ORIGINAL RESEARCH

IFN-γ, SCF, MIP1b and IL-16 Were Associated with Risk of Diabetic Nephropathy: A Mendelian Randomization Study

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Background: The impact of inflammatory factors on the risk of diabetic nephropathy (DN) is inconsistent. Two-sample Mendelian randomization (MR) analyses were used to detect the causal role of inflammatory factors in DN risk.

Methods: Inflammatory factor GWAS summary data were collected from a meta-analysis including 8,293 Finnish participants, and DN information was extracted from a GWAS of 213,746 individuals from FinnGen. The MR Pleiotropy Residual Sum and Outlier (MR-PRESSO) outlier test was used for the removal of horizontal pleiotropic outliers. Multivariable MR analysis was also used to adjust for pleiotropy.

Results: IFN-γ [OR_{IVW}: 1.33; 95% CI: 1.09–1.63; p=0.005] and SCF [OR_{IVW}: 1.25, 1.02–1.52; $p = 0.027$] were associated with an increased risk of DN. MIP1b [OR_{IVW}: 0.92; 95% CI: 0.85–0.98; $p = 0.022$] and IL-16 [OR_{IVW}: 0.89, 0.81–0.99; $p = 0.043$] showed negative associations with the risk of DN. We validated our MR results with MR-PRESSO analyses. Significant horizontal pleiotropy was not found. Moreover, in the multivariable MR analysis, the associations between cytokines and DN risk remained.

Conclusion: Our MR results based on genetic data contribute to a better understanding of the pathogenesis of DN and provide evidence for a causal effect of inflammatory factors on DN. These findings support targeting specific inflammatory factors to alleviate DN risk.

Keywords: causal association, diabetic nephropathy, inflammatory factors, Mendelian randomization

Introduction

Diabetes-related complications are considered one of the most challenging health problems worldwide. Diabetic nephropathy (DN) is one of the most frequent and severe microvascular complications of diabetes mellitus, which develops in approximately one-third of patients with diabetes and is a more frequent primary cause of end-stage renal failure.[1] Risk factors for DN are classified into modifiable and nonmodifiable factors. The modifiable factors include obesity, hypertension, hyperglycemia, dyslipidemia,[2] and smoking.[3] The second group comprises age, sex, ethnicity,[4] and genetic variants (CARS, FRMD3, viz. NLRP3, INPPL1, PIK3C2G, NRXN3, and TBC1D4).[5]

Inflammatory molecules play a key role in glomerular and tubulointerstitial damage associated with urinary albumin excretion (UAE) among patients with diabetes.[6,7] These molecules can also recruit inflammatory cells (monocytes, neutrophils, and lymphocytes) to accumulate in the kidney, which is associated with the development and progression of DN.[8,9] There are no available treatments to prevent the development of DN. Assessing the causal role of inflammatory factors in DN risk may help in protecting patients from the development and progression of renal damage.

The existence of a potential causal association between exposure and outcome can be estimated by using the Mendelian randomization (MR) approach with genetic variants.[10,11] We used two-sample MR analyses to explore the potential causal associations between inflammatory factors and the risk of DN. Our findings will help identify the important role of

inflammatory factors in the development and progression of DN and provide potential implications for the pathogenesis, diagnosis and therapy of DN.

Methods

Study Design

The present study followed the Strengthening the Reporting of Observational Studies in Epidemiology using Mendelian Randomization (STROBE-MR) guidelines. A brief description of the study design is displayed in Figure 1.

Data Source

The GWAS summary data for 41 inflammatory factors were obtained from a meta-analysis on three independent cohorts (YFS, FINRISK1997 and FINRISK2002), including 8,293 Finnish participants (Supplementary Table 1). DN data were extracted from a GWAS of 213,746 individuals of European ancestry from FinnGen (3,282 cases vs 210,463 controls). All data were downloaded from the MRC Integrated Epidemiology Unit (IEU) open GWAS database (https://gwas.mrcieu.ac.uk/datasets/).[12]

Instrumental Variables (IVs)

Since only a few independent genetic variants reached genome-wide significance ($p < 5 \times 10^{-8}$), we employed a less stringent threshold (5×10^{-6}) to obtain more SNPs for inflammatory factors. We clumped these SNPs with linkage disequilibrium (LD) r2 < 0.01. We deleted weak IVs with an F value less than 10.[13] The MR-Pleiotropy Residual Sum and Outlier (MR-PRESSO) test was used for the removal of horizontal pleiotropic outliers.[14] SNPs missing in the outcome were replaced by proxies with LD R2 >0.8.

