A Mendelian randomization study of circulating uric acid and type 2 diabetes

Running title: Mendelian randomization uric acid and diabetes

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Abstract

We aimed to investigate the causal effect of circulating uric acid concentrations on type 2 diabetes risk. A Mendelian randomization study was performed using a genetic score with 24 uric acid associated loci. We used data of the EPIC-InterAct case-cohort study, comprising 24,265 individuals of European ancestry from eight European countries. During a mean (SD) follow-up of 10 (4) years, 10,576 verified incident type 2 diabetes cases were ascertained. Higher uric acid associated with higher diabetes risk following adjustment for confounders, with a HR of 1.20 (95% CI: 1.11, 1.30) per 59.48 µmol/L (1 mg/dL) uric acid. The genetic score raised uric acid by 17 µmol/L (95% CI: 15, 18) per SD increase, and explained 4% of uric acid variation. Using the genetic score to estimate the unconfounded effect found that a 59.48 µmol/L higher uric acid concentration did not have a causal effect on diabetes (HR 1.01, 95% CI: 0.87, 1.16). Including data from DIAGRAM consortium, increasing our dataset to 41,508 diabetes cases, the summary OR estimate was 0.99 (95% CI: 0.92, 1.06). In conclusion, our study does not support a causal effect of circulating uric acid on diabetes risk. Uric acid lowering therapies may therefore not be beneficial in reducing diabetes risk.
Introduction

Elevated serum uric acid concentrations have been associated with higher diabetes risk in observational studies (1;2). Meta-analyses reported 6-17% higher diabetes risk with every 59.48 µmol/L (1 mg/dL) higher uric acid concentration (1;2). If this observed association were found to be causal, uric acid lowering therapies could be used in diabetes prevention. However, whether uric acid causes diabetes is still a matter of debate (3;4). Uric acid concentrations are closely linked to other diabetes risk factors such as obesity, which makes it difficult to determine the independent effects of uric acid when limited to observational analysis alone (3;4). Evidence from human intervention studies on the effect of uric acid lowering therapy on glucose metabolism is very limited and inconsistent (5-7).

The concept of Mendelian randomization, i.e. using genetic variants as instrumental variable, can be applied to test and estimate the causal effects of risk factors on disease outcomes (8). Since alleles are randomly allocated during gamete formation, the association of a genetic variant with risk of a disease outcome is unlikely to be confounded by other factors. Also, reverse causality is abrogated. Three meta-analyses together identified 31 loci associated with uric acid (9-11). Variants at such loci can be used as genetic instruments, to estimate the unconfounded effect of uric acid on diabetes risk. Only one Mendelian randomization study on uric acid and diabetes risk has been previously performed (12), and reported no evidence for a causal effect. That study used a small number of SNPs (8 identified in the first meta-analyses (9)), and used different studies to estimate the association between the genetic score and diabetes, the association between the genetic score and uric acid, and the association between uric acid and diabetes (i.e. the three sides of the Mendelian randomization triangle (13)).

In the present study, we aimed to estimate the unconfounded effect of uric acid on diabetes risk, using a multi-locus Mendelian randomization approach. We performed instrumental
variable estimation within the same study, using data on genetic variants in 24 uric acid
associated loci, and measured uric acid concentrations among 24,265 individuals, including
10,576 incident type 2 diabetes cases. We then bolstered the sample size by including
summary-level data from the DIAGRAM consortium, bringing our total number of diabetes
cases to 41,508.

Subjects and methods

Study population

The EPIC-InterAct study is a large, prospective case-cohort study involving individuals from
eight European countries (Denmark, France, Germany, Italy, the Netherlands, Spain, Sweden,
and the United Kingdom [UK]; 26 study centers), which is nested within the European
Prospective Investigation into Cancer and Nutrition (EPIC)(14). The majority of participants
were aged 35 to 70 years and were recruited between 1991 and 2000, mainly from the general
population. The EPIC-InterAct study, drawn from a total cohort of 340,234 individuals
comprising 3.99 million person–years of follow-up, was designed to investigate the interplay
between genetic and lifestyle factors and type 2 diabetes risk(15). A total of 12,403 verified
incident cases of type 2 diabetes were identified. A center-stratified, random subcohort of
16,154 individuals was selected for analysis. Because of the random selection, this subcohort
also included a random set of 778 individuals who had developed incident type 2 diabetes
during follow-up. All participants gave written informed consent, and the study was approved
by the local ethics committees and the Internal Review Board of the International Agency for
Research on Cancer.
For the observational part of this analysis, we excluded participants with missing uric acid (1,873) or co-variable (n=1,641) data, leaving 24,265 (10,576 cases, 14,364 subcohort participants, including 675 cases in the subcohort) participants for analyses. For the instrumental variable analysis, we excluded participants with missing uric acid (1,875), genetic (n= 8,634; including 4,063 from Denmark, since at the time of analysis, genetic data were not yet available from the Danish cohort), BMI (n=141) or biomarker (n=11) data, leaving 17,118 (7,319 cases, 10,235 subcohort participants, including 436 cases in the subcohort) participants for analyses.

