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Role of CD33 basophils in mediating the effect of lipidome on chronic kidney disease

A 2-sample, 2-variable, bidirectional Mendelian randomization analysis

Qi Li, MM[a], Haoyu Chen, MD[a], Hui Gao, MD[b], Xiaona Wei, MD[b,*], Dong Bian, MM[b], Linlin Zheng, MD[a], Hongyu Wei, MD[a], Wanqing Wang, MM[a], Yashi Wang, MM[a], Wanying Deng, MM[a]

Abstract

This study aimed to investigate the causal relationship between lipidomes and chronic kidney disease (CKD) and identify and quantify the role of immune cells as a potential mediator. Using summary-level data from a genome-wide association study, a 2-sample Mendelian randomization (MR) analysis of genetically predicted lipidomes (7174 cases) and CKD (406,745 cases) was performed. Furthermore, we used 2-step MR to quantitate the proportion of the effect of immune cells traits–mediated lipidomes on CKD. The MR analysis revealed a causal relationship between lipidomes and CKD, with different lipidomes either increasing or decreasing the risk of CKD. Immune cells may serve as intermediaries in the pathway from lipidomes to CKD. Our study indicates that CD33 on basophils accounts for 3.23% of the reduced risk associated with triacylglycerol (53:3) levels in CKD. In conclusion, our study has identified a causal relationship between lipidomes and CKD, as well as the mediating role of CD33 on basophils. However, other risk factors like potential mediators require further investigation. In clinical practice, particular attention should be paid to lipidomic changes, especially triacylglycerol, in patients with CKD.

Abbreviations: AC = absolute cell, CKD = chronic kidney disease, DC = dendritic cells, GWAS = genome-wide association studies, IV = instrumental variable, IVW = inverse-variance weighting, MR = Mendelian randomization, MR-PRESSO = MR pleiotropy residual sum and outliers, OR = odds ratio, SNP = single-nucleotide polymorphism, Treg = regulatory T.

Keywords: chronic kidney disease, immune cell, lipidome, Mendelian randomization

1. Introduction

Chronic kidney disease (CKD) is an increasingly serious public health issue worldwide, characterized by the gradual loss of kidney function, often accompanied by structural damage.[1] Furthermore, CKD is an incurable progressive disease that advances slowly, and it may not present noticeable symptoms in its early stages, leading to many patients being diagnosed only in the later stages of the illness.[2] According to the latest survey, CKD is a significant global public health issue, with a median prevalence rate as high as 9.5% worldwide.[3] Currently, the incidence of CKD is on the rise globally, with its prevalence and disease burden continuously increasing over the past few decades, making it a major health challenge worldwide.[4] This situation is closely related to the worsening environmental pollution, as well as the prevalence of chronic diseases such as diabetes and hypertension.[5–7] In addition, due to the complexity of CKD mechanisms involving various biological processes, as CKD progresses, patients experience a progressive decline in kidney function, leading to complications such as anemia, bone disease, and cardiovascular diseases, and may eventually progress to end-stage kidney disease, requiring kidney replacement therapy.[8] Consequently, investigating the pathogenesis of CKD is of great significance for guiding proactive and effective prevention strategies.

QL, HC, and HG contributed to this article equally.

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The authors have no conflicts of interest disclose.

The data that supports the findings of this study is available from the corresponding author upon reasonable request. Furthermore, the relevant data for exposure factors and outcome variables can be accessed from publicly available databases. Chronic kidney disease data is sourced from the Finngen database (https://storage.googleapis.com/finngen-public-data-r10/summary_stats/). Immune cell and liposome data can be obtained from the GWAS (Genome-Wide Association Studies，https://gwas.mrcieu.ac.uk/) database.

Ethical clearance was not required for this study as no patients were involved in the development of the research questions or their outcome measures. In addition, only secondary analyses using published genome-wide association studies summary statistics in the public domain were conducted for this study.

Supplemental Digital Content is available for this article.

[a] School of Graduate, Hebei University of Chinese Medicine, Hebei, Shijiazhuang, China, [b] Department of Nephropathy, Hebei Province Hospital of Chinese Medicine, Hebei, Shijiazhuang, China.

** Correspondence: Xiaona Wei, Department of Nephropathy, Hebei Province Hospital of Chinese Medicine, Hebei, Shijiazhuang, China (e-mail: wxncam@163.com).*

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Lipids are a class of important and highly diverse molecules that can perform and regulate many key functions, including redox balance, energy storage, intracellular and extracellular signal transduction, acute and chronic inflammation, and metabolic homeostasis.[9] It is currently believed that dysregulation of lipid oxidation, lipid uptake, and fat generation is considered one of the causes of CKD.[10] For instance, the reduction and disappearance of fatty acid oxidation can lead to renal tubular damage and fibrosis. A study has found that fatty acid oxidation and its corresponding regulatory genes are downregulated in mouse models of renal tubular interstitial fibrosis and renal tubular interstitial fibrosis.[11] CKD is also associated with metabolic disorders of phosphatidylcholine, and the dysregulation of phosphatidylcholine metabolism is related to the pathological severity of CKD.[12] In addition, lipid metabolism disorders in human kidney cells are paralleled by the accumulation of hydroperoxides of lipids such as triglycerides, phosphatidylcholine, and phosphatidylethanolamine.[13] A large cohort study found that lipid components such as high-density lipoprotein cholesterol and triglyceride lipids in metabolites are one of the possible causes driving adverse cardiac events.[14] Thus, lipids are closely linked to the development and progression of CKD. However, due to the lack of related research, it is currently unknown which specific lipid has a definitive causal relationship with CKD.

In addition, immunity is highly likely to mediate the link between lipids and CKD. Current research has found that lipids play a role in regulating immune responses in a variety of diseases. For example, changes in the lipidome of macrophages can coordinate macrophage polarization and survival in tumors by inducing endoplasmic reticulum stress responses, thereby maintaining the host's antitumor immunity.[15] Methyltransferases can act as epigenetic regulators of T cell–mediated autoimmunity by remodeling phospholipids, thereby serving as a brake and potentially offering treatment for neuroinflammatory diseases.[16] CKD, as a chronic condition, inherently has significant associations with immune cells. For instance, circulating innate lymphoid cell subsets are markedly increased in various types of CKD and are associated with clinical pathological characteristics.[17] The localization changes of kidney-resident macrophages can reflect the dysregulation of the local immune system after acute injury, and the imbalance of macrophages may increase the risk of CKD.[18] In conclusion, lipids may potentially affect the onset and progression of CKD by modulating the immune system, although there is currently a lack of clear and specific research in this area.

