The causal relationship between circulating leukocytes and kidney function: A Mendelian randomization study

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Research Article

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Abstract

Background

Several studies proposed that inflammatory response strongly correlated with kidney function and the progression of the chronic kidney disease (CKD), both in terms of its onset and course as well as any ensuing consequences.

Objectives

To investigate the potential causal relationship of the five subtypes of leukocytes count (monocytes, lymphocytes, neutrophils, eosinophils, and basophils) with CKD and kidney function by employing Mendelian randomization (MR) analysis.

Methods

At the genome-wide significance level, single-nucleotide polymorphisms correlated to major white blood cell types were identified. Large-scale genome-wide association studies with sample sizes of 44,266, 86,640, 58,284, and 23,210 provided summary-level data for CKD, eGFR, and urine albumin-to-creatinine ratio (uACR), respectively. The inverse variance weighted (IVW) method was used for primary MR analysis, and additional sensitivity approach were carried out to evaluate the robustness.

Results

We discovered that a higher genetically determined monocyte count was causally associated with an increased genetically predicted eGFR level (beta = 0.0035; 95% CI: 0.0013–0.0057; P = 1.45×10^{-3}) and uACR level (beta_{IVW} = 0.017; 95%CI: 0.008–0.027, P = 5.5 × 10^{-4}). Sensitivity analyses employing different approaches revealed comparable associations, while MR-Egger regression revealed no indication of pleiotropy. In addition, we observed that was lymphocyte count (beta_{IVW} = 0.018; 95%CI: 0.004–0.033, P = 1.1 × 10^{-2}) and neutrophil count (beta_{IVW} = 0.018; 95%CI: 0.001–0.035, P = 3.9 × 10^{-2}) were positively associated with uACR, while the association remained non-significant after Bonferroni correction.

Conclusion

Our research implicates peripheral white blood cells, specifically monocytes, lymphocytes, and eosinophils, to the kidney function damage, underscoring the necessity for mechanistic investigations to discover these associations.
The estimated glomerular filtration rate (eGFR) is typically used to measure kidney function and chronic kidney disease (CKD), a condition in which the function and structure of the kidney are adversely affected by heterogeneous disorders [1], is defined as when it falls below 60 mL/min/1.73 m² for at least 90 days [2, 3], to differentiate the acute kidney injury or transient fluctuations in kidney function unrelated to kidney damage from CKD [4]. In recent years, a growing body of research has shown that multiple systems within the body are affected by CKD, which is associated with cardiovascular disease [5–8], Cerebrovascular Disease [9, 10], Central and peripheral arterial diseases [8], and so on. In the 21st century, CKD has been identified as one of the leading causes of worldwide mortality [11–13]. Several factors, including population growth, aging, diabetes, hypertension, and increased mortality, have all contributed to the dramatic increase in global CKD incidence, prevalence, mortality, and DALYs since 1990 [14]. Between 1990 and 2017, CKD increased global mortality rates by 41.5% [15], occupying the place of 19th leading cause of years of life lost in 1990 and 12th place in 2017. Globally, forecasts suggest it will become the fifth leading cause of years of life lost by 2040 [16]. Taking action to target deteriorating renal function and diagnosing it early are crucial. To limit the future burden of healthcare, it is imperative to identify novel risk factors and subsequent prophylactic and treatment options for CKD, given current challenges.