Diabetes

Ascertainment and verification of incident diabetes has been described in detail elsewhere(15). In short, incident diabetes cases were identified through self-report, linkage to primary care registers, secondary care registers, medication use and hospital admissions and mortality data. Information from any follow-up visit or external evidence with a date later than the baseline visit was used. To increase the specificity of the case definition, we sought further evidence for all cases with information on incident type 2 diabetes from ≥2 independent sources at a minimum, including individual review of medical records. Participants were followed-up for occurrence of diabetes until the 31st of December 2007.

Uric acid and other biomarkers

Non-fasting blood samples were taken at baseline. Laboratory measures were carried out by the Stichting Huisartsen Laboratorium Groep (Etten-Leur, the Netherlands) on serum (except for participants in the Umea center (Sweden), where only plasma samples were available) or erythrocyte samples that had been previously frozen at either in ultra-low temperature freezers.
at −80°C or in liquid nitrogen. Serum uric acid, triglycerides, glucose and HDL were measured using a Cobas® enzymatic assay (Roche Diagnostics, Mannheim, Germany) on a Roche Hitachi Modular P analyser. Erythrocyte HbA1c was measured using Tosoh (HLC-723G8) ion exchange high-performance liquid chromatography on a Tosoh G8.

**Genotyping and construction of the genetic score**

DNA was extracted from buffy coat from a citrated blood sample using standard procedures on an automated Autopure LS DNA extraction system (Qiagen, Hilden, Germany) with PUREGENE chemistry (Qiagen). In total, 8,536 (3,942 cases, 4,859 subcohort participants, including 265 cases in the subcohort) participants were genotyped with a customised version of the CardioMetabochip (CardioMetabochip+; Illumina, San Diego, CA, USA), using a Sequenom iPLEX array (Sequenom, San Diego CA, USA). The remaining participants (n=8,582; 2,941 cases, 5,812 subcohort participants, including 171 cases in the subcohort) were genotyped with the Illumina 660W quad chip (Illumina, San Diego, CA, USA), using TaqMan (Applied Biosystems, Carlsbad, CA, USA). Missing genotypes for participants genotyped with the Illumina 660W quad chip were imputed by assigning the mean genotype at each locus for cases and non-cases separately, for individuals successfully genotyped. In total, genotypes for 15 out of 24 SNPs were imputed. We selected SNPs that passed the significance threshold of $P < 5 \times 10^{-8}$ in three large-scale GWAS meta-analyses of uric acid (9-11) that were identified from searching PubMed with key words ‘GWAS’ and ‘uric acid or urate’. No SNPs were in linkage disequilibrium with each other. The alleles were coded 0, 1, 2, according to the number of uric acid raising alleles. We then calculated a genetic score by summing the number of risk alleles. To take into account that effect sizes of individual SNPs differ, we calculated a weighted genetic score, by weighing the individual SNPs by their
effect on uric acid, using estimates from the previously published GWAS meta-analyses(9-11). **Online supplementary table 1** provides an overview of the SNPs included in the genetic score, and weights assigned to each SNP.

**Co-variables**

Baseline information on lifestyle, diet and medical history were obtained from self-administered questionnaires. Weight and height were recorded by trained health professionals during a visit to a study center. Presence of hypertension was defined based on self reported diagnosis and/or use of medication. Physical activity was assessed by questionnaire and classified into inactive, moderately inactive, moderately active, and active, according to the Cambridge Physical Activity Index(16). Glomerular filtration rate (eGFR) was estimated using the Chronic Kidney Disease Epidemiology Collaboration equation, with creatinine standardized to the Roche enzymatic method(17).