Mendelian randomization (MR) refers to an analytical method for assessing the causal relationship between exposures or risk factors and clinically relevant outcomes.[19,20] MR can reduce the impact of unmeasured errors or confounding factors while avoiding reverse causality through Mendelian inheritance laws.[21] This research group utilizes a comprehensive 2-sample, 2-directional MR analysis to evaluate the causal relationship and strength between the lipidome and CKD and employs mediating MR to observe the intermediary role of immune cells between these. Through this study, we have gained a deeper understanding of the complex interplay among these biomarkers and their potential mechanisms preceding CKD. This knowledge can inform future basic research and provide valuable insights for clinical preventive practices targeting CKD.

2. Materials and methods

2.1. Design of study

In this study, we explored the causal relationship between the lipidome and CKD using the 2-sample bidirectional MR approach. The design is illustrated in Figure 1. Moving forward, we will utilize the mediation MR approach to investigate the mediating role of immune cells in how lipidomes influence CKD.[22] In our research, single-nucleotide polymorphisms (SNPs) were defined as instrumental variables (IVs).[23,24] All MR analyses adhere to the following assumptions: the relevance assumption, which indicates a stable and significant correlation between genetic variation and exposure factors; the independence assumption, which states that confounders affecting the relationship between exposure factors and outcomes do not correlate with genetic variation; and the exclusion restriction assumption, which asserts that exposure factors are the only pathway through which genetic variation

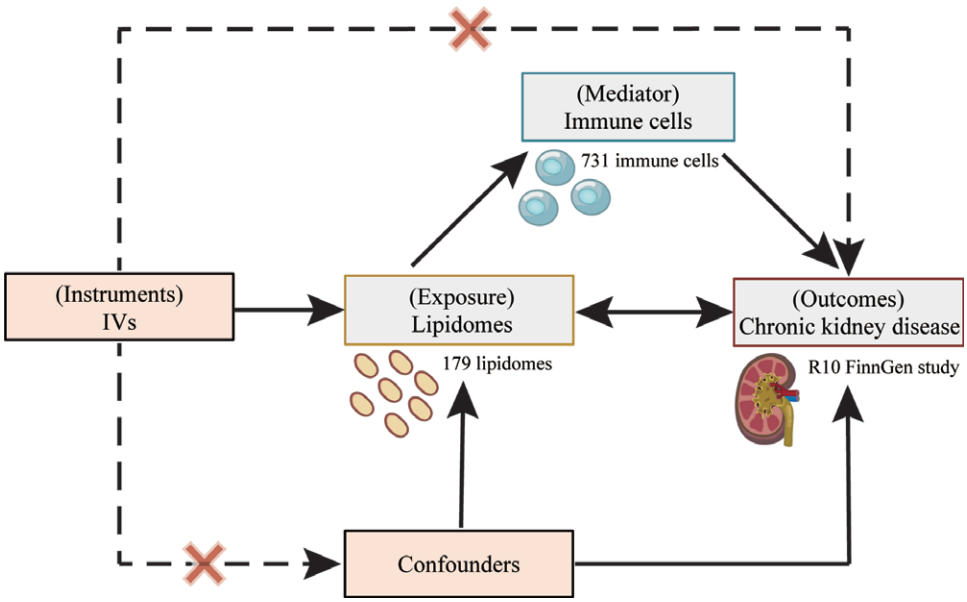

Figure 1. Bidirectional and mediation MR analysis assumptions and design. Initially, forward MR is conducted to investigate the causal relationship between 179 lipidomes and CKD. Subsequently, forward MR is performed to explore the causal relationship between 731 immune cell traits and CKD. Thereafter, forward MR is applied to the lipidomes and immune cells that were positively identified to establish their causal relationships. Second, reverse MR between lipidomes and CKD is conducted to rule out potential reverse associations. Finally, mediation MR analysis is performed to detect potential mediating immune traits. CKD = chronic kidney disease, IV = instrumental variable, MR = Mendelian randomization.

affects outcomes, not via other routes. The schematic diagram is shown in Figure 2A.

2.2. Summary of data sources

The aggregate lipidomics data were extracted from a curated genome-wide association studies (GWAS) database derived from the GeneRISK cohort, encompassing a comprehensive analysis of 179 distinct lipid species in 7174 individuals (4642 females and 2624 miles) from Southern Finland (accession numbers GCST90277238–GCST90277416).[25] This study employed shotgun lipidomics to perform mass spectrometry analysis of lipids in the samples, covering 13 defined lipid classes, including 4 major lipid categories: triglycerides, glycerophospholipids, sphingolipids, and sterols. The original research publication provides a detailed methodology, including protocols for sample collection, stringent quality control measures, and the inductive techniques utilized.

The summary information of each immune cell comes from GWAS statistical data. The information is publicly available from the GWAS Catalog (accession number is GCST90001391–GCST90002121).[26] This compilation encapsulates the findings from GWAS that have delved into various traits of immune cells, encompassing a comprehensive set of 731 immune phenotypes. The analyses were conducted using flow cytometry within a broad population cohort and scrutinized several key metrics: absolute cell (AC) counts for 118 different cell types, median fluorescence intensity as an indicator of surface antigen levels across 389 parameters, morphological parameters for 32 distinct cellular features, and relative cell counts for 192 cell populations. The research extended its scope to include a detailed analysis of T cells, B cells, and natural killer cells, the maturation stages of T cells, regulatory T (Treg) cells, dendritic cells (DCs), B cells, monocytes, and a myeloid cell panel. The GWAS estimation for this study was meticulously carried out by examining Sardinian sequences on a reference panel comprising 3514 individuals, probing for associations with 20,143,392 SNPs and 1,688,858 indels (insertions and deletions of DNA sequences).

The CKD data for CKD came from the R10 dataset published by the FinnGen study,[27] with a total sample size of 406,745 a case group of 10,039.

