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Genetic Evidence for a Normal-Weight "Metabolically Obese" Phenotype Linking Insulin Resistance, Hypertension, Coronary Artery Disease, and Type 2 Diabetes

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The mechanisms that predispose to hypertension, coronary artery disease (CAD), and type 2 diabetes (T2D) in individuals of normal weight are poorly understood. In contrast, in monogenic primary lipodystrophy—a reduction in subcutaneous adipose tissue—it is clear that it is adipose dysfunction that causes severe insulin resistance (IR), hypertension, CAD, and T2D. We aimed to test the hypothesis that common alleles associated with IR also influence the wider clinical and biochemical profile of monogenic IR. We selected 19 common genetic variants associated with fasting insulin–based measures of IR. We used hierarchical clustering and results from genome-wide association studies of eight nondisease outcomes of monogenic IR to group these variants. We analyzed genetic risk scores against disease outcomes, including 12,171 T2D cases, 40,365 CAD cases, and 69,828 individuals with blood pressure measurements. Hierarchical clustering identified 11 variants associated with a metabolic profile consistent with a common, subtle form of lipodystrophy. A genetic risk score consisting of these 11 IR risk alleles was associated with higher triglycerides ($\beta = 0.018$; $P = 4 \times 10^{-29}$), lower HDL cholesterol ($\beta = -0.020$; $P = 7 \times 10^{-37}$), greater hepatic steatosis ($\beta = 0.021$; $P = 3 \times 10^{-4}$), higher alanine transaminase ($\beta = 0.002$; $P = 3 \times 10^{-5}$), lower sex-hormone-binding globulin ($\beta = -0.010$; $P = 9 \times 10^{-13}$), and lower adiponectin ($\beta = -0.015$; $P = 2 \times 10^{-26}$). The same risk alleles were associated with *lower* BMI (per-allele $\beta = -0.008$; $P = 7 \times 10^{-8}$) and increased visceral-to-subcutaneous adipose tissue ratio ($\beta = -0.015$; $P = 6 \times 10^{-7}$). Individuals carrying ≥17 fasting insulin–raising alleles (5.5% population) were slimmer (0.30 kg/m^2) but at increased risk of T2D (odds ratio [OR] 1.46; per-allele $P = 5 \times 10^{-13}$), CAD (OR 1.12; per-allele $P = 1 \times 10^{-5}$), and increased blood pressure (systolic and diastolic blood pressure of 1.21 mmHg [per-allele $P = 2 \times 10^{-5}$] and 0.67 mmHg [per-allele $P = 2 \times 10^{-4}$], respectively) compared with individuals carrying ≤9 risk alleles (5.5% population). Our results provide genetic evidence for a link between the three diseases of the "metabolic syndrome" and point to reduced subcutaneous adiposity as a central mechanism.

Some individuals are at increased risk of metabolic diseases, including hypertension, coronary artery disease (CAD), and type 2 diabetes (T2D) despite a normal BMI. These individuals are often referred to as "metabolically obese, normal weight" (1,2). The mechanisms that cause an adverse metabolic phenotype in individuals of normal weight are poorly understood. One possible mediator is insulin resistance (IR), which was proposed 25 years ago as a potential link among hypertension, CAD, and T2D and which led to the idea of a "metabolic syndrome" (3).

Three broad categories of unusually severe IR exist caused by mutations in single genes. The mechanisms underlying these monogenic disorders are better understood (4) compared with the "common" IR for which genome-wide association studies (GWAS) have identified numerous associated alleles. The first category is lipodystrophy, a partial or complete lack of subcutaneous fat, most often caused by mutations in genes involved in fat cell differentiation or function (e.g., *PPARG* [5]) or, rarely, by more distal insulin signaling defects (*AKT2* [6]). Lipodystrophy is characterized by varying degrees of adipose

tissue deficiency, severe dyslipidemia (high triglycerides, low HDL cholesterol [HDL-C]), severe fatty liver, low adiponectin, low sex-hormone-binding globulin (SHBG), and an increased risk of hypertension, CAD, and T2D (7–9). The uncoupling of indices of adiposity from severe metabolic disease in lipodystrophy is one of the key pieces of evidence for the notion that expandable, metabolically flexible adipose tissue is essential for health (9). The second category includes syndromes (such as those caused by leptin deficiency) in which IR is driven by a primary effect on energy homeostasis, leading to severe obesity and a biochemical profile resembling the "common" metabolic syndrome. These disorders are usually characterized by hyperphagia from an early age and extreme childhood obesity and are most commonly attributable to mutations affecting key components of hypothalamic neurocircuitry (10). The third category includes syndromes caused by loss-of-function mutations in the insulin receptor called "receptoropathies." These disorders are characterized by extremely high insulin levels but lack dyslipidemia and fatty liver disease and often exhibit normal or raised levels of plasma adiponectin and SHBG (11), quite unlike common IR.

