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ORIGINAL ARTICLE

Assessing the causal association between smoking behavior and risk of gout using a Mendelian randomization study

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Abstract

This study aimed to examine whether smoking behavior is causally related to gout. Summary statistics of publicly available data from genome-wide association studies (GWAS) of smoking behavior ($n = 85{,}997$) served as the exposure dataset, while meta-analysis results of 14 studies including 2115 cases and 67,259 controls of European descent served as the outcome dataset. The data were subjected to two-sample Mendelian randomization (MR) analysis using the inverse-variance weighted (IVW), weighted median, and MR-Egger regression methods. Five single-nucleotide polymorphisms (SNPs) from GWAS of smoking behavior were selected as instrumental variables (IVs) to improve inference: *CHRNA3* (rs1051730), *PDE1C* (rs215614), *CYP2A6* (rs4105144), *CHRNB3* (rs6474412), and *CYP2B6* (rs7260329). The IVW data did not support a causal association between smoking behavior and gout (beta = − 0.035, SE = 0.036, $p = 0.333$). MR-Egger regression indicated that directional pleiotropy did not bias the result (intercept = 0.021; $p = 0.560$). MR-Egger analysis revealed no causal association between smoking behavior and gout (beta = − 0.074, SE = 0.070, $p = 0.366$). The weighted median approach did not support a causal association between smoking behavior and gout (beta = − 0.043, SE = 0.040, $p = 0.279$). Cochran's Q test indicated no evidence of heterogeneity between IV estimates based on individual variants. The results of "leave one out" analysis demonstrated that no single SNP drove the IVW point estimate. MR estimates using IVW, weighted median, and MR-Egger analysis were consistent and did not support a causal inverse association between smoking behavior and gout.

Keywords Gout · Mendelian randomization · Smoking

Introduction

Gout is an inflammatory disorder characterized by hyperuricemia and deposition of monosodium urate crystals in intra-articular and peri-articular locations, resulting in episodic gout flares, gouty arthropathy, tophi formation, and urolithiasis [1]. Although the etiology of gout is not fully understood, it is clear that gout has both genetic and environmental components [2, 3]. The primary cause of gout is hyperuricemia due to excess urate production or impaired renal excretion of uric acid. Increased levels of hyperuricemia are correlated with a higher incidence of gout [4].

Many environmental factors have been suggested to induce gout [2]. Hyperuricemia can be influenced by risk factors such as age, obesity, sex, hypertension, kidney disease, alcohol intake, and cigarette smoking [2]. Among these factors, smoking has been reported to have a potentially protective association with gout, although the evidence is inconsistent [5–7]. Smoking is considered to impact the immune system and may have both pro-inflammatory and anti-inflammatory effects [8]. Smoking is associated with lower serum urate concentrations, possibly by inactivating xanthine oxidase via the cyanides in cigarettes [9]. However, observational studies are prone to bias such as reverse causation interpretations and residual confounding, preventing a clear understanding of the effect of smoking on gout [10, 11]. Cigarette smoking is correlated with lower body mass, alcohol intake, and higher prevalence of hypertension, dyslipidemia, and kidney disease, which can influence the risk of gout [12–14].

Mendelian randomization (MR) is a technique that uses genetic variants as instrumental variables (IVs) to assess whether an observational association between a risk factor

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and outcome is consistent with a causal effect [15]. MR analysis can be used to evaluate whether genetically increased smoking behavior is associated with a decreased risk of gout. Two-sample MR estimates causal effects between datasets for the exposure and outcome variable measured in independent samples, which is a very useful method for situations in which it is difficult to measure the exposure and outcome in the same set of individuals [16]. However, MR has not been applied to explore causal effects of smoking on gout risk. Therefore, the aim of the present study was to examine whether smoking behavior is causally associated with gout using two-sample MR analysis.