2.3. IV selection and data harmonization

The significance level of the IV for each lipidome and immune cell was set to 1×10^{-5}, according to recent studies.[28,29] These SNPs (linkage disequilibrium r^2 threshold < 0.1 within 500 kb distance) were trimmed using the clump function in the R package TwoSampleMR,[30] where linkage disequilibrium r^2 was a control calculated according to the 1000 Genomes Project as a reference plate. For CKD, the data were screened and analyzed to enhance the reliability of the results ($P < 5 \times 10^{-8}$) by referring to multiple publications and developing stricter criteria.[31,32]

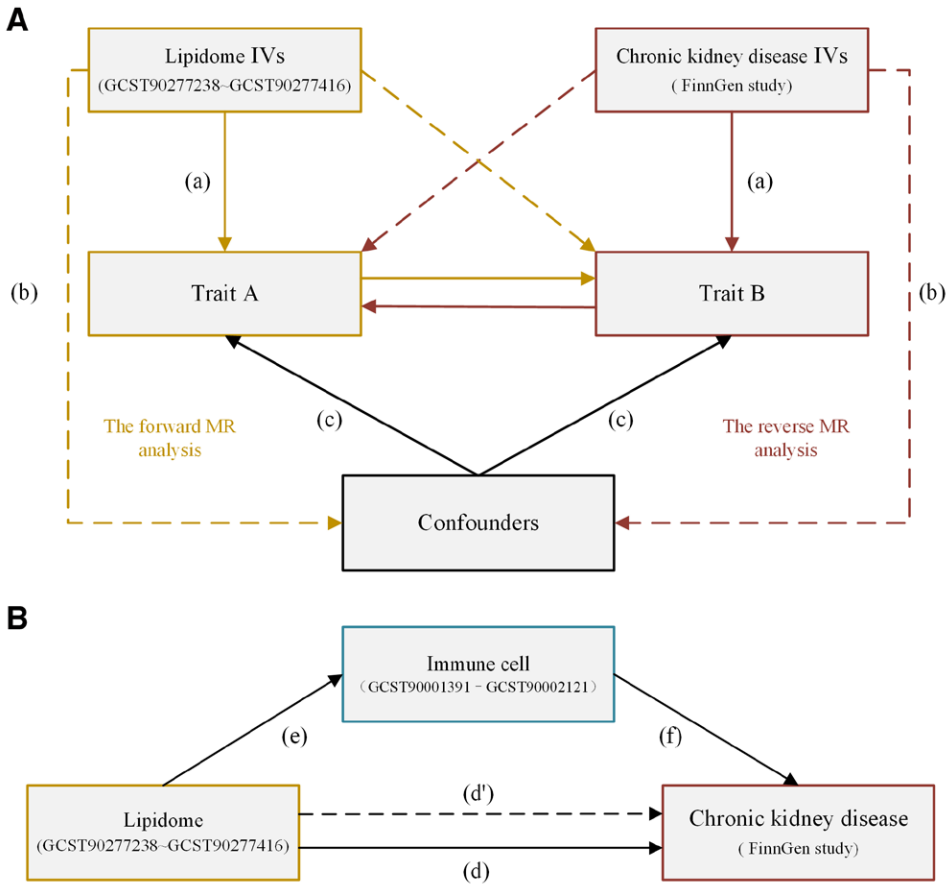

Figure 2. Schematic diagram. (A) a: The stable and significant correlation between genetic variation and exposure factors. b: The confounders affecting the relationship between exposure factors and outcomes do not correlate with genetic variation. c: Exposure factors are the only way in which genetic variation affects outcomes, not through other routes. (B) d: The arrow represents the total effect of the lipidome on chronic kidney disease. d′: The dashed arrow represents the direct effect of the lipidome on chronic kidney disease after accounting for the mediating effect of immune cells is removed. e: The arrow represents the effect of the lipidome on immune cells. f: The arrow represents the effect of the immune cell on chronic kidney disease. IV = instrumental variable, MR = Mendelian randomization.

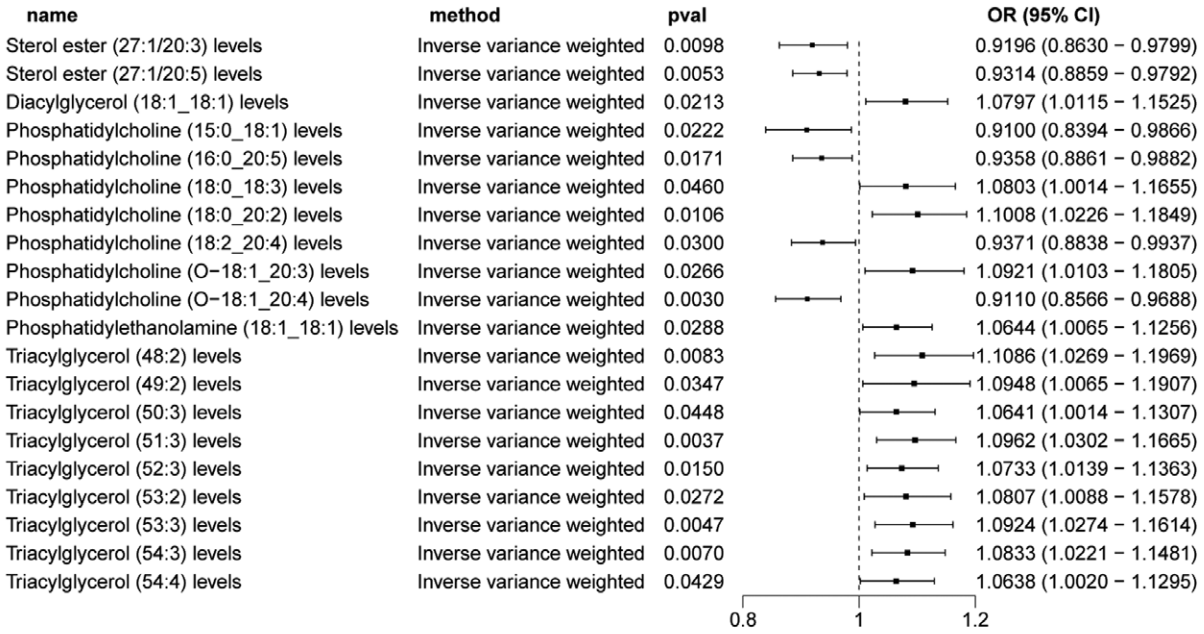

Figure 3. Forest plot of the MR analysis of lipidomes on CKD. CI = confidence interval, CKD = chronic kidney disease, IV = instrumental variable, MR = Mendelian randomization, OR = odds ratio.

