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Integrative Analysis by Mendelian Randomization and Large-Scale Single-Cell Transcriptomics Reveals Causal Links between B Cell Subtypes and Diabetic Kidney Disease

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Keywords
Diabetic kidney disease · Circulating immune cells · Mendelian randomization · Single-cell disease relevance score · Immune cell infiltration analysis · B cell

Abstract

Introduction: The increasing incidence of diabetic kidney disease (DKD) and the challenges in its management highlight the necessity for a deeper understanding of its pathogenesis. While recent studies have underscored the substantial impact of circulating immunity on the development of diabetic microvascular complications such as retinopathy and neuropathy, research on circulating immunity in DKD remains limited. ***Methods:*** This study utilized Mendelian randomization analysis to explore the potential independent causal relationships between circulating immune cells and DKD pathogenesis. Additionally, a combination of single-cell disease relevance score (scDRS) and immune cell infiltration analysis was employed to map the circulating immunity landscape in DKD patients. ***Results:*** Ten immune traits, including 5 of B cells, 2 of T cells, 2 of granulocytes, and one of monocytes, were defined to be associated with the pathogenesis of DKD. Notably, *IgD$^-$CD27$^-$ B cell Absolute Count* (IVW: OR, 1.102 [1.023–1.189], $p = 0.011$) and *IgD$^-$CD24$^-$ B cell Absolute Count* (IVW: OR, 1.106 [1.030–1.188], $p = 0.005$) were associated with promoting DKD pathogenesis, while *CD24$^+$CD27$^+$ B cell %B cell* (IVW: OR, 0.943 [0.898–0.989], $p = 0.016$) demonstrated a protective effect against DKD onset. The presence of B cell-activating factor receptor (BAFF-R) on *CD20$^-$CD38$^-$ B cell* (IVW: OR, 0.946 [0.904–0.989], $p = 0.015$) and *BAFF-R on IgD$^-$CD38$^+$ B cell* (IVW: OR, 0.902 [0.834–0.975], $p = 0.009$) also indicated a potential role in preventing DKD. scDRS analysis revealed that two main subsets of B cells, naïve B and memory B cells, had a higher proportion of DKD-related cells or a higher scDRS score of DKD phenotype, indicating their strong association with DKD. Furthermore, immune infiltrate deconvolution analysis showed a notable decrease in the circulating memory B cells and class-switched memory B cells in DKD patients compared to those of DM patients without DKD. ***Conclusion:*** Our study revealed the causal

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relations between circulating immunity and DKD susceptibility, particularly highlighted the potential roles of B cell subtypes in DKD development. Further studies addressing the related mechanisms would broaden the current understanding of DKD pathogenesis.

Introduction

Diabetes mellitus (DM) has surfaced as a pressing healthcare concern with substantial implications for global health [1, 2]. The diabetic kidney disease (DKD), characterized by renal involvement in DM, stands as a prevalent complication whose advancement ranks as the leading contributor to end-stage renal disease internationally [3]. Prior research underscores environmental metabolic disturbances, including hyperglycemia, hypertension, and hemodynamic discrepancies, as fundamental risk elements for DKD [4, 5]. Nonetheless, despite meticulous regulation of glucose, blood pressure, and cholesterol levels, roughly 35% of individuals with DM still succumb to DKD development [3–6]. Furthermore, there are instances where DM patients enduring long-term hyperglycemia do not contract DKD, pointing to potential unique pathogenetic mechanisms in renal engagement beyond DM-associated risk factors [7]. Current studies have revealed that distinct genetic profiles correlate with diverse metabolic, immunological, and hemodynamic characteristics, culminating in variable DM prognoses; however, conclusive data affirming a genetic predisposition to DKD is yet scarce.

Recent research has unveiled that various immune cells penetrate the kidney, playing a pivotal role in exacerbating kidney dysfunction in DKD [8–10]. This infiltration is now recognized as a crucial factor in the progression of kidney damage. Moreover, genome-wide association studies (GWAS) have identified specific genes associated with DKD susceptibility, including *AFF3* [11], *RGMA-MCTP2* [11], and *CDCA7-SP3* [12]. These discoveries underscore the kidney's vulnerability to autoimmune [13, 14] and inflammatory [15, 16] responses in the context of DKD. Fascinatingly, recent investigations have highlighted the significance of circulating immunity in the pathogenesis of other diabetes-related microvascular complications, such as diabetic retinopathy and neuropathy [17, 18]. Utilizing Mendelian randomization (MR) studies derived from GWAS data, researchers have discovered associations between certain cytokines and the risk of severe diabetic conditions. Specifically, TNF-receptors and IL-8 have been linked to an increased risk of severe diabetic retinopathy [17, 18], while interferon-gamma has been associated with a higher risk of diabetic neuropathy. In contrast, IL-9 and stem cell factor have been shown to potentially mitigate diabetic neuropathy risks [18]. Given these insights, delving further into the relationship between circulating immunity and DKD development presents a compelling avenue for research. Such exploration could significantly enhance our comprehension of the mechanisms underlying kidney involvement in diabetes, paving the way for novel therapeutic approaches for DKD.

The efficacy of MR studies in assessing the causal impact of genetic variants on disease development is increasingly acknowledged in the scientific community [19–21]. Paralleling randomized controlled trials (RCTs), MR analysis serves as an instrumental tool to examine potential causative links between genetically proxied environmental exposures and diseases, while eliminating confounding factors [22]. In the present investigation, we employed MR analysis to probe the possible causative relationship between circulating immune cells and the onset of DKD. This analysis particularly accentuated the bidirectional importance of B cells in DKD development, further substantiated with a single-cell disease relevance score (scDRS) for enhanced validation. Additionally, our immune infiltration deconvolution analysis has revealed a marked reduction in memory B cells and class-switched memory B cells in individuals with DKD as opposed to diabetic patients without kidney involvement. This comprehensive methodology addresses the previously overlooked role of circulating immune cells – especially memory B cells – in the pathogenesis of DKD, thereby enriching our understanding and potentially informing future therapeutic strategies.

Material and Methods

Study Design

Two-sample MR analysis was conducted to evaluate the causal relationships between 731 types of immune cells and the risk of DKD. The exposure of MR analysis was GWAS data of circulating immune cells obtained from a population of 3,757 Sardinian individuals [23], and the outcome was determined as "Diabetic nephropathy (more control exclusions)" from the FinnGen database [24], which contains GWAS data from 4,111 DKD patients and 308,539 controls without DM. To compare the differential circulating immune cells that have significant causal links between DKD and DM, outcomes of "Type 1 diabetes without complications" and "Type 2 diabetes without complications" from the FinnGen database were also selected. Adhering to the principles of MR studies, three fundamental hypotheses were followed [25].

First of all, the genetic instrumental variable (IV) should be strongly associated with exposure. Second, it must be independent of the confounders. Finally, this genetic variation is the only pathway to affect the outcome through the exposure. Based on these assumptions, we selected strongly correlated single nucleotide polymorphisms (SNPs), conducted MR analysis, and excluded results that did not pass sensitivity tests (Fig. 1).

To validate the significant circulating immune cell types strongly associated with DKD pathogenesis, MAGMA was used to infer the top genes that have hereditary susceptibility with DKD. These genes were then composed as a gene set indicating DKD correlation, which was further input into scDRS to calculate the DKD scores at single-cell level on 5 publicly available PBMC datasets of single-cell RNA sequencing [26–30]. Cell types with higher scDRS scores and cells with significant p value calculated by scDRS were considered to be strongly related to DKD pathogenesis (Fig. 1). After obtaining the key cell types that have causal links with DKD, we determined the proportion alteration of different types of circulating immune cells in DKD compared to DM, through CIBERSORT or xCell immune deconvolution analysis of PBMC RNA gene-expression data of healthy controls (HCs), DM and DKD patients (Fig. 1) (online suppl. Table 1; for all online suppl. material, see https://doi.org/10.1159/000539689). The gene-expression data were obtained from the Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo/) [31].

IVs of MR Study

The data of 731 immune traits were attained from the GWAS Catalog (GCST0001391 to GCST0002121). This original GWAS was performed in a population of 3,757 Sardinians individuals, in which over 20 million SNPs and 1.6 million indels were imputed in the sequence-based reference panel [23]. 731 immune traits were attained from this cohort, including 539 immune traits in help of flow cytometry and 192 relative cell counts. Flow cytometry therein identified cells through absolute cell counts (n = 118), median fluorescence intensities of surface antigens (n = 389), as well as morphological parameters (n = 32). Those immune cells were classified into seven panels, encompassing dendritic cells, monocytes, myeloid cells, TBNK (lymphocytes: $CD3^+$, corresponding to T cells, and $CD3^-$, including B cells [$CD19^+$] and natural killer cells [$CD16^+$ or $CD56^+$]), B cells, maturation stages of T cells, and regulatory T cells panels [23].