Statistical Analysis

The inverse variance weighted (IVW) method was administered as the major statistical method, which is to combine the specific ratio estimates using each SNP.[15] The IVW method uses an asymptotic estimation of the standard error of causal ratio for each variable; this is known to underestimate the true changes in estimation, especially when asymptotic estimation is weak.[16] MR-PRESSO was also performed to validate the IVW results. MR results were reported as odds ratios (ORs) with 95% confidence intervals (CIs). The statistical power of MR was calculated using an online website
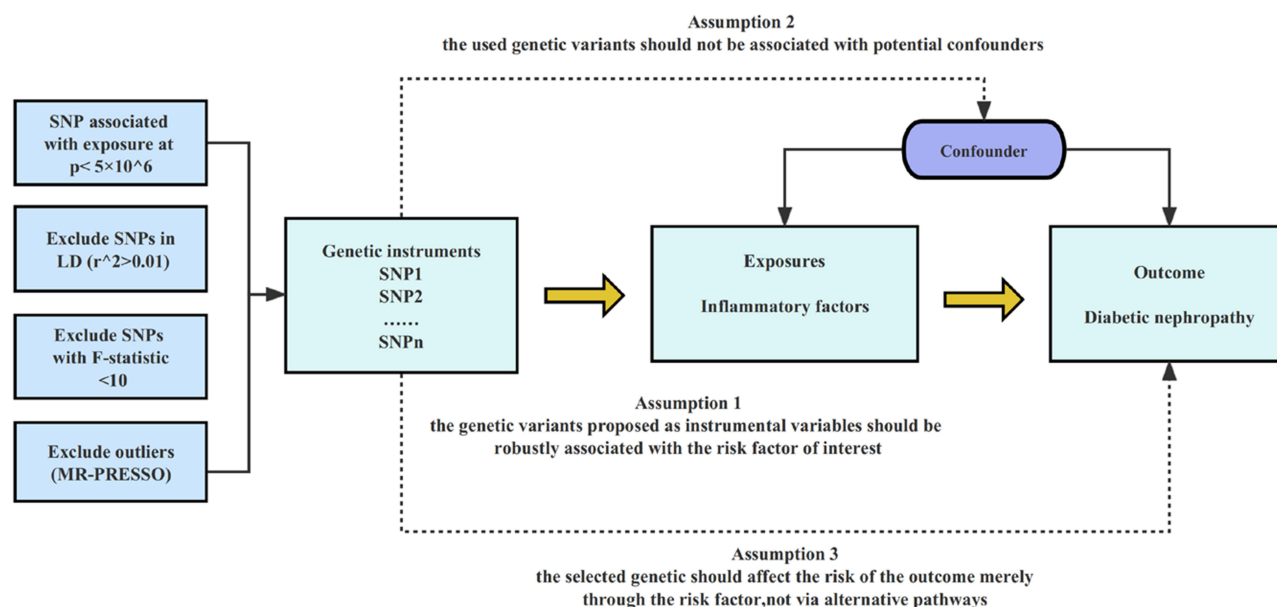
Figure 1 Overview and assumptions of the Mendelian randomization study design. The MR design was used to explore the causal association between inflammatory factors and DN risk. The MR design satisfies three major assumptions: 1). SNPs are strongly correlated with inflammatory factors; 2). SNPs are irrelevant to the confounder; 3). SNPs affect DN merely via exposure.

