

# The Uric Acid and Gout have No Direct Causality With Osteoarthritis: A Mendelian Randomization Study

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**Objective.** To examine whether uric acid level or gout is causally associated with the risk of osteoarthritis. **Methods.** We performed a two-sample Mendelian randomization (MR) analysis using inverse-variance weighted (IVW), MR-Egger regression, and weighted median methods. We used the publicly available summary statistics datasets of uric acid level or gout genome-wide association studies (GWASs) as the exposure, and a GWAS in 3,498 patients with osteoarthritis in the arcOGEN study and 11,009 controls of European ancestry as the outcome. **Results.** Six single nucleotide polymorphisms (SNPs) from the GWAS data on uric acid level and 12 SNPs from the GWAS data on gout were selected as instrumental variables (IVs). The IVW analysis did not support a causal association between uric acid level or gout and risk of osteoarthritis ( $\beta = -0.026$ , standard error [SE] = 0.096,  $p = 0.789$ ;  $\beta = -0.018$ , SE = 0.025,  $p = 0.482$ ). MR-Egger regression revealed no causal association between uric acid level or gout and risk of osteoarthritis ( $\beta = 0.028$ , SE = 0.142,  $p = 0.852$ ;  $\beta = -0.056$ , SE = 0.090,  $p = 0.548$ ). Similarly, no evidence of a casual association was provided by the weighted median approach ( $\beta = 0.004$ , SE = 0.064,  $p = 0.946$ ;  $\beta = -0.005$ , SE = 0.025,  $p = 0.843$ ). **Conclusion.** The results of MR analysis demonstrates that uric acid level and gout may be not causally associated with the increased risk of osteoarthritis. Considering MR study is not susceptible to bias from unmeasured confounders or reverse causation, the epidemiological evidence for an association between uric acid level or gout and a higher risk of osteoarthritis may be due to residual confounding or reverse causation rather than direct causality. (*J Rheum Dis* 2020;27:88-95)

**Key Words.** Uric acid, Gout, Osteoarthritis, Mendelian randomization analysis

## INTRODUCTION

Osteoarthritis is the most common disease of joints, characterized by a progressive degeneration of articular cartilage, joint pain, and immobility [1]. It is a major cause of pain and disability in elderly people, and its prevalence continues to increase worldwide [2]. The health burden of osteoarthritis is growing with increase in the average longevity worldwide. Therefore, a comprehensive understanding of the risk factors associated with the occurrence of osteoarthritis is needed. Hyperuricemia is a condition in which the serum urate level exceeds the limit of urate solubility (6.8 mg/dL), which reflects supersaturation of the extracellular fluid with urate, and pre-

disposes the body to gout [3]. Gout is an inflammatory disorder characterized by hyperuricemia and effects of urate crystal deposition, including episodic gout flares, gouty arthropathy, tophi, and urolithiasis [3]. Hyperuricemia leads to the deposition of monosodium urate (MSU) crystals in tissues, and increase in the levels of hyperuricemia is correlated with increasing incidences of gouty arthritis [4].

Epidemiological studies have demonstrated that higher serum uric acid levels and gout are associated with an increased risk of osteoarthritis, suggesting uric acid and gout as possible risk factors for osteoarthritis [5,6]. The relationship among hyperuricemia, gout, and osteoarthritis may be explained by the finding that MSU crystal

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deposition on/in cartilage may create local mechanical and/or inflammatory damage, promoting osteoarthritis development, and that uric acid transporters are present on human articular chondrocytes, suggesting that these cells may internalize uric acid with potential pro-oxidant effects [7]. However, the shared risk factors due to obesity and aging represent a common feature among hyperuricemia, gout and osteoarthritis [8]. Hyperuricemia and gout may predispose joints to osteoarthritis, and osteoarthritis-caused cartilage damage may promote MSU deposition; further these conditions may trigger or result in shared inflammatory cascades. Observational studies are prone to biases, such as reverse causation interpretations and residual confounding, preventing a clear understanding of the effects of uric acid level and gout on osteoarthritis.

