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RESEARCH ARTICLE

Zhang et al: Genome-wide pleiotropic analysis

Genomic correlation, shared loci, and causal link between obesity and diabetic microvascular complications: A genome-wide pleiotropic analysis

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ABSTRACT

Observational studies have identified a connection between obesity and microvascular complications in diabetes, yet the genetic contributions to their co-occurrence remain incompletely understood. Our research aims to explore the shared genetic architecture underlying both conditions. We employed Linkage Disequilibrium Score Regression (LDSC) and Local Analysis of [co]Variant Association (LAVA) to assess genetic correlations between obesity and diabetic microvascular complications. To validate shared genetic regions, we conducted pleiotropic analysis under the composite null hypothesis (PLACO), functional mapping and annotation (FUMA), and colocalization analysis. Additionally, we applied Multimarker Analysis of GenoMic Annotation (MAGMA), Summary-based Mendelian Randomization (SMR), multi-trait colocalization, and enrichment analysis to identify potential functional genes and pathways. Finally, Mendelian Randomization (MR) and Latent Causal Variable (LCV) analysis were used to clarify causal and pleiotropic relationships across trait pairs. Among 21 trait pairs analyzed, 15 exhibited significant global genetic correlations, and 97 regions showed significant local correlations. PLACO identified 3,659 to 20,489 potentially pleiotropic single nucleotide polymorphisms (SNPs) across 15 trait pairs, with 828 lead SNPs detected via FUMA. Colocalization analysis confirmed 52 shared loci. Gene-based analysis identified seven unique candidate pleiotropic genes, with ribosomal protein S26 (*RPS26*) emerging as the strongest shared gene. MR and LCV analyses supported a causal relationship between body mass index (BMI) and diabetic kidney disease (DKD). Our findings highlight a shared genetic basis linking obesity with diabetic microvascular complications. These insights offer potential pathways for understanding the mechanisms driving their comorbidity and may inform more integrated therapeutic approaches.

Keywords: Shared genetic architecture; obesity; diabetic microvascular complications; global genetic correlation

INTRODUCTION

Diabetic microvascular complications have been identified as an important cause of death in patients with diabetes [1]. Diabetic kidney disease (DKD), diabetic retinopathy (DR) and diabetic neuropathy (DN) are the hallmark manifestations of microvascular complications in patients with diabetes mellitus, frequently co-occurring within the affected patient cohort [2]. DKD can rapidly progress to end-stage kidney disease, and currently, there is no specific and effective drug treatment to address this issue [3]. In addition, DR can lead to vision loss, significantly affecting the patients' quality of life [4]. DN, which includes both peripheral and autonomic forms, is a prevalent complication in the diabetic population, often manifesting with a 'stocking and glove' distribution of sensory symptoms and potentially affecting vital organs such as the heart, kidneys, and bladder [5]. Obesity emerges as a pivotal determinant in the etiology of diabetes mellitus and a significant exacerbator of its attendant microvascular and macrovascular complications [6]. Weight management stands as a fundamental strategy for mitigating the risk of microvascular complications among individuals with diabetes [7], with the strategic optimization of lipid profiles further augmenting this effect [8,9]. Research has shown that combining lifestyle intervention and early medication treatment can preserve microvascular function to some extent in prediabetic patients [10]. Furthermore, a study has indicated that Roux-en-Y gastric bypass (RYGB) surgery can alleviate proteinuria in patients with type 2 diabetes mellitus (T2DM) and obesity who have early-stage chronic kidney disease [11]. Therefore, the American Diabetes Association (ADA) recognizes that behavioral changes, medication interventions, and surgical options are crucial for achieving weight loss and mitigating the harms caused by obesity in individuals with T2DM [12]. In conclusion, the close relationship between obesity and diabetic microvascular complications has been well established, but the specific mechanisms still require further research and elucidation. Genetic evidence strongly supports weight management as an important means to prevent diabetic microvascular complications, independent of glucose lowering [13]. This provides us with a new perspective to explore the possibility of a shared genetic basis between obesity and diabetic microvascular complications.

Obesity and diabetic microvascular complications are thought to have a significant genetic foundation. Large-scale Genome-Wide Association Studies (GWAS) have uncovered numerous genetic markers associated with both ailments, providing substantiation for this standpoint [14,15]. The domain of research investigating the interplay between genetics and illnesses has made notable strides. Genetic links have been confirmed for various disorders,

like the relationship between Body Mass Index (BMI) and Polycystic Ovary Syndrome [16], as well as the correlation between multiple sclerosis and inflammatory bowel diseases [17]. However, until now, there has been limited exploration into the genetic connection between obesity and diabetic microvascular complications. Despite the intricate, multifactorial nature of these diseases, genetic factors play an essential role in their initiation and progression. In-depth exploration of specific genetic loci associated with genetic correlations is crucial for understanding the genetic basis of diseases and devising more effective prevention and treatment strategies [18].

In this comprehensive genome-wide shared genetic study, we conducted an extensive comparative analysis of seven obesity-related traits (BMI, waist-to-hip ratio [WHR], waist-to-hip ratio adjusted for body mass index [WHRadjBMI], low-density lipoprotein cholesterol [LDL-C], high-density lipoprotein cholesterol [HDL-C], total cholesterol [TC], triglycerides [TG]) and three types of diabetic microvascular complications (DKD, DR and DN). Our aim was to explore potential shared genetic factors between them, using various statistical genetics methodologies. Initially, we investigated the global and local genetic correlations among each pair of traits. Subsequently, a comprehensive array of methodologies, including Pleiotropic analysis under composite null hypothesis (PLACO), Functional Mapping and Annotation (FUMA), colocalization, Multimarker Analysis of GenoMic Annotation (MAGMA), summary data-based Mendelian randomization (SMR) and multi-trait colocalization, was employed to identify pleiotropic variants and genes. Finally, the potential causality or pleiotropy behind these diseases were further explored by utilizing Mendelian randomization (MR) and Latent Causal Variable (LCV). A flowchart of the main analytic steps is provided in Figure 1.

MATERIALS AND METHODS

GWAS Data sets

The obesity-related information primarily originated from two main sources. The first dataset included BMI, WHR, and WHRadjBMI data, which resulted from a comprehensive meta-analysis using information from the UK Biobank and the GIANT consortium [19]. This particular study stands as the most extensive genome-wide association analysis on obesity to date, encompassing approximately 700,000 individuals with European heritage [19]. The second dataset originates from summarized statistics provided by the Global Lipids Genetics Consortium, encompassing 1,654,960 participants from five distinct genetic ancestry

groups[20]. For our research, we specifically employed the GWAS summarized data for the four lipid characteristics (LDL-C, HDL-C, TC, and TG) from a European cohort. The GWAS researchers adjusted for age, age squared, sex, principal components, and study-specific covariates to consider potential influences on the results[20]. The original data for diabetic microvascular complications comes from the Finnish Biobank Alliance (FinnGen) version 9 data. The genetic association cohorts for DKD, DN, and DR consist of 4,111 cases, 2,843 cases, and 10,413 cases respectively, contrasted against 308,539, 271,817, and 308,633 controls, respectively [21]. DKD, DR and DN were identified using ICD-10 codes. All the datasets used in our study have been publicly accessible (Table S1).