In this study, we tested the hypothesis that common alleles associated with IR influence the metabolic outcomes of monogenic forms of IR, including increased risk of hypertension, CAD, and T2D. We aimed to use the subclinical phenotypes that characterize different forms of monogenic IR to determine whether mechanistically informative subphenotypes of common IR may be identified. Recent GWAS have identified 19 common genetic variants associated with indices of IR based on fasting plasma insulin concentration (12,13). One of these variants is in the *FTO* gene, and the allele associated with greater risk of IR is associated with higher BMI and an adverse metabolic profile (12). This pattern is closely similar to that of severe obesity. A second common variant, near the *IRS1* gene, is associated with IR, lower total body fat content, lower subcutaneous fat amount, dyslipidemia, lower adiponectin, and increased risk of diabetes and CAD (14). This pattern is closely similar to that of "lipodystrophic" IR, but quite unlike the severe obesity or "receptoropathy" patterns.

We show that a cluster of 11 variants among 19 common genetic variants associated with indices of IR are collectively associated with metabolic features similar to those of lipodystrophy, including a *lower* BMI, higher visceral-to-subcutaneous adipose tissue ratio, and a predisposition to T2D, hypertension, and CAD. This finding provides genetic evidence for an association between reduced subcutaneous adiposity and BMI and increased risk of "obesity-associated" diseases.

RESEARCH DESIGN AND METHODS

Selection of Genetic Variants

We selected the 19 independent single nucleotide polymorphisms associated with fasting insulin as reported in

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the most recent Meta-Analysis of Glucose and Insulin Consortium (MAGIC) GWAS (12) (Supplementary Table 1). Of these 19 variants, 14 were detected at genome-wide significance before correction for BMI, and 5 only reached significance after correcting for BMI. All were at least nominally associated with uncorrected fasting insulin at $P < 1.19 \times 10^{-5}$. Although not the optimum measure of IR, collectively these variants are associated with gold standard measures of IR (15). For comparison, we selected the 32 variants associated with BMI from the Genetic Investigation of ANthropometric Traits (GIANT) consortium (16) (Supplementary Table 2).

Metabolic Traits

We used summary statistics data from publicly available GWAS (Table 1).

Study Design

Selection of Nondisease Metabolic Traits

We selected eight traits known to be nondisease markers of the three different subtypes of monogenic IR (Table 1). These traits were HDL-C (17) and triglyceride levels (17) to represent dyslipidemia; BMI (16) and visceral-to-subcutaneous adipose tissue ratio (18) to represent adiposity; computed tomography (CT)-measured hepatic steatosis (19) and plasma levels of the liver enzyme alanine transaminase (ALT) (20) to represent the spectrum of nonalcoholic fatty liver disease; and circulating levels of the fat cell–derived protein adiponectin (21) and circulating levels of the liver-derived protein SHBG (22).

Selection of Metabolic Disease Outcome

We selected six metabolic diseases or disease-related outcomes known to be increased in some forms of monogenic IR. These outcome traits represent the diseases that the metabolic syndrome tries to predict: carotid intima-media thickness (cIMT) and carotid plaque as surrogates of atherosclerosis (23); diastolic and systolic blood pressure to represent hypertension (24); and CAD (25) and T2D (26). Further details of disease outcomes are available in Supplementary Table 3.

Cluster Analysis of Fasting Insulin–Associated Genetic Variants Using Nondisease Metabolic Traits

We used a hierarchical clustering analysis and the eight nondisease traits to group fasting insulin variants into those likely to predispose to IR through similar mechanisms. This method is used for clustering gene expression profiles to discover coregulated and functionally related genes or to identify subtypes of related samples (27) and is similar to principal components analyses. This approach was recently used to group 37 genetic variants associated with T2D into those with primary effects on glucose sensing, β-cell dysfunction, or IR (28). We used the summary statistics of the 19 fasting insulin variants (12) from the GWAS of the 8 nondisease metabolic traits (Table 1) and aligned all effects to the fasting insulin increasing alleles. We used the "pvclust" package in R, which performs agglomerative hierarchical clustering and bootstrapping, to estimate the stability and significance of the resulting clusters (29). In this method, variants are assigned to clusters based on associations with a similar set of phenotypes. We used Euclidean metrics in the algorithm to calculate pairwise distance between the effect sizes on biomarker levels of the variants as input data. We used "ward" as a cluster method. The *P* values in this method represent the relative frequency of how often the variants are in the same cluster.