Mendelian randomization (MR) is a technique that uses genetic variants as instrumental variables (IVs) to assess whether an observational association between a risk factor and an outcome is consistent with a causal effect [9]. However, to date, MR has not been used to explore the causal effects of uric acid level and gout on the risk of osteoarthritis. The aim of this study was to examine, using a two-sample MR analysis, whether uric acid level and gout are causally associated with the risk of osteoarthritis.

## MATERIALS AND METHODS

### Data sources and selection of genetic variants

We searched NHGRI-EBI GWAS (<https://www.ebi.ac.uk/gwas>), a comprehensive catalog of reported associations from published GWAS studies. We used publicly available summary statistics datasets from uric acid level or gout GWASs. For the exposure dataset, we used publicly available summary statistics datasets from a meta-analysis of GWASs on uric acid levels from 14 studies with a total 28,141 participants of European descent [10], and three GWASs on gout (European: n=968 cases and 15,506 controls [11]; Japanese: n=945 cases and 1,213 controls, followed by 1,048 cases and 1,334 controls [12]), and replicated the results using a dataset from a GWAS with 1,396 cases and 1,268 controls from Caucasian and New Zealand Polynesian populations [13]. We used summary statistics from a GWAS based on 3,498 patients with osteoarthritis of the knee and hip in the arcOGEN study and 11,009 controls from the UK or European descent, as an outcome [14]. A two-sample MR study uses genetic variants associated with uric acid level or gout as

IVs to improve the inference. We obtained summary statistics (beta coefficients and standard errors [SE]) for single-nucleotide polymorphisms (SNPs) associated with uric acid level and gout as IVs from uric acid level and gout GWASs, and utilized the summary data of SNPs from osteoarthritis GWAS as an outcome. The ‘top hits’ from a GWAS can be used to define genetic instruments for exposures in MR analyses. The selection criteria for IVs were as follows; 1) Association p-values of SNPs with exposure should be less than a threshold of  $p < 2.04 \times 10^{-5}$  in GWAS dataset of exposure. 2) The SNPs from exposure dataset also should be present in GWAS dataset of outcome.

### Statistical analysis for Mendelian randomization

MR analysis requires genetic variants to be related to, but not potential confounders of, an exposure [15]. First, we assessed the independent association of SNPs with uric acid level or gout. Second, we examined the association between each SNP and the risk of osteoarthritis. Third, we combined these findings to estimate the uncompounded causal association between uric acid level or gout and osteoarthritis risk using MR analysis. We performed two-sample MR, which is a method used to estimate the causal effect of an exposure (uric acid level and gout) on outcomes (osteoarthritis) using summary statistics from different GWASs [16], to assess the causal relationships between uric acid level or gout and osteoarthritis risk.

The IVW method uses a meta-analysis approach to combine the Wald ratio estimates of the causal effect obtained from different SNPs, and provides a consistent estimate of the causal effect of the exposure on the outcome when each of the genetic variants satisfies the assumptions of an instrumental variable [17]. Although the inclusion of multiple variants in an MR analysis results in increased statistical power, it has the potential to include pleiotropic genetic variants that are not valid IVs [16]. To explore and adjust for pleiotropy (association of genetic variants with more than one variable), the weighted median and MR-Egger regression methods were used. MR-Egger regression analysis tests and accounts for the presence of unbalanced pleiotropy by introducing a parameter for this bias, through incorporation of summary data estimates of causal effects from multiple individual variants, making it robust to invalid instruments [18]. MR-Egger method performs a weighted linear regression of the gene-outcome coefficients on the gene-exposure coefficients [18]. The slope of this regression represents the causal effect

estimate, and the intercept can be interpreted as an estimate of the average horizontal pleiotropic effect across the genetic variants [19]. The weighted median estimator provides a consistent estimate of the causal effect, even when up to 50% of the information contributing to the analysis comes from genetic variants that are invalid IVs [20]. The weighted median estimator has the advantage of retaining greater precision in the estimates than those from the MR-Egger analysis [20]. Tests were considered statistically significant at  $p < 0.05$ . All MR analyses were performed on MR Base platform [21].

