

Integrative genomic analysis implicates limited peripheral adipose storage capacity in the pathogenesis of human insulin resistance

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Abstract

Insulin resistance is a key mediator of obesity-related cardiometabolic disease, yet the mechanisms underlying this link remain obscure. Using an integrative genomic approach, we identify 53 genomic regions associated with insulin resistance phenotypes (higher fasting insulin adjusted for BMI, lower HDL cholesterol and higher triglycerides) and provide evidence that their link with higher cardiometabolic risk is underpinned by an association with *lower* adipose mass in peripheral compartments. Using these 53 loci, we show a polygenic contribution to familial partial lipodystrophy-type 1, a severe form of insulin resistance, and highlight shared molecular mechanisms between common/mild and rare/severe insulin resistance. Population-level genetic analyses combined with experiments in cellular models implicate CCDC92, DNAH10 and L3MBTL3 as previously unrecognised molecules influencing adipocyte differentiation. Our findings support the notion that limited storage capacity of peripheral adipose tissue is an important aetiological component in insulin-resistant cardiometabolic disease and highlight genes and mechanisms underpinning this link.

1 **Introduction**

2
3 Insulin resistance, usually defined as an impaired ability of insulin to maintain normal
4 glucose metabolism and initially manifested by higher levels of circulating insulin, is
5 positively associated with adiposity and is a key mediator of the link between obesity and its
6 adverse impact on metabolic and cardiovascular disease.¹⁻⁸ Given the current global epidemic
7 of metabolic disease, there is an urgent need for improved understanding of the mechanisms
8 that link over-nutrition to insulin resistance in the general population.⁷⁻¹⁰

9 Among individuals stratified on the basis of overall adiposity, there is considerable
10 variation in the extent of adverse metabolic sequelae,¹¹ demonstrating the importance of other
11 factors in the aetiology of insulin resistance and its complications. Indeed, while insulin
12 resistance often coexists with obesity, severe forms of insulin resistance develop without
13 obesity or in association with generalized or regional lack of adipose tissue, i.e.
14 lipodystrophy.¹² In lipodystrophies,¹³ it has been proposed that the impaired capacity of
15 peripheral adipose tissue to expand under the challenge of a positive energy balance leads to
16 lipid accumulation at ectopic sites (e.g. liver, skeletal muscle, pancreas) and eventually to
17 overt diabetes.^{12,14} The notion of “adipose overflow” or “limited adipose tissue
18 expandability”¹⁵⁻¹⁹ is supported (i) by the metabolic disturbances seen in rare, monogenic
19 lipodystrophies and their dramatic amelioration in response to dietary calorie restriction²⁰ or
20 leptin replacement,^{21,22} and (ii) by a series of elegant rodent studies including those in which
21 adipose tissue capacity was expanded by fat transplantation in lipodystrophic mice²³ or by
22 over-expressing adiponectin,²⁴ or where partially lipodystrophic mice were energetically
23 challenged by rendering them leptin deficient.^{25,26} However, the relevance of this model to
24 the general population remains uncertain.

25 Some initial human genetic insights into more prevalent forms of insulin resistance are
26 available. Genome-wide studies of gold-standard measures of insulin resistance have been

1 limited by sample size,²⁷ but multiple genomic loci have been associated with fasting insulin
2 levels, a widely-measured marker of insulin resistance.^{28,29} A subset of these loci were
3 associated with higher triglycerides and lower high-density lipoprotein (HDL) cholesterol,²⁸
4 which are hallmarks of insulin resistance.^{13,30} These loci were later validated for their
5 association with insulin resistance,³¹ suggesting that the combined association with this triad
6 of phenotypes could help identify specific genetic determinants of insulin resistance.

7 Given the availability of large-scale genome-wide association data on lipid traits and
8 fasting insulin,^{28,29,32} we undertook an integrative genomic approach to characterise genetic
9 and molecular mechanisms underpinning insulin resistance at a given level of adiposity and
10 its role in cardiometabolic disease in the general population.

Results

Associations with insulin resistance phenotypes at 53 independent genomic regions

We combined genome-wide association results for fasting insulin adjusted for body mass index,^{22,23} HDL-cholesterol and triglyceride levels³² from up to 188,577 individuals to identify loci associated with a phenotypic pattern indicative of insulin resistance (**Online Methods, Supplementary Figures 1-2 and Supplementary Table 1**). After aligning the association results of ~2.4 million single nucleotide polymorphisms (SNPs) to the insulin-raising allele, 630 SNPs from 53 1Mb-genomic regions were associated with higher fasting insulin, higher triglycerides and lower HDL-cholesterol ($p < 0.005$ for each phenotype, expected probability of association under null hypothesis $p = 3.1 \times 10^{-08}$; **Online Methods, Supplementary Figures 3-4**). These 53 genomic regions included 10 loci previously implicated in insulin resistance,³¹ and an additional 43 loci (**Supplementary Table 2**). A subset of 25 of the 53 loci had previously been associated with HDL cholesterol or triglyceride levels at genome-wide significance,³² while 28 had not (**Supplementary Table 2**). We first investigated the associations of these loci in a completely independent sample of 6,101 individuals and found that genetic risk scores comprising the 53 lead SNPs were strongly associated with higher fasting insulin, higher triglycerides and lower HDL-cholesterol (**Supplementary Figure 5**). We next asked whether these variants were associated with “gold-standard” measures of insulin sensitivity. Having a greater number of risk-alleles from the 53-SNP, 43-SNP or 28-SNP (excluding loci previously implicated in insulin resistance and lipid traits, respectively) genetic scores was strongly associated with (a) lower insulin sensitivity measured by euglycemic clamp or insulin suppression test in 2,764 individuals²⁷ (p -value for 53-SNP genetic score = 4.3×10^{-06} ; **Table 1**) and (b) lower insulin sensitivity index in 4,769 individuals with a frequently-sampled oral glucose tolerance test³³ (p -value for 53-SNP genetic score = 7.3×10^{-10} ; **Table 1**).

Genetic predisposition to insulin resistance via the 53 loci confers higher risk of cardiometabolic disease but lower levels of peripheral adiposity

We next investigated associations of the 53 genomic regions with a range of continuous metabolic traits and disease outcomes. In 45,836 cases and 230,358 controls, the 53-SNP genetic score was associated with a higher risk of type 2 diabetes (odds ratio [OR] per standard deviation [SD] of the genetic score [i.e. ~4.5 alleles], 1.12; 95% confidence interval [CI], 1.11-1.14; $p=9.2 \times 10^{-61}$; **Table 1**). In studies with available individual-level data, we saw no difference in associations across sex or body mass index (BMI) strata (**Supplementary Table 3**). Genetically-predicted insulin resistance was also associated with a higher risk of coronary heart disease (**Table 1**). The associations with type 2 diabetes (OR, 1.10; $p=9.0 \times 10^{-32}$) and coronary heart disease (OR, 1.04; $p=9.7 \times 10^{-07}$) remained after removing 13 loci that were previously shown to be associated with either of the two diseases at genome-wide significance.^{34,35} Association estimates were also consistent after removing the 25 loci previously associated with HDL cholesterol or triglyceride levels at genome-wide significance (**Table 1**). We also observed an association with coronary heart disease in 5,369 cases of coronary heart disease and 106,969 controls from the UK Biobank study not previously included in genome-wide discovery analyses of insulin or lipid traits (OR, 1.09; $p=5.3 \times 10^{-09}$). Individually, 30 of the 53 lead SNPs were associated with higher type 2 diabetes risk ($p<0.05$; **Supplementary Table 4**), including a novel association at genome-wide significance for rs718314 near *ITPR2* (OR per allele, 1.06; $p=6.8 \times 10^{-09}$). We found an enrichment of loci associated with higher risk of both type 2 diabetes and coronary heart disease, including those encompassing the proximal insulin signalling *INSR*, *IRS1* and *PIK3R1* genes (11/53 loci associated with both diseases at $p<0.05$; two-tailed binomial probability of observing this proportion of loci by chance $p=8.5 \times 10^{-22}$; **Supplementary Table 5**).