Materials and methods

Data sources and selection of genetic variants

We searched the NHGRI-EBI GWAS catalog (https://www.ebi.ac.uk/gwas/), which is a comprehensive catalog of reported associations from published genome-wide association studies (GWAS). As the exposure dataset, we used publicly available summary statistics (total $n = 85{,}997$) of GWAS meta-analyses to determine the association of the number of cigarettes smoked per day (CPD) in smokers ($n = 31{,}266$) and smoking initiation ($n = 46{,}481$) using samples from the ENGAGE Consortium, with replication in the Tobacco and Genetics and Glaxo Smith Kline consortia cohorts ($n = 45{,}691$ smokers), and a third sample of European ancestry ($n = 9040$) [17]. The outcome dataset included summary statistics datasets of the meta-analysis of gout comprising 14 studies including 2115 cases and 67,259 controls of European ancestry [18]. A two-sample MR study using genetic variants associated with gout as IVs was also performed to improve inference. We obtained summary statistics (beta coefficients and standard errors) for five single-nucleotide polymorphisms (SNPs) associated with smoking behavior as IVs from smoking behavior GWAS [17]. Additionally, we utilized summary data for five SNPs from gout GWAS as the outcome dataset [19].

Statistical analysis for MR

MR analysis requires genetic variants that may be related to, but are not potential confounders of, an exposure variable [20]. First, we assessed the independent association of four SNPs with smoking behavior. Second, we examined the association between each SNP and the risk of gout. Third, we combined these findings to estimate the uncompounded causal association between smoking behavior and gout risk by MR analysis. As described above, we performed two-sample MR to estimate the causal effect of exposure (smoking) on the outcome (gout) using summary statistics from different GWAS [21] to assess the causal relationships between smoking behavior and gout risk, using summary data from smoking behavior and gout GWAS with five SNPs as IVs.

The inverse variance-weighted (IVW) method uses a meta-analysis approach to combine Wald ratio estimates of the causal effect obtained from associations of different SNPs and provides a consistent estimate of the causal effect of exposure on the outcome when each genetic variant satisfies the assumptions of an IV [22]. Although including multiple variants in MR analysis results in increased statistical power, pleiotropic genetic variants that are not valid IVs may also be included [21]. To explore and adjust for such pleiotropy effects (i.e., association of genetic variants with more than one variable), weighted median and MR-Egger regression methods were performed. MR-Egger regression analysis tests and accounts for the presence of unbalanced pleiotropy by introducing a parameter for this bias by incorporating summary data estimates of the causal effects with multiple individual variants, and has been shown to be robust against invalid instruments [23]. The MR-Egger procedure performs weighted linear regression of the gene-outcome coefficients on gene-exposure coefficients [23]. 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 all genetic variants [24]. The weighted median estimator then provides a consistent estimate of the causal effect, even when up to 50% of the information contributing to the analysis is derived from genetic variants that are invalid IVs [25]. Use of the weighted median estimator increases the precision of the estimates compared to MR-Egger analysis [25]. All tests were considered statistically significant at $p < 0.05$. All MR analyses were performed using the MR-Base platform [26].

Heterogeneity and sensitivity testing

We assessed heterogeneities among SNPs using Cochran's Q-statistics and funnel plots [27]. We also performed a "leave one out" analysis to investigate whether the causal association observed was driven by a unique SNP. Additionally, subgroup analysis was performed using only IV SNPs at a genome-wide significance level for the sensitivity test.

Results

Studies included in the meta-analysis

Instrumental variables for MR

We selected five SNPs from smoking behavior GWAS as IVs [17]. These SNPs were in the genes *CHRNA3* (rs1051730), *PDE1C* (rs215614), *CYP2A6* (rs4105144), *CHRNB3* (rs6474412), and *CYP2B6* (rs7260329)

Table 1 Instrumental SNPs from smoking behavior and gout GWAS

Instrumental SNP	Effect allele	Gene	Exposure (smoking behavior)				Outcome (gout)					
			Unit	Beta	SE	*p* value	Case	Control	Unit	Beta	SE	*p* value
rs1051730	A	*CHRNA3*	CPD increase	0.800	0.051	2.00E−69	2115	67,259	log odds	−0.033	0.034	0.340
rs215614	G	*PDE1C*	CPD increase	0.220	0.041	2.00E−07	2115	67,259	log odds	0.020	0.033	0.558
rs4105144	C	*CYP2A6*	CPD increase	0.390	0.061	2.00E−12	2115	67,259	log odds	−0.023	0.043	0.599
rs6474412	T	*CHRNB3*	CPD increase	0.290	0.051	1.00E−08	2115	67,259	log odds	−0.030	0.040	0.463
rs7260329	G	*CYP2B6*	CPD increase	0.200	0.041	6.00E−06	2115	67,259	log odds	0.015	0.036	0.679