Routine blood tests measure white blood cells (WBC) and categorize them into five subtypes: basophils, eosinophils, lymphocytes, monocytes, and neutrophils [17]. Hospitalization, cardiovascular events, and a host of other adverse outcomes are linked to higher total white blood cell counts, a novel predictor of mortality in kidney disease patients, following infection or inflammation [18–20]. Multiple granules are found in the cytoplasm of PMNLs, which are named after their lobulated nuclei. Granulocytes can be subdivided into three groups based on the staining behavior of their granules: eosinophilic, basophilic, and neutrophilic [21]. CKD patients’ morbidity and mortality are increased by impaired PMNL functions, which lead to an increased risk of bacterial infections [22]. As neutrophils become activated, ROS are released, leading to oxidative stress. As a result of protease release, endothelial dysfunction is caused and proinflammatory cytokines are released that further exacerbate the inflammatory response. The development of fibrotic changes in the kidneys is caused by all these mechanisms [23]. In HES, renal affection occurs mainly late, primarily as a result of vascular events resulting from thromboembolism or atheroembolism [24]. It is plausible that higher monocyte counts are a marker that precedes or is involved in the pathway mediating the development and progression of CKD, based on epidemiologic findings that higher monocyte counts predict future CKD and its progression [25]. A reduction in polarization toward proinflammatory phenotypes in T lymphocytes caused by the positive type 1 angiotensin (AT1) receptors signal protects the kidney from hypertensive damage [26]. As a mechanism of preventing autoimmune responses by modulating innate immune functions and lymphocyte homeostasis, autophagy is renoprotective in epithelial renal cells and podocytes in a variety of models of acute kidney injury, glomerular disease, and aging [27, 28]. In CKD, HD cannot restore leukocyte autophagy activation, which is impaired by the disease [29].
In genetic epidemiology, Mendelian randomization (MR) is a method for inferring causality between exposure and outcome from genetic variations, especially single nucleotide polymorphisms (SNPs) [30]. The majority of epidemiologic analyses conducted on CKD and kidney function are based on traditional methods (e.g., cross-sectional, case-control, cohort). However, they are subject to various limitations, such as confounding and reverse causation bias, which can affect the estimates of effect [31, 32]. As genotypes are genetically determined at conception and cannot be altered, MR can overcome these biases. The potential causal relationship of circulating WBC subtypes with CKD and kidney function has not yet been established in MR studies. Therefore, our study aimed to determine if genetically predicted WBCs were related to CKD using the two-sample MR method.

**Methods**

As shown in Fig. 1, our study design is summarized. An institutional review board was not needed since the data we used were extracted from published studies and public databases.

2.1 | WBC count GWAS data

Based on genome-wide association studies (GWASs) published in the literature, we collected summary statistics on WBC subtype counts. The hitherto largest GWAS summary statistics were obtained from the Blood Cell Consortium (BCX) meta-analysis, the largest study being UKBB (N = 562132) [33]. Supplementary Table S1 provides detailed information on the outcome of GWASs. The WBC counts summary statistics were downloaded from http://www.mhi-humangenetics.org/en/resources/.

A total of 524, 519, 430, 456, and 207 single nucleotide polymorphisms (SNPs) with genome-wide significance (P < 5 × 10^{-8}) and a clumping at a linkage disequilibrium (LD) threshold of r^2 < 0.01 were used in the analyses as instrumental variables (IVs) for monocytes, lymphocytes, neutrophils, eosinophils, and basophils, respectively (Table S2-S6). To exclude weak instrumental variable bias, the calculated F-statistics for five subtypes of white blood cells used to quantify the IVs ranged from 22 to 230, which is consistent with the hypothesis of F > 10 for MR analyses [34].

2.2 | CKD GWAS data

A meta-analysis of 41,395 cases and 439,303 controls, the largest GWAS of CKD to date, was conducted by the CKDGen Consortium, combining data from 23 participating studies. All the participants were of European ancestry. Since no index variants were reported in the original GWAS, we identified independent SNPs at the genome-wide significance level (P < 5 × 10^{-8}) using clumping at an LD threshold of r^2 < 0.01. The relevant GWAS of eGFR encompassed 567,460 European ancestry samples from 54 cohorts [35]. We further adopted another eGFR GWAS for MR analysis from the Within family GWAS consortium (https://www.withinfamilyconsortium.com/cohorts/), containing 76,511 European ancestry samples, to minimize the false positive results. The specially chosen urine albumin-to-creatinine ratio (uACR) related
data was derived from the large-scale GWAS meta-analyses of uACR genome-wide association studies conducted by Teumer et al. [36], comprising 51,861 cases and 297,093 healthy controls selected by the CKDgen Consortium. The GWAS study was limited to individuals with European ancestry to minimize the possibility of population stratification bias.