### Heterogeneity and sensitivity test

We assessed heterogeneities among SNPs using Cochran's Q-statistics and funnel plots [22]. We also performed a "leave one out" analysis to investigate the possibility that

the causal association was driven by a single SNP.

## RESULTS

### Studies included in the meta-analysis

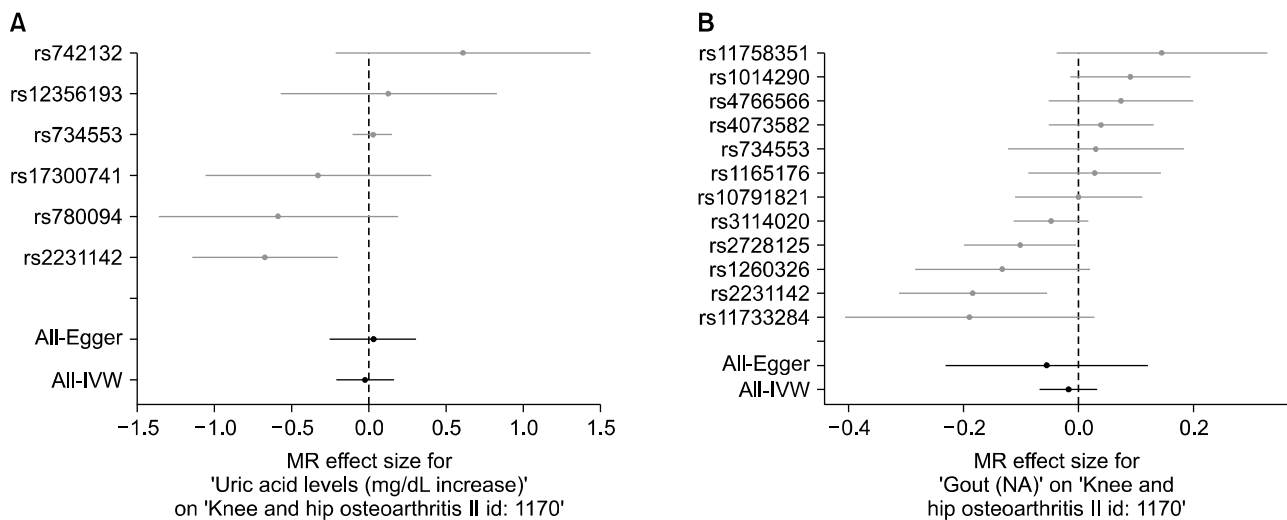
#### 1) Instrumental variables for Mendelian randomization

We selected six SNPs as IVs from the GWAS data on uric acid level and 12 SNPs from the GWAS data on gout as IVs to improve the inference (Table 1, Figure 1). Two of the IVs (rs2231142, rs734553) were associated with both uric acid level and gout (Table 1). The selected IVs were associated with uric acid level and gout at genome-wide significance, except for rs10791821, 11733284, and rs734553 in gout (Table 1).