Table 1—Source of summary statistic data from GWAS of metabolic traits and their changes in monogenic forms of IR

Trait	Consortia	Maximum *N* (all or cases vs. controls)	Reference	Monogenic obesity*	Monogenic lipodystrophy*	Monogenic receptoropathy*
Nondisease metabolic traits						
SHBG	CHARGE	21,000	(22)	–	–	0/+
HDL-C	GLGC	99,900	(17)	–	–	0
Adiponectin	ADIPOGEN	29,346	(21)	–	–	0/+
BMI	GIANT	123,865	(16)	+	–	0
VATSAT ratio	VATGen	10,557	(18)	+	+	0
CT-measured hepatic steatosis	GOLD	7,176	(19)	+	+	0
ALT	—	55,474	(20)	+	+	0
Triglyceride	GLGC	96,598	(17)	+	+	0
Metabolic disease and disease-related outcomes						
T2D	DIAGRAM	12,171 vs. 56,862	(26)	+	+	+
CAD	CARDIoGRAM	40,365 vs. 63,714	(25)	+	+	0
Systolic blood pressure	ICBP	69,828	(24)	+	+	0
Diastolic blood pressure	ICBP	69,816	(24)	+	+	0
cIMT	CHARGE	31,210	(23)	+	+	0
Carotid plaque	CHARGE	25,179	(23)	+	+	0

VATSAT, visceral-to-subcutaneous adipose tissue. *+ refers to high in condition, – refers to low in condition, and 0 refers to not changed.

Meta-analysis of Fasting Insulin– and BMI-Associated Genetic Variants Against Nondisease Metabolic Traits and Metabolic Disease Outcomes

We calculated genetic risk scores of variants that clustered together by meta-analyzing summary statistics of genotype-phenotype associations across the variants for a given trait. This method has been described and validated previously (30). We repeated this analysis for the 32 variants known to be associated with BMI. We compared per-allele effects of fasting insulin clusters to each other and to per-allele effects of the BMI variants using Z test. We considered a conservative nominal $P \leq 0.001$ as significant, corresponding to a Bonferroni correction of 42 tests (14 traits tested [8 nondisease metabolic traits and 6 metabolic disease outcomes] against 3 clusters).

Sensitivity Analyses

We performed three sets of sensitivity analyses to test whether weighting changes the association of each genetic risk score with nondisease marker and disease outcome traits. We calculated the genetic risk score using summary statistics of genotype-phenotype associations weighted in three different ways: *1*) by each variant's corresponding effect size with the primary trait (fasting insulin) as weight; *2*) by each variant's corresponding effect size with the primary trait (fasting insulin) using the formula used before (21),

$$beta_{riskallelescore} = \frac{\sum_{i=1}^{k} w_i \beta_i {s_i}^{-2}}{\sum_{i=1}^{k} {w_i}^2 {s_i}^{-2}}$$

$$se(beta_{riskallelescore}) = \sqrt{\frac{1}{\sum_{i=1}^{k} {w_i}^2 {s_i}^{-2}}},$$

where β_i refers to the effect of variant$_i$ on the outcome data, s_i is the associated SE estimate, and w_i is the effect of variant$_i$ on fasting insulin; and *3*) by weighting variants and taking into account allele frequencies,

$$w_i = 2 * EAF_i * NEAF_i * {\beta_i}^2$$

where EAF_i is the effect allele frequency of the variant$_i$ and $NEAF_i$ is the frequency of the other allele for variant$_i$.

RESULTS

A Cluster of 11 Fasting Insulin–Associated Genetic Variants Resembles the Metabolic Profile of Monogenic Lipodystrophic IR