2.3 | Mendelian randomization analysis

The effect alleles of the exposure and outcome datasets were placed on the same reference strand after harmonization [37]. Corrections were made to SNPs with incorrect but unambiguous strand references, while deletions were made to those with ambiguous strand references. By using the R package 'TwoSampleMR', summary statistics for exposures were processed to select valid MR instruments. The causal relationship was examined with the use of two-sample MR analyses based on summary statistics and “MRPRESSO” packages in the R software version. Three core assumptions underpin the MR study: (1) IVs and exposure are strongly associated; (2) there are no confounding factors that affect the IVs; and (3) there are no alternative pathways for instruments to influence the outcome other than the exposure of interest. The multiplicative random effects meta-analysis of the effect of all exposure SNPs on outcome was undertaken using the inverse-variance weighted (IVW) method for the primary MR analyses [38]. To complement IVW estimates, MR-Egger and weighted median methods were employed, as they provided more robust estimates in a wider range of scenarios, but were less efficient [39]. As part of the comparison with the main IVW estimates, several sensitivity analyses have been conducted. Cochran's Q heterogeneity test was used to detect vertical pleiotropy, which occurs when a trait is downstream of the genetic variant but follows the same pathway as the exposure. One of the main assumptions of MR is violated by horizontal pleiotropy, in which some or all instruments for a trait act via an alternative pathway from exposure. By using the weighted median method, the overall causal effect can be estimated consistently when more than half of the IVs are valid, reducing its bias as compared with the IVW estimate [40]. Instrumental SNPs were tested for multi-effects using the MR-Egger method. The directional pleiotropy was measured by examining the intercept of the MR-Egger regression [41]. In addition, by eliminating possible outliers from the MR pleiotropy residual sum and outlier test (MR-PRESSO), we were able to detect and correct individual SNPs contributing to horizontal pleiotropy [42]. If the percentage of horizontal pleiotropy variants is less than 10%, it has less bias and precision to a greater degree than IVW and MR-Egger [42]. MR estimates were also analyzed with leave-one-out analysis to determine whether a single SNP biases the result.

Multiple statistical tests were corrected using the Bonferroni method. A P-value < 3.33 × 10^{-3} (P = 0.05 / (five exposures × three outcomes) was applied to prove statistical significance. It was regarded as suggestive evidence for a possible causal association if the P-value exceeded the Bonferroni-corrected threshold but was lower than 0.05.

Results
Based on the MR analyses, Fig. 2 illustrates the association of leukocytes with CKD, eGFR, and uACR. For these instrumental variables, the F-statistics for the selected SNPs are above 10, indicating that these instrumental variables are subject to a weak instrument bias with a low probability. Across all instrumental variables in the heterogeneity test between each of the cell subtypes and CKD, Cochran's Q values exceeded 0.05, so we chose the fixed-effect IVW model for MR analysis. The causal relationship of genetically predicted levels of all five white blood cell subpopulations for CKD risk was not confirmed (monocyte count: $\text{OR}_{\text{IVW}} = 0.84$, 95%CI: 0.67–1.05, $P = 0.13$; lymphocyte count: $\text{OR}_{\text{IVW}} = 1.08$, 95%CI: 0.83–1.40, $P = 0.58$; neutrophil count: $\text{OR}_{\text{IVW}} = 1.23$, 95%CI: 0.92–1.64, $P = 0.16$; basophil count: $\text{OR}_{\text{IVW}} = 0.68$, 95%CI: 0.44–1.05, $P = 0.08$; eosinophil count: $\text{OR}_{\text{IVW}} = 1.02$, 95%CI: 0.79–1.31, $P = 0.90$).

