Article

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Investigating Circulating Metabolites and Proteins as Mediators of the Link Between Diabetes and Cardiovascular-Kidney-Metabolic Diseases

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Background: Cardiovascular-Kidney-Metabolic (CKM) diseases, including coronary heart disease, atherosclerosis, and chronic kidney disease, are significant causes of mortality among diabetic patients. Although diabetes is known to influence these diseases, the underlying biological pathways remain unclear.

Methods: We performed a two-sample Mendelian randomization (MR) analysis to investigate the relationships between type 1 diabetes (T1D), type 2 diabetes (T2D) and CKM traits. Two-step MR analyses were conducted to explore potential pathways involving circulating inflammatory proteins and metabolites. Cross-trait linkage disequilibrium score regression (LDSC) was employed to investigate the genetic association and colocalization analyses were used to assess shared genetic architecture. Additional sensitivity analyses were performed, including exclusion of variants within the major histocompatibility complex/human leukocyte antigen (MHC/HLA) region for T1D.

Results: MR analyses suggested that genetically predicted T2D was associated with increased risks of multiple CKM traits, including coronary heart disease (OR = 1.16, $p = 2.01 \times 10^{-5}$) and myocardial infarction (OR = 1.17, $p = 1.46 \times 10^{-4}$), with consistent results across sensitivity analyses. In contrast, initial associations between T1D and CKM diseases were attenuated and no longer statistically significant after excluding variants within the MHC/HLA region, indicating that these associations were largely driven by this immune-related locus. Two-step MR analyses identified several circulating metabolites associated with both T2D and CKM diseases, suggesting that metabolic factors (e.g., 1-(1-enyl-palmitoyl)-2-linoleoyl-GPC) may partially explain the association between T2D and CKM risk (mediation proportions ranging from 4.56% to 29.31%). For T1D, circulating inflammatory proteins and metabolites were also associated with CKM traits; however, these findings are better interpreted as reflecting shared immune and metabolic pathways rather than causal mediation.

Conclusion: Our findings support a potential causal role of T2D in the development of CKM diseases, partly through circulating metabolites. In contrast, the associations observed for T1D appear to be largely driven by the HLA/MHC region and likely reflect shared immune-mediated genetic architectures rather than a direct causal effect. These results provide insight into distinct biological pathways linking different forms of diabetes with CKM diseases.

Keywords: diabetes; Cardiovascular-Kidney-Metabolic diseases; inflammatory cytokines; metabolomics; mendelian randomization

Introduction

Epidemiological data indicate that diabetes ranks as the seventh most prevalent cause of death in America, while projections suggest the global diabetic population could escalate to 693 million by 2045 [1]. Prior investigations highlight Cardiovascular-Kidney-Metabolic (CKM) disorders, including ischemic cardiac events, brain vessel hardening, and prolonged renal impairment, as primary mortality drivers in this demographic [2,3]. Notably, among elderly individuals, CKM-related fatalities exceed 60%, creating tremendous socioeconomic and medical challenges [4]. Although the specific mechanisms remain unclear, several studies suggest that diabetes is associated with CKM diseases, potentially through circulating metabolites and inflammatory factors [5,6].

In recent years, Mendelian randomization (MR) has become increasingly popular for studying potential causal relationships. Fundamentally, this methodology relies on the natural assortment of genetic variants strongly associated with a specific trait, utilizing them as proxies [7]. Through evaluating how these genetic instruments impact both exposure factors and resulting phenotypes, researchers can infer likely causal pathways [8]. A primary benefit of this genetic approach is its high resilience against environmental confounders and backward causation, primarily because genotypes are permanently fixed prior to birth [9].

For the present investigation, our team hypothesized that inflammatory proteins and circulating metabolites may be involved in the biological pathways linking diabetes and CKM traits. Initially, a two-sample MR was exe-

cuted to assess direct connections between diabetes status and CKM traits. Then, a two-step mediatory framework was performed to explore how diabetes may be linked to CKM traits through inflammatory proteins and circulating metabolites, shedding light on the mechanisms underlying this association.

Materials and Methods

Study Design

The present study adheres to the STROBE-MR guidelines [10] (**Supplementary Table 1**). A schematic of the core MR assumptions and the research design are presented in Fig. 1. First, the genetic instrumental variables should be significantly related to diabetes. Second, any variants associated with potential confounding factors must be removed. Third, the instrumental variables must influence the outcome (CKM diseases) solely through the exposure (diabetes), without affecting the outcome via any alternative biological pathways [9]. Single nucleotide polymorphisms (SNPs) were used as instrumental variables.

Data Sources

Comprehensive information about the included datasets can be found in **Supplementary Table 2**. For the exposures, type 1 diabetes (T1D) (9266 cases and 15,574 controls) [11] and type 2 diabetes (T2D) (242,283 cases and 1,569,734 controls) [12] were included. For the outcomes, the definition and selection of CKM diseases were guided by the 2023 American Heart Association (AHA) Presidential Advisory on CKM Health [2]. To comprehensively capture the CKM continuum, we selected the FinnGen study [13] endpoints representing the metabolic/vascular risk component (e.g., hypertension), the kidney component (e.g., chronic kidney disease), and the broad cardiovascular component, encompassing atherosclerotic/ischemic events, structural heart diseases/heart failure, arrhythmias, and systemic thromboembolic/vascular diseases. A complete list of the included CKM phenotypes can be found in **Supplementary Table 2**. For the mediating factors, inflammatory protein markers were derived from Zhao *et al.* [14], while circulating metabolite profiles originated from Chen *et al.* [15]. Metabolite ratios were excluded from subsequent analyses. Public genome-wide association studies (GWASs) summary-level data were used rather than individual-level genetic data; therefore, no additional ethical approval was required. Importantly, to prevent potential bias in causal estimates, we rigorously ensured that there were no sample overlaps among the datasets used for exposures, mediators, and outcomes. Specifically, the summary statistics for CKM diseases were exclusively sourced from the FinnGen consortium, which represents an independent cohort with no overlap with the populations used for the GWASs of diabetes, inflammatory proteins, or metabolites.

Genetic Instrument Selection

Variants reaching robust genomic significance thresholds ($p < 5 \times 10^{-8}$) served as genetic proxies. PLINK aggregation method was conducted to measure the linkage disequilibrium between the SNPs for each exposure variable. After clumping using a threshold of $r^2 < 0.001$, a genomic window of 10,000 kilobases remained. The instrument strength was verified for individual variants, completely removing any proxy scoring under an F-value of 10. Besides, SNPs correlated with confounders (Body mass index, lipid traits, smoking and alcohol consumption) were excluded using the LDtrait tool [16]. Although this step was intended to reduce potential bias from selected known confounders, it does not fully exclude horizontal pleiotropy.

Notably, for T1D, a substantial proportion of associated variants are located within the major histocompatibility complex/human leukocyte antigen (MHC/HLA) region, which is characterized by extensive linkage disequilibrium and structural pleiotropy across immune-related traits. Therefore, the potential influence of region-specific pleiotropy was considered in subsequent sensitivity analyses.

We identified cis-acting quantitative trait loci for circulating inflammatory proteins by applying criteria of independence and genome-wide significance ($r^2 < 0.001$, $p < 5 \times 10^{-8}$). Using the same thresholds, SNPs associated with circulating metabolites were also screened. After this selection procedure, a total of 62 inflammatory proteins and 649 metabolites were included in the final analysis.