Using the 8 nondisease metabolic traits of monogenic IR and all 19 common fasting insulin associated genetic variants (12), we identified 11 genetic variants that together could be distinguished statistically from the remaining 8 variants (*P* of the robustness of the branching event = 96%) (Fig. 1). This cluster of 11 common variants included those in or near the genes *IRS1*, *GRB14*, *ARL15*, *FAM13A*, *LYPLAL1*, *PEPD*, *PDGFC*, *RSPO3*, *PPARG*, *TET2*, and *ANKRD55*. The 11-variant genetic risk score was associated with all 8 nondisease markers of monogenic IR. As expected, given the traits used to define the cluster, fasting insulin–raising alleles were associated with higher triglycerides ($\beta = 0.018$; $P = 4 \times 10^{-29}$), lower HDL-C ($\beta = -0.020$; $P = 7 \times 10^{-37}$), greater hepatic steatosis ($\beta = 0.021$; $P = 3 \times 10^{-4}$) higher ALT ($\beta = 0.002$; $P = 3 \times 10^{-5}$), lower SHBG ($\beta = -0.010$; $P = 9 \times 10^{-13}$), and lower adiponectin ($\beta = -0.015$; $P = 2 \times 10^{-26}$). In contrast to this adverse metabolic profile, the fasting insulin–raising alleles were associated with *lower* BMI (per-allele $\beta = -0.008$; $P = 7 \times 10^{-8}$) (Table 2). The fasting insulin–raising alleles were also associated with increased visceral-to-subcutaneous adipose tissue ratio ($\beta = -0.015$; $P = 6 \times 10^{-7}$) (Table 2). The association with visceral-to-subcutaneous adipose tissue ratio was primarily driven by reduced subcutaneous adipose tissue (-0.014; 2×10^{-6}). As an example, these effects meant that the 5.5% of individuals carrying ≥17 fasting insulin–raising alleles were 0.30 kg/m^2 of BMI slimmer but had triglyceride levels 0.16 SD units higher and HDL levels 0.18 SD units lower, compared with the 5.5% of individuals carrying ≤9 fasting insulin–raising alleles. Note that the units for each biomarker may be different depending on the source of GWAS data (Table 2).

A second cluster of five genetic variants (*P* of the robustness of the branching event = 98%) included those in or near the genes *YSK4*, *UKRF1BP1*, *TCF7L2*, *IGF1*, and *HIP1* (Fig. 1) but was not associated with any of the nondisease markers of monogenic IR (all *P* values > 0.001) except higher BMI ($\beta = 0.012$; $P = 3 \times 10^{-6}$) (Supplementary Table 4) and so was not studied further. Variants in or near *FTO*, *GCKR*, and *PPP1R3B* did not cluster with other variants, most likely because of their primary effects on BMI, triglycerides, and HDL-C, respectively (Fig. 1; Supplementary Table 4). The variant in *FTO* provides a proof-of-principle example of how a BMI-associated variant can be separated from variants associated with fasting insulin through mechanisms other than higher BMI. The *GCKR* variant shows how the use of multiple metabolic phenotypes can be used to separate out different mechanisms—we know this variant is likely to operate through a particular mechanism linking glucose to lipid metabolism (31) in the liver and it was individually associated with multiple liver-based phenotypes (Supplementary Table 4).

A Cluster of 11 Fasting Insulin Associated Variants Resembling Lipodystrophy Is Associated With Metabolic Disease Outcomes

The genetic risk score consisting of the 11 fasting insulin variants was associated with 4 of the 6 metabolic disease outcomes, in directions consistent with the lipodystrophy-like phenotype. The 11-variant genetic risk score was associated with a higher risk of T2D (per-allele odds ratio