Meanwhile, we calculated the proportion of phenotypic variation explained and *F*-statistics for each IV to assess IV strength and avoid weak instrument bias.

2.4. 2-sample bidirectional MR analysis

The analyses were conducted utilizing R software, specifically version 4.3.2, which can be accessed at the Comprehensive R Archive Network website (https://cran.r-project.org/). We investigated the causal interplay between 179 liposomes, 731 immunophenotypes, and CKD. In addition, we explored the causal links between the 731 immune phenotypes and CKD. Primarily, we utilized the "TwoSampleMR" package[30] to execute a suite of methodological approaches including inverse-variance weighting (IVW),[33] weighted median-based,[34] MR-Egger,[35] and model-based analyses.[36] The MR-Egger regression algorithm was implemented to mitigate the potential biases introduced by horizontal pleiotropy.[35] The presence of a significant intercept term in the MR-Egger regression would indicate the existence of horizontal pleiotropy. To further refine our analysis and exclude any outliers that could significantly skew the estimation outcomes, we applied the MR pleiotropy residual sum and outliers (MR-PRESSO) technique using the MR-PRESSO package.[24] To visualize our findings, we generated scatter plots and forest plots for a clear and comprehensive representation of the data.

2.5. Mediation analysis

We further conducted a mediation MR analysis to explore whether immune cells mediate the causal pathway from the lipidome to the outcomes of CKD (as shown in Figure 2B). The total effect can be decomposed into an indirect effect (via the mediator) and a direct effect (without mediation). The total effect of the lipidome on CKD (represented as d in Figure 2B) can be divided into the direct effect of the lipidome on CKD (indicated as d′ in Figure 2B) and the indirect effect mediated by immune cells (depicted as e × f in Figure 2B).

3. Results

3.1. Exploring the causal impact of lipidomes onset on CKD

First, we conducted a forward MR analysis of 179 lipidomes with CKD. We used an IVW selection criteria with a threshold of $P < 5e{-}5$ for the exposure factor and a $P < .05$ for the outcome factor, resulting in 22 findings. Subsequently, to obtain more reliable data, we applied Bayesian weighted Mendelian randomization[37] for a secondary screening, using a threshold of $P < .05$, which yielded 20 results. Finally, we identified 20 lipidome species associated with the risk of CKD.

According to the IVW method, we obtained odds ratios (ORs) and *P* value for 20 lipidome species associated with CKD are as follows: Sterol ester (27:1/20:3) levels (OR: 0.9196 [0.8630–0.9799], *P* = .0098); sterol ester (27:1/20:5) levels (OR: 0.9314 [0.8859–0.9792], *P* = .0053); diacylglycerol (18:1_18:1) levels (27:1/20:3) levels (OR: 1.0797 [1.0115–1.1525], *P* = .0213); phosphatidylcholine (15:0_18:1) levels (OR: 0.9100 [0.8394–0.9866], *P* = .0222); phosphatidylcholine (16:0_20:5) levels (OR: 0.9358 [0.8861–0.9882], *P* = .0171); phosphatidylcholine (18:0_18:3) levels (OR: 1.0803 [1.0014–1.1655], *P* = .0460); phosphatidylcholine (18:0_20:2) levels (OR: 1.1008 [1.0226–1.1849], *P* = .0106]; phosphatidylcholine (18:2_20:4) levels (OR: 0.9371 [0.8838–0.9937], *P* = .0300); phosphatidylcholine (O-18:1_20:3) levels (OR: 1.0921 [1.0103–1.1805], *P* = .0266); phosphatidylcholine (O-18:1_20:4) levels (OR: 0.9110 [0.8566–0.9688], *P* = .0030); phosphatidylethanolamine (18:1_18:1) levels [OR: 1.0644 [1.0065–1.1256], *P* = .0288); triacylglycerol (48:2) levels (OR: 1.1086 [1.0269–1.1969], *P* = .0083); triacylglycerol (49:2) levels (OR: 1.0948 [1.0065–1.1907], *P* = .0347); triacylglycerol (50:3) levels (OR: 1.0641 [1.0014–1.1307], *P* = .0448); triacylglycerol (51:3) levels (OR: 1.0962 [1.0302–1.1665], *P* = .0037); triacylglycerol (52:3) levels (OR: 1.0733 [1.0139–1.1363], *P* = .0150); triacylglycerol (53:2) levels (OR: 1.0807 [1.0088–1.1578], *P* = .0272); triacylglycerol (53:3) levels (OR: 1.0924 [1.0274–1.1614], *P* = .0047); triacylglycerol (54:3) levels (OR: 1.0833 [1.0221–1.1481], *P* = .0070); triacylglycerol (54:4) levels (OR: 1.0638 [1.0020–1.1295], *P* = .0429). The results are presented in the

