Inhibition of interleukin 6 signalling and renal function: a Mendelian randomization study

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Abstract

Inhibition of interleukin 6 (IL-6) signalling has been proposed as a potential cardioprotective strategy for patients with chronic kidney disease (CKD) but the direct renal effects of IL-6 inhibition are not established. A Mendelian randomization (MR) study was performed to investigate the association of genetically proxied inhibition of IL-6 signalling with estimated glomerular filtration rate (eGFR), CKD and blood urea nitrogen (BUN). Inverse variance weighted MR was used as the main analysis, with sensitivity analyses performed using simple median, weighted median and MR-Egger methods. There was no evidence for an association of genetically proxied inhibition of IL-6 signalling (scaled per unit decrease in natural log transformed C-reactive protein) with log eGFR (0.002, 95% confidence interval -0.004 – 0.008), BUN (0.088, 95% confidence interval -0.003 – 0.019) and CKD (odds ratio 1.018, 95% confidence interval 0.899 – 1.153). These findings suggest that inhibition of IL-6 signalling is unlikely to have a direct effect on renal function.

Introduction

Cardiovascular disease (CVD) accounts for half of all deaths in end-stage renal failure and the burden of CVD in chronic kidney disease (CKD) is not fully explained by traditional risk factors.[1] This suggests that alternative pathways may be implicated in the disproportionately high CVD risk in patients with declining renal function.[2] CKD is recognised as a low-grade but persistent inflammatory state, with raised levels of inflammatory biomarkers such as interleukin-1β (IL-1β) interleukin-6 (IL-6), tumour necrosis factor α (TNF-α) and C-reactive protein (CRP).[3] Inflammation plays a critical role in atherosclerosis and it is possible that the inflammatory milieu of CKD contributes to the excessive risk of CVD in CKD.[4,5]Inflammatory markers such as high-sensitive CRP and IL-6 are predictive of cardiovascular events and IL-6 levels are independent predictors of CVD and mortality in patients with CKD.[6,7] Inhibition of IL-1β and IL-6 have shown promising results in lowering cardiovascular events.[8,9] There are now plans to trial the IL-6 signalling inhibitor, ziltivekimab for reduction of CVD in patients with CKD.[10] However, it has not been established whether direct IL-6 signalling inhibition has an impact on renal function.

Mendelian randomization (MR) employs genetic polymorphisms as instrumental variables to study the effect of an exposure on an outcome.[11] MR is less susceptible to confounding due to the balancing of environmental factors at conception with the independent assortment and random allocation of genetic variants. For a valid MR study, the following assumptions must hold: the genetic proxy must be associated with the exposure, the genetic variant only affects the outcome through the exposure of interest with no horizontal pleiotropic effect, the genetic variant is not associated with any known confounder affecting the exposure and the outcome.[11]In principle, a valid MR study represents an endogenous randomised controlled trial based on the randomisation of genetic variants at conception. Applied to drug development, MR provides an *in-silico*platform to predict adverse drug consequences and explore drug repurposing, minimising confounding and reducing potential for reverse causality.[12] Pre-clinical MR studies could also reduce the exposure of trial participants to potentially

harmful compounds and enable us to determine whether new drug targets are suitable to be trialled among vulnerable populations, such as patients with CKD.[12] Considering the growing interest of IL-6 inhibition in patients with CKD, the aim of the present study was to investigate the effect of inhibition of IL-6 signalling on renal function by MR methods.

Methods

A two-sample MR study was conducted to investigate the association of genetically proxied inhibition of IL-6 signalling with different measures of renal function: estimated glomerular filtration rate (eGFR), CKD and blood urea nitrogen (BUN). Two-sample refers to the fact that the instrument-exposure and instrument-outcome estimate are obtained from two different genome wide association study (GWAS), increasing the statistical power of the MR study.

