during suspended respiration, which were used to position a single-slice multiecho sequence through the liver.

Published GWAS

We used a published GWAS of subcutaneous and visceral fat distribution as measured by CT scan or MRI (35).

RESULTS

Fourteen Alleles Identified as Associated With Favorable Adiposity

Using a three-step approach, we characterized 14 genetic variants associated with favorable adiposity. Of these variants, seven were previously known to be associated with a favorable adiposity phenotype: those in/near *PPARG*, *LYPLAL1*, *GRB14*, *IRS1*, *PEPD*, *FAM13A*, and *ANKRD55*; five were known to be associated with a relevant trait but not confirmed as having a favorable adiposity phenotype (those in/near *TRIB1*, *KLF14/MKLN1*, *DNAH10*, *VEGFA/C6orf223*, and *AEBP2/PDE3A*), and two were novel (those in/near *MAFF* and *CITED2*) (Supplementary Table 2). Twelve of the 14 variants had not been associated previously with body fat % at genome-wide levels of statistical confidence.

In the first step (Supplementary Fig. 1), we performed a GWAS of body fat % in 442,278 individuals in the UK Biobank. We identified 620 variants at $P < 5 \times 10^{-8}$. In the second step, we used published GWAS statistics from 7 circulating biomarkers of metabolic health and identified 33 of these 620 variants as associated with a multivariable metabolic phenotype. This approach identifies alleles associated with metabolic traits after accounting for the phenotypic correlation between higher adiposity and these metabolic traits (Supplementary Tables 3 and 4 and Supplementary Fig. 2). For example, this approach has more power to detect alleles paradoxically associated with higher adiposity but with a favorable metabolic profile because the model accounts for the population-level correlation between higher adiposity and an adverse metabolic profile. The resulting 33 alleles also included some associated very strongly with higher BMI and an adverse metabolic profile, such as the allele in the *FTO* gene, most likely because adjusting for body fat % in the model does not fully account for the adverse metabolic effects of lifelong higher adiposity. We therefore undertook a third step where we further refined the phenotypic characteristics of these variants by performing a clustering analysis. This approach led to the clustering of 14 alleles associated with favorable adiposity as defined by association with higher body fat %, HDL-C, SHBG, and adiponectin levels and lower triglycerides, alanine transaminase, and fasting insulin levels (Supplementary Fig. 3). We validated the effect of the 14 favorable adiposity alleles together in a genetic score on levels of metabolic biomarkers using five independent studies: NEO, EXTEND, GS:SFHS, TÜF, and IMI-DIRECT (Supplementary Table 5).

A Genetic Score of Favorable Adiposity Alleles Was Associated With Lower Risk of Cardiometabolic Disease Outcomes

Carrying additional favorable adiposity alleles was associated with higher body fat % and higher BMI but lower risk of type 2 diabetes, hypertension, and heart disease (Table 1). For example, the 10% of subjects carrying the most favorable adiposity alleles had ~1.04% higher body fat % (95% CI 0.95, 1.13; $P = 6 \times 10^{-115}$) and 0.4 kg/m^2 higher BMI (0.32, 0.45; $P = 3 \times 10^{-29}$) but a lower risk of type 2 diabetes (odds ratio [OR] 0.66 [95% CI 0.61, 0.72]; $P = 7 \times 10^{-23}$), lower risk of hypertension (OR 0.87 [0.84, 0.90]; $P = 1 \times 10^{-19}$), and lower risk of heart disease (OR 0.84 [0.80, 0.89]; $P = 6 \times 10^{-10}$) compared with the 10% of subjects carrying the fewest favorable adiposity alleles (data from UK Biobank) (Fig. 1). These effects were similar in men and women and when we removed the seven

Table 1—The effect of favorable adiposity genetic score on measures of adiposity and cardiometabolic disease outcome in the UK Biobank study