Cross-Trait Linkage Disequilibrium Score Regression Analysis

By applying linkage disequilibrium score regression (LDSC) to GWAS summary statistics, we assessed the genetic association (rg) between diabetes and CKM [17,18]. This method allowed us to calculate SNP-based heritability (h^2) for each phenotype and the SNP-based coheritability between the two traits. Because the GWAS dataset consisted of individuals of European descent, linkage disequilibrium panel were referenced using data from the 1000 Genomes Project [19]. In the current study, it included two exposures and 42 outcomes, resulting in a total of 84 tests. A Bonferroni-corrected significance threshold of $p < 0.05/84$ ($p < 5.95 \times 10^{-4}$) was applied [20].

Main Analysis

A two-sample MR framework was used to examine the potential causal effect of diabetes on CKM traits. Three MR methods including inverse variance weighted (IVW), Weighted Median and MR Egger were employed, with IVW being the primary MR analysis method. The IVW model was selected based on heterogeneity [7]. A multiplicative random-effects model was used when $p < 0.05$, and a fixed-effects model otherwise. For analyses with only one SNP, the Wald ratio method was applied [9]. Bonfer-

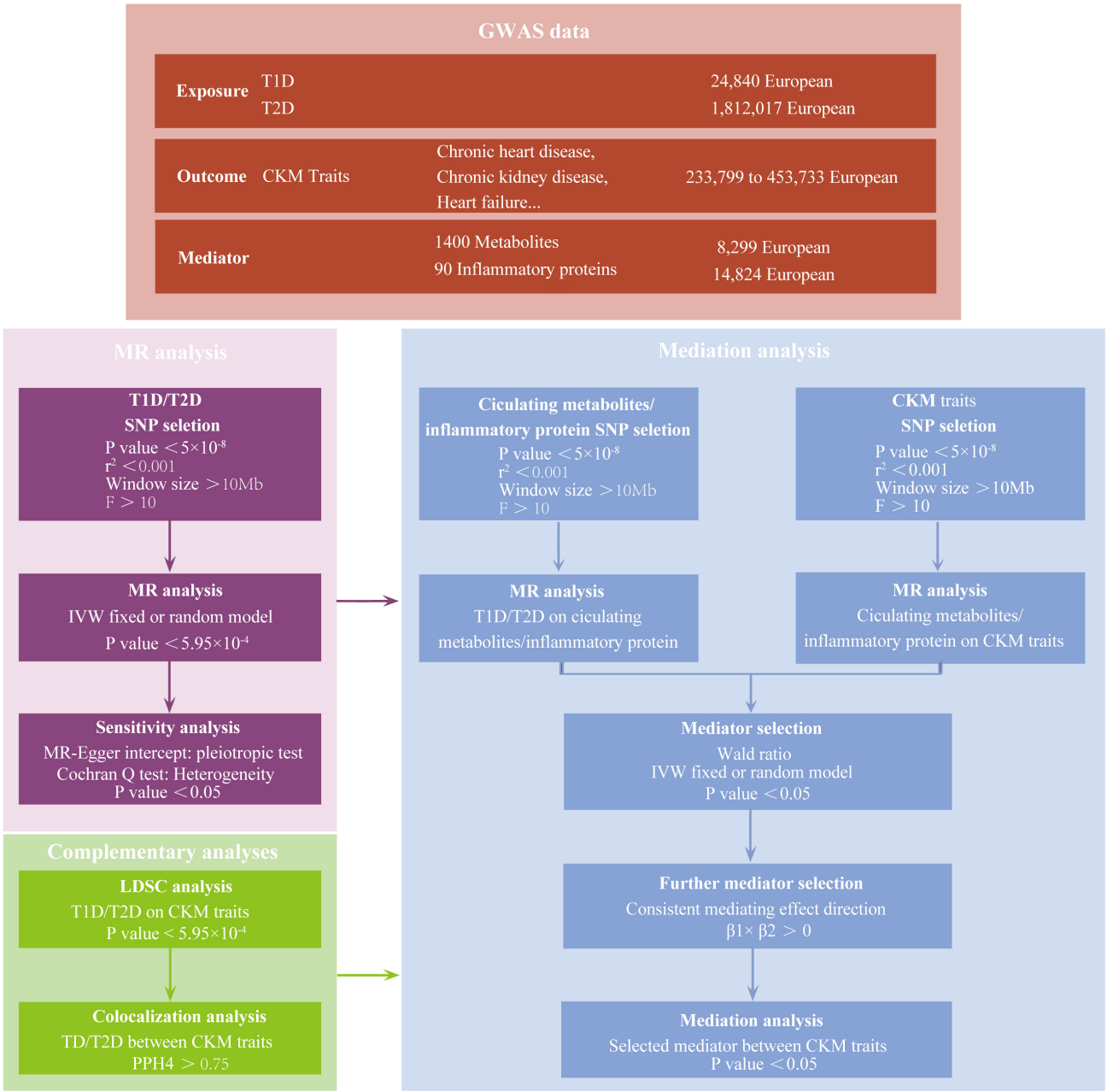

Fig. 1. Study flowchart. Abbreviation: CKM, Cardiovascular-Kidney-Metabolic; IVW, Inverse variance weighted; LDSC, linkage disequilibrium score regression; MR, Mendelian randomization; SNP, Single nucleotide polymorphism; T1D, type 1 diabetes; T2D, type 2 diabetes.

roni correction for multiple testing was conducted [20], and the significance threshold was set at 0.05/84 ($p < 5.95 \times 10^{-4}$).

Colocalization Analyses

The purpose of the colocalization analysis was to identify variants potentially shared by distinct traits and to determine whether a shared causal variant within the region influences both traits. This analysis employed a Bayesian model to determine the posterior probabilities of five hypotheses (PPH): (1) no association (H0), (2) only trait 1 shows an association (H1), (3) only trait 2 shows an association (H2), (4) two distinct causal variants are linked to the two traits (H3), and (5) a single causal variant is linked to both traits (H4). We employed colocalization methods to investigate the potential interactions between diabetes and CKM. These analyses were conducted using the R package "coloc", with strong evidence for a shared causal variant between the two traits defined as PPH4 >0.75.

Two-Step Mediation Analysis

Potential biological pathways were explored using a two-step MR design. First, the effect of diabetes on circulating inflammatory proteins or metabolites was estimated as $\beta 1$. Second, the effect of these mediators on CKM diseases was estimated as $\beta 2$. The indirect effect was calculated as the product of $\beta 1$ and $\beta 2$ [9], and its standard error was derived using the delta method [21]. Only indirect effects with directions consistent with the total effect were considered. The mediation proportion was rigorously estimated as the indirect effect divided by the sum of the direct effect (derived from the primary MR) and the indirect effect. Because this was an exploratory analysis, $p < 0.05$ was considered statistically significant.

Given the potential influence of MHC/HLA-driven pleiotropy on T1D, the two-step MR results for T1D were interpreted as exploratory evidence for shared biological pathways rather than definitive evidence of causal mediation. In contrast, the corresponding analyses for T2D were interpreted in the context of the primary MR findings.

Importantly, because the exposures and outcomes are binary diseases while the mediators are continuous omics traits, causal estimates were kept on their original summary statistic scales (log-odds for binary traits, standard deviation units for continuous traits). Thus, both the indirect and the total effects are expressed on the log-odds scale, and the mediation proportion was estimated as the indirect effect divided by the total effect, including both the direct and indirect components.