Consistent with previous studies [23, 32, 33], SNPs were extracted with the statistical significance of $p < 1 \times 10^5$. In order to adjust the weak instrument bias, F statistics were calculated in the formula $F = \frac{R^2 \times (N-2)}{1-R^2}$, in which

$$R^2 = \frac{2 \times \beta^2 \times \mathrm{EAF} \times (1 - \mathrm{EAF})}{2 \times \beta^2 \times \mathrm{EAF} \times (1 - \mathrm{EAF}) + 2 \times \mathrm{SE}^2 \times \mathrm{N} \times \mathrm{EAF} \times (1 - \mathrm{EAF})}$$

In this estimation, N refers to total sample size of population in the selected exposure, while EAF represents effect allele frequency. β and SE, respectively, mean the effect size and the standard error in exposure GWAS [34–36]. Threshold of F statistics was set at F>10 to fetch available IVs. We also used the clumping procedure in PLINK to prune those SNPs, with parameters of linkage disequilibrium (LD) refer to R^2 threshold < 0.001 within 10,000 kb distance.

Outcome Variables of MR Study

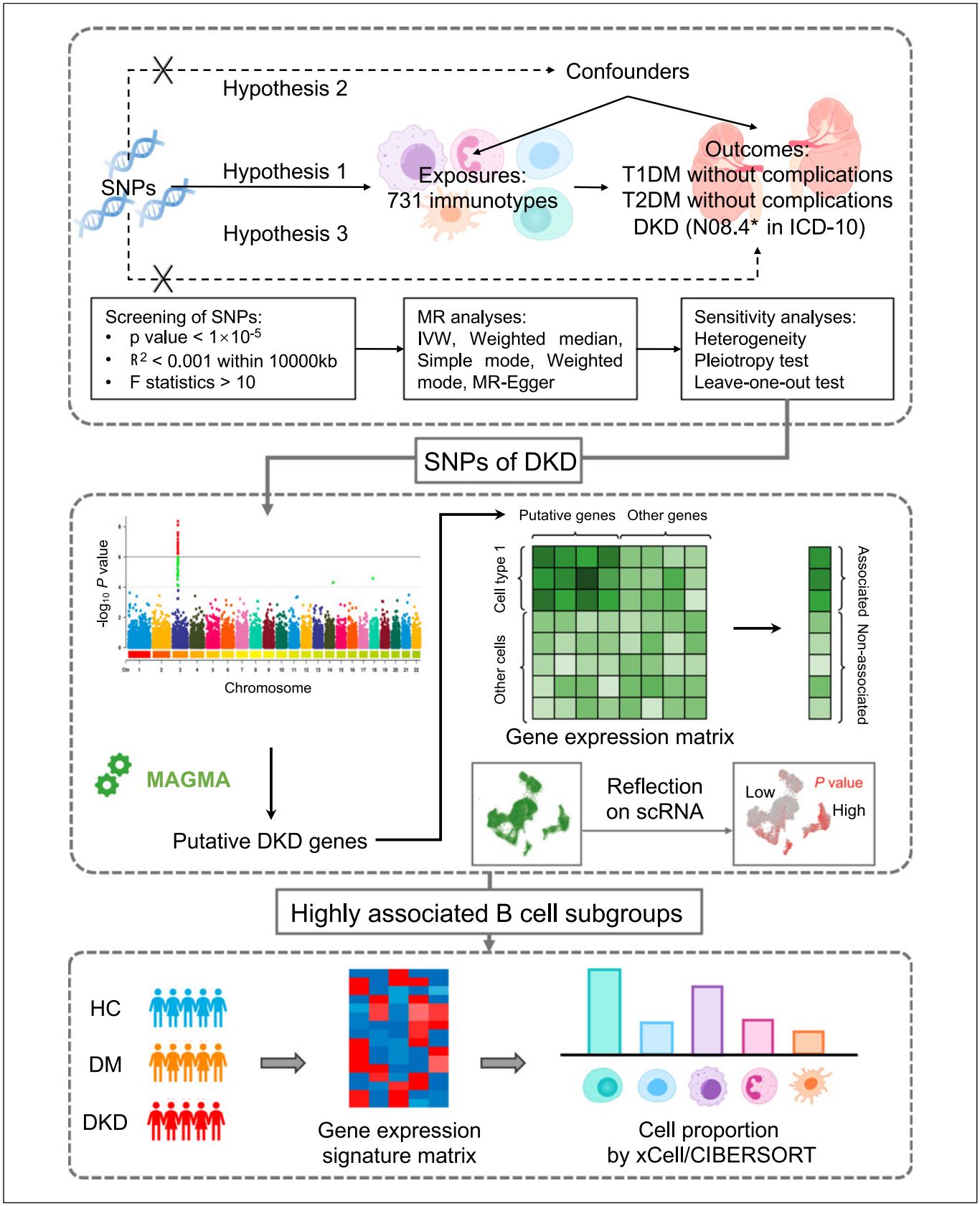
The DKD outcome of our study was selected from the FinnGen population version 9 (https://www.finngen.fi/en). FinnGen project enriches genomic information and national healthcare data of over 300,000 Finnish individuals [24]. This R9 version dataset was released to the public in 2023, with 2,272 disease endpoints identified. Our outcome in FinnGen R9, namely "Diabetic nephropathy (more control exclusions)," include 4,111 cases and 308,539 controls. The endpoint was defined as N08.3* (glomerular disorders in DM Source) in International Classification of Diseases-10 (ICD-10). Moreover, people with diabetes were excluded from the control population. For outcome of DM without any complications, we chose publicly available "Type 1 diabetes without complications" and "Type 2 diabetes without complications" data from FinnGen population version 7. The type 1 DM outcome GWAS data contains 7,205 cases and 255,466 controls. The endpoint was defined as E10.9 (type 1 diabetes without complications) in ICD-10. While the type 2 DM outcome data includes 24,905 cases and 255,466 controls, the endpoint was defined as E11.9 (type 2 diabetes without complications) in ICD-10.

Single-Cell Disease Relevance Score

The same GWAS data of DKD in the MR analysis was used to conduct MAGMA (V 1.10) analysis for exploring the association between DKD phenotype and the gene-level information. MAGMA was performed with a window of 10 kb upstream and downstream of each gene, accounting for LD. Genes associated with increasing genetic risk of DKD phenotype were then resolved, obtained, and further processed as gene sets of DKD phenotype by the "munge-gs" function in the scDRS package (V1.0.2). Five publicly available large-scale single-cell transcriptomics data containing ~2,500,000 PBMCs were downloaded. Among them, datasets of COvid-19 Multi-omics Blood ATlas (COMBAT) Consortium [26] (*Cell*, 2022), Jin et al. [27] (*iScience*, 2021), and Yoshida et al. [28] (*Nature*, 2022) were obtained through CZI CELLxGENE collections (https://cellxgene.cziscience.com/collections). Hao et al. [29] (*Cell*, 2021) was downloaded from the SeuratData package. Oelen et al. [30] (*Nature Communication*, 2022) was downloaded from https://eqtlgen.org/sc/datasets/1m-scbloodnl.html. The original cell type annotation and uniform manifold approximation and projection (UMAP) reduction by authors was used directly. The processed gene set of DKD phenotype was scored in each single-cell dataset by "compute-score" function in the scDRS package. Visualization of DKD phenotype score or DKD positive cells (defined by p value <0.05 in scDRS analysis) was performed by Seurat (V5.0.1) [37].

Acquisition of Transcriptomic Data

The human PBMC transcriptomic microarrays were obtained from GEO database, including population of DKD, DM without DKD (DM group), and HC. In the GSE142153 dataset, total RNA was extracted from 23 individuals with type 2 DM and DKD, along with 10 HC [38]. For GSE9006 [39], GSE29142 [40], GSE55098 [41], and GSE156035 [42], total RNA was attained from patients of diabetes without DKD and respective control groups. These four datasets comprised 93, 9, 12, and 20 samples respectively for DM, and 24, 10, 10, and 20 samples, respectively, for control. Detailed information of each PBMC GEO dataset is available in online



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supplementary Table 1. Gene-expression values were log2 transformed, and the batch effects were removed using the Combat function of sva R package [43].

Immune Cell Infiltration Analysis

We employed xCell and CIBERSORT algorithms to weigh the genes associated with immune cells and quantify their enrichment status. The xCell algorithm was used to infer 64 immune and stromal cell types [44]. Similarly, twenty-two kinds of hematopoietic cells were supported in CIBERSORT for speculating cell types [45]. The proportion of cell types was displayed using 1,000 permutations. Comparisons between groups were conducted by Wilcoxon-Mann-Whitney Test.

Statistical Analysis

The whole MR analysis was performed in R 4.3.2 version software (http://www.Rproject.org) using the TwoSampleMR package (version 0.5.8). In order to determine the causal association between circulating immune traits and DKD, the inverse variance-weighted (IVW) [46], simple mode [47], weighted median-based [48], weighted mode-based [47], MR Egger regression [49] methods were selected for causal effects. To avoid the pleiotropic effects bias, MR-Egger regression intercept and MR-PRESSO analyses were performed for horizontal pleiotropy detection and high-precision outlier test, respectively [50]. IVW and MR-Egger methods were also utilized for the evaluation of heterogeneity that might arise due to variations in analytical platforms, experimental methodologies, study cohorts, and other factors. Leave-one-out method of sensitivity analysis was finally conducted to assess individual genetic variations one at a time, validating the robustness, and reliability of the analysis [51].