**Statistical analysis**

Associations of individual SNPs with uric acid were assessed with linear regression, among the participants in the subcohort. Uric acid was modelled per 59.48 µmol/L (1 mg/dL), SNPs were modelled per uric acid increasing allele (additive model), and associations were adjusted for study center. Associations of individual SNPs and the uric acid related genetic score (per SD increase) with incident diabetes were examined with modified Cox regression that accounted for case-cohort design (Prentice-weighted model(18)), adjusted for study center. We calculated country specific HRs, and used random-effects meta-analysis to calculate a pooled HR. We investigated associations of the uric acid related genetic score (per SD increase) with potential confounders using linear regression for continuous and logistic regression for dichotomous confounders.
For the observational association of uric acid and incident diabetes, we estimated country-specific HRs and pooled them through meta-analysis. We used $I^2$ to quantify heterogeneity between countries. Interactions with sex, age and BMI were tested within each country by including interaction terms in the multivariable models. Country-specific estimates were pooled as described above. For the instrumental variable estimate of uric acid on diabetes risk, we used the weighted genetic score to estimate the unconfounded effect of a 59.48 µmol/L (1 mg/dL) increase in uric acid on diabetes risk. We applied the two stage control function estimator approach (19) for this instrumental variable estimate. Instrumental variable estimates were adjusted for study center, and in a second model sex and BMI were added. Country-specific estimates were pooled as described above. The analyses were repeated in strata of sex, age, BMI, and duration of follow-up. Furthermore, we generated instrumental variable estimates of uric acid on glycemic traits (non-fasting glucose and HbA1c) as described above. Proportional Hazard assumptions were inspected visually using log-minus-log plots, with no deviations detected.

Sensitivity analyses
Analyses were repeated after excluding participants with HbA1c >6.5% (N=22,146 for observational analysis and 15,380 for instrumental variable analysis). Furthermore, the observational association of uric acid and diabetes was estimated in the population used for the instrumental variable analysis (N=17,118 instead of 24,265). Moreover, we re-analysed the instrumental variable estimate of uric acid on diabetes risk using the non-weighted genetic score, excluding SNPs that were not statistically significantly associated with uric acid in our study, excluding proxy SNPs with $r^2 < 0.80$, and excluding SNPs (rs7345553; rs2231142) with the strongest effects on uric acid (Online supplementary table 1).
We estimated the power for the Mendelian randomization analysis at a 2-sided alpha of 0.05 based on the sample size and proportion of cases, strength of the genetic instrument, and the expected causal hazards ratio using the online tool mRnd (http://glimmer.rstudio.com/kn3in/mRnd/)(20).

**Incorporation of publicly available data from MAGIC and DIAGRAM to bolster power**

In order to maximize power, we additionally incorporated data made publicly available by GWAS consortia. For fasting glucose (n=58,074) and HOMA-IR (n=37,073), we used data from the MAGIC consortium, which is a collaborative effort that combined data from multiple GWAS to identify genetic determinants that impact on glycemic and metabolic traits. Participants were of European ancestry, and genotyped with the Metabochip(21). Data are publicly available at: http://www.magicinvestigators.org/. For diabetes, we used data from DIAGRAM consortium, which meta-analysed genetic variants on Metabochip in 34,840 diabetes cases and 114,981 controls from 37 studies (22). All studies participating in DIAGRAM included both men and women; participants were mainly of European ancestry; the mean age varied from 43 to 72 years and the mean study-level BMI varied from 25.9 to 33.4 kg/m$^2$ among diabetes cases, and from 22.3 to 28.3 kg/m$^2$ among controls. Data are publicly available at http://diagram-consortium.org/downloads.html.

For DIAGRAM, we selected the same 24 SNPs (either directly or in LD>0.85) and extracted the ORs and accompanying standard errors. Diabetes estimates were meta-analysed with odds ratios from InterAct (after excluding EPIC-Norfolk, which contributes to DIAGRAM) using fixed-effects meta-analysis on the log scale, to generate a summary estimate for each SNP and diabetes risk. We then used pooled SNP-diabetes effect estimates (including up to 41,508 diabetes cases) and external weights from uric acid GWAS (Online supplementary table 1) for
instrumental variable analysis. In MAGIC, exactly the same process was repeated but without meta-analysing MAGIC and InterAct (given that fasting glucose and HOMA-IR are not quantified in InterAct). We generated instrumental variable estimates for each SNP by dividing each SNP-trait effect estimate by the corresponding SNP-uric acid estimate. The analysis took into account the uncertainty in both the SNP-trait and SNP-uric acid estimates by using the delta method to estimate standard errors of instrumental variable ratio estimates(23). We then pooled instrumental variable estimates across SNPs using fixed-effects meta-analysis to generate the summary causal effects.

All analysis were performed using Stata 13.1 (StataCorp, College Station, Texas, USA).

Results

The mean (SD) age in the subcohort was 52 (10) years, and 65% was men. The mean (SD) uric acid concentration was 280 (77) µmol/L among the subcohort and 333 (83) µmol/L among diabetes cases (Table 1). Mean uric acid ranged from 327 µmol/L in Italy and Sweden to 351 µmol/L in Spain among males, and from 241 µmol/L in Germany to 261 µmol/L in the Netherlands among women.

