Epidemiological Studies of Uric Acid: A Mini-review with a Focus on Mendelian Randomization

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Abstract

This article aims to summarize the recent epidemiological studies that have been conducted on the potential effects of urate (uric acid) on diseases, specifically focusing on studies that used a Mendelian randomization approach. As is generally the case with cardiometabolic diseases, urate epidemiology has resorted to Mendelian randomization to disentangle causal relationships. The Mendelian randomization approach utilizes genetic variants as an instrumental variable to address whether a given biomarker has a causal effect on the disease, or whether it is simply a non-causal marker (perhaps a consequence of the disease); thus, this approach allows addressing causality in the presence of potential confounding factors. Most Mendelian randomization studies on urate have suggested modest or negligible degrees of causal effect of urate on many diseases and that reverse causality may explain the associations of urate with cardiometabolic biomarkers, such as adiposity, which are repeatedly observed in conventional epidemiology. Conflicting results have been reported partly due to the use of different sets of genetic variants, which emphasizes the importance of physiological and epidemiological characterizations of individual genetic variants and codified proteins. With improved sets of genetic variants and methods to infer causal effects in the presence of invalid genetic variants (for example, those with pleiotropy), further Mendelian randomization analyses may uncover subtle causal effects of urate on cardiovascular and kidney outcomes.

Urate as a predictive biomarker

Uric acid (urate) is an organic anion and is the final product of purine catabolism in humans and higher primates. Urate is a powerful antioxidant that has been considered a factor for the longevity of humans and great apes, who lack urate oxidase activity [1]. The medical relevance of the serum urate concentration has long been studied with both experimental models and epidemiological approaches. A number of studies have shown that higher serum urate levels correlate with increased risk of gout, cardiometabolic outcomes [2], and chronic kidney disease [3]. However, the extent of the causal effects of serum urate on health outcomes other than gout is not yet clear, which makes it difficult to evaluate the clinical importance of controlling urate levels [4,5]. In epidemiological studies on urate, confounding factors have increasingly been considered more problematic, as multivariate regression models adjusted for conventional confounding factors tend to show attenuation of the association between urate and vascular outcomes compared to unadjusted models [6]. To name one example, in the prospective study (named the Reykiavik study), Wheeler et al. reported that the odds ratio (OR) for coronary heart disease was 1.39 (95% confidence interval (CI), 1.20 to 1.61) in males with the top third of baseline serum uric acid levels compared to those in the bottom third; however, this value declined to 1.12 (CI, 0.94 to 1.33) after adjusting for smoking and other established risk factors [6]. Such findings lead researchers to wonder whether the apparent effects of urate on diseases are false and due to confounding with other risk factors. Reverse causation has also been found to confound the epidemiological analyses of urate, specifically regarding kidney disease.

On the other hand, experimental studies have shown that the metabolism of urate is tightly linked to the metabolism of sugar and lipids. An increase in urate levels within hepatocytes upregulates fructokinase activity, which mediates fructose-induced hepatic steatosis [7]. Further, intracellular urate inhibits adenosine monophosphate kinase activity and activates adenosine monophosphate dehydrogenase activity, causing enhanced gluconeogenesis [8]. Moreover, despite the anti-oxidant activity of extracellular urate, urate is known to act as a pro-oxidant inside the cell, whereby it stimulates NADPH oxidase and increases oxidative stress. In this article, we do not elaborate on this topic in detail, as it has already been reviewed and covered by many articles, including that of El Din et al. [8].

Despite such findings in support of the causal effect of urate on diseases, many recent analyses that use the Mendelian randomization technique show no or only modest evidence in support of causality. In this article, after a brief introduction on Mendelian randomization, we summarize the results of studies using Mendelian randomization. In later sections, we discuss the implications of these results in terms of each disease category, such as hypertension, cardiovascular disease (CVD), and kidney disease. As we do not cover experimental studies in this article, we suggest readers refer to excellent recent review articles that were recently published on experimental studies, such as Cortese et al. [2] and El Din et al. [8]. We also refer readers to Gul and Zager, which is an excellent review article on the pathophysiological role of urate metabolism in kidney disease [3].

Mendelian randomization

Mendelian randomization has drawn attention in epidemiology, as this approach allows probing causality [9,10]. This approach is based on the way that genetic variants (alleles) are randomly assigned during meiosis and can predispose or expose their subjects to differential levels of risk factors, which enables an analysis that is somewhat alike to a randomly controlled clinical trial. If genetic variants are strongly associated with the risk factor of interest (urate, in this case), such variants may allow us to examine a possible causal relationship between the risk factor and outcome (for example, cardiovascular risk). The variants of single nucleotide polymorphism (SNP) loci that are utilized in this way are known as instrumental variables.

The validity of this approach rests on three assumptions and thus requirements [9]: i) the genetic variant strongly associates with the risk factor of interest (urate, in our case); ii) the genetic instrument associates exclusively with the risk factor of interest; and iii) the effect of the instrument on disease outcomes is mediated exclusively by the risk factor of interest. Thus, a prerequisite to perform MR is that there is at least one SNP that is exclusively associated with the outcome (such as cardiovascular events) only via their effect on the exposure and not via alternate pathways.

A recent trend is the use of a genetic risk score instead of single SNP. This procedure mitigates potential pleiotropic effects attributable to single genetic variants. Another feature of recent MR research is that when multiple SNPs can be used as instrumental variables, they are incorporated into a two-stage least squares analysis; the first stage associates the SNP genotype (or genetic risk score) to exposure and the second stage associates the exposure to outcome. One of the standard ways to derive a genetic risk score is to use the inverse-variance weighted (IVW) method [10]. A positive aspect of MR research is the recent advent of genome-wide association studies (GWAS), which provides several publicly available datasets on SNP-trait associations [11]. This has enabled *two-sample summary data MR*; if study 1 measured the association of a SNP with a risk factor (urate, in this context), and study 2 measured the association of the same SNP with an outcome (e.g., cardiovascular risk), then it is possible to study the causal effect of the risk factor on the outcome using the two independent studies.

