The serum uric acid concentration is not causally linked to diabetic nephropathy in type 1 diabetes

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Previous studies have shown a relationship between uric acid concentration and progression of renal disease. Here we studied causality between the serum uric acid concentration and progression of diabetic nephropathy in 3895 individuals with type 1 diabetes in the FinnDiane Study. The renal status was assessed with the urinary albumin excretion rate and estimated glomerular filtration rate (eGFR) at baseline and at the end of the follow-up. Based on previous genomewide association studies on serum uric acid concentration, 23 single nucleotide polymorphisms (SNPs) with good imputation quality were selected for the SNP score. This score was used to assess the causality between serum uric acid and renal complications using a Mendelian randomization approach. At baseline, the serum uric acid concentration was higher with worsening renal status. In multivariable Cox regression analyses, baseline serum uric acid concentration was not independently associated with progression of diabetic nephropathy over a mean follow-up of 7 years. However, over the same period, baseline serum uric acid was independently associated with the decline in eGFR. In the cross-sectional logistic regression analyses, the SNP score was associated with the serum uric acid concentration. Nevertheless, the Mendelian randomization showed no causality between uric acid and diabetic nephropathy, eGFR categories, or eGFR as a continuous variable. Thus, our results suggest that the serum uric acid concentration is not causally related to diabetic nephropathy but is a downstream marker of kidney damage.

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Diabetic nephropathy is a common complication in type 1 diabetes, affecting approximately one-third of the patients.[1] Characteristic of the condition are persistent urinary albumin excretion and a progressive decline in renal function.[2] Indeed, diabetic nephropathy is a major factor leading to dialysis and renal transplantation. Importantly, however, diabetic nephropathy is also associated with premature death.[3] In order to alleviate the long-term burden related to renal failure, it is important to discover factors associated with its development and progression.

A number of epidemiologic studies have shown a connection between serum uric acid concentration and progression of diabetic nephropathy in type 1 diabetes. Jalal *et al.*,[4] for example, reported that during a 6-year follow-up period, elevated baseline serum uric acid concentrations predicted the development of albuminuria. Similarly, Hovind *et al.*[5] observed that uric acid was associated with subsequent development of macroalbuminuria, but not microalbuminuria, during 18-year follow-up. Moreover, Ficociello *et al.*[6] reported that the baseline uric acid concentration was associated with the development of an early decrease in glomerular filtration rate (GFR) in a 6-year follow-up. In their study, uric acid was not, however, associated with an increase in urinary albumin excretion rate (AER).

In epidemiologic studies, causal inference and confounding pose challenges to the accurate interpretation of the results. In such studies, randomization, as customarily conducted in randomized, controlled trials, may not be applicable due to the study design or ethical reasons. To tackle this shortcoming, mendelian randomization, a form of genetic randomization study has been used to assess causality between an outcome and a given biological variable. This is done by studying the relationship between the outcome and a genetic determinant of the biological variable of interest. The idea behind Mendelian randomization is that the alleles derived from both parents are randomly assigned to gametes during meiosis. Therefore, the process is unaffected by confounding factors. Large-scale meta-analyses of genomewide association studies (GWASs) have revealed multiple genetic risk factors associated with elevated serum uric acid concentrations, with the strongest effect seen at rs12498742 in the *SLC2A9* gene encoding a transporter that mediates urate flux across the renal proximal tubule.[7,8]

Although of the genetic variants, rs734553 in the *SLC2A9* gene (also known as *GLUT9*), was used in the Mendelian randomization setting to show a causal link between serum uric acid and progression of chronic kidney disease (CKD) in the general (mainly nondiabetic) population,[9] another Mendelian randomization study, based on genetic variation in uric acid transporters, reported a possible causal link between increased serum uric acid and improved kidney function in healthy men (i.e., in the opposite direction).[10] Furthermore, in populations not focusing on individuals with diabetes, the observed causal associations between serum uric acid concentration and various vascular outcomes are mixed. Kleber *et al.*[11] reported that high uric acid is causally related to adverse cardiovascular outcomes, especially that of sudden cardiac death. Palmer *et al.*,[12] on the other hand, observed no causality between uric acid and ischemic heart disease or blood pressure.

Mendelian randomization has not previously been used to assess the relationship between the serum uric acid concentration and the development of diabetic nephropathy in type 1 diabetes. In the current study, we therefore aimed to assess the causality between the serum uric acid concentration and the progression of diabetic nephropathy in patients with type 1 diabetes using a Mendelian randomization approach.

RESULTS

At baseline, 2524 participants had normal AER, 540 had microalbuminuria, 536 had macroalbuminuria, and 295 had end-stage renal disease (ESRD) (Table 1, Figure 1). The median (interquartile range) baseline serum uric acid concentrations in the respective groups were 214 μmol/l (182–252), 239 μmol/l (199–276), 323 μmol/l (256–398), and 336 μmol/l (278–400) (correlation coefficient between the renal status and serum uric acid concentration $r = 0.481$, $P < 0.001$) (Figure 2).

Progression of albuminuria

Of the 3600 participants without ESRD at baseline, prospective data on renal status were available for a total of 3384 individuals (94%). At the end of the mean follow-up period of 7 ± 4 years, kidney disease had progressed in 521 of these participants (15.4%) (Table 2). Compared with those whose renal status remained unchanged during the follow-up, those whose renal status declined had longer diabetes duration, higher body mass index, higher systolic blood pressure, higher diastolic blood pressure, higher serum uric acid concentration, lower estimated glomerular filtration rate (eGFR), higher HbA_{1c}, higher total cholesterol, lower high-density lipoprotein cholesterol, and higher triglyceride concentration at baseline. Moreover, the progressors were more frequently men.