	Fourteen SNPs			Seven additional SNPs			
Trait/disease and analysis	Effect	95% CI	*P* value	Effect	95% CI	*P* value	*n* (patients vs. control subjects)
Body fat %							
All	0.17	0.169, 0.171	6×10^{-263}	0.15	0.149, 0.151	1×10^{-105}	443,000
Women	0.15	0.148, 0.152	3.5×10^{-116}	0.14	0.138, 0.142	8.9×10^{-52}	240,882
Men	0.19	0.188, 0.192	1×10^{-165}	0.16	0.158, 0.162	3×10^{-61}	202,118
BMI (kg/m^2)							
All	0.040	0.039, 0.041	3.6×10^{-45}	0.045	0.044, 0.047	4.5×10^{-30}	449,359
Women	0.041	0.039, 0.042	3×10^{-28}	0.047	0.045, 0.049	1.9×10^{-19}	243,797
Men	0.039	0.038, 0.041	1.6×10^{-22}	0.042	0.040, 0.045	6×10^{-14}	205,528
Type 2 diabetes (OR)							
All	0.954	0.948, 0.960	4×10^{-44}	0.966	0.957, 0.975	1.9×10^{-13}	14,371 vs. 428,017
Women	0.950	0.939, 0.961	3×10^{-18}	0.962	0.946, 0.977	2×10^{-6}	4,713 vs. 236,073
Men	0.960	0.948, 0.964	5×10^{-26}	0.966	0.955, 0.978	1×10^{-8}	9,076 vs. 192,344
Heart disease (OR)							
All	0.984	0.980, 0.989	3×10^{-14}	0.982	0.976, 0.988	1.5×10^{-9}	37,741 vs. 318,892
Women	0.987	0.980, 0.994	0.0003	0.981	0.971, 0.991	0.0003	12,270 vs. 184,550
Men	0.982	0.977, 0.987	2×10^{-11}	0.983	0.975, 0.990	2.6×10^{-6}	25,363 vs. 134,433
Hypertension (OR)							
All	0.987	0.985, 0.989	1×10^{-33}	0.989	0.986, 0.992	3×10^{-13}	241,691 vs. 206,525
Women	0.988	0.985, 0.991	2×10^{-16}	0.989	0.985, 0.993	3×10^{-7}	114,713 vs. 128,623
Men	0.985	0.981, 0.988	1.7×10^{-19}	0.987	0.983, 0.992	1.6×10^{-7}	126,978 vs. 77,902
Systolic blood pressure (mmHg)							
All	−0.173	−0.174, −0.172	9×10^{-46}	−0.139	−0.141, −0.138	3.6×10^{-16}	450,075
Women	−0.163	−0.165, −0.162	1×10^{-22}	−0.134	−0.136, −0.132	1×10^{-8}	244,183
Men	−0.206	−0.208, −0.205	7.9×10^{-27}	−0.161	−0.163, −0.159	2×10^{-9}	205,892
Diastolic blood pressure (mmHG)							
All	−0.074	−0.075, −0.073	7×10^{-24}	−0.085	−0.087, −0.083	1×10^{-16}	449,322
Women	−0.078	−0.080, −0.077	1.6×10^{-14}	−0.093	−0.095, −0.091	1×10^{-10}	243,732
Men	−0.073	−0.074, −0.071	1.9×10^{-10}	−0.081	−0.083, −0.079	3.5×10^{-7}	205,590

Effects are per carrying an additional adiposity allele.