Results

Description and Statistical Power of Circulating Immune Cells and Risk of DKD

In this study, SNPs were meticulously selected with significance threshold of $p < 1 \times 10^{-5}$ and F>10. Clumping method was implemented to address LD in these IVs. To ensure consistency in the effect alleles between the exposure and outcome variables, harmonizing process was undertaken. Within the framework of MR analysis, the IV weighted (IVW) method was employed with the significance criterion of $p < 0.05$. A total of 33 out of 731 immune traits were filtered after this screening.

To evaluate the reliability of our findings, we carried out an extensive series of sensitivity analyses. By employing the heterogeneity tests of Egger's regression and the IVW methods, we systematically excluded immune traits with p values below 0.05. Should either method fail, we would consider this indicative of SNP heterogeneity. A total of 29 immune traits successfully passed this test. Following the application of two distinct methods for assessing horizontal pleiotropy, specifically MR-Egger and MR-PRESSO, no traits were excluded. This substantiates that all IVs included are robustly linked to the outcome solely through the risk factor. The final step in our sensitivity analysis involved a leave-one-out test using the IVW algorithm, which determined the final count of retained cells. In this phase, 18 immune trait exposures were excluded. The remaining 11 exposures include four distinct cell types: B cells, T cells, monocytes, and granulocytes (Fig. 2).

Two-Sample MR Reveals Causal Effect of B Cells on DKD

To explore the relationship between circulating immune cells and DKD, we aggregated findings from five distinct methods: IVW, weighted median, simple mode, weighted mode, and MR Egger. Among all the exposures that successfully cleared the sensitivity tests, the number of SNPs ranged from 13 to 32 (Fig. 2).

Notably, 6 out of 11 exposures were on the B cell panel. *IgD^-CD27^- B cell Absolute Count* (IVW: OR, 1.102 [1.023–1.189], $p = 0.011$) and *IgD^-CD24^- B cell Absolute Count* (IVW: OR, 1.106 [1.030–1.188], $p = 0.005$) showed a promoting pathogenic effect on DKD. Accordingly, *$CD24^+CD27^+$ B cell %B cell*, showed a protective effect against DKD development (IVW: OR, 0.943 [0.898–0.989], $p = 0.016$). In addition, B cell-activating factor receptor (*BAFF-R*) *on $CD20^-CD38^-$ B cell* (IVW: OR, 0.946 [0.904–0.989], $p = 0.015$) and *BAFF-R on IgD^-CD38^+ B cell* (IVW: OR, 0.902 [0.834–0.975], $p = 0.009$) suggested a role of BAFF-R in preventing DKD onset. In addition, *CD20 on IgD^+CD24^+ B cell* (IVW: OR, 0.923 [0.868–0.983], $p = 0.012$) likewise showed protective effects in DKD (Fig. 2). Apart from B cells, we also revealed potential roles of T cells, monocytes, and granulocytes in DKD development. It was found that *$CD62L^-HLA\ DR^{++}$ monocyte Absolute Count* facilitated the development of DKD (IVW: OR, 1.123 [1.025–1.230], $p = 0.013$). Similarly, granulocyte panel such as *Granulocyte Absolute Count* (IVW: OR, 1.101 [1.029–1.178], $p = 0.005$] and *CD80 on granulocyte* (IVW: OR, 1.057 [1.013–1.103], $p = 0.011$) demonstrated a pro-pathogenic role through IVW test (Fig. 2).

In order to define the immune cell types specific for DKD pathogenesis rather than for DM, we performed screening MR analysis between the 731 immune traits

Fig. 1. Study design of MR analysis between circulating immune cells and DKD. IVs, instrumental variants; SNP, single nucleotide polymorphism; DKD, diabetic kidney diseases; IVW, inverse variance-weighted.

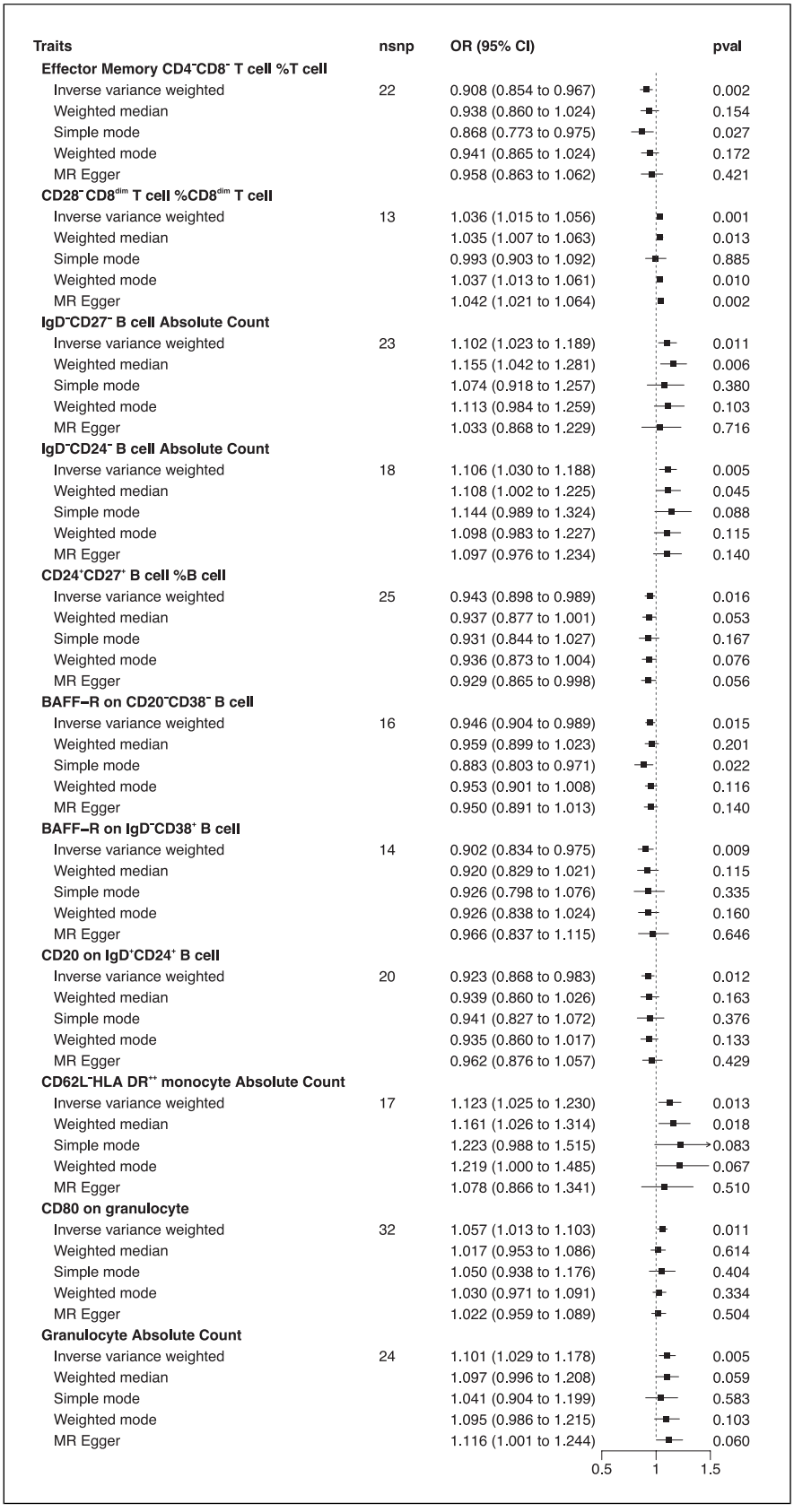


Fig. 2. Forest plot showed MR results on causal effects between immune cell traits and DKD. IVW, inverse variance-weighted; OR, odd ratio; CI, confidence interval.