Of those with normal AER at baseline, the renal status of a total of 202 individuals (8.6%) deteriorated to microalbuminuria or worse during the follow-up period (Figure 1). The renal status progressed in 95 individuals (19.5%) and 224 individuals (41.9%) with microalbuminuria and macroalbuminuria at baseline, respectively. In the unadjusted Cox regression analyses, baseline serum uric acid concentration was associated with the progression from normal AER to microalbuminuria ($P = 0.047$), from microalbuminuria to macroalbuminuria ($P = 0.031$), and from macroalbuminuria to ESRD ($P < 0.001$) (Table 3). After adjusting for confounding factors (normal AER to microalbuminuria: triglyceride concentration, HbA_{1c}, sex, and diastolic blood pressure; microalbuminuria to macroalbuminuria: triglyceride concentration, HbA_{1c}, and sex; macroalbuminuria to ESRD: HbA_{1c}, diastolic blood pressure, and eGFR), baseline serum uric acid concentration was, however, no longer associated with the progression (respective P values: $P = 0.648$, $P = 0.133$, and $P = 0.054$).

Decrease in eGFR

Of the 2455 participants with baseline eGFR stages 1 and 2, the renal status of a total of 139 individuals (5.7%) deteriorated to stages 3 to 5 during the follow-up period. In the multivariable Cox regression analysis, the serum uric acid

Table 1 | Baseline characteristics of study subjects categorized by renal status at baseline

	Normal AER $N = 2524$ (64.8%)	Microalbuminuria $N = 540$ (13.9%)	Macroalbuminuria $N = 536$ (13.7%)	ESRD $N = 295$ (7.6%)	All $N = 3895$	P value[a]
Men (%)	48.1	59.4	58.2	60.0	52.0	<0.001
Age (yr)	37 (27–47)	39 (30–49)	41 (34–50)	46 (41–52)	39 (30–48)	<0.001
Diabetes duration (yr)	17 (9–27)	25 (17–33)	28 (22–34)	34 (29–40)	22 (13–31)	<0.001
BMI (kg/m^2)	24.6 (22.6–26.6)	25.5 (23.1–28.0)	25.6 (23.3–28.6)	23.6 (21.1–26.4)	25.0 (23.0–27.0)	<0.001
Systolic blood pressure (mm Hg)	129 (119–140)	135 (124–148)	142 (130–157)	150 (136–167)	132 (121–145)	<0.001
Diastolic blood pressure (mm Hg)	79 (71–85)	80 (74–88)	82 (77–90)	84 (73–91)	80 (72–86)	<0.001
Insulin dose (units/kg per day)	0.67 (0.53–0.82)	0.70 (0.56–0.90)	0.65 (0.53–0.79)	0.67 (0.51–0.83)	0.67 (0.53–0.83)	<0.001
Serum uric acid (μmol/l)	214 (182–252)	239 (199–276)	323 (256–398)	336 (278–400)	233 (192–281)	<0.001
eGFR (ml/min per 1.73 m^2)	94 (82–107)	89 (75–105)	58 (36–77)	NA	90 (75–105)[b]	<0.001
HbA_{1c} (mmol/mol)	65.0 (56.3–74.9)	71.6 (61.7–80.3)	72.7 (63.9–85.8)	67.2 (56.3–77.0)	67.2 (57.4–77.0)	<0.001
HbA_{1c} (%)	8.1 (7.3–9.0)	8.7 (7.8–9.5)	8.8 (8.0–10.0)	8.3 (7.3–9.2)	8.3 (7.4–9.2)	<0.001
Total cholesterol (mmol/l)	4.8 (4.2–5.4)	5.0 (4.4–5.6)	5.2 (4.6–5.9)	4.9 (4.1–5.7)	4.9 (4.3–5.5)	<0.001
HDL cholesterol (mmol/l)	1.33 (1.11–1.58)	1.28 (1.06–1.53)	1.19 (0.95–1.42)	1.22 (0.93–1.53)	1.30 (1.08–1.56)	<0.001
Triglycerides (mmol/l)	0.94 (0.72–1.28)	1.08 (0.82–1.56)	1.41 (1.03–2.04)	1.40 (0.98–1.90)	1.03 (0.77–1.46)	<0.001

AER, albumin excretion rate; BMI, body mass index; eGFR, estimated glomerular filtration rate; ESRD, end-stage renal disease; NA, not available.
Data are presented as frequency (%) for categorical variables and median (interquartile range) for continuous nonnormally distributed variables.
[a]*P* value represents the difference among the 4 groups of renal status.
[b]Values represent median (interquartile range) values of participants with normal AER, microalbuminuria, and macroalbuminuria.