Following a formulation by Burgess and peers, we will proceed to brief the framework of MR analysis. X denotes the risk factor of interest (urate level in our case) and Y denotes the outcome (for example CVD). Thus, Mendelian randomization can be represented as:

$$\beta_{Yj} = \alpha_j + \theta_j \beta_{Xj} ,$$

where α_j represents the effect of jth genetic variants on the outcome (CVD, in this case) that is not mediated via the risk factor (urate, in this case). θ_j is the causal effect of the risk factor on the outcome. If the jth genetic variant is pleiotropic, $\alpha_j \neq 0$. In the IVW method, θ_j is estimated as the IVW ratio of the two association estimates: one is the estimate of β_{Yj} (i.e., association of the outcome Y on the genetic variant) and the other is the estimate of β_{Xj} (the association of the risk factor X on the genetic variant). For more details see ref [12].

GWAS are currently being conducted by several researchers, which expands the number of genetic variants with different traits. If all such variants are valid instruments (i.e., $\alpha_j = 0$ for all j), the IVW method is a reasonable approach. However, it is possible that a substantial number of genetic variants are invalid for the risk factor of interest due to association with confounders (violating ii) above) or the issue of pleiotropy that affects the outcome directly (violating iii)). Several approaches to reduce the influences of invalid instruments have been proposed. In the MR-Egger, the above formulation is generalized such that a non-zero value for the intercept is introduced, instead of the assumption that $\alpha_j = 0$ for all j [12]. The MR-PRESSO method sequentially removes outliers out of the candidate instruments from the analysis until all the remaining genetic variants have similar estimates [13]. Such an approach is based on the assumption that when the causal estimates from each instrumental variable (i.e., variant-specific causal estimates) are compared, the estimates should venture to become similar to one another if the instrumental variables are valid. Recently developed methods based on such "plurality of valid instruments" include the contamination mixture method [14]. This method is a likelihood–based method: different values of the causal effect are tested consequently and the causal effect that maximizes the profile likelihood is taken as the estimate, where the profile likelihood is computed by essentially considering all the configurations of each genetic variant as valid or invalid. For the profile likelihood computation, the authors assume that the causal estimates based on valid instruments will normally distribute around the true value of the causal effect, whereas the estimates based on invalid instruments will distribute around zero, with a large standard deviation. This method can identify groups of variants that have similar causal estimates, which assists in the identification of causal mechanisms. The latter paper demonstrates the potential usefulness of this aspect using an example of an MR analysis performed on HDL-cholesterol effects on coronary heart disease risk [14]. The method not only detected two separate groups of variants, which suggests distinct protective effects of HDL-cholesterol on coronary heart disease risk, but also assisted in identifying several variants that share a feature that is associated with platelet traits (specifically, platelet distribution width).

From the viewpoint of urate biology, however, it is not clear to what extent such approaches accurately represent the relationship of genetic variants to both urate and the outcome. For example, we cannot exclude the possibility that highly influential genetic variants, in terms of raising serum urate levels, tend to show high reduction of intracellular fructose levels and lipid synthesis, thereby exerting a protective effect on cardiometabolic outcomes. If such a situation holds for multiple urate transporters, then the InSIDE assumption (i.e., the assumption that pleiotropic effects α_j are independently distributed based on genetic associations with the risk factor β_{Xj}) does not hold and bias may become significant regardless of whether these methods are used. For this reason, it seems that both experimental approaches focusing on molecular

processes and MR analyses that focus on individual genetic variants are warranted to further improve the MR analysis-based inference on the causal role of urate.

MR-based studies examining the causal effect of urate on diseases

This section briefly reviews the results of the MR analyses that address the role of urate in diseases. Despite strong associations of measured serum urate levels with a number of pathological conditions in many ordinary multivariate regression studies, most MR studies demonstrate that genetically predicted urate is not associated with hypertension, diabetes mellitus (DM), or coronary artery disease. As such, the causal role of urate in diseases has been poorly supported by MR studies, although there are some exceptions.

Early MR analyses of urate mainly used genetic variants of *SLC2A9* (also known as *GLUT9*) as an instrumental variable. *SLC2A9* is a transporter that mediates urate flux across the renal proximal tube. *SLC2A9* is a major genetic regulator of serum urate levels, and recent GWAS have shown a strong association of *SLC2A9* variants with serum urate levels, which explain about 1.2–6.0% of the variance of serum urate [11,15]. The studies that used genetic variants of *SLC2A9* as a sole instrumental variable include Parsa et al. [16] and McKeigue et al. [17]. Parsa et al. showed that elevated serum urate plays a causal role in hypertension [16]. However, Sedaghat et al. used a genetic risk score based on 30 urate-associated genetic variants and showed that urate has the effect of lowering blood pressure; 1-SD increase in the genetic risk score was associated with 0.75 mm Hg lower systolic blood pressure (SBP) (95% CI, −1.31 to −0.19) [18]. As we soon discuss, a SNP in the *SLC2A9* gene (rs12498742) shows a significant interaction with diuretics treatment in relation to blood pressure [18], which calls for a careful interpretation of analyses that involve treatment with diuretics.