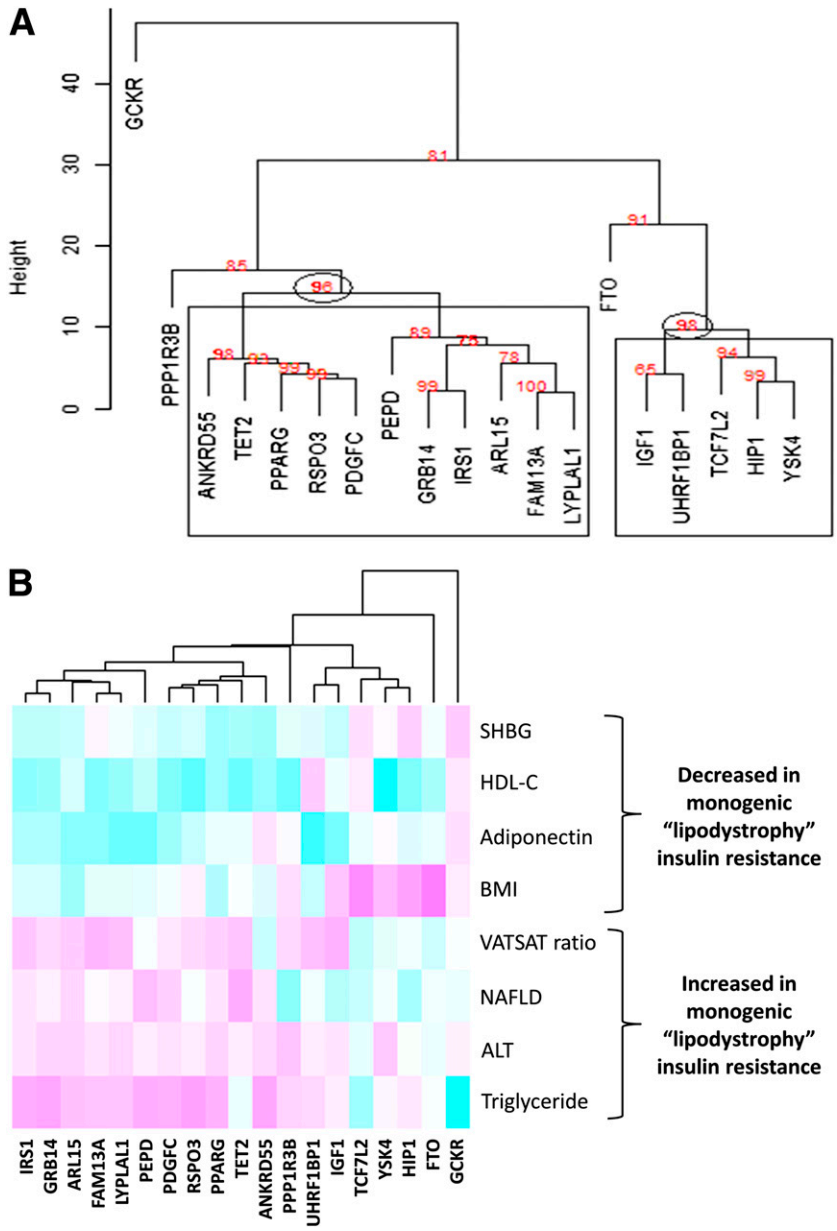

Figure 1—Cluster analysis of fasting insulin variants using eight traits known to be nondisease metabolic traits of monogenic IR, including those representing dyslipidemia (HDL and triglyceride), adiposity (BMI and visceral-to-subcutaneous adipose tissue ratio), fatty liver (CT-measured hepatic steatosis and the liver enzyme ALT), and adiponectin and SHBG levels. The dendrogram (*A*) shows that 11 variants and 5 variants are grouped in two significant clusters (the approximate unbiased values = 96% [$P = 0.04$] and 98% [$P = 0.02$], respectively). The heatmap (*B*) shows this cluster is consistent with monogenic lipodystrophic IR; the stronger the green color, the stronger the effect of the insulin-raising allele with reduced trait levels; the stronger the pink color, the stronger the effect of the insulin-raising allele with higher trait levels. VATSAT, visceral-to-subcutaneous adipose tissue ratio; NAFLD, nonalcoholic fatty liver disease.

[OR] = 1.043; 95% CIs 1.031, 1.055; $P = 5 \times 10^{-13}$) and CAD (per-allele OR = 1.013; 95% CIs 1.007, 1.019; $P = 1 \times 10^{-5}$), and higher systolic (β = 0.135; 95% CIs 0.072, 0.198; $P = 2 \times 10^{-5}$) and diastolic blood pressure (β = 0.075; 95% CIs 0.036, 0.114; $P = 2 \times 10^{-4}$) but was not associated with cIMT or risk of carotid plaques (*P* values > 0.001) (Fig. 2; Table 2). As an example, these effects meant that individuals carrying ≥17 fasting insulin–raising alleles (5.5% of the population) were 0.30 kg/m^2 slimmer but had an estimated OR of T2D and coronary heart disease of 1.46 and 1.12, respectively, and an estimated increase in systolic and diastolic blood pressure of 1.21 and 0.67 mmHg, respectively, compared with individuals carrying ≤9 fasting insulin–raising alleles (5.5% of the population) (Supplementary Fig. 1*A* and *C*). Sensitivity analyses using three different methods to weight individual variants provided very similar results (Supplementary Table 5), and exclusion of the previously

Table 2—Results of genotype risk score analysis of the 11 "lipodystrophy-like" variants