While insulin resistance is often considered secondary to higher adiposity, at the 53 loci we observed associations with *lower* body fat percentage, BMI and hip circumference (**Figure 1A, Supplementary Figures 6 and 7**). The larger magnitude of association with measures of body fat rather than with glycaemic phenotypes is consistent with a primary effect of many variants on adipose tissue mass (**Figure 1A, Supplementary Figures 6 and 7**).

By follow-up studies using dual-energy X-ray absorptiometry (DEXA) measures in 12,848 individuals, we found that the most marked association of the genetic score was with lower levels of gynoid and leg fat mass (**Figure 1B**). Individuals in the highest quintile of the 53-SNP genetic score had an average of 712 grams *less* leg fat mass compared to individuals in the bottom quintile (**Figure 1C**), which accounted for the majority of the overall body fat association (**Supplementary Figure 8**). The association with lower levels of leg fat was accompanied by a higher hazard of incident type 2 diabetes (**Figure 1C**). In 9,150 participants from the EPIC-Norfolk cohort who gained weight during a median follow-up of 3.7 years, carrying a greater number of the 53 risk alleles was inversely associated with change in hip circumference, adjusted for the amount of weight gained (i.e. individuals carrying more alleles were less likely to deposit extra mass in their gluteal region for a given increase in body mass; β in cm of hip circumference per SD of genetic score, -0.07; $p=0.027$; **Supplementary Note**). Overall, these association analyses suggest that individuals genetically predisposed to insulin resistance via the 53 loci have a relative inability to expand their peripheral fat compartment when challenged by a positive energy balance and that this incapacity results in higher cardiometabolic disease risk. We also found that the 53-SNP genetic score was associated with higher levels of alanine aminotransferase and gamma-glutamyl transferase (**Supplementary Table 6**), which suggests that the failure to store lipid in gluteofemoral adipose tissue may be accompanied by hepatic lipid deposition.

The 53-SNP genetic score was associated with greater waist circumference (**Figure 1A**), but not with trunk adipose tissue (**Figure 1B**), indicating that the association with body fat distribution and cardiometabolic disease was largely driven by the association with lower levels of peripheral adipose tissue (**Figure 1 and Supplementary Figures 6-8**). Among the 53 lead SNPs, 17 were within 500kb of a waist-to-hip ratio (WHR) associated SNP³⁶ (**Supplementary Figure 6**). Consistent with DEXA analyses, the associations with WHR at this subset of overlapping loci were largely driven by an association with lower hip circumference, rather than higher waist circumference (**Supplementary Figure 6 and Supplementary Table 4**).

Our large-scale meta-analyses allowed the investigation of individual-SNP associations with both adiposity and metabolic risk. At eight of the 53 loci, the lead SNP was associated with *lower* total body fat percentage or hip circumference at genome-wide significance ($p < 5 \times 10^{-8}$), including a novel association of the insulin-raising G allele of rs4976033 near *PIK3R1* with lower body fat percentage ($p = 3.0 \times 10^{-9}$; see **Figure 1D and Supplementary Figure 9**). Seven of the eight adiposity-lowering alleles at these loci were associated with a higher risk of type 2 diabetes ($p < 0.05$; **Figure 1D**).

Role of common variants in the genetic basis of a severe form of lipodystrophy

Given the strong association with insulin resistance but lower levels of peripheral adiposity, we hypothesized that the polygenic predisposition to insulin resistance imparted by the 53 loci might contribute to the pathogenesis of familial partial lipodystrophy-type 1 (FPLD1). When compared to women from the population-based Fenland study, women diagnosed with FPLD1 displayed markedly lower levels of leg fat mass for a given fat mass in the rest of the body (**Figure 2A**). Whilst the name of the condition implies Mendelian inheritance, using exome sequencing in 9 FPLD1 cases we did not identify likely candidate

causal genes (**Online Methods and Supplementary Table 7**). When compared with 5,296 unrelated women from the UKHLS study in a case-control analysis, 37 patients with FPLD1 had a higher burden of the 53 risk alleles (OR per SD of genetic score in logistic regression analyses adjusted for age and the first 10 genetic principal components, 1.70; 95% CI, 1.21-2.39; $p=0.0021$, **Figure 2B**; $p_{\text{permutation}}=0.0020$, see **Online Methods**). Also, the phenotypes observed in FPLD1 patients in comparison to obese women from the Fenland study mirrored the association pattern of the 53-SNP genetic score (**Supplementary Table 8**). FPLD1 women had a more severe leg fat phenotype compared to that expected from the relationship between the 53 SNP score and leg fat mass in the Fenland study (**Figure 2C**), suggesting that additional genetic and environmental factors contribute to determining this extreme phenotype.

Prioritisation of putative effector genes, cell-types and tissues

We prioritised putative effector genes at the 53 loci by integrating data about physical proximity, expression quantitative trait locus (eQTL) mapping, functional annotation and previous knowledge about genes causing monogenic forms of insulin resistance (summarised in **Supplementary Table 2**; see also **Supplementary Tables 9-11** for details). Putative effector genes included five with well-established roles in proximal insulin signalling (**Supplementary Table 2 and Figure 3A**). Other candidates included *LPL*, encoding the key lipolysis regulator lipoprotein lipase (**Supplementary Table 2 and Figure 3B**). The insulin-lowering, minor allele of rs1011685 (near *LPL*) is on the same haplotype ($r^2=1$) as a gain-of-function,³⁷ protein-truncating allele in *LPL* (p.Ser447*; rs328; minor-allele frequency [MAF], 9.9%). The p.Ser447* gain-of-function variant was recently reported to be associated with lower risk of coronary heart disease,³⁸ while an independent loss-of-function missense variant of *LPL*³⁹ (p.Asp36Asn; rs1801177; MAF, 1.9%) was associated with higher risk.³⁸ Here, we

found that the p.Ser447* gain-of-function variant is associated with greater insulin sensitivity, lower fasting glucose, lower levels of liver markers and protection from type 2 diabetes (OR per allele, 0.93; $p=1.6 \times 10^{-05}$; **Figure 3B and Supplementary Figure 10**). Conversely, the p.Asp36Asn loss-of-function variant in *LPL* is associated with a higher risk of type 2 diabetes (OR per allele, 1.11; $p=0.0086$; **Figure 3B and Supplementary Figure 10**). Thus, recent findings of an allelic series of *LPL* variants implicating lipoprotein lipase as a putative therapeutic target in heart disease³⁸ are now complemented by a directionally consistent observation for type 2 diabetes, compatible with a role for impaired lipoprotein lipase-mediated lipolysis in insulin resistance and type 2 diabetes.