Sensitivity Analysis

Sensitivity analyses included the evaluation of heterogeneity and pleiotropy. Cochran Q statistic was conducted to analyze the heterogeneity, and a *p*-value below 0.05 was considered indicative of significant heterogeneity [9]. Pleiotropy was analyzed by MR-Egger intercept, where a non-significant MR-Egger intercept was interpreted as indicating no evidence of directional pleiotropy at the global level, but not as excluding balanced pleiotropy or region-specific structural pleiotropy. Because many genetic instruments for T1D are located within the MHC/HLA region, we performed a prespecified leave-HLA-out sensitivity analysis excluding all SNPs in the extended MHC region (Chr 6: 25–35 Mb) to assess whether the observed T1D associations were independent of this immune-related locus.

All analyses were conducted in R (Version 4.3.0; R Foundation for Statistical Computing, Vienna, Austria). The MendelianRandomization (v0.9.0) and TwoSampleMR (v0.6.1) packages were used for MR analyses, the forestploter package was used to create forest plots, and ggplot2 (v3.3.6) was used for dot plots and heatmaps.

Results

Strength of Genetic Instruments

SNPs correlated with T1D and T2D are shown in **Supplementary Tables 3,4**. The F-statistics of all SNPs were more than 10. LDtrait tools eliminated SNPs correlated with confounding factors. Finally, the 38 and 105 SNPs of T1D and T2D were retained as instrumental variables after excluding ambiguous and palindromic SNPs.

Effect of T1D on CKM Diseases

MR analysis initially suggested that T1D genetic liability was associated with atherosclerosis, chronic kidney disease, diseases of arteries, arterioles, and capillaries (DOAAC), hypertension, coronary heart disease, and myocardial infarction (Fig. 2A and **Supplementary Table 5**). Direction of effects remained consistent when other methods were applied (Fig. 2A and **Supplementary Table 5**). No significant results were seen in LDSC analysis (**Supplementary Fig. 1** and **Supplementary Table 6**), likely reflecting the focal nature of T1D genetic architecture. The MR-Egger intercept test did not detect evidence of directional pleiotropy (**Supplementary Table 7**). However, given the known concentration of T1D-associated variants within the HLA/MHC region, these findings were interpreted with caution. Colocalization analysis showed that T1D shared common causal variants with atherosclerosis, coronary heart disease, chronic kidney disease, DOAAC, myocardial infarction (Fig. 3, **Supplementary Table 8**). Notably, a detailed examination of the genomic coordinates revealed that the colocalization signals between T1D and atherosclerosis, chronic kidney disease, and DOAAC originated from a single shared immune locus, specifically the HLA region. In contrast, the shared common variants for coronary heart disease and myocardial infarction were mapped to an independent locus on Chromosome 1 (approx. 113.5–115.0 Mb).

To further evaluate whether these associations were driven by the HLA region, we repeated the MR analyses after excluding SNPs within the extended HLA region (Chr6: 25–35 Mb). After the removal of the HLA-region variants, all previously observed T1D–CKM associations were attenuated and no longer statistically significant (**Supplementary Table 9**). These findings suggest that the apparent associations between T1D genetic liability and CKM diseases were largely driven by the HLA/MHC region and are more consistent with a shared immune-mediated genetic architecture than with a direct causal effect of T1D on CKM diseases.

Effect of T2D on CKM Diseases

There were potential associations between T2D and increased risks of angina pectoris, atherosclerosis, coronary atherosclerosis, coronary heart disease, DOAAC, hypertension, and myocardial infarction (Fig. 2B and **Supplemen-**

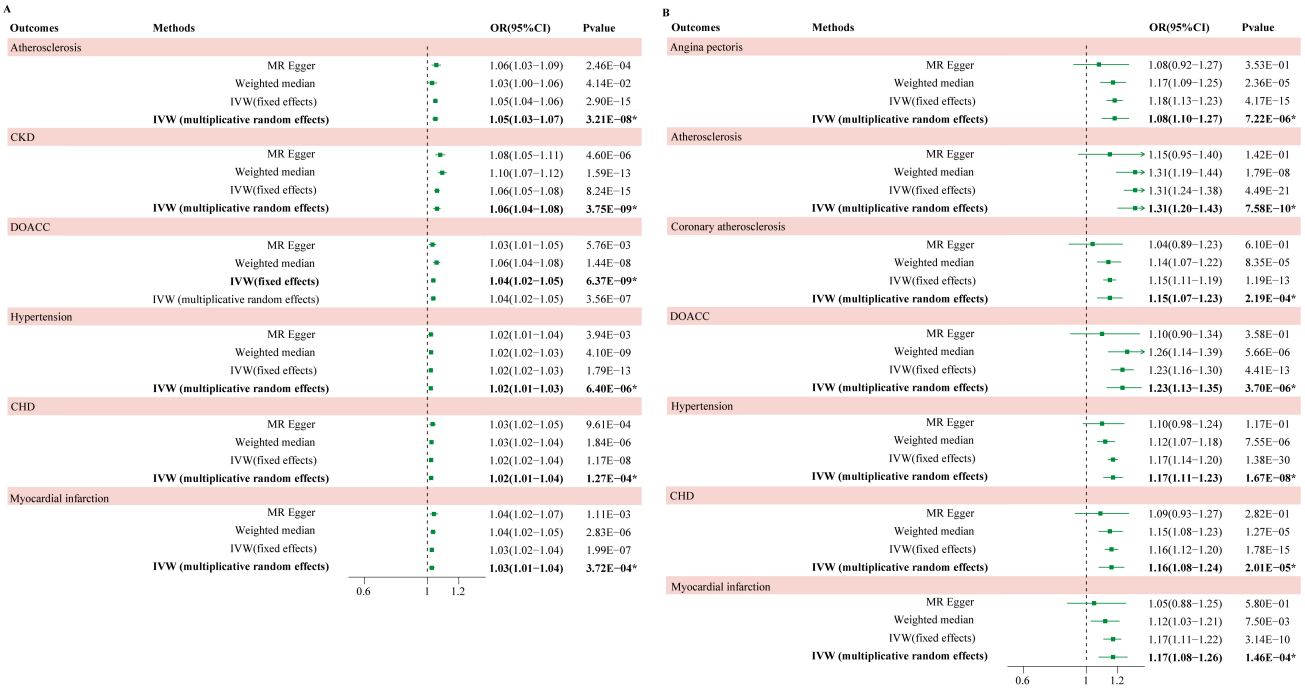

Fig. 2. Mendelian randomization analysis between diabetes and CKM traits. Forest plot depicting two-sample Mendelian randomization estimates for the associations between T1D (A), T2D (B) and CKM traits. Estimates from the IVW method, which was used as the primary analysis, are shown together with supporting results from complementary MR methods. Squares correspond to point estimates and horizontal lines indicate 95% CI. Statistically significant associations in the primary IVW analysis are marked with asterisks (*) and the text is bolded, based on the Bonferroni-corrected threshold of $p < 5.95 \times 10^{-4}$. Abbreviation: CHD, Coronary heart disease; CKD, Chronic kidney disease; CKM, Cardiovascular-Kidney-Metabolic; CI, Confidence interval; DOAAC, Diseases of arteries, arterioles, and capillaries; IVW, Inverse variance weighted; MR, Mendelian randomization; OR, Odds ratio; T1D, Type 1 diabetes; T2D, Type 2 diabetes.

tary Table 10), which were consistent with the findings from the LDSC analysis (**Supplementary Fig. 1** and **Supplementary Table 11**). For all the significant positive associations identified above, the MR-Egger intercept test indicated no evidence of directional pleiotropy ($p > 0.05$), supporting the robustness of the observed associations. Although potential pleiotropy was observed for a few traits (Right bundle-branch block, $p = 0.03$), these traits did not show significant causal relationships in the primary IVW analysis, and thus did not affect our final conclusions regarding the positive findings (**Supplementary Table 12**). Colocalization analysis showed that T2D shared common causal variants with angina pectoris, atherosclerosis, coronary atherosclerosis, coronary heart disease, DOAAC, hypertension, and myocardial infarction (Fig. 4, **Supplementary Table 13**).

