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Causal Assessment of Serum Urate Levels in Cardiometabolic Diseases Through a Mendelian Randomization Study

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ABSTRACT

BACKGROUND Although epidemiological studies have reported positive associations between circulating urate levels and cardiometabolic diseases, causality remains uncertain.

OBJECTIVES Through a Mendelian randomization approach, we assessed whether serum urate levels are causally relevant in type 2 diabetes mellitus (T2DM), coronary heart disease (CHD), ischemic stroke, and heart failure (HF).

METHODS This study investigated 28 single nucleotide polymorphisms known to regulate serum urate levels in association with various vascular and nonvascular risk factors to assess pleiotropy. To limit genetic confounding, 14 single nucleotide polymorphisms exclusively associated with serum urate levels were used in a genetic risk score to assess associations with the following cardiometabolic diseases (cases/controls): T2DM (26,488/83,964), CHD (54,501/68,275), ischemic stroke (14,779/67,312), and HF (4,526/18,400). As a positive control, this study also investigated our genetic instrument in 3,151 gout cases and 68,350 controls.

RESULTS Serum urate levels, increased by 1 SD due to the genetic score, were not associated with T2DM, CHD, ischemic stroke, or HF. These results were in contrast with previous prospective studies that did observe increased risks of these 4 cardiometabolic diseases for an equivalent increase in circulating urate levels. However, a 1 SD increase in serum urate levels due to the genetic score was associated with increased risk of gout (odds ratio: 5.84; 95% confidence interval: 4.56 to 7.49), which was directionally consistent with previous observations.

CONCLUSIONS Evidence from this study does not support a causal role of circulating serum urate levels in T2DM, CHD, ischemic stroke, or HF. Decreasing serum urate levels may not translate into risk reductions for cardiometabolic conditions. (J Am Coll Cardiol 2016;67:407–16) © 2016 by the American College of Cardiology Foundation.

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ABBREVIATIONS AND ACRONYMS

CHD = coronary heart disease

GRS = genetic risk score

HF = heart failure

MR = Mendelian randomization

SNP = single nucleotide polymorphism

T2DM = type 2 diabetes mellitus

Uric acid is the end product of purine metabolism and circulates in the blood as the anion urate. Blood levels of uric acid are causally associated with gout, as implicated by evidence from randomized clinical trials using urate-lowering therapies (1). In 1923, Kylin initially described a constellation of metabolic disturbances that included hypertension, hyperglycemia, and elevated uric acid levels. Since then, circulating levels of serum uric acid have been reported to be positively correlated with several vascular risk factors including blood pressure, lipids, kidney function, and other metabolic traits (2). A number of prospective epidemiological studies have associated increased serum uric acid levels and elevated risk for type 2 diabetes mellitus (T2DM) (3), coronary heart disease (CHD) (4–7), ischemic stroke (8,9), and heart failure (HF) (10,11).

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No large-scale intervention studies, however, have evaluated urate-lowering therapies for metabolic and

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Dr. Danesh is a consultant with Takeda; a member of the Novartis Cardiovascular & Metabolic Advisory Board; a member of the International Cardiovascular and Metabolism Research and Development portfolio committee at Novartis; a member of the Merck Sharp & Dohme UK Atherosclerosis Advisory Board; a member of Sanofi Advisory Board; and has received funding from the British Heart Foundation, BUPA Foundation, diaDexus, European Research Council, European Union, Evelyn Trust, Fogarty International Centre, GlaxoSmithKline, Merck, National Heart, Lung, and Blood Institute, National Health Service Blood and Transplant, National Institute for Health Research, National Institute of Neurological Disorders and Stroke, Novartis, Pfizer, Roche, Sanofi, Takeda, The Wellcome Trust, UK Biobank, University of British Columbia, and the UK Medical Research Council. Studies participating in the METASTROKE consortium: *The MGH Genes Affecting Stroke Risk and Outcome Study (MGH-GASROS).* GASROS was supported by the National Institute of

vascular outcomes. In the absence of such evidence, it remains unknown whether circulating uric acid is an independent causal factor for cardiometabolic conditions and whether lowering urate levels might offer therapeutic utility in these disorders.

