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Abstract: Abstract
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Findings: In meta-analysis of 17 prospective observational studies (166 486 individuals; 9 784 CHD events) a 1 standard deviation (SD) higher

urate concentration was associated with an odds ratio (OR) for CHD of 1·07 (95% confidence interval [CI], 1·04, 1·10) after adjustment. The corresponding OR estimates from the conventional, multivariable adjusted, and Egger MR analysis (198 598 individuals; 65 877cases; 58 studies) were 1·18 (95%CI: 1·08, 1·29), 1·10 (95%CI, 1·00, 1.22), and 1.05 (95%CI: 0.92, 1.20) respectively, per 1-SD increment in plasma urate.
Interpretation: Conventional and multivariate MR analysis implicates a causal role for urate in the development of CHD, but these estimates may be inflated by hidden pleiotropy. MR-Egger, which has less statistical power, but accounts for hidden pleiotropy suggests the true effect of urate on CHD could include the null. These results may help investigators determine the priority of trials of urate lowering for CHD prevention as compared to other potential interventions.
Funding: The UCLEB consortium is supported by funding from NIHR