Exploratory Two-Step MR Analysis of T1D-Related Inflammatory Proteins and Metabolites

We further explored potential biological pathways linking T1D genetic liability and CKM traits using a two-step MR framework.

For circulating inflammatory proteins, we identified significant associations between T1D and 10 inflammatory proteins, as well as between 23 inflammatory proteins and T1D-related CKM outcomes (Fig. 5A, **Supplementary Tables 14,15**). Among these, five inflammatory proteins were associated with both T1D and CKM diseases. After applying direction-consistency criteria, IL-15Rα, CCL19 and MMP10 were retained for further analysis (Fig. 5B). Subsequent quantitative analysis revealed that only 2 proteins (IL-15Rα and CCL19) demonstrated statistically significant pathway effects ($p < 0.05$) connecting T1D liability to CKM outcomes (atherosclerosis and hypertension, respectively) (Table 1).

For circulating metabolites, significant associations were observed between T1D and 80 metabolites, and between 279 metabolites and CKM outcomes (Fig. 5C, **Supplementary Tables 16,17**). Among these, 40 metabolites were associated with both T1D and CKM diseases. After direction-consistency filtering, 20 metabolites were retained for downstream analyses (Fig. 5D). Subsequent quantitative analysis revealed that only 3 metabolites (3,4-dihydroxybutyrate, N-alpha-acetylornithine, and N6-carbamoylthreonyladenosine) demonstrated statistically significant pathway effects ($p < 0.05$) connecting T1D liability to CKM outcomes (atherosclerosis, DOAAC and hypertension) (Table 1). However, because the primary

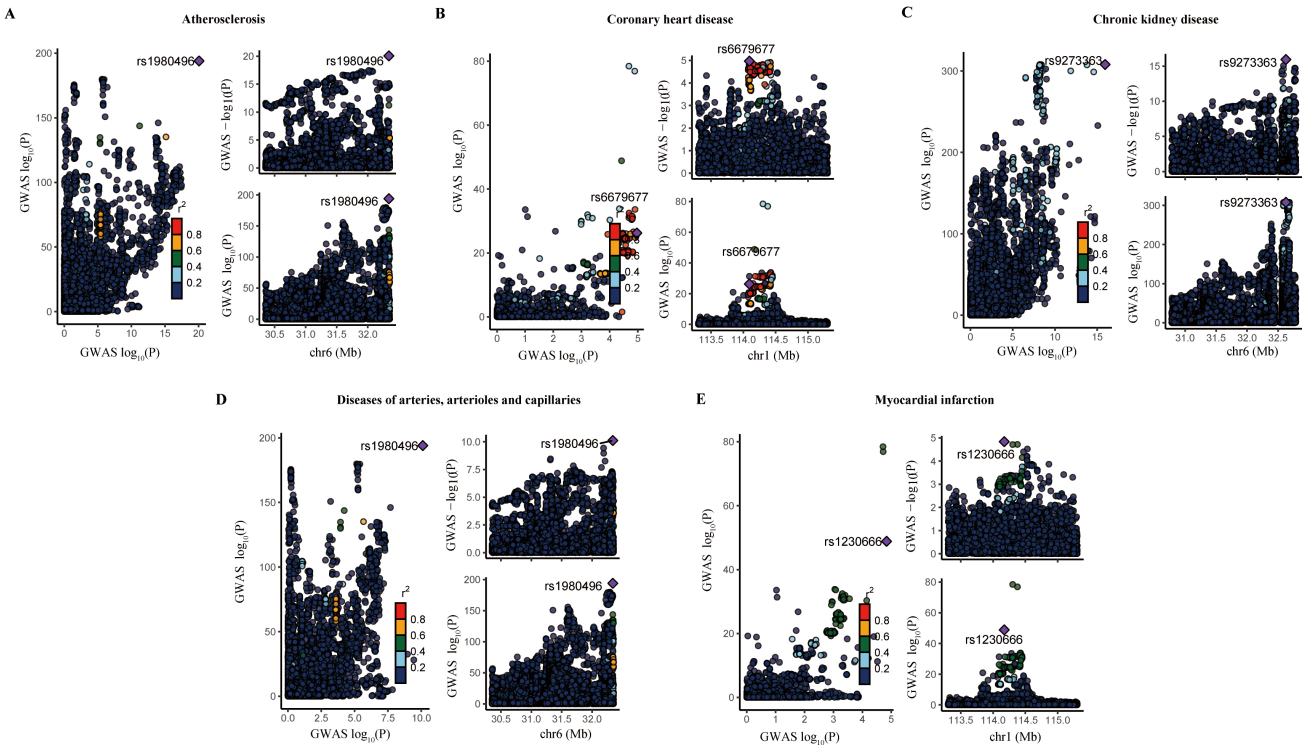

Fig. 3. Colocalization analysis of T1D with CKM traits. Colocalization analysis showed that T1D shared common variants with atherosclerosis (A), coronary heart disease (B), chronic kidney disease (C), diseases of arteries, arterioles, and capillaries (D), myocardial infarction (E). Abbreviation: Chr, Chromosome; CKM, Cardiovascular-Kidney-Metabolic; GWAS, Genome wide association studies; T1D, Type 1 diabetes.