SNP, single-nucleotide polymorphism; *GWAS*, genome-wide association studies; *Beta*, beta coefficient; *SE*, standard error; *CHRNA3*, cholinergic receptor nicotinic alpha 3 subunit; *PDE1C*, phosphodiesterase 1C; *CYP2A6*, cytochrome P450 family 2 subfamily A member 6; *CHRNB3*, cholinergic receptor nicotinic beta 3 subunit; *CYP2B6*, cytochrome P450 family 2 subfamily B member 6; *CPD*, cigarettes smoked per day

(Table 1, Fig. 1). Except for rs215614 and rs7260329, the SNPs were found to be associated with smoking behavior at a genome-wide significance level and were inversely associated with gout. However, none of these SNPs were nominally significantly associated with gout (Table 1).

Mendelian randomization results

The IVW method showed no evidence to support a causal association between smoking behavior and gout (beta = −0.035, SE = 0.036, $p = 0.333$) (Table 2, Figs. 1 and 2). The intercept represents the average pleiotropic effect

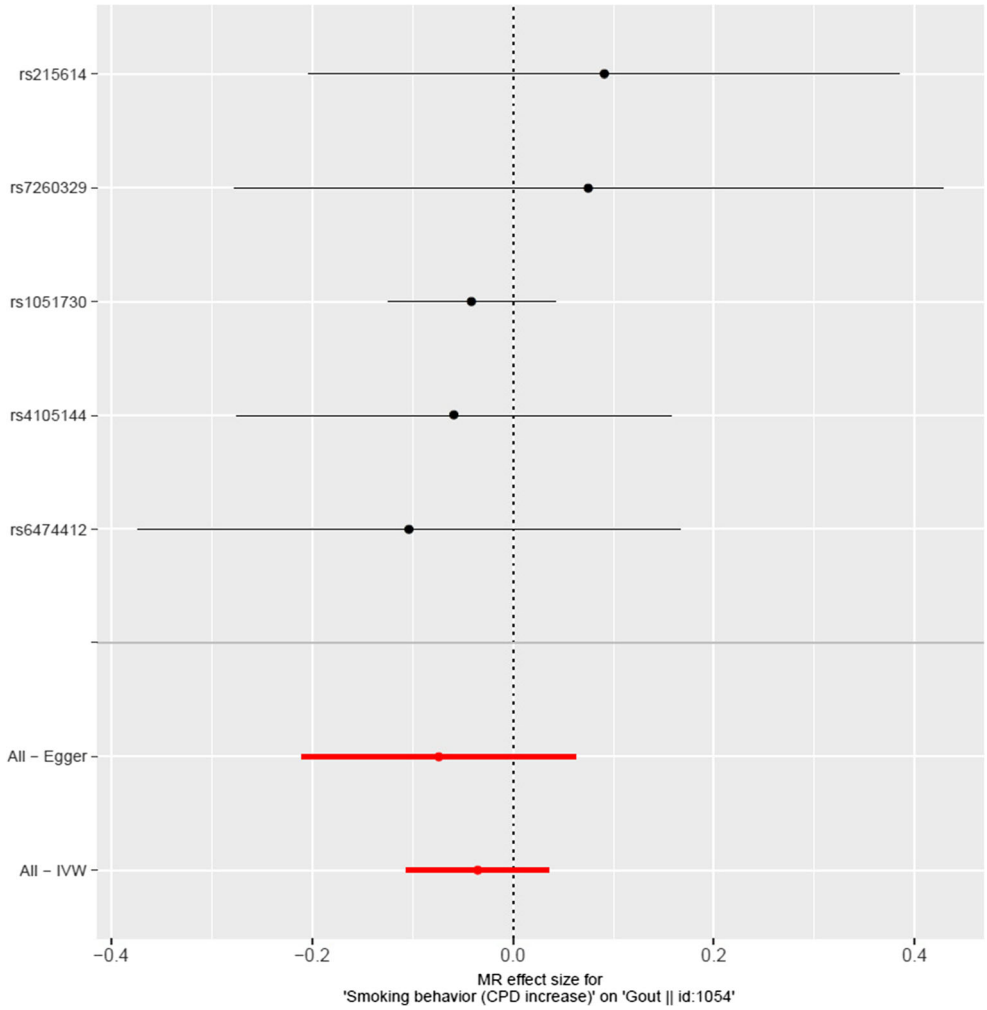

Fig. 1 Forest plot of the causal effects of smoking behavior-associated single-nucleotide polymorphisms on gout