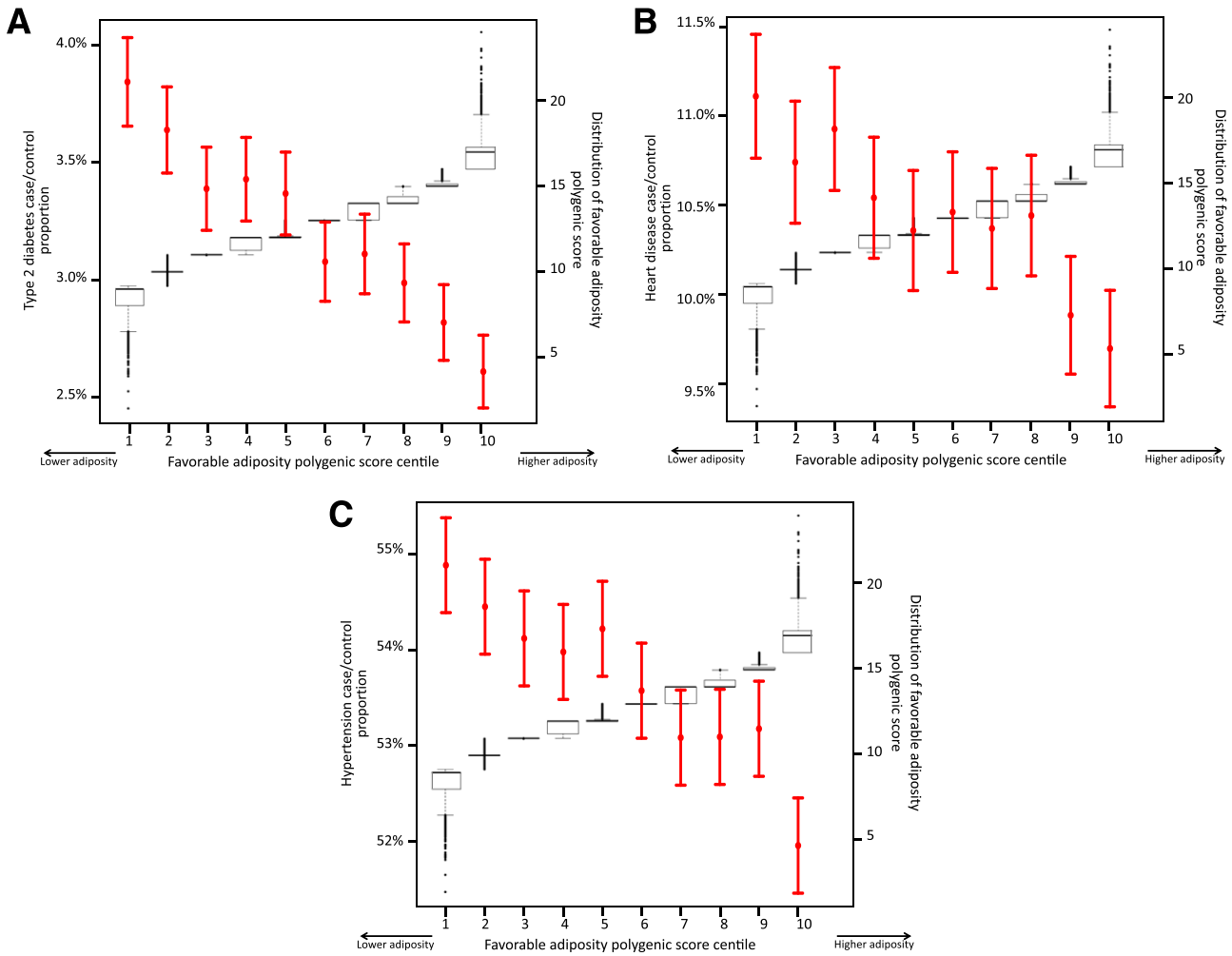

Figure 1—Carrying more favorable adiposity alleles was associated with higher adiposity but lower risk of type 2 diabetes (*A*), heart disease (*B*), and hypertension (*C*). We divided individuals from UK Biobank into 10 centiles on the basis of their favorable adiposity genetic score (*x*-axis). The distribution of favorable adiposity genetic score is shown in black and the case/control proportion is shown in red per each centile.

known favorable adiposity variants from the analysis (Table 1). These associations were similar when using data from published GWAS (Supplementary Table 6). For each of the 14 individual variants, except that at the AEBP2 locus, the body fat %–increasing allele was associated with at least one of lower risk of type 2 diabetes, lower risk of heart disease, or lower diastolic or systolic blood pressure in UK Biobank (Supplementary Fig. 4). In published GWAS data, the exceptions were the variants at the *AEBP2* and *MAFF* loci (Supplementary Table 6).

Individual Favorable Adiposity Alleles Were Associated With Heterogeneous Effects on Waist-to-Hip Ratio

Five of the individual 14 variants were previously identified as associated with waist-to-hip ratio (36). Previous studies have pointed out that the disease-protective effect of these alleles is likely to be due to their association with redistribution of the extra fat into the lower body (defined by lower waist-to-hip ratio). We therefore examined the alleles' association with waist-to-hip ratio in more detail. Carrying more favorable adiposity alleles was associated with lower waist circumference ($P = 3.7 \times 10^{-5}$) but higher hip circumference ($P = 2.3 \times 10^{-109}$) in women. However, in men, carrying more favorable adiposity alleles was associated with higher waist circumference ($P = 1.7 \times 10^{-40}$), higher hip circumference ($P = 1.8 \times 10^{-53}$), and no effect on waist-to-hip ratio (Supplementary Table 7). These associations were robust when limiting the variants to the seven not previously identified as having a favorable adiposity phenotype (Supplementary Table 7). The individual variants were associated with heterogeneous effects on waist-to-hip ratio. Most notably, for the two variants in/near *PPARG* and *ANKRD55*, the favorable adiposity allele was not associated with lower waist-to-hip ratio in women, and for *ANKRD55*, it was associated with higher waist-to-hip ratio (Fig. 2).