Category/trait	Unit	Per-allele β	95% CI	*P*	*N*
Nondisease metabolic traits of monogenic IR					
SHBG (BMI adjusted)	Natural log	−0.010	−0.012, −0.008	**9×10^{-13}**	21,000
HDL-C	SD	−0.020	−0.024, −0.016	**7×10^{-37}**	99,900
Adiponectin (BMI adjusted)	log	−0.015	−0.017, −0.013	**2×10^{-26}**	29,346
BMI	SD	−0.008	−0.012, −0.004	**7×10^{-8}**	123,865
VATSAT ratio	z-score	0.015	0.009, 0.021	**6×10^{-7}**	10,557
CT-measured hepatic steatosis	SD	0.021	0.009, 0.033	**3×10^{-4}**	7,176
ALT	$\log_{10}$	0.002	0.001, 0.003	**3×10^{-5}**	55,474
Triglyceride	SD	0.018	0.014, 0.022	**4×10^{-29}**	96,598
Metabolic disease and disease-related outcomes					
T2D	OR	1.043	1.031, 1.055	**5×10^{-13}**	12,171 vs. 56,862
CAD	OR	1.013	1.007, 1.019	**1×10^{-5}**	40,365 vs. 63,714
Systolic blood pressure (BMI adjusted)	mmHg	0.135	0.072, 0.198	**2×10^{-5}**	69,828
Diastolic blood pressure (BMI adjusted)	mmHg	0.075	0.036, 0.114	**2×10^{-4}**	69,816
cIMT	log	0.000	−0.002, 0.002	0.70	31,210
Carotid plaques	OR	1.005	0.991, 1.019	0.50	25,179

The 8 nondisease metabolic traits were used to select the 11 variants, so associations are not independent of the clustering process. The metabolic disease outcomes were not used in the clustering process and so represent independent tests. VATSAT, visceral-to-subcutaneous adipose tissue. *P* values ≤ 0.001 are in bold.

reported variant near *IRS1* did not appreciably change the results (Supplementary Table 6).

Comparing the Cluster of 11 Fasting Insulin Variants Resembling Lipodystrophy to the 32 Known BMI Variants

We next compared the per-allele effects of the cluster of fasting insulin–associated variants with the per-allele effects of the known 32 BMI variants. They were both associated with nondisease metabolic traits, with two notable differences. *1*) The cluster of 11 fasting insulin variants was associated with higher visceral-to-subcutaneous adipose tissue ratio, whereas the 32-variant BMI group was associated with lower visceral-to-subcutaneous adipose tissue ratio (Supplementary Table 4). *2*) The 32-variant BMI cluster was not associated with CT-measured hepatic steatosis (Supplementary Table 4). Compared with the 32-BMI-variant risk score, the 11 variant fasting insulin cluster was more strongly associated with risk of T2D (BMI per-allele OR = 1.021 [1.016–1.028], $P = 1 \times 10^{-12}$; the 11-variant cluster per-allele OR = 1.043 [1.031–1.055], $P = 5 \times 10^{-13}$; *P* of difference = 0.002) but did not have detectably different per-allele associations with CAD (Supplementary Fig. 1*B* and *D*; Supplementary Table 4). As with the fasting insulin cluster, the 32-BMI-variant score was not associated with cIMT or carotid plaque.

DISCUSSION

We have shown that common alleles associated with IR (as measured by fasting insulin) are associated with metabolic outcomes of monogenic forms of IR. Most notably, a cluster of 11 common genetic variants show a pattern of metabolic trait associations consistent with a common, subtle "lipodystrophy-like" phenotype, conferring an increased risk of hypertension, T2D, and CAD despite a lower BMI. Our findings are consistent with "adipose expandability" hypotheses (32), which propose that the expandability of adipose tissue is limited and that it is the exceeding of this intrinsic storage capacity, which is partly genetically determined, which contributes to common forms of IR. More broadly, our results suggest that there is a partly genetic basis to the widely reported "metabolically obese, normal-weight" phenotype (1,2).

Our study has a number of implications. First, our results provide evidence that the 11 variants associated with a lipodystrophy-like metabolic phenotype exert their effect through a primary role in adipose tissue. Consistent with this notion, the genetic loci involved include *PPARG*, which is the most important transcription factor in the control of adipogenesis (33,34) and which harbors dominant negative mutations in a Mendelian subtype of partial lipodystrophy featuring reduced body fat and severely dyslipidemic IR (35). Thus, although our findings do not prove causality in the observed associations, they indicate that future in vitro and in vivo functional studies could start with adipose tissue–based hypotheses and the genes located near the 11 variants highlighted by our cluster analysis.

Second, we have shown that using phenotypic features of monogenic diseases may be an informative method to help understand likely mechanisms of more common, less penetrant alleles. We show that common fasting insulin–associated variants identified by GWAS may be clustered into discrete subgroups using a panel of nondisease markers that discriminate different forms of monogenic severe IR (9). These markers included circulating factors such as lipids, adiponectin, and SHBG as well as adiposity. The approach is analogous to that recently used to cluster T2D variants into different groups according to subclinical phenotypes (28).