Table 2 MR estimates from each method of examining the causal effect of smoking behavior on gout risk

MR method	Number of SNPs	Beta	SE	Association *p* value	Cochran Q statistic	Heterogeneity *p* value
Inverse variance weighted	5	− 0.035	0.036	0.333	1.045	0.903
MR Egger	5	− 0.074	0.070	0.366	0.966	0.809
Weighted median	5	− 0.043	0.040	0.279	1.391*	0.846*

MR, Mendelian randomization; *SNP*, single-nucleotide polymorphism; *Beta*, beta coefficient; *SE*, standard error
*Maximum-likelihood method

across the genetic variants (average direct effect of a variant with the outcome). An intercept differing from zero (MR-Egger test) is evidence of directional pleiotropy. MR-Egger regression revealed that directional pleiotropy was unlikely to have biased the result (intercept = 0.021; $p = 0.560$). MR-Egger analysis revealed no causal association between smoking behavior and gout (beta = − 0.074, SE = 0.070, $p = 0.366$) (Table 2, Figs. 1 and 2). The weighted median approach yielded no evidence of a causal association between smoking behavior and gout (beta = − 0.043, SE = 0.040, $p = 0.279$) (Table 2, Fig. 2). The MR estimates determined using the IVW, weighted median, and MR-Egger regression methods were consistent. Therefore, the MR analysis results did not support a causal inverse association between smoking behavior and gout.

Heterogeneity and sensitivity tests

Cochran's Q test indicated no evidence of heterogeneity among the IV estimates based on the individual variants (Table 2). The results of "leave one out" analysis demonstrated that no single SNP was driving the IVW point estimate for gout (Fig. 3). Additionally, when the MR analysis was limited to only three SNPs (rs1051730, rs4105144, and rs6474412) at the genome-wide significance level, the results remained non-significant (IVW beta = − 0.048, SE = 0.038, $p = 0.207$).