In the MR analysis of the causal relationship of each type of leukocytes with eGFR or uACR, the presence of heterogeneity was detected by the Cochran Q-test and MR-PRESSO (p-value in the global heterogeneity test < 0.001) after removing outliers, so we chose the random-effects IVW approach. As shown in Fig. 3, genetically predicted quintiles of all cell subtypes were found to be causally associated with eGFR risk only by the IVW method (monocyte count: $\beta = 0.0035$; 95% CI: 0.0013–0.0057; $P = 1.45 \times 10^{-3}$). The sensitivity analysis excluding palindromic SNPs showed similar results. MR-Egger regression ($\beta = 0.0042$; 95% CI: 0.0007-1 = 0.0076; $P = 1.85 \times 10^{-2}$) or weighted median ($\beta = 0.0032$; 95% CI: 0.0006–0.0059; $P = 1.79 \times 10^{-2}$) approaches yielded directionally consistent causal estimates. However, MR analysis of the rest of four subtypes of leukocytes with eGFR indicated no statistically significant association existing (lymphocyte count: $\beta_{\text{IVW}} = 0.0031$, 95%CI: -0.003-0.0064, $P = 0.0031$; neutrophil count: $\beta_{\text{IVW}} = -0.0014$, 95%CI: -0.0051-0.0023, $P = 0.4625$; basophil count: $\beta_{\text{IVW}} = -0.0018$, 95%CI: -0.0074-0.0064, $P = 0.5363$; eosinophil count: $\beta_{\text{IVW}} = 0.0033$, 95%CI: 0.0006–0.0065, $P = 0.0460$). Of note, an increased level of monocyte count ($\beta_{\text{IVW}} = 0.017$; 95% CI: 0.008–0.027, $P = 5.5 \times 10^{-4}$) was the only significantly associated factor with genetically predicted uACR. In the meanwhile, the potential causal association of lymphocyte count ($\beta_{\text{IVW}} = 0.018$; 95% CI: 0.004–0.033, $P = 1.1 \times 10^{-2}$) and neutrophil count ($\beta_{\text{IVW}} = 0.018$; 95% CI: 0.001–0.035, $P = 3.9 \times 10^{-2}$) with uACR were identified. Furthermore, no causal correlation was detected for eosinophil count and lymphocyte count with uACR. These negative results were confirmed by the MR-PRESSO and the weighted median. MR-Egger regression also revealed no evidence of horizontal pleiotropy.

The directional pleiotropy was identified in the MR analysis between the basophil count and uACR (intercept = $1.19 \times 10^{-3}$, SE = $5.85 \times 10^{-4}$, $P = 0.043$), which means the pleiotropic IVs may influence the outcome as a result of exposure to other pathways. No significant intercept was discovered in the other aforementioned MR analyses, indicating the null findings of the directional pleiotropy. Additionally, a leave-one-out analysis was performed to determine whether any single SNP significantly violated the causal estimate, indicating that monocyte count and eGFR are positively associated genetically.

There was no single variant found that influenced the IVW association as determined by the leave-one-out sensitivity analysis (Figure S1-S3). The effects of all individual IVs on leukocyte counts and levels of
kidney function were visualized with further inspection of the funnel plots and scatter plots, supporting the robustness of the results (Figure S4-S9).

To further comprehend the causal correlations between kidney function and blood cell counts, we investigated the interaction of kidney function traits with leukocyte traits in the reverse direction using kidney function-associated variants as IVs. There was no indication that the CKD index and kidney function index were associated with the blood cell counts (Table S7-S9; Table S14-S16).

Discussion

In our opinion, this is the first genetic study that systematically assesses the genetic architecture linking five circulating WBC subtypes, CKD, and kidney function. By adjusting for shared genetic architecture, MR allowed us to assess WBC counts as a causal effect. In comparison with the other four WBC subtypes, monocyte count consistently showed more pronounced associations with kidney function. Increased circulating eosinophil and lymphocyte counts potentially affect uACR with a protective causal effect, according to the evidence of our analyses.