McKeigue et al. showed that an elevation in the serum urate levels is not causal for metabolic syndrome [17]. An influential study focusing on the urate-lipid metabolism relationship was Lyngdoh et al., which examined the relationship between urate and metabolic syndrome-related parameters [19]. This study performed bidirectional MR analyses; one direction is based on the serum urate-associated SNP in *SLC2A9* and the other direction is based on the adiposity genes *FTO*, *MC4R*, and *TMEM18*. While the genetic variants of the adiposity genes showed causal effects on serum urate levels, no evidence was found for the causal impact of serum urate on adiposity, suggesting that the elevated serum urate is a consequence rather than a cause of adiposity. Palmer et al. conducted two cohorts each comprising 4,890 and 2,282 ischemic heart disease cases, respectively, in Denmark and showed no evidence of a causal effect of elevated urate on the risk of ischemic heart disease or hypertension [20]. This study also used only one SNP (rs7442295) in the *SLC2A9* gene as the instrumental variable. Their additional analyses that address BMI did not support a causal effect of urate on BMI. They also conducted bidirectional MR using the SNPs known to associate with

BMI and observed a causal effect of BMI on urate levels, which agrees with the findings of Lyngdoh et al. [19].

After these studies were published, Merriman and coworkers reported an intriguing set of results on renal functions. They showed that MR-based urate analyses could lead to diverse results depending on the genes used as the instrumental variables [21]. The authors applied a genetic risk score based on SNPs in genes for urate transporters (*SLC2A9*, *SLC17A1*, *SLC22A11*, *SLC22A12*, and *ABCG2*) to participants of longitudinal cohorts, and observed that elevated urate has a causal effect for *improved* renal function, contrary to the consensus of urate as a risk factor for kidney disease [21]. Thus, different genes from which SNPs were used as an instrument led to finding diverse effects on renal function. This suggests the violation of the third assumption that is required for MR, which is the assumption that the effects of the instrument on an outcome should be only mediated by the intermediate variable. Merriman and coworkers further used a similar set of genes/SNPs to analyze causal effect of urate on triglycerides (TG) and found no evidence for the causal role of urate in increasing serum TG levels [22]. This study also analyzed individual genetic variants and showed a causal effect of *SLC2A9* and *SLC22A11* (also called *OAT4*) variants in *reducing* serum TG. Among the solute carrier (SLC) superfamily, SLC22 constitutes a group of organic anion/urate transporters. We proceed to discuss this further below.

Given the potential problem of relying on a small number of urate-associated genes as instrumental variables, MR-based urate research has evolved to utilize many urate-associated genes. This approach was expedited by genome-wide association studies (GWAS), such as, Yang et al. [23] and Köttgen et al. [11]. Köttgen et al. covered the data of >140,000 individuals and identified 28 loci that were significantly associated with serum urate levels [11]. Using available information on genetic variants, Sluijs et al. [24] applied a genetic score based on 24 urate-associated loci to a cohort study comprising 24,265 European participants, among whom 10,576 developed type 2 DM during the follow-up period. Strikingly, while conventional multivariate statistics show a causative effect of urate, their MR analysis shows no causal effect of circulating urate on DM risk.

Out of the 28 urate-associated SNPs identified by Köttgen et al. [11], in Kleber et al. 8 SNPs were selected that did not show an apparent association with other major risk markers, and applied the genetic risk scores to a cross-sectional and cohort analyses of 3,060 patients who were hospitalized for coronary angiography [15]. The genetic risk score based on eight SNPs (namely GRS8) did not show an association with biochemical markers, such as blood lipids, blood glucose, blood pressure, or estimated glomerular filtration rate (eGFR). Importantly, GRS8 did not show association with coronary artery disease, which argues against the hypothesis that urate is the independent cause for atherosclerosis [15]. Nonetheless, after multivariate adjustment for established risk scores, the causal hazard ratio per 1-mg/dL of genetically predicted urate remained significant: 1.77 (CI, 1.12 to 2.81) for cardiovascular mortality and 2.41 (CI, 1.16 to 5.00) for sudden cardiac death.

A meta-analysis by White et al. showed a modest level of causal effect of urate on coronary heart disease [25]. Their MR analysis using 31 SNPs derived from GWAS, after multivariate adjustment, also showed modest causality, though MR-Egger [26] showed this effect was not significant.

Keenan et al. [27] used a genetic risk score based on 14 SNPs that showed no association with any of the 50 vascular and non-vascular risk factors, excluding urate. Using primarily only summary-level datasets such as DIAGRAM (DIAbetes Genetics Replication And Meta-analysis consortium) and CARDIoGRAM (Coronary Artery Disease Genetics consortium), this study analyzed relatively large numbers of cases/controls of type 2 DM, coronary heart disease, ischemic stroke, and heart failure, of which all participants were of European or South Asian ancestry. Once again, the results did not support a causal role of serum urate in type 2 DM, coronary heart disease, ischemic stroke, or heart failure.

More recently, for examining the potential causal effect of rate on incident type 2 DM, Keerman et al. performed MR analysis against 15,195 participants using a genetic risk score that was based on 15 SNPs. No significant associations were observed between the genetic score and diabetes risk during the mean follow-up of 4.5 years [28].

A few MR studies focused on the effect of urate on kidney disease. In a cohort analysis by Testa et al., 755 patients with chronic kidney disease (CKD), ranging from stages 2 to 5, were analyzed [29]. A polymorphism (specifically, T allele in rs734553) in $SLC2A9$ ($GLUT9$) was shown to be a predictor of renal outcomes (>30% decrease in GFR, dialysis or transplantation). Intriguingly, no such relationship was found with measured serum urate levels. As the authors suggest, this may suggest a possible important role of long-term exposure to high urate levels, beyond the examined period during which the study occurred. However, given the results from Hughes et al., which argue against the causal role of a SLC2A9-mediated elevation of serum urate in kidney disease, the findings of this study should be interpreted carefully [21]. We will discuss this issue in the section on urate and renal disease.