Genetic variants for downregulated IL-6 signalling were selected as independent (linkage disequilibrium $r^2<$ 0.1) single-nucleotide polymorphism (SNPs) within 300 kB of the IL-6 receptor gene (*IL6R*) that strongly associated with circulating CRP ($p < 5\times10^{-8}$ with independent associations as described by Georgakis et al[8]), a downstream target of IL-6 signalling. The association between the IL6R genetic variants and CRP level were taken from the summary data of the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Inflammation Working Group GWAS of 204,402 individuals of European ancestry.[13] In this GWAS, association estimates are expressed as change in natural log transformed CRP (mg/L). The variance in CRP levels explained by the genetic variants,R^2 , was calculated using the formula: $R^2 = [2 \times MAF \times (1–MAF) \times \beta^2]/var(log(CRP))$, where MAF is the minor allele frequency and β is the effect estimate of the SNP on CRP levels. F-statistics, a measure of instrument strength in MR, were calculated using the formula: $F = ?^2\times(?-2)/(1-?^2)$ whereR^2 is the variance of CRP explained by the specific variant and N the number of individuals in the GWAS analysis.

Summary GWAS data from the Chronic Kidney Disease Genetics (CKDGen) Consortium meta-analyses were used for the primary outcome associates of log eGFR (61 studies, 765,348 participants), BUN (33 studies, 416,178 participants), CKD (30 studies, 64,164 cases and 561,055 controls).[14] Log eGFR was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation in adults and using the Schwartz formula for participants who were 18 years or younger. CKD was constructed as a binary outcome based on an eGFR $<$ 60 ml min^{-1} per 1.73 m^2. BUN was calculated as 2.8 x blood urea (mg/dl).

Data for the exposure and outcome were harmonised according to the effect allele and no exclusions were made for palindromic variants. Random-effects inverse-variance weighted method was used as the primary MR analysis. To assess potential pleiotropy, we conducted sensitivity analyses using the simple median, weighted median and MR-Egger methods. The median methods are robust even with up to 50% of the contribution to the MR estimates coming from invalid instrumental variables.[15] MR-Egger provides robust estimates even when all instrumental variables are invalid, as long as the INstrument Strength Independent of Direct Effect (INSIDE) assumption holds - that any pleiotropic effect of the variants on the outcome are independent of the strength of their association with the exposure.[15]The estimated MR-Egger intercept is indicative of the average pleiotropic effect of the variants used.[15] We tested for such pleiotropy by assessing whether our intercept was significantly different from zero.[15] Results are presented as effect estimates and corresponding 95% confidence intervals in forest plots. For eGFR and BUN respectively, estimates represent the change in log eGFR or blood urea nitrogen per genetically predicted decrease in natural log of CRP. For CKD the results are expressed as odds ratio of CKD, with 95% confidence intervals. All data analyses were performed by the "TwoSampleMR" package version 4.26 in R software.

Results

Seven SNPs were used as instrumental variables to represent genetically proxied inhibition of IL-6 signalling (table 1). The F-statistic for these genetic exposure associations ranged between 73.16 and 694.37, indicating strong associations between the IL6-R variant and CRP level (table 1). Outcome summary data was accessed from the CKDGen Consortium and details about the data source, population ancestry and beta coefficient units are described in table 2.

There was no strong evidence for an association of genetically proxied inhibition of IL-6 inhibition with log eGFR (0.002, 95% confidence interval -0.004 – 0.008), BUN (0.088, 95% confidence interval -0.003 – 0.019) and CKD (odds ratio 1.018, 95% confidence interval 0.899 – 1.153). The results were consistent across all measurements of kidney function (Figure 1, appendix I). MR-Egger estimates were also consistent across all metrics of kidney function. The intercept did not show evidence of pleiotropy for the eGFR, BUN or CKD ($p = 0.645$, $p = 0.486$ and $p = 0.387$ respectively).