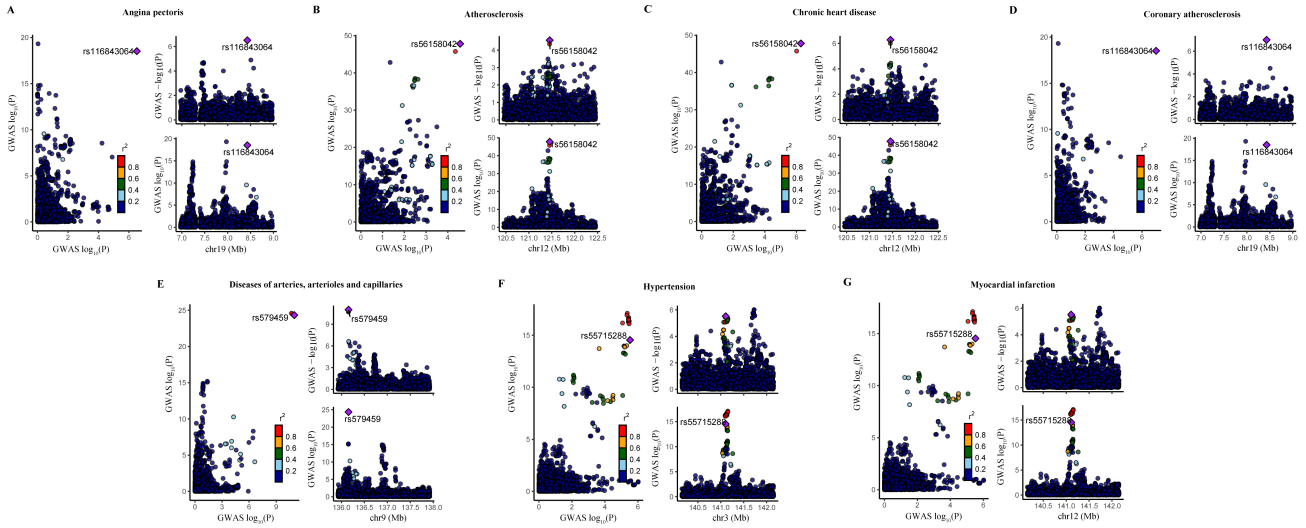

Fig. 4. Colocalization analysis of T2D with CKM traits. Colocalization analysis showed that T2D shared common variants with angina pectoris (A), atherosclerosis (B), coronary atherosclerosis (C), coronary heart disease (D), diseases of arteries, arterioles, and capillaries (E), hypertension (F), and myocardial infarction (G).

T1D–CKM associations were no longer significant after excluding the HLA region, these findings should not be interpreted as evidence of a causal mediation pathway. Instead, they likely reflect shared immune and metabolic pathways underlying both T1D and CKM diseases. In addition, because no formal multiple-testing correction was applied in this exploratory screening framework, these candidate proteins and metabolites should be interpreted cautiously.

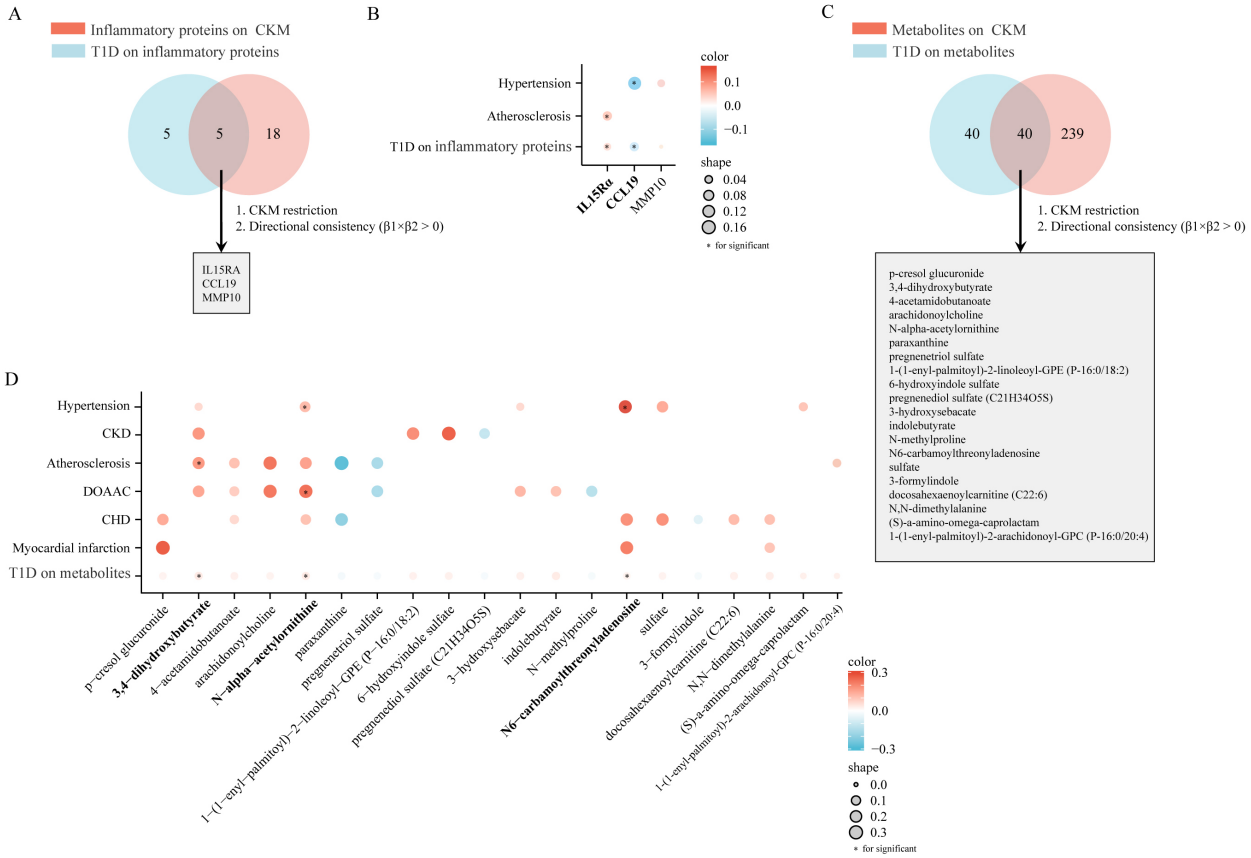

Fig. 5. Exploratory associations between T1D genetic liability and CKM traits mediated by circulating inflammatory proteins and metabolites. Venn diagram showing circulating inflammatory proteins associated with both T1D and CKM traits (A). Dot plot illustrating the associations between genetically predicted T1D and circulating proteins levels, as well as the associations between proteins and CKM traits (B). Venn diagram showing circulating metabolites associated with both T1D and CKM traits (C). Dot plot illustrating the associations between genetically predicted T1D and circulating metabolites, and between these metabolites and CKM traits (D). The downward arrows in panels (A) and (C) denote the stringent two-step filtering pipeline applied to the overlapping candidates from the Venn diagrams. The reduction in numbers is the result of applying: (1) CKM restriction (ensuring the specific outcome was significantly associated with T1D liability in the primary MR); (2) Directional consistency ($\beta1 \times \beta2 > 0$), ensuring the pathway aligns with the overarching direction. The x-axis in panels (B) and (D) represents the inflammatory proteins or metabolites. The bottom row of the y-axis indicates associations with T1D genetic liability ($\beta1$), while the remaining rows represent associations with CKM outcomes ($\beta2$). The size of the circles reflects the magnitude of the effect estimates ($\beta1$ or $\beta2$). The color indicates the direction of the estimates ($\beta1$ or $\beta2$), red for positive and blue for negative. Statistically significant results are displayed in bold. Note: These analyses are exploratory. Because the primary associations between T1D and CKM traits were not supported after excluding variants within the HLA/MHC region, the observed relationships should be interpreted as reflecting shared biological pathways rather than causal mediation. Abbreviation: CHD, Coronary heart disease; CKD, Chronic kidney disease; CKM, Cardiovascular-Kidney-Metabolic; DOAAC, Diseases of arteries, arterioles, and capillaries; T1D, type 1 diabetes.

Two-Step MR Analysis of T2D-Related Inflammatory Proteins and Metabolites

We further explored the potential biological pathways linking T2D to CKM diseases using a two-step MR framework.

For circulating inflammatory proteins, significant associations were observed between T2D and seven inflammatory proteins, as well as between 23 inflammatory proteins and T2D-related CKM outcomes (Fig. 6A, **Supplementary Tables 15,18**). Among these, TGFB1 and TNFSF12 were associated with both T2D and CKM diseases. However, after applying direction-consistency criteria, no inflammatory proteins were retained for downstream analyses.