Observational association of uric acid and diabetes

In the observational analysis, uric acid was associated with higher diabetes risk, with a HR of 1.51 (95%CI: 1.42, 1.62) per 59.48 µmol/L (1 mg/dL) uric acid. After adjustment for confounders, the observed association attenuated but remained present, with a corresponding HR of 1.20 (95%CI: 1.11, 1.30) in the multivariable model. BMI was the largest contributor to this attenuation (Table 2). Additional adjustment for red meat and vitamin C did not alter
the findings (HR 1.22 [95%CI: 1.11, 1.34]). The association remained consistent when we
explored the association using the population selected for the instrumental variable analysis
(HR multivariable model: 1.25 [95%CI: 1.13, 1.38]). Excluding participants with HbA1c
＞6.5% yielded a multivariable HR of 1.26 (95%CI: 1.17, 1.36).

Although all country specific HRs directed towards a higher diabetes risk with higher uric
acid concentrations, there was substantial heterogeneity between countries ($I^2$ 70%, P-value
0.001; Online supplementary figure 1). Heterogeneity remained present when the analyses
were stratified by age, sex, and BMI with no significant interactions for age and sex (P-values
for interaction 0.16 and 0.77, respectively) and borderline significant (P-value 0.06) for BMI
with no substantially different results in BMI strata; data not shown). After excluding Sweden
from the analysis, heterogeneity attenuated substantially, with $I^2$ of 48% (P-value 0.07), and
the association remained present (HR 1.17 [95%CI: 1.09, 1.25]).

**Associations of individual SNPs and genetic score with uric acid and diabetes**

Individual uric acid associated SNPs were all directly associated with uric acid, with the
strongest association for rs734553 on locus SLC2A9 (Table 3). The individual SNPs were
generally not associated with diabetes risk (Table 3).

The mean (SD) uric acid associated genetic score was 1.55 (0.25) in both the subcohort and
diabetes cases, and normally distributed among the study participants. A one SD higher
genetic score associated with a 17 μmol/L (95%CI: 15, 18) higher uric acid concentration
(Online supplementary table 2). The genetic score explained 4% of the proportion of
variance of uric acid (F-statistic 462). The genetic score did not associate with diabetes risk
(HR: 1.01 [95%CI: 0.97, 1.05] per SD higher genetic score; Online supplementary figure
2).
Association of genetic score with potential confounders or mediators

The uric acid associated genetic score was associated with higher triglyceride concentrations (Beta: 0.01 mmol/L [95%CI: 0.001, 0.02] per SD higher genetic score) and a borderline association was identified with vitamin C intake and physical activity. Remaining potential confounders or mediators were not associated with the genetic score (Online supplementary table 3).

Instrumental variable analysis of uric acid and diabetes

Using the uric acid associated genetic score to estimate the unconfounded effect of uric acid (per 59.48 µmol/L [1 mg/dL]) on diabetes showed no evidence for an effect (HR 1.01 [95%CI: 0.87, 1.16]). There was no substantial heterogeneity between countries ($I^2$ 16%, P-value 0.31; Online supplementary figure 3). This did not materially change after further adjustment for sex and BMI (Table 2). No differential effects were found in subgroups based on sex, age, BMI and duration of follow-up (Online supplementary table 4). Furthermore, there was no evidence for an effect of uric acid on glycemic traits (Online supplementary table 5).

Excluding participants with HbA1c >6.5% yielded a HR of 1.02 (95%CI: 0.89, 1.17). Using the non-weighted genetic score as the instrumental variable instead of the weighted genetic score yielded a HR of 0.96 (95%CI: 0.71, 1.30). Excluding SNPs from the weighted genetic score that were not associated with uric acid in our study did not change our findings (HR 1.02 [95%CI: 0.89, 1.17]), and neither did excluding proxy SNPs with $r^2 < 0.80$ (HR 0.99...
Adjustment for triglycerides, vitamin C and physical activity did not materially alter the estimate (HR 0.97 [95%CI: 0.82, 1.15]).

Inclusion of DIAGRAM, increasing our dataset to 41,508 diabetes cases yielded a summary causal estimate of OR 0.99 (95%CI: 0.92, 1.06) (Table 2; Online supplementary figure 4).

Using this combined dataset, exclusion of the two SNPs that most strongly associated with circulating uric acid (rs734553 in SLC2A9 and/or rs2231142 in ABCG2) did not alter the summary estimate (Online supplementary table 6).