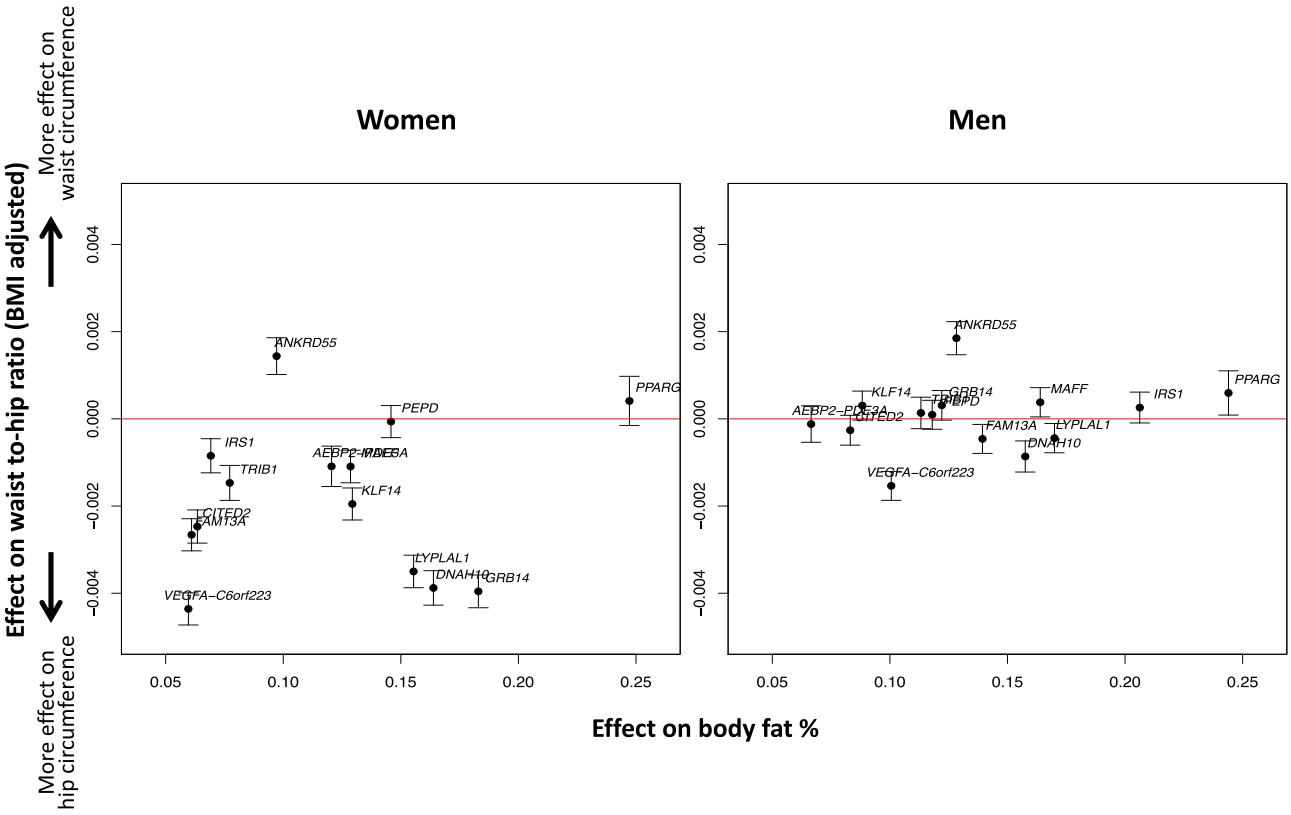

Figure 2—The individual variants were associated with heterogeneous effects on waist-to-hip ratio. Most notably, for two variants, those in/near *PPARG* and *ANKRD55*, the favorable adiposity allele was not associated with lower waist-to-hip ratio in women, and for *ANKRD55*, it was associated with higher waist-to-hip ratio. For 11 variants (those in/near *IRS1*, *TRIB1*, *CITED2*, *FAM13A*, *VEGFA*, *AEBP2*, *KLF14*, *LYPLAL1*, *DNAH10*, *MAFF*, and *GRB14*), the favorable adiposity allele was associated with lower waist-to-hip ratio in women, whereas for the variant in/near *PEPD*, there was no clear association with waist-to-hip ratio in either sex. Data are from UK Biobank population.

Favorable Adiposity Alleles Were Associated With Less Liver Fat and More Abdominal Subcutaneous Fat

We next investigated the associations between the favorable adiposity variants and MRI measures of subcutaneous, visceral, and liver fat using data from 9,510 individuals and four studies: the first wave of UK Biobank imaging data (n = 5,045), NEO (n = 2,236), IMI-DIRECT (n = 1,323), and TÜF (n = 906). A fifth set of data did not include liver fat and came from a published meta-analysis of 13 studies with abdominal MRI or CT scans of 18,332 individuals (35).