For circulating metabolites, significant associations were observed between T2D and 107 metabolites, and between 285 metabolites and CKM outcomes (Fig. 6B, **Supplementary Tables 17,19**). Among these, 44 metabolites were associated with both T2D and CKM outcomes. After applying direction-consistency criteria,

Table 1. Exploratory pathway analysis linking T1D genetic liability to CKM traits through circulating inflammatory proteins and metabolites.

Exposure	Outcome	Effect estimate (β)	p-value
IL-15Rα	Atherosclerosis	0.0020	3.86×10^{-2}
CCL19	Hypertension	0.0066	2.95×10^{-2}
3,4-dihydroxybutyrate	Atherosclerosis	0.0058	3.23×10^{-2}
N-alpha-acetylornithine	DOAAC	0.0076	3.11×10^{-2}
N-alpha-acetylornithine	Hypertension	0.0037	2.82×10^{-2}
N6-carbamoylthreonyladenosine	Hypertension	0.0061	3.42×10^{-2}

Note: The effect estimate (β) represents the product of two-step MR coefficients ($\beta 1 \times \beta 2$). Because the associations between T1D and CKM diseases were no longer significant after excluding variants within the HLA/MHC region, these estimates are presented for exploratory purposes only and should not be interpreted as evidence of causal mediation. Instead, they reflect potential shared biological pathways linking T1D genetic liability and CKM diseases. Importantly, while a larger number of candidate proteins and metabolites passed the initial directional consistency filters (as depicted in Fig. 5B,D), only the subset of molecular effectors that demonstrated statistically significant combined pathway effects ($p < 0.05$) were retained for presentation in this final summary table.

Abbreviations: CKM, Cardiovascular-Kidney-Metabolic; DOAAC, Diseases of arteries, arterioles and capillaries; IL-15Rα, Interleukin-15 receptor alpha; CCL19, C-C motif chemokine 19; MR, Mendelian randomization; T1D, type 1 diabetes.

28 metabolites were retained for downstream analyses (Fig. 6C). Subsequent mathematical derivation of the indirect effect revealed that only a specific subset [1-(1-enyl-palmitoyl)-2-linoleoyl-GPC (P-16:0/18:2), 1-(1-enyl-palmitoyl)-2-oleoyl-GPC (P-16:0/18:1), cholate, N-oleoylserine, Sphingomyelin (d17:1/16:0, d18:1/15:0, d16:1/17:0), Sphingomyelin (d18:1/20:2, d18:2/20:1, d16:1/22:2), Sphingomyelin (d18:2/16:0, d18:1/16:1), Sphingomyelin (d18:2/23:0, d18:1/23:1, d17:1/24:1), Sphingomyelin (d18:2/24:1, d18:1/24:2)] demonstrated statistically significant causal mediation (Table 2). These findings suggest that circulating metabolites may partially explain the association between T2D and CKM traits. Besides, given the exploratory nature of the screening and the lack of formal multiple-testing correction, these candidates require independent validation.

Discussion

In the present study, using multi-omics and MR methods, we examined the correlation between diabetes and CKM diseases. We also assessed the potential role of circulating inflammatory factors and metabolites in this relationship. The results suggest that diabetes is associated with CKM diseases, such as coronary heart disease, DOAAC, and hypertension, and that inflammatory factors and metabolites may be involved in the biological pathways linking diabetes with CKM diseases. For T2D, the findings were consistent with a potential causal role in CKM diseases, whereas for T1D, the observed associations appeared to be more consistent with shared immune-mediated mechanisms.

CKM diseases have emerged as a major focus of clinical research in recent years, particularly in the assessment of cardiovascular risks and kidney damage in diabetic patients [22]. Extensive literature confirms that poor glycemic control drastically magnifies cardiovascular vulnerability, predisposing patients to arterial blockages, systemic hypertension, and ischemic events [2]. Additionally, diabetes and chronic kidney disease are closely interconnected, with diabetes representing an important risk factor for chronic kidney disease [23]. In the United States, the cost of treating CKM diseases in patients with diabetes is much higher than the cost of treating diabetes alone [24]. In this study, we also found that diabetes is associated with CKM traits, such as coronary heart disease, atherosclerosis, hypertension, and chronic kidney disease. Therefore, studying the mechanisms by which diabetes contributes to cardiovascular risk and kidney damage, and identifying potential therapeutic targets, is of great importance.

The biological mechanisms linking diabetes with cardiovascular and kidney diseases are complex. Studies have reported that diabetes not only leads to elevated blood glucose levels but also causes inflammation, dyslipidemia, such as high triglycerides, promotes endothelial cell dysfunction, and accelerates atherosclerosis, thereby increasing the risk of cardiovascular diseases and stroke [25]. Additionally, the inflammatory and hyperglycemic state in diabetes can cause microvascular damage in the kidneys, and lipid deposition within renal tubular cells can further trigger inflammation and damage [26]. Metabolomics is an emerging biological technology that has gained significant attention in recent years because of its distinct advantages over other methods [27]. It enables the identification of novel

Table 2. Two-step MR analysis of circulating metabolites linking T2D and CKM traits.

Exposure	Outcome	Effect estimate (β)	p-value	Proportion explained (%)
1-(1-enyl-palmitoyl)-2-linoleoyl-GPC (P-16:0/18:2)	Coronary heart disease	0.0526	1.13×10^{-3}	26.04%
1-(1-enyl-palmitoyl)-2-linoleoyl-GPC (P-16:0/18:2)	DOAAC	0.0646	2.72×10^{-3}	23.61%
1-(1-enyl-palmitoyl)-2-linoleoyl-GPC (P-16:0/18:2)	Angina pectoris	0.0685	6.71×10^{-4}	29.31%
1-(1-enyl-palmitoyl)-2-linoleoyl-GPC (P-16:0/18:2)	Coronary atherosclerosis	0.0559	8.62×10^{-4}	28.93%
1-(1-enyl-palmitoyl)-2-linoleoyl-GPC (P-16:0/18:2)	Myocardial infarction	0.0504	3.90×10^{-3}	24.62%
1-(1-enyl-palmitoyl)-2-linoleoyl-GPC (P-16:0/18:2)	Hypertension	0.0351	2.44×10^{-2}	18.59%
1-(1-enyl-palmitoyl)-2-oleoyl-GPC (P-16:0/18:1)	Hypertension	0.0127	2.89×10^{-2}	7.60%
cholate	Angina pectoris	0.0311	4.68×10^{-2}	15.86%
cholate	Coronary atherosclerosis	0.0284	4.29×10^{-2}	17.13%
N-oleoylserine	Coronary atherosclerosis	0.0099	4.29×10^{-2}	6.73%
N-oleoylserine	Atherosclerosis	0.0193	1.91×10^{-2}	6.71%
N-oleoylserine	DOAAC	0.0192	2.04×10^{-2}	8.40%
N-oleoylserine	Coronary heart disease	0.0129	1.83×10^{-2}	7.93%
Sphingomyelin (d17:1/16:0, d18:1/15:0, d16:1/17:0)	Hypertension	0.0074	3.62×10^{-2}	4.56%
Sphingomyelin (d17:1/16:0, d18:1/15:0, d16:1/17:0)	Angina pectoris	0.0108	4.46×10^{-2}	6.11%
Sphingomyelin (d18:1/20:2, d18:2/20:1, d16:1/22:2)	DOAAC	0.0298	2.45×10^{-2}	12.47%
Sphingomyelin (d18:1/20:2, d18:2/20:1, d16:1/22:2)	Atherosclerosis	0.0281	2.76×10^{-2}	9.46%
Sphingomyelin (d18:2/16:0, d18:1/16:1)	Coronary heart disease	0.0306	2.37×10^{-2}	16.98%
Sphingomyelin (d18:2/23:0, d18:1/23:1, d17:1/24:1)	Hypertension	0.0189	1.61×10^{-2}	10.94%
Sphingomyelin (d18:2/24:1, d18:1/24:2)	Hypertension	0.0533	3.83×10^{-2}	25.74%