(sb452.shinyapps.io/power/). MR-Steiger analysis was used to monitor the direction of the potential causal effect between inflammatory factors and DN risk. Cochran's Q-test was performed to detect heterogeneity between IVs. If heterogeneity existed, the IVW random-effect model was used. The MR-PRESSO global test and pleiotropy test function of the TwoSampleMR package were used to detect horizontal pleiotropy. The robustness of the MR analysis was assessed by leave-one-out analysis. TwoSampleMR and MR-PRESSO packages were used for statistical tests. A *p*-values below 0.0012 (0.05/41) was considered as strong evidence of associations using Bonferroni method to correct for multiple testing. We also selected SNPs for positive associations to perform multivariable MR analyses to adjust for pleiotropy. A *p* value of 0.05 was the threshold to indicate statistical significance.
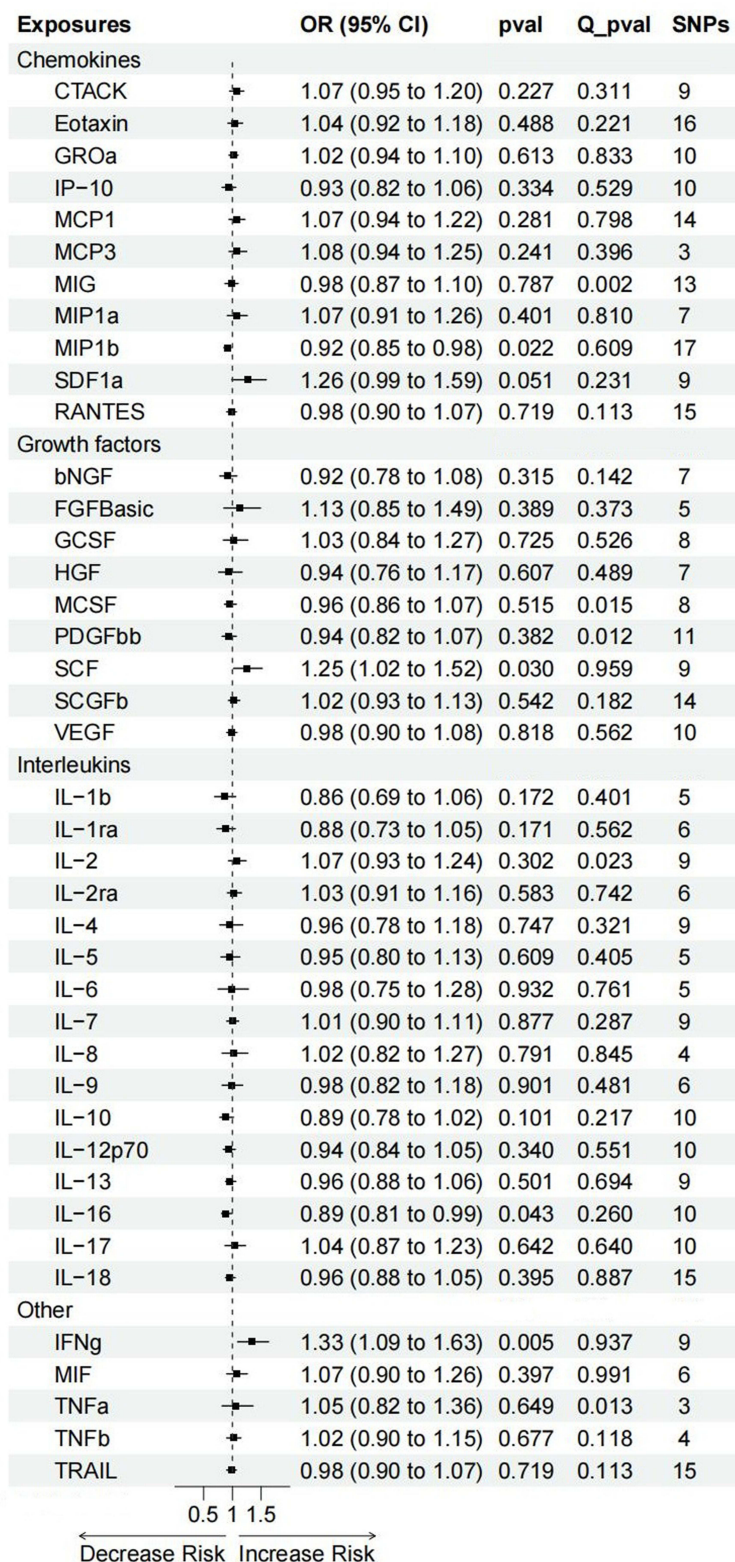
Results