Human genetic data can be used to directly test the hypothesis of causality between uric acid and clinical endpoints. In particular, Mendelian randomization (MR) studies assess causal inference by using genetic alleles as unbiased proxies for circulating biomarkers (12). MR studies are based on the random assortment of genetic alleles during meiosis that can confer advantages similar to a randomized controlled trial by investigating the relationship between genetic alleles that are exclusively associated with a biomarker of interest and disease risk (13). Previously, such an approach has been used to assess the causality of low- and high-density lipoprotein cholesterol (14), triglycerides (15), lipoprotein(a) (16,17), fibrinogen (18), and C-reactive protein in CHD (19).

This study's objective is to test the hypothesis that serum urate levels are causally associated with cardiometabolic conditions by applying an MR study design. We integrated information on genetic variants related to serum urate, 50 potential confounders, and risk of disease outcomes. In contrast to previously published genetic reports on serum urate–related genetic variants and disease risk (20–23), the current study investigates >10 times more CHD cases and examines, for the first time, risks of stroke and HF conferred by genetically raised serum urate levels. It also systematically evaluates pleiotropy, enabling reliable assessment of any possible moderate causal effect of serum urate levels on any of the 4 major cardiometabolic outcomes.

METHODS

STUDY DESIGN. Our study had 3 interrelated components. First, we selected single nucleotide polymorphisms (SNPs) previously discovered in genome-wide association studies of serum urate levels. Second, we conducted genetic analyses in relation to a panel of 50 vascular and nonvascular risk factors and identified SNPs that did not exhibit pleiotropy (i.e., SNPs exclusively associated with circulating urate levels, but not with other cardiometabolic traits that might confound our interpretation). For these analyses, we queried publicly available resources and genome-wide association data available from 18,828 subjects of PROMIS (Pakistan Risk of Myocardial Infarction Study), a case-control study in urban Pakistan (24). Third, we used a genetic risk score (GRS) comprised of SNPs exclusively associated with serum urate levels to evaluate the potential causal role of circulating urate levels in T2DM, CHD, ischemic stroke, and HF through an MR approach.

URATE GENETIC VARIANTS AND ASSESSMENT OF PLEIOTROPY. All of the 28 urate SNPs included in the current analyses were in linkage equilibrium ($r^2 = 0$, based on participants of European, South Asian, and East Asian ancestries in the International HapMap Project phase II and phase III) (25). Each SNP was evaluated for associations with 50 vascular and nonvascular traits in up to 18,828 PROMIS participants (24). Information from publicly available genome-wide association studies databases was also used to assess associations of these SNPs with blood pressure traits in up to 134,433 participants (Global BPgen Consortium) (26); with major lipids in up to 100,000 participants (Global Lipids Genetics

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Consortium) (27), with anthropometric traits in up to 183,727 participants (Genetic Investigation of ANthropometric Traits) (28–30); and with glycemic traits in up to 46,368 nondiabetic participants (Meta-Analyses of Glucose and Insulin-related traits Consortium) (31–34). Pleiotropy was declared at a nominal p value of <0.01. Only nonpleiotropic SNPs were used to construct a urate-specific GRS. We used additive linear regression models to interrogate the urate GRS in association with a range of traits in PROMIS.

ASSOCIATION WITH DISEASE OUTCOMES. For each of the 28 SNPs, summary effect estimates in association with T2DM, CHD, ischemic stroke, and HF were obtained from various consortia, including DIAGRAM (DIAbetes Genetics Replication And Meta-analysis consortium) (35), CARDIoGRAM (Coronary ARtery DIsease Genome-wide Replication and Meta-analysis consortium) (36), C4D (Coronary Artery Disease Genetics consortium) (36), METASTROKE (METASTROKE Collaboration) (37), and CHARGE-Heart Failure studies (Cohorts for Heart and Aging Research in Genomic Epidemiology consortium) (38). DIAGRAM data were downloaded from their website; other data were acquired by contacting investigators within each consortium. We maximized study power by obtaining further data on participants who did not contribute to any of these consortia previously, thus increasing sample size for CHD, ischemic stroke, and HF by up to 25% (Online Table 1). Effect sizes and errors from consortia data and study-specific effect sizes and errors from additional studies (Online Table 1) were combined via meta-analysis (inverse-variance fixed-effect model). In the final analyses, data were available on 26,488 T2DM cases and 83,964 controls; 54,501 CHD cases and 68,275 controls; 14,779 ischemic stroke cases and 67,312 controls; and 4,553 HF cases and 19,985 controls. Effect estimates in association with prevalent gout were obtained from GUGC (Global Urate Genetics Consortium) involving 3,151 gout cases and 68,350 controls (39). All participants were of self-reported European or South Asian ancestry. Individual studies within each consortium obtained written informed consent from participants and received approval from the relevant ethics boards.