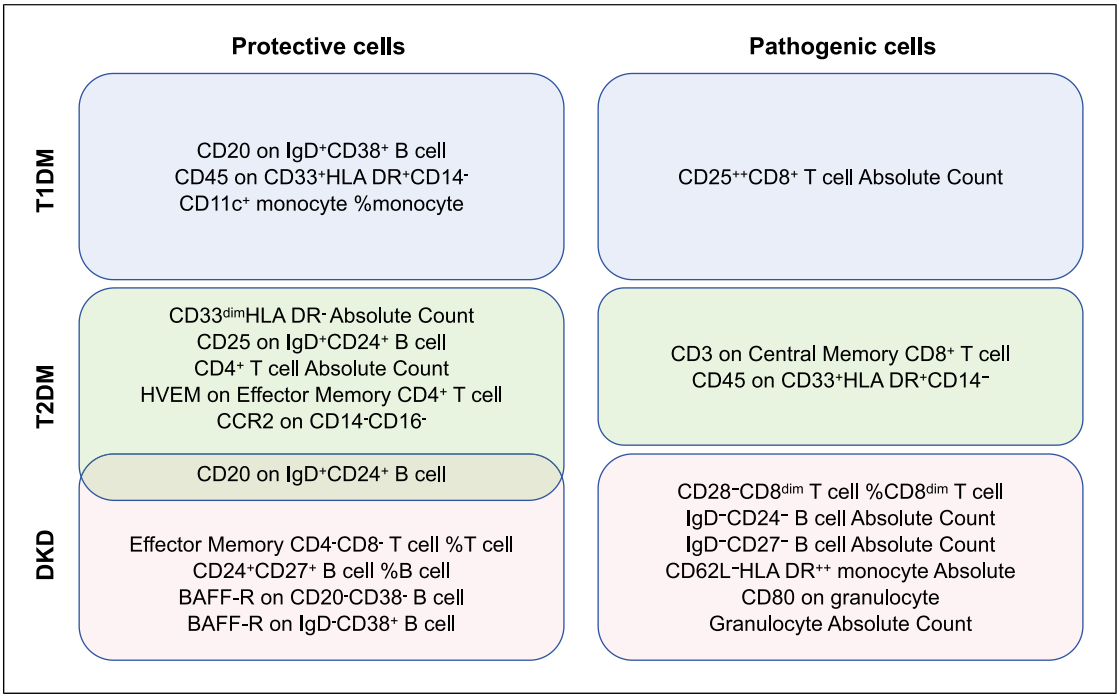
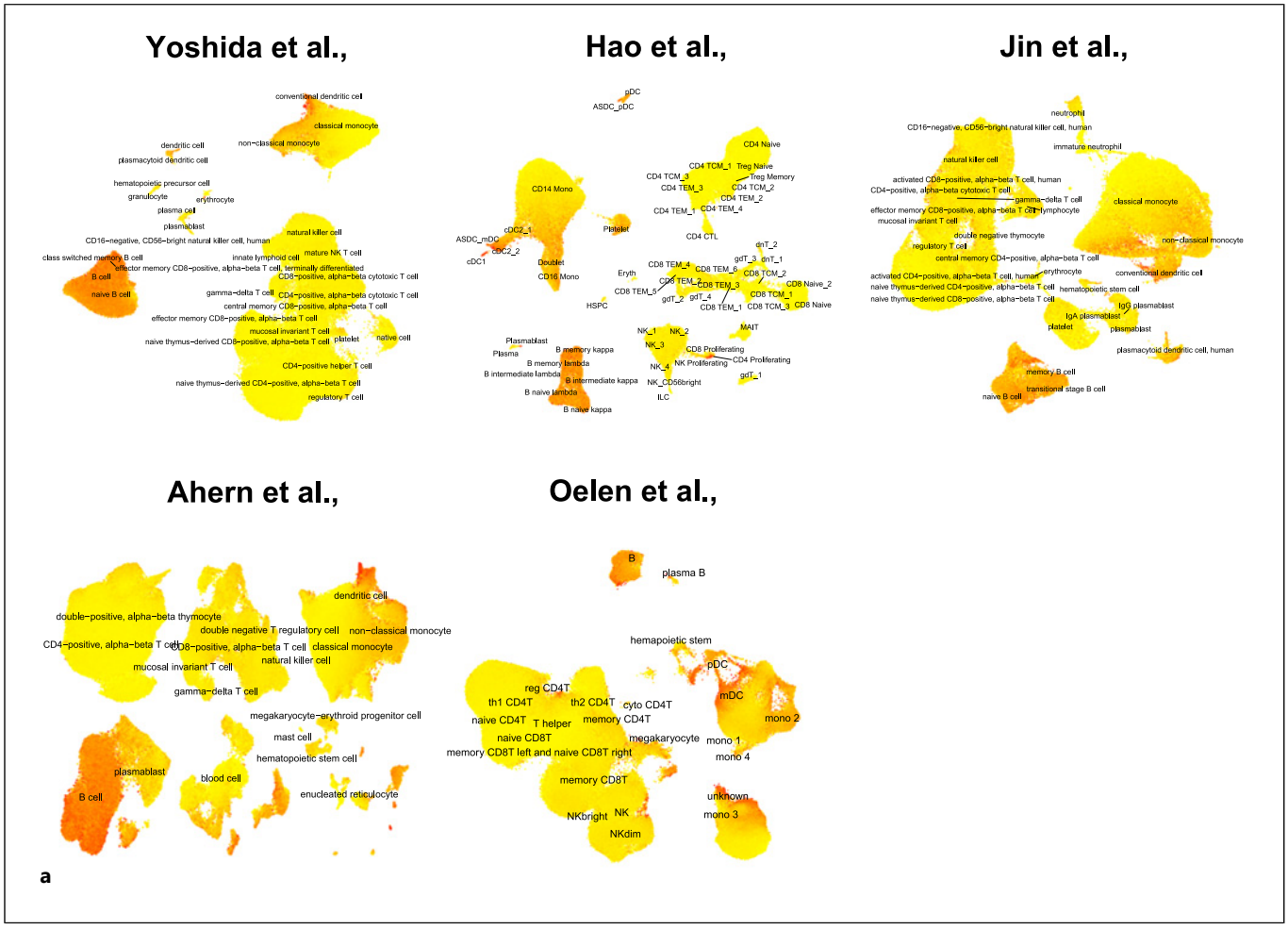


Fig. 3. Venn plot demonstrating the intersection and union set of DKD- and DM-associated immune traits.

and DM. As shown in online supplementary Figure 1, 1 of the 5 exposures was on the B cell panel, as was demonstrated in the forest plot of type 1 DM. *CD20 on IgD$^+$CD38$^+$ B cell* showed protective effects in type 1 DM (IVW: OR, 0.955 [0.920–0.991], $p = 0.015$) (online suppl. Fig. 1). For type 2 DM, 2 of 7 were subtypes of B cells. Both *CD20 on IgD$^+$CD24$^+$ B cell* (IVW: OR, 0.964 [0.938–0.991], $p = 0.010$) and *CD25 on IgD$^+$CD24$^+$ B cell* (IVW: OR, 0.978 [0.965–0.992], $p = 0.002$) showed protective effects in type 2 DM (online suppl. Fig. 2). By grouping positive immune traits into protective and pathogenic categories, Venn diagrams were utilized to show the intersection and union set of DKD- and DM-associated immune traits (Fig. 3). This allowed identification of distinct circulating immune cell types that have causal links to DKD. Except for *CD20 on IgD$^+$CD24$^+$ B cell* trait that was shared by DKD and type 2 DM, other 10 positive immune traits were all specific to DKD, suggesting their potential roles in the development of kidney injury under DM conditions (Fig. 3).

Large-Scale Single-Cell Integrative Analysis Reveals Correlations of B Cells and DKD

To validate the MR results, we asked whether similar cell types related to DKD susceptibility could be resolved in human PBMC single-cell transcriptomics. Therefore, scDRS analysis was introduced to link the DKD GWAS data to human PBMC single-cell transcriptomics. For an adequate validation, five publicly available large-scale PBMC scRNA-seq datasets containing a total of ~2,570,000 human PBMCs with previous annotation and UMAP reduction were obtained and used for scDRS scoring, as was shown in Figure 4a. Cells identified to be significantly associated with DKD phenotype by the scDRS analysis were highlighted in the UMAP plot (Fig. 4b). By large-scale analysis of these datasets, we constructed a comprehensive atlas of human PBMCs associated with DKD phenotype. Obviously, B cells, dendritic cells, monocyte, and T cell subsets were identified as DKD-related cell types, in line with each other among the five datasets and in accordance with previous MR results. Subsequent proportion quantification of DKD relevant or irrelevant cells in each cell type was visualized by stacked bar plot in decreasing order. The scDRS score distribution in each cell type was also quantified as was shown in the violin plot. B cell cluster and two main subsets of B cells, naïve B and memory B cells had higher proportion of DKD-related cells or scDRS score of DKD phenotype, suggesting their strong association with DKD phenotype. This result further provided quantitative evidence and highlighted associations between B cells or B cell subpopulations and DKD phenotype (Fig. 4c–g).