Among the 53 loci, three contained genes at which rare mutations have been previously implicated in severe monogenic forms of insulin resistance (i.e. *PPARG*, *PIK3RI*, *INSR*; **Figure 3C**), which is more than what expected by chance given the prevalence of monogenic insulin resistance genes in the genome (observed percentage 0.54% [3 out of a total of 553 genes in the 53 loci], expected percentage 0.064%, two-tailed binomial $p=0.0056$). The *PIK3RI* gene encodes regulatory subunits of a critical kinase involved in proximal insulin signalling and rare, loss-of-function mutations in this gene are associated with SHORT syndrome, a dysmorphic condition characterised by short stature, partial lipodystrophy and insulin resistance.⁴⁰⁻⁴³ To date, such mutations have been identified in few families worldwide and data from Exome Aggregation Consortium show this gene to have decreased tolerance of missense variation ($Z=2.42$) and to be particularly intolerant of loss-of-function mutations (pLI=1; Exome Aggregation Consortium, Cambridge, MA, URL: <http://exac.broadinstitute.org> accessed 22nd March 2016). Here, we provide evidence that a common variant near *PIK3RI*, which accounts for almost half of all alleles in the general population (MAF=49%), is associated with subtle effects on insulin resistance, lower body fat percentage, higher risk of type 2 diabetes and coronary heart disease (**Supplementary**

Figure 9). This association pattern partially overlaps with that reported for *PIK3R1* mutations and SHORT syndrome (**Supplementary Table 12**). We also found that the common rs8101064 allele T in *INSR*, encoding the insulin receptor, was associated with insulin resistance and higher risk of type 2 diabetes (OR per allele, 1.08; $p=0.020$), but not with body fat percentage ($p=0.16$), consistent with the fact that patients with heterozygous loss-of-function mutations in the *INSR* are frequently insulin resistant but are not commonly lipodystrophic.¹³

We assessed the overlap of lead SNPs and their proxies ($r^2>0.8$) with functional regulatory annotations across 98 cell types from the NIH Epigenome Roadmap (**Online Methods**) and, consistent with phenotypic associations, identified substantial overlap with adipose tissue active enhancer elements (31 of 53 loci overlapped adipose tissue active enhancer elements; observed percentage=58.4% of loci, expected=30.1%, binomial $p=2.1 \times 10^{-05}$; **Online Methods and Figure 3D**). Furthermore, combined pathway analyses with integration of large-scale gene expression data⁴⁴ implicated adipocytes as likely effector cell-type underlying observed associations (**Figure 3E**). In subcutaneous adipocyte eQTL data from 1,064 individuals of the EUROBATs and GTex projects (**Online Methods and Supplementary Table 10**), we observed evidence of eQTL associations with nearby genes ($p<10^{-06}$) at 21 loci including 14 with supportive evidence of co-localisation of lead phenotypic and eQTL associations (**Online Methods and Supplementary Table 10**).

Experimental validation of putative effector genes in cellular adipogenesis models

We sought to experimentally validate the role of five putative effector genes (*IRS1*, *CCDC92*, *DNAH10*, *L3MBTL3* and *FAM13A*) across four loci which showed associations with (a) expression of nearby transcripts in subcutaneous adipocytes, (b) lower peripheral adiposity and (c) higher metabolic disease risk (**Supplementary Note, Supplementary**

1 **Figure 11, Supplementary Tables 10 and 13).** We used short interfering RNA (siRNA) to
2 reduce mRNA levels for these five genes in OP9-K cells, an adipocyte model which shows
3 rapid differentiation in response to adipogenic stimuli.⁴⁵ Knockdown of the *IRS1*, *CCDC92*,
4 *DNAH10* and *L3MBTL3* genes significantly reduced both mRNA of the target gene (**Figure**
5 **4A** top graph) and lipid accumulation (**Figure 4A** bottom graph and **Figure 4B**). These
6 results were directionally consistent with the association of the insulin-raising alleles at these
7 loci with lower expression of these genes in subcutaneous adipocytes and with lower levels of
8 peripheral fat (**Figure 4C**). Knockdown of *FAM13A* reduced mRNA (**Figure 4A** top graph),
9 but did not significantly affect pre-adipocyte lipid accumulation (**Figure 4A** bottom graph
10 and **Figure 4B**). In contrast to the other four genes, the risk allele at *FAM13A* was associated
11 with higher mRNA expression of this gene in subcutaneous adipocytes (**Figure 4C**).

Discussion

Our data implicate the impaired capacity to adequately expand the peripheral adipose tissue compartments in human insulin resistance and related disease at the population level. These results substantially augment existing evidence^{31,36,46-49} by clarifying the extent and relevance of adipose tissue dysfunction to cardiometabolic disease and by providing novel mechanistic insights into its underpinning biology.

Our results are consistent with the existence of dozens of genomic regions at which common genetic variation affects cardiometabolic disease risk via subtle “lipodystrophy-like” mechanisms. While these common genetic mechanisms have individually small effects, their cumulative effect is large and relevant to a large fraction of the population. For instance, we observed ~700 grams difference in leg fat mass between the top and bottom 20% of the population distribution of risk alleles. We also show a polygenic contribution to an extreme phenotype, referred to as FPLD1 or Köbberling-type lipodystrophy, illustrating the contribution of common alleles to severe forms of insulin resistance. At given loci (e.g. *PIK3R1*), we found that genetic variants at the two extremes of the allele frequency spectrum result in corresponding consequences at the extremes of the phenotypic severity spectrum. These findings strongly concur with the notion that molecular and pathophysiologic mechanisms first described in severe forms of lipodystrophic insulin resistance are relevant to the general population. While a centripetal distribution of body fat is a well-recognised risk factor for metabolic and cardiovascular disease,⁵⁰⁻⁵⁴ there is considerable confusion about the underlying mechanisms and relative importance of lower peripheral fat versus higher central adiposity. Whilst supportive of a role for central fat accumulation, different lines of evidence from this study suggest a role for impaired peripheral fat deposition in insulin-resistant cardiometabolic disease in the general population. These include strong associations with gluteo-femoral adiposity, overlap with regulatory regions in adipose tissue and with genomic

1 loci known to cause lipodystrophies, as well as functional characterisation in adipocytes.
2 These findings provide evidence from large-scale human genetics studies which add to a
3 body of research about the links between subcutaneous and lower-body adipocyte phenotypes
4 and a favourable metabolic profile.^{19,55-57}

5 By combining population-scale association studies with eQTL data from adipose tissue
6 and experimental evidence from murine cellular models, we provide evidence that specific
7 risk loci influence adipose gene expression resulting in impaired adipogenesis, reduced
8 peripheral fat depots and ultimately increased cardiometabolic disease risk. For the
9 *L3MBTL3*, *DNAH10* or *CCDC92* genes, evidence presented in this study provides the first
10 link with impaired adipocyte differentiation capacity. *L3MBTL3* recognises methylated
11 lysine residues on histone tails⁵⁸ and previous genome-wide anthropometric studies have
12 implicated this locus in adult height and length at birth.⁵⁹⁻⁶¹ At the chromosome 12q24 locus,
13 our analyses are consistent with the implication of both *CCDC92* and *DNAH10* in impaired
14 adipogenesis. *CCDC92* is a coiled coil domain protein which interacts with proteins in the
15 centriole/ciliary interface.⁶² *DNAH10* encodes one of the heavy chains of the dynein arms of
16 the motile cilia and it is, therefore, surprising that its product appears to have cell autonomous
17 effects on adipocyte biology. However, an essential splice site mutation in *DNAH10* has
18 previously been reported to co-segregate with abnormal circulating HDL-cholesterol levels in
19 a family,⁶³ while the locus containing both *CCDC92* and *DNAH10* has been associated with
20 circulating levels of large HDL particles,⁶⁴ further supporting an unexpected role for these
21 proteins in metabolism.