STATISTICAL ANALYSES. All 28 SNPs used in the current analyses have been previously shown to be associated with serum urate levels at a p value of $<5 \times 10^{-8}$ (39). The association of each SNP with each cardiometabolic outcome was evaluated with a fixed-effects, inverse-variance, weighted meta-analysis using beta(s) and SE(s) obtained from consortia and studies listed in Online Table 1. SNPs found exclusively associated with serum urate levels were used in a genetic score as an instrument for MR analyses (39,40). The impact of the urate genetic score on disease risk was calculated using methods described previously (41,42). Briefly, under the assumptions that SNPs are unlinked and the effects of each SNP are log additive on uric acid levels, using an MR framework (12,13), a causal effect (alpha) between a biomarker and outcome can be estimated by

$$\alpha = \left(\sum_j \beta w s^{-2}\right) \Big/ \left(\sum_j w^2 s^{-2}\right)$$

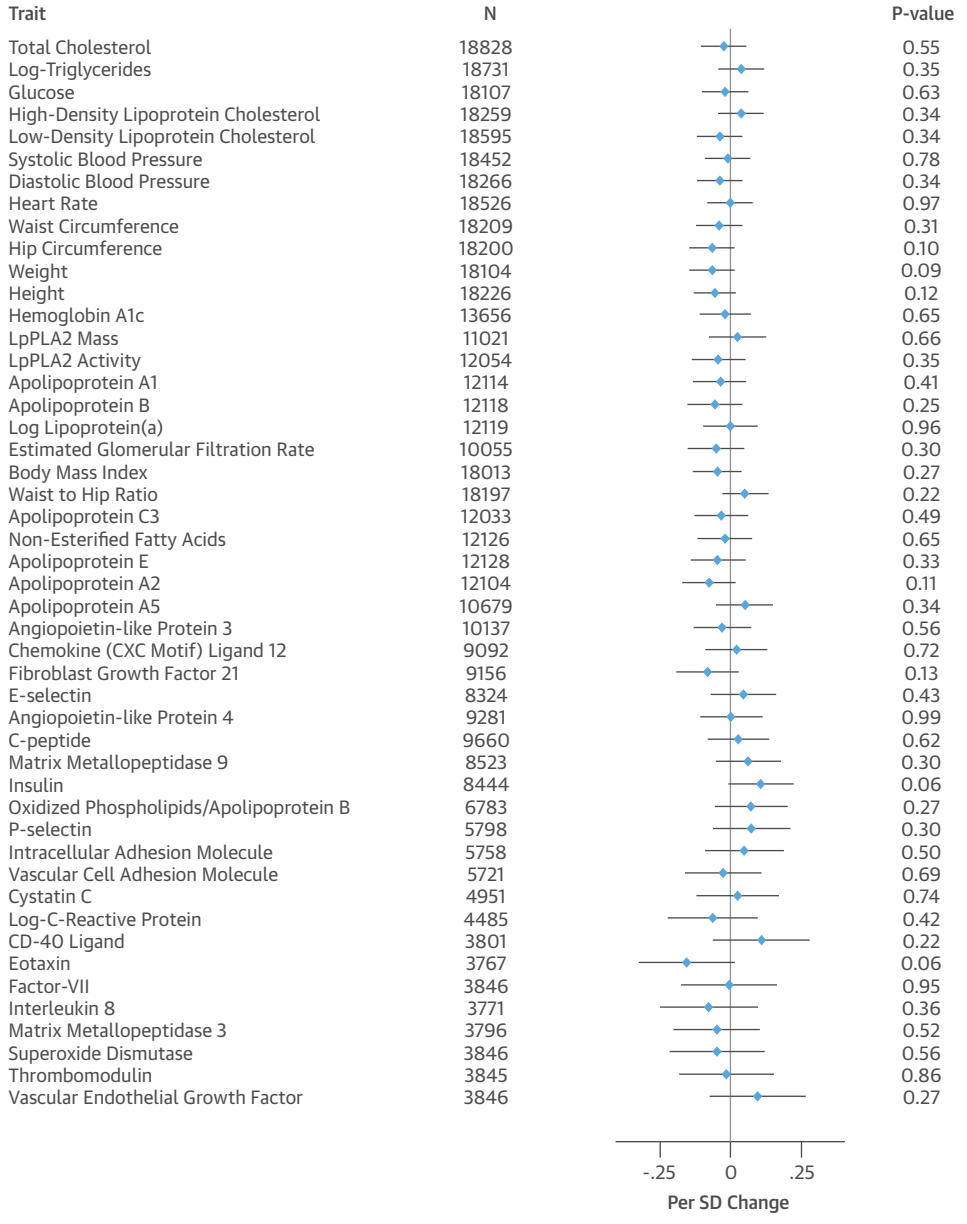
where for all j SNPs, β represents the estimated natural log odds effect of the *j*-th SNP on the endpoint of interest, s represents the standard error on the log odds effect of the *j*-th SNP on the endpoint, and w represents a weight for the SNP on the outcome. Each SNP was weighted using the reported estimated effect of the SNP on uric acid levels (in SD units). SE for alpha-hat was calculated by taking the square root of the reciprocal of the denominator, as previously described (42). A simulation approach was used to estimate the power to identify or exclude causal effects of the urate genetic score on each tested outcome (Online Appendix) (43). All analyses were conducted in STATA (StataCorp LP, College Station, Texas), R (The R Foundation), SNPTEST (University of Oxford), or PLINK (Harvard University).