Power calculation

Power calculations for our Mendelian randomization analysis are shown in Online supplementary table 7. In InterAct, we had 100% power to detect a HR of 1.51, 68% power to detect a HR of 1.20, and 31% power to detect the same effect estimate when we excluded rs734553. Inclusion of DIAGRAM increased power to detect a HR of 1.2 for all sensitivity analyses to over 90% (Online supplementary Table 7), meaning that the estimates derived from the combined analysis (InterAct and DIAGRAM) were well powered for all scenarios.

Discussion

In this large European case-cohort study, we found a 20% higher diabetes risk per 59.48 µmol/L (1 mg/dL) higher circulating uric acid concentration in multivariable observational analysis. Instrumental variable analysis did not confirm this association, and suggests no evidence of a causal effect of circulating uric acid on diabetes risk.
The results of the observational analysis are in line with previous reports\(^1;2\). Two previous meta-analyses showed 6-17% higher diabetes risk per 59.48 \(\mu\)mol/L (1 mg/dL) uric acid. We found a 20% higher risk per 59.48 \(\mu\)mol/L (1 mg/dL) which is comparable to the previous studies. However, residual confounding and/or reverse causality may explain these associations, since we did not find evidence for such an association in instrumental variable analysis. The results of our instrumental variable analysis generally agree with previous studies. First of all, our findings are in agreement with the previously performed Mendelian randomization study of uric acid and diabetes, that included fewer uric acid associated loci and used different studies to estimate the three sides of the Mendelian randomization triangle\(^12\). Moreover, a study of Yang et al.\(^{11}\) showed no association of a genetic score for uric acid with plasma glucose concentrations, in line with our results. Studies that used a genetic uric acid score or \textit{SLC2A9} as instrumental variable also suggested a bystander role for uric acid in other metabolic and cardiovascular traits, namely metabolic syndrome\(^{24;25}\), ischemic heart disease\(^{26}\), markers of subclinical atherosclerosis\(^{27}\), markers of adiposity\(^{28}\), and triglycerides\(^{29}\). For blood pressure, the results are mixed, with reports of no effect\(^{26}\), reducing effects\(^{30;31}\), and increasing effects\(^{32}\) (\textit{Online supplementary Table 8}).

There are observations that support a potential causal role of uric acid, whereas others suggest a bystander role. First of all, hyperinsulinemia decreases renal excretion of uric acid, leading to increased blood concentrations of uric acid\(^3\), supporting a bystander role. Furthermore, sub-clinical chronic inflammation may precede the development of diabetes\(^{33}\), and uric acid generation may be increased as a result of oxidative stress. Support for a causal role comes from a recent study showing that intestinal knockdown of uric acid resulted in hyperuricemia and development of metabolic syndrome in mice\(^{34}\). Moreover, there are reports that xanthine oxidase inhibitors (pharmacological agents used to lower uric acid) may improve
endothelial function, what may reduce insulin resistance(3). However, it has been suggested that this may represent an additional effect of enzyme inhibition that is unrelated to uric acid, since therapies other than xanthine oxidase inhibitors that reduce uric acid concentrations did not show the same benefits to endothelial function(7;35). Inhibition of xanthine oxidase may improve endothelial function by reduction of oxidative stress instead of lowering of uric acid (7).

Strengths of our study are its large sample size (especially including data from DIAGRAM, which provided a cumulative total of over 40,000 diabetes cases and bolstered our power for sensitivity analyses), heterogeneous European population, and availability of a comprehensive range of potential confounders. Moreover, uric acid concentrations were available for all participants, and were measured centrally to optimize comparability of uric acid concentrations among participants. Furthermore, our findings showed robustness in sensitivity analysis. A potential limitation of our study includes that the genetic score explained only 4% of variation in uric acid. The percentage of explained variation is very comparable to previous Mendelian randomization studies(36), and the corresponding F-statistic was high, indicating we are unlikely to suffer from weak instrument bias(13). Second, our study investigated the effect of circulating uric acid in blood, and does not necessarily also reflect effects of intracellular uric acid. Individual SNPs in the gene score may have differential effects on uric acid concentration by body compartment(34;37). Despite this, it is not plausible there will be common pleiotropy among the individual SNPs included in the score, and any pleiotropic roles of SNPs should be balanced out by use of a polygenic score(38). Third, our study population was of European ancestry, which limits generalizability to populations of other ancestries.

Mendelian randomization studies are a valid way to explore evidence for causality, given that certain assumptions are met. First, there has to be a strong association between the
instrumental variable and risk factor of interest. All SNPs used in this study have previously been shown to be strongly associated with uric acid concentrations in large meta-analyses of genome wide association studies (9-11). Nevertheless, some SNPs did not associate with uric acid in our study. However, when we excluded those SNPs from the genetic score, the null-association remained present. Moreover, we strengthened our instrumental variable by using a genetic score of multiple uric acid associated SNPs. No SNPs were in linkage disequilibrium with each other, which justifies combining those SNPs.