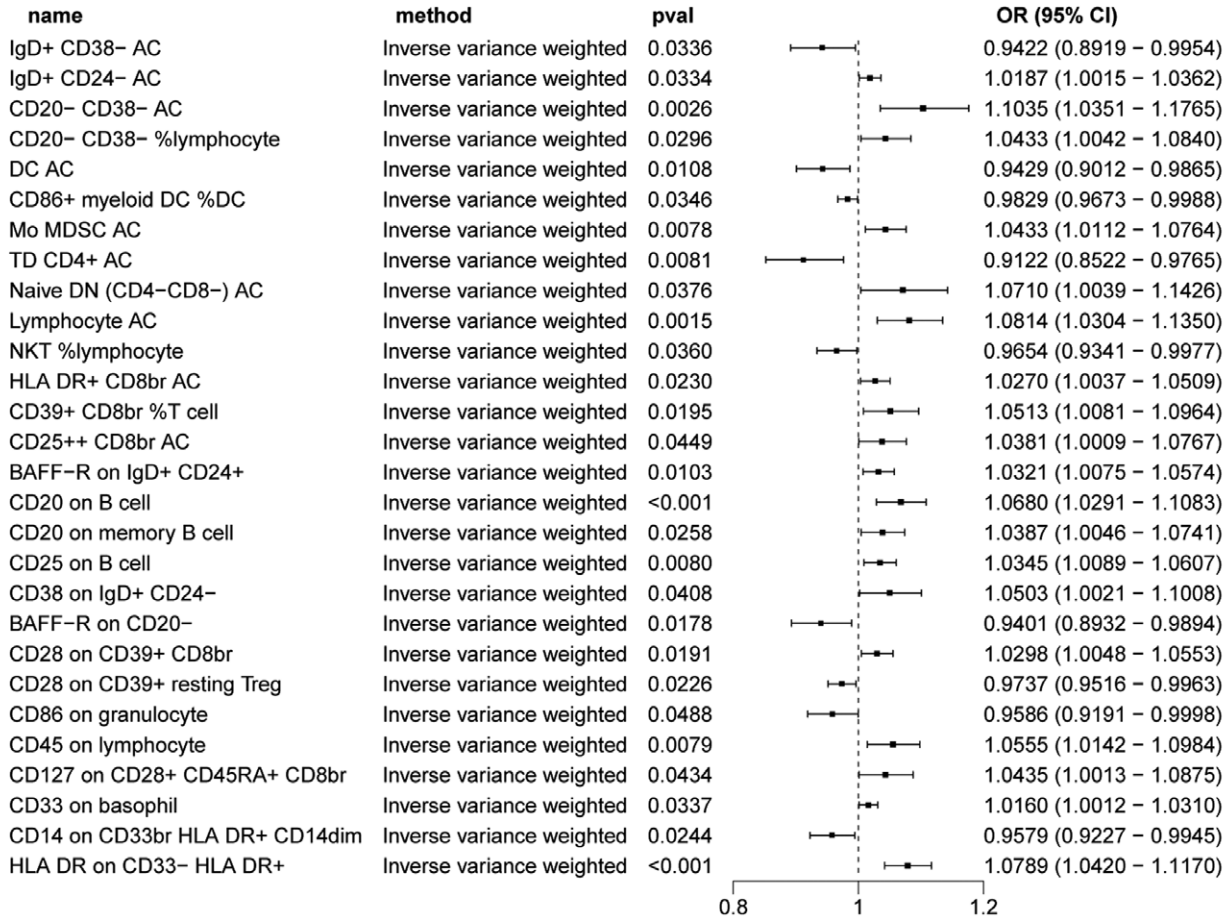

Figure 4. Forest plot of the MR analysis of immune cell traits on CKD. CI = confidence interval, CKD = chronic kidney disease, MR = Mendelian randomization, OR = odds ratio.

information from Figure 3. The results from other methods mostly demonstrated similar outcomes (Supplementary Table S1, Supplemental Digital Content, https://links.lww.com/MD/O818). In addition, the possibility of horizontal pleiotropy of these 20 associations was excluded by the MR-Egger intercept and MR-PRESSO global tests. The robustness of the observed causal association was also demonstrated by sensitivity analysis (Supplementary Table S2, Supplemental Digital Content, https://links.lww.com/MD/O819). Scatter plots and funnel plots also demonstrate the stability of the results (Supplementary Figures S1 and S2, Supplemental Digital Content, https://links.lww.com/MD/O820).

3.2. Exploring the causal impact of immune cell traits onset on CKD

Next, a positive MR analysis was conducted on 731 immune cell traits in relation to CKD. The IVW method was used as the primary selection criterion, with the exposure traits selected based on a $P < 5e{-}5$ in the IVs analysis. This resulted in the identification of 28 immune cells that are causally associated with the development of CKD. According to the IVW method, we obtained OR and *P* value for 28 immune cells associated with CKD are as follows: IgD+ CD38− AC(OR: 0.9422 [0.8919–0.9954], *P* = .0336); IgD+ CD24− AC (OR: 1.0187 [1.0015–1.0362], *P* = .0334); CD20− CD38− AC (OR: 1.1035 [1.0351–1.1765], *P* = .0026); CD20− CD38− %lymphocyte (OR: 1.0433 [1.0042–1.0840], *P* = .0296); DC AC (OR: 0.9429 [0.9012–0.9865], *P* = .0108); CD86+ myeloid DC %DC (OR: 0.9829 [0.9673–0.9988], *P* = .0346); monocytic myeloid-derived suppressor cells AC (OR: 1.0433 [1.0112–1.0764], *P* = .0078); TD CD4+ AC (OR: 0.9122 [0.8522–0.9765], *P* = .0081); naive DN (CD4–CD8–) AC (OR: 1.0710 [1.0039–1.1426], *P* = .0376); lymphocyte AC; (OR: 1.0814 [1.0304–1.1350], *P* = .0015); NKT %lymphocyte (OR: 0.9654 [0.9341–0.9977], *P* = .0360); HLA DR+ CD8br AC (OR: 1.0270 [1.0037–1.0509], *P* = .0230); CD39+ CD8br %T cell (OR: 1.0513 [1.0081–1.0964], *P* = .0195); CD25++ CD8br AC (OR: 1.0381 [1.0009–1.0767], *P* = .0449); BAFF-R on IgD+ CD24+ (OR: 1.0321 [1.0075–1.0574], *P* = .0103); CD20 on B cell (OR: 1.0680 [1.0291–1.1083], *P* = .0005); CD20 on memory B cell (OR: 1.0387 [1.0046–1.0741], *P* = .0258); CD25 on B cell (OR: 1.0345 [1.0089–1.0607], *P* = .0080); CD38 on IgD+ CD24− (OR: 1.0503 [1.0021–1.1008], *P* = .0408); BAFF-R on CD20− (OR: 0.9401 [0.8932–0.9894], *P* = .0178); CD28 on CD39+ CD8br (OR: 1.0298 [1.0048–1.0553], *P* = .0191); CD28 on CD39+ resting Treg (OR: 0.9737 [0.9516–0.9963], *P* = .0226); CD86 on granulocyte (OR: 0.9586 [0.9191–0.9998], *P* = .0488); CD45 on lymphocyte (OR: 1.0555 [1.0142–1.0984], *P* = .0079); CD127 on CD28+ CD45RA+ CD8br (OR: 1.0435 [1.0013–1.0875], *P* = .0434); CD33 on basophil (OR: 1.0160 [1.0012–1.0310], *P* = .0337); CD14 on CD33br HLA DR+ CD14dim (OR: 0.9579 [0.9227–0.9945], *P* = .0244); HLA DR on CD33− HLA DR+ (OR: 1.0789 [1.0420–1.1170], *P* = .00001). The results are presented in the information from Figure 4. The results from other methods mostly demonstrated similar outcomes (Supplementary Table S3, Supplemental Digital Content, https://links.lww.com/MD/O821). In addition, the possibility of horizontal pleiotropy of