RESULTS

URATE VARIANTS. Of the 28 SNPs related to serum urate levels, 14 variants had pleiotropic associations at a p value <0.01 with at least 1 vascular or nonvascular trait (Online Tables 2 and 3). The remaining 14 nonpleiotropic SNPs were used in a genetic score weighted for the reported urate effect estimate of each SNP. The weighted GRS was not associated with any vascular or nonvascular trait at a p value <0.01 (**Figure 1**).

Of the 14 urate-specific SNPs, 9 variants were associated with increased risks of gout but none of the variants were associated with T2DM, CHD, ischemic stroke, or HF at a p value <0.01 (**Figures 2 and 3**). Most notably, the SNP at the *SLC2A9* locus, which was associated with the largest increases in serum urate level (0.37 mg/dl) and risk of gout (odds ratio [OR]: 1.56; 95% confidence interval [CI]: 1.45 to 1.68; $p = 1.9 \times 10^{-31}$), was not associated with any of the cardiometabolic outcomes. Of the 14 pleiotropic SNPs, we found 1 SNP at the *ATXN2* locus to be significantly associated with increased risk of CHD (OR: 1.06; 95% CI: 1.03 to 1.08; $p = 6.5 \times 10^{-6}$) and ischemic stroke (OR: 1.08; 95% CI: 1.04 to 1.11; $p = 4.4 \times 10^{-6}$) (Online Table 4). The variant at the *VEGFA* locus was significantly associated with decreased risk of T2DM