In 2017, Ahola et al. conducted a longitudinal study (~7 years follow-up) consisting of 3,384 patients with type 1 DM using a genetic risk score that is based on 29 urate-associated SNPs. No causality was shown between urate and diabetic nephropathy based on albuminuria or eGFR [30]. Thus, for kidney disease, Testa et al. showed positive causality of urate in the general population [29]. However, Ahola et al. did not show such causality in patients with diabetes, which they suggest could be due to the roles that urate play in the processes that lead to nondiabetic renal disease compared to the insignificant role that it plays in diabetic nephropathy [30]. It should also be noted that the Testa et al. only used $SLC2A9$ genetic variants as the instrumental variable.

Testa et al. extended their study to three high risk cohorts that consist of 755 patients with CKD, 353 patients with type 2 DM and coronary artery disease, and 119 patients enrolled after a myocardial infarction [31]. In all cohorts, the T allele of rs734553 in $GLUT9$ was associated with the risk of incident cardiovascular events. Specifically, the allele was shown to predict a doubling of risk of incident cardiovascular events in patients at high cardiovascular risk, which supports the causal role of urate in atherosclerosis. As these analyses are based on a relatively small size of participants, further analyses on the causal role of high urate in high-risk patients is warranted.

More recently, using a dataset with a large sample size (N>400,000), Jordan et al. conducted seven distinct MR analyses to examine the potential causal effect of serum urate in eGFR and CKD risk [32]. None of these analyses showed a causal effect of urate. Their additional analysis, which excluded 2 SNPs with the most significant effects on urate ($SLC2A9$ and $ABCG2$), also showed no causal effect of urate. They further stratified the population by sex and age, but still observed no causality. Their MR analysis using only one SNP (rs12498742) in $SLC2A9$ likewise showed no causality.

In a MR study in 3,734 Chinese participants (of the general population), Liu et al. used a genetic risk score based on four SNPs located in three genes ($ABCG2$, $SLC2A9$ and $SLC17A1$) and showed no association with potential confounding factors— including BMI, total cholesterol, BUN, and fasting blood glucose [33]. In this study, MR analyses toward various subpopulations that were stratified in several schemes were performed. The two-stage least square regression (genetic risk score to urate, and urate to renal function) showed that serum urate was not a risk factor for renal function in men, though serum urate was strongly associated with serum creatinine and eGFR among women. They also showed that, in participants who were smokers or under 65 years old, or who had high fasting blood glucose (FBG) levels or normal levels of eGFR, serum urate had a causal effect of increasing serum creatinine and reducing eGFR.

Recently, Efstathiadou et al. [34] utilized large datasets and performed 2-sample MR analyses to assess the potential role urate plays in cognitive function, Alzheimer's disease, coronary heart disease, myocardial infarction, and ischemic stroke, including its subtypes (cardioembolic stroke, small-vessel disease, and large-artery atherosclerotic stroke). The results demonstrate a causal effect of genetically determined serum urate levels in these diseases, despite the associations observed in many observational studies. However, the same group extended their study to incorporate updated methods and variants, after which their data showed support for an effect of serum urate on the risk of coronary heart disease, peripheral artery disease, and stroke. Importantly, this also suggests that the causality may partly be mediated though elevated SBP [35]. A 1-SD increase in genetically-predicted serum urate was associated with an increased risk of coronary heart disease (OR 1.19, CI 1.10 to 1.30). The latter study used new methods, such as the contamination mixture methods and MR-PRESSO.

Recent MR analyses also focused on the potential role of urate in neurological disorders. The causal role of urate in Alzheimer's disease [36] and the protective effect of urate in Parkinson disease [37,38] were addressed, although, to our knowledge, none of these MR analyses observed a significant role of urate. In terms of PD, observational analyses suggest that a high plasma urate is associated with lower risk of PD.

Another unique MR study on urate is Kobylecki et al. [39], which analyzed 86,210 participants, and showed that elevated plasma urate was both observationally and genetically associated with high cancer incidence and high all-cause mortality. This study used a SNP from *SLC2A9* as the sole instrumental variable.

Urate and disease -- impact of Mendelian randomization studies

Numerous epidemiological, molecular, and animal studies have established the association of serum urate with CKD [2], metabolic syndrome [40], hypertension [41], and coronary artery disease [42]. In the following sections, we discuss some MR studies and focus on their impact on the epidemiology of urate.

Urate association with hypertension and cardiovascular disease

Compared to other diseases, tremendous evidence supports the association of urate with hypertension. Experimental and epidemiological studies on the urate role in asymptomatic atherosclerotic damage (in terms of carotid intima-media thickness, arterial stiffness, endothelial function, and so on), including studies that report negative results, have been reviewed in Cortese et al. [2]. Recent epidemiological studies in support of the urate role in hypertension include Kuwabara et al. [43], Wang et al. [45] and Ohyama et al. [44]. However, the directionality of the association remains debated.

MR-based analysis by Palmer et al. did not demonstrate a positive causal relationship between urate and systolic blood pressure (SBP) or diastolic blood pressure (DBP), as we had discussed previously [20]. The previously mentioned MR study by Kleber et al., which included 3,060 patients who were hospitalized for coronary angiography, also found no causal relationship between urate and hypertension [15]. On the other hand, several MR-based studies demonstrated a causal effect of urate on blood pressure, although with conflicting results [16,18]. In Parsa et al., rs16890979 (Val253Ile) in *SLC2A9* was used. Two-stage regression MR demonstrated a causal effect of urate on elevated SBP and DBP, though importantly no effect on BMI and TG. Mallamaci et al. showed that rs7345555 variant in *SLC2A9* is associated with SBP in cardiovascular complication-free individuals [46]. In Sedaghat et al., a genetic risk score based on 30 SNPs was used [18]. Intriguingly, higher genetic risk scores, which are associated with high serum urate levels, were associated with lower SBP and DBP. It should be noted, however, that adjusting for serum urate levels did not change the results, which suggests that this association is unlikely to be mediated by urate [18]. Similarly, Yang et al., found a negative association (with borderline significance) between SBP and a urate genetic risk score that is based on 8 genetic variants [23].