STATISTICAL ANALYSIS

Identification of genetic correlations

LDSC is a commonly used method to estimate the genetic correlation between different traits through summary statistics from GWAS. In this study, we utilized LDSC to estimate the genetic correlation between obesity-related traits and microvascular complications of diabetes[22]. We preprocessed the data by performing single nucleotide polymorphisms (SNPs) filtering using HapMap3, which helps ensure the quality and consistency of the SNPs used. The genetic correlation estimates (rg) range from -1 to 1, with the absolute value of rg closer to 1 indicating a strong genetic correlation between the two traits and implying a significant shared genetic basis. Conversely, as rg approaches 0, the genetic correlation becomes weaker. It's generally considered that $rg > 0.1$ is indicative of a meaningful genetic correlation between the two traits. For the LDSC-derived *P*-value, we applied the Bonferroni correction, defining statistical significance as $P < 0.002$ (0.05/21). In the whole-genome assessment, it's possible to overlook local regional correlations between two traits. Therefore, we employed the Local Analysis of [co]Variant Association (LAVA) method to gain deeper insights into the shared genetic factors in specific genomic regions between two diseases [23]. This approach divides the human genome into 2495 independent segments of approximately 1 Mb each, allowing for precise evaluation of the associations between genetic variations within specific regions and the traits. Applying a Bonferroni correction, we considered a correlation significant if the *P*-value was below the threshold of 0.00002 (0.05/2495).

Identification of Pleiotropic regions

PLACO is a novel statistical method used for identifying pleiotropic SNPs between two traits

[24]. Its core concept involves testing SNP against a composite null hypothesis that it's associated with either one or none of the traits. It employs the multiplication of two sets of Z-statistics as input and breaks down the pleiotropic composite null hypothesis into three sub-scenarios, along with an alternative hypothesis representing pleiotropic associations. In contrast to traditional SNP association analyses, PLACO, through the use of composite hypotheses, avoids the false positive results caused by SNP imbalances between two traits. Following PLACO analysis, SNPs ($P < 5\times10^{-8}$) are defined as significant pleiotropic variants, potentially exerting substantial effects across multiple traits. However, PLACO does not directly indicate which specific SNPs contribute most to the observed linkage disequilibrium (LD). To further identify SNPs that may significantly impact specific phenotypes, we utilized FUMA, which integrates information on LD and other genetic data to pinpoint lead SNPs by analyzing the aforementioned results. The analysis was conducted with an LD threshold of $R^2 < 0.1$ within a 1 Mb window [25]. We undertook a comprehensive colocalization analysis using the locus containing aforementioned lead SNPs in order to investigate potential connections between genetic variations and a diverse range of phenotypic characteristics. Colocalization analysis strictly adhere to five mutually exclusive hypotheses, which includes H0: no association with any traits, H1 and H2: a locus being associated with only one trait, H3: association with both traits but at separate causal variants, and H4: association with both traits at a shared causal variant. If the posterior probability of H4 (PPH4) value exceeds 0.95, we can confidently conclude that this locus exhibits shared genetic effects across different traits [26].

Functional annotation and enrichment analysis

MAGMA software serves as a valuable tool for gene-based analysis of GWAS. This approach involves aggregating the collective associations of multiple SNPs within entire gene regions, while accounting for the influence of LD between SNPs，which efficiently mapped the associated SNPs to their corresponding genes, providing a foundation for thorough genome annotation. We adhered to the default settings of the FUMA software and integrated the SNPs *P*-values obtained from previous PLACO analysis, facilitating the execution of the MAGMA analysis [27]. Moreover, we utilized SMR, which integrates genetic variation from GWAS with biological data such as gene expression or abundance of protein, to explore the associations between gene or protein and target traits [28]. In this study, we chose SNPs from 8 different GWAS datasets as instrumental variables, each representing distinct traits like BMI, WHR, WHRadjBMI, TG, HDL-C, DKD, DR and DN. These SNPs from GWAS datasets were then subjected to joint analysis with expression quantitative trait loci (eQTL) in blood, kidney and

pancreas as well as protein quantitative trait loci (pQTL) in blood, aiming to gain a deeper understanding of their roles in different tissues [29,30]. To assess whether there are collinearity form intergenic linkage effects in the observed relationships between QTL and traits, we employed the heterogeneity in dependent instruments（HEIDI）test. When interpreting the results, we introduced a Bonferroni-corrected *P*-value (SMR) threshold by dividing 0.05 by the number of results in each group, and used a HEIDI value greater than 0.05 as the decision criterion. To further enhance the causality of the SMR findings, we employed the HyPrColoc (hypothesis prioritization for multi-trait colocalization) method to explore the potential existence of a shared genetic influence that contributes to these traits simultaneously. This method offers the advantages of hypothesis prioritization and enumerating causal configurations, efficiently managing various traits. It allows for rapid analysis of extensive trait sets while focusing on a limited set of plausible causal configurations and consider results with PPH4 > 0.7 to be extremely stringent [31]. In addition, to gain insights into the potential biological functions of the genes associated with the colocalization results and the intersection with MAGMA-identified genes, we conducted both Gene Ontology (GO) terms and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses using the clusterProfiler and pathview package, with a significance threshold of $P < 0.05$ [32,33].

Assessment causal and pleiotropy relationship

MR and LCV were utilized to investigate the causal and pleiotropic relationships between various shape pairs, providing two different viewpoints. MR used genetic variants as instrumental variables to evaluate the possible causal relationships between environmental factors or behaviors and diseases. The underlying hypothesis posited that a robust association between a genetic variant and a particular exposure, coupled with the exposure's correlation to disease risk, suggested a probable causal influence of the variant on the propensity for disease manifestation[34,35]. The Cochran's Q test was used to assess the heterogeneity of individual causal effects and MR-Egger's intercept was also utilized to evaluate horizontal pleiotropy. LCV provided a more refined framework for dissecting the causal structure behind genetic correlations by introducing a latent causal variable. If a trait exhibited a strong genetic correlation with this latent causal variable, it was considered to have a partial genetic causal effect on another trait at the genetic level. Furthermore, LCV, through quantifying the Genetic Causality Proportion (GCP) metric, evaluated the genetic causality between traits, where a GCP nearing 1 signified a predominant genetic causal influence and approaching 0 indicated a diminished causal relationship due to the significant role of pleiotropy, with the GCP's sign concurrently indicating the directionality of the causal effect. A GCP value surpassing the threshold of 0.7 was often interpreted as denoting a substantive genetic causal effect, with the majority of genetic correlation likely being propelled by causal mechanisms[35]. Both MR and LCV methodologies worked together to differentiate between pleiotropy and true causal relationships, offering a more comprehensive understanding of the genetic connections underlying complex diseases. To avoid the false positive caused by multiple testing, the Bonferroni correction test has been applied.