Second, the instrumental variable must be independent of potential confounders (confounders in the association between uric acid and diabetes). To test this, we examined the associations of the genetic score with potential confounders, and found an association with triglycerides. However, it can be debated whether this is a true confounder, or downstream consequence of uric acid pathways. Moreover, since we did not find an association of uric acid and diabetes in instrumental variable analysis, it is not likely that this is explained by the higher risk of hypertriglyceridemia in individuals with a high genetic score. Indeed, when we additionally adjusted the instrumental variable estimate of uric acid on diabetes risk for triglycerides, the null-effect remained. The observed higher triglyceride concentrations suggests that, although uric acid may not be causally involved in development of diabetes, there may be a separate causal role for uric acid in this metabolic disorder.

Third, the instrumental variable affects the outcome only through the risk factor of interest. This assumption is untestable, and should be considered using information on the underlying biology. None of the SNPs used in this study were in linkage disequilibrium with loci known to influence diabetes risk (22;39;40), which strengthens this assumption. Moreover, the vast majority of SNPs identified in the meta-analysis of Kolz et al. (9) were involved in regulating urate transport across cell membranes, which suggests that these SNPs directly influence uric acid levels. However, SLC2A9, the strongest uric acid associated locus, does not only
transport uric acid, but also glucose and fructose (41), and exchanges uric acid for glucose (42), leaving room for possible pleiotropy. Moreover, SLC2A9 has recently been shown to have differential effects on urinary and intestinal secretion of uric acid in mouse, suggesting a rise serum uric acid due to reduced urinary secretion could be counterbalanced by increased intestinal secretion and decreased portal vein levels (34). Similar contrasting roles have been reported for ABCG2 (37). A sensitivity analysis excluding the SNPs in these loci did not alter the result (Online supplementary table 6).

In conclusion, our study does not support the hypothesis that circulating uric acid has a causal effect on diabetes risk. Our findings therefore suggest that increased uric acid concentrations are a consequence of an adverse metabolic profile, rather than a cause of diabetes, and that uric acid has limited value as therapeutic target in preventing diabetes.
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None of the authors declares a conflict of interest.


33. Pradhan, AD, Manson, JE, Rifai, N, Buring, JE, Ridker, PM: C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. JAMA 286:327-334, 2001


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</tr>
<tr>
<td>BMI, kg/m²</td>
<td>26.0 (4.3)</td>
<td>30.0 (4.8)</td>
</tr>
<tr>
<td>Systolic blood pressure, mmHg</td>
<td>131 (20)</td>
<td>143 (20)</td>
</tr>
<tr>
<td>Diastolic blood pressure, mmHg</td>
<td>81 (11)</td>
<td>87 (11)</td>
</tr>
<tr>
<td>Prevalent hypertension</td>
<td>19</td>
<td>39</td>
</tr>
<tr>
<td>Uric acid, µmol/L</td>
<td>280 (77)</td>
<td>333 (83)</td>
</tr>
<tr>
<td>Triglycerides mmol/L, median (IQR)</td>
<td>1.1 (0.8, 1.6)</td>
<td>1.7 (1.2, 2.5)</td>
</tr>
<tr>
<td>eGFR, mL/min/1.73m²</td>
<td>100 (20)</td>
<td>95 (20)</td>
</tr>
<tr>
<td>Non-HDL cholesterol, mmol/L</td>
<td>4.4 (1.2)</td>
<td>5.0 (1.2)</td>
</tr>
<tr>
<td>Non-fasting glucose, mmol/L</td>
<td>5.0 (1.3)</td>
<td>6.4 (2.6)</td>
</tr>
<tr>
<td>HbA1c, % (mmol/mol)</td>
<td>5.5 [0.5] (36 [5])</td>
<td>6.2 [1.0] (44 [11])</td>
</tr>
</tbody>
</table>
* N = 10,235 subcohort participants and 7,319 incident type 2 diabetes cases; values are mean (SD) or %, unless otherwise indicated; BMI: body mass index; eGFR: estimated glomerular filtration rate; HDL: high-density lipoproteins.
Table 2. Observational and instrumental variable estimates for the association of circulating uric acid concentrations with incident type 2 diabetes*