Third, our results raise the possibility that individuals carrying large numbers of fasting insulin–raising alleles at these 11 loci represent human "in vivo" models of adipose tissue dysfunction. Although individually the common alleles

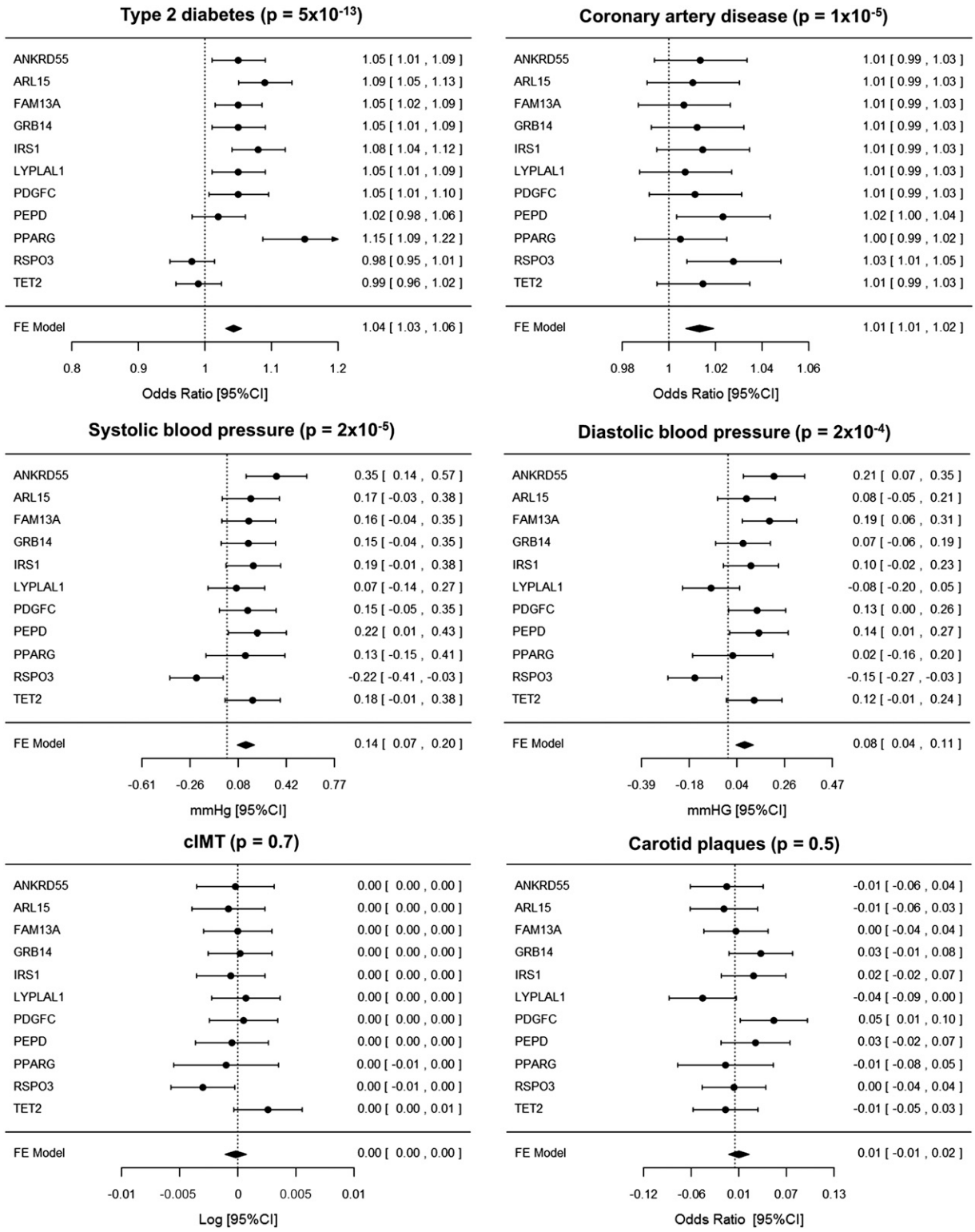

Figure 2—Forest plots of the effect of the 11 "lipodystrophy-like" variants on six metabolic disease outcomes. The *x*-axis is the effect size per fasting insulin–increasing alleles on each trait. The dashed line is the null effect. For cIMT, actual effects ranged from −0.003 to 0.003 but here are shown rounded to two decimal places. FE, fixed effect.

have very subtle effects, ~5.5% of people in the general population will have an ~1.46 increased odds of T2D and 1.12 increased odds of CAD compared with people from the other end of the distribution of these alleles, but these people will have lower BMIs. More in depth studies of the fat distribution and adipose tissue morphology and function

of these individuals may lead to further understanding of the BMI-independent mechanisms of metabolic disease.