RESULTS

Overall and local genetic correlation

The overall genetic correlation results indicate that BMI, WHR, WHRadjBMI, TG and HDL-C exhibit moderate positive correlations with both diabetic microvascular complications. Among them, BMI and WHR display the strongest correlation with DKD (r_g = 0.47, *P* = 4.725e-26; r_g = 0.47, *P* = 4.40e-22). Additionally, TG demonstrates a negative genetic correlation with both DKD, DR and DN (r_g = -0.42, *P* = 8.062e-16; r_g = -0.31, *P* = 2.181e-14; r_g = -0.27, *P* = 8.41e-09). However, TC demonstrates nearly negligible correlation with DKD, DR and DN (r_g

= -0.01, $P = 0.704$; $r_g = -0.02$, $P = 0.547$), similar to the results observed for LDL-C ($r_g = -0.05$, $P = 0.301$; $r_g = -0.04$, $P = 0.358$; $r_g = 0.05$, $P = 0.257$). In summary, among the 21 pairs of traits, only 15 pairs exhibited positive results (Table 1, Figure S1 and Table S2). LAVA analysis identified 97 significant local genetic correlations among 21 pairs of traits. Both LDL-C and HDL-C exhibit the highest local genetic correlations with DKD, contributing 10 of these significant correlations. Whereas, the global genetic correlation analysis did not identify a significant relationship between LDL-C and either DR or DKD. In the global genetic correlation analysis, a positive relationship was observed between WHRadjBMI and DKD, While there was no evidence of local genetic correlation between WHRadjBMI and DKD in the results from the LAVA analysis. Surprisingly, despite the less satisfactory overall genetic correlation for TC-DKD/DR/DN, there are a total of 7, 9 and 9 significant local genetic correlations (Figure 2 and Table S3).

Pleiotropic regions validation

Within the scope of these 15 distinct traits, PLACO analysis revealed a spectrum of potential pleiotropic SNPs ranging from 3659 to 20,489, with a total of 37,738 unique SNPs (Figure S2). Subsequent meticulous scrutiny using FUMA identified a subset of 828 independent SNPs, representing instances of pleiotropy. Among these, the HDL-C and DR trait pair exhibited the highest abundance with 103 lead SNPs, while the TG-DN trait pair displayed the lowest count of 25 lead SNPs. Additionally, among these lead SNPs, rs429358 concurrently influenced 11 pairs of traits, while rs7903146 impacted 7 pairs of traits (Table S4). According to ANNOVAR's categorization as a facet of FUMA's capabilities, among the 828 lead SNPs, 41.7% were found to be intronic variants, while 34.6% were intergenic variants. Exonic variants, which included 8 noncoding RNA exonic variants, made up only 5.4% of the total. Furthermore, there were 20 UTR3 variants (2.4%) and 6 UTR5 variants (0.7%) (Table S4). Subsequent colocalization analysis unveiled 52 loci with strong colocalization signals, all surpassing a PPH4 threshold of 0.95. Notably, among these loci, 10 were associated with DKD, 40 with DR, and 2 with DN. Within these loci, rs10938397 emerged as a pivotal candidate locus, linked to DR, and exhibiting concurrent evidence of associations with BMI, HDL-C, and WHR. Moreover, another SNP, rs429358, demonstrated colocalization evidence in both DKD and DR, displaying significant correlations with HDL-C, WHR, and WHRadjBMI traits. Similarly, rs7144011 showed a significant association with DR, and further analysis revealed its associations with a range of obesity-related characteristics, including BMI, HDL-C, TG, and WHR (Table 2, Figure S3 and Table S5).

Shared gene function and enrichment analysis

In this study, we employed various analytical methods to delve into potential shared genetic influences among multiple traits. Firstly, we identified a total of 4164 pleiotropic genes through MAGMA analysis, of which 88 overlapped with genes in the region of the colocalization analysis results (Table S6). Notably, several genes such as APOE, PVRL2, and TOMM40 exhibited significance across all seven pairs of traits, followed by APOC1 being implicated in five pairs of traits. Subsequently, we conducted SMR analyses on GWAS data, eQTL data (including whole blood, kidney, and pancreas), and whole-blood pQTL data, leading to the discovery of 879 shared genes and proteins. In the eQTL analysis, we found 348, 91 and 259 shared genes in the non-MHC regions of whole blood, kidney, and pancreas, respectively. Particularly in kidney tissue, C4A was identified as a shared gene across multiple traits, encompassing WHR and DR/DKD/DN, TG and DR/DKD/DN, as well as BMI and DR/DKD/DN. Additionally, we observed the presence of XXbac-BPG254F23.7 as shared between HDL-C and DR/DKD/DN, and intriguingly, RPS26 emerged as a shared gene not only between TG and DR but also between HDL-C and DR/DKD. Notably, it's important to highlight that previous colocalization analyses focusing on HDL-C and DR had already pinpointed RPS26 as one of the shared genetic variants. Similarly, in pancreas tissue, RPS26 was identified as a shared gene between TG and DR. However, no shared genes were identified in blood tissue (Table S7). Using pQTL data, MANBA (associated with the corresponding protein) was exclusively found in the BMI-DR pair (Table S8). Lastly, in the multi-trait colocalization analysis, we discovered a series of shared genes across different trait pairs, including JAZF1, NCR3LG1, RP1-239B22.5, SUOX, ZBTB20, IKZF4, and NIPSNAP1 (Table S9). However, the most surprising finding was the identification of RPS26 between HDL-C and DR, as well as in eQTL (kidney) analyses. This result was consistently validated across multi-trait colocalization analysis, SMR analysis, and previous colocalization analyses. Moreover, all three analytical methods supported the lead SNP: rs11171739.

In the GO enrichment analysis, we identified 311 enriched biological process (BP) pathways, 33 enriched cellular component (CC) pathways, and 57 enriched molecular function (MF) pathways. Such as the "regulation of insulin secretion" (GO:0050796, P = 1.89e-07) pathway exhibited the most significant enrichment in BP, while "chylomicron" (GO:0042627, P = 3.98e-04) and "MAP kinase kinase activity" (GO:0004708, P = 8.39e-04) showed significant enrichment in CC and MF, respectively (Figure 3A). Additionally, we conducted KEGG pathway enrichment analysis and identified 13 significantly enriched pathways, with "Insulin

secretion" (KEGG: hsa04911, P = 2.41-3e) displaying significant enrichment (Figure 3B).

