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Mendelian randomization analysis associates increased serum urate, due to genetic variation in uric acid transporters, with improved renal function

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Increased serum urate predicts chronic kidney disease independent of other risk factors. The use of xanthine oxidase inhibitors coincides with improved renal function. Whether this is due to reduced serum urate or reduced production of oxidants by xanthine oxidase or another physiological mechanism remains unresolved. Here we applied Mendelian randomization, a statistical genetics approach allowing disentangling of cause and effect in the presence of potential confounding, to determine whether lowering of serum urate by genetic modulation of renal excretion benefits renal function using data from 7979 patients of the Atherosclerosis Risk in Communities and Framingham Heart studies. Mendelian randomization by the two-stage least squares method was done with serum urate as the exposure, a uric acid transporter genetic risk score as instrumental variable, and estimated glomerular filtration rate and serum creatinine as the outcomes. Increased genetic risk score was associated with significantly improved renal function in men but not in women. Analysis of individual genetic variants showed the effect size associated with serum urate did not correlate with that associated with renal function in the Mendelian randomization model. This is consistent with the possibility that the physiological action of these genetic variants in raising serum urate correlates directly with improved renal function. Further studies are required to understand the mechanism of the potential renal function protection mediated by xanthine oxidase inhibitors.

Kidney International (2014) **85,** 344–351; doi:10.1038/ki.2013.353; published online 18 September 2013

KEYWORDS: creatinine; Mendelian randomization; renal; renal function decline; urate; uric acid transporter

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Received 10 February 2013; revised 1 July 2013; accepted 25 July 2013; published online 18 September 2013

The cause–effect relationship between hyperuricemia and renal function is unclear. Epidemiological studies demonstrate that elevated serum urate (SU) is a modest predictor of chronic kidney disease, independent of other classical risk factors.[1,2] The relationship between elevated SU and reduced renal function is supported by clinical intervention studies where urate-lowering treatment, predominantly with the xanthine oxidase inhibitor allopurinol, improves renal function and slows progression of chronic kidney disease.[3] In animals, hyperuricemia induces renal inflammation, preglomerular arterial disease and glomerular hypertension,[4–6] nephropathies that can be ameliorated and reversed by xanthine oxidase inhibition.[6] However, the clinical significance and independence of these observations remain to be clarified.[7] With regard to the epidemiological evidence, the observed relationship may be confounded by factors that have not been identified and measured and that may be extensively interrelated.[8] The beneficial effects of allopurinol on renal function in human clinical trials and experimental studies could be mediated via effects of its active metabolite, oxypurinol, on inhibition of production of oxidants by xanthine oxidase[9] and subsequent improvement of endothelial function,[10] additional or alternative to inhibition of urate production. Disentangling and further understanding the basis of the SU and renal function relationship and the mechanism of protection of renal function by xanthine oxidase inhibition is important given the increasing clinical trial focus on the use of urate-lowering drugs for renal function.[11–14]

Genetic variants in renal (SLC2A9, SLC17A1, SLC22A11, SLC22A12, ABCG2) and gut (ABCG2) uric acid transporter variants collectively explain, depending on sex, 3–5% of the variance in SU levels[15] and are associated with gout.[15–18] Variants in 18 other genes have also recently been associated with SU levels, with two pathways identified—glucose metabolism and inhibins-activins signaling pathways.[19] Genetic variants in these additional pathways account for $<2\%$ of the variance in SU concentrations.[19] The glucose metabolism pathway may affect urate levels through metabolism of fructose, which is known to elevate SU levels.[20] How the inhibins–activins pathway influences SU is unclear.

Mendelian randomization (also known as instrumental variables analysis) is a statistical genetics approach that allows the disentangling of cause and effect in the presence of potential confounding. This technique has previously been used to demonstrate that increased SU is a consequence and not a cause of adiposity,[21,22] that elevated SU is causative of hypertension[23] and that elevated SU is not causal of type 2 diabetes[24] or metabolic syndrome.[25] This analysis assumes that inherited genetic risk variants for one phenotype are naturally randomized at conception with respect to a second phenotype, and can therefore be used as instrumental variables to control confounding, and are not influenced by health outcomes, so that associations are not affected by reverse causality.[26,27] A Mendelian randomization approach has previously been applied to the relationship between SU and renal function (estimated glomerular filteration rate (eGFR)), with no association between a urate genetic risk score and eGFR.[15] However, this was done by simply regressing the genetic risk score (the instrumental variable) against eGFR. An alternative method, two-stage least squares,[28] regresses the instrumental variable (e.g. genetic risk score for SU) against the outcome variable (e.g. eGFR) using changes in measure (e.g. SU) directly attributable to the instrumental genetic risk score variable. This approach would be expected to be less confounded than the straightforward regression approach. Here we used Mendelian randomization by the two-stage least squares approach to study the relationship between SU and renal function.