FIGURE 1 **Association of Urate Genetic Score With Potential Confounders**

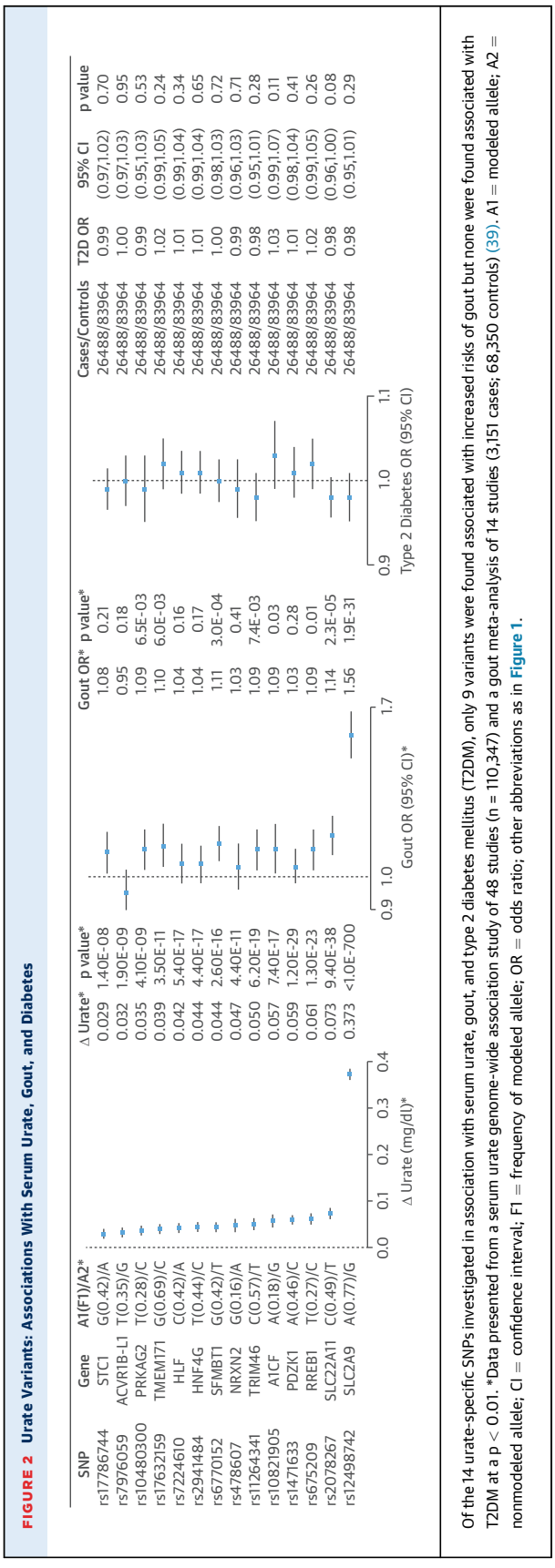


Of the 28 single nucleotide polymorphisms (SNPs) related to serum urate levels, 14 variants had pleiotropic associations at a $p < 0.01$ with at least 1 vascular or nonvascular trait. The remaining 14 nonpleiotropic SNPs were used to calculate a genetic score by using individual participant data in the PROMIS (Pakistan Risk of Myocardial Infarction Study) participants. The genetic score was weighted for the reported urate effect estimate of each SNP. The genetic score was subsequently used in analyses with 50 traits in the PROMIS participants and was not found associated with any trait at a $p < 0.01$. LpPLA2 = lipoprotein-associated phospholipase A2.

(OR: 0.93; 95% CI: 0.89 to 0.96; $p = 1.0 \times 10^{-4}$) but increased serum urate levels (Online Table 5).

URATE GENETIC SCORE AND DISEASE OUTCOMES. For a 1 SD increase in serum uric acid levels, the OR of gout conferred by genetic score was 5.84 (95% CI: 4.56 to 7.49; $p = 4.2 \times 10^{-44}$), which was directionally consistent with the observed OR of 2.12 (95% CI: 1.90 to 2.33) for gout in epidemiological studies (44). However, a 1 SD increase in serum urate due to the genetic score had no relationship with T2DM

FIGURE 2 Urate Variants: Associations With Serum Urate, Gout, and Diabetes



Of the 14 urate-specific SNPs investigated in association with serum urate, gout, and type 2 diabetes mellitus (T2DM), only 9 variants were found associated with increased risks of gout but none were found associated with T2DM at a $p < 0.01$. *Data presented from a serum urate genome-wide association study of 48 studies (n = 110,347) and a gout meta-analysis of 14 studies (3,151 cases; 68,350 controls) (39). A1 = modeled allele; A2 = nonmodeled allele; CI = confidence interval; F1 = frequency of modeled allele; OR = odds ratio; other abbreviations as in Figure 1.

(OR: 0.95; 95% CI: 0.86 to 1.05; p = 0.28), CHD (OR: 1.02; 95% CI: 0.92 to 1.12; p = 0.73), ischemic stroke (OR: 0.99; 95% CI: 0.88 to 1.12; p = 0.93), or HF (OR: 1.07; 95% CI: 0.88 to 1.30; p = 0.51) (**Central Illustration**). In further subsidiary analysis, a GRS comprised of all 28 urate-related SNPs was not associated with the 4 cardiometabolic outcomes (Online Table 6). A score based on the 14 urate-related variants with pleiotropic effects was also not associated with stroke or HF (Online Table 7). However, this score was nominally associated with T2DM, though in a direction opposite of epidemiological expectation, and weakly associated with CHD. We posit that these weak associations are explained by strong, confounding associations of these SNPs with blood pressure, cholesterol, triglycerides, obesity, glucose, insulin, and insulin resistance (Online Table 3). These null associations are in contrast to data from observational epidemiological studies which have previously shown that equivalent increases in serum urate levels are associated with increased risks of T2DM (OR: 1.25; 95% CI: 1.13 to 1.37) (3), CHD (OR: 1.06; 95% CI: 1.03 to 1.09) (4), ischemic stroke (OR: 1.17; 95% CI: 1.00 to 1.37) (8), and HF (OR: 1.19; 95% CI: 1.17 to 1.21) (10).