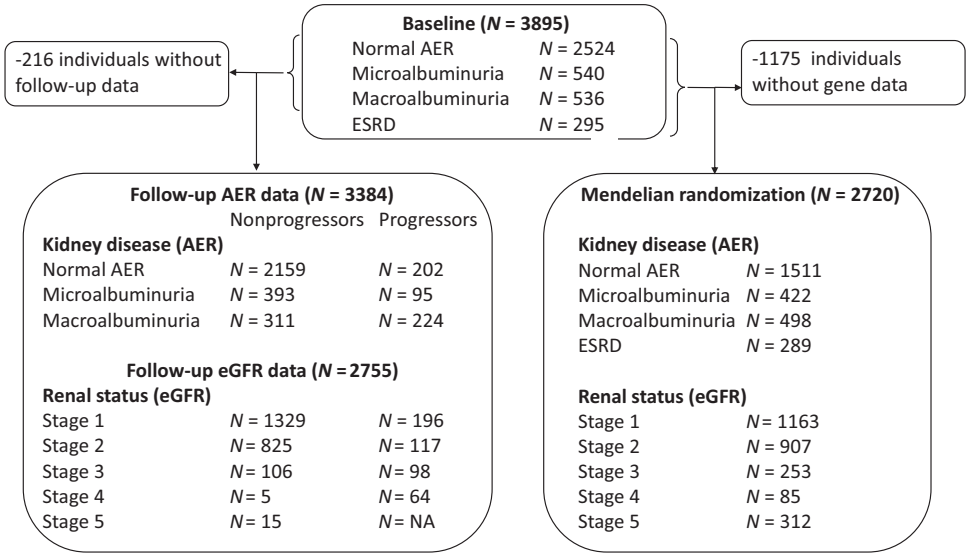

Figure 1 | Flow chart of the study population. AER, albumin excretion rate; eGFR, estimated glomerular filtration rate; ESRD, end-stage renal disease.

concentration was significantly associated with renal deterioration in both the unadjusted model and the model adjusted for confounding factors (CKD groups 1/2 to 3/4/5: triglyceride concentration, HbA_{1c}, systolic blood pressure, diabetes duration, and eGFR; CKD groups 1/2/3 to 4/5: triglyceride concentration, HbA_{1c}, systolic blood pressure, age, diabetes duration, and eGFR) ($P < 0.001$) (Table 3).

Of the 2659 participants with baseline eGFR stages 1 to 3, the renal status of a total of 154 individuals (5.8%) deteriorated to stages 4/5 during the follow-up period. In the multivariable Cox regression analysis, serum uric acid concentration was significantly associated with the renal deterioration ($P < 0.001$, unadjusted and adjusted).

Of the 204 participants with baseline eGFR stage 3, the renal status of a total of 98 individuals (48.0%) deteriorated to stages 4/5 during follow-up. In the multivariable Cox regression analysis, serum uric acid concentration was significantly associated with renal deterioration in the unadjusted ($P < 0.001$) and adjusted (HbA_{1c} and eGFR) ($P = 0.009$) models.

Finally, of the 257 participants with macroalbuminuria and eGFR groups 1 to 3 at baseline, the renal status of a total of 142 individuals (55.3%) deteriorated to stages 4 to 5 during follow-up. Also in this population, serum uric acid concentration in the Cox regression analyses was associated with renal deterioration ($P < 0.001$, unadjusted and adjusted).

Genetic risk score for serum uric acid

For the purpose of performing Mendelian randomization, we constructed a single nucleotide polymorphism (SNP) score for serum uric acid based on the 29 SNPs previously robustly associated with serum uric acid levels ($P < 5 \times 10^{-8}$),[7] of which 23 were found to be of high quality in our GWAS data (Supplementary Table S1). The SNP score was strongly associated with the baseline serum uric acid concentration ($P = 3.0 \times 10^{-13}$; F-statistic = 54), supporting the use of the SNP score in the Mendelian randomization (F-statistics ≥10 are considered sufficient for analysis).[13] The proportion of the variance of serum uric acid explained by the SNP score (R^2) was 1.9%, of which 1.0% was attributable to rs12498742 in *SLC2A9* alone. A total of 17.4% variance was explained when adjusting the model for age and sex (i.e., somewhat higher than the 7% estimated for the 29 in the general population).[7] Despite a strong association with serum uric acid, the SNP score was not associated with any of the AER- or eGFR-based nephropathy classes (P values >0.05) or with eGFR as a continuous variable ($P = 0.74$); neither was the score associated with any of the covariates listed in Table 1 (P values > 0.05).

Mendelian randomization analysis

Next we tested the causality between serum uric acid and diabetic nephropathy with the Mendelian randomization approach using the SNP score as the instrumental variable. In the cross-sectional regression analyses, serum uric acid was strongly associated ($P < 2 \times 10^{-16}$) with all tested AER- and eGFR-based nephropathy categories (Table 4, Figure 2). On the contrary, the Mendelian randomization indicated no causal effect of serum uric acid on the albuminuria-based or the CKD group–based nephropathy status (P values >0.05) evaluated with the control function estimator method[14]; similarly, no causal effect of serum uric acid was observed for eGFR as a continuous variable ($P = 0.63$, evaluated with the 2-stage least-squares method[15]) (Table 4). The strongest known genetic factor for serum uric acid, rs12498742, is located in *SLC2A9,* which encodes a transporter protein that stimulates the uptake of uric acid from the urine into the blood. The same protein also has an opposing effect of transporting uric acid from blood into the intestine. Because of this potential dual effect of the single strongest genetic factor, we repeated the Mendelian randomization excluding