22 Our results have preventive and therapeutic implications for cardiovascular and
23 metabolic disease. First, they suggest that attempts to develop pharmacological agents acting
24 on the molecular mechanisms of obesity are likely to reduce cardiometabolic risk if they
25 reduce calorie intake (e.g. by acting on appetite) or reduce ectopic fat deposition in tissues

1 such as the liver, muscle and pancreas, but not if they impair adipogenesis or peripheral fat
2 deposition. Agents that promote adipocyte differentiation and increase peripheral adipose
3 mass via action on the peroxisome proliferator-activated receptor gamma have powerful
4 antidiabetic actions,^{65,66} and in some cases have a beneficial effect on cardiovascular
5 outcomes,^{67,68} although some of these agents have been reported to have an adverse
6 cardiovascular safety profile.⁶⁹ An early but vital challenge in the translation of genetic
7 findings towards therapeutic insight is the ability to identify likely effector transcripts
8 underlying genetic associations. In the current study, we identify putative effectors of genetic
9 associations and the tissues in which they operate. We also demonstrate that these genetic
10 variants often affect the risk of type 2 diabetes and coronary disease in a consistent direction,
11 which suggests that targeting these pathways may satisfy current regulatory requirements that
12 anti-diabetic agents should not be associated with unacceptable cardiovascular risk.⁷⁰ This is
13 particularly true of findings from both gain- and loss-of-function variants in the *LPL* gene and
14 risk of type 2 diabetes, which are directionally consistent with those previously reported for
15 the same mutations and risk of heart disease.³⁸ Notably, the directional concordance for risk
16 of heart disease and type 2 diabetes is in contrast to genetic evidence for other lipid-lowering
17 agents (e.g. cholesterol-lowering variants near the molecular target of statins).⁷¹ In the
18 context of a growing body of evidence linking lipolysis and heart disease risk,^{38,72-75} these
19 data suggest that enhancing lipoprotein lipase activity may also become a viable preventive
20 or therapeutic strategy in type 2 diabetes.

21 In interpreting the results of this study, it is important to note that combining multiple
22 genetic-association analyses to gain insights about a latent unmeasured phenotype (i.e. insulin
23 resistance) is not immediately comparable with a univariate genome-wide association study
24 of a trait (e.g. fasting insulin). However, we validated these genetic variants as being strongly
25 associated with “gold-standard” measures of insulin sensitivity, with multiple insulin-

1 resistance related diseases, including a severe form of insulin-resistant partial lipodystrophy,
2 and showed overlap with monogenic insulin resistance genes. Thus, approaches to leverage
3 additional sources of evidence to prioritise genomic variation (such as multiple phenotypes or
4 putative functional class⁷⁶) represent a powerful use of extant genetic association results to
5 advance understanding of biology previously intractable to conventional strategies.

6 Our results were based on genome-wide analyses of fasting insulin adjusted for BMI,^{28,29}
7 and we did not identify loci with a primary effect on higher adiposity and secondary
8 association with insulin resistance (e.g. *FTO*). Our approach was more likely to identify loci
9 influencing insulin resistance at a given level of adiposity. Prompted by the strong association
10 pattern of the genetic scores and variants, we focused on evaluation of mechanisms linking
11 lower levels of peripheral adiposity with insulin resistance. The importance of adipose
12 function as a prominent driver of common insulin resistance is highlighted by the observation
13 that half of all variants associated with fasting insulin at genome-wide significance (without
14 adjustment for BMI) in a previous study²⁸ were included in our genetic score. However, our
15 results do not preclude the presence nor diminish the importance of other mechanisms
16 underlying insulin resistance.^{5,77} Indeed, the associations we observe of the genetic score with
17 central fat, visceral fat and liver enzymes would be further strengthened after adjustment for
18 overall adiposity. While we conclude that our findings implicate a primary effect on impaired
19 adipose function and a secondary effect on insulin resistance, we cannot entirely exclude the
20 possibility of the reverse, nor that there are pleiotropic contributions to the associations.

21
22

Conclusions

Collectively, our findings support the notion that limited capacity of peripheral adipose tissue to store surplus energy is implicated in human insulin resistance and related cardiometabolic disease in the general population. Furthermore, we highlight putative effector genes, tissues and mechanisms underpinning this link.

Data Access Statement

This research has been conducted using the UK Biobank resource. Data on glycemic traits were contributed by the MAGIC consortium investigators. Associations with type 2 diabetes were obtained from the DIAGRAM (DIAbetes Genetics Replication And Meta-analysis) consortium investigators. Data on coronary heart disease / myocardial infarction have been contributed by the CARDIoGRAMplusC4D consortium investigators. Data on body mass index, waist, hip, waist-to-hip ratio were contributed by the GIANT consortium investigators. Data about triglycerides and high-density lipoprotein cholesterol were contributed by the Global Lipids Genetics Consortium investigators. We are very grateful to the GENESIS consortium for provision of summary statistic results for clamp- or insulin suppression test-based insulin resistance. The authors would like to thank the Exome Aggregation Consortium and the groups that provided exome variant data for comparison. A full list of contributing groups can be found at <http://exac.broadinstitute.org/about>. Raw exome sequence data from FPLD1 individuals and family members is available from the European Genome-Phenome Archive (<https://www.ebi.ac.uk/ega/home>, see full accession codes in Supplementary Table 7). Understanding Society: The UK Household Longitudinal Study is led by the Institute for Social and Economic Research at the University of Essex and funded by the Economic and Social Research Council. The survey was conducted by NatCen and the genome-wide scan data were analysed and deposited by the Wellcome Trust Sanger Institute. Information on how to access the data can be found at <https://www.understandingsociety.ac.uk/>. Genome-wide genotyping data of 5,296 unrelated women from UKHLS is publically available through the European Genome-phenome Archive (Dataset Accession: EGAD00010000891).

Data download:

MAGIC consortium (<http://www.magicinvestigators.org/>)
GLGC consortium (<http://csg.sph.umich.edu/abecasis/public/lipids2013/>)
GIANT (<https://www.broadinstitute.org/collaboration/giant/>)
DIAGRAM consortium (<http://diagram-consortium.org/>)
CARDIoGRAMplusC4D (<http://www.cardiogramplusc4d.org/>)
Exome Aggregation Consortium (<http://exac.broadinstitute.org/about>)
European Genome-Phenome Archive (<https://www.ebi.ac.uk/ega/home>)

Study websites:

Fenland (<http://www.mrc-epid.cam.ac.uk/research/studies/fenland/>)
EPIC-Norfolk (<http://www.srl.cam.ac.uk/epic/>)
EPIC-InterAct (<http://www.inter-act.eu/>)
UK Biobank (<http://www.ukbiobank.ac.uk/>)
Understanding Society: The UK Household Longitudinal Study (<https://www.understandingsociety.ac.uk/>)

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Author Contributions

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The authors report no conflict of interest relative to this study.