**Table 1.** Instrumental SNPs from uric acid level (A) or gout (B) and osteoarthritis GWASs

A. Uric acid levels										
Instrumental SNP	Effect allele	Gene	Exposure (uric acid)			Outcome (osteoarthritis)				
			Beta	SE	p-value	Case (n)	Control (n)	Beta	SE	p-value
rs12356193	A	SLC16A9	0.080	0.014	$1.00 \times 10^{-8}$	3,498	11,009	0.010	0.029	0.657
rs17300741	A	SLC22A11	0.060	0.008	$7.00 \times 10^{-14}$	3,498	11,009	-0.020	0.022	0.259
rs2231142	T	ABCG2	0.140	0.022	$1.00 \times 10^{-10}$	3,498	11,009	-0.094	0.034	0.006
rs734553	T	SLC2A9	0.400	0.013	$1.00 \times 10^{-192}$	3,498	11,009	0.010	0.026	0.679
rs742132	A	SCGN	0.050	0.009	$9.00 \times 10^{-9}$	3,498	11,009	0.030	0.021	0.169
rs780094	T	GCKR	0.050	0.008	$1.00 \times 10^{-9}$	3,498	11,009	-0.030	0.020	0.190
B. Gout										
Instrumental SNP	Effect allele	Gene	Exposure (gout)			Outcome (osteoarthritis)				
			Beta	SE	p-value	Case (n)	Control (n)	Beta	SE	p-value
rs1014290	T	SLC2A9	0.451	0.042	$7.00 \times 10^{-26}$	3,498	11,009	0.041	0.024	0.075
rs10791821	G	MAP3K11	0.451	0.084	$1.00 \times 10^{-7}$	3,498	11,009	0.000	0.026	0.973
rs1165176	G	SLC17A1	0.351	0.057	$1.00 \times 10^{-9}$	3,498	11,009	0.010	0.021	0.717
rs11733284	A	NIPAL1	0.215	0.045	$9.00 \times 10^{-7}$	3,498	11,009	-0.041	0.024	0.099
rs11758351	G	HIST1H2BF	0.336	0.058	$2.00 \times 10^{-8}$	3,498	11,009	0.049	0.031	0.088
rs1260326	T	GCKR	0.307	0.043	$2.00 \times 10^{-12}$	3,498	11,009	-0.041	0.024	0.089
rs2231142	T	ABCG2	0.513	0.075	$3.00 \times 10^{-12}$	3,498	11,009	-0.094	0.034	0.006
rs2728125	C	ABCG2	0.713	0.046	$7.00 \times 10^{-54}$	3,498	11,009	-0.073	0.036	0.043
rs3114020	C	ABCG2	0.637	0.051	$9.00 \times 10^{-35}$	3,498	11,009	-0.030	0.021	0.193
rs4073582	G	CNIH-2	0.507	0.086	$6.00 \times 10^{-9}$	3,498	11,009	0.020	0.024	0.259
rs4766566	T	CUX2	0.412	0.046	$4.00 \times 10^{-20}$	3,498	11,009	0.030	0.026	0.201
rs734553	T	SLC2A9	0.329	0.065	$2.00 \times 10^{-7}$	3,498	11,009	0.010	0.026	0.652

SNP: single nucleotide polymorphism, GWAS: genome-wide association study, Beta: beta coefficient, SE: standard error, SLC16A9: Solute Carrier Family 16 Member 9, SLC22A11: Solute Carrier Family 22 Member 11, ABCG2: ATP Binding Cassette Subfamily G Member 2 (Junior Blood Group), SLC2A9: Solute Carrier Family 2 Member 9, SCGN: Secretagogen, EF-Hand Calcium Binding Protein, GCKR: Glucokinase Regulator, MAP3K11: Mitogen-Activated Protein Kinase Kinase Kinase 11, SLC17A1: Solute Carrier Family 17 Member 1, NIPAL1: NIPA-Like Domain Containing 1, HIST1H2BF: Histone Cluster 1 H2B Family Member F, CNIH-2: Cornichon Family AMPA Receptor Auxiliary Protein 2, CUX2: Cut-Like Homeobox 2.



**Figure 1.** Forrest plot of the causal effects of uric acid level (A) or gout (B)-associated SNPs on osteoarthritis. SNP: single nucleotide polymorphism, MR: Mendelian randomization, IVW: inverse-variance weighted, Na: not available.