Note: The effect estimate (β) represents the product of the two-step MR coefficients ($\beta 1 \times \beta 2$). The proportion explained was calculated as the ratio of the indirect effect to the total effect. These results are interpreted in the context of the primary MR findings supporting a potential causal effect of T2D on CKM traits. Furthermore, while 28 metabolites successfully passed the initial directional consistency filter (as illustrated in Fig. 6C), only the subset of metabolites that achieved statistically significant indirect causal mediation ($p < 0.05$) were included in this final quantitative summary.

Abbreviations: CKM, Cardiovascular-Kidney-Metabolic; DOAAC, Diseases of arteries, arterioles and capillaries; MR, Mendelian randomization; T2D, Type 2 diabetes.

disease biomarkers, uncovers metabolic pathways linked to disease mechanisms, and provides a direct reflection of the biochemical activities and overall state of an organism [28]. Through MR and leveraging existing metabolomics data [15], we investigated the potential role of circulating metabolites in the relationship between diabetes and CKM diseases. Our findings suggest that 1-(1-enyl-palmitoyl)-2-linoleoyl-GPC (P-16:0/18:2), N-oleoylserine, N-alpha-acetylornithine and several sphingomyelin may play a significant role in the cardiovascular complications triggered by diabetes.

In this study, we found that diabetes was associated with decreased circulating levels of 1-(1-enyl-palmitoyl)-2-linoleoyl-GPC (P-16:0/18:2) and N-oleoylserine, which are consistent with previous findings [29,30]. Previous studies suggested that 1-(1-enyl-palmitoyl)-2-linoleoyl-GPC (P-16:0/18:2), N-oleoylserine play protective roles in the heart and vascular tissues, and low levels are associated with greater severity and poor prognosis of cardiovascular diseases [29,30]. Similarly, our results show that reduced levels of circulating 1-(1-enyl-palmitoyl)-2-linoleoyl-GPC (P-16:0/18:2) and N-oleoylserine are associated with increased risks of coronary heart disease, DOAAC, myocardial infarction, and atherosclerosis. Cardiovascular diseases are often linked to oxidative stress and inflammatory responses [31,32]. A decrease in 1-(1-enyl-palmitoyl)-2-linoleoyl-GPC (P-16:0/18:2) may weaken the ability of cardiomyocyte and vascular endothelial cells to combat oxidative stress, exacerbating inflammation and promoting disease progression [33]. Additionally, N-oleoylserine and 1-(1-enyl-palmitoyl)-2-linoleoyl-GPC (P-16:0/18:2) are involved in regulating membrane fluidity and signaling, which is crucial for the proper function of cardiomyocyte and vascular endothelial cells [34,35]. The reduced levels of 1-(1-enyl-palmitoyl)-2-linoleoyl-GPC (P-16:0/18:2) and N-oleoylserine could increase the risk of cardiovascular diseases such as atherosclerosis [29,35].

Xu *et al.* [36] reported significantly lower serum sphingomyelin levels in individuals with impaired fasting glucose or T2D compared to healthy controls. Consistently, we also found that diabetes reduces circulating sphingomyelin levels, and that the reduction of sphingomyelin may contribute to the association between T2D and CKM traits. Several studies on the association between circulating sphingomyelin levels and the incidence of cardiovascular diseases have yielded inconsistent results. Fernandez *et*