An analysis of cross-sectional data of 4581 cohort participants without cardiovascular disease was conducted by Ganda et al. [43], which was the first report on the relationship between monocyte number and renal function indicators. There was a significant increase in the odds of having higher monocyte counts in quintile 5, relative to quintile 1, where the average eGFR was 80.54 ml/min per 1.73 m², as compared with quintile 1, with an average eGFR of 68.84 ml/min per 1.73 m². An observational cohort study of 1,594,700 veterans of the United States found that monocyte count is linearly related to adverse renal outcomes such as over 30% reduction in eGFR, doubling of serum creatinine, and risk of end-stage renal disease (ESRD) progression [25]. Others have reported similar associations between renal function measurements and monocyte count [44, 45]. However, our MR analysis showed the opposite, with monocytes playing a protective role in eGFR elevation. A family of four structurally related chemokines known as monocyte chemoattractant proteins (MCPs) are essential for the transmigration of monocytes. Chen et al. discovered that a decrease in MCP causes neutrophil recruitment and that there is an inverse relationship between the number of neutrophils and monocytes [46]. Binnetoğlu et al. observed 1000 patients with chronic kidney disease and found that neutrophils were pronouncedly positively correlated with 24-hour proteinuria severity and occurrence [47]. There was a significant increase in neutrophils in patients with chronic renal failure, and they were associated with an increased level of tumor necrosis factor-α (TNF-α) and interleukin (IL)-6, indicating a possible pathway in which neutrophils affect kidney function [48]. As evidenced by Huang et al., a significant relationship between neutrophil and monocyte counts with proteinuria was found among 12,225 normal, healthy individuals who underwent hospital examinations [49]. In addition, Cormican et al. discovered that only intermediate monocytes (IMs) with high human leukocyte antigen (HLA)-DR expression levels increased in individuals with CKD compared with healthy controls, indicating different monocyte subpopulations may play different roles in the process of influencing eGFR [50].
It is yet unknown how white blood cells, particularly lymphocytes, eosinophils, and monocytes, could contribute to uACR. Mateusz et al. Notably, recent studies have shown a causal relationship between eosinophil counts lymphocyte counts, and blood pressure [51]. In addition, Mendelian analysis also confirmed a two-way causal association between proteinuria and BP [52]. It could suggest that eosinophils and lymphocytes influence uACR through BP, however, it's also feasible that eosinophils and lymphocytes influence BP through separate causal pathways that involve uACR.

Proteinuria is associated with high levels of cytokines released by certain T lymphocyte subsets in patients with kidney disease [53]. Measurement of a cluster of differentiation 40 ligand (CD40L) on T follicular helper cells (TFH) found that CD40L levels in patients with kidney disease were significantly lower than in normal people [54]. IL-17, the main cytokine secreted by T helper cell (Th) 17, a newly discovered CD4 + T cell subpopulation, is highly expressed in the kidney. By reducing the expression of Podophysin proteins in podocytes and causing podocyte apoptosis, Th17, and IL-17 may contribute to kidney disease pathogenesis [55].

Patients with chronic kidney disease, particularly those with chronic kidney failure, commonly experience proteinuria. The development of glomerulosclerosis and fibrogenesis, which play a vital role in the progression of several diseases of the kidney, is associated with proteinuria, a fundamental finding in renal impairment [56]. Proteinuria often occurs due to impaired glomerular filtration barriers or irreversible tubular dysfunction. Infiltration of inflammatory cells into the renal interstitium is correlated with the degree of albuminuria. Early inflammatory response and advanced immunoinflammatory activity will damage the glomerular capillary filtration barrier, resulting in albuminuria in patients [57, 58]. Due to persistent inflammation and proteinuria, the CD40 receptors of T lymphocytes migrate from the basement membrane to the renal tubular wall, where they bind to T lymphocytes and release inflammatory factors, causing renal impairment [59].