Causal and pleiotropy inference

To delve into the causal relationships between diabetic microvascular complications (DKD, DR and DN) and obesity-related traits (HDL-C, TG, BMI, WHRadjBMI, and WHR), we employed a bidirectional MR approach with the Inverse Variance Weighted (IVW) method as the primary analytical tool. The results show clear causal relationships in only six pairs: BMI-DKD (P_{IVW} = 5.12e-11, OR = 1.68[1.44, 1.97]), BMI-DR (P_{IVW} = 4.76e-13, OR = 1.44[1.30, 1.58]), WHRadjBMI-DKD (P_{IVW} = 7.43e-06, OR = 1.47[1.24, 1.74]), WHRadjBMI-DR (P_{IVW} = 5.96e-07, OR = 1.32[1.18, 1.47], WHR-DN (P_{IVW} = 1.10e-07, 0R = 1.81[1.45, 2.25]) and WHRadjBMI-DN (P_{IVW} = 8.8e-04, 0R = 1.38[1.14, 1.67]). Additionally, all the aforementioned results have been subjected to heterogeneity (P > 0.05) and pleiotropy testing (P > 0.05). Notably, no evidence of reverse causation was observed among these factors (Figure 4 and Table S10). To fortify the integrity of our findings, we judiciously employed LCV. This rigorous approach reaffirmed the causal nexus between BMI and DKD (P = 1.55e-4, GCP = 0.75), substantiating the robustness of this association. In contrast to the MR findings, there is also a strong genetic causality between HDL-C and DN (P = 4.71e-14, GCP = 0.82) (Table S11).

DISCUSSION

To the best of our knowledge, this study represents the first comprehensive genome-wide study delving into the pleiotropic associations underpinning the co-occurrence of obesity and microvascular complications in diabetes. We employed a multifaceted array of statistical methodologies to rigorously assess genetic correlations, pleiotropic genetic variants and loci, as well as explore potential shared geneset and causal relationships, and relevant biological pathways. These research results help eliminate confounding factors introduced by observational studies, elucidate the etiology and comorbidity patterns between obesity and diabetic microvascular complications, thus reducing the complexity of disease prevention and management.

We explored the genetic connections between obesity-related traits and microvascular complications in diabetes, and found that there is incomplete concordance between the global genome and specific genomic regions. To illustrate, the correlation between TC/LDL-C and diabetic microvascular complications is generally weak across the global genome but became

noteworthy in specific genetic regions. This phenomenon likely reflects the heterogeneity of genetic architecture underlying complex traits, where multiple local genomic regions may harbor correlation signals with opposing directions or weak effect sizes. These signals counteract each other in global analyses, leading to an overall diluted correlation that fails to reach statistical significance. For instance, certain loci may simultaneously carry both pro-disease and protective alleles, or distinct biological pathways may exert antagonistic effects on the phenotype. The strength of local analytical approaches lies in their capacity to resolve such counteracting effects, unmasking region-specific associations obscured by global methods. This precision enables the identification of biologically relevant targets for mechanistic studies, offering insights into context-dependent genetic contributions that are otherwise averaged out in genome-wide analyses.

In addition, in our global genetic correlation analysis, we noted that the genetic association between BMI and DKD stands out prominently, which was in harmony with the results obtained from MR and LCV analyses. Notably, the GCP for the BMI-DKD relationship is 0.75, surpassing the threshold of 0.7, suggesting that the genetic connection between BMI and DKD is largely driven by a causal relationship, with limited influence from pleiotropy. Furthermore, with a positive GCP value and MR results indicating the absence of a reverse causality between BMI and DKD, we can reasonably infer a causal pathway: genes affect trait1 (BMI), which subsequently impacts trait2 (DKD). These findings are consistent with epidemiological research, where a substantial systematic review and meta-analysis encompassing 20 cohort studies have identified BMI as an independent risk factor for DKD. Specifically, for every 5 kg/m^2 increase in BMI, there is a 16% rise in the risk of DKD[36]. These results further underline the genetic association between BMI and DKD stemming from a causal relationship.

Utilizing LDSC, we identified a significant genetic correlation between HDL-C and DN, with LCV results being consistent. However, MR analysis did not detect a significant causal effect. This discrepancy likely stems from methodological differences: MR relies on genetic variants as instruments and assumes effect proportional to exposure, while LCV considers genetic correlations and models pleiotropy. LCV's flexible instrument requirements and efficient use of sample data give it an edge in statistical power, especially with smaller samples. Moreover, LCV identifies partial causal components missed by MR by quantifying the genetic causality proportion, particularly in complex genetic networks.

In the course of our exploration into the common genetic framework, we undertook a thorough investigation of pleiotropy, encompassing both the SNP and gene levels. To identify pleiotropic SNPs associated with specific traits, we employed a systematic and rigorous analytical

approach. Initially, we performed an initial screening using PLACO, which enabled the identification of pleiotropic SNPs, laying the foundation for subsequent analyses. Subsequently, we employed FUMA to identify 828 lead SNPs carefully filtering out those in LD. Our colocalization analysis ultimately confirmed the existence of 52 loci with a high level of evidence. Furthermore, we employed a combination of methodologies, including MAGMA, SMR, and multivariate colocalization, to successfully identify 102 genes associated with these 52 loci, with 48 of them being unique. Amongst these shared genes, we consider RPS26, along with its lead SNP: rs11171739, to be the most strongly substantiated pleiotropic risk gene.

Ribosomal Protein S26 (RPS26) plays an important role in the biogenesis of ribosomes through its involvement in the processing of pre-ribosomal RNA. RPS26 exerts a significant regulatory influence on the conformational stability and transcriptional activity of p53, a critical mediator of cellular responses to stress and guardian of the genome. Experimental evidence suggests that both the overexpression and the reduction of RPS26 levels can culminate in the enhanced stabilization of p53, which in turn triggers a series of downstream cellular responses, including cell cycle arrest and the induction of programmed cell apoptosis[37]. Within the framework of diabetes mellitus pathology, the p53 assumes a predominantly deleterious role in cellular function and metabolic regulation. The aberrant accumulation of p53 within the cytoplasm of pancreatic β-cells has been associated with the disruption of Parkin-mediated mitophagy. This perturbation culminates in mitochondrial dysfunction, which is directly implicated in the etiology of impaired insulin secretion[38]. Moreover, p53 contributes to autophagic impairment in renal tubules by inducing the expression of miR-214, which subsequently suppresses the key autophagy-initiating protein kinase, unc-51–like autophagy-activating kinase 1 (ULK1)[39]. Recent study indicates that the regulation of p53 stability by O-GlcNAc modification may play a role in controlling hyperglycemia-induced cell death in retinal pericytes[40]. On the other hand, RPS26 orchestrates the survival of T-cells in a p53-dependent manner. Intriguingly, murine experiments have revealed heightened expression of RPS26 in T lymphocytes, and the deletion of RPS26 in T cells provokes peripheral T-cell instability and impedes thymic T-cell development[41]. This observation is intimately tied to the pathogenesis of diabetes mellitus, given the pivotal role of the immune system in the evolution of this condition. Additionally, multiple studies has demonstrated the close association between p53 and obesity, with p53 being involved in important pathways related to lipid metabolism, energy balance, and hormone sensitivity[42–44]. Consequently, we postulate that RPS26 may influence diabetic microvascular complications and obesity through mechanisms involving the activation and stabilization of p53. To investigate RPS26's regulatory role in the p53