<table>
<thead>
<tr>
<th>Analysis</th>
<th>Diabetes cases, N</th>
<th>HR (95% CI) per 59.48 µmol/L (1 mg/dL) increase in circulating uric acid</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Observational</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adjusted for center, age and sex</td>
<td>10,576</td>
<td>1.51 (1.42, 1.62)</td>
</tr>
<tr>
<td>Adjusted for center, age, sex, BMI</td>
<td>10,576</td>
<td>1.25 (1.18, 1.33)</td>
</tr>
<tr>
<td>Multivariable model†</td>
<td>10,576</td>
<td>1.20 (1.11, 1.30)</td>
</tr>
<tr>
<td><strong>Instrumental variable using InterAct</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adjusted for center</td>
<td>7,319</td>
<td>1.01 (0.87, 1.16)</td>
</tr>
<tr>
<td>Adjusted for center, age, sex, BMI</td>
<td>7,319</td>
<td>0.96 (0.76, 1.20)</td>
</tr>
<tr>
<td><strong>Instrumental variable using InterAct and DIAGRAM</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Combined analysis</td>
<td>41,508</td>
<td>0.99 (0.92, 1.06)</td>
</tr>
</tbody>
</table>

* For observational associations, N = 24,265 with 10,576 incident type 2 diabetes cases, estimates were pooled HR (95%CI) derived from random effects meta-analysis. For instrumental variable associations in InterAct, N = 17,118 with 7,319 incident type 2 diabetes cases, estimates were derived from two stage control function estimator approach analysis, and were pooled with random effects meta-analysis. For instrumental variable association using InterAct and DIAGRAM, N= 41,508 diabetes cases, and 123,974 controls. † Adjusted for study center, sex, age (as underlying time scale), BMI, systolic blood pressure, prevalent hypertension, nonHDL cholesterol (total – HDL cholesterol), triglycerides, eGFR, alcohol consumption, smoking status, highest educational level, and level of physical activity.
Table 3. Associations of individual uric acid related SNPs with circulating uric acid and incident type 2 diabetes