Discussion

This MR study did not provide evidence to suggest that genetically proxied inhibition of IL-6 signalling has an effect on renal function. IL-6 inhibitors such as tocilizumab are currently licenced for use in rheumatoid arthritis, juvenile arthritis and more recently were under investigation for treatment of excessive inflammation in patients with severe acute respiratory syndrome coronavirus 2 infection.[16] Atherosclerotic cardiovascular disease is an inflammatory disorder and both MR studies and randomised controlled-trials suggest that inhibition of IL-6 signalling reduces risk of cardiovascular outcomes (coronary artery disease, atrial fibrillation) and thromboembolic events.[8,9] Given the disproportionate burden of cardiovascular disease in patients with CKD and the inflammatory nature of both these conditions, there is growing interest in repurposing IL-6 inhibitors to treat CVD in CKD.[10] Our current findings support that pharmacological IL-6 inhibition would be unlikely to have a direct adverse effect on renal function.

The findings of this MR study are in line with an earlier study of renal function in patients with rheumatoid arthritis and renal insufficiency receiving tocilizumab therapy.[17] However, this observational study is small (120 participants), had large numbers of patient stopping or switching therapy (60% switching biological therapy) and may be susceptible to confounding factors (patients receiving IL-6 inhibition were older and had rheumatoid arthritis for longer).[17] The present MR study adds support to the initial pharmacovigilance surveys, and further is less prone to confounding and reverse causality. Furthermore, manufacturer notes for the tocilizumab (tradename: Actemra) suggest that no dose adjustment is required for patients with mild renal impairment, however, cautions that the drug has not been studied in patients with moderate to severe dysfunction.[18] This highlights a potential limitation of available clinical trial data - despite over 10% of patients in the developed world having renal impairment, patients with CKD are excluded from up to 75% of all randomised-controlled trials.[19]MR may help in evaluating the safety of drugs *in-silico* prior to trials in vulnerable patients. For example, MR drug safety studies have in the past substantiated the causal relationship between statin therapy and the increased risk of diabetes.[20] Furthermore, MR can provide more immediate drug safety information compared to usual pharmacovigilance strategies such as the Medicines and Healthcare Products Regulatory Agency yellow card system.

Our study has a number of strengths. To ensure the strength of the genetic-variant mimicked the strength of pharmacological inhibition of IL-6, the variant-exposure association was scaled to a reduction in CRP levels.[8] In an attempt to minimise the bias related to pleiotropic effects of variants, instrumental variables were selected based on their proximity to the *IL6R* gene and relation to biomarkers of IL-6 signalling. In addition, the MR-Egger method did not provide evidence to suggest biasing pleiotropy. Furthermore, the consistency of our results across different MR methods and different measurements of renal dysfunction further substantiates the null findings. Our study also has limitations. It is important to interpret our findings within the context of an MR study, which considers genetically proxied inhibition of IL-6 signalling, rather than the effect of a discrete clinical intervention. Our approach looks at IL-6 signalling in isolation, and it is possible that pharmacological IL-6 inhibitors could have off-target effects (aside from IL-6R signalling) on other renal or extra-renal pathways which may ameliorate or exacerbate renal function indirectly. There are also the possibility of drug-drug interactions that cannot be accounted for in the present MR analysis.

In conclusion, this study is consistent with the hypothesis that inhibition of IL-6 signalling does not adversely affect renal function, supporting this approach as a therapeutic opportunity for reducing the risk of CVD in patients with CKD.

References

1. Longenecker JC, Coresh J, Powe NR, Levey AS, Fink NE, Martin A, et al. Traditional cardiovascular disease risk factors in dialysis patients compared with the general population: the CHOICE Study. J Am Soc Nephrol [Internet]. 2002 Jul 1 [cited 2020 Sep 24];13(7):1918–27. Available from: http://www.ncbi.nlm.nih.gov/pubmed/10703676

2. Herzog CA, Asinger RW, Berger AK, Charytan DM, Diez J, Hart RG, et al. Cardiovascular disease in chronic kidney disease. A clinical update from Kidney Disease: Improving Global Outcomes (KDIGO). Kidney Int [Internet]. 2011 Sep 2 [cited 2020 Sep 24];80(6):572–86. Available from: https://www.sciencedirect.com/science/article/pii/S0085253815551053