In a MR-PheWAS analysis by Li et al. [47], analysis with the MR inverse-variance weighted (IVW) method showed significant causal OR for two phecode-defined disease categories, 'essential hypertension' and 'hypertensive disease' (causal OR being 1.08 with 95% CI 1.03 to 1.14). However, this is a quite small causal OR. Moreover, MR-Egger showed a non-significant causal relationship.

It is important to address two issues that potentially confound the relationship between urate and blood pressure. First, confounding variables by adiposity/obesity-related factors may venture to be problematic. As obesity and BMI have enormous causal effect on both urate and blood pressure, it should be important to carefully examine whether the genetic variants used for urate MR analysis has any effect on adiposity/obesity. Another issue is the use of diuretics; treatment with diuretics can modulate both blood pressure as well as serum urate levels. Hyperuricemia is known to be a side effect of diuretics [48]. Moreover, as shown by Sedaghat et al. [18], the association between the urate genetic score and blood pressure was more pronounced in those who were using diuretics. In the case of Sedaghat et al., compared to other SNPs, an SNP in *SLC2A9* (rs12498742) showed a more pronounced level of interaction with diuretics treatment in relation to SBP. It is likely that those patients who carry the risk allele in the urate-associated genes (involving *SLC2A9*) respond more favorably to diuretics, which leads to the occurrence of hyperuricemia as a side effect of diuretics [18].

Overall, it seems premature to draw conclusions about the potential causal effect of urate on cardiovascular disease. Although none of the studies by Keenan et al. [27], Kleber et al. [15], White et al. [25], and Efstathiadou et al. [34] showed a clear causal effect of urate on CVD, Gill et al. showed a causal role of genetically predicted urate on cardiovascular disease [35]. Given that Gill et al. used a large dataset and new set of genetic variants, it is possible that the previous studies showing no causal effect will have suffered from limited statistical power and certain pleiotropic effects from the genetic variants, which may conceal the effects of urate on cardiovascular outcomes [35]. Thus, although it is likely that the causal effect is modest, it is still possible that further analyses show more pronounced causal effects of urate on cardiovascular disease.

Urate and obesity/adiposity

It has been well-established that serum urate associates with obesity [40,50,51]. Although we did not cover this in the present article, several epidemiological studies suggest results consistent with the hypothesis that urate is a causative factor for obesity and metabolic syndrome [19,40]. However, the issue of reverse causality was not fully addressed in conventional multivariate regression analyses. Indeed, obesity/adiposity has been increasingly implicated as a causal factor for hyperuricemia. In studies addressing this reverse causality issue, hyperinsulinemia/insulin resistance, which tends to accompany obesity, has been shown to reduce urate clearance, thereby elevating serum urate levels. For example, Tsunoda et al. showed that amelioration of insulin resistance by a low-energy diet or troglitazone led to a decrease in serum urate levels in overweight hypertensive patients, which underscores the causative role of hyperinsulinemia or insulin resistance in hyperuricemia [52]. Moreover, several

epidemiological studies show that weight gain can predict the development of hyperuricemia, suggesting a causative role of adiposity in hyperuricemia [19].

Several studies that use genetic variants have supported the causal role of adiposity in increased urate levels (reverse causality). For example, the effect of BMI on urate levels was analyzed in Brandstatter et al., which used SNPs in *SLC2A9* and showed that this association is influenced by sex and BMI [53]. As such, the association between genotypes of SNPs in *SLC2A9* and urate levels was more pronounced in women compared to men, and modified by BMI, such that an increase in BMI amplified the effects of genetic variants on urate levels. As discussed prior, Lyngdoh et al. used a bidirectional MR to examine the causality between serum urate and adiposity, and reported results that suggest the elevated serum urate is a consequence rather than a cause of adiposity [19]. MR analysis by Rasheed et al. provided no evidence for the causal effect of urate on increased levels of TG [22]. Intriguingly, their data supported the view that elevated urate has a causal role in *lowering* serum TG. This study used SNPs from *SLC2A9*, *ABCG2*, *SLC17A1*, *SLC22A11*, and *SLC22A12*. This unexpected relationship may be partly caused by the characteristics of *SLC2A9* that encode a transporter whose activity is under the influence of the presence of hexose and/or SLA22A11 (OAT4), which was unexpectedly shown in their preceding study to have a causal effect in *protecting* renal function [21]. As the authors discuss, these SNPs may have functional effects on lipid metabolism, irrespective of the effect on serum urate levels, thereby violating assumption iii for selecting a MR instrumental variable. Lipid metabolism-related effects that are not mediated by urate per se have been proposed for the SLC2A9 transporter. For this transporter, urate transport is modified by fructose and glucose. Witkowska et al. used the oocyte expression system to show that the presence of extracellular hexose can increase the influx of urate through this transporter (SLC2A9a and SLC2A9b), with fructose showing more pronounced effects than glucose [54]. Thus, once dietary fructose enters cells via various transporters, it may potentiate the influx of urate. It is possible that the SLC2A9 activity required to raise urate will lower TG levels by influencing the availability of sugar for TG synthesis. This consideration involves the difficulty of performing a MR analysis without violating the three assumptions. Nonetheless, MR analyses using individual genetic variants that were demonstrated by Merriman and coworkers demonstrated the potential of a MR study to provide biological insights into the genes used as instrumental variables— which may spur further studies in urate biology [21].