<table>
<thead>
<tr>
<th>Gene</th>
<th>Chr</th>
<th>SNP</th>
<th>Uric acid raising / other allele</th>
<th>Beta (95%CI) for uric acid concentrations *</th>
<th>P-value †</th>
<th>HR (95%CI) for incident diabetes ‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>GCKR</td>
<td>2</td>
<td>rs780094</td>
<td>T/C</td>
<td>0.05 (0.02, 0.09)</td>
<td>0.01</td>
<td>0.98 (0.93, 1.03)</td>
</tr>
<tr>
<td>SLC2A9</td>
<td>4</td>
<td>rs734553</td>
<td>T/G</td>
<td>0.36 (0.32, 0.40)</td>
<td>&lt; 0.001</td>
<td>1.02 (0.95, 1.09)</td>
</tr>
<tr>
<td>ABCG2</td>
<td>4</td>
<td>rs2231142</td>
<td>T/G</td>
<td>0.19 (0.13, 0.25)</td>
<td>&lt; 0.001</td>
<td>0.93 (0.86, 1.01)</td>
</tr>
<tr>
<td>LRRC16A</td>
<td>6</td>
<td>rs742132</td>
<td>A/G</td>
<td>0.04 (0.001, 0.08)</td>
<td>0.04</td>
<td>1.00 (0.95, 1.06)</td>
</tr>
<tr>
<td>RREB1</td>
<td>6</td>
<td>rs675209</td>
<td>T/C</td>
<td>0.08 (0.04, 0.12)</td>
<td>&lt; 0.001</td>
<td>1.03 (0.98, 1.08)</td>
</tr>
<tr>
<td>SLC16A9</td>
<td>10</td>
<td>rs12356193</td>
<td>A/G</td>
<td>0.06 (0.01, 0.11)</td>
<td>0.01</td>
<td>1.03 (0.97, 1.09)</td>
</tr>
<tr>
<td>SLC22A11</td>
<td>11</td>
<td>rs17300741</td>
<td>A/G</td>
<td>0.09 (0.05, 0.12)</td>
<td>&lt; 0.001</td>
<td>1.00 (0.96, 1.05)</td>
</tr>
<tr>
<td>PDZK1</td>
<td>1</td>
<td>rs12129861</td>
<td>G/A</td>
<td>0.03 (0.004, 0.08)</td>
<td>0.03</td>
<td>1.04 (0.97, 1.12)</td>
</tr>
<tr>
<td>SLC17A1</td>
<td>6</td>
<td>rs1183201</td>
<td>T/A</td>
<td>0.07 (0.04, 0.11)</td>
<td>&lt; 0.001</td>
<td>0.97 (0.93, 1.01)</td>
</tr>
<tr>
<td>SLC22A12</td>
<td>11</td>
<td>rs505802</td>
<td>C/T</td>
<td>0.05 (0.01, 0.09)</td>
<td>0.01</td>
<td>1.00 (0.91, 1.09)</td>
</tr>
<tr>
<td>INHBC</td>
<td>12</td>
<td>rs1106766</td>
<td>C/T</td>
<td>0.06 (0.02, 0.11)</td>
<td>0.01</td>
<td>1.07 (1.01, 1.13)</td>
</tr>
<tr>
<td>ORC4L</td>
<td>2</td>
<td>rs2307394</td>
<td>C/T</td>
<td>0.03 (-0.01, 0.06)</td>
<td>0.15</td>
<td>0.99 (0.93, 1.05)</td>
</tr>
<tr>
<td>SFMBT1</td>
<td>3</td>
<td>rs6770152</td>
<td>G/T</td>
<td>0.05 (0.01, 0.09)</td>
<td>0.01</td>
<td>1.06 (1.00, 1.13)</td>
</tr>
<tr>
<td>VEGFA</td>
<td>6</td>
<td>rs729761</td>
<td>G/T</td>
<td>0.07 (0.03, 0.11)</td>
<td>&lt; 0.01</td>
<td>0.92 (0.87, 0.97)</td>
</tr>
<tr>
<td>BAZ1B</td>
<td>7</td>
<td>rs1178977</td>
<td>A/G</td>
<td>0.05 (0.01, 0.10)</td>
<td>0.02</td>
<td>1.00 (0.92, 1.09)</td>
</tr>
<tr>
<td>Gene</td>
<td>Position</td>
<td>SNP ID</td>
<td>Allele</td>
<td>Beta (95% CI)</td>
<td>P-value</td>
<td>Hazard Ratio (95% CI)</td>
</tr>
<tr>
<td>--------</td>
<td>----------</td>
<td>------------</td>
<td>---------</td>
<td>----------------</td>
<td>---------</td>
<td>----------------------</td>
</tr>
<tr>
<td>PRKAG2</td>
<td>7</td>
<td>rs10480300</td>
<td>T/C</td>
<td>0.06 (0.03, 0.10)</td>
<td>0.001</td>
<td>1.00 (0.95, 1.05)</td>
</tr>
<tr>
<td>STC1</td>
<td>8</td>
<td>rs17786744</td>
<td>G/A</td>
<td>0.04 (0.01, 0.08)</td>
<td>0.02</td>
<td>0.97 (0.93, 1.02)</td>
</tr>
<tr>
<td>OVOL1</td>
<td>11</td>
<td>rs642803</td>
<td>C/T</td>
<td>0.03 (-0.01, 0.06)</td>
<td>0.16</td>
<td>1.00 (0.95, 1.06)</td>
</tr>
<tr>
<td>ATXN2</td>
<td>12</td>
<td>rs653178</td>
<td>C/T</td>
<td>0.03 (-0.01, 0.06)</td>
<td>0.16</td>
<td>1.00 (0.95, 1.06)</td>
</tr>
<tr>
<td>UBE2Q2</td>
<td>15</td>
<td>rs1394125</td>
<td>A/G</td>
<td>0.003 (-0.03, 0.04)</td>
<td>0.86</td>
<td>0.99 (0.94, 1.03)</td>
</tr>
<tr>
<td>IGF1R</td>
<td>15</td>
<td>rs6598541</td>
<td>A/G</td>
<td>0.07 (0.03, 0.10)</td>
<td>0.001</td>
<td>1.03 (0.98, 1.08)</td>
</tr>
<tr>
<td>NFAT5</td>
<td>16</td>
<td>rs7193778</td>
<td>C/T</td>
<td>0.06 (0.01, 0.12)</td>
<td>0.02</td>
<td>1.02 (0.95, 1.09)</td>
</tr>
<tr>
<td>MAF</td>
<td>16</td>
<td>rs7188445</td>
<td>G/A</td>
<td>0.03 (-0.01, 0.07)</td>
<td>0.16</td>
<td>0.98 (0.92, 1.04)</td>
</tr>
<tr>
<td>BCAS3</td>
<td>17</td>
<td>rs2079742</td>
<td>T/C</td>
<td>0.02 (-0.02, 0.07)</td>
<td>0.30</td>
<td>1.01 (0.96, 1.08)</td>
</tr>
</tbody>
</table>

* Beta obtained from linear regression with uric acid modeled per 59.48 μmol/L (1 mg/dL) increase, and SNPs modeled per uric acid increasing allele (additive model), adjusted for study center, among 10,235 subcohort participants; † P-value for association uric acid related SNPs with uric acid concentrations; ‡ HR and 95%CI obtained from random effects meta-analysis using modified Cox regression, adjusted for study center, among 17,118 participants of which 7,319 were incident diabetes cases.