The genetic score of favorable adiposity alleles was associated with lower visceral-to-subcutaneous adipose tissue volume ratio ($P = 2 \times 10^{-14}$) in both men and women. This effect was driven by an association with more subcutaneous fat ($P = 2 \times 10^{-14}$) (Table 2 and Fig. 3). All 14 individual genetic variants were associated with higher subcutaneous adipose tissue, 7 at $P < 0.05$ (in/near *DNAH10*, *FAM13A*, *GRB14*, *KLF14*, *LYPLAL1*, *IRS1*, and *PPARG*). Nine individual favorable adiposity alleles were associated with lower visceral-to-subcutaneous adipose tissue volume ratio, all at $P < 0.05$ (in/near *CITED2*, *DNAH10*, *FAM13A*, *KLF14*, *LYPLAL1*, *IRS1*, *PPARG*, *TRIB1*, and *VEGFA*) (Supplementary Fig. 4 and Supplementary Table 8). Paradoxically, the favorable adiposity alleles in/near *ANKRD55* and *PEPD* were associated with higher visceral-to-subcutaneous adipose tissue volume ratio (P = 0.001 and 0.02, respectively).

The genetic score of favorable adiposity was associated with lower liver fat in women ($P = 6.3 \times 10^{-9}$) but was not associated with liver fat in men (P = 0.8) (Table 2 and Fig. 3). These effects were robust when limiting the variants to the seven not previously identified as having a favorable adiposity phenotype (Table 2). For 11 individual variants, the allele associated with higher subcutaneous fat was associated with lower liver fat, four with $P < 0.05$ (in/near *CITED2*, *GRB14*, *PPARG*, and *TRIB1* (Supplementary Fig. 4 and Supplementary Table 8).

Sensitivity Analysis of Liver Fat

We performed three sensitivity analyses to assess whether the effect of favorable adiposity alleles on lower liver fat was affected by menopause, inclusion of patients with type 2 diabetes, or alcohol consumption. First, menopause leads to a redistribution of adipose tissue toward more central obesity and an android phenotype (37,38). To study whether the association with liver fat in women was influenced by menopausal status, we divided women

Table 2—The effect of favorable adiposity genetic score on MRI/CT scan measures of abdominal adipose tissue using data from five studies

	Fourteen SNPs				Seven additional SNPs			
Analysis	β	95% CI	*P* value	*P* het	β	95% CI	*P* value	*P* het
SAT (L)								
All	0.054	0.042, 0.067	2×10^{-14}	0.36	0.048	0.029, 0.067	9.6×10^{-7}	0.38
Women	0.032	0.016, 0.048	6×10^{-5}	0.55	0.032	0.010, 0.054	3×10^{-3}	0.89
Men	0.051	0.035, 0.067	2.5×10^{-11}	0.16	0.045	0.022, 0.064	4.9×10^{-5}	0.35
VAT (L)								
All	0.005	−0.007, 0.014	0.4	0.69	−0.002	−0.016, 0.011	0.84	0.94
Women	−0.007	−0.018, 0.005	0.2	0.28	−0.009	−0.025, 0.005	0.21	0.6
Men	0.011	0.000, 0.020	0.05	0.05	0.007	−0.009, 0.020	0.43	0.34
VATSAT ratio								
All	−0.005	−0.007, −0.004	2×10^{-14}	0.15	−0.005	−0.008, −0.004	4×10^{-9}	0.75
Women	−0.005	−0.007, −0.003	1×10^{-10}	0.46	−0.006	−0.008, −0.004	9×10^{-8}	0.93
Men	−0.004	−0.005, −0.002	7×10^{-7}	0.03	−0.004	−0.006, −0.002	4×10^{-4}	0.16
Liver fat (%)								
All	−0.087	−0.124, −0.051	5.6×10^{-6}	0.015	−0.060	−0.115, −0.009	0.02	0.015
Women	−0.170	−0.225, −0.110	6.3×10^{-9}	0.26	−0.133	−0.216, −0.055	1×10^{-3}	0.57
Men	−0.005	−0.055, 0.041	0.8	0.16	0.000	−0.069, 0.069	0.99	0.086

Effects are per carrying an additional adiposity allele. *P* het, *P* value of heterogeneity test across the five studies; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue; VATSAT, visceral-to-subcutaneous adipose tissue.