3. Spoto B, Mattace-Raso F, Sijbrands E, Leonardis D, Testa A, Pisano A, et al. Association of IL-6 and a functional polymorphism in the IL-6 gene with cardiovascular events in patients with CKD. Clin J Am Soc Nephrol [Internet]. 2015 Feb 6 [cited 2020 Sep 24];10(2):232–40. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25492254

4. Libby P, Ridker PM, Hansson GK. Inflammation in Atherosclerosis: From Pathophysiology to Practice. J Am Coll Cardiol [Internet]. 2009 Dec 1 [cited 2020 Sep 24];54(23):2129–38. Available from: https://www.sciencedirect.com/science/article/pii/S073510970903006X

5. Zoccali C, Mallamaci F, Tripepi G. Inflammatory proteins as predictors of cardiovascular disease in patients with end-stage renal disease. Nephrol Dial Transplant [Internet]. 2004 Aug 1 [cited 2020 Sep 24];19(suppl_5):v67–72. Available from: https://academic.oup.com/ndt/article-lookup/doi/10.1093/ndt/gfh1059

6. Bermudez EA, Rifai N, Buring J, Manson JE, Ridker PM. Interrelationships Among Circulating Interleukin-6, C-Reactive Protein, and Traditional Cardiovascular Risk Factors in Women. Arterioscler Thromb Vasc Biol [Internet]. 2002 Oct [cited 2020 Sep 24];22(10):1668–73. Available from: https://www.ahajournals.org/doi/10.1161/01.ATV.0000029781.31325.66

7. Honda H, Qureshi AR, Heimburger O, Barany P, Wang K, Pecoits-Filho R, et al. Serum albumin, C-reactive protein, interleukin 6, and fetuin a as predictors of malnutrition, cardiovascular disease, and mortality in patients with ESRD. Am J Kidney Dis [Internet]. 2006 Jan [cited 2020 Sep 24];47(1):139–48. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16377395

8. Georgakis MK, Malik R, Gill D, Franceschini N, Sudlow CLM, Dichgans M, et al. Interleukin-6 Signaling Effects on Ischemic Stroke and Other Cardiovascular Outcomes: A Mendelian Randomization Study. Circ Genomic Precis Med [Internet]. 2020 Jun [cited 2020 Sep 24];13(3):e002872. Available from: https://www.ahajournals.org/doi/10.1161/CIRCGEN.119.002872

9. Ridker PM, Everett BM, Thuren T, MacFadyen JG, Chang WH, Ballantyne C, et al. Antiinflammatory Therapy with Canakinumab for Atherosclerotic Disease. N Engl J Med [Internet]. 2017 Sep 21 [cited 2020 Sep 24];377(12):1119–31. Available from: http://www.nejm.org/doi/10.1056/NEJMoa1707914

10. Kakkar R, Lo L, Kling D, Devalaraja M, Davidson M. Effects of Ziltivekimab (ZILTI), a Novel Anti-Interleukin-6 Monoclonal Antibody, on Markers of Inflammation and Cardiovascular Risk in Patients With Chronic Kidney Disease on Hemodialysis. Circulation. 2019;

11. Davies NM, Holmes M V, Davey Smith G. Reading Mendelian randomisation studies: a guide, glossary, and checklist for clinicians. BMJ [Internet]. 2018 Jul 12 [cited 2020 Sep 24];362:k601. Available from: http://www.ncbi.nlm.nih.gov/pubmed/30002074

12. Walker VM, Davey Smith G, Davies NM, Martin RM. Mendelian randomization: a novel approach for the prediction of adverse drug events and drug repurposing opportunities. Int J Epidemiol [Internet]. 2017 [cited 2020 Sep 24];46(6):2078–89. Available from: http://www.ncbi.nlm.nih.gov/pubmed/29040597

13. Ligthart S, Vaez A, Vosa U, Stathopoulou MG, de Vries PS, Prins BP, et al. Genome Analyses of >200,000 Individuals Identify 58 Loci for Chronic Inflammation and Highlight Pathways that Link Inflam-

mation and Complex Disorders. Am J Hum Genet [Internet]. 2018 Nov 1 [cited 2020 Sep 24];103(5):691–706. Available from: https://www.sciencedirect.com/science/article/pii/S0002929718303203