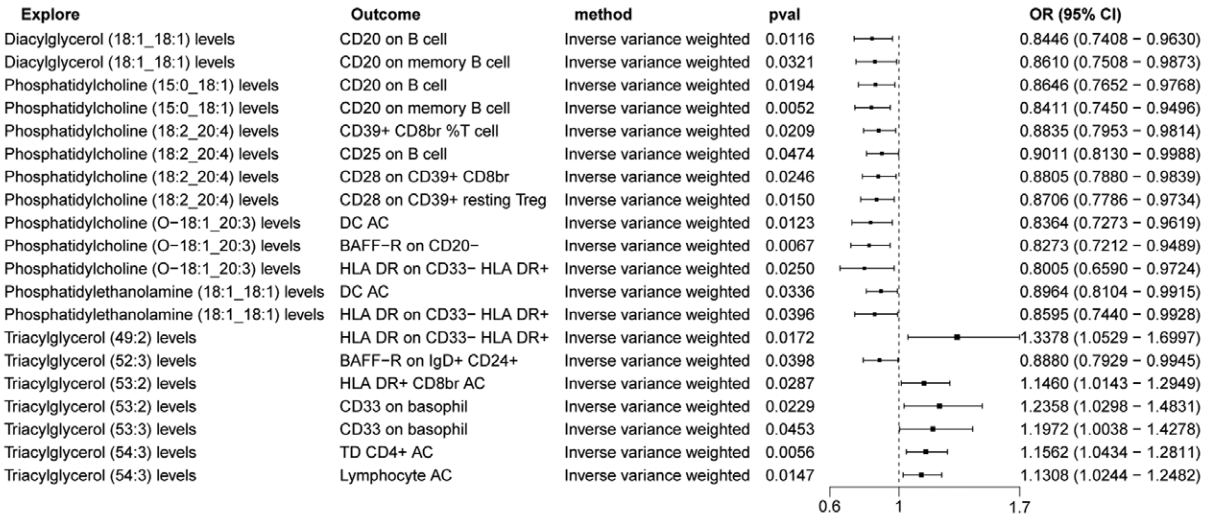

Figure 5. Forest plot of the MR analysis of lipidomes on immune cell traits. CI = confidence interval, MR = Mendelian randomization, OR = odds ratio.

these 28 associations was excluded by the MR-Egger intercept and MR-PRESSO global tests. The robustness of the observed causal association was also demonstrated by sensitivity analysis (Supplementary Table S4, Supplemental Digital Content, https://links.lww.com/MD/O822). Scatter plots and funnel plots also demonstrate the stability of the results (Supplementary Figures S3 and S4, Supplemental Digital Content, https://links.lww.com/MD/O823).

3.3. Exploring the causal impact of lipidomes onset on immune cell traits

Subsequently, the forward MR analysis was performed on the 20 selected lipidomes and 28 immune cells. The inclusion criteria for the exposure traits were $P < 5e{-}5$ in the IVs analysis, and for the outcome traits, the criteria were $P < 5e{-}8$. Ultimately, we used the IVW analysis method as the primary selection criterion with $P < .05$. Finally, we identified the relationships between 20 of lipidomes and immune cells. In the end, we selected 20 relationships between lipidomes and immune cells, and the results are as follows: diacylglycerol (18:1_18:1) levels for CD20 on B cell (OR: 0.8446 [0.7408–0.9630], $P = .0116$); diacylglycerol (18:1_18:1) levels for CD20 on memory B cell (OR: 0.8610 [0.7508–0.9873], $P = .0321$); phosphatidylcholine (15:0_18:1) levels for CD20 on B cell (OR: 0.8646 [0.7652–0.9768], $P = .0194$); phosphatidylcholine (15:0_18:1) levels for CD20 on memory B cell (OR: 0.8411 [0.7450–0.9496], $P = .0052$); phosphatidylcholine (18:2_20:4) levels for CD39+ CD8br %T cell (OR: 0.8835 [0.7953–0.9814], $P = .0209$); phosphatidylcholine (18:2_20:4) levels for CD25 on B cell (OR: 0.9011 [0.8130–0.9988], $P = .0474$); phosphatidylcholine (18:2_20:4) levels for CD28 on CD39+ CD8br (OR: 0.8805 [0.7880–0.9839], $P = .0246$); phosphatidylcholine (18:2_20:4) levels for CD28 on CD39+ resting Treg (OR: 0.8706 [0.7786–0.9734], $P = .0150$); phosphatidylcholine (O-18:1_20:3) levels for DC AC (OR: 0.8364 [0.7273–0.9619], $P = .0123$); phosphatidylcholine (O-18:1_20:3) levels for BAFF-R on CD20– (OR: 0.8273 [0.7212–0.9489], $P = .0067$); phosphatidylcholine (O-18:1_20:3) levels for HLA DR on CD33– HLA DR+ (OR: 0.8005 [0.6590–0.9724], $P = .0250$); phosphatidylethanolamine (18:1_18:1) levels for DC AC (OR: 0.8964 [0.8104–0.9915], $P = .0336$); phosphatidylethanolamine (18:1_18:1) levels for HLA DR on CD33– HLA DR+ (OR: 0.8595 [0.7440–0.9928], $P = .0396$); triacylglycerol (49:2) levels for HLA DR on CD33– HLA DR+ (OR: 1.3378 [1.0529–1.6997], $P = .0172$); triacylglycerol (52:3) levels for BAFF-R on IgD+ CD24+ (OR: 0.8880 [0.7929–0.9945], $P = .0398$); triacylglycerol (53:2) levels for HLA DR+ CD8br AC (OR: 1.1460 [1.0143–1.2949], $P = .0287$); triacylglycerol (53:2) levels for CD33 on basophil (OR: 1.2358 [1.0298–1.4831], $P = .0229$); triacylglycerol (53:3) levels for CD33 on basophil (OR: 1.1972 [1.0038–1.4278], $P = .0453$); triacylglycerol (54:3) levels for TD CD4+ AC (OR: 1.1562 [1.0434–1.2811], $P = .0056$]; triacylglycerol (54:3) levels for Lymphocyte AC (OR: 1.1308 [1.0244–1.2482], $P = .0147$). The results are presented in the information from Figure 5. The results from other methods mostly demonstrated similar outcomes (Supplementary Table S5, Supplemental Digital Content, https://links.lww.com/MD/O824). In addition, the possibility of horizontal pleiotropy of these 20 associations was excluded by the MR-Egger intercept and MR-PRESSO global tests. The robustness of the observed causal association was also demonstrated by sensitivity analysis (Supplementary Table S6, Supplemental Digital Content, https://links.lww.com/MD/O825). Scatter plots and funnel plots also demonstrate the stability of the results (Supplementary Figures S5 and S6, Supplemental Digital Content, https://links.lww.com/MD/O826).

3.4. Exploring the causal impact of CKD onset on lipidomes

Next, based on the results from the previous step, we conducted a reverse MR analysis on the remaining 10 lipidomes to assess potential inverse associations between these lipidomes and CKD. Using the IVW method with IVs selection criteria $P < 5e{-}8$ and outcome selection criteria $P \geq .05$, we ultimately found that there were no statistically significant inverse associations between 10 lipidomes and CKD. The results are as follows: diacylglycerol (18:1_18:1) levels (OR: 0.8873 [0.7688–1.0240], $P = .1020$); phosphatidylcholine (15:0_18:1) levels (OR: 0.9269 [0.7693–1.1169], $P = .4251$); phosphatidylcholine (18:2_20:4) levels (OR: 0.9612 [0.8340–1.1077], $P = .5841$); phosphatidylcholine (O-18:1_20:3) levels (OR: 1.0020 [0.8307–1.2087], $P = .9830$); phosphatidylethanolamine (18:1_18:1) levels (OR: 0.9916 [0.8793–1.1182], $P = .8904$); triacylglycerol (49:2) levels (OR: 0.9620 [0.8451–1.0952], $P = .5585$); triacylglycerol (52:3) levels (OR: 0.9201 [0.7876–1.0750], $P = .2943$); triacylglycerol (53:2) levels (OR: 0.9445 [0.8374–1.0654], $P = .3529$); triacylglycerol (53:3) levels (OR: 0.9479 [0.8187–1.0975], $P = .4741$); triacylglycerol (54:3) levels (OR: 0.9447

[0.8388–1.0640], $P = .3484$). The results are presented in the information from Figure 6. These results indicate that the causal relationship between these 10 lipidomes and CKD is reliable.

3.5. Immune cells mediate the association between liposomes and CKD

Finally, we analyzed the role of immune cells as mediators for liposomes in the occurrence of CKD. On the basis of the aforementioned research findings, we selected CD33 on basophils in immune cells as a mediator to study its role in mediating the effect of triacylglycerol (53:3) levels on CKD. Through mediation MR analysis, we have determined that the β value for the effect of triacylglycerol (53:3) levels on CD33 on basophils is 0.1799739 (β1), and the β value for the effect of CD33 on basophils on CKD is 0.0158538 (β2). On the basis of calculations, the calculated β value for the mediating effect of CD33 on basophils in the relationship between triacylglycerol (53:3) levels and CKD is 0.002853271, accounting for 3.23% of the total effect. The findings are elegantly displayed within the data depicted in Figure 7.

4. Discussion

Over the past decade, the application of mass spectrometry has made it possible to analyze the lipidomes of plasma and tissues. This allows for a broader investigation of lipids beyond traditional measurements such as total cholesterol, lipoproteins, and triglycerides, which do not consider the chemical diversity and complexity of lipidomes. Recently, a significant amount of research has discovered that the occurrence and progression of CKD are accompanied by extensive changes in the lipidomes.[10,38,39] Indeed, it is important to note that the current evidence is primarily based on observational studies, and the research findings may be influenced by confounding factors. Our study aimed to elucidate the causal relationship between lipidomes and CKD. On the basis of the existing global genomic research, we seek to demonstrate whether this causal relationship is mediated through immune cells. Our research findings indicate that the increase in triacylglycerol (53:3) levels is associated with an increased risk of CKD, and 3.2% of this effect is mediated through CD33 on basophils.

Triacylglycerol, as an important component of lipids, is associated with cardiovascular diseases, metabolic disorders, and aging.[40–42] Current research has found a certain relationship between triacylglycerol and the occurrence and progression of kidney-related diseases. The accumulation of excessive lipids such as triacylglycerol can exacerbate diabetes-related microvascular and macrovascular diseases, increase glomerular damage, promote tubulointerstitial fibrosis, and accelerate the progression of diabetic kidney disease.[43] In clinical practice, lipid-lowering medications are often used to improve lipid metabolism in patients with CKD and prevent cardiovascular complications such as atherosclerosis.[44] In addition, triacylglycerol

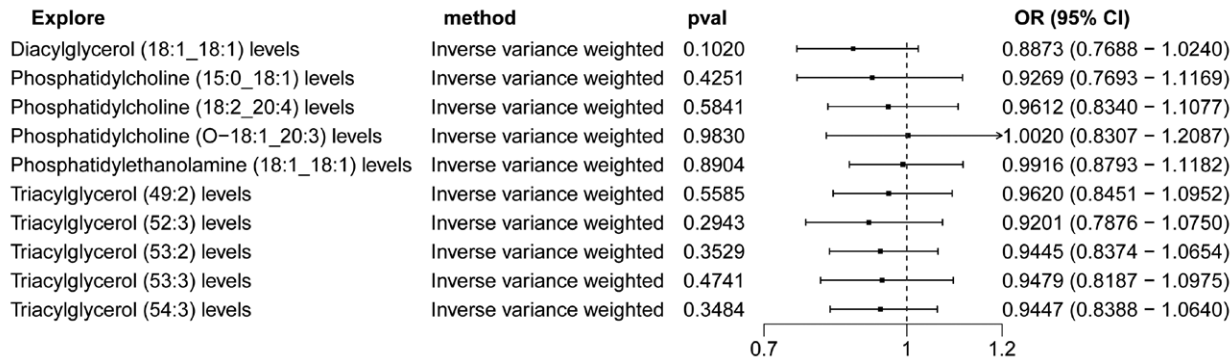

Figure 6. Forest plot of the MR analysis CKD of on lipidomes. CI = confidence interval, CKD = chronic kidney disease, MR = Mendelian randomization, OR = odds ratio.

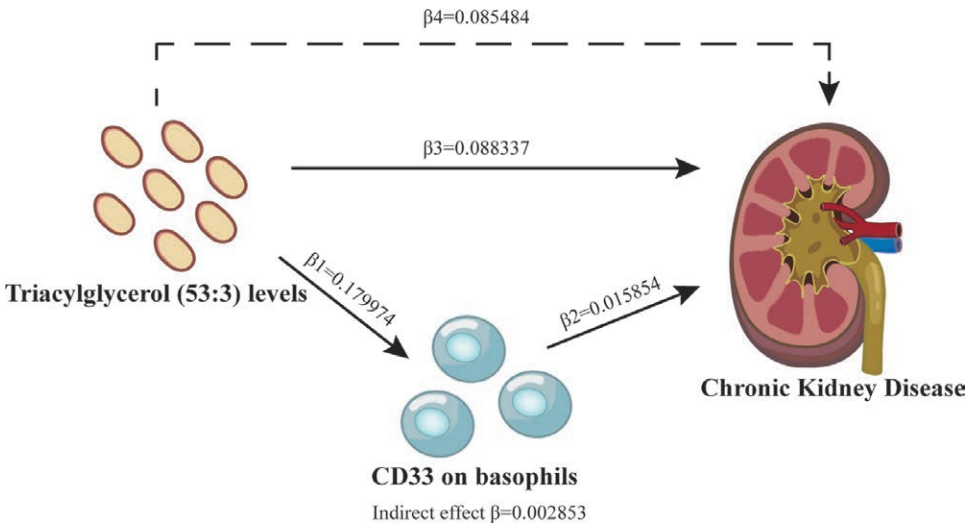

Figure 7. Plot of mediation analysis results in MR. MR = Mendelian randomization.

is significantly associated with the risk of end-stage renal disease. Triglycerides account for nearly half of the total association between body mass index and end-stage renal disease in the general population.[45] However, currently, there is no research that has reported a causal relationship between triglycerides and the risk of CKD. Our research findings indicate a positive correlation between triacylglycerol and the risk of CKD, with triacylglycerol (53:3) levels showing the most significant statistical differences.

Both innate and adaptive immunity play crucial roles in renal injury and repair processes.[46] Interactions between renal cells and immune cells may initiate a vicious cycle leading to irreversible kidney tissue damage. Consequently, we have also focused on the link between immune cells and CKD.[47] From the 731 immune cell traits, we conducted a screening process and identified through mediation analysis that CD33-positive basophils may be a key intermediary in the pathway by which triacylglycerols contribute to CKD. Basophils, an important immune subset in renal fibrosis, can induce inflammation by modulating the release of interleukin-6 and the recruitment of helper T cells.[48] Furthermore, a study suggests that alterations in both innate and adaptive immune pathways observed in hemodialysis patients may be associated with changes in the activity of basophils in peripheral blood.[49] In addition, current research indicates that triacylglycerols have immunomodulatory effects. Triacylglycerol can provide fatty acids to the body, which not only serve as an energy source but also play a role in the intersecting metabolism and immune response, thereby influencing immune response reactions.[50] Research utilizing data from the UK Biobank cohort has found a positive correlation between triacylglycerol levels and various white blood cell levels, including basophils. This suggests that triacylglycerol might be directly involved in white blood cell generation.[51] Similarly, a national health and nutrition examination survey conducted in the United States revealed a positive correlation between triacylglycerol levels and basophil count.[52] Thus, the impact of triacylglycerols on CKD may occur through their influence on the immune profile mediated by basophils.

These studies confirm our data results, and our research further focuses on CD33 basophils, indicating the mediating effect of this type of immune cell in the impact of triacylglycerol on CKD. Our results contribute to a deeper understanding of the interplay between lipids, immunity, and the underlying mechanisms preceding CKD. They provide direction for future basic research and offer valuable insights into clinical practice and management of CKD. However, this study still has several limitations. First, the existing database mainly includes 7 immune cell traits and does not encompass all immune cells. It is necessary to wait for future data on new immune cell traits to supplement the existing database. Second, the GWAS datasets utilized in this study for lipidomics and CKD were derived from Finnish individuals, while the immune cell trait data predominantly originated from Sardinian residents, both of which are part of the European population. Although the sample size is sufficiently large to mitigate the impact of genetic variation and render these results applicable to other populations, future studies should expand the participant pool for additional validation to prevent potential biases. Third, even though measures have been taken to identify and eliminate outlier variants, we cannot rule out the possibility that pleiotropy at the level of lipidomics may affect our results. More in-depth research is needed to verify the complexity of the associations. Fourth, the mediating effect observed is relatively modest, suggesting that this pathway's contribution to explaining the causal relationship between lipidomics and CKD is limited. This may be attributed to the multifactorial and multipathway etiology of CKD, where a single intermediary molecule or pathway cannot fully elucidate the intricate pathological process. Consequently, future studies should consider incorporating a wider array of biological markers and intermediate pathways to gain a more comprehensive understanding of the mechanisms underlying CKD development.

5. Conclusion

In conclusion, our study has identified a causal relationship between lipidomics and CKD, as well as the mediating role of CD33 on basophils. However, other risk factors like potential mediators require further investigation. In clinical practice, particular attention should be paid to lipidomic changes, especially triacylglycerol, in patients with CKD.

Author contributions

Conceptualization: Qi Li, Haoyu Chen.
Data curation: Qi Li, Haoyu Chen, Dong Bian, Linlin Zheng, Hongyu Wei, Wanqing Wang, Yashi Wang, Wanying Deng.
Formal analysis: Qi Li, Haoyu Chen, Dong Bian, Linlin Zheng.
Methodology: Qi Li, Haoyu Chen, Dong Bian, Linlin Zheng.
Visualization: Qi Li, Haoyu Chen, Hongyu Wei, Wanqing Wang, Yashi Wang.
Writing – original draft: Qi Li, Haoyu Chen.
Supervision: Hui Gao, Xiaona Wei.
Writing – review & editing: Hui Gao, Xiaona Wei, Dong Bian, Linlin Zheng.
Validation: Wanqing Wang, Yashi Wang, Wanying Deng.

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