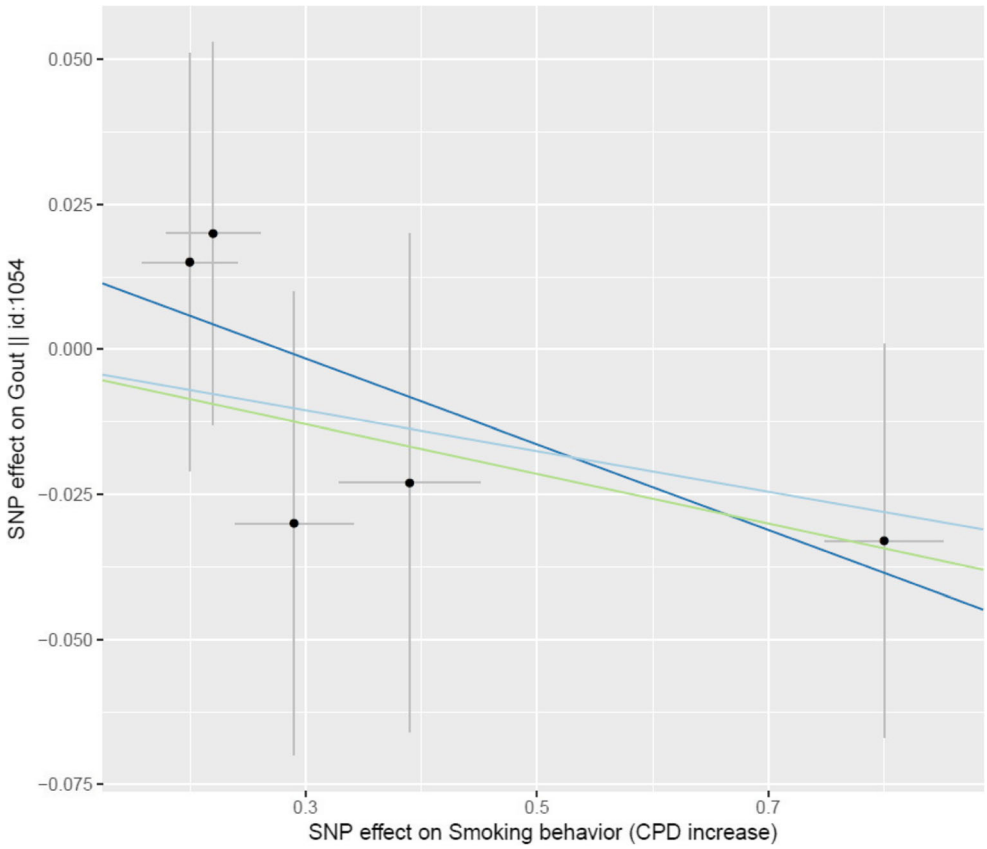

Fig. 2 Scatter plots of genetic associations with smoking behavior against the genetic associations with gout (gout). The slopes of each line represent the causal association for each method. The blue line represents the inverse variance-weighted (IVW) estimate, the green line represents the weighted median estimate, and the dark blue line represents the MR-Egger estimate

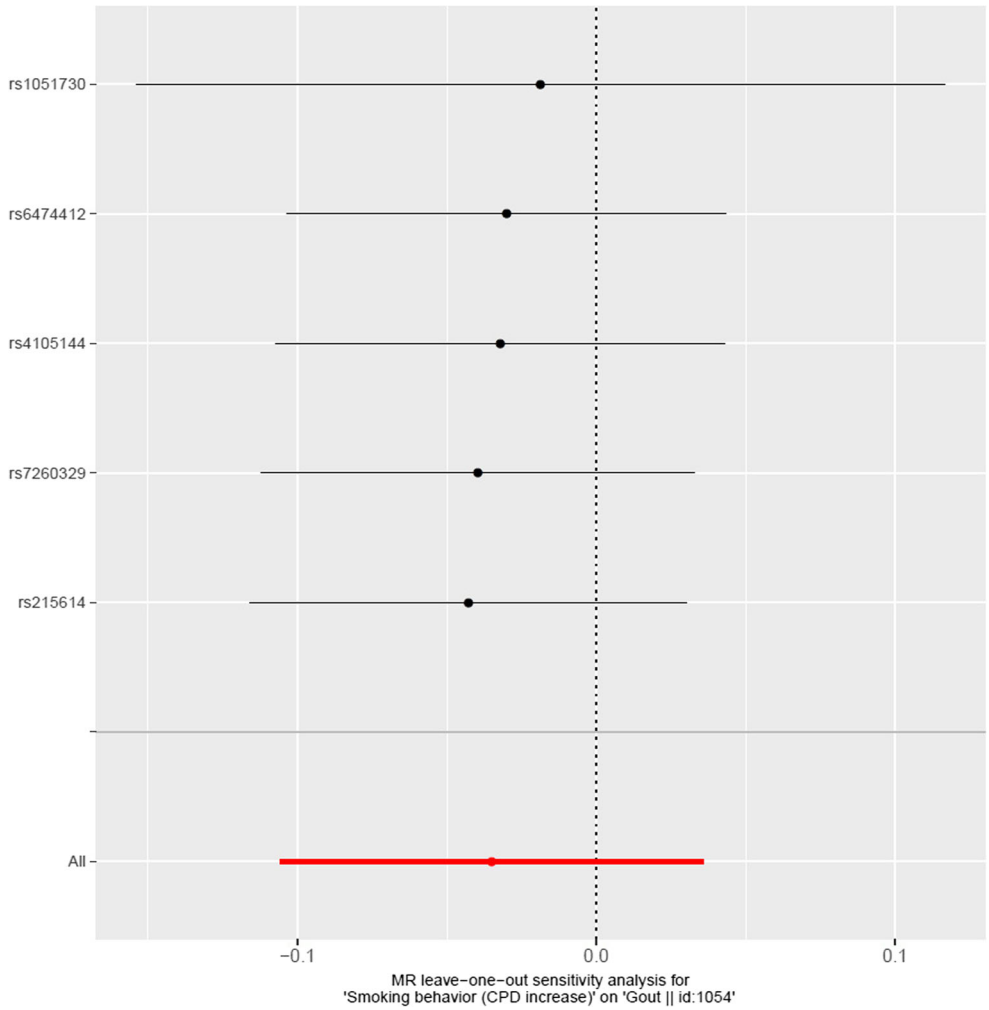

Fig. 3 "Leave one out" analysis to investigate whether the causal association was driven by a unique single-nucleotide polymorphism (SNP)