14. Wuttke M, Li Y, Li M, Sieber KB, Feitosa MF, Gorski M, et al. A catalog of genetic loci associated with kidney function from analyses of a million individuals. Nat Genet [Internet]. 2019 Jun [cited 2020 Sep 24];51(6):957–72. Available from: http://www.nature.com/articles/s41588-019-0407-x

15. Burgess S, Thompson SG. Interpreting findings from Mendelian randomization using the MR-Egger method. Eur J Epidemiol [Internet]. 2017 May 19 [cited 2020 Sep 24];32(5):377–89. Available from: http://link.springer.com/10.1007/s10654-017-0255-x

16. Guaraldi G, Meschiari M, Cozzi-Lepri A, Milic J, Tonelli R, Menozzi M, et al. Tocilizumab in patients with severe COVID-19: a retrospective cohort study. Lancet Rheumatol [Internet]. 2020 Aug 1 [cited 2020 Sep 24];2(8):e474–84. Available from: http://www.ncbi.nlm.nih.gov/pubmed/32835257

17. Mori S, Yoshitama T, Hidaka T, Hirakata N, Ueki Y. Effectiveness and safety of tocilizumab therapy for patients with rheumatoid arthritis and renal insufficiency: a real-life registry study in Japan (the ACTRA-RI study). Ann Rheum Dis [Internet]. 2015 Mar [cited 2020 Sep 24];74(3):627–30. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25561361

18. Food and Drug Administration U. FDA: Tocilizumab (Actemra) Product Information Sheet [Internet]. 2013. [cited 2020 Sep 24]. Available from: https://www.accessdata.fda.gov/drugsatfda_docs/label/2013/125276s092lbl.pdf

19. Charytan D, Kuntz RE. The exclusion of patients with chronic kidney disease from clinical trials in coronary artery disease. Kidney Int [Internet]. 2006 Dec [cited 2020 Sep 24];70(11):2021–30. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17051142

20. Swerdlow DI, Preiss D, Kuchenbaecker KB, Holmes M V, Engmann JEL, Shah T, et al. HMG-coenzyme A reductase inhibition, type 2 diabetes, and bodyweight: evidence from genetic analysis and randomised trials. Lancet [Internet]. 2015 Jan 24 [cited 2020 Sep 24];385(9965):351–61. Available from: https://www.sciencedirect.com/science/article/pii/S0140673614611831

Table 1:

Instrumental variables: Variants employed as instrumental variables to proxy inhibition of IL-6 signalling. Associations with C-reactive protein are detailed.

SNP	Effect Allele	Other Allele	Effect allele frequency	Beta	Standard error	P value	R^2
rs73026617	t	c	0.097	0.0474	0.0068	3.16E-12	3.58E-
rs12083537	a	g	0.193	0.0643	0.0053	7.14E-34	1.17E-
rs4556348	t	c	0.148	0.0541	0.0067	6.77E-16	6.71E-
rs2228145	a	c	0.36	0.0899	0.0042	1.21E-101	3.39E-
rs11264224	a	c	0.193	0.0465	0.0057	3.41E-16	6.12E-
rs12059682	t	c	0.196	-0.0441	0.0049	2.26E-19	5.57E-
rs34693607	c	g	0.184	0.0368	0.0057	1.07E-10	3.70E-

Table 1 shows the summary data for the variants that proxy IL-6 signalling inhibition. SNP: single nucleotide polymorphism. Beta is the unit change in natural log transformed CRP (mg/L) per copy increment in the effect allele. R^2 represents the variance in CRP explained by the respective genetic variant. F-statistic measures the strength of the instrumental variable with the exposure. Variants identified and associated well with IL-6 signalling inhibition in a previous Mendelian randomization study exploring IL-6 inhibition and effects on ischaemic stroke and cardiovascular outcomes.[8]

Table 2:

Data Sources for exposure and outcomes

Data	Data Source	Population Ancestry	Sample size	Exposure definition	Adjustments
Genetic variants in or near IL-6R associated with variation in CRP	CHARGE Inflammation Working Group[13]	European	204,402	Change in natural log transformed CRP (mg/L) per copy increment of the effect allele	Age, sex, population structure. Estimates scaled for the effect of Tocilizumab (8 mg/kg) on CRP levels between 4 – 24 weeks after administration.
Blood urea nitrogen (BUN)	Chronic Kidney Disease Genetics Consortium meta-analysis (n=33 studies)[14]	Trans-ancestry	416,178	Change in BUN per copy increment of effect allele	Age, sex, population structure
Estimated glomerular filtration rate (eGFR)	Chronic Kidney Disease Genetics Consortium meta-analysis (n=61 studies)[14]	Trans-ancestry	765,348	Change in log eGFR per copy increment of effect allele	Age, sex, population structure
Chronic Kidney Disease Binary outcome eGFR > 60 ml min^{-1} per 1.73 m^2.	Chronic Kidney Disease Genetics Consortium meta-analysis (n=30 studies)[14]	Trans-ancestry	64,164 cases 561,055 controls	Log odds ratio for CKD per copy increment of effect allele	Age, sex, population structure

Table 2 describes the source, population ancestry, sample size and exposure definitions for the GWAS studies used in the present MR analysis.

Figure 1:

Effects of genetically proxied IL-6 signal inhibition on renal function

Chronic kidney disease

Log estimated glomerular filtration rate

Effects of genetically proxied IL-6 signal inhibition on renal function

Blood urea nitrogen

Forest plots showing the causal estimates for chronic kidney disease (CKD), log eGFR (estimated glomerular filtration rate) and blood urea nitrogen (BUN) with different meta-analysis methods (simple median, weighted median, inverse-variance weighted, MR-Egger). eGFR and BUN are presented as causal estimates with 95% confidence interval. CKD is described as the odds ratio (95% confidence interval) of having CKD given genetically proxied IL-6 signalling inhibition. Full results table described in appendix I.

Appendix I:

Table 3: Pooled estimates for the association of genetically proxied inhibition of IL-6 signalling with measures of renal function

Chronic Kidney Disease: effects of genetically proxied IL-6 inhibition on Chronic

Kidney Disease

Method	Estimate (standard error)	Odds ratio (95% Confidence Interval)	P value
Inverse-variance weighted	0.018 (-0.063)	1.018 (0.899 – 1.153)	0.782
Simple median	0.046 (-0.100)	1.047 (0.862 – 1.274)	0.643
Weighted median	-0.011 (-0.077)	0.989 (0.850 – 1.151)	0.892
MR-Egger	-0.150 (-0.203)	0.861 (0.578 – 1.283)	0.462
MR-Egger intercept	0.011 (-0.013)	1.011 (0.986 – 1.035)	0.387

Log estimated glomerular filtration rate: association of genetically proxied inhibition of IL-6 signalling with log odds of estimated glomerular filtration rate

Method	Estimate (standard error)	95% confidence interval	P value
Inverse-variance weighted	0.002 (-0.003)	-0.004 – 0.008	0.593
Simple median	0.002 (-0.004)	-0.005 – 0.010	0.569
Weighted median	0.001 (-0.003)	-0.005 – 0.007	0.669
MR-Egger	-0.003 (-0.011)	-0.024 – 0.018	0.779
MR-Egger intercept	0.000 (-0.001)	-0.001 – 0.002	0.645

Blood urea nitrogen: effects of genetically proxied IL-6 inhibition on blood urea nitrogen

Method	Estimate (standard error)	95% confidence interval	P value
Inverse-variance weighted	0.008 (-0.006)	-0.003 – 0.019	0.144
Simple median	0.004 (-0.009)	-0.014 – 0.022	0.645
Weighted median	0.003 (-0.007)	-0.010 – 0.017	0.609
MR-Egger	-0.004 (-0.018)	-0.040 – 0.032	0.833
MR-Egger intercept	0.001 (-0.001)	-0.001 – 0.003	0.486

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