**Table 2.** The MR estimates of the causal effect of uric acid level (A) and gout (B) on osteoarthritis risk, derived using different methods

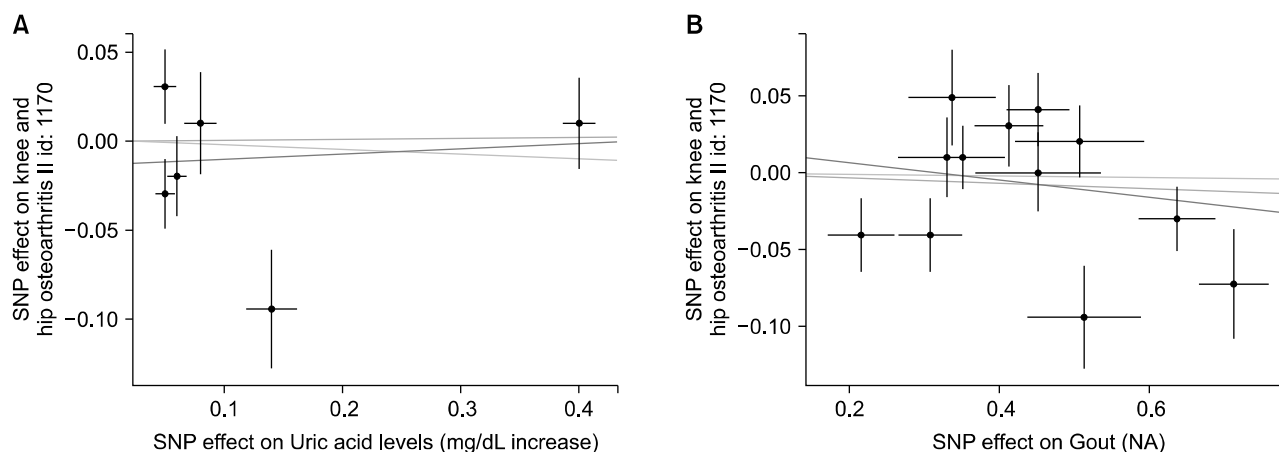
A. Uric acid level						
MR method	Number of SNP	Beta	SE	Association p-value	Cochran Q statistic	Heterogeneity p-value
Inverse variance weighted	6	-0.026	0.096	0.789	13.02	0.232
MR-Egger	6	0.028	0.142	0.852	12.09	0.166
Weighted median	6	0.004	0.064	0.946	na	na
B. Gout						
MR method	Number of SNP	Beta	SE	Association p-value	Cochran Q statistic	Heterogeneity p-value
Inverse variance weighted	12	-0.018	0.025	0.482	26.53	0.005
MR-Egger	12	-0.056	0.090	0.548	26.03	0.004
Weighted median	12	-0.005	0.025	0.834	na	na

MR: Mendelian randomization, SNP: single nucleotide polymorphism, Beta: beta coefficient, SE: standard error, na: not available.