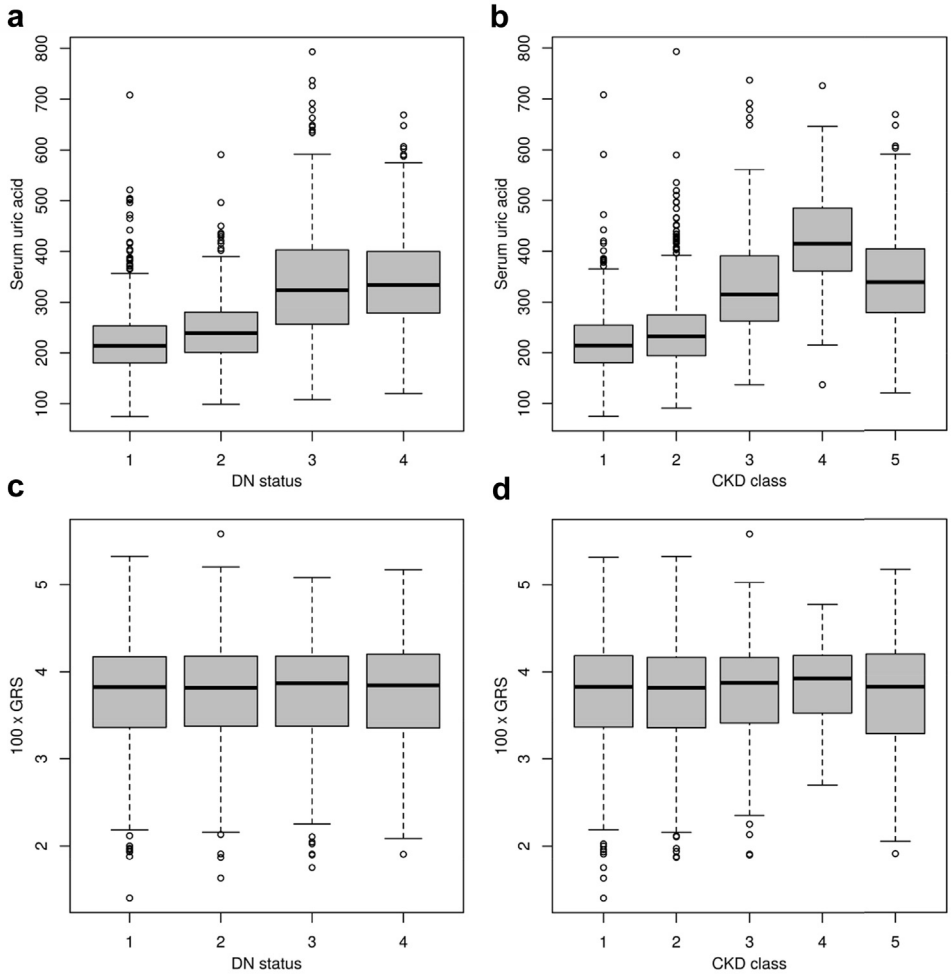

Figure 2 | Box plots showing that baseline serum uric acid concentration (a,b), but not the genetic risk score (GRS) for serum uric acid levels (c,d), differs by diabetic nephropathy (DN) and chronic kidney disease (CKD) classes. (**a,c**) Grouped by DN status (1, normal albumin excretion rate; 2, microalbuminuria; 3, macroalbuminuria; 4, end-stage renal disease). (**b,d**) Grouped by CKD class. Serum uric acid: untransformed serum uric acid concentration, μmol/l. The GRS is multiplied by 100. Figures are based on the 2720 patients in the Mendelian randomization analysis.

rs12498742 from the SNP score. Even after exclusion, the SNP score was strongly associated with serum uric acid ($P = 4.1 \times 10^{-7}$, *F*-statistic = 25.7), and results of all Mendelian randomization analyses remained nonsignificant.

DISCUSSION

In the current study, we evaluated the role of serum uric acid concentration on the progression of diabetic nephropathy. In our cross-sectional analyses, we observed that serum uric acid concentration was higher in more advanced renal disease, the progressors had higher baseline uric acid concentrations, and the calculated SNP score was associated with the serum uric acid concentration. In line with previous studies,[4–6] during the 7-year follow-up, the baseline serum uric acid concentration strongly predicted the progression of albuminuria and CKD in unadjusted Cox regression models. However, uric acid concentration was independently associated with the decrease in eGFR but not the progression of albuminuria. Observations in prospective studies do not, however, implicate potential causal relationships. Therefore, we evaluated the causality between serum uric acid and diabetic nephropathy with Mendelian randomization. Contrary to the positive associations with progression of renal disease in studies by us and others and to the previously reported positive causal association with progression of CKD in 755 mainly nondiabetic subjects,[9] uric acid concentration was not causally related to the development of diabetic nephropathy in the Mendelian randomization, as evaluated by either AER- or eGFR-based nephropathy categories or by eGFR as a continuous variable.