pathway, co-immunoprecipitation (co-IP) and p53-responsive luciferase assays should be performed to assess protein interaction and transcriptional modulation. In vivo studies using RPS26 transgenic/knockout mice can evaluate its pathophysiological relevance through blood glucose monitoring, insulin sensitivity tests, and histological analysis of diabetic complications. In vitro, overexpression and knockdown cell models are constructed by transfecting RPS26 overexpression plasmids or siRNA. Western Blot is used to detect p53 protein levels under different RPS26 expression states.

In addition to the conspicuous pleiotropic effects exhibited by RPS26, it's noteworthy that several genes identified through multivariate colocalization analysis also demonstrate substantial effects. However, it's essential to acknowledge that these genes did not surpass the stringent SMR threshold, primarily due to the formidable correction pressure imposed by their extensive pleiotropy. Zinc finger protein 1（JAZF1）, predominantly expressed within pancreatic tissues, is regarded as a pivotal regulator of glucose and lipid metabolism. It interacts with critical pathways such as Adenosine Monophosphate（AMP）, AMP-activated protein kinase（AMPK）and mitogen-activated protein kinase（MAPK）, exerting anti-glycemic, anti-lipidemic, and anti-inflammatory actions[45,46]. Similarly, ZBTB20, another zinc finger protein abundantly expressed in pancreatic β-cells, exerts its influence by inhibiting the transcription of Fructose-1,6-bisphosphatase (FBP)-1[47]. This regulatory mechanism regulates β-cell function and contributes to the stability of glucose homeostasis. Additionally, ZBTB20 also plays a significant role in hepatic *de novo* lipogenesis (DNL) for the regulation of whole-body lipid metabolism. Nipsnap1 has been extensively studied for its role in recruiting autophagy-related proteins to participate in the process of mitochondrial autophagy in the outer membrane of mitochondria. This mechanism is of paramount importance in regulating diabetes and its complications[48,49]. When faced with chronic cold exposure, impairment or inhibition of Nipsnap1 may potentially compromise cellular DNL and mitochondrial lipid beta-oxidation capacity[50]. IKZF4 and SUOX have been confirmed as susceptibility loci for diabetes[51,52]. However, research linking them to obesity remains limited and incomplete. Based on current research, NCR3LG1 is exclusively associated with various tumor diseases[53,54] and RP1-239B22.5 has received very limited attention, with only one study indicating higher expression levels in late-stage cancer[55].

In enrichment analysis, we can observe the regulation of insulin hormone secretion emphasized both in the BP domain of GO and in the KEGG. This suggests that controlling blood sugar levels is a primary task in reducing microvascular complications in diabetes. Furthermore, the

analysis results indicate that enrichment of CC is mainly concentrated in areas related to lipid metabolism, further highlighting the association between obesity and microvascular complications in diabetes. In terms of MF enrichment, it covers various aspects such as signal transduction, ion transport, protein kinase activity, calcium-dependent processes, ATPase coupling, and more. These signaling pathways play critical roles in processes like cell proliferation, differentiation, survival, and apoptosis. Their abnormal activity can lead to cellular dysfunction, metabolic disorders, abnormal insulin secretion, vascular changes, and so on[56–58]. For example, the MAPK family, which includes ERKs (extracellular-signal-regulated kinases), JNKs (Jun amino-terminal kinases), and p38/SAPKs (stress-activated protein kinases), plays crucial roles in both diseases through various mechanisms, including inducing inflammation, interfering with insulin signaling, impacting lipid metabolism, and affecting pancreatic islet function[59–61].

While our study has yielded important findings, it's essential to acknowledge several limitations that need consideration. Firstly, despite utilizing large-scale GWAS data for obesity-related traits, the data available for DKD, DR and DN remain limited, which could potentially impact the comprehensiveness of our research findings. Secondly, all our data sources are derived from individuals of European descent, which may restrict the generalizability of our study results to other population groups. Future studies should include a broader range of ancestries to fully understand the genetic architecture of the traits under investigation. Lastly, to gain a more in-depth understanding of the functional and mechanistic roles of shared risk genes in the microvasculature of diabetes and obesity, further in vitro and in vivo studies can be pursued. These studies should consider tissue specificity, including but not limited to whole blood, kidney, and pancreas.

CONCLUSION

In summary, our study has identified significant genetic correlations between obesity and microvascular complications in diabetes, and we have successfully identified shared risk SNPs and genes, with RPS26 demonstrating the strongest genetic evidence. Furthermore, we have explored causal and pleiotropic relationships in detail, providing important insights into the genetic mechanisms underlying these traits. These findings provide robust support for further research into the pathogenesis and therapeutic approaches for these traits.

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Conflicts of interest: Authors declare no conflicts of interest.

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Data availability: GWAS summary statistics for obesity：
http://csg.sph.umich.edu/willer/public/glgc-lipids2021/ and
https://zenodo.org/record/1251813#.ZGCbP7JBztU

GWAS summary statistics for diabetic microvascular complications：
https://www.finngen.fi/en/access_results

The pQTL summary data can be found at http://nilanjanchatterjeelab.org/pwas/

The codes used in this study can be found at:

LDSC: https://github.com/bulik/ldsc.

LAVA: https://github.com/josefin-werme/LAVA

PLACO: https://github.com/RayDebashree/PLACO.

COLOC: https://github.com/chr1swallace/coloc.

SMR: https://cnsgenomics.com/software/smr/#Overview.

FUMA: https://fuma.ctglab.nl/celltype/.

TwoSampleMR: https://mrcieu.github.io/TwoSampleMR/.

LCV: https://github.com/lukejoconnor/LCV.

All the data and code are accessible in public databases and open for public access. Further inquiries can be directed to the corresponding author.

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TABLES AND FIGURES WITH LEGENDS

Table 1. Genome-wide genetic correlation between diabetic microvascular complications and obesity-related traits.