For a 1 SD change in serum urate levels due to genetic score, our study was statistically powered at >80% with a 5% alpha rate to assess ORs of 1.15 for T2DM, 1.17 for ischemic stroke, 1.10 for CHD, and 1.24 for HF.

We conducted sensitivity analyses and investigated, in the same study population, the associations of the previously published urate-related SNPs with serum urate levels and CHD risk. In the 7 studies analyzed, we found highly significant associations for uric acid levels by the 3 risk scores that we used in the main analyses earlier (Online Figure 1A) whereas no association was observed between any of the risk scores investigated and CHD risk in the same studies (Online Figure 1B). We further restricted our analyses to 3 studies in which we investigated the association of: 1) serum urate levels with CHD risk; 2) SNPs with serum urate levels; and 3) SNPs with CHD risk. Although we found highly significant associations between circulating serum urate levels and CHD risk (Online Figure 2A) and highly significant associations between SNPs and serum urate levels (Online Figure 2B), no association was observed for any of 3 urate-related GRSs with CHD risk (Online Figure 2C). These sensitivity analyses provide further validation to the "2-stage" MR experiment used earlier. Further, in analyses stratified by ethnicity, similar null results were obtained for participants of European or South Asian origin (Online Tables 8 to 10).

DISCUSSION

Contrary to epidemiological studies in humans in which higher serum urate levels correlate with increased risk of cardiometabolic outcomes, the MR analyses reported here provided no evidence of causal associations between circulating urate levels and risks of T2DM, CHD, ischemic stroke, or HF (**Central Illustration**). First, we analyzed all SNPs associated with circulating urate levels across a range of vascular and nonvascular traits to assess pleiotropy, and identified 14 exclusively associated with serum urate levels. Second, a genetic score combining these nonpleiotropic variants exclusively increased uric acid levels and risk of gout. Third, none of the urate-specific SNPs individually or combined as a genetic score associated with any cardiometabolic outcome. Fourth, a genetic risk score comprised of all 28 SNPs known to regulate serum urate levels was not associated with any cardiometabolic outcome.

The current study raised doubts about the etiological relevance of serum uric acid in cardiovascular and metabolic diseases as suggested by prior epidemiological and model systems studies (3–11,45), which may have observed increased uric acid levels to associate with higher risk of cardiometabolic diseases due to residual confounding or reverse causality. Moreover, no large-scale randomized control trials have been conducted using targeted interventions to lower serum urate levels (e.g., xanthine-oxidase inhibition inhibition) for the primary prevention of cardiometabolic endpoints, although an ongoing trial is evaluating the role of xanthine-oxidase inhibitors in patients with HF (46). Prior studies have suggested a role for urate-lowering therapies in reducing blood pressure in adolescents with hyperuricemia, ameliorating exercise capacity in patients with chronic stable angina, improving endothelial function in patients with HF, and making other biochemical parameters more favorable in patients with stable disease (47–49). Such evidence, however, was generated through studies conducted in populations with prevalent and stable disease and did not assess the association of urate reduction with primary cardiometabolic events (i.e., stroke, CHD, diabetes, or HF). Moreover, these prior studies do not address the etiological relevance of urate reduction in the prevention of primary cardiometabolic events in healthy participants. In contrast, findings from this report suggested that uric acid lowering may not succeed in primary prevention of metabolic and vascular events, consistent with a recent study that showed initiation of xanthine oxide inhibitors in patients with gout was not associated with a change in cardiovascular disease risk (50).