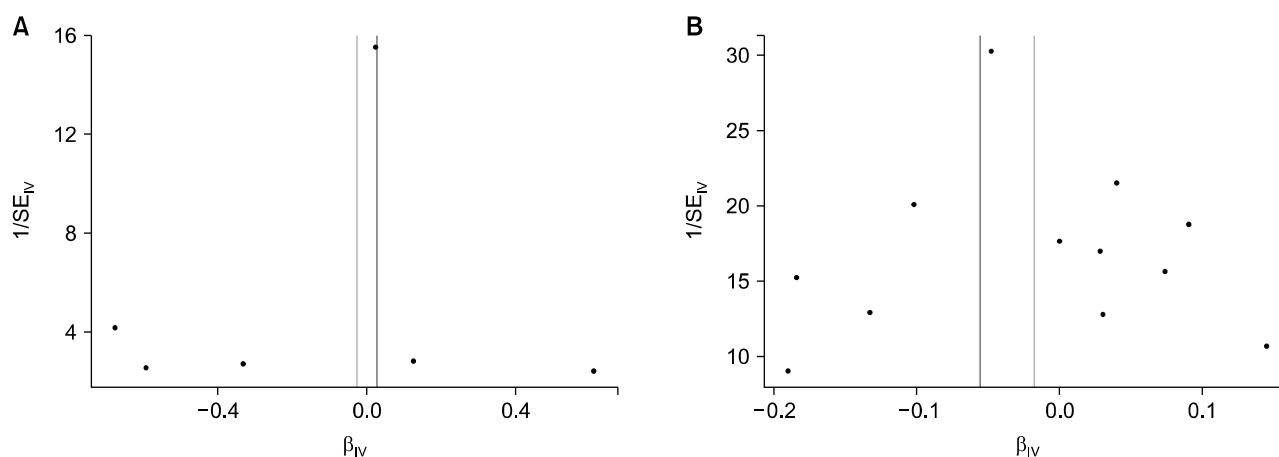
## 2) Mendelian randomization results

The IVW method yielded no evidence to support a causal association between uric acid level or gout and the risk of osteoarthritis (beta=-0.026, SE=0.096, p=0.789; beta=-0.018, SE=0.025, p=0.482) (Table 2, Figures 1, 2). In the MR-Egger test, the intercept represents the average pleiotropic effect across the genetic variants, i.e., the average direct effect of a variant on the outcome. If the intercept differs from zero, it serves as an evidence of directional pleiotropy. MR-Egger regression revealed that directional pleiotropy was unlikely to be biasing uric acid level (intercept=-0.013, p=0.610) or uric acid results (intercept=0.018, p=0.668), while yielding no causal as-

sociation between uric acid level or gout and the risk of osteoarthritis (beta=0.028, SE=0.142, p=0.852; beta=-0.056, SE=0.090, p=0.548) (Table 2, Figures 1, 2). In addition, the weighted median approach provided no evidence of a causal association between uric acid level or gout and the risk of osteoarthritis (beta=0.004, SE=0.064, p=0.946; beta=-0.005, SE=0.025, p=0.843) (Table 2, Figures 1, 2). We performed subgroup analysis using exposure dataset of European. However, the MR result from European did not change significantly the overall MR result.



**Figure 2.** Scatter plots of genetic associations of uric acid level (A) or gout (B) against the genetic associations of osteoarthritis. The slopes of each line represent the causal association for each method. Blue line represents the IVW estimate, green line represents the weighted median estimate, and dark blue line represents the MR-Egger estimate. IVW: inverse-variance weighted, SNP: single nucleotide polymorphism, MR: Mendelian randomization, Na: not available.



**Figure 3.** Funnel plot to assess the heterogeneity of the causal effects of uric acid level (A) or gout (B)-associated SNPs on osteoarthritis. Blue line represents the IVW estimate, and dark blue line represents the MR-Egger estimate. SNP: single nucleotide polymorphism, IVW: inverse-variance weighted, MR: Mendelian randomization, SE: standard error,  $\beta$ : beta coefficient.

### 3) Heterogeneity and sensitivity test

Cochran's Q-test indicated no evidence of heterogeneity between IV estimates based on the individual variants of uric acid level, but not gout (Table 2). The funnel test yielded symmetry, indicating no evidence of heterogeneity in the MR analyses of uric acid level and gout (Figure 3). Results from the "leave one out" analysis demonstrated that no single SNP was driving the IVW point estimate.

## DISCUSSION

Hyperuricemia and gout have been hypothesized to be associated with the development of osteoarthritis [23].

However, it remains unclear whether hyperuricemia and gout have a causal relationship with osteoarthritis, particularly because the previously reported associations between hyperuricemia or gout and osteoarthritis may be affected by the bias or confounding factors inherent to observational studies, reverse causation, limited number of studies with small sample sizes, and selection bias [24,25]. To investigate this, we employed three different estimation methods (inverse variance weighting method, weighted median method, and MR-Egger regression) for the MR analyses. The results of these methods were consistent with each another, yielding no causal associations between uric acid level or gout and osteoarthritis.