Trait1	Trait2	r_g	SE	*P*-value
DKD	BMI	0.47	0.0447	4.73e-26
DKD	WHR	0.47	0.0488	4.40e-22
DKD	WHRadjBMI	0.22	0.0437	5.07e-07
DKD	LDL-C	-0.05	0.0447	3.01e-01
DKD	HDL-C	0.43	0.0473	2.17e-19
DKD	TC	-0.01	0.0378	7.04e-01
DKD	TG	-0.42	0.0519	8.06e-16
DR	BMI	0.38	0.0348	1.28e-27
DR	WHR	0.40	0.0347	8.54e-31
DR	WHRadjBMI	0.20	0.0321	6.72e-10
DR	LDL-C	-0.04	0.0382	3.58e-01
DR	HDL-C	0.32	0.0412	6.19e-15
DR	TC	-0.02	0.0324	5.47e-01
DR	TG	-0.31	0.0402	2.18e-14
DN	BMI	0.36	0.0487	1.45e-13
DN	WHR	0.33	0.0425	5.01e-15
DN	WHRadjBMI	0.13	0.0368	3.01e-04
DN	LDL-C	0.05	0.0453	2.57e-01
DN	HDL-C	0.29	0.0475	7.47e-10
DN	TC	0.05	0.0403	1.97e-01
DN	TG	-0.27	0.0464	8.41e-09

r_g, genetic correlation; SE, standard error; BMI, Body Mass Index; WHR, waist-to-hip ratio; WHRadjBMI, waist-to-hip ratio adjusted for body mass index; LDL-C, low-density lipoproteins cholesterol; HDL-C, high-density lipoprotein cholesterol; TC, total cholesterol; TG, triglycerides; DKD, diabetic kidney disease; DR, diabetic retinopathy; DN, diabetic neuropathy.

Table 2. 52 Colocalized loci identified by colocalization analysis

Trait pair	Lead SNP	CHR	Locus boundary*	PP.H4	Trait pair	Lead SNP	CHR	Locus boundary*	PP.H4
BMI-DKD	rs7903146	10	114722134-114818754	0.991	TG-DR	rs9379084	6	7231843-7231843	0.980
BMI-DKD	rs76895963	12	4384844-4384844	1.000	TG-DR	rs7451008	6	20641336-20727570	0.982
BMI-DR	rs10938397	4	45068929-45193147	0.996	TG-DR	rs1708302	7	28142088-28209953	0.967
BMI-DR	rs849135	7	28142088-28209953	0.977	TG-DR	rs3802177	8	118184783-118220270	0.957
BMI-DR	rs6602411	10	10255003-10264200	0.993	TG-DR	rs10811661	9	22132076-22136489	0.989
BMI-DR	rs7903146	10	114729482-114867427	0.977	TG-DR	rs7903146	10	114729482-114818754	0.994
BMI-DR	rs1557765	11	17368013-17421886	0.987	TG-DR	rs10765572	11	92668975-92708710	0.957
BMI-DR	rs76895963	12	4384844-4384844	1.000	TG-DR	rs76895963	12	4384844-4384844	1.000
BMI-DR	rs12885454	14	29680331-29777492	0.965	TG-DR	rs7144011	14	79833494-79945162	0.979
BMI-DR	rs7144011	14	79703248-79945162	0.980	TG-DR	rs483082	19	45232161-45524119	0.967
HDL-C-DKD	rs429358	19	45337918-45523583	0.999	WHR-DKD	rs429358	19	45392254-45424351	0.999
HDL-C-DR	rs11708067	3	122936084-123131254	0.957	WHR-DR	rs11705729	3	185488303-185538006	0.955
HDL-C-DR	rs10938397	4	45164637-45187622	0.996	WHR-DR	rs10938397	4	45164637-45187622	0.995
HDL-C-DR	rs1574285	9	4282536-4296430	0.980	WHR-DR	rs1513272	7	28142088-28209953	0.986
HDL-C-DR	rs11171739	12	56368078-56584247	0.984	WHR-DR	rs7144011	14	79833494-79945162	0.984
HDL-C-DR	rs3184504	12	111662984-113218868	0.995	WHR-DR	rs9923544	16	53797908-53848561	0.951
HDL-C-DR	rs7144011	14	79833494-79945162	0.979	WHR-DR	rs429358	19	45386467-45428234	1.000

HDL-C-DR	rs151249695	15	38909425-38909425	0.997	WHRadjBMI-DKD	rs9356744	6	20635719-20727570	0.962
HDL-C-DR	rs429358	19	45324138-45623467	1.000	WHRadjBMI-DKD	rs429358	19	45392254-45424351	0.998
TG-DKD	rs7766070	6	20652717-20703952	0.974	WHRadjBMI-DR	rs112256201	3	50599511-50724724	0.959
TG-DKD	rs7903146	10	114729482-114817009	0.996	WHRadjBMI-DR	rs4686696	3	185488303-185538006	0.959
TG-DKD	rs76895963	12	4328521-4384844	1,000	WHRadjBMI-DR	rs1513272	7	28142088-28256240	0.981
TG-DKD	rs695399	22	29889324-30082569	0.951	WHRadjBMI-DR	rs1002226	11	17368013-17421886	0.980
TG-DR	rs28408152	3	115063640-115102814	0.966	WHRadjBMI-DR	rs7310615	12	111826477-112906415	0.961
TG-DR	rs11716713	3	185488303-185538006	0.959	WHRadjBMI-DR	rs429358	19	45388500-45424351	0.999
BMI-DN	rs7903146	10	114749734-114817009	0.970	TG-DN	rs7903146	10	114749734-114817009	0.986

SNP, single nucleotide polymorphism; CHR, chromosome; PP.H4, posterior probability of H4; BMI, Body Mass Index; WHR, waist-to-hip ratio; WHRadjBMI, waist-to-hip ratio adjusted for body mass index; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides; DKD, diabetic kidney disease; DR, diabetic retinopathy; DN, diabetic neuropathy.

*Locus boundary displays the region (start-end) defined by FUMA analysis.