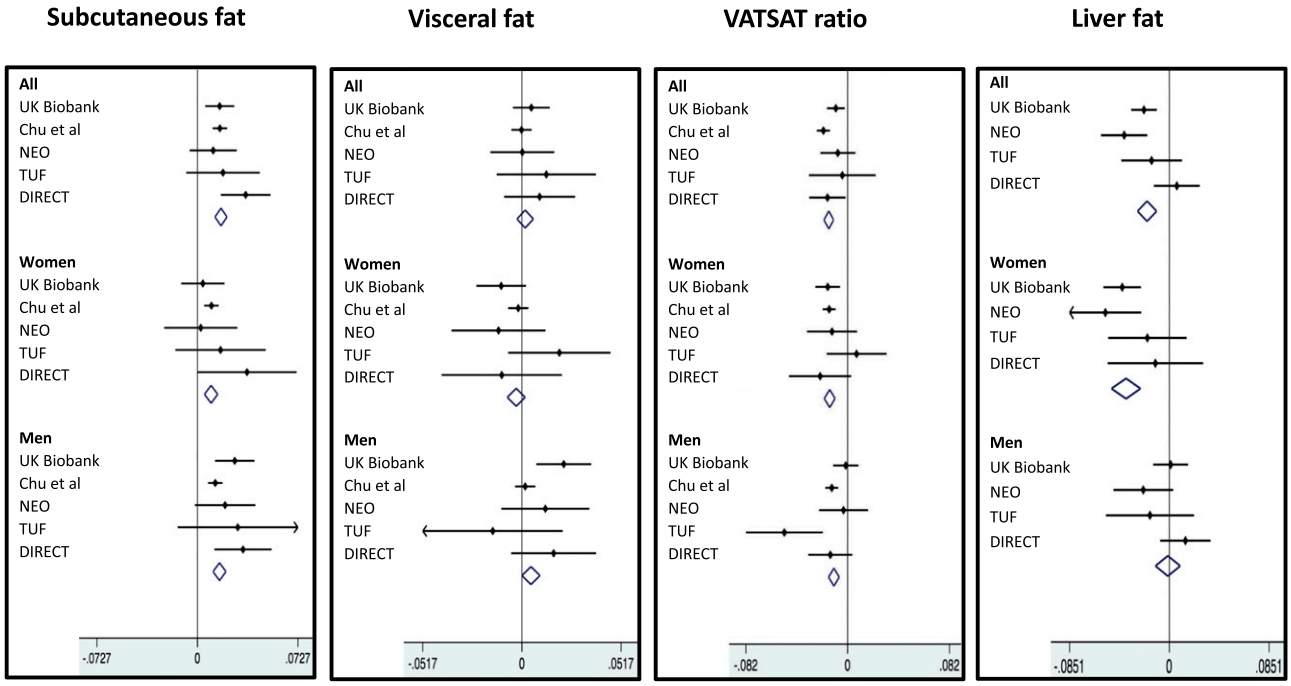

Figure 3—The effect of favorable adiposity genetic score on MRI/CT scan measures of abdominal adipose tissue using data from five studies: UK Biobank, Chu et al. (35), NEO, TÜF, and DIRECT. The *x*-axis is the effect size per carrying an additional favorable adiposity allele. VATSAT, visceral-to-subcutaneous adipose tissue.

from the UK Biobank and TÜF studies into pre- and postmenopausal status. The association between favorable adiposity alleles and lower liver fat in premenopausal women was twice that (−0.258% [95% CI −0.223, −0.293]; P = 0.002; n = 433) of postmenopausal women (−0.124% [−0.106, −0.142]; P = 0.002; n = 2,356), but the difference was not statistically meaningful ($P_{\text{difference}}$ = 0.14) (Supplementary Table 9).

Second, fatty liver disease is very common (>50%) in patients with type 2 diabetes (39). To check whether inclusion of patients with type 2 diabetes affected the association with liver fat, we ran the tests in UK Biobank subjects, excluding those with type 2 diabetes (n = 222) from the analysis of liver fat. The association of favorable adiposity alleles with liver fat remained similar after exclusion of patients with type 2 diabetes in all, men, and women (all $P_{\text{difference}} > 0.7$) (Supplementary Table 10).

Third, the most common cause of increased fat in the liver is alcohol consumption, which is more prevalent in men (40,41). To study whether the lack of association with liver fat in men was due to greater alcohol consumption, we assessed the effect of favorable adiposity alleles on liver fat in men defined as heavy, moderate, and nondrinkers on the basis of self-report alcohol questionnaires. The favorable adiposity alleles were not associated with liver fat in any of the three groups (Supplementary Table 11).