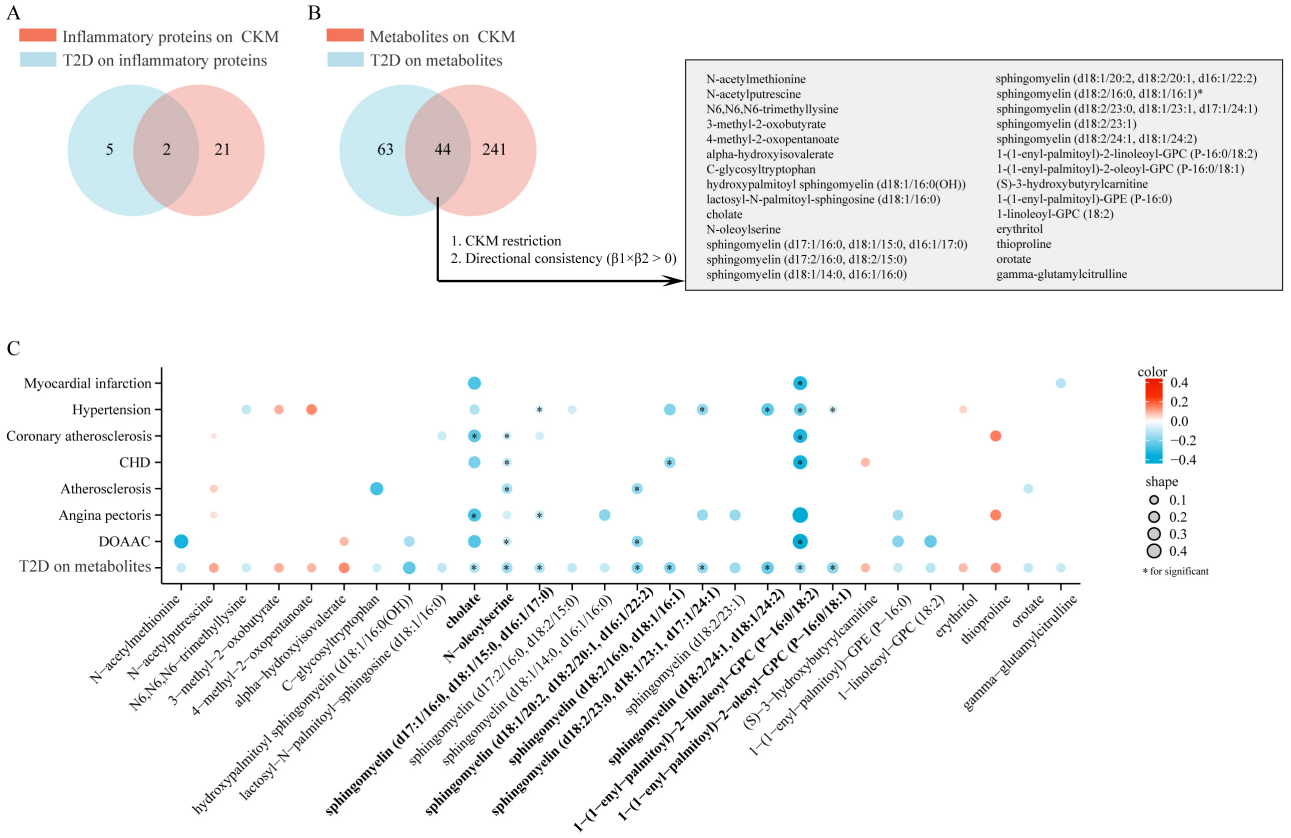

Fig. 6. Two-step MR analysis linking T2D and CKM traits through circulating inflammatory proteins and metabolites. Venn diagram shows 2 circulating inflammatory proteins linked to both T2D and CKM traits (A). Venn diagram shows 44 circulating metabolites linked to both T2D and CKM traits (B). Dotplot for effect of T2D on the levels of 28 circulating metabolites and their effect on related CKM traits (C). The downward arrows in panels (A) and (B) denote the stringent two-step filtering pipeline applied to the overlapping candidates from the Venn diagrams. The reduction in numbers is the result of applying: (1) CKM restriction (ensuring the specific outcome was significantly associated with T2D liability in the primary MR); (2) Directional consistency ($\beta 1 \times \beta 2 > 0$), ensuring the pathway aligns with the overarching direction. The x-axis of the figure represents the mediator variables. The bottom row of the y-axis indicates the effect of T2D on the metabolites ($\beta 1$), while the remaining rows of the y-axis represent the effect of metabolites on the outcomes ($\beta 2$). The size of the circles in the figure reflects the magnitude of the single-step causal estimates ($\beta 1$ or $\beta 2$), while the color indicates the direction of the single-step causal estimates ($\beta 1$ or $\beta 2$), red for positive and blue for negative. Statistically significant results are displayed in bold. Abbreviation: CHD, Coronary heart disease; CKM, Cardiovascular-Kidney-Metabolic; DOAAC, Diseases of arteries, arterioles, and capillaries; T2D, type 2 diabetes.

al. [37] reported that Sphingomyelin (38:2) was the only subtype associated with an increased risk of cardiovascular disease; however, Yeboah *et al*. [38] found that high plasma sphingomyelin levels were not associated with an increased risk of coronary heart disease. Interestingly, our study found that certain sphingomyelin subtypes may actually reduce the risk of cardiovascular disease. This differential, subtype-specific effect is likely driven by their structural diversity, specifically their carbon chain length and saturation patterns. Existing lipidomic literature suggests that while long-chain saturated sphingolipids are often pro-inflammatory and promote endothelial apoptosis [39], very long-chain species tend to be cardioprotective by maintaining vascular membrane integrity [40]. Furthermore, the presence of unsaturated double bonds enhances cell membrane fluidity. This increased fluidity prevents the rigidification of lipid rafts, thereby dampening inflammatory receptor clustering and protecting against vascular endothelial dysfunction [41]. Thus, our findings provide genetic evidence supporting the paradigm that the biological impact of sphingomyelins is tightly regulated by acyl chain length and desaturation.

Contemporary literature posits that autoimmune-driven diabetes accelerates vascular decline primarily via systemic immune dysregulation [42,43]. Previous studies have also shown that circulating IL-15Rα levels are elevated in T1D, which may be related to the abnormal immune system activation and chronic low-grade inflammation associated with the disease [44,45]. Studies have further reported that plasma IL-15 and IL-15Rα levels are

higher in patients with coronary artery disease compared to healthy patients [46]. IL-15Rα may exacerbate inflammatory responses in the arterial intima by promoting inflammation and recruiting immune cells, thereby contributing to the formation and progression of atherosclerotic plaques. In the current study, we found that circulating IL-15Rα is associated with atherosclerosis, and that T1D genetic liability was associated with increased circulating IL-15Rα levels. However, because the primary associations between T1D and CKM diseases were no longer significant after excluding variants within the HLA/MHC region, these findings should not be interpreted as evidence that T1D directly causes atherosclerosis through IL-15Rα. Rather, they suggest that T1D and CKM diseases may share immune-related mechanisms involving inflammatory pathways such as IL-15Rα. This finding enriches our understanding of the potential biological links between T1D and atherosclerosis. However, further functional and genetic studies are needed before therapeutic implications can be drawn. Importantly, our leave-HLA-out sensitivity analysis showed that the T1D–CKM associations were largely attenuated after excluding the HLA region, further indicating that the observed T1D findings are primarily driven by this immune-related locus rather than reflecting a direct causal effect. This interpretation is also consistent with the discrepancy observed between MR and LDSC results. While MR initially suggested associations, no significant genetic correlations were detected in the LDSC analysis. This difference likely reflects the distinct methodological frameworks and the unique genetic architecture of T1D. LDSC evaluates genome-wide polygenic overlap and typically excludes the MHC region due to its complex linkage disequilibrium structure. Given that T1D genetic susceptibility is heavily concentrated within the HLA region rather than distributed across the genome, LDSC may fail to capture this dominant signal. Taken together, these results suggest that the relationship between T1D and CKM diseases is unlikely to reflect a widespread polygenic causal effect, but is more consistent with a shared immune-related genetic architecture concentrated within the HLA/MHC region. Therefore, the LDSC and MR findings should be viewed as complementary, jointly supporting an immune-mediated rather than causal interpretation of the T1D–CKM association.

Our study has several limitations. First, although MR-Egger intercept tests did not detect evidence of directional pleiotropy, residual pleiotropy cannot be fully excluded, particularly for T1D due to the complex linkage disequilibrium structure and structural pleiotropy within the HLA region. Second, the genetic variants used to proxy diabetes reflect its long-term effects and may not accurately represent the short-term impacts of the condition. Third, since our research was conducted within a European population, additional studies are needed to confirm these findings in other populations. Besides, in this study, we used a p-value threshold of $<5 \times 10^{-8}$ to select inflammatory proteins and metabolites for the mediation analysis. While this approach was intended to ensure that the instrumental variables were sufficiently strong, reduce weak instrument bias, control for false positives due to multiple testing, and enhance the reliability of causal inference, most candidates were excluded from the analysis. As a result, it is possible that some metabolic and inflammatory factors playing a mediating role in the relationship between diabetes and CKM were not included in the analysis. Finally, the two-step MR analyses involved screening a large number of correlated inflammatory proteins and metabolites. Although the Bonferroni correction may be overly conservative in this setting, we did not apply a formal multiple-testing correction. Therefore, some of the identified candidate mediators may represent false-positive findings, and these exploratory results should be interpreted cautiously until replicated in independent datasets or supported by functional evidence.

Conclusion

In conclusion, this study provides multi-omics insights into the relationships between diabetes and CKM diseases and their underlying biological mechanisms. Our findings support a potential causal role of T2D in the development of CKM diseases, with circulating metabolites likely partly explaining these observed associations. In contrast, the associations observed for T1D appear to be largely driven by the HLA/MHC region and are more consistent with shared immune-mediated genetic mechanisms than with a direct causal effect. These findings provide genetic evidence for the heterogeneous mechanisms linking different forms of diabetes with CKM diseases and may offer clues for future research and the development of potential therapeutic targets.

Availability of Data and Materials

All datasets used in this study are included in the article. Further data used and analyzed are available from the corresponding author upon reasonable request.

Author Contributions

YXW and BL designed the research study. YXW performed the research. NL collected and analyzed the data. BL and NL have been involved in drafting the manuscript and all authors have been involved in revising it critically for important intellectual content. All authors gave final approval of the version to be published. All authors have participated sufficiently in the work to take public responsibility for appropriate portions of the content and agreed to be accountable for all aspects of the work in ensuring that questions related to its accuracy or integrity.

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.24976/Discov.Med.202638208.113.

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