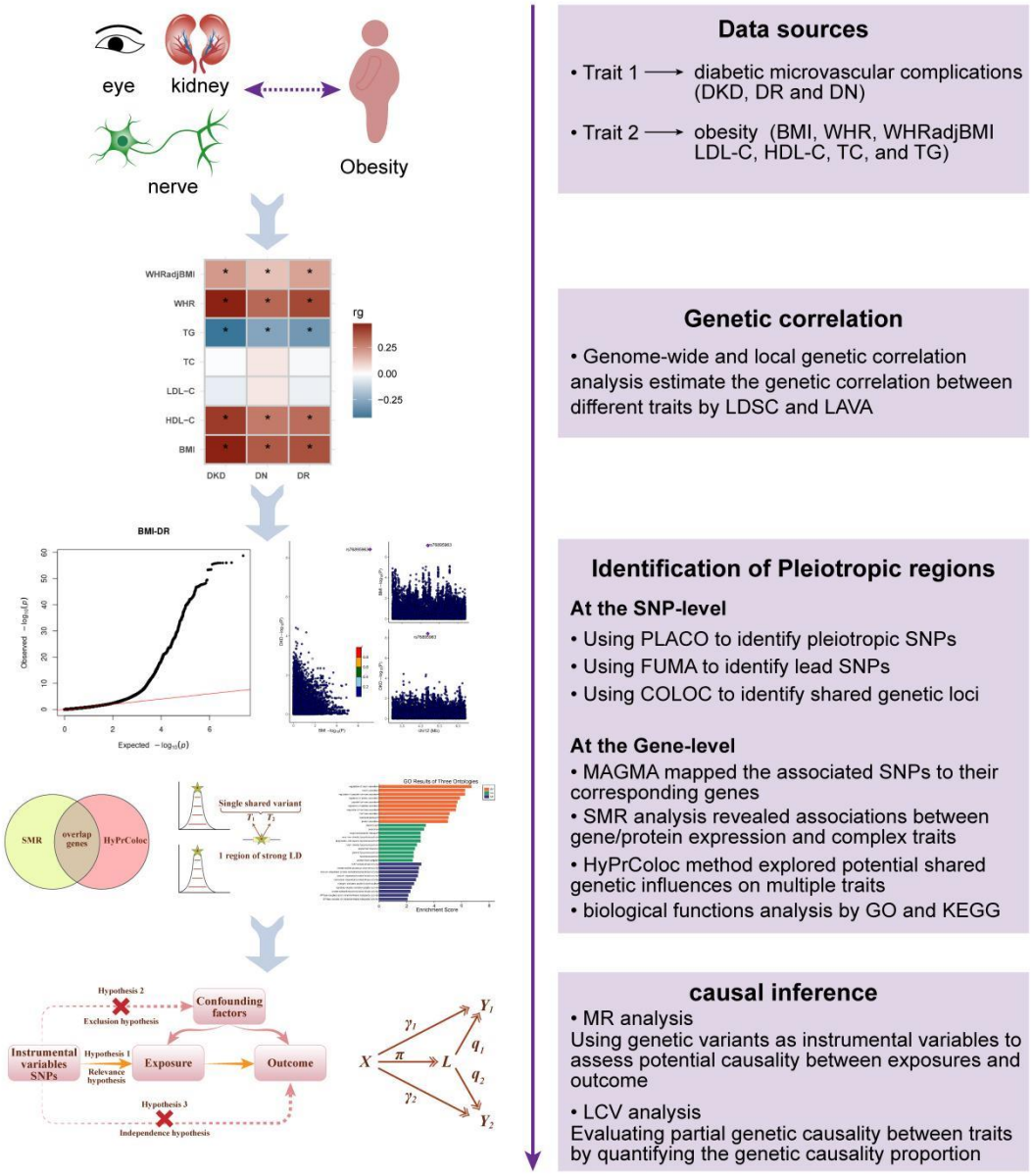

Figure 1. Overview of statistical analyses performed in the study. GWAS summary statistics on 7 obesity-related traits and diabetic microvascular complications were retrieved. First, we investigated the global and local genetic correlations among each pair of traits. Subsequently, we used a series of comprehensive approaches to identify pleiotropic variants and genes. Finally, the potential causality or pleiotropy behind these diseases were further explored. LDSC,

Linkage Disequilibrium Score; LAVA, Local Analysis of [co]Variant Association; PLACO, Pleiotropic analysis under composite null hypothesis; FUMA, Functional mapping and annotation of genetic associations; MAGMA, Multimarker Analysis of GenoMic Annotation; SMR, Summary-based Mendelian Randomization; MR, Mendelian Randomization; LCV, Latent Causal Variable; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; DKD, diabetic kidney disease; DR, diabetic retinopathy; DN, diabetic neuropathy; BMI, Body Mass Index; WHR, waist-to-hip ratio; WHRadjBMI, waist-to-hip ratio adjusted for body mass index; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides.