GWAS elucidated several genetic variants associated with elevated urate levels, but the molecular mechanisms for the coded proteins to regulate urate levels are only partially understood. SLC22 constitutes a group of organic anion/urate transporters. SLC22A12 (URAT1) has antiporter activity, and lactate serves as a substrate for the antiporter activity to increase urate absorption [55]. SLC22A11 (OAT4) urate reabsorption is transactivated by intracellular dicaroboxylates (i.e., molecules containing two carboxyl groups) [56]. SLC17 is a family known as Na^+/phosphate cotransporters (NPTs),

but to our knowledge, the molecular mechanism for how SLC17A1 transports urate, and whether the transport of other substrates is related to urate transport activity, is not understood well. Similarly, although the impact of genetic variants of *ABCG2* are known, whether the activity toward urate is related to the activity toward other substrates is not fully understand for ABCG2. For further information, we suggest referring to Xu et al. [57] and He et al. [58].

Increased recent studies that utilize genetic risk cores based on SNPs in multiple genes have also been conducted, but have supported no causal role of serum urate in adiposity. For example, when Kleber et al. checked the 28 urate SNPs related to serum urate levels [11,15], some SNPs showed associations with LDL-cholesterol, TG, or BMI; however, it was possible to choose eight SNPs that show no pleiotropy with such risk factors and thus show no association with BMI or LDL-cholesterol. Similarly, Keenan et al. checked pleiotropy and identified 14 nonpleiotropic SNPs; further, the weighted genetic risk score showed no association with any vascular or nonvascular trait, including TG, LDL-cholesterol, and BMI [27].

Together, these findings suggest that serum urate does not have significant causal effect on adiposity. The causality in the opposite direction— that is the causal role of obesity/adiposity in hyperuricemia— is clear. Thus, it seems reasonable to consider that urate has no or very weak causal effect on obesity/adiposity in the general population. As Kleber et al. used a cohort composed of patients who were hospitalized for coronary angiography, and identified the urate-related SNPs with genetic risk scores that showed no pleiotropy, it is possible that even for patient populations with atherosclerosis, there is no or negligibly weak causal effect of urate on adiposity [15].

MR analysis examining urate causality for diabetes

Association of hyperuricemia and type 2 DM has been demonstrated in a number of studies, with several epidemiological and experimental studies that implicate the causal role of urate in developing diabetes [40]. However, there has been inconsistency among studies as discussed in Li et al. [59]. The recent studies that did not support the causal effect of high urate on DM include a cohort study by Li et al. [59]. This study showed that elevated serum XO activity, rather than urate concentration, was associated with an increased risk of developing type 2 DM in multivariate models that mutually adjust XO and urate. Importantly, in the study by Tsai et al. involving 739 patients with hyperuricemia, patients with urate levels of <6mg/dL had high prevalence of DM compared to those with urate of ≥ 6mg/dL, which is contrary to the positive correlation between serum urate and incident DM [60].

Thus far, MR studies have not supported the causal role of urate in diabetes. Pfister et al. showed a non-significant causal effect of urate on type 2 DM risk [61]. As previously mentioned, MR analysis by Sluijs et al. did not show any evidence of a causal effect [24]. Further, the genetic risk score used in Kleber et al. did not reveal an association with blood glucose [15]. Further, in the MR-based study by Keenan et al.,

the causal role of serum urate in type 2 DM was not supported [27].

Thus, MR studies are generally against the causal role of urate in type 2 DM. However, it should be noted that the activities of at least several urate transporters have been shown to have antiporter activities and rather loose substrate specificities. This causes a situation in which urate transport has influence on the transport of sugar and metabolic intermediates as well [62]. Moreover, as the urate transporters comprise the group that is a major determinant of urate levels, genetic variants from genes coding these transporters may alter the intracellular-extracellular equilibrium of urate. As Johnson et al. discussed, this issue needs to be carefully examined, because insulin resistance and gluconeogenesis are considered as processes modulated by intracellular urate, rather than extracellular [63]. Thus, to avoid incorrect conceptions regarding the role of urate in diabetes, analyses of physiology and the metabolism of urate and sugar metabolites in molecular details have become of high relevance.

Recent focus has shifted to the potential effect of urate on vascular complications, mortality, and comorbidities in patients with DM. Elevated serum urate was found to be an independent predictor of vascular complications and mortality in type 2 DM patients. For example, in 2013, Xu et al. conducted a meta-analysis that included 9 eligible articles and over 20,000 patients with type 2 DM [64]. The results supported the idea that elevated serum urate is an independent predictor of vascular complications and mortality in patients with type 2 DM. As we discussed in the following sections, it is possible that soon more MR approaches be utilized in cohort studies on participants with DM.

MR analysis of urate in kidney diseases

Epidemiological studies have shown that elevated serum urate is a modest predictor of CKD [65-67]. Recent studies supported the hypothesis that hyperuricemia is associated with a greater decline in renal function and a higher risk of progression to kidney failure [e.g., 60]. However, as the mini-review part of the latter paper discuss, several studies show no association between hyperuricemia and the progression of CKD. In addition to such conflicting results, the limitations of conventional multivariate regression analysis present problems; it cannot remove unmeasured or unknown confounding variables and thus cannot provide progress on the causality issue. Reverse causality is another challenge, especially because though serum urate is considered a factor in the progression of renal disease, renal dysfunction may raise serum urate concentration [68]. Clinical studies have also shown that urate-lowering drugs improve renal function and slow progression of CKD. However, while allopurinol has shown beneficial effects on renal function, its effect may be mediated primarily by inhibiting the production of oxidants by xanthine oxidase, and subsequent improving of endothelial cell function. [69].