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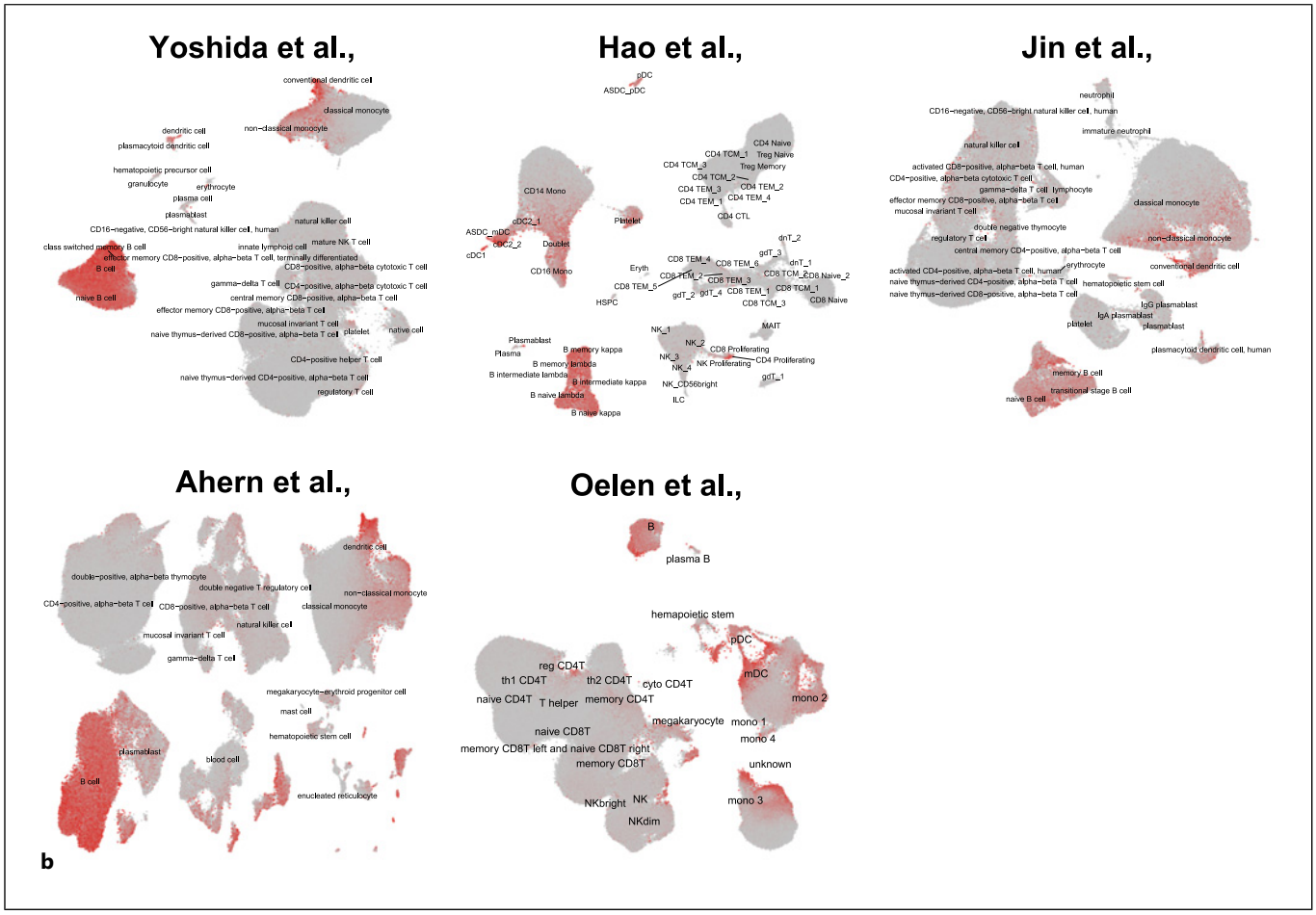
Deciphering Circulating Immune Cell Type Alteration in DM to DKD Progression

Finally, to study whether alterative composition of circulating immune cells that have causal links to DKD could play a role in DM to DKD progression, we asked whether circulating B cells or B cell subpopulations were upregulated or downregulated in DKD compared to DM. Five RNA microarray datasets encompassing a total of 74 HCs, 134 individuals with DM, and 23 patients with DKD were selected and integrated to remove the batch effect (online suppl. Table 1) (online suppl. Fig. 3). Immune infiltrate deconvolution analysis by CIBERSORT and xCell were then performed. Our CIBERSORT analysis detected significant reduction in memory B cells in the DKD group, compared to both HC and DM groups. In parallel, xCell analysis demonstrated a notable decrease in memory B cells and class-switched memory B cells in PBMCs of DKD patients, in contrast to DM patients without DKD (Fig. 5). These results indicate a potential deficiency of memory B cells in DKD patients versus DM patients without kidney implications.

Discussion

Despite strict adherence to medication and dietary regimes, approximately 35% of individuals diagnosed with DM are likely to develop DKD [5, 6]. Unfortunately, the limited efficacy of existing treatments means a significant proportion of DKD patients will progress to end-stage renal disease [4]. Therefore, it is critical to delve into the pathogenesis of DKD in order to devise novel therapeutic strategies. This study represents the first exploration of the causal links between total circulating human immune cells and DKD utilizing MR methods. Our findings underscore the pivotal roles played by memory



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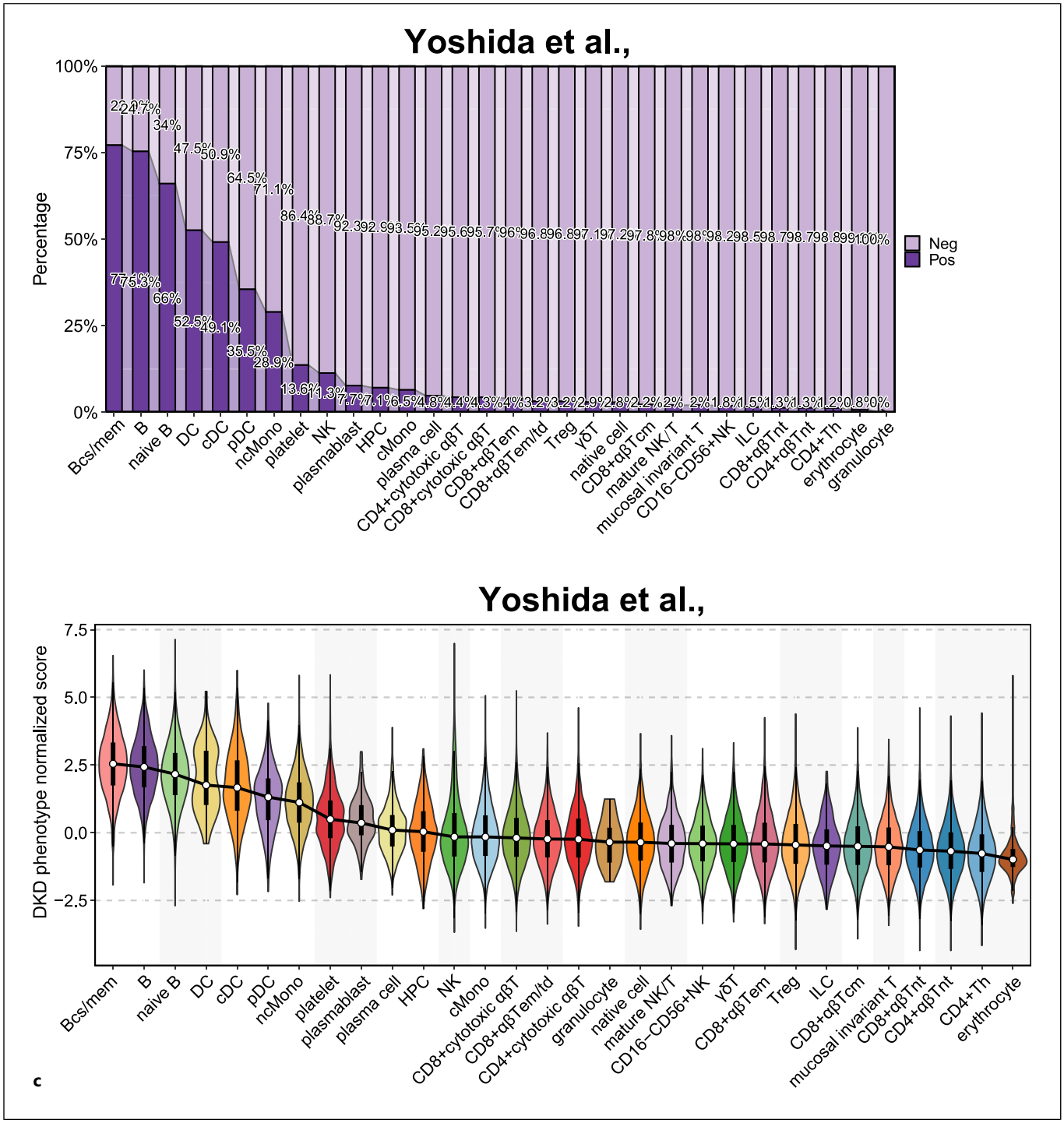
B cells in the pathogenesis of DKD and, through further analysis utilizing scDRS, identified specific pathogenic and protective subpopulations. Notably, *CD24^{+}CD27^{+} B cells*, *BAFF-R on CD20^{-}CD38^{-} B cells* and *BAFF-R on IgG^{-}CD38^{+} B cells* were identified as having protective potential against DKD. Conversely, *IgD^{-}CD27^{-} B cells* and *IgD^{-}CD24^{-} B cells* were found to be pathogenic in the context of DKD. Moreover, immune infiltrate deconvolution analysis revealed a significant reduction in memory B cells within the PBMCs of DKD patients. By adopting a comprehensive investigative approach, this study bridges the knowledge gap concerning the role of circulating immune cells, particularly memory B cells, in the development of DKD.