RESULTS

Using a genetic risk score (Table 1) as an instrumental variable for SU, we conducted Mendelian randomization using the two-stage least squares approach for renal function with the uric acid transporter genetic risk score as instrumental variable (Table 2). Two-stage least squares is a form of Mendelian randomization, as is a standard genetic association study between a marker and phenotype, with the advantage that the two-stage least squares approach provides a quantitative effect of exposure (urate) on outcome (renal function). Mean SU, serum creatinine (SCr) and eGFR according to SU genetic risk score is shown in Supplementary Table S1 online. As genetic risk score increased, SCr decreased considerably more in males (SCr ranged from 103.05 to 84.66 μmol/l) than in females (range 80.44 to 79.56 μmol/l). There was evidence for a causal role for SU in determining SCr and eGFR in males (Table 2; $\beta = 45.06$ μmol/l decrease in SCr and 39.26 ml/min per 1.73 m^2 increase in eGFR from each unit increase in SU attributable to the genetic risk score, $P = 0.020$ and 0.045, respectively). However, the direction of effect was inverse to that predicted from the SU vs. eGFR and SCr regression analyses (Table 2; Durbin–Hausman $P < 1 \times 10^{-4}$). Thus, using a uric acid transporter genetic risk score as an instrumental variable, there was evidence that increased SU caused by genetic variation in uric acid transporters improved renal function in males.

Table 1 | Association between uric acid transporter genetic risk score and SU

	SU transporter genetic risk score	
	F-statistic[a]	R^2 [a]
ARIC		
Overall	114.81	0.0215
Men	63.05	0.0256
Women	99.25	0.0335
FHS		
Overall	73.32	0.0259
Men	27.56	0.0215
Women	94.73	0.0593
Combined		
Overall	190.74	0.0231
Men	90.6	0.0242
Women	185.14	0.0409

Abbreviations: ARIC, Atherosclerosis Risk in Communities; FHS, Framingham Heart Study; SU, serum urate.
Single-nucleotide polymorphisms rs11942223, rs2231142, rs1183201, rs2078267, and rs3825018 were used for both ARIC and FHS for SLC2A9, ABCG2, SLC17A1, SLC22A11, and SLC22A12, respectively.
[a]F-statistic represents the strength, and R^2 the percent variance in SU explained, of the association between the genetic risk score and SU.

We next used linear regression to further characterize the association of the uric acid transporter genetic risk score with renal function in sex-specific SU quartiles (Table 3; Supplementary Table S1 online). This revealed correlation of an increase in uric acid transporter genetic risk score with improved renal function in hyperuricemic men and women (SCr; $\beta = -1.057$, $P = 5 \times 10^{-4}$ and $\beta = -0.630$, $P = 0.009$, respectively).

The use of an instrumental variable in Mendelian randomization requires that it fulfills three assumptions.[26] First, the uric acid transporter genetic risk score is strongly associated with SU, with F-statistics being considerably greater than 10 and explaining >2% of the variance in SU (Table 1), indicating an adequate instrumental variable.[29] Second, that the instrumental variable is independent of the factors that confound the association of SU and renal function. This can be partly verified by testing the instrumental variable for association by linear regression with the measured confounders adjusted for here (Supplementary Table S2 online)—there was no evidence for association of the uric acid transporter instrumental variable with any of the confounders tested. Third, that the uric acid transporter instrumental variable has an effect on renal function solely via SU (i.e. there are no pleiotropic effects). This was verified to a limited extent by adjusting the Mendelian randomization analysis with covariates (age, sex, systolic and diastolic blood pressure, body mass index) that could potentially influence the association between SU and renal function. However, it is not possible to fully verify that the third assumption is satisfied given the likelihood that other pleiotropic effects exist (refer Discussion). To check for a role of population stratification, we also included the first two eigenvalues from genome-wide single-nucleotide