Genetic Support for a Causal Role of Insulin Resistance on Circulating Branched-Chain Amino Acids and Inflammation

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OBJECTIVE

Insulin resistance has deleterious effects on cardiometabolic disease. We used Mendelian randomization analyses to clarify the causal relationships of insulin resistance (IR) on circulating blood-based metabolites to shed light on potential mediators of the IR to cardiometabolic disease relationship.

RESEARCH DESIGN AND METHODS

We used 53 single nucleotide polymorphisms associated with IR from a recent genome-wide association study (GWAS) to explore their effects on circulating lipids and metabolites. We used published summary-level data from two GWASs of European individuals; data on the exposure (IR) were obtained from meta-GWASs of 188,577 individuals, and data on the outcomes (58 metabolic measures assessed by nuclear magnetic resonance) were taken from a GWAS of 24,925 individuals.

RESULTS

One-SD genetically elevated IR (equivalent to 55% higher geometric mean of fasting insulin, 0.89 mmol/L higher triglycerides, and 0.46 mmol/L lower HDL cholesterol) was associated with higher concentrations of all branched-chain amino acids (BCAAs)—isoleucine (0.56 SD; 95% CI 0.43, 0.70), leucine (0.42 SD; 95% CI 0.28, 0.55), and valine (0.26 SD; 95% CI 0.12, 0.39)—as well as with higher glycoprotein acetyls (an inflammation marker) (0.47 SD; 95% CI 0.32, 0.62) ($P < 0.0003$ for each). Results were broadly consistent when using multiple sensitivity analyses to account for potential genetic pleiotropy.

CONCLUSIONS

We provide robust evidence that IR causally affects each individual BCAA and inflammation. Taken together with existing studies, this implies that BCAA metabolism lies on a causal pathway from adiposity and IR to type 2 diabetes.

The obesity pandemic is a public health crisis leading to a dramatic surge in the incidence of type 2 diabetes mellitus (T2DM) and related diseases (e.g., cardiovascular diseases) (1). Adiposity, particularly visceral adiposity (2), is associated with insulin resistance (IR) and subsequent T2DM. Recent genetic studies using the Mendelian randomization (MR) approach have shown adiposity traits (such as general adiposity, indexed by BMI, and central adiposity, indexed by waist-to-hip ratio [WHR]) to show causal relationships with blood pressure, lipids, coronary heart disease (CHD), stroke, and diabetes (3–6). Furthermore, such studies have demonstrated that adiposity traits causally influence IR (3,4,6). IR is the clinical state of a reduced sensitivity to insulin, typically manifested as

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elevated levels of fasting insulin and often accompanied by higher levels of circulating triglycerides (TGs) and lower levels of HDL cholesterol (HDL-C) (7).

Exploring the molecular mechanism by which IR leads to T2DM may help to identify biomarkers that could mediate the relationship and provide novel opportunities for disease prevention. Recent studies have suggested that branched-chain amino acids (BCAAs) might play a role in the development of T2DM. Prospective observational studies show that higher levels of circulating BCAAs are positively associated with markers of IR (8) and risk of incident T2DM (9,10). Recent genetic studies have also implicated the metabolism of BCAAs in the development of diabetes (11).

IR is a complex trait that can be assessed by different metrics, including clamp/insulin suppression test (gold standard), insulin sensitivity test (based on oral glucose tolerance test [OGTT]), HOMA-IR, and fasting insulin. The GENESIS Consortium has published a genome-wide association study (GWAS) of insulin sensitivity measured by clamp/insulin suppression test in a modest number of subjects (N = 5,624) (12). However, the statistical power limits the findings of this study. Other metrics that can be more easily measured, such as fasting insulin or HOMA-IR, are often used in large-scale genetic and epidemiological studies. In the GWAS of fasting insulin conducted by Scott et al. (13) (up to 108,557 individuals), they also tested the associations of insulin-associated single nucleotide polymorphisms (SNPs) with lipid traits. They found that majority of the insulin-associated SNPs were associated with HDL-C and/or TGs, and this pattern was not observed for those SNPs associated with fasting glucose or 2-h glucose. Subsequently, a genetic instrument was built for IR that used the 19 SNPs associated with fasting insulin and restricted the instrument to those SNPs that were also associated with TGs and HDL-C (14). This instrument was recently adopted by Mahendran et al. (15), and the results suggest that IR might be causal for circulating concentrations of BCAAs. More recently, Lotta et al. (7) considerably expanded the set of SNPs associated with three components of IR (higher fasting insulin, higher TGs, and lower HDL-C), identifying 53 such SNPs, and found that the SNPs in aggregate also associated with risks of CHD and T2DM.

In this study, we aim to *1*) assess the causal effects of IR on BCAAs using these 53 SNPs recently identified from across the genome that associated with higher fasting insulin, higher TGs, and lower HDL-C (7); *2*) use multiple instruments and multiple sensitivity analysis as a means to detect and correct for potential genetic pleiotropy in order to ensure reliable findings; *3*) expand the outcome measures from BCAAs to a comprehensive panel of amino acids (including alanine, glutamine, tyrosine, and phenylalanine), lipoprotein subclasses, fatty acids, glycolysis-related measures, and one inflammatory marker, which are established or emerging biomarkers for T2DM and cardiovascular diseases; and *4*) provide an overview of the potential causal pathways and mediator roles that IR places in the underlying association of adiposity with T2DM by incorporating our findings into multiple strands of genetic evidence.

RESEARCH DESIGN AND METHODS

We used published summary-level data from two GWASs of European individuals (7,16). Data on the exposure (IR) were obtained from meta-analysis of GWASs (meta-GWASs) of up to 188,577 individuals (7), and data on the outcome (58 circulating metabolic measures) were taken from a GWAS of up to 24,925 individuals (16). Characteristics of these GWASs are reported in Supplementary Tables 1 and 2.

Generation of Genetic Instruments

We used the 53 SNPs associated with an IR phenotype from Lotta et al. (7). In brief, Lotta et al. (7) conducted a meta-GWAS to identify SNPs that associated with an IR phenotype of *1*) higher fasting insulin adjusted for BMI; *2*) higher TGs; and *3*) lower HDL-C at $P < 0.005$ for each trait. The combined association with the triad of phenotypes have been proposed as a means to characterizing the genetic architecture of IR (7). This meta-GWAS identified 53 SNPs, of which a subset of 25 loci had been previously associated with TGs or HDL-C at genome-wide significance, whereas the remaining 28 had not.

We used the 53 SNPs to generate a genetic instrument for IR. To conduct the MR analyses (17), we needed to obtain the association of SNPs with the exposure (IR) and also the associations with outcomes (metabolic measures). Lotta et al. (7) did not provide β or SE for the associations of individual SNPs with the IR phenotype. To generate our own SNP to exposure estimate, we took the absolute value of the standardized β coefficient for each of the 53 SNP associations with the individual components of the composite IR phenotype (i.e., fasting insulin adjusted for BMI, TGs, and HDL-C) and meta-analyzed the estimates together using a fixed-effect inverse-variance weighted (IVW) method (data sources provided in Supplementary Table 3). We used this meta-analyzed value as the SNP-exposure estimate for the summary-level MR analyses. Supplementary Fig. 1 shows the associations of the 53 individual SNPs for our IR trait with the three individual components. Most of the SNPs fell in a straight line (with a slope equal to 1), suggesting a similar contribution of the three traits to the composite IR phenotype with the exception of rs1011685 (near *LPL*), which had a much weaker effect on insulin adjusted for BMI. We therefore conducted sensitivity analyses in which rs1011685 was excluded from the instrument.

Two-Sample MR Analysis

We used data from Kettunen et al. (16) to obtain SNP associations with metabolic measures. Summary data for 58 measures were used in this study, including 14 lipoprotein subclasses, 3 lipoprotein size measures, 9 total lipids, apolipoprotein A-1, apolipoprotein B, 10 fatty acid–related measures, 9 amino acids, 1 inflammation marker (glycoprotein acetyls [GlycA]), and several other measures. These metabolic measures were quantified by a high-throughput nuclear magnetic resonance (NMR) metabolomics platform using primarily fasting serum samples with an ~1:1 male-to-female ratio and age span of 20–60 years (Supplementary Table 2). We used a conventional IVW MR analysis in which the SNP to outcome estimate is regressed on the SNP to exposure, with the *y*-axis intercept forced through the origin. The data used for the MR analyses are presented in Supplementary Tables 3 and 4.

Sensitivity Analyses

As the conventional IVW MR approach can be vulnerable to unbalanced horizontal pleiotropy (18), we conducted MR-Egger, weighted median, and weighted mode-based MR analyses, which allow relaxation of some of the instrumental variable assumptions. The characteristics of these different MR methods are summarized in Supplementary Table 5. Overall, use of several MR methods that each make different assumptions on the amount and

type of genetic confounding is a useful strategy to assess the robustness of findings to potential violations of the instrumental variable assumptions (19).

In addition to the 53-SNP instrument, we *1*) removed the rs1011685 (near *LPL*), which, as described above, did not show consistent associations across individual phenotypes of IR; *2*) used the 28 SNPs reported in Lotta et al. (7) that were not in loci previously associated with TGs or HDL-C at genome-wide significance; and *3*) used 12 SNPs associated with fasting insulin (BMI adjusted) reported by the MAGIC investigators (13). As fasting insulin is another marker of IR, consistent results of the primary analysis and sensitivity analysis (item 3 on list above) would provide further confidence in concluding the causal role of IR on the circulating metabolites. Further, sensitivity analysis (items 2 and 3 on list above) are helpful in assessing the contribution of primarily lipid-associated SNPs on the causal effect estimates. In addition, to quantify whether the genetic instruments for IR associated with BMI, we regressed the associations of SNPs with IR against the associations of SNPs with BMI using summary-level data from the GIANT consortium (20). A final step was to remove SNPs from the 53-SNP instrument that individually associated with BMI at $P < 0.001$ using GIANT summary statistics (20) in order to clarify whether this materially altered the MR effect estimates.

Genetic effect estimates are presented as SD differences in metabolite concentrations per one-SD genetically higher IR. To gain insight into the association of the genetic instrument with its individual components, we quantified the association of a one-SD higher genetically elevated IR on fasting insulin from the MAGIC consortium (13), and the blood lipids HDL-C and TGs from the Global Lipids Genetics Consortium (21). We used a two-sided $P < 0.001$ (0.05/58; multiple testing correction) to denote evidence of an association.

All analyses were conducted in R.

RESULTS

The associations of the 53 SNPs with each of the metabolic measures are shown in Fig. 1. As expected, all of the SNPs associated with higher BMI-adjusted insulin

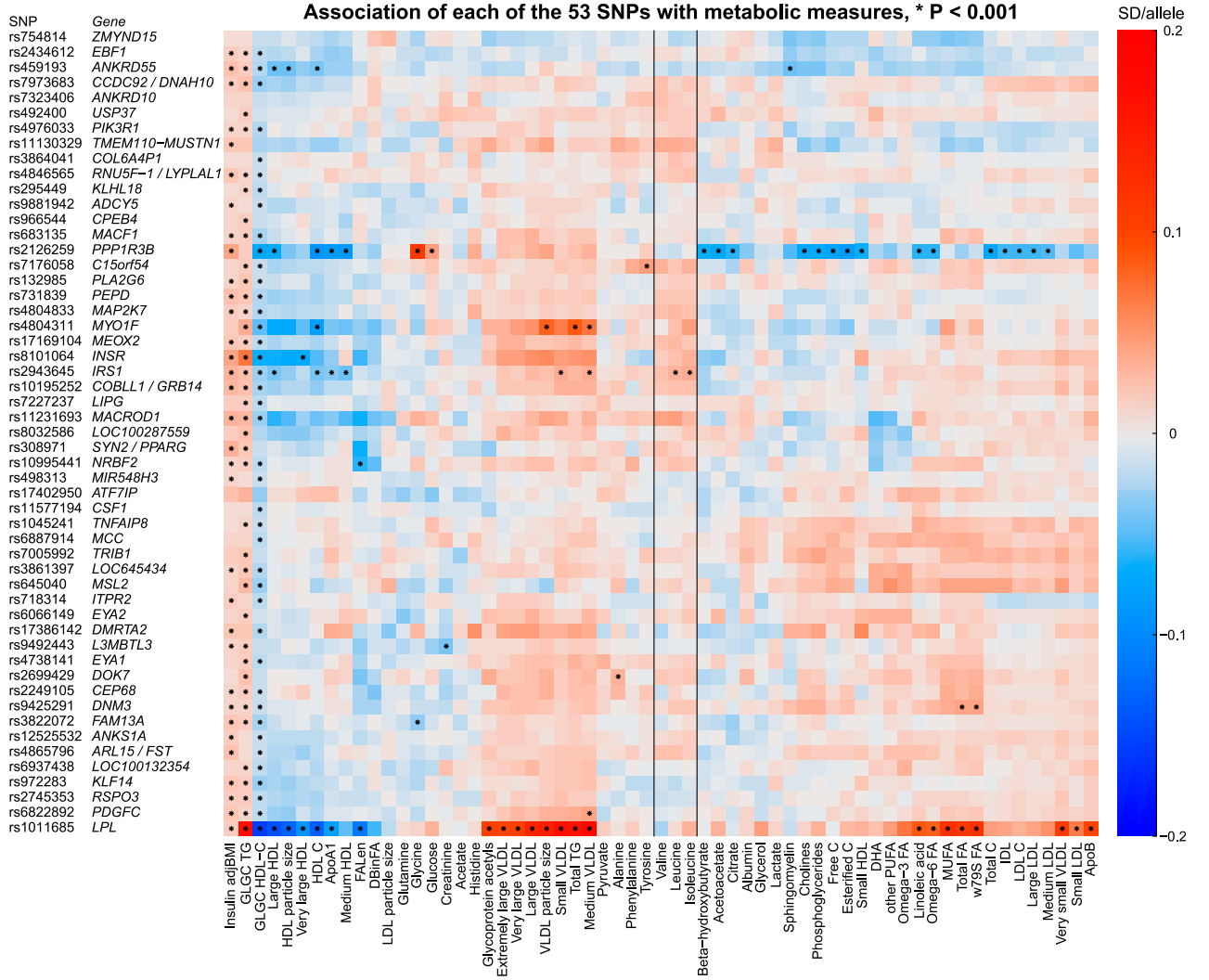

Figure 1—Heat map of the 53 SNPs and their associations with 58 circulating biomarkers. The units are reported as an SD difference in metabolic measure per IR-increasing allele. Lipoprotein measures without further specification refer to total lipid concentrations. IR was defined as a triad of higher fasting insulin (BMI adjusted), higher TGs, and lower HDL-C. Metabolic measures were quantified by the high-throughput NMR metabolomics platform using primarily fasting serum samples. Apo, apolipoprotein; DBinFA, the average number of double bonds in fatty acids; DHA, docosahexaenoic acid; FALen, the average fatty acid chain length; IDL, intermediate-density lipoprotein; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; w79S FA, n-7, n-9, and saturated fatty acids.

and TGs and lower HDL-C. There were also general trends of the SNPs to associate with higher VLDL and lower HDL traits.

Causal effect estimates of IR, proxied by the 53-SNP instrument, on the individual metabolic traits are illustrated in Fig. 2. The association magnitudes (βs), SEs, and corresponding *P* values are reported in Supplementary Table 6. A one-SD genetically higher IR was associated with 55% (95% CI 50, 60) higher fasting insulin adjusted for BMI, 0.89 mmol/L (95% CI 0.85, 0.93) higher TGs, and 0.46 mmol/L (95% CI 0.44, 0.48) lower HDL-C. In addition, there were clear associations of the genetic instrument for IR with higher concentrations of all VLDL subclasses with more moderate associations with intermediate-density lipoprotein and LDL subclasses. In contrast, the associations were inverse for most HDL subclasses. The genetic instrument was positively associated with VLDL and negatively with HDL particle size. These findings corroborate the characteristics of the instrument as devised by Lotta et al. (7). Similarly, we identified positive associations of the genetic instrument with circulating fatty acids, including monounsaturated and n-3 fatty acids. Interestingly, the genetic instrument only weakly associated with an increase in NMR-quantified glucose, a finding in keeping with the observation by Lotta et al. (7) using the MAGIC data. As reported in the original study (7), the genetic instruments were negatively associated with BMI (Supplementary Table 7).

We identified strong positive associations of genetically higher IR with the BCAAs isoleucine, leucine, and valine. These estimates correspond to a 0.56-SD (95% CI 0.43, 0.70) higher isoleucine, 0.42-SD (0.28, 0.55) higher leucine, and 0.26-SD (0.12, 0.39) higher valine per 1-SD higher IR. Weaker associations were noticed with the other amino acids. In addition, genetically higher IR was positively associated with GlycA, an inflammation marker (0.47 SD; 95% CI 0.32, 0.62).

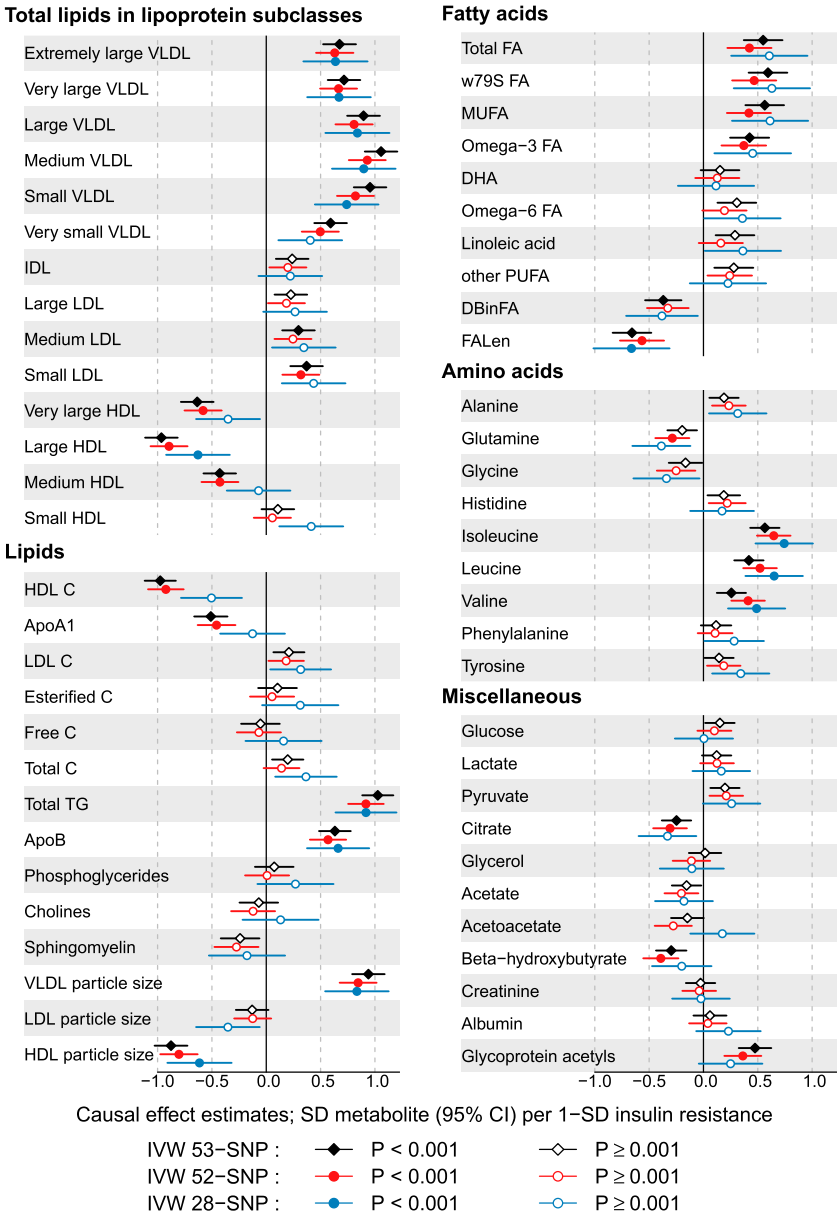

Figure 2—Forest plot of the causal effect estimates of IR on circulating metabolic measures. Estimates are derived from IVW MR analyses. The three instruments are 53 SNPs identified from Lotta et al. (7) (black diamonds), 52 SNPs removing an outlier variant rs1011685 (near *LPL*) (red circles), and 28 SNPs in loci not previously associated with HDL-C and TGs at genome-wide significance (blue circles). Open and closed symbols indicate $P \geq 0.001$ and $P < 0.001$, respectively. Units are given as SD difference in metabolic measures per one-SD genetically higher IR. Apo, apolipoprotein; DBinFA, the average number of double bonds in fatty acid; DHA, docosahexaenoic acid; FALen, the average fatty acid chain length; IDL, intermediate-density lipoprotein; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; w79S FA, n-7, n-9, and saturated fatty acids.

Sensitivity Analyses

Most of the associations identified for the 53-SNP instrument were replicated with the 28-SNP instrument (limited to those SNPs that were not in loci of prior GWAS hits for TGs or HDL-C) (Fig. 2) as well as the 12-SNP instrument (identified in a GWAS of fasting insulin adjusted for BMI) (Supplementary Fig. 2). The associations were also consistent when rs1011685 near *LPL* was removed from the 53-SNP instrument (Fig. 2). Removal of six SNPs associated with BMI ($P <$ 0.001) had no material effect on the MR estimates (data not shown).

To investigate the robustness of these MR estimates to potential confounding by genetic pleiotropy, we also investigated the association of the 53-SNP instrument with the BCAAs and GlycAs using MR-Egger, weighted median, and weighted mode-based estimators. Discordance of the point estimates was noticed across the methods, predominantly because of the inclusion of the rs1011685 variant that had minimal effects on insulin adjusted for BMI (Supplementary Table 8). Because MR approaches can be vulnerable to the inclusion of such outliers, we

repeated the sensitivity analyses excluding rs1011685, which led to estimates across all MR methods that were comparable to the IVW approach (Fig. 3 and Supplementary Table 8).

The intercepts of MR Egger were of generally small magnitude (absolute values ≤0.01, far smaller than the corresponding β coefficients) with little or no evidence that they departed from zero, providing little evidence for the presence of genetic pleiotropy (Supplementary Table 9). Because the MR-Egger estimate of the causal effect (obtained from the slope of the regression line) can be underestimated when the assumption of no measurement error of the exposure is violated, the heterogeneity index (I^2) was used to detect the extent of this potential violation (22). Results remained consistent when simulation extrapolation–adjusted MR-Egger was used to correct potential errors of the SNP to exposure estimates (Supplementary Table 8).

Pathways

Fig. 4 and Supplementary Table 10 illustrate the current evidence base for various pathways leading from adiposity to T2DM. Prior MR studies have shown that general adiposity (measured by BMI) and central adiposity (measured by WHR adjusted for BMI) causally influence fasting insulin, HDL-C, and TGs (4). BMI has been previously shown to influence BCAAs (23), and in this study, we show that both BMI and WHR (adjusted for

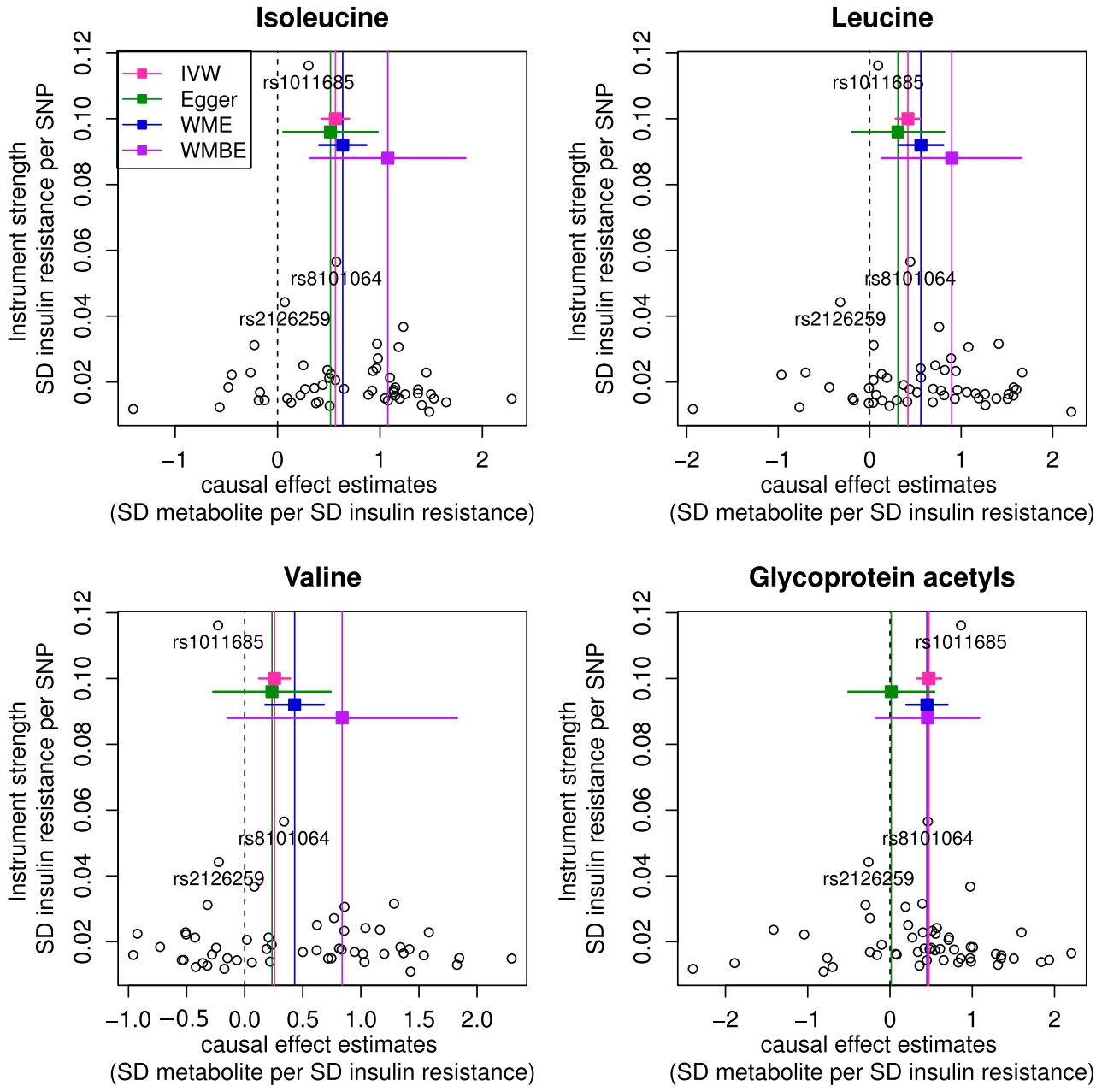

Figure 3—Funnel plots for the three BCAAs and GlycAs showing the causal effect estimates. IVW refers to the conventional IVW method (using 53 SNPs; pink vertical lines), Egger to the MR-Egger (using 52 SNPs; green vertical lines), WME to the weighted median estimator (using 52 SNPs; blue vertical lines), and WMBE to the weighted mode-based estimator (using 52 SNPs; purple vertical lines). For the results shown for MR-Egger, WME, and WMBE, the outlier SNP rs1011685 near *LPL* was removed. The 95% CIs for each method are shown as the corresponding colored horizontal lines. Each individual black circle shows the causal effect estimate using the individual SNP as the instrument.

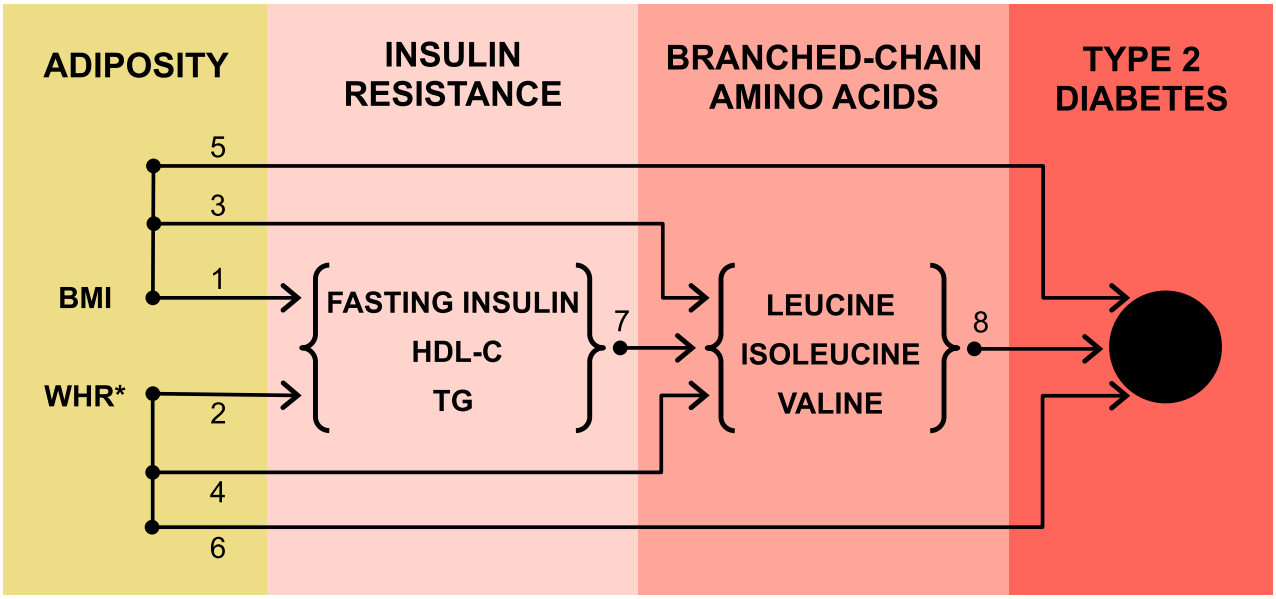

Figure 4—Strands of evidence from multiple genetic studies supporting a causal pathway from adiposity, through IR and BCAAs, to diabetes. Sources of evidence: *1*, Holmes et al. (3) and Dale et al. (4); *2*, Dale et al. (4) and Emdin et al. (6); *3*, Würtz et al. (23) and current study (Supplementary Fig. 3); *4*, current study (Supplementary Fig. 4); *5*, Holmes et al. (3), Dale et al. (4), and Lyall et al. (5); *6*, Emdin et al. (6) and Dale et al. (4); *7*, current study (Fig. 2) and Mahendran et al. (15); and *8*, Lotta et al. (11). For details of these studies and the MR estimates provided, see Supplementary Table 10. *Refers to adjustment with BMI.

BMI) affect these traits (Supplementary Figs. 3 and 4). Both BMI and WHR causally affect diabetes (4). Our study shows that IR affects BCAAs, and, together with a prior study providing genetic support of BCAAs metabolism in T2DM (11), the various sources of data support a causal pathway that is from adiposity to IR to BCAAs to diabetes.

CONCLUSIONS

Our study provides genetic evidence in support of higher levels of IR leading to an elevation in circulating BCAAs. Within the context of other studies, our findings support the hypothesis that the metabolism of BCAAs may be a mediator that is downstream of adiposity and IR on the causal pathway to T2DM. If true, then this not only has important etiological relevance, but also could point toward potential novel opportunities for disease treatment and prevention.

These findings for IR and BCAAs are consistent with a recent paper by Mahendran et al. (15) in which 10 IR-associated SNPs were used to quantify the association with a composite measure of BCAAs in a one-sample MR setting of ~1,300 individuals. However, the selection of SNPs into the 10-SNP instrument may induce bias, as the instrument was enriched for GWAS hits of fasting insulin that were also associated with TGs and HDL-C (18). In this study, a more robust approach was taken to instrument derivation by selecting >50 SNPs across the genome, which have recently been identified using a hypothesis-free approach to show directionally consistent associations with a triad of phenotypes that mark IR; this 53-SNP instrument was used to infer the causality of IR using a two-sample MR design with little overlap between datasets and with data on ~180,000 individuals for the SNP to exposure (IR) estimates and data on ~25,000 individuals for the SNP to outcome (metabolic markers) estimates. The consistent results that we report derived from multiple genetic instruments and multiple MR sensitivity analyses provide robust evidence that IR impacts on BCAAs in a cause-and-effect manner. Particularly, as IR can be measured by various metrics (e.g., a triad of the phenotypes as defined in this study and also by fasting insulin alone), the consistent results of the 53-SNP instrument (a genetic proxy for the IR triad) and 12-SNP instrument (a genetic proxy for fasting insulin alone) across the metabolic profile strengthen the evidence base for a causal role of IR and potentially validates the biological meaning of IR as defined by a complex phenotype characterized by higher insulin, higher TGs, and lower HDL-C.

Interventional studies provide orthogonal support for our findings that obesity and IR causally affect circulating BCAAs. Multiple longitudinal studies have shown that BCAA levels were reduced after various insulin-sensitizing interventions, including weight-loss surgery through gastric bypass, pioglitazone therapy, or physical exercise (24–26). Also, a reduction in BCAA concentrations was observed following the secretion of insulin during OGTT, with individuals with IR showing less BCAA suppression (i.e., higher BCAA concentrations) following OGTT (27). Prospective studies have identified circulating BCAAs to be predictive of incident T2DM, and a recent genetic study found that the metabolism of BCAAs is likely causally linked to T2DM (11). Triangulating these sources of evidence provides support for the hypothesis that circulating BCAAs may mediate the relation from adiposity and IR to T2DM. In contrast, observational studies have reported that higher dietary intake of BCAAs is associated with an improved cardiometabolic risk profile, including a lower risk of T2DM (28,29). However, dietary BCAAs, both measured in absolute terms or as a percentage of total protein, are only weakly correlated with circulating concentrations of BCAAs (28,29). There is also evidence that the expression of enzymes involved in BCAA catabolism (e.g., branched-chain α-ketoacid dehydrogenase [BCKD]) is reduced in obese

individuals and those with diabetes (11,30). BCKD is responsible for the rate-limiting step of BCAA catabolism, and BCKD can be activated by its regulatory phosphatase encoded by *PPM1K*. Individuals with T2DM have reduced upregulation of *PPM1K* in skeletal muscle during OGTT (11). Consistent with this, after weight-loss surgery, BCKD concentrations are increased, leading to a commensurate reduction in BCAAs (24,30). Thus, elevated circulating BCAA levels observed in obese individuals and those with diabetes could arise from impaired BCAA catabolism (11). Putting these strands of evidence together, it is plausible that pharmacotherapies to improve or restore the function of BCAA catabolism may represent a means to prevent T2DM. However, further studies are required to understand the exact role of BCAA metabolism in the etiology of T2DM.

In contrast to the strong effects of IR on the BCAAs, we noticed a generally weaker effect of IR on alanine, glutamine, and aromatic amino acids (phenylalanine and tyrosine). Each of these biomarkers has been associated with the risk of IR, hyperglycemia, T2DM, and cardiovascular diseases (8,10,31). Although imprecise estimates were observed for these measures in the MR analyses reported in this study, the consistent results from different instruments on these traits merit further investigation in larger datasets to clarify whether these represent causal relationships.

The association that we identify of IR with GlycA is novel. GlycA is a marker of both acute-phase and chronic inflammation and has been linked to neutrophil activity (32). GlycA reflects circulating levels of various inflammatory glycoproteins (primarily α-1-acid glycoprotein and haptoglobin) and is also associated with a wide range of inflammatory cytokines (32). Prospective observational studies have identified positive associations of GlycA with cardiovascular disease, T2DM, and premature mortality (33,34). A role for inflammation in the development of T2DM has been proposed for many years on account of the observational associations among higher concentrations of biomarkers of inflammation, such as C-reactive protein, interleukin-1, interleukin-6, and the risk of T2DM (35,36). Although recent MR studies have so far failed to provide evidence in support of this hypothesis (36), it remains plausible that such causal pathways (from inflammation to T2DM) exist and that larger studies and/or investigations of other inflammatory markers and pathways may identify a causal role of inflammation in T2DM. Therefore, GlycA could represent a biomarker either involved in or correlated to an inflammation pathway involved in the etiology of T2DM. A causal role of inflammation in vascular disease is gaining traction given recent findings from genetic studies in humans of the interleukin-6 receptor and CHD (37) and more recently in phase III clinical trials of anti-inflammatory drugs for the treatment of CHD (CANTOS trial of canakinumab, a monoclonal antibody to interleukin-1β) (38). Of note, a previous study has suggested that BMI has a causal impact on circulating concentrations of GlycA (23) (as we also report in Supplementary Fig. 3 for BMI and Fig. 4 for WHR), and this study provides clarification on the potential causal pathway, showing that IR is also causal for GlycA. However, elucidating the causal role of GlycA in cardiometabolic disease remains challenging using an MR approach because at present, the identified genetic variants associated with GlycA are limited in number (16), thus hindering our ability to answer this important question. Larger GWAS of GlycA may facilitate this endeavor.

Strengths of this study include *1*) a comprehensive genetic instrument for an IR phenotype using findings from a recent GWAS (7); *2*) characterizing and validating the genetic instrument for IR with a repertoire of biomarkers of TG and HDL-C metabolism; *3*) use of multiple sensitivity analyses (both in the derivation of the genetic instruments and their application to state-of-the-art MR methodologies) that provided robust and consistent evidence; *4*) quantifying the causal effects of IR on each of the three BCAAs individually; *5*) adding important new information on the effect of IR on an inflammation marker; and *6*) a data summation that provides evidence of a causal pathway from adiposity through IR and BCAA to T2DM.

Limitations include *1*) analyses were conducted at the summary level, and we could not investigate associations by subgroups (e.g., of age or sex), meaning that it is not possible to test whether these associations are modified by age; *2*) our analyses were conducted using European datasets that may hamper their translational relevance to non-Europeans; however, risk factors for disease tend to show similar relationships across geographical regions (39), and emerging studies are providing evidence that shows the genetic architecture for common diseases is likely similar across ethnic groups (40); *3*) a meta-GWAS of three traits was used to proxy IR, which may not include other traits related to IR and may have limited clinical relevance, although in the original paper by Lotta et al. (7), associations were identified for diabetes and heart disease; and *4*) meta-GWAS may select SNPs on the basis of pleiotropy (i.e., by their very selection, they associate with higher fasting insulin, higher TGs, and lower HDL-C), and thus, SNPs may tag heterogeneous pathways, some of which may result in unbalanced horizontal pleiotropy (18). Against this are the consistent associations across the different genetic instruments, their stability to various MR sensitivity analyses (with each MR approach having its own assumptions on the amount and type of genetic pleiotropy) (Supplementary Table 5), and the general consistency with a prior study that used a weaker instrument in a much smaller dataset (15). Finally, the instruments were derived from a meta-GWAS that included fasting insulin adjusted for BMI; conditioning on a trait in discovery GWAS can induce collider bias, as evidenced by the negative association of the instruments with BMI. However, this negative association with BMI would be expected to diminish the association of the genetic instruments with BCAA that we report (and also diminish the association with T2DM and CHD reported by Lotta et al. [7] rather than augment it) and therefore is unlikely to result in major bias in the MR estimates we report. Further, removal of six SNPs that were associated with BMI (at $P < 0.001$ using GIANT summary statistics) had no material impact on the causal estimates derived from MR.

In conclusion, our findings provide new information in support of a causal role of IR on BCAAs and inflammation. Taken together with recent findings from complimentary studies, these data suggest BCAA metabolism may lie on a causal pathway from adiposity and IR to T2DM.

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