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Figure Legends

Figure 1. Combined associations with detailed anthropometry and metabolic disease risk at the 53 genomic loci. *Panel A:* association of the 53-SNP genetic score with anthropometric and glycaemic traits in meta-analyses of genetic association studies. Body mass index (BMI), waist-to-hip ratio (WHR), waist and hip circumference data are from the GIANT consortium and the UK Biobank study. Body fat percentage data are from the UK Biobank, EPIC-Norfolk and Fenland studies. Fasting plasma glucose (FPG), 2 hour glucose and HbA1c data are from the MAGIC consortium. Squares with error bars represent the per-allele beta coefficients in standard deviation units and their 95% confidence intervals. *Panel B:* association of genetic scores with compartmental body masses. Data are from 12,848 participants of the Fenland and EPIC-Norfolk studies who underwent a DEXA scan. Squares with error bars represent the per-allele beta coefficients in standard deviation units and their 95% confidence intervals. *Panel C:* association with lower levels of leg fat mass and higher hazard of incident type 2 diabetes by quintiles of the 53-SNP genetic risk scores. Associations are reported for individuals in the exposed category compared with the bottom quintile (reference category). Associations with leg fat mass are from 9,747 participants of the Fenland study and are reported on the left. Associations with incident type 2 diabetes are from 7,420 incident cases and 9,267 controls of the InterAct study and are reported on the right. Squares represent the beta coefficients in grams of leg fat mass (left plot) or the hazard ratio (HR) for incident type 2 diabetes (right plot) in each category compared with the lowest quintile. Error bars represent the 95% confidence intervals of these estimates. *Panel D:* associations of individual lead SNPs at eight loci with waist, hip circumference (left) and type 2 diabetes (right). Loci were selected on the basis of their genome-wide significant association with hip circumference or body fat percentage (i.e. *PIK3R1*). Waist and hip circumference analyses are from a meta-analysis of the GIANT and UK Biobank studies. Type 2 diabetes analyses are from a meta-analysis of the DIAGRAM, InterAct and UK Biobank studies. Squares with error bars represent the per-allele beta coefficients in standard deviation units of waist and hip circumference (left plot) or the per-allele odds ratio (OR) of type 2 diabetes (right plot). Error bars represent the 95% confidence intervals of these estimates. *Detailed associations at the *PIK3R1* locus, which was primarily associated with lower body fat percentage, are presented in *Supplementary Figure 9*.

Figure 2. Associations at the 53 genomic loci with familial partial lipodystrophy type 1 (FPLD1). *Panel A:* distribution of leg fat mass as a function of the fat mass of the rest of the body (from DEXA) in women of the Fenland study at the extreme quintiles (Q) of the 53-SNP genetic score and in 14 FPLD1 subjects. Q1 represents a low genetic burden, whereas Q5 a high genetic burden. Lines of fit are plotted for each group. *Panel B:* histograms of the distribution of risk alleles in the FPLD1 subjects and in control women from the UKHLS study. *Panel C:* bi-dimensional box plots of the distribution of leg fat mass as a function of the distribution of the number of risk alleles in women of the Fenland study at the extreme quintiles (Q) of the 53-SNP genetic score and in FPLD1 subjects. Q1 represents a low genetic burden, whereas Q5 a high genetic burden. Each rectangle represents a group of individuals. For each dimension, the two sides of the rectangle represent the interquartile range and the central line the median. Data for obese women from Fenland were plotted to show the relationship between genetic risk and levels of leg fat in a group of women with a similar body mass index to that of FPLD1 patients.

Figure 3. Putative effector genes, tissues and cell types. *Panel A:* schematic representation of some established components of the insulin signalling pathway with stars reporting the location in the pathway of putative effector genes, with their respective lead single nucleotide polymorphism listed. *Panel B:* associations of gain- and loss-of-function genetic variants in the *LPL* gene with type 2 diabetes. The reference number in parenthesis refers to the study reporting the association with triglycerides and coronary heart disease (see reference number 38 of this manuscript).³⁸ *Panel C:* summary of evidence about links between genetic variants, lipodystrophy, insulin resistance, and type 2 diabetes at different levels of the population phenotypic distribution. *Rare syndromes caused by autosomal dominant *INSR* mutations are not usually associated with lipodystrophy and the *INSR* rs8101064 polymorphism is not associated with body fat percentage. *Panel D:* overlap of the 53 loci (lead SNPs plus proxy variants in $r^2 > 0.8$) with chromatin state annotations from the NIH Roadmap. *Panel E:* DEPICT's annotation of cell types and tissues on the basis of expression patterns in 37,427 human microarray samples. The y-axis represents the $-\log_{10}(\text{p-value})$ for enrichment of signal in a cell or tissue type attributed by DEPICT. The horizontal broken line represents the multiple-test corrected threshold of statistical significance (Bonferroni $p=0.00072$).

Figure 4. Experimental knockdown of putative effector genes in cellular adipogenesis models and comparisons with phenotypic associations. *Panel A:* results of experimental knockdown in OP9-K cells. Full circles represent the difference of the means from knockout experiments of a given gene compared with control experiments ($n=4-7$). Error bars represent the 95% confidence intervals of the difference of the means. Top

graph: effect on mRNA levels of knockdown experiments of target genes using short interfering RNA (siRNA) in OP9-K cells. The two-tailed t-test p-values for differences in means were: *IRS1*, $p=4.6 \times 10^{-06}$; *CCDC92*, $p=2.7 \times 10^{-09}$; *DNAH10*, $p=2.4 \times 10^{-06}$; *L3MBTL3*, $p=4.6 \times 10^{-06}$; *FAM13A*, $p=2.4 \times 10^{-05}$. Bottom graph: effect on lipid accumulation in siRNA knockdown experiments. The two-tailed t-test p-values for differences in means were: *IRS1*, $p=0.0047$; *CCDC92*, $p=1.2 \times 10^{-05}$; *DNAH10*, $p=0.0027$; *L3MBTL3*, $p=0.00013$; *FAM13A*, $p=0.92$. *Panel B*: illustrative images showing florescence microscopy from lipid accumulation experiments. Red indicates adipored staining of neutral lipid, blue is hoechst staining of nuclei. *Panel C*: Association of the risk (insulin-raising) allele of the lead single nucleotide polymorphism in or near each of the putative effector genes with (a) the expression of the corresponding gene in subcutaneous adipocytes in the EUROBATs project (top graph in the panel); (b) hip circumference in a meta-analysis of GIANT and UK Biobank (mid graph); and (c) type 2 diabetes in a meta-analysis of InterAct, DIAGRAM and UK Biobank (bottom graph). Full circles represent the $-\log_{10}(p\text{-value})$ for the association of the insulin-raising allele multiplied by the direction of the beta coefficient (i.e. a “directional” $-\log_{10}(p)$). For graphic display purposes, the $-\log_{10}(p\text{-value})$ for the association with type 2 diabetes of the rs2943645-T allele near *IRS1* is represented as 10 instead of 16.9.

Tables

Table 1. Association with “gold-standard” insulin resistance measures, type 2 diabetes and coronary heart disease of genetic scores comprising lead polymorphisms at identified loci. Results are displayed for genetic scores comprising either (a) the lead SNP at each of the 53 associated loci or (b) the lead SNP at each of the 43 additional loci identified in this study after removing 10 previously implicated in insulin resistance or (c) the lead SNP at each of the 28 loci not previously associated with levels of HDL cholesterol or triglycerides.