As shown in Figure 2, IFN-γ [OR_{IVW}: 1.33; 95% CI: 1.09–1.63; *p*= 0.005] and SCF [OR_{IVW}: 1.25, 1.02–1.52; *p* =0.027] showed a causal relationship with an increased risk of DN. MIP1b [OR_{IVW}: 0.92; 95% CI: 0.85–0.98; *p* = 0.022] and IL-16 [OR_{IVW}: 0.89, 0.81–0.99; *p* = 0.043] showed a causal relationship with a decreased risk of DN. There was a strong trend toward a positive association between SDF1a and the risk of DN [OR_{IVW}: 1.26, 0.99–1.59; *p* = 0.051]. The same associations were observed using the MR-PRESSO method (Supplementary Table 2). Neither IVW nor MR-PRESSO found any significance for multiple testing. Significant horizontal pleiotropy was not found in our study. In multivariable IVW analyses, We also observe the same associations (SCF [OR_{IVW}: 1.32, 1.10–1.59; *p* = 0.003]; IL-16 [OR_{IVW}: 0.90, 0.82–0.99; *p* = 0.031]; MIP1b [OR_{IVW}: 0.92; 95% CI: 0.86–0.98; *p* = 0.010]; IFN-γ [OR_{IVW}: 1.20; 95% CI: 1.01–1.43; *p* =0.043]).

Discussion

In this study, we conducted an MR analysis to assess the causal association of inflammatory factors with DN. We successfully identified four upstream regulators of DN. Higher IFN-γ and SCF were associated with an increased risk of DN, while higher MIP1b and IL-16 were shown to possibly decrease the risk of DN. Our results confirmed the hypothesis that inflammatory factors play an important role in the development of DN.

First, we explicate the surprisingly positive causality between IFN-γ and DN. IFN-γ secretion is considered to be a powerful upstream event for many inflammatory responses and a major mediator for the activation or release of other inflammatory cytokines, such as TNF-α and the IL-1 families.[17] Elevated serum levels of IFN-γ have been observed in type-2 diabetes patients with nephropathic complications compared with those of healthy controls.[18,19] IFN-γ mRNA expression was significantly increased in diabetic mouse kidneys compared with those of controls.[20] Second, SCF is a novel endothelial permeability factor.[21] SCF/c-kit signaling promotes the recruitment of endothelial progenitor cells and contributes to neovascularization.[22,23] Neovascularization has been implicated in the genesis of diverse diabetic complications, such as retinopathy and nephropathy.[24] Increased expression of SCF protein and mRNA in DN model rats was positively correlated with the infiltration degree of mast cells, which may aggravate renal tubulointerstitial fibrosis in DN rats.[25] Third, IL-16 may contribute to regulatory T-cell (Treg) expansion for immunosuppressive effects.[26] In T1D, peripheral immunocompetent cells are defective in IL-16 secretion.[27] Lower expression of CD4+CD25+Foxp3+ Tregs was associated with microalbuminuria and macroalbuminuria in type-2 diabetes patients.[28] Adoptive transfer of CD4 +FoxP3+ Tregs significantly improved diabetic nephropathy by limiting the proinflammatory milieu.[29] Fourth, MIP-1b is a chemokine also known as CCL4. MIP-1b plays an important role in the immunoregulatory process, recruiting Tregs into inflammatory sites.[30] Tregs deficient in CCL4 expression were impaired in their ability to suppress experimental autoimmune encephalomyelitis in murine models.[31] CCL4 was significantly lower in patients with type-1 diabetes mellitus (DM) than it was in control participants.[32] A protective role of CCL4 was suggested in a nonobese diabetic (NOD) mouse model.[33]

There are a few study limitations that should be considered. First, our study was based on the available GWAS data. There are no data to infer the differential effects of sex on the causal association of inflammatory factors with DN. Sexual dimorphism may play a key role in the pathogenesis of DN.[34] Second, the study population was limited to individuals of European ethnicity. Our results cannot be generalized to other populations. Race/ethnicity is classified as a susceptibility risk factor for DN.[1] Third, MR results could reflect the lifelong effect of inflammatory factors on DN. However, it may be directly affected in adult life due to many unknown factors. Fourth, we only obtained GWAS data 41 inflammatory