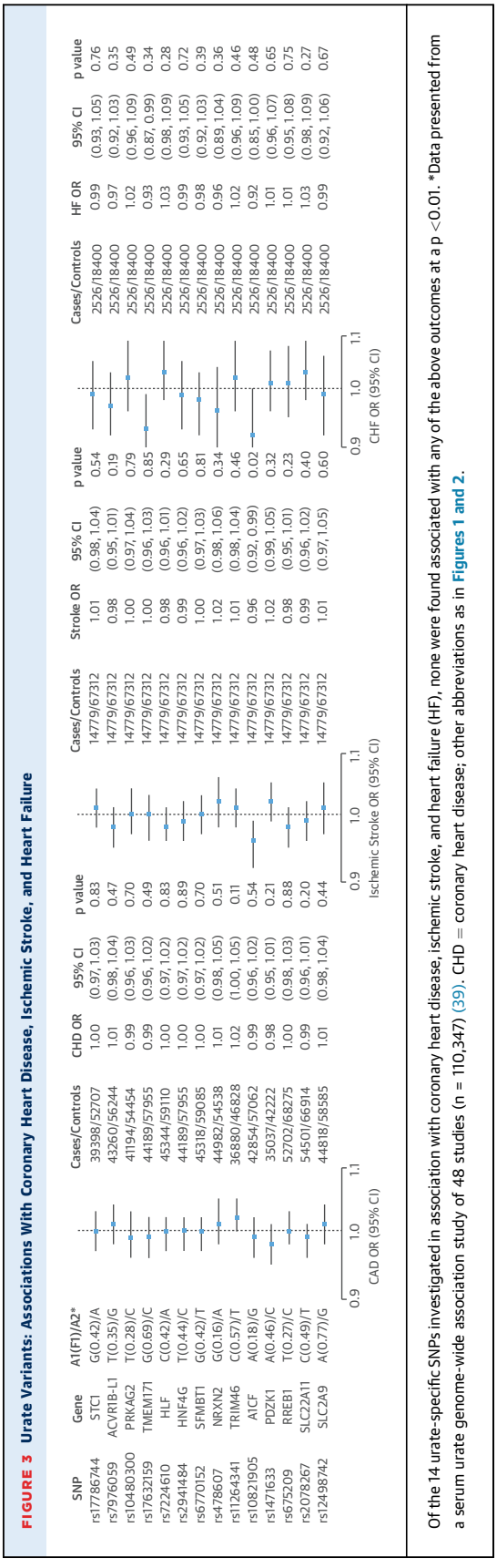
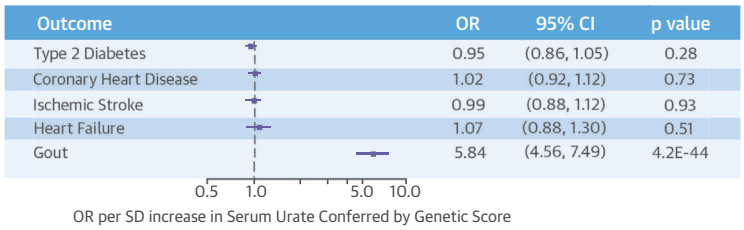


FIGURE 3 Urate Variants: Associations With Coronary Heart Disease, Ischemic Stroke, and Heart Failure

Of the 14 urate-specific SNPs investigated in association with coronary heart disease, ischemic stroke, and heart failure (HF), none were found associated with any of the above outcomes at a $p < 0.01$. *Data presented from a serum urate genome-wide association study of 48 studies (n = 110,347) (39). CHD = coronary heart disease; other abbreviations as in **Figures 1 and 2**.

CENTRAL ILLUSTRATION **Urate Genetic Score: Association of Genetically Raised Urate With Cardiometabolic Outcomes**



Keenan, T. et al. J Am Coll Cardiol. 2016; 67(4):407–16.

A genetic score was created using single nucleotide polymorphisms exclusively associated with serum urate levels. For a 1 SD increase in serum uric acid levels, the odds ratio (OR) of gout conferred by the urate-specific genetic score was 5.84 (95% confidence interval [CI]: 4.56 to 7.49), which was directionally consistent with the observed OR of 2.12 (95% CI: 1.90 to 2.33) for gout in epidemiological studies. However, a 1 SD increase in serum urate due to the genetic score had no relationship with type 2 diabetes, coronary heart disease, ischemic stroke, or heart failure. SD = 1.427 mg/dl urate.

Our findings were consistent with a prior report that evaluated variation at the *SLC2A9* gene in association with ischemic heart disease that found no evidence of an association between genetically lowered uric acid and CHD or blood pressure (21). The current study extended these prior findings by evaluating all variants associated with uric acid systematically, exploring pleiotropy for all uric-acid related variants, investigating other cardiometabolic outcomes (i.e., T2DM, stroke, and HF), and assessing >7-fold more CHD cases (54,501 in the current report vs. 7,172 in the prior report). Thus, it provided an analysis adequately powered to assess urate variants and genetic scores known to have modest effects on urate levels.