Given such discussions, MR has become an important approach to gain insight into the potential causality of urate. As previously mentioned, the MR analysis by Testa et al. shows a causal role of urate on the decline of renal function in

the general population [31]. However, the MR analysis by Hughes et al. demonstrates that increased UA has a causal effect for *improved* renal function [21]. The authors applied a genetic risk score based on SNPs in genes for UA transporters (*SLC2A9*, *SLC17A1*, *SLC22A11*, *SLC22A12*, and *ABCG2*) to the participants of the longitudinal cohorts [21]. Strikingly, while ordinary regression showed associations between urate and both serum creatinine and eGFR, which indicates the predictive value of urate for renal function, a two-stage regression of the MR analysis showed that increased serum urate caused by genetic variation *improved* renal function in males. Moreover, in both hyperuricemic men and women, an increase in the genetic risk score was found to correlate with improved renal function. Why was such an improved correlation with improvement seen? As the authors discuss, the genetic risk marker based on five uric transporters may suffer from violations of the third assumption, which is that the genetic score (instrumental variable) has an effect only through serum urate. It is possible that genotypes of the transporters directly influence the renal function, rather than serum urate. These results are shown in Hughes et al. [21], and the findings underscore the importance of a careful choice of instrumental variables.

Moreover, we will now compare these two studies. Testa et al. demonstrated the T variants of rs734553 of SLC2A9 are associated with high serum urate levels in the general population; further, they showed that the T variants predict CKD progression in patients with CKD stage 2–5 [31]. However, Hughes et al. showed no causal effect of elevated serum urate, and instead suggested the *benefit* of SLC2A9-mediated high serum urate levels on renal function [21]. Why did such discrepancy occur? First, it should be noted that Hughes et al. used cohorts of the "Atherosclerosis Risk in Communities and Framingham Heart Study (FHS)," which were composed of patients with high risk for atherosclerosis, though the patients with kidney diseases were excluded. In contrast, Testa et al. used the patient population with CKD stage 2–5. Focusing on patients with CKD may lead to a complex situation because glomerulonephritis and tubulointerstitial nephritis may be differentially affected depending on *SLC2A9* (and other transporters) activities. In fact, urinary protein was 0.7 in patients with the T allele, higher than 0.3 in the patients without the T allele. This raises the possibility that, even among patients of a similar CKD stage, glomerulonephritis, which associates with proteinuria, may be more important (dominant) in T allele patients, while tubulointerstitial nephritis-dominant CKD is more important in patients without T allele. The rationale for this idea is that SLC2A9 and other transporters function in a renal tubule, and therefore, patients with high activity of SLC2A9-mediated urate reabsorption may have a lesser degree of tubulointerstitial nephritis compared to patients with low activity of SLC2A9 within the same CKD stage. When the CKD stage is the same, the latter patients may be in a more advanced stage of glomerulonephritis and thus proteinuria. In any case, such consideration suggests potential challenges of MR analysis. Testa et al. analyzed the direct association between events of kidney disease and the T allele, but no two-stage regression was performed, likely due to the small size of

the analyzed population [31]. This is another weakness of this study, which makes it difficult to form an argument on the role of urate in the progression of CKD.

What about the potential urate causality in diabetic nephropathy? It has been shown that lowering of serum urate levels with a drug was associated with reduced risk of renal outcomes during a follow-up of patients with type 2 DM. However, as we discussed earlier, Ahola et al. did not observe causal effects of urate in patients with diabetes in a larger-scale MR study [30]. This was an unexpected result, as it has generally been considered that the elevated serum urate is a risk factor for progression of renal disease in patients with diabetes [e.g., 70,71].

Thus, the results of the MR studies that address urate causality in kidney diseases are conflicting. Given also the controversial results of conventional multivariate regression [60], future MR studies may well be directed toward analyses of subpopulations. For example, in Tsai et al. [60], patients with and without proteinuria showed differences; the effect of hyperuricemia on the decline of eGFR was more pronounced among patients without proteinuria compared to those with proteinuria. This led the authors to consider that, for patients with proteinuria, the glomerulonephritis is the dominant process, and urate plays rather minor roles, whereas for those without proteinuria, the underlying pathological processes are more likely to be tubulointerstitial nephritis, which is directly worsened by urate. Such considerations may lead to an MR analysis in patients with hyperuricemia and without proteinuria. Causal effect of urate may become evident in such a MR analysis. Another interesting issue would be the potential association of the urate-associated genetic variants and the activity of urate excretion. It is also important to examine the interaction of drugs including diuretics with the genetic variants of urate-associated genes [18]. Such analyses will form an important basis for future studies on the influence of genetic variants on urate metabolism.

Urate causality in cardiovascular and renal diseases in diabetic patients

Urate has been considered a risk factor for macrovascular disease, such as carotid atherosclerosis, lower limb arteriosclerosis, coronary atherosclerosis, myocardial infarction, and stroke. As we have seen, several MR-based studies have addressed causal effects of urate on cardiovascular diseases in the general population. On the other hand, it has not been fully studied whether urate plays a causal role in the cardiovascular outcomes of patients with DM.

In conventional observational studies, several studies addressed the association of urate levels with cardiovascular outcome. For example, based on a cohort of 1,540 participants with type 2 DM, Panero et al. showed a non-significant hazard ratio for cardiovascular mortality [72]. In a study by Ong et al., the serum urate levels did not predict cardiovascular mortality in 1,268 patients in Australia with type 2 DM [72]

Only a limited number of MR analyses on patients with type 2 DM patients have been published. Yan et al. used MR to address the question on whether urate is an independent risk factor for diabetic macrovascular disease (carotid

atherosclerosis, lower limb arteriosclerosis, coronary atherosclerosis, myocardial infarction, and stroke) on patients with type 2 DM in China [74]. In male patients with type 2 DM, even measured urate did not associate with macrovascular diseases. In female patients, a genetic risk score that was derived from SNPs in *SLC2A9, ABCG2*, and *SLC22A12*, and consisting of 3,207 patients with type 2 DM, observed a causal relationship between the genetic score and diabetic macrovascular disease (OR=1.184) after adjustment for the parameters including blood pressure, eGFR, BMI, and duration of diabetes. However, the two-step regression led to a small causal effect (OR=1.016) found, raising the possibility that the genetic variants used may have been related to other (non-urate) pathways that influence the macrovascular outcome.