DISCUSSION

We characterized 14 genetic variants associated with favorable adiposity. The current study adds to previous studies (6,7,9,10) in several ways. First, we outlined a new approach that leads to the identification of more favorable adiposity variants. Second, we provide more clarity about which individual alleles are likely favorable adiposity alleles and how they affect metabolic traits and diseases. Third, we used MRI data that strongly suggest that these variants have a collective effect on lower liver fat as well as higher subcutaneous fat but little detectable effect on visceral fat. Finally, we provide a template for detecting alleles with apparently paradoxical effects on adiposity and disease on the basis of a wide variety of publicly accessible GWAS data. In addition, the current results strengthen previous observations, including that the favorable adiposity effect is not driven by altered body shape in men detectable by waist-to-hip ratio (6).

Of the 14 variants detected, 12 had been associated with at least one metabolic trait, including fasting insulin (those in/near *LYPLAL1*, *GRB14*, *IRS1*, *FAM13A*, *ANKRD55*, and *PEPD* [42]), lipid levels (those in/near *GRB14*, *IRS1*, *KLF14*, *TRIB1*, and *DNAH10* [16]), adiponectin (those in/near *TRIB1*, *DNAH10*, and *AEBP2* [17]), and alanine transaminase (*TRIB1* [20]). However, only two were known to be associated with body fat % (those in/near *GRB14* and *IRS1* [15]) at genome-wide levels of statistical confidence. Our data provide several insights about individual variants. First, the alleles at *PPARG*, *GRB14*, and *IRS1* are associated with higher body fat % but lower liver fat and lower risk of type 2 diabetes. Second, the allele in *ANKRD55* is paradoxically associated with higher visceral fat but lower risk of type 2 diabetes. In

agreement with this finding, this variant is in high linkage disequilibrium ($R^2 = 0.97$) with another variant (rs459193) found to associate with lower waist circumference but higher 2-h glucose levels (43). Third, the allele in *TRIB1* is associated with higher body fat %, lower visceral fat, lower liver fat, and lower risk of heart disease and hypertension, but it does not have any detectable effect on type 2 diabetes. Fourth, four variants we previously noted as favorable adiposity were not detected in this study. These variants (in/near *PDGFC*, *PEPD*, *RSPO3*, and *TET2*) may alter body fat distribution or other aspects of body composition without altering overall body fat % and, hence, were not detected at $P < 5 \times 10^{-8}$ in stage 1.

A key question is whether the favorable adiposity effect is entirely due to preferential storage of the excess adiposity in the lower body as previously proposed (36,44). We made two general observations. First, despite similar effects on higher body fat % and lower risk of disease in each sex, the protective effect in men was not characterized by preferentially more fat in the lower body as estimated by waist-to-hip ratio and consistent with our previous observation (6). Second, the individual variants were associated with heterogeneous effects on waist-to-hip ratio even within women. For example the allele in/near *ANKRD55* was associated with favorable adiposity but higher waist-to-hip ratio in women.

Having established that the favorable adiposity effect is not driven by preferential storage of fat in the lower body, as estimated by waist-to-hip ratio in men, we examined more detailed measures of fat redistribution using MRI data. The association with lower liver fat was detected only in women. Our sensitivity analyses did not find hormonal differences owing to menopause, alcohol consumption, or type 2 diabetes as possible explanations for sex differences. We would expect the favorable adiposity alleles to be associated with liver fat in nondrinkers or moderate drinkers if the alcohol intake in men confounded the association. However, the analysis stratified by alcohol intake in men did not show an association. The lack of association with visceral fat suggests that these alleles were not protecting from disease because of lower visceral fat. This observation is consistent with some studies that showed that lower ectopic fat accumulation in the liver may be more important than visceral fat in protection from risk of type 2 diabetes (45). A caveat to this conclusion is that we used a marker of liver fat, alanine transaminase, as one of the metabolic biomarkers to identify the variants, and therefore our findings will be biased toward those variants that affect liver fat more than visceral fat.

Our approach provides a framework for identifying additional alleles with apparently paradoxical effects on adiposity and disease. A previous study used a simple and effective approach by taking published GWAS data and selecting all variants associated with higher fasting insulin adjusted for BMI, lower HDL-C, and higher triglycerides at $P < 0.005$ for each of the three traits (9). However, this approach has a couple of limitations. First, it applies an arbitrary cutoff for the three traits, and second, it does not use information from other biomarkers. We combined GWAS of seven metabolic biomarkers and used a multivariate test that does not require individual trait associations to reach a certain statistical threshold. We showed that our method performs well because it was able to identify the seven variants previously known to be associated with favorable adiposity as well as seven additional variants that we then validated in independent GWAS data. Furthermore, by including SHBG, adiponectin, and alanine transaminase in the model, we had more power to detect favorable adiposity variants (Supplementary Table 12).