Exposures		OR (95% CI)	pval	Q_pval	SNPs
Chemokines					
CTACK		1.07 (0.95 to 1.20)	0.227	0.311	9
Eotaxin		1.04 (0.92 to 1.18)	0.488	0.221	16
GROa		1.02 (0.94 to 1.10)	0.613	0.833	10
IP−10		0.93 (0.82 to 1.06)	0.334	0.529	10
MCP1		1.07 (0.94 to 1.22)	0.281	0.798	14
MCP3		1.08 (0.94 to 1.25)	0.241	0.396	3
MIG		0.98 (0.87 to 1.10)	0.787	0.002	13
MIP1a		1.07 (0.91 to 1.26)	0.401	0.810	7
MIP1b		0.92 (0.85 to 0.98)	0.022	0.609	17
SDF1a		1.26 (0.99 to 1.59)	0.051	0.231	9
RANTES		0.98 (0.90 to 1.07)	0.719	0.113	15
Growth factors					
bNGF		0.92 (0.78 to 1.08)	0.315	0.142	7
FGFBasic		1.13 (0.85 to 1.49)	0.389	0.373	5
GCSF		1.03 (0.84 to 1.27)	0.725	0.526	8
HGF		0.94 (0.76 to 1.17)	0.607	0.489	7
MCSF		0.96 (0.86 to 1.07)	0.515	0.015	8
PDGFbb		0.94 (0.82 to 1.07)	0.382	0.012	11
SCF		1.25 (1.02 to 1.52)	0.030	0.959	9
SCGFb		1.02 (0.93 to 1.13)	0.542	0.182	14
VEGF		0.98 (0.90 to 1.08)	0.818	0.562	10
Interleukins					
IL−1b		0.86 (0.69 to 1.06)	0.172	0.401	5
IL−1ra		0.88 (0.73 to 1.05)	0.171	0.562	6
IL−2		1.07 (0.93 to 1.24)	0.302	0.023	9
IL−2ra		1.03 (0.91 to 1.16)	0.583	0.742	6
IL−4		0.96 (0.78 to 1.18)	0.747	0.321	9
IL−5		0.95 (0.80 to 1.13)	0.609	0.405	5
IL−6		0.98 (0.75 to 1.28)	0.932	0.761	5
IL−7		1.01 (0.90 to 1.11)	0.877	0.287	9
IL−8		1.02 (0.82 to 1.27)	0.791	0.845	4
IL−9		0.98 (0.82 to 1.18)	0.901	0.481	6
IL−10		0.89 (0.78 to 1.02)	0.101	0.217	10
IL−12p70		0.94 (0.84 to 1.05)	0.340	0.551	10
IL−13		0.96 (0.88 to 1.06)	0.501	0.694	9
IL−16		0.89 (0.81 to 0.99)	0.043	0.260	10
IL−17		1.04 (0.87 to 1.23)	0.642	0.640	10
IL−18		0.96 (0.88 to 1.05)	0.395	0.887	15
Other					
IFNg		1.33 (1.09 to 1.63)	0.005	0.937	9
MIF		1.07 (0.90 to 1.26)	0.397	0.991	6
TNFa		1.05 (0.82 to 1.36)	0.649	0.013	3
TNFb		1.02 (0.90 to 1.15)	0.677	0.118	4
TRAIL		0.98 (0.90 to 1.07)	0.719	0.113	15

0.5 1 1.5

Decrease Risk Increase Risk

Figure 2 Associations between genetically predicted inflammatory factors and DN risk.

factors from a meta-analyses involving 8293 individuals[35] and some essential inflammatory factors are not included in our study. TGF-β/Smad signaling plays a critical role in the development of DN.[36] Finally, our results were based on the reported GWAS summary data noted above. Using other data may yield different results.

Conclusion

In conclusion, this MR study suggested that there was a causal effect of inflammatory factors on the risk of DN. Four common inflammatory factors, IFN-γ, SCF, MIP1b and IL-16, should be noted and emphasized in the pathogenesis and treatment of DN. Further study is needed to validate our findings in a prospective manner.

Data Sharing Statement

Only publicly available data were used in our study, and data sources are described in the Methods/Supplementary material.

Ethics Statement

Our study was based on publicly available GWAS summary-level data and all original studies have been approved by their Institutional Review Boards or local ethics committees. Besides, our study did not involve individual-level data and was deemed not to require ethical approval after consultation with the Ethics Committee of ZhongDa Hospital.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare no conflicts of interest in this work.

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