The M1/M2 phenotype, with its mutually antagonistic functions, is produced following macrophage polarization. The former is activated by Interferon and lipopolysaccharide, which secretes cytokines that inhibit tumor growth and promote pathogen clearance. IL-4 and IL-13 stimulate the latter, which is associated with anti-inflammatory and tissue repair functions [60, 61].

Challenges can be encountered when interpreting estimates derived from MR. The first limitation was that we found significant differences only among individuals with European ancestry. Future investigations are expected to examine the relationships between white blood cell count and CKD risk among people of other ancestries since white blood cell count and CKD risk exhibit significant racial differences [45]. The assumptions underlying MR may be untestable, especially if potential confounding variables remain unmeasured. The presence of pleiotropy can be observed when genetic variants independently affect traits other than those investigated. MR-Egger and weighted median approaches do not completely protect findings from pleiotropic effects when used individually, but the consistency of effect estimates derived from multiple sensitivity analyses reinforces the plausibility of true causal effects. Thirdly, Two-sample MR assumes that exposure has a linear effect on outcome in GWAS summary data, but there is
no way to completely reveal other types of underlying relationships, such as U-shaped relationships. As white blood cell count phenotypes and kidney functions were mainly investigated by population-based cross-sectional studies, the effects of genetic variables may not reflect a longitudinal pattern. Further research should be conducted to clarify the relationship between white blood cell count changes and alterations in kidney function levels.

**Conclusion**

In summary, the current study identifies causal effects of monocyte counts on eGFR and uACR through a genetic analysis of European ancestry. The molecular mechanism of the potential causal links of lymphocyte count and neutrophil count on uACR is possibly related to blood pressure fluctuation and several immune responses in the human body. Management targeting circulating leukocyte counts may mitigate the damage to kidney function. Further investigation is required to clarify the molecular processes and identify particular pathways through which monocyte count, lymphocyte count, and neutrophil count may prevent the damage of kidney function.

**Declarations**

**Ethics approval and consent to participate**

Ethical approval was obtained in all original studies.

**Consent for publication**

Consent to participate was obtained in all original studies.

**Availability of data and materials**

GWAS summary data for blood cell counts from Blood Cell Consortium is available at https://www.withinfamilyconsortium.com/cohorts/; GWAS summary statistics for the CKD, eGFR, and uACR from CKDGen Consortium is available at https://www.finngen.fi/en/access results; GWAS summary statistics for the eGFR from the Within family GWAS consortium is available at https://www.withinfamilyconsortium.com/cohorts/.

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**Competing Interests**

The authors have no relevant financial or non-financial interests to disclose.

**Author Contributions**
X.L. and H.Y. contributed to the study conception and design. Data collection and analysis were performed by X.L. Visualization was accomplished by X.L. The first draft of the manuscript was written by X.L. H.Y. commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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References


7. Vallon V, Verma S: **Effects of SGLT2 Inhibitors on Kidney and Cardiovascular Function.** *Annual review of physiology* 2021, **83**:503-528.


49. Huang ZS, Chen YM, Wu KD, Chen MF: Higher peripheral neutrophil and monocyte counts are independent indicators of the presence and severity of proteinuria in apparently normal adults. *Internal medicine journal* 2010, 40(1):30-36.


**Figures**
Figure 1

Flowchart of Mendelian randomization analyses demonstrated the causal relationship between leukocyte counts on CKD and kidney function. CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; uACR, urine albumin-to-creatinine ratio; MR-PRESSO, MR Pleiotropy RESidual Sum and Outlier
Figure 2

Examining the relationship between white blood cell counts and chronic kidney disease using Mendelian randomization. 95% confidence intervals (95%CI) are indicated by the error bars, while the boxes denote the point estimate of the causal effects. CKD, chronic kidney disease
Figure 3

Examining the relationship between white blood cell counts and kidney function using Mendelian randomization. 95% confidence intervals (95%CI) are indicated by the error bars, while the boxes denote the point estimate of the causal effects. eGFR, estimated glomerular filtration rate; uACR, urine albumin-to-creatinine ratio

Supplementary Files

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