GWAS have pinpointed several gene variants proximal to pro-inflammatory cytokines including AFF3 [11], RGMA-MCTP2 [11], and CDCA7-SP3 [12]. These variants demonstrate a noteworthy positive correlation with the development of DKD. Existing literature underscores the pivotal influence of peripheral immune mechanisms on other diabetic microvascular afflictions, such as diabetic retinopathy and neuropathy [17, 18]. Nevertheless, research exploring the association between systemic immune cell populations and DKD etiology remains scant. Our MR analysis constitutes an inaugural effort to delineate a putative causal association between systemic immune cells and DKD, intimating that these cells, particularly diverse memory B cell subsets, may exert pathogenic or protective influences. Intriguingly, this influence seems kidney-specific, evidenced by the predominant impact of T cells, rather than memory B cells on DM, corroborating previous findings [19]. Additionally, deconvolution of immune infiltrates has revealed a significant reduction in memory B cell frequencies in patients with DKD relative to those with DM but without DKD. It is well-documented that metabolic



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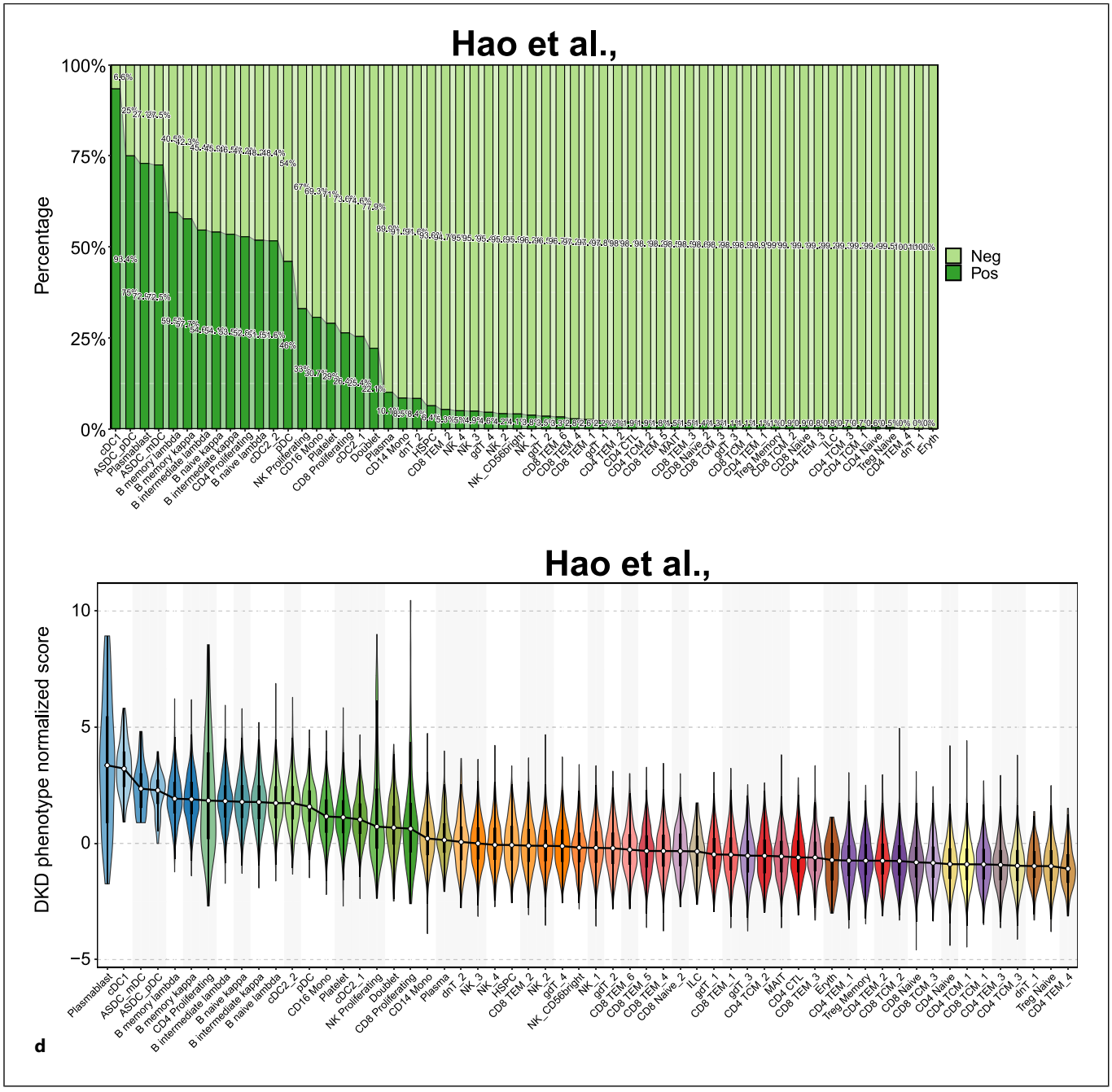
disturbances in DM, such as hyperglycemia and the production of AGEs, instigate chronic inflammation, thereby accelerating the progression of various organ sequelae, including DKD [52]. Recognizing the causative links between immune cells in circulation and the onset of DKD, as suggested by our study, points to an immunological predisposition for renal involvement in DM, with memory B cells potentially playing an instrumental



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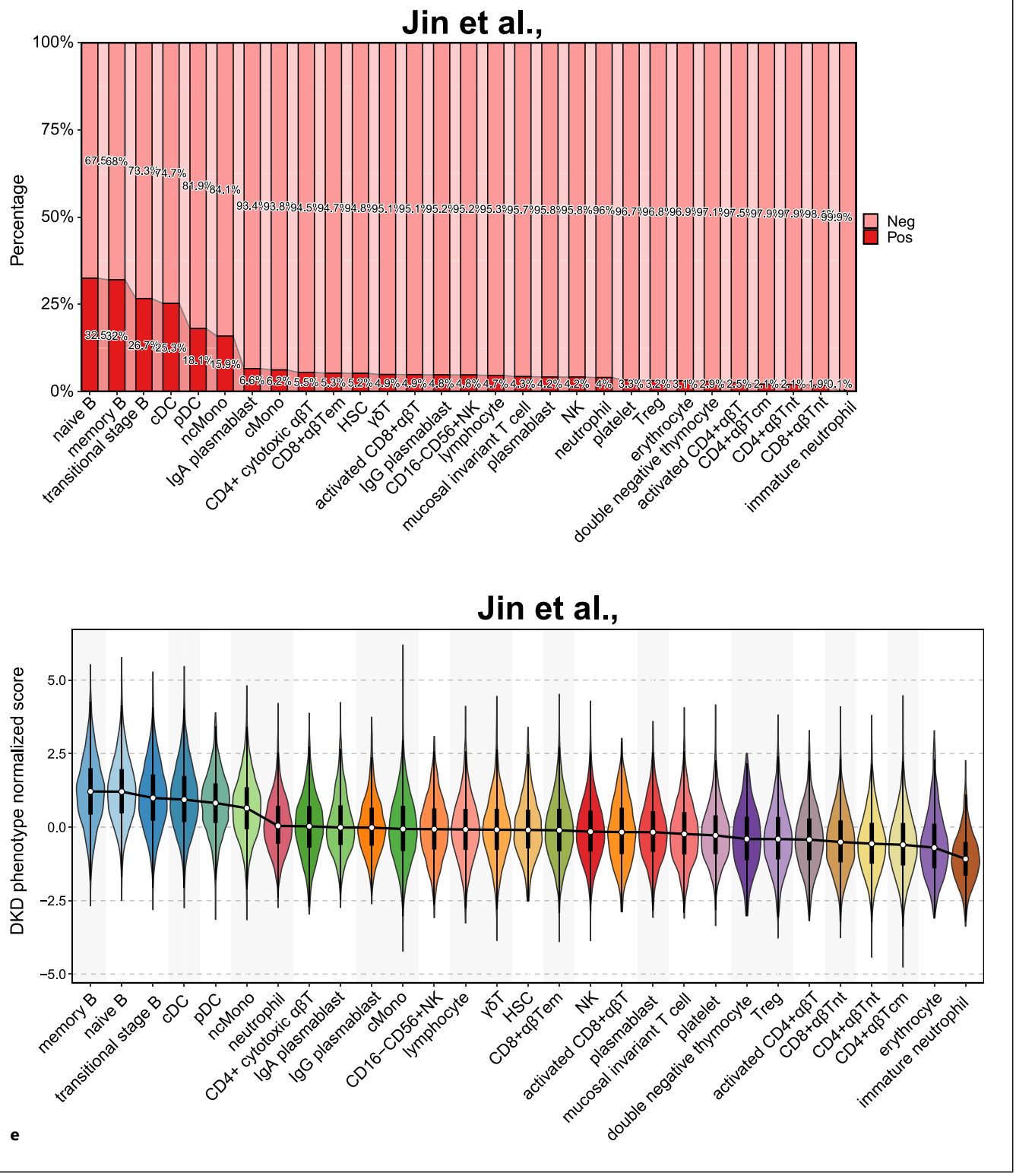
role in modulating the renal immunological and inflammatory responses in diabetic contexts.