Exposure	Outcome	Sample size, N or N cases and N controls	Beta or odds ratio	SE or 95% CI	p-value
Association with “gold-standard” measures of insulin sensitivity					
53-SNP score	Insulin sensitivity ^a	2,764	-0.09	0.019	4.3 x 10 ⁻⁰⁶
43-SNP score			-0.08	0.022	4.6 x 10 ⁻⁰⁴
28-SNP score			-0.09	0.022	2.6 x 10 ⁻⁰⁵
53-SNP score	Insulin sensitivity index ^b	4,769	-0.10	0.016	7.3 x 10 ⁻¹⁰
43-SNP score			-0.08	0.018	4.6 x 10 ⁻⁰⁵
28-SNP score			-0.09	0.027	0.0010
Association with disease endpoints					
53-SNP score	Type 2 diabetes	45,836 cases and 230,358 controls	1.12	1.11, 1.14	9.2 x 10 ⁻⁶¹
43-SNP score			1.09	1.08, 1.11	7.6 x 10 ⁻²⁹
28-SNP score			1.11	1.09, 1.13	1.9 x 10 ⁻²⁵
53-SNP score	Coronary heart disease	63,746 cases and 130,681 controls	1.05	1.04, 1.06	1.8 x 10 ⁻¹³
43-SNP score			1.04	1.03, 1.06	5.7 x 10 ⁻⁰⁸
28-SNP score			1.04	1.02, 1.06	1.2 x 10 ⁻⁰⁵

Abbreviations: N, number of participants; SE, standard error; CI, confidence interval; SNP, single nucleotide polymorphism. Beta coefficients are in standardised units per standard deviation of the 53-SNP genetic score (i.e. 4.5 alleles); odds ratios are per standard deviation of the 53-SNP genetic score (i.e. 4.5 alleles). The association with insulin sensitivity is from 2,764 participants of the GENESIS consortium²⁷ and the association with the insulin sensitivity index is from 4,769 participants of the MAGIC consortium who underwent a frequently sampled oral glucose tolerance test (OGTT)³³; the association with type 2 diabetes is from the InterAct, DIAGRAM and UK Biobank studies; the association with coronary heart disease is from the CARDIoGRAM and the C4D consortia.

a In MAGIC, insulin sensitivity index (ISI) = 10,000/√ (fasting plasma glucose (mg/dl) × fasting insulin × mean glucose during OGTT (mg/dl) × mean insulin during OGTT).³³

b In GENESIS, insulin sensitivity was measured by clamp or insulin suppression test using study-specific parameters (e.g. glucose disposal or M-value) which were then standardised before meta-analysis.²⁷

Online Methods

Study design

We integrated the results of genome-wide analyses on insulin and lipid phenotypes with the aim of identifying genetic variants associated with an insulin resistance phenotypic pattern (**Supplementary Figures 1-4**). We then investigated the mechanistic links of genetic variation at 53 identified genomic regions with cardiometabolic diseases by integrating analyses of: (a) cardiometabolic traits and outcomes from up to 451,193 individuals; (b) detailed continuous metabolic traits from 12,848 deeply-phenotyped individuals; (c) genetic and clinical features from 37 individuals diagnosed with familial partial lipodystrophy type 1; (d) gene expression from over 100 separate eQTL datasets and (e) siRNA mediated knockdown of putative effector genes in experimental adipogenesis models (**Supplementary Table 1 and Supplementary Figures 1-2**).

Participating studies

Lists of phenotypes, participating studies and sample sizes for each analysis are in **Supplementary Table 1 and Supplementary Figures 1-2**. Details about participants and cohorts with individual-level genotype data are in **Supplementary Table 14**. Ethical approvals were obtained at each study site and informed consent was obtained from all participants.

The Fenland study is a population-based cohort study of 12,435 participants without diabetes born between 1950 and 1975. Participants were recruited from general practice surgeries in Cambridge, Ely and Wisbech (United Kingdom) and underwent detailed metabolic phenotyping and genome-wide genotyping.

EPIC-Norfolk is a prospective cohort study of 25,639 individuals aged between 40 and 79 and living in the Norfolk county in the United Kingdom⁷⁸ at recruitment. EPIC-Norfolk is

1 a constituent cohort of the European Prospective Investigation of Cancer (EPIC).⁷⁹ A total of
2 3,101 participants with available dual energy X-ray absorptiometry (DEXA) were included in
3 analyses of detailed anthropometry, while 9,150 participants were included in analyses of
4 change in hip or waist circumference in individuals who gained weight during follow-up.

5 EPIC-InterAct is a case-cohort study nested within the EPIC study, a cohort study of
6 519,978 European participants.⁸⁰ During an average of 8 years of follow-up, 12,403
7 individuals who were free of diabetes at baseline were identified incident type 2 diabetes
8 cases.⁸⁰ InterAct has also defined a randomly-selected subcohort of 16,154 individuals free of
9 diabetes at baseline.⁸⁰ Data on 15,357 individuals with available genotyping and not
10 overlapping with DIAGRAM³⁴ were included.

11 UK Biobank is a population-based cohort study of ~500,000 people aged between 40-69
12 years who were recruited in 2006-2010 from several centres across the United Kingdom.⁸¹
13 Associations with prevalent type 2 diabetes were estimated in 111,016 individuals (4,586
14 cases and 106,430 controls) of the initial UK Biobank dataset. We also used the UK Biobank
15 data for anthropometry analyses and for a sensitivity analysis of prevalent coronary heart
16 disease (i.e. self-reported myocardial infarction or angina) in 5,369 cases and 106,969
17 controls.

18 The United Kingdom Household Longitudinal Study (UKHLS; also known as
19 Understanding Society) is a longitudinal panel survey of 40,000 households representative of
20 the population of the United Kingdom. Participants were surveyed annually since 2009 and
21 contributed information relating to their socioeconomic circumstances, attitudes, and
22 behaviours via a computer assisted interview. For a subset of individuals who took part in a
23 nurse health assessment, blood samples were taken and genomic DNA analysed.

1 In addition to individual-level genotyping data, we used genome-wide meta-analyses
2 results on a variety of cardiometabolic traits and disease endpoints (**Supplementary Table 1**
3 **and Supplementary Figures 1-2**).

4 5 *Detailed anthropometric analyses*

6 In the Fenland and EPIC-Norfolk studies, body composition was determined by dual-
7 energy X-ray absorptiometry (DEXA) using a Lunar Prodigy advanced fan beam scanner
8 (GE Healthcare, Bedford, UK) using the encore software version 14.10.022 (GE Healthcare,
9 Bedford UK). Participants were scanned by trained operators using standard imaging and
10 positioning protocols. The coefficient of variation for scanning precision, calculated from 30
11 consecutive scans, was 2% for total fat mass. The enCORE software was used to demarcate
12 regional boundaries. All the images were manually processed by one trained researcher, who
13 corrected demarcations according to a standardized procedure. Boundaries of body regions
14 are described in details in the **Supplementary Note**. In the UK Biobank study, body fat
15 percentage was estimated by bio-impedance using the Tanita BC418MA body composition
16 analyser.