Results of a number of previous studies, with a follow-up period ranging from 6 to 18 years, have suggested that serum uric acid concentration is associated with the progression of diabetic nephropathy in type 1 diabetes.[4–6,16] Common to these studies is that they all evaluated the association between baseline uric acid concentration and progression of renal impairment while taking into account a number of known confounding factors. Moreover, 1 study showed that lowering

Table 2 | Baseline characteristics of study subjects divided into nonprogressors and progressors of diabetic nephropathy during the follow-up

	Nonprogressors $N = 2863$ (84.6%)	Progressors $N = 521$ (15.4%)	*P*
Men (%)	49.6	61.8	<0.001
Age (yr)	37 (28–48)	39 (31–48)	0.063
Diabetes duration (yr)	19 (11–29)	23 (15–31)	<0.001
BMI (kg/m^2)	24.8 (22.8–26.9)	25.2 (22.7–27.9)	0.004
Systolic blood pressure (mm Hg)	130 (120–141)	137 (125–152)	<0.001
Diastolic blood pressure (mm Hg)	79 (72–85)	82 (76–89)	<0.001
Insulin dose (units/kg per day)	0.67 (0.53–0.83)	0.68 (0.54–0.84)	0.130
Serum uric acid (μmol/l)	223 (187–264)	267 (209–376)	<0.001
eGFR (ml/min per 1.73 m^2)	91 (78–106)	74 (39–97)	<0.001
HbA_{1c} (mmol/mol)	66.1 (56.3–76.0)	77.7 (66.1–89.1)	<0.001
HbA_{1c} (%)	8.2 (7.3–9.1)	9.2 (8.2–10.3)	<0.001
Total cholesterol (mmol/l)	4.8 (4.2–5.4)	5.2 (4.5–5.8)	<0.001
HDL cholesterol (mmol/l)	1.31 (1.10–1.57)	1.20 (0.97–1.45)	<0.001
Triglycerides (mmol/l)	0.97 (0.74–1.34)	1.34 (0.97–2.06)	<0.001

BMI, body mass index; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein.
Data are presented as frequency (%) for categorical variables and median (interquartile range) for continuous nonnormally distributed variables.

of serum uric acid concentration with drugs was associated with a reduced risk of renal outcomes during a 3.4-year follow-up in patients with type 2 diabetes.[17] In the current analyses, the progressors also had a higher serum uric acid concentration at baseline. Higher baseline uric acid concentration did not, however, remain significant in the multivariate model, suggesting that, at least in our population, other variables play a stronger role in the progression of renal failure.

Because the lack of prediction in the multivariate models could be due to multicollinearity with other covariates, we assessed whether the serum uric acid concentration is causally related to diabetic nephropathy using the Mendelian randomization approach. These Mendelian randomization analyses suggested that the serum uric acid concentration does not have any causal effect on the diabetic kidney complications, but is rather a downstream marker of the kidney damage. This is a novel observation because no other previous studies used the Mendelian randomization approach to study the causal association between serum uric acid and diabetic nephropathy. Although 1 previous Mendelian randomization study of serum uric acid and CKD was performed in a much smaller study population (755 mainly nondiabetic subjects) and was based on only 1 genetic variant rather than an SNP score, they found a positive causal association between serum uric acid and CKD.[9] The controversial results might reflect different pathophysiologic mechanisms behind diabetic nephropathy and CKD in the general population or might be due to potential pleiotropic effects of the particular SNP used in the previous study (rs734553 in *SLC2A9*), possibly affecting some other

Table 3 | Association between baseline serum urate concentration and renal deterioration over the follow-up period: Cox regression analyses

Progression	Model	HR (95% CI)	*P* value
Normal AER: microalbuminuria	Unadjusted	1.480 (1.004–2.182)	0.047
	Adjusted[a]	1.110 (0.710–1.735)	0.648
Microalbuminuria: macroalbuminuria	Unadjusted	1.852 (1.059–3.237)	0.031
	Adjusted[b]	1.594 (0.866–2.932)	0.133
Macroalbuminuria: ESRD	Unadjusted	4.433 (3.239–6.065)	<0.001
	Adjusted[c]	1.539 (0.992–2.387)	0.054
CKD groups 1/2 to groups 3/4/5	Unadjusted	7.584 (4.966–11.581)	<0.001
	Adjusted[d]	2.793 (1.748–4.461)	<0.001
CKD groups 1/2/3 to groups 4/5	Unadjusted	18.803 (13.369–26.444)	<0.001
	Adjusted[e]	3.037 (1.920–4.805)	<0.001
CKD group 3 to groups 4/5	Unadjusted	4.651 (2.827–7.651)	<0.001
	Adjusted[f]	2.232 (1.221–4.082)	0.009
Macroalbuminuria and CKD groups 1/2/3 to groups 4/5	Unadjusted	3.374 (2.253–5.052)	<0.001
	Adjusted[c]	2.472 (1.616–3.782)	<0.001

AER, albumin excretion rate; CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; ESRD, end-stage renal disease; HR (95% CI), hazard ratio and 95% confidence interval, per 1 unit increase in $\log_2$ serum uric acid (i.e., odds ratio for doubling of the serum uric acid).
[a]Adjusted for triglyceride concentration, HbA_{1c}, sex, and diastolic blood pressure.
[b]Adjusted for triglyceride concentration, HbA_{1c}, and sex.
[c]Adjusted for HbA_{1c}, diastolic blood pressure, and eGFR.
[d]Adjusted for triglyceride concentration, HbA_{1c}, systolic blood pressure, diabetes duration, and eGFR.
[e]Adjusted for triglyceride concentration, HbA_{1c}, systolic blood pressure, age, diabetes duration, and eGFR.
[f]Adjusted for HbA_{1c} and eGFR.