Finally, if more lipodystrophy-like variants are identified through ongoing genetic studies, testing such variants in combination may have clinical utility in identifying individuals at high genetic risk of "adipose failure." These individuals have previously been termed the metabolically obese, normal weight (1,2). In such people, it may be appropriate to use lower BMI-based thresholds for access to adipose offloading therapies, including oral weight loss medications and bariatric surgery, as they have the potential to develop metabolic disease at a lower BMI.

Our study has some notable strengths and some limitations. The main strength is that we used a genetic approach to dissect the associations between multiple metabolic traits. While we cannot be certain of the primary trait involved, genetic variants are far less susceptible to confounding and bias than most nongenetic measures, and so our results provide strong support for a causal link between the traits tested. The second main strength is that we used a wider panel of phenotypic features than IR alone as a basis for subgrouping fasting insulin–associated variants while, critically, constraining this panel to those markers that have shown utility in discriminating different forms of monogenic IR.

There are some important limitations to our results. Firstly and most importantly, the associations we observed, while statistically very robust, are very subtle in terms of effects on metabolic traits. More than 50,000 individuals were required to identify the variants associated with fasting insulin, and so they explain only a small proportion of the variation in IR and the other metabolic traits we have examined. Hence our results do not provide evidence that the "polygenic lipodystrophy-like" phenotype is the primary mechanism of IR and its link to metabolic disease but instead establish the principle that reduced adipose expandability is one mechanism that links these traits in the general population. Further genetic discoveries will help us understand the relative role of this mechanism. A second limitation was that our hierarchical clustering approach was based on eight nondisease markers that are correlated with each other, and many of the same studies and individuals contributed to multiple phenotypes. Further studies with individual level data will be needed to able to account for these correlations. However, using two correlated, but not perfectly correlated measures, such as adiponectin and SHBG, will increase the resolution of the clustering approach, as recently demonstrated in a similar approach to cluster T2D variants by correlated glycemic traits. A third limitation is that the fasting insulin–associated variants were ascertained from truncated distributions of glycemic traits because the MAGIC study excluded individuals with fasting glucose above 7.0 mmol/L to limit the possibility of confounding effects from diabetes disease processes and treatments. This ascertainment issue may explain why the diabetes risk allele at *TCF7L2* and possibly other alleles may be associated with apparently greater insulin sensitivity—carriers of T2D risk alleles will need to be subtly more insulin sensitive to remain nondiabetic, for example. It is therefore reassuring that *TCF7L2* does not cluster with the 11 "lipodystrophy-like" variants. A fourth main limitation is that some of the phenotypes used were not gold standard measures. We used genetic variants associated with fasting insulin, which is not the best measure of IR, but we note that the genetic risk score we used is strongly associated with more direct measures of IR in the accompanying article (15). We also used cIMT and carotid plaque as surrogates for atherosclerosis, and it is well-known that these traits are suboptimal measures of atherosclerosis (23). The lack of association between the cluster of 11 fasting insulin variants and cIMT or carotid plaque could be due to reduced statistical power due to sample size or the intra-individual variation and measurement error involved in these phenotypes. Alternatively, the lack of association could be because the fasting insulin–associated variants predispose to CAD through pathways other than those captured by cIMT and carotid plaque measures.

In summary, the group of genetic variants associated with a "lipodystrophy-like" phenotype provides evidence that subtle genetically influenced higher visceral-to-subcutaneous adipose tissue ratio, fasting insulin, and dyslipidemia in combination can increase the risk of hypertension, CAD, and T2D in the absence of increased BMI. Our results provide genetic evidence for a link between the three diseases of the "metabolic syndrome." Our results may help elucidate the mechanistic pathways of how common genetic loci are linked to IR and the mechanisms behind how some individuals can remain relatively healthy despite obesity while others are susceptible to heart disease and diabetes despite relative leanness.

Our results highlight the potential role of adipose tissue dysfunction as one of the underlying mechanisms for IR, hypertension, T2D, and CAD.

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the study and wrote the first draft of the manuscript. T.M.F. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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