17 18 *Association of genetic variants with insulin resistance phenotypes*

19 A dyslipidaemic pattern with higher triglyceride levels and lower HDL cholesterol is
20 considered characteristic of the clinical presentation of insulin resistance¹³ and has been used
21 to specifically identify individuals with insulin resistance.³⁰ In a previous large-scale genome-
22 wide discovery of genetic determinants of fasting insulin levels, among 19 fasting insulin-
23 associated loci we identified ten that were strongly associated with higher triglycerides and
24 lower HDL cholesterol.²⁸ This pattern of association was used to refine loci which were then
25 validated as being associated with “gold-standard” measures of insulin resistance.³¹ Loci

1 associated with insulinaemia but not with lipid traits included *TCF7L2*,²⁸ which is primarily
2 implicated in insulin secretion, rather than resistance.⁸² These findings suggested that the
3 combined association with this triad of phenotypes could help identify specific genetic
4 determinants of insulin resistance.

5 With this background, we systematically triangulated the results of the association of
6 ~2.4M single nucleotide polymorphisms (SNPs) with fasting insulin adjusted for body mass
7 index (FIadjBMI; from up to 108,557 participants of the MAGIC consortium),^{28,29} HDL-
8 cholesterol, and triglycerides (from up to 188,577 participants of the Global Lipids Genetics
9 Consortium)³² using publicly available genome-wide results (**Supplementary Figures 1-4**).
10 For FIadjBMI analyses, we used metabochip association results²⁸ when available. We aligned
11 alleles across the three phenotypes such that the effect allele was the insulin-raising allele.
12 We took forward for further analysis all SNPs associated with higher FIadjBMI, higher
13 triglycerides and lower HDL cholesterol at $p < 0.005$ for each of the three traits. The prior
14 probability for association of a given SNP with the three traits and in the pre-specified
15 directional concordance under the null hypothesis corresponds to $0.005 * 0.0025 * 0.0025 = 3.1$
16 $\times 10^{-08}$. In each 1 Mb locus, we retained the lead SNP for association with fasting insulin for
17 further analysis (**Supplementary Figure 4**).

18 Fasting insulin analyses adjusted for BMI were chosen because we were interested in
19 identifying genetic determinants of insulin resistance for a given level of adiposity. It has
20 been proposed that adjusting genetic association analyses for covariates such as BMI might
21 result in a bias known as “collider bias”.^{83,84} Therefore, we assessed the association of the 53
22 lead SNPs for a bias in the identification of variants primarily associated with BMI and
23 artificially associated with fasting insulin, but found no evidence on such bias
24 (**Supplementary Figure 12**).

1 *Statistical methods*

2 We studied the association of individual SNPs and of genetic scores with continuous
3 metabolic traits and endpoints. Where individual level genotype data was available, the
4 associations of genetic variants or scores (i.e. the sum of effect alleles) with outcomes were
5 estimated using multivariable linear, logistic or Cox regression models. For result-level
6 association data, we used the inverse-variance weighted method developed by Burgess et al.,
7 assigning a weight of 1 to each SNP, to approximate the association of an unweighted genetic
8 risk score.⁸⁵ Statistical analyses were conducted using STATA v14.1 (StataCorp, College
9 Station, Texas 77845 USA), R v3.2.2 (The R Foundation for Statistical Computing), and
10 METAL.²⁹ All p-values presented in the study are two-tailed p-values.

12 *Analyses in a severe form of partial lipodystrophy and insulin resistance*

13 We studied the clinical and genetic characteristics of 37 women with familial partial
14 lipodystrophy type 1 (FPLD1; also called Köbberling-type familial partial lipodystrophy).

15 All cases were referred to the insulin resistance/lipodystrophy specialist centre led by
16 Drs Semple, Savage and O’Rahilly in Cambridge. FPLD1 is currently a clinical diagnosis
17 used to describe predominantly women with selective paucity of limb adipose tissue, central
18 obesity, severe insulin resistance, and a higher risk of type 2 diabetes.^{86,87} As some women
19 with lipodystrophy due to loss-of-function mutations in *PPARG* manifest a similar phenotype
20 (i.e. FPLD3), mutations in this gene were excluded in all FPLD1 cases included in this study.
21 The biochemical and anthropometric phenotype of the 37 FPLD1 patients was compared with
22 that of female participants of the Fenland study. In these analyses we compared the
23 phenotypes of the 37 FPLD1 patients with those of all Fenland study women (**Figure 2**) and
24 with those of obese Fenland study women (**Figure 2 and Supplementary Table 8**), who
25 have a similar BMI to that of FPLD1 women.

To understand the genetic basis of FPLD1, we carried out exome sequencing analyses in 18 individuals from 9 pedigrees with FPLD1 without identifying any clear candidate mutations or genes. Sequencing, variant calling and annotation were performed as described previously (Family 2;⁸⁸ Families 1 and 3–9 as part of the UK10K project).⁸⁹ Calls were annotated with 1000 Genomes allele frequencies and the NCBI dbSNP database build 132 (ftp://ftp.ncbi.nih.gov/snp/organisms/human_9606/). Variants were defined as potentially functional if they were non-synonymous, resulted in loss or gain of a stop codon or a frameshift, or occurred within essential splice sites. Those unlikely to have a functional impact were removed, as were all variants found with a MAF >1% in individuals of European descent from the 1000 Genomes Phase 1v3 (<ftp://ftp.1000genomes.ebi.ac.uk/vol1/ftp/release/20110521/>) or the NHLBI ESP Exomes (URL: <http://evs.gs.washington.edu/EVS/>; January 2012). Further filtering was then implemented to retain only variants in genes seen in multiple patients.

We compared the burden of the 53-SNP genetic score in the 37 FPLD1 patients with that of 5,296 unrelated control women from UKHLS. Genome-wide genotyping of UKHLS women was performed using the Illumina Infinium HumanCoreExome-12 v1.0 BeadChip. Genotype calling was performed using the Illumina GenCall software. Genome-wide genotyping of FPLD1 patients was performed using the Illumina® Infinium CoreExome-24v1.0 chip. Prior to imputation, the following quality control criteria were applied for exclusion of SNPs in PLINK⁹⁰ (version 1.07): (1) minor allele frequency <0.01; (2) Hardy-Weinberg equilibrium $p < 1 \times 10^{-6}$; (3) call rate <99%; (4) differential missingness between cases and controls $p < 1 \times 10^{-6}$; (5) SNPs showing differential genotyping between the CoreExome-24v1.0 and CoreExome-12v1.0 chips. Samples were excluded prior to imputation in PLINK based on the following criteria: (1) call rate <95%; (2) autosomal heterozygosity >3 standard deviations from the mean; (3) pairwise identity by descent was

calculated and one individual was removed for every pair of individuals with a $\pi\text{-hat} > 0.05$, with preference given to retaining female samples; (4) identity assessed by the concordance between the genome-wide and Fluidigm® genotypes at 24 sites (excluding individuals with concordance $< 90\%$); (5) ethnic outliers based on a principal components analysis. Imputation was performed using the 1000 Genomes Phase 3 reference panel using SHAPEIT2⁹¹ (version 2.r644) and IMPUTE2⁹² (version 2.3.1). FPLD1 cases and UKHLS controls were imputed together. In an analysis of the genetic principal components derived from genome-wide genotyping (defined based on the combined sample), the 37 FPLD1 patients clustered with UKHLS control women (**Supplementary Figure 13**). Association analyses were performed in R by logistic regression adjusting for age and the first 10 genetic principal components. We also derived a permutation-based null comparator by performing 100,000 permutations of randomly selecting 53 SNPs ($> 1\text{Mb}$ apart) from genome-wide analyses of FPLD1 status adjusted for age and principal components and performing summary statistic Mendelian randomisation.⁸⁵ Of 100,000 iterations, 201 had a p-value less than our observed association ($p_{\text{permutation}} = 2.01 \times 10^{-03}$).