The identification of favorable adiposity alleles highlights genes that may be targets for novel insulin sensitizing agents. The allele in *PPARG* provides an important proof of principle because thiazolidinediones are peroxisome proliferator–activated receptor-γ agonists and appear to lower glucose levels despite increasing the patient's weight by activating adipocyte differentiation, which redistributes fat away from the liver toward an expanded subcutaneous depot (46,47). The variants identified in our study do not identify which genes they are acting through; however, previous studies have suggested some strong candidates. For example, *TRIB1* encodes a protein critical for adipose tissue maintenance and suppression of metabolic disorders (48). Mice lacking Trib1 show diminished adipose tissue mass and increased lipolysis, even when on a normal diet (48). GWAS in humans have implicated *TRIB1* in lipid metabolism (16) and regulation of hepatic lipogenesis (20). Higher levels of vascular endothelial growth factor A in mice can facilitate healthy expansion of adipose tissue and protect from lipotoxicity and metabolic disease (49). *CITED2* is required for optimal peroxisome proliferator–activated receptor-γ activation (50). *FAM13A* encodes a protein enriched in mature adipocytes and plays an important role in the insulin signaling cascade (51) by protecting insulin receptor substrate 1 from degradation (51). The proteins encoded by *IRS1* and *CCDC92* are associated with adipogenesis, lipid accumulation, and adipocyte differentiation ability (9,51). Functional studies have suggested that *DNAH10* is involved in adipocyte differentiation capacity (9). *KLF14* is a master regulator of gene expression in adipose tissue (52) associated with adipocyte cell size in humans (53). *MAP3K1* regulates expression of *IRS1* (54). *LYPLAL1*, as a triglyceride lipase, is overexpressed in subcutaneous adipocytes of obese individuals to maintain triglyceride metabolism (55). The regulation of Grb14 expression in adipose tissue may play a physiological role in insulin sensitivity (56). *AEBP2* regulates a gene encoding a fatty acid–binding protein.

The current study had a number of limitations. First, we used seven metabolic biomarkers from published GWAS in our multivariate analysis. The sample size for each GWAS was different: ranging from 21,800 individuals from the GWAS of SHBG to 99,900 from the GWAS of

lipids. These differences, caused by using GWAS meta-analysis data from different studies, will have limited our power and led to less accurate estimates of the correlation among phenotypes compared with having the same sample size for all phenotypes. Second, the published GWAS of biomarkers were performed in men and women together rather than in a sex-specific way. Because men and women have different body fat distribution, it seems necessary to perform the discovery of favorable adiposity variants in men and women separately when data become available. Third, we used bioimpedance measures of body fat % as a measure of adiposity in the discovery step. This measure of adiposity is imprecise and not as accurate in calculating body fat % in obese individuals or people with higher muscle mass (57). However, its availability in 442,278 individuals meant that it represented a powerful data set from which to start (58). Finally, individual variants had subtle effect sizes. All variants were associated with at least one disease, with the body fat percent–increasing allele associated with lower risk except the one at the *AEBP2* locus, although this variant had a paradoxical effect on adiposity and metabolic biomarkers with significant association between body fat %–increasing allele and higher adiponectin ($P = 4.76 \times 10^{-8}$), higher HDL-C ($P = 2.83 \times 10^{-6}$), and lower triglycerides ($P = 0.003$) (Supplementary Table 4).

To yield a better understanding of how favorable adiposity protects against cardiometabolic disease, more studies are warranted. First, testing the association of favorable adiposity variants with pancreatic fat as a potential cause of β-cell dysfunction will be important to inform the associations with type 2 diabetes. Second, substantial ethnic differences exist in diabetes risk by BMI, with South Asians having a much higher risk of type 2 diabetes for a given BMI than Europeans (59). Study of the genetics of favorable adiposity in various ethnic groups may provide important insights into the mechanisms underpinning the significant ethnic differences in diabetes risk.

In summary, the current study provides additional genetic evidence that the balance of subcutaneous-to-ectopic liver fat is an important factor for type 2 diabetes, heart disease, and hypertension. This finding is consistent with data from monogenic forms of lipodystrophy and the importance of an expandable subcutaneous adipose tissue as a protective disease mechanism and limited adipose storage capacity as a risk mechanism (on the basis of the opposite alleles) as proposed in previous studies (60–62).

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