Table 4 | Mendelian randomization analysis of the effect of serum uric acid on diabetic kidney disease with AER- and eGFR-based definitions and comparison with the corresponding cross-sectional regression analyses

Comparison	No. of cases	No. of controls	Mendelian randomization		Cross-sectional regression[a]	
			OR or β (95% CI)[b]	*P* value	OR or β (95% CI)[b]	*P* value
Albuminuria-based comparisons						
miA/maA/ESRD versus noA	1209	1511	1.18 (0.32–4.37)	0.801	13.54 (10.71–17.11)	$<2 \times 10^{-16}$
maA/ESRD versus noA/miA	787	1933	1.57 (0.34–7.29)	0.565	35.91 (26.68–48.34)	$<2 \times 10^{-16}$
ESRD versus noA/miA/maA	289	2431	2.12 (0.3–15.2)	0.455	9.69 (7.26–12.93)	$<2 \times 10^{-16}$
maA/ESRD versus noA	787	1511	1.52 (0.28–8.36)	0.631	45.24 (32.59–62.81)	$<2 \times 10^{-16}$
ESRD versus noA	289	1511	2.27 (0.21–24.35)	0.497	100.92 (60.11–169.42)	$<2 \times 10^{-16}$
eGFR-based comparisons						
CKD groups 3/4/5 versus groups 1/2	650	2070	2.31 (0.44–12.21)	0.801	48.76 (35.21–67.52)	$<2 \times 10^{-16}$
CKD groups 4/5 versus groups 1/2/3	397	2323	2.48 (0.39–16.01)	0.565	24.57 (17.96–33.6)	$<2 \times 10^{-16}$
CKD groups 4/5 versus group 1	397	1163	2.35 (0.21–25.82)	0.455	154.63 (90.91–263.01)	$<2 \times 10^{-16}$
eGFR, continuous	2720		2.24 (−17.29 to 21.77)	0.631	−40.15 (−42.35 to −37.94)	$<2 \times 10^{-16}$

AER, albumin excretion rate; CI, confidence interval; CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; ESRD, end-stage renal disease; maA, macroalbuminuria; miA, microalbuminuria; noA, normal albumin excretion rate; OR, odds ratio per 1-unit increase in $\log_2$ serum uric acid (i.e., odds ratio for doubling of the serum uric acid).

[a]Cross-sectional regression was calculated with logistic regression for case-control variables and with linear regression for eGFR as a continuous variable. Models are not adjusted for covariates.

[b]Effect size estimate is given for case-control variables as OR; for eGFR as continuous variable; effect size estimate is given as β, representing the increase of eGFR per 1-unit increase in $\log_2$ serum uric acid.

confounding factors for CKD. Of note, the variant was not associated with serum uric acid in that study. Similar to our study, Hughes *et al.*[10] used a genetic risk score to investigate the causality between serum uric acid and renal function, using Mendelian randomization.[10] Importantly, the study population did not exclusively include patients with diabetes. In that study, a uric acid transporter genetic risk score was not associated with renal function in their combined population or in women, but it was associated with improved renal function in men rather than with reduced renal function, as would be expected based on previous literature. The authors suggest that the observed renal protection might be due to the activity of the uric acid transporters rather than to serum uric acid concentrations.

The genetic background of serum uric acid concentration has been studied intensively, and a large meta-analysis of >140,000 individuals of European ancestry identified 29 loci affecting the level of serum uric acid.[7] We were able to extract 23 of these loci of high quality from our genomewide genetic data, including the 2 strongest variants near the *SLC2A9* and *ABCG2* genes. The *F*-statistic of 53.7, describing the strength of the instrumental variable (i.e., the SNP score), indicated that the SNP score was well powered for the analysis (values >10 are considered sufficient for Mendelian randomization).[18] Therefore, the lack of causal association is unlikely to be a false-negative finding. Furthermore, we used the SNP score instead of single SNPs in order to maximize the statistical power and to mitigate any potential pleiotropic effects addressable to single genetic variants. As a sensitivity test, our negative results remained when the analyses were repeated after excluding from the SNP score the strongest single variant in *SLC2A9*, as the encoded transporter also has an opposite effect of transporting uric acid from blood to the intestine, in addition to its main role of stimulating the uptake of uric acid from the urine into the blood.[19]

If uric acid is not causally related to the deterioration of diabetic renal disease, the question arises why lowering of serum uric acid was associated with a reduced risk of renal outcomes in patients with type 2 diabetes.[17] The answer may be that uric acid is only a minor player in the multifaceted cascade leading to renal disease, but that the drugs used to lower uric acid have other effects beyond lowering uric acid. Moreover, as 1 Mendelian randomization study suggests that serum uric acid is a causal risk factor for CKD in the general population,[9] it might be that serum uric acid plays a role only in the processes leading to nondiabetic renal disease rather than in pure diabetic nephropathy. Although a substantial proportion of patients with type 2 diabetes and kidney disease do not have diabetic nephropathy but have renal impairment due to other reasons similar to the general population (such as aging, overweight, and hypertension), the vast majority of patients with type 1 diabetes have histologic lesions typical of diabetic nephropathy,[20] potentially explaining why no causal association was seen between uric acid and renal impairment in our study population. Ongoing studies addressing this issue will provide important new data in order to understand the true role of uric acid in diabetic kidney disease.

Our study has both strengths and limitations. We studied a fairly large population of genetically homogeneous and well-characterized patients, which provides a reasonably good basis to study the phenomenon. In Mendelian randomization, pleiotropy, a condition in which a single gene influences several traits, may be of concern. In case the selected SNP would be associated not only with the uric acid concentration but also with another variable that also has an effect on the diabetic nephropathy, the results would be biased. The use of an SNP score does, however, dilute the effect of a single variant pleiotropy. In addition, the use of the SNP score increases the statistical power over using single variants. Finally, our study is descriptive and therefore does not give any insight into the potential mechanisms behind the progression of diabetic nephropathy. More research is required to assess the role of uric acid in the diabetic complications.