MR eliminates, or at least minimizes, the possibility of

the bias inherent to observational studies due to residual confounding or reverse causality [26]. The assumption in MR is that the genetic variant associated with exposure is not associated with confounding variants and that the genetic variant influences the outcome only through the risk factor, thus, implying a causal effect of exposure on the outcome [27]. MR is a powerful tool for dissecting causal relationships between exposures and outcomes, owing to its minimization of residual confounding. However, MR studies are susceptible to bias resulting from pleiotropy [28]. Genetic variants may be associated with multiple phenotypes, a phenomenon known as ‘pleiotropy’, which may cause a confounded estimate from MR, potentially leading to biased causal estimates [29]. Although the inclusion of multiple variants in MR analysis typically leads to increased statistical power, it can also potentially lead to the inclusion of pleiotropic genetic variants that are not valid IVs [30]. Therefore, sensitivity analysis is needed to test the validity of conclusions from MR analysis. To eliminate pleiotropy, we employed a weighted median estimator, which provides valid estimates even if 50% of the SNPs are not valid instruments [20], and MR-Egger regression, which tests for unbalanced pleiotropy and estimates the causal effect of an exposure on an outcome [18]. Based on the intercept P-values obtained in this study, the MR-Egger approach showed no evidence of unbalanced pleiotropy. Use of the MR-Egger method may result in loss of precision and power, but the results of this analysis are supported by the results of the weighted median estimator analysis and similar to the IVW estimates, thus providing additional confidence in these findings. Genetic variants in MR are treated similarly to studies in a meta-analysis. Each genetic variant contributes an estimate of the causal effect and a pooled estimate is calculated based on all the genetic variants using a formula from the meta-analysis in MR. Thus, pooled estimate from MR is considered more accurate than estimate from each genetic variant by increasing the number of instruments, although the finding that the individual SNPs in ABCG2 are associated with OA are not negligible.

Although observational studies have provided evidence of association between uric acid level or gout and osteoarthritis [5,6], the results of our MR analysis suggest otherwise. Instead, the findings of the present study suggest that previous observational epidemiological associations may have been the result of confounding or reverse causation. In addition, our findings indicate the need for

investigations into discrepancies between observational evidence and the absence of causal evidence. Such discrepancies could be accounted for by residual confounds in observational studies, which are unable to fully control for it.

The present study has several limitations. First, our analysis utilized a relatively small number of SNPs as IVs and may have limited power for detecting associations. The six SNPs associated with uric acid level explain only a relatively small proportion of the variation in vitamin D concentration [31]. This limits the statistical power for detection of a weak association between genetically predicted uric acid levels and osteoarthritis. The statistical power can be increased and a more precise causal estimate can be obtained by combining multiple genetic variants [32]. Second, the osteoarthritis data were from participants of European ancestry. As causality may depend on ethnicity, further MR studies based on other populations are needed. Third, only the osteoarthritis of knee and hip were included in this MR analysis. Osteoarthritis in other sites needs to be further explored. Fourth, since prevalence of gout and osteoarthritis are quite different according to sex, consideration of sex is needed. Unfortunately, we could not conduct the MR analysis based on sex, because the data from exposure and outcome did not provide the according to gender. Nevertheless, this meta-analysis has some strengths. First, although the uric acid level has been studied as a potential biomarker for osteoarthritis, to our knowledge, an MR analysis has never been performed. Therefore, this is the first MR study investigating the potential role of uric acid and gout in the development of osteoarthritis, yielding no association between genetically predicted uric acid level or gout and osteoarthritis. Second, using a large genetic data of osteoarthritis risk (consisting of 3,498 patients with osteoarthritis of the knee and hip and 11,009 controls) enabled us to test our study hypothesis more precisely, which would have not been possible with individual-level data from a smaller study.

## CONCLUSION

In conclusion, the results of our MR analysis demonstrates that uric acid level and gout may be not causally associated with the increased risk of osteoarthritis. Considering MR study is not susceptible to bias from unmeasured confounders or reverse causation, the epidemiological evidence for an association between uric

acid level or gout and a higher risk of osteoarthritis may be due to residual confounding or reverse causation rather than direct causality. Well-designed epidemiological and MR studies using more variants that explain a greater proportion of uric acid level or gout are warranted to confirm or rule out its causal relationship with osteoarthritis.

## CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported..

## AUTHOR CONTRIBUTIONS

Y.H.L. was involved in conception and design of study, acquisition of data, analysis and interpretation of data, drafting the manuscript, and revising the manuscript. G.G.S. was involved in conception and design of study, analysis and interpretation of data, and drafting the manuscript.

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