Memory B cells differentiate from naive B cells by their prolonged lifespan, enhanced capacity for a rapid and vigorous response upon stimulation, and the expression of somatically mutated and affinity-matured immunoglobulin (Ig) genes [53]. Within the adult human B cell population, approximately 40% are memory B cells [53], comprising diverse subsets including IgG, IgA, IgE, IgD$^-$only, IgM$^-$only, and IgM$^+$IgD$^+$ memory B cells, in addition to splenic marginal zone B cells and regulatory B (Breg) cells [54]. The MR analysis conducted in our study identified *CD24$^+$CD27$^+$ B cells*, representing a subset of Breg cells characterized by high interleukin-10 (IL-10)



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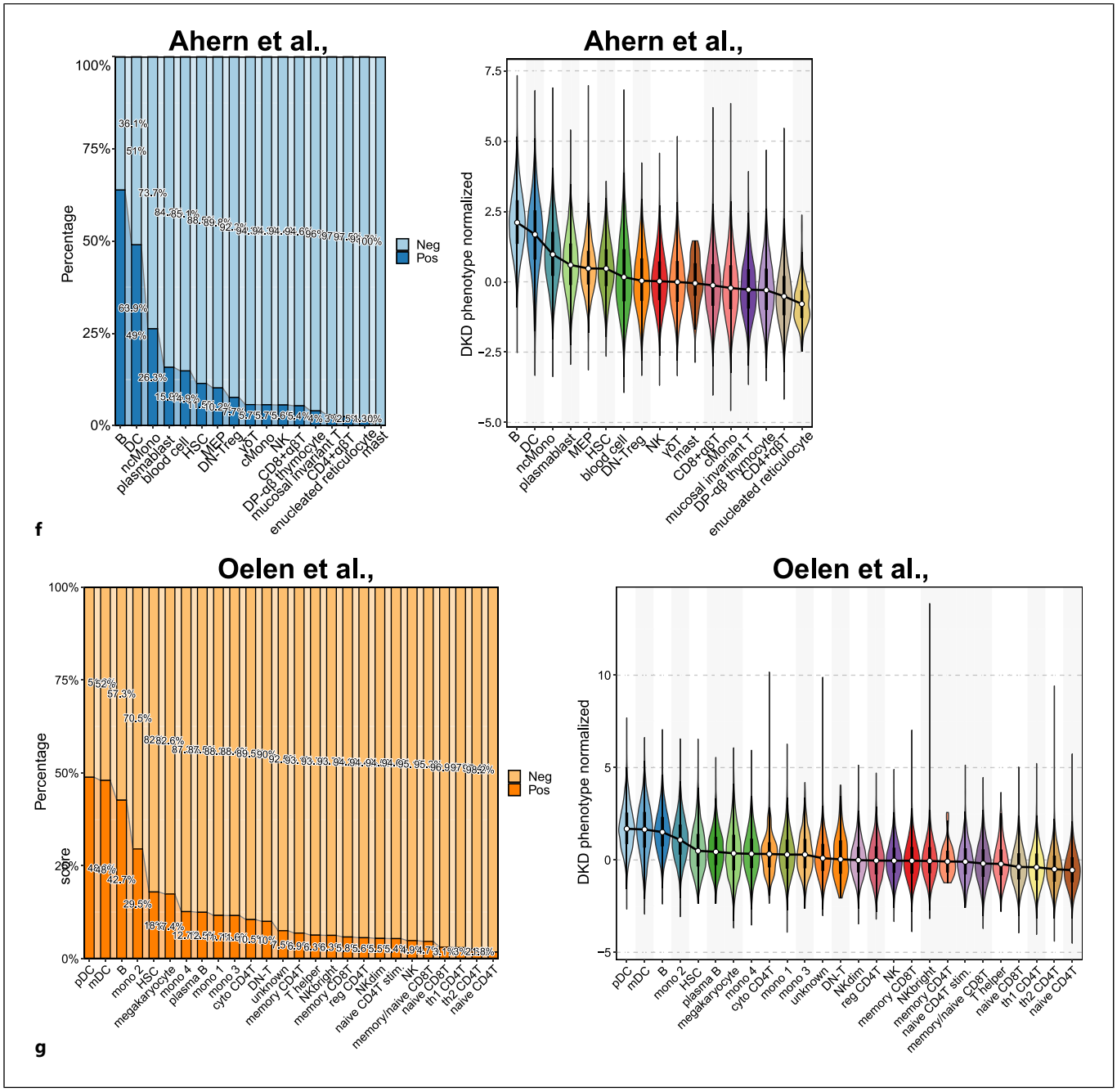

Fig. 4. Identification of association between DKD phenotype and B cells or B cell subsets by scDRS scoring analysis. **a** UMAP plots demonstrating DKD phenotype score by scDRS analysis of each cell in 5 datasets. **b** UMAP plots displaying cells significantly associated with DKD phenotype identified by scDRS as were shown in the red color. Cells that were not significantly associated with DKD phenotype were in gray color. **c–g** Stacked bar plots depicting the proportion of DKD relevant or irrelevant cells from 5 datasets in each cell type in decreasing order. Violin plots showing the scDRS score in each cell type from 5 datasets in decreasing order.

secretion and commonly referred to as B10 cells, as a protective element in DKD. It is well-documented that B10 cells can attenuate excessive inflammation by inhibiting TNF-α, IFN-γ, and IL-17 in autoimmune conditions largely through their production of IL-10 [52, 55–57], and the depletion of B10 cells exacerbates symptoms of autoimmune diseases in murine models [56]. Under diabetic conditions, metabolic derangements

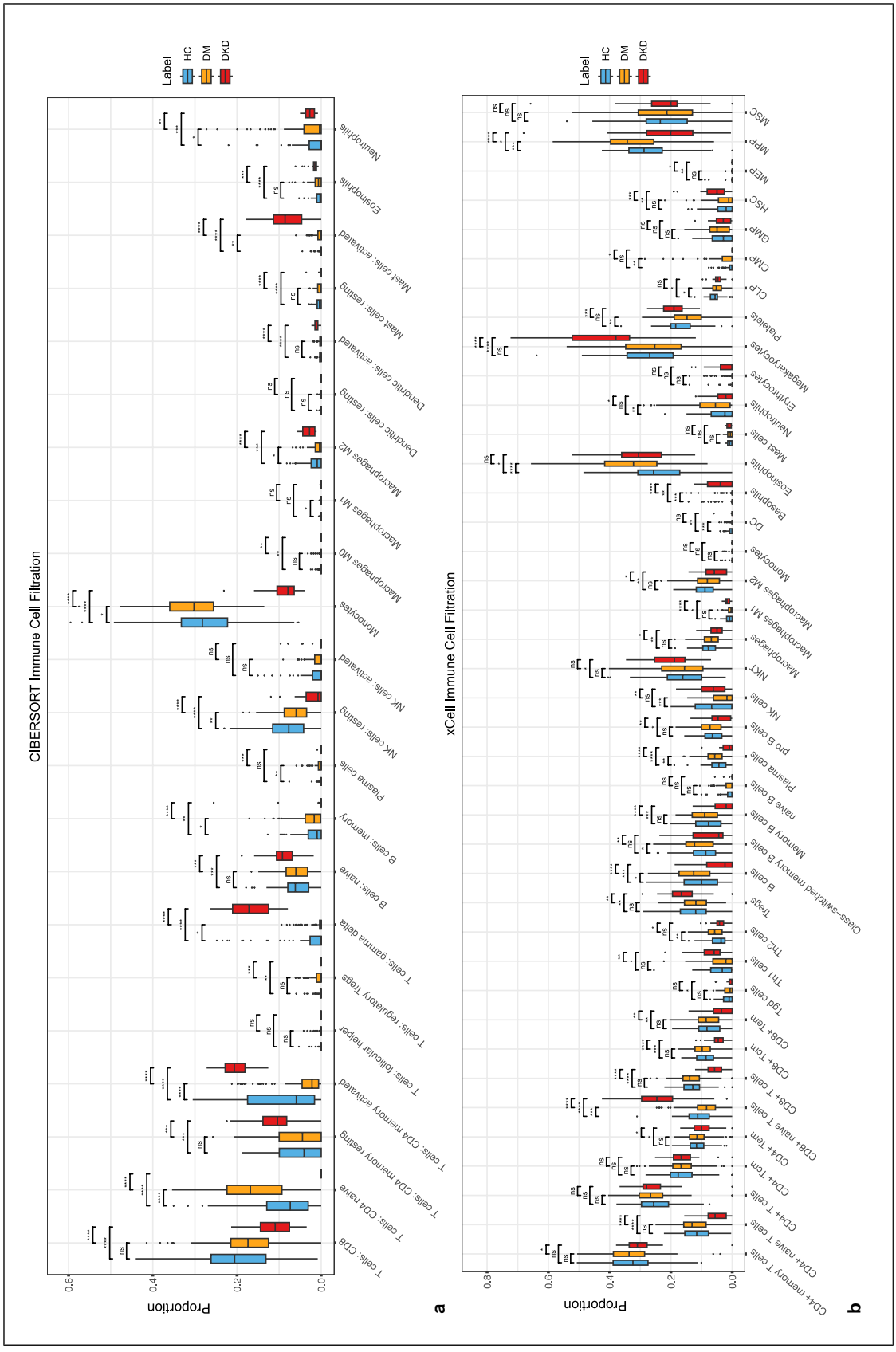

Fig. 5. Immune cell infiltration analysis of PBMC gene-expression data from DM and DKD patients. Box plots exhibiting the infiltration proportion of immune cells through different analysis methods, CIBERSORT (**a**) and xCell (**b**). Wilcoxon-Mann-Whitney test was used for comparisons differences between groups (ns indicates no significance, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, $****p < 0.0001$).