In summary, serum uric acid does not seem to be a clinically useful predictor of the disease progression beyond other known risk factors.

METHODS

Subjects were participants in the Finnish Diabetic Nephropathy Study (FinnDiane), a prospective study to investigate the factors associated with diabetic complications in type 1 diabetes. Included in the current analyses were all participants with serum uric acid measurements at baseline and known urinary AERs at baseline and at the end of the follow-up period. Individuals taking gout medication 1 year before the uric acid measurement were excluded. In all, data were available from 3895 Caucasian participants with type 1 diabetes (52% men; mean age ± SD, 39 ± 12 years at baseline). The study protocol was approved by the Ethics Committee of the Hospital District of Helsinki and Uusimaa, and patients gave written informed consent before participation.

Serum uric acid concentration was measured at baseline using an enzymatic method. Serum lipid and lipoprotein concentrations were measured as previously described.[21] HbA_{1c} was determined locally using standardized assays. Patient height and weight were measured while wearing light-weight clothing. From these measurements, body mass index was calculated (kg/m^2). After at least a 10-minute rest, blood pressure was measured with patients in the sitting position and was repeated after 2 minutes, and the mean of these measurements was used in the analyses.

Progression of renal disease was based on the assessment of deterioration in the levels of AER and eGFR during the follow-up period. AER was measured in at least 2 of 3 timed 24-hour or overnight urine collections. Patients were classified as follows: normal AER (<20 μg/min or <30 mg/24 h), microalbuminuria (AER ≥20 and <200 μg/min or ≥30 and <300 mg/24 h), macroalbuminuria (AER ≥200 μg/min or ≥300 mg/24 h), or ESRD (undergoing dialysis or having had a kidney transplant). The eGFR was calculated using the CKD-EPI formula.[22] Patients were divided into CKD groups 1 to 5 based on their eGFR values (group 1: eGFR ≥90 ml/min per 1.73 m^2; group 2: eGFR ≥60 and <90 ml/min per 1.73 m^2; group 3: eGFR ≥30 and <60 ml/min per 1.73 m^2; group 4: eGFR ≥15 and <30 ml/min per 1.73 m^2; group 5: <15 ml/min per 1.73 m^2 or ESRD). For patients with ESRD, eGFR was set at 10 ml/min per 1.73 m^2 for the analyses requiring a continuous eGFR value. Patients were followed for an average of 7 ± 4 years. At the end of the follow-up period, the renal status was reevaluated, and patients were, accordingly, divided into progressors and nonprogressors based on the newly assessed AER level and CKD group. Progression, defined by AER or eGFR level, was assumed if an individual advanced from healthier AER or eGFR level to one of more advanced levels, respectively.

Among the FinnDiane participants, with serum uric acid and albuminuria–based diabetic nephropathy group defined at baseline, GWAS data were available for 2720 subjects. GWAS genotyping, quality control, and imputation have been previously described.[23] Based on a previous GWAS on serum uric concentration,[7] 29 SNPs were selected for the SNP score. The estimated allele doses of the 29 SNPs were converted to the most likely genotypes using a probability threshold of 0.9 for the genotype calling. After filtering the SNPs with genotyping rate <0.95 or $P < 0.01$ for Hardy-Weinberg equilibrium, 23 SNPs of good quality remained (Supplementary Table S1). The SNP score was calculated by weighting the risk allele count by the reported per-allele effect,[7] and the score was scaled by the number of available SNPs.

Statistical analyses

Descriptive results are reported as percentages for categorical data and median (interquartile range) for continuous data as they were non-normally distributed. The respective statistical comparisons between progressors and nonprogressors were conducted using the χ^2 test and Kruskal-Wallis test. The correlation between serum uric acid concentration and renal status at baseline was studied with Spearman's ρ. Multivariate Cox regression was used to study whether baseline serum uric acid concentration was associated with progression of diabetic nephropathy. Baseline variables that remained significant in the multivariate Cox regression analyses, before inclusion of serum uric acid concentration into the models, were used as confounding factors. The association between the SNP score and the serum uric acid concentration was tested by linear regression. The Mendelian randomization approach was used to assess causality between serum uric acid concentration and diabetic nephropathy. Mendelian randomization is a form of instrumental variable analysis. We used the 2-stage least-squares method to analyze the effect of eGFR as a continuous variable.[15] For case-control phenotypes, we used the control function estimator method, which is deemed more suitable for binary outcomes than the 2-stage least-squares (2SLS) method and better controls the effect of covariates that are not included in the model.[14] Statistical analyses were performed with IBM SPSS Statistics for Windows, Version 19.0 (IBM Corp., Armonk, NY), plink v.1.09 (https://www.cog-genomics.org/plink2/),[24] and R software (http://cran.r-project.org/; "sem" package for 2SLS).

DISCLOSURE

Per-Henrik Groop has received research grants from Eli Lilly and Roche and is an advisory board member for AbbVie, AstraZeneca, Boehringer-Ingelheim, Cebix, Eli Lilly, Janssen, Medscape, MSD, and Novartis. He also has received lecture fees from AstraZeneca, Boehringer-Ingelheim, Eli Lilly, Genzyme, MSD, Novartis, Novo Nordisk, and Sanofi. However, none of the above are relevant to the article. All the other authors declared no competing interests.