Discussion

Epidemiologic studies suggested that smoking is associated with a lower risk of gout in men, although there are also conflicting findings [6, 7]. It is possible that smoking impacts serum urate and gout risk via mechanisms in which cigarette smoking causes oxidative stress, leading to the depletion of uric acid, which is one of the most important antioxidant scavengers of reactive oxygen species [28], and/or that cyanide in smoking may inhibit xanthine oxidase for urate formation in purine metabolism [9]. The causal relationship between smoking and gout remains unclear because previously reported associations may have been influenced by biases or confounding factors inherent to observational studies, such as reverse causation interpretation, a low number of studies of small sizes, and selection bias [10, 29]. Thus, to clarify this association, we evaluated causality using three different estimation methods (IVW method, weighted median method, and MR-Egger regression) for MR analyses. Our study did not indicate that the inverse associations between gout and Alzheimer's disease are causal. The MR estimates using IVW, MR Egger, and weighted median analysis were consistent and do not support a causal inverse association between smoking behavior and gout.

MR minimizes the possibility of the bias inherent to observational studies due to residual confounding or reverse causality [30]. However, MR studies are susceptible to bias from pleiotropy (association of genetic variants with more than one variable) [31]. Although including multiple variants in MR analysis typically leads to increased statistical power, it also may include pleiotropic genetic variants that are not valid IVs [32]. Therefore, appropriate sensitivity analysis is essential for testing the validity of any conclusions drawn from an MR study. Here, to eliminate the influence of pleiotropy, we employed a weighted median estimator, which provides valid estimates even if 50% of the SNPs are not valid instruments [25]. We also used MR-Egger regression to test for unbalanced pleiotropy and determine a causal estimate of the influence of exposure on the outcome [23]. Our results using all three approaches were consistent, and the MR-Egger approach showed no evidence of unbalanced pleiotropy as indicated by the intercept p value. The MR-Egger method results

in a loss of precision and power, but our weighted median estimator results were similar to the IVW estimator, providing additional confidence in these findings.

The present study has several limitations. First, our analysis included a relatively small number of SNPs as IVs and thus may have had limited power in detecting an association. The statistical power can be increased and a more precise causal estimate can be obtained by combining multiple genetic variants [33]. Second, genetic variants have only a modest effect on a given exposure (smoking behavior) because they may only explain a very small proportion of the overall variance in a particular exposure [34]. Thus, very large numbers of cases are required to accurately detect a causal relationship for the outcome of interest. Third, it is important to ensure that there is a strong relationship between the genetic variant and an exposure. "Weak instrument bias," which refers to a genetic variant that does not have a sufficiently strong association with the exposure, may affect MR analysis [35]. However, the sensitivity test performed to limit our analysis to SNPs at a genome-wide significance level did not change the results. Fourth, the gout dataset was based on a study that included participants of European ancestry. As causality may depend on ethnicity and selection bias, further MR studies of other populations are needed. Fifth, epidemiologic data showed that cigarette smoking is associated with a decreased risk of gout, particularly among men [6, 7]. The association between cigarette smoking and gout is not discernible among women. We could not analyze our MR based on gender. Nevertheless, this meta-analysis also has strengths. Although smoking has been investigated as a potential risk factor for gout, this is the first MR to assess this association. It is important that estimates of both gene-exposure and gene-outcome associations are available for each variant considered. Thus, this is the first study to determine the causal relationship between smoking behavior and gout.

In conclusion, the MR analysis results do not support inverse causal associations between smoking behavior and gout development. Epidemiological evidence for a relationship between smoking behavior and decreased risk of gout does not appear to be causal. However, we cannot rule out small associations. Well-designed epidemiological studies and MR studies using more variants that explain a greater proportion of smoking behaviors are warranted to confirm or rule out the causal relationship.

Compliance with ethical standards

Disclosures None.

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