facilitate the formation of AGEs, which accumulate in the renal glomeruli and tubules, instigating cellular apoptosis and inflammation [58–61]. Previous research has suggested that IL-10 could confer protection against AGE-induced apoptosis by diminishing oxidative stress via the suppression of NF-κB activation in the Schwann cells of diabetic neuropathy [62]. However, the observed significant reduction in Breg cell populations in patients with DKD, compared to those with DM and HCs [63, 64], may lead to a decrease in B10 cells and a subsequent reduction in IL-10 secretion among DKD patients, potentially facilitating the onset of kidney injury through an unmitigated inflammatory response. Deciphering the role of *CD24$^+$CD27$^+$ B cells* in individual patients may pave the way for the development of precision therapies aimed at individuals genetically at risk for DKD.

Our investigation has elucidated that the presence of *BAFF-R on CD20$^-$CD38$^-$ B cells* or *BAFF-R on IgG$^-$CD38$^+$ B cells* may exert protective roles in the context of DKD. *CD20$^-$CD38$^-$ B cells* are delineated as a transitional cohort of preplasmablasts pivotal in the differentiation trajectory of human memory B cells toward plasma cells [65], whereas *IgG$^-$CD38$^+$ B cells* epitomize a subclass of immature B cells or plasmablasts [66], with both subsets being classified as immature. BAFF-R, the receptor for B cell-activating factor (BAFF), is instrumental in facilitating the maturation of transitional B cells and in prolonging the viability of mature B cells [67–69], potentially fostering the maturation or survival of *CD20$^-$CD38$^-$* or *IgG$^-$CD38$^+$ B cells*. Despite the paucity of research focusing on the implications of these cellular phenotypes in DKD patients, existing literature indicates a reduction in BAFF-R-expressing B cells in patients with T1DM [70]. A diminution in *BAFF-R on CD20$^-$CD38$^-$ B cells* or *BAFF-R on IgG$^-$CD38$^+$ B cells* may impede the maturation process of these cells, thereby affecting the functionality and longevity of memory B cells. Experimental studies on diabetic NOD mice have demonstrated that short-term administration of BAFF-R-Fc, a BAFF-R analog, augments the population of Breg lymphocytes (B10 cells) that secrete IL-10, thereby inhibiting the progression of T1DM [71]. These insights propose that *BAFF-R on CD20$^-$CD38$^-$ B cells* or *BAFF-R on IgG$^-$CD38$^+$ B cells* may synergize with B10 cells to modulate immune responses in DKD pathogenesis. As of the present time, there exists a paucity of research explicitly concentrating on the elucidation of BAFF-R's roles within the two aforementioned cellular types. Consequently, it is imperative that additional investigations are undertaken to substantiate its potential functionality.

IgD$^-$CD27$^-$ B cells and *IgD$^-$CD24$^-$ B cells* have been identified as pathogenic for DKD in the current MR analysis. The subset of *IgD$^-$CD27$^-$ B cells*, commonly known as "double negative" memory B cells, has been observed to increase in older individuals and in autoimmune conditions such as systemic lupus erythematosus [72]. These cells contribute to disease pathogenesis through the secretion of pro-inflammatory cytokines like TNF-a and cytotoxic granzyme B [73, 74], and by migrating to inflammatory sites via the expression of CXCR3 and CCR6 [75]. Although direct evidence linking the *IgD$^-$CD27$^-$ B cells* to DKD is lacking, analogous disease insights suggest they may play a role in DKD by promoting inflammation. *IgD$^-$CD24$^-$ B cells*, on the other hand, are indicative of memory switched B cells or plasmablasts [76]. Previous MR studies have highlighted *CD20 on IgD$^-$CD24$^-$ B cells* as a risk factor for Parkinson's disease [77] and *BAFF-R on IgD$^-$CD24$^-$ B cells* as a risk factor for alopecia areata [78]. However, the precise role of *IgD$^-$CD24$^-$ B cells* in DKD is still unclear, and no studies have been conducted to elucidate their function in this context. Further investigation is required to elucidate the multifaceted roles of B cell subtypes in the pathogenesis of DKD, potentially guiding the development of novel therapeutic strategies for DKD.

Our investigation, encompassing multi-omics interrogation, concedes certain limitations that warrant recognition. Primarily, constraints stemming from the data provenance precluded the execution of a MR analysis contrasting DM and DKD within an identical cohort. Nevertheless, availing ourselves of two iterations of the FinnGen repository, we instigated distinct MR inquiries comparing DM and DKD against populations devoid of DM. These inquiries unveiled that predisposition factors pertinent to DKD diverge from those implicated in DM. Significantly, the MR delineations pertaining to DM align with antecedent analyses conducted across variegated cohorts [19], which serves to substantiate the reliability of our discoveries. Second, MR analysis serves as a pivotal technique for elucidating causal relationships between exposures and outcomes. Nonetheless, it is imperative to recognize the constraints of MR in addressing various potential confounders, such as pleiotropy, population stratification, LD, canalization, measurement inaccuracies, and dynastic effects. To surmount these obstacles, our investigation executed comprehensive leave-one-out sensitivity analyses, heterogeneity assessments utilizing IVW and MR-Egger methods, and pleiotropy evaluations through MR-Egger regression intercept and MRPRESSO. Furthermore, to fortify the credibility of our causal inferences,

we applied five distinct analytical strategies, encompassing IVW, simple mode, weighted median-based, weighted mode-based, and MR-Egger regression methodologies. Third, whilst the dataset, encompassing 731 immune traits, demonstrates formidable statistical significance thereby bolstering the reliability of the genetic correlations identified, integrating data derived from a more heterogeneous array of populations in the GWAS catalog would serve to mitigate biases idiosyncratic to specific populations. As such, additional external validations should be pursued upon availability of pertinent datasets. Fourth, the GEO dataset may exhibit biases and limitations that could potentially impact the validity of immune deconvolution analyses. These include issues pertaining to data fidelity, intra-sample variability, and inter-batch inconsistencies. In order to enhance the precision of the data, we implemented a variety of strategies aimed at reducing potential biases, which are detailed in the methods section of this paper. In conclusion, while MR scrutiny insinuates a contributory relation of immune cells vis-à-vis DKD, corroborative experimental probings remain imperative for the corroboration of these insights.

Through the confluence of data from MR and scDRS analyses, our investigation has elucidated a causative connection between circulating immune cells and DKD, underscoring the critical function of various B cell subsets in the etiology of DKD. Such revelations enable a more nuanced understanding of the mechanisms by which immune cells exacerbate the development of DKD. The exploration into the diverse B cell factions and their precise influences on DKD paves the way for the identification of innovative pharmacological targets. Concentrating efforts on immune-modulating therapies that selectively engage specific B cell cohorts represents an avant-garde approach to mitigating DKD. Collectively, our research indicates a fertile domain for the development of tailored therapeutic modalities in the context of DKD. However, the translation of these scientific findings into efficacious medical interventions demands supplemental investigative endeavors, clinical validations, and a meticulous contemplation of the attendant implementation challenges.

Statement of Ethics

An ethics statement was not required for this study type since no human or animal subjects or materials were used.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

Yuan Ma, Jing Ji, Xintong Liu, Lingyi Xu, and Qingqing Zhou collected and analyzed the data. Yuan Ma and Zehua Li drafted the manuscript. Zehua Li conceived the study and revised the manuscript. Xizi Zheng revised the manuscript. Li Yang conceived and supervised the study, interpreted the data, and revised the manuscript. All authors read and approved the final manuscript.

Data Availability Statement

All data generated or analyzed during this study are publicly available and all included in this article and its supplementary material files. The access of all data is provided in the methods and materials session. Further inquiries can be directed to the corresponding author.

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