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AUTHOR CONTRIBUTIONS

All authors have taken part in the design of the work, acquired data, and interpreted the results. AJA and NS drafted the manuscript, and other authors revised it. All authors approved the final version.

SUPPLEMENTARY MATERIAL

Supplementary Table S1. SNPs previously associated with serum uric acid ($P < 5 \times 10^{-8}$) and their genotyping quality in FinnDiane.
Supplementary Text. The physicians and nurses at each center participated in the collection of patients

Supplementary material is linked to the online version of the paper at www.kidney-international.org.

REFERENCES

1. Hovind P, Tarnow L, Rossing K, et al. Decreasing incidence of severe diabetic microangiopathy in type 1 diabetes. *Diabetes Care*. 2003;26:1258–1264.
2. Hovind P, Rossing P, Tarnow L, et al. Progression of diabetic nephropathy. *Kidney Int*. 2001;59:702–709.
3. Groop PH, Thomas MC, Moran JL, et al. The presence and severity of chronic kidney disease predicts all-cause mortality in type 1 diabetes. *Diabetes*. 2009;58:1651–1658.
4. Jalal DI, Rivard CJ, Johnson RJ, et al. Serum uric acid levels predict the development of albuminuria over 6 years in patients with type 1 diabetes: findings from the Coronary Artery Calcification in Type 1 Diabetes study. *Nephrol Dial Transplant*. 2010;25:1865–1869.
5. Hovind P, Rossing P, Tarnow L, et al. Serum uric acid as a predictor for development of diabetic nephropathy in type 1 diabetes: an inception cohort study. *Diabetes*. 2009;58:1668–1671.
6. Ficociello LH, Rosolowsky ET, Niewczas MA, et al. High-normal serum uric acid increases risk of early progressive renal function loss in type 1 diabetes: results of a 6-year follow-up. *Diabetes Care*. 2010;33:1337–1343.
7. Köttgen A, Albrecht E, Teumer A, et al. Genome-wide association analyses identify 18 new loci associated with serum urate concentrations. *Nat Genet*. 2013;45:145–154.
8. Kolz M, Johnson T, Sanna S, et al. Meta-analysis of 28,141 individuals identifies common variants within five new loci that influence uric acid concentrations. *PLoS Genet*. 2009;5:e1000504.
9. Testa A, Mallamaci F, Spoto B, et al. Association of a polymorphism in a gene encoding a urate transporter with CKD progression. *Clin J Am Soc Nephrol*. 2014;9:1059–1065.
10. Hughes K, Flynn T, de Zoysa, et al. Mendelian randomization analysis associates increased serum urate, due to genetic variation in uric acid transporters, with improved renal function. *Kidney Int*. 2014;85:344–351.
11. Kleber ME, Delgado G, Grammer TB, et al. Uric acid and cardiovascular events: a Mendelian randomization study. *JASN*. 2015;26:2831–2838.
12. Palmer TM, Bordestgaard BG, Benn M, et al. Association of plasma uric acid with ischaemic heart disease and blood pressure: mendelian randomisation analysis of two large cohorts. *BMJ*. 2013;347:f4262.
13. Staiger D, Stock J. Instrumental variables regression with weak instruments. *Econometrica*. 1997;65:557–586.
14. Palmer TM, Sterne JA, Harbord RM, et al. Instrumental variable estimation of causal risk ratios and causal odds ratios in Mendelian randomization analyses. *Am J Epidemiol*. 2011;173:1392–1403.
15. Fox J. Simultaneous equation models and two-stage least squares. *Sociological Method*. 1979;10:130–150.
16. Krolewski AS, Niewczas MA, Skupien J, et al. Early progressive renal decline precedes the onset of microalbuminuria and its progression to macroalbuminuria. *Diabetes Care*. 2014;37:226–234.
17. Miao Y, Ottenbros SA, Laverman GD, et al. Effect of a reduction in uric acid on renal outcomes during losartan treatment: a post hoc analysis of the reduction of endpoints in non-insulin-dependent diabetes mellitus with the Angiotensin II Antagonist Losartan Trial. *Hypertension*. 2011;58:2–7.
18. Lawlor DA, Harbord RM, Sterne JA, et al. Mendelian randomization: using genes as instruments for making causal inferences in epidemiology. *Stat Med*. 2008;27:1133–1163.
19. DeBosch BJ, Kluth O, Fujiwara H, et al. Early-onset metabolic syndrome in mice lacking the intestinal uric acid transporter SLC2A9. *Nat Commun*. 2014;5:4642.
20. Fioretto P, Mauer M. Histopathology of diabetic nephropathy. *Semin Nephrol*. 2007;27:195–207.
21. Thorn LM, Forsblom C, Fagerudd J, et al. Metabolic syndrome in type 1 diabetes: association with diabetic nephropathy and glycemic control (the FinnDiane study). *Diabetes Care*. 2005;28:2019–2024.
22. Levey AS, Stevens LA, Schmid CH, et al. A new equation to estimate glomerular filtration rate. *Ann Intern Med*. 2009;150:604–612.
23. Sandholm N, Salem RM, McKnight AJ, et al. New susceptibility loci associated with kidney disease in type 1 diabetes. *PLoS Genet*. 2012;8:e1002921.
24. Chang CC, Chow CC, Tellier LC, et al. Second-generation PLINK: rising to the challenge of larger and richer datasets. *Gigascience*. 2015;4:7-015-0047-8 (eCollection).