What about the role of urate in diabetic nephropathy? Several cohort studies have shown that serum urate is an independent risk factor that predicts the decline of renal function in patients with type 1 DM as well as those with type 2 DM. For example, [75-78]. We suggest a couple of excellent review articles [67,79].

However, the results of Ahola et al. argue that the elevated serum urate has no causal effect on the diabetic kidney complication in patients with DM [30]. As the authors mentioned, this may be due to urate potentially playing a role only in the processes that lead to nondiabetic renal disease, but an insignificant role in diabetic nephropathy. In any case, this finding highlighted the disparity between MR analysis and conventional observational studies, as observational studies have stressed the association of urate and patients with diabetes. This disparity may be partly caused by the confounding variables of obesity and/or adiposity. As aforementioned, Lyngdoh et al. demonstrated that urate is largely downstream of adiposity and/or obesity, and the apparent effect of urate on kidney disease is likely to be confounded by elevated adiposity [19]. In support of such interpretations, the MR study by Todd et al. showed a causal role of adiposity and/or obesity in diabetic kidney disease [80]. In MR studies with over 6,000 participants with type 1 DM, Todd et al. used a genetic risk score that was based on 32 BMI–related loci as an instrumental variable, and showed its causal link to macroalbuminemia in end-stage renal disease (ESRD) or diabetic kidney disease defined as presence of macroalbuminemia or ESRD [80]. Moreover, van Zuydam et al. performed a set of association analyses using several genetic risk scores and phenotypes [81], and observed significant associations for several combinations. For example, a genetic risk score for increased BMI was associated with all diabetic kidney diseases. This study also provided some support for the causal effect of insulin resistance to diabetic kidney disease. Other recent studies on diabetic kidney disease and adiposity and/or obesity include Taira et al. [82], in which a GWAS meta-analysis for diabetic nephropathy showed a significant association of rs56094641 in *FTO* with susceptibility to diabetic nephropathy in Japanese patients with type 2 DM. Genetic variants in *FTO* have been repeatedly shown to be associated with adiposity and/or obesity [83 Loos Yeo 10 51]. A *FTO* variant at rs17817449, which is in the linkage disequilibrium to rs56094641, has

been shown to be associated with ESRD [84]. Thus, it is likely that *FTO* variants play a causal role on CKD/ESRD via mechanisms that are mediated by adiposity and/or obesity.

Overall, it is likely that obesity and/or adiposity is a strong confounding factor in the association between serum urate and diabetic kidney diseases. Without further stratification, serum urate levels are likely to have no or negligible causal effect on decreased renal function in patients with type 2 DM. However, by focusing on patient subgroups to reduce heterogeneity in terms of type and kidney disease stage, it is still possible that the causal role of urate may emerge for some stage of diabetic kidney disease.

Perspectives

As of present, MR analyses on the causal effect of urate generally produced modest or negligible causality in hypertension, cardiovascular, kidney disease, and diabetes. However, the choice of genetic variants has profound influence on the results of MR studies. This notion is supported by the recent study by Gill et al., in which the standard IVW approach led to positive causality in cardiovascular disease, implying the importance of choice of genetic variant [35]. Improved methods to manage invalid variants are important for MR analyses on urate, given that the modest level of causality should be examined.

In medical science, MR analysis is used to assess the causality of a marker of interest on diseases, and when successful can provide important insights for considering targets of drug development. Nonetheless, MR analyses of urate so far have provided valuable information in the domain of molecular physiology. That is, MR analysis using individual SNPs as instrumental variables (e.g., those from transporter genes) demonstrated the merits of MR analysis in acquiring pathophysiological insights. For instance, in Sedaghat et al., an SNP of *SLC2A9* (rs12498742), compared to other SNPs, showed a more pronounced level of interaction with diuretics treatment in relation to systolic blood pressure [18]. Thus, it is likely that many urate-related genes are differentially linked to the salt sensitivity or efficacy of diuretics and other drugs. Although such issues are serious challenges for MR analysis, the association of urate-associated genetic variants with transporter activity and drug efficacy is of upmost importance for future studies on urate epidemiology.

Such considerations suggest that, while use of a genetic score that is based on a combination of multiple SNPs from different genes has benefits to dilute the effects of specific genes with pleiotropy, it is also useful to check the impact of each individual genetic variant on the causal effect of urate. This is especially because transporters, such as SLC2A9 and SLC22A11, are influential determinants that have not been fully characterized in terms of sugar metabolism activity and renal function. Further, how exactly lipid metabolism is linked to urate metabolism is not yet fully understood. As Burgess et al. demonstrated in their example on HDL-cholesterol [14], detailed analyses on the route through which the biomarker of interest exerts a causal effect on the outcome may venture into a promising approach in pathophysiological understanding.

Further, it seems to be increasingly important to apply MR to subpopulations, such as patients with a specific disease or participants stratified according to gender, age, ethnicities, or other properties. Such considerations are especially important for kidney disease, as some patient subgroups, such as those with IgA nephropathy, DM, and transplants, are demonstrated to have a clear association that is consistent with the causal effects of urate [60]. It would also be important to perform MR analyses that consider the types of renal dysfunction. Specifically, reduced kidney function, as reflected by eGFR and ESRD, and dysfunction of the glomerular filtration barrier, as reflected by albuminuria, develop independently, and thus may be subject to the distinct influences of urate levels and genetic variants of urate transporters.

Conflict of Interest

Authors declare that they have no conflict of interest.

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