We observed that one serum urate SNP in the *ATXN2* gene, which was pleiotropic for major lipid, glycemic, and anthropometric traits (thus excluded from our score-based MR analysis), appeared to be associated with risks of CHD and ischemic stroke at nominal levels of significance. This SNP is located in a high-frequency (~40%) long-range (1.6 Mb) haplotype, previously described to be associated with a range of other traits including type 1 diabetes, celiac disease, and elevated platelet counts. This haplotype is speculated to have arisen from a selective sweep specific to Europeans ~3,400 years ago when high-density human settlements were expanding in that region of the world (33). In analyses restricted to participants of South Asian ancestry, we did not find this variant to be associated with major lipids in 37,000 participants or with risks of CHD (9,000 cases and 9,000 controls) or ischemic stroke (3,500 cases and 5,000 controls). Because of the high pleiotropic nature of this locus and specificity to populations of European ancestry, it is unlikely that the *ATXN2* locus leads to CHD by increasing serum urate levels.

STUDY LIMITATIONS. Potential limitations of this study should be considered. First, while analyses on HF in the current study were underpowered (Online Table 9), the concordance of the null findings observed for all cardiometabolic outcomes tend to suggest a lack of a major etiological role of serum urate levels in HF. Second, we evaluated only 50 traits to assess pleiotropy for uric acid SNPs and did not conduct measurements for all possible biological traits; however, we conducted analyses using both single SNPs and a GRS in association with cardiometabolic outcomes. Importantly, we also conducted analyses for a variant, rs12498742, that imparts the strongest effect on uric acid levels (Online Table 10) and is located in an intron of the *SLC2A9* gene that encodes for a glucose and urate transporter in the kidney, hence providing biological plausibility to our hypothesis. We did not find this variant to be associated with any other trait apart from circulating urate levels; hence enabling MR analyses using this variant only. We did not find rs12498742 to be associated with any cardiometabolic outcome despite the fact that MR analyses with this variant were sufficiently powered (Online Table 9).

Third, nonpleiotropic variants in addition to the SLC2A9 variant explained only 15.3% of the variance in serum urate levels (Online Table 10). However, none of them were associated with any of the investigated cardiometabolic endpoints in our large-scale analyses, casting further doubt on serum urate as a causal factor. Fourth, as suggested by our power calculations (Online Table 9), although we were able

to exclude effects imparted by a 1 SD change in serum urate levels on disease risk, which are weak to modest and consistent with prior epidemiological studies (3-11) (Online Table 9), our analyses may not have detected very weak disease risk estimates (e.g., OR for CHD < 1.10).

Fifth, while our assessment of causality was limited to SNPs that are observed to be non-pleiotropic, it can be argued that the loci that do exhibit pleiotropy can mediate the disease. We ruled out the latter possibility by demonstrating that risk scores comprised of all 28 SNPs or 14 pleiotropic SNPs were not associated with any cardiometabolic outcomes. Finally, although we had access to only summary-level data, preventing adjustment for factors acting as potential mediators between genotypes and disease risk, MR analyses on summary-level data have been shown to achieve results similar to the methods that have used individual participant data (14-19). Moreover, analyses with gout provided a positive control and reinforced the findings observed for other outcomes.

CONCLUSIONS

Our MR analyses did not support a causal role of circulating serum urate concentrations in cardiometabolic conditions. Our results suggested that lowering serum urate levels may not translate into risk reductions for T2DM, CHD, ischemic stroke, or HF events.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: Although elevated serum uric acid levels have been associated with an increased risk of cardiometabolic diseases, a causal link has not been established. The results of a large Mendelian randomization study suggest that lowering serum urate levels may not translate into reductions in the risks of type 2 diabetes, coronary heart disease, ischemic stroke, or heart failure.

TRANSLATIONAL OUTLOOK: Genetic studies that take advantage of the random assortment of alleles during meiosis can save time and resources, minimize bias, and inform clinical practice when data from prospective clinical trials are not available to provide evidence for causality.

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APPENDIX For supplemental text, tables, and figures, please see the online version of this article.