Prioritisation of putative effector genes

We sought to determine the putative effector genes at the 53 loci. We combined information on (a) physical proximity, (b) eQTL data from over 100 repositories, (c) functional and regulatory annotations and (d) previous knowledge about causal genes for monogenic forms of insulin resistance.

For physical proximity analysis, we reviewed genes within a 1 Mb-window of each lead SNP and generated regional association plots using LocusZoom.⁹³ For eQTL analysis, we analysed both publicly available and unpublished datasets (see below). For functional annotation, we used the gene and the tissue/cell type prioritisation functions of the integrative

software DEPICT,⁴⁴ in order to gain insights about putative effector genes, tissues and cell types. We also looked for nonsynonymous variants in linkage disequilibrium with the lead SNP ($r^2 > 0.8$) in European ancestry populations using Haploreg v3.⁹⁴

We assessed the overlap of identified loci with chromatin state definitions of active enhancers and active promoters for 98 cell types from the NIH Epigenome Roadmap project, including a total of 196 genomic annotations. For each of the 53 lead SNPs, we identified the set of proxy variants in high linkage disequilibrium (CEU $r^2 > 0.8$) using SNAP⁹⁵ and defined a ‘locus’ as a lead SNP plus its proxies. We then calculated the number of loci where at least one variant at the locus overlapped a given annotation. We determined enrichment in the overlap at the 53 loci compared to an expected distribution built from randomly selected matched loci. First, we identified all variants with genome-wide significant association ($p < 5 \times 10^{-8}$) to any trait in Europeans from the GWAS catalog.⁹⁶ We then pruned this set of variants (using a CEU r^2 threshold of 0.1), resulting in a list of independent trait-associated variants. For each of these variants, we constructed a background ‘locus’ as the set of proxy variants in high linkage disequilibrium (CEU $r^2 < 0.8$). For each of the 53 loci, we then selected a locus from the background set matched on total number of proxy variants, total genomic distance covered, and distance of the midpoint to the closest gene transcription start site. We then re-calculated the overlap of each annotation in the set of matched background loci. We obtained the expected overlap for each annotation by averaging over 1 million permuted background locus sets. We then tested for enrichment of each annotation with a binomial test using the observed number of overlapping loci, total number of loci and expected percentage of overlapping loci.

We compiled a list of 13 genes (*PPARG*, *INSR*, *PIK3R1*, *TBC1D4*, *LMNA*, *PLIN1*, *AKT2*, *CIDEA*, *AGPAT2*, *BSCL2*, *CAVI*, *PTRF*, *PCYT1A*) known to cause monogenic forms of insulin resistance from the literature¹³ and used that to look for overlap with genes in

1 identified regions. Two experts in the clinical care of patients with monogenic insulin
2 resistance (Drs Semple and Savage) reviewed the curated list.

4 *Analysis of eQTLs in multiple tissues*

5 Using a curated collection of over 100 separate eQTL datasets, we queried whether the
6 53 lead SNPs or their proxies ($r^2 > 0.8$) were associated with transcript expression in a wide
7 range of tissues. Proxy SNPs in linkage disequilibrium in European ancestry populations
8 were identified using SNAP.⁹⁵ For this study, we considered all associations below a p-value
9 cut-off of 1×10^{-6} . A general overview of a subgroup of >50 eQTL datasets has been
10 published,⁹⁷ with specific citations for the >100 datasets included in the current query
11 provided in the **Supplementary Note**.

13 *Specific analysis of eQTLs in subcutaneous adipose tissue*

14 We analysed in depth the association of lead SNPs with gene expression in subcutaneous
15 adipose tissue using two subcutaneous adipose tissue cis-eQTL datasets. The first dataset was
16 generated by the EUROBATs consortium and consists of samples from well-phenotyped
17 healthy female twins (n=766) with eQTLs derived as described previously.⁹⁸ We also used
18 the subcutaneous adipose tissue data (n=298) generated by the GTEx consortium (version
19 6),⁹⁹ which were obtained from www.gtexportal.org on 20/11/15. GTEx results were limited
20 to GENCODE “protein_coding” and “lincRNA” biotype transcripts, and only variants with a
21 minor allele frequency >0.01 were used. Linkage disequilibrium statistics between the index
22 SNP (lead SNP for fasting insulin at the locus) and the most significant expression SNP for
23 the gene were calculated in PLINK 1.9 using 1000 Genomes phase 1 version 3 European
24 ancestry samples.⁹⁰ We also assessed the regulatory trait concordance (RTC) value for SNPs
25 associated with gene expression in adipose tissue, in order to assess the likelihood of co-

1 localisation of signal with the lead eQTL signal at that region.¹⁰⁰ In brief, if the index variant
2 and the eQTL do tag the same causal variant, it is expected that removing the genetic effect
3 of the index variant will have a significant consequence on the eQTL association. To this end,
4 the RTC method assesses the likelihood of a shared functional effect between a GWAS
5 variant and an eQTL by quantifying the change in the statistical significance of the eQTL
6 after correcting for the genetic effect of the index variant and comparing its correction impact
7 to that of all other SNPs in the interval. We considered an RTC of ≥ 0.8 or high linkage
8 disequilibrium between the lead eQTL SNP and trait-associated SNP ($r^2 > 0.8$) to be
9 supporting evidence of co-localisation.

11 *Functional studies in mouse OP9-K cells*

12 We sought to experimentally validate candidate causal genes at loci associated with
13 lower levels of peripheral adiposity, higher risk of type 2 diabetes and with gene expression
14 in subcutaneous adipose tissue (**Supplementary Note and Supplementary Figure 11**). We
15 studied the effects of gene knockdown using siRNA on adipogenic differentiation in murine
16 OP9-K cell lines. The mouse OP9-K cell line used in this study is a model suitable for mid-
17 throughput screening of genes influencing adipogenesis. OP9-K cells are clonal cells derived
18 from mouse stromal OP9 cells obtained from the bone marrow, that accumulate large lipid-
19 droplets after 72 hours of adipogenic stimulation.⁴⁵ OP9-K cells were grown and
20 differentiated using oleic acid-containing differentiation media as described previously.⁴⁵ For
21 siRNA transfections, 2.5×10^4 cells per well were cultured in the 24-well dish. After 24hrs,
22 cells were transfected with smartpool siRNA (from Dharmacon) against each gene using
23 Optifect reagent as per the manufacturer protocol. On the following day, differentiation of
24 OP9-K cells into adipocytes was initiated by replacing the media with 500 μ L of
25 differentiation media. After 48hrs of differentiation induction, differentiated cells were

1 stained with adipored to assess lipid accumulation using fluorescent spectroscopy. For
2 quantitative-PCR, total RNA was isolated from differentiated OP9-K cells and cDNA was
3 synthesized using TaqMan® Fast Cells-to-CT™ Kit (Applied Biosystems) according to
4 manufacturer's instructions. Quantitative real-time PCR analysis was performed on a TaqMan
5 ABI Prism 7900 Sequence Detector System (Applied Biosystems). Expression results were
6 analysed relative to GAPDH mRNA content in the same sample.

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