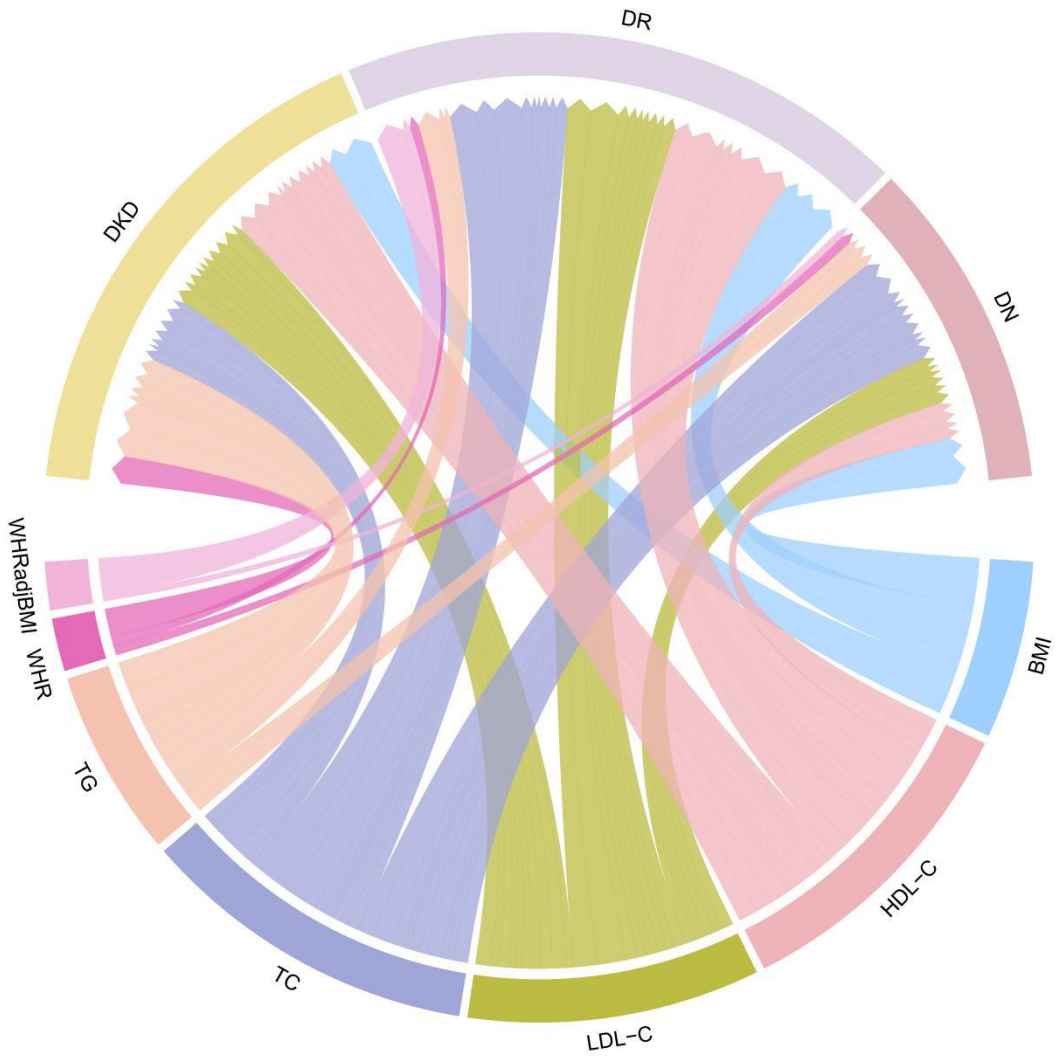

Figure 2. Local genetic correlations between obesity-related traits and diabetic microvascular complications. Distinct colors are used to represent different traits, while the width of the connecting bands (chords) reflects the strength of the relationships between genes. A wider band indicates a stronger correlation between traits, while a narrower band signifies a weaker correlation. Only correlations meeting $P < 0.00002$ (0.05/2495) are displayed. DKD, diabetic kidney disease; DR, diabetic retinopathy; DN, diabetic neuropathy; BMI, Body Mass Index; WHR, waist-to-hip

ratio; WHRadjBMI, waist-to-hip ratio adjusted for body mass index; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides.

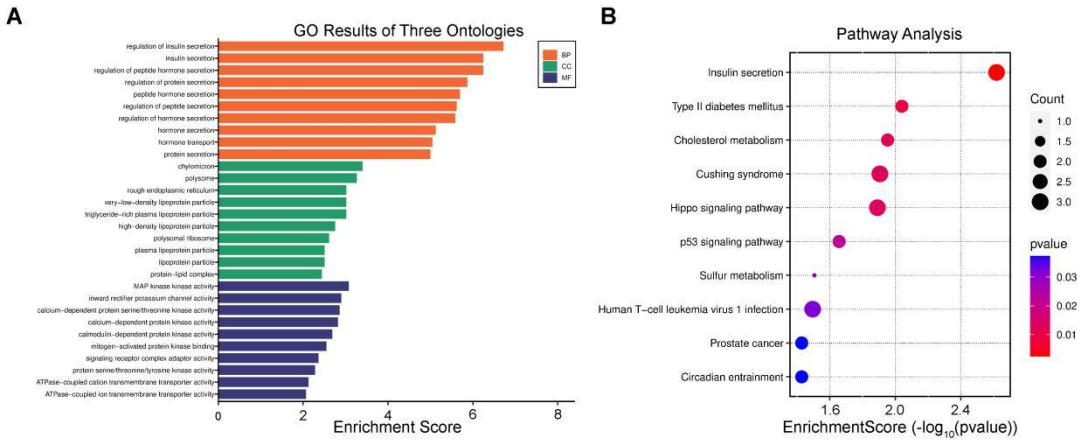

Figure 3. The Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis of genes in the region of the colocalization analysis results. (A) Different colors are used to represent the three main categories of Gene Ontology terms: Biological Process (BP), Cellular Component (CC), and Molecular Function (MF). Each bar in the chart corresponds to enrichment score of the GO term within the respective category. (B) The x-axis represents enrichment score, and y-axis represents different biological pathways. The size of circle represents gene count, with larger circles indicating more genes and smaller circles indicating fewer genes. Circle colors indicate *P*-values, with blue representing higher *P*-values (less significant) and red representing lower *P*-values (more significant).

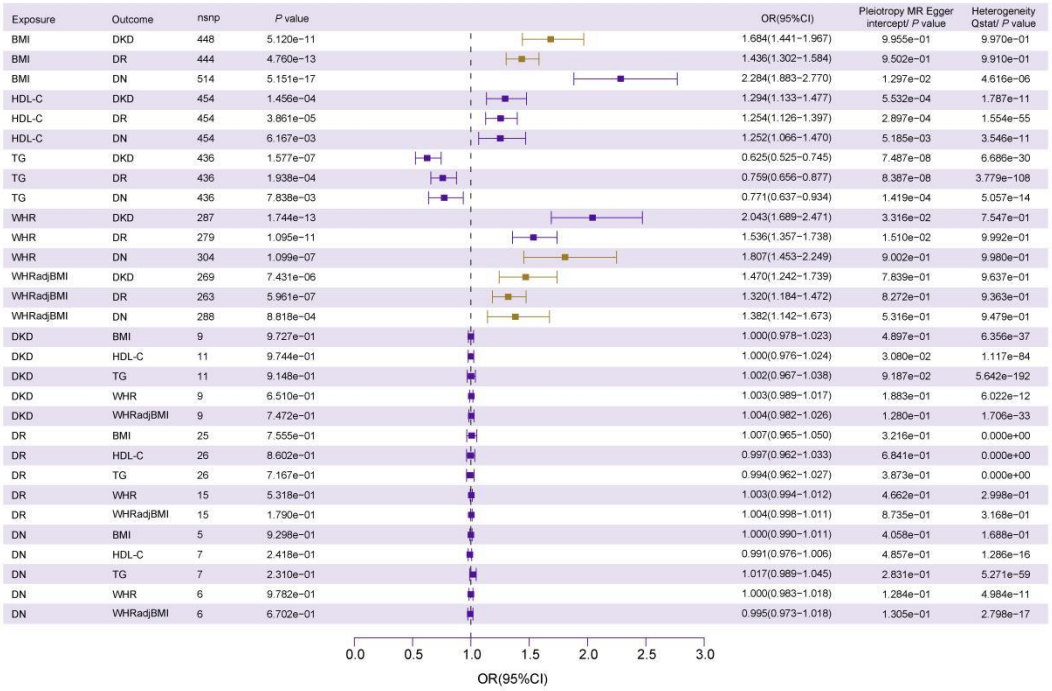

Figure 4. Summary of bi-directional MR analyses between obesity-related traits and diabetic microvascular complications. Error bars represent the 95% confidence intervals for the associated MR estimates. The primary method for *P*-value calculation is the IVW method. The MR-Egger intercept and Cochrane's Q test were used to assess pleiotropy and heterogeneity. A significant MR-Egger intercept ($P < 0.05$) suggests pleiotropic effects, while a significant Cochrane's Q test ($P < 0.05$) indicates heterogeneity. DKD, diabetic kidney disease; DR, diabetic retinopathy; DN, diabetic neuropathy; BMI, Body Mass Index; WHR, waist-to-hip ratio; WHRadjBMI, waist-to-hip ratio adjusted for body mass index; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides

SUPPLEMENTAL DATA

Supplemental data are available at the following link:

https://www.bjbms.org/ojs/index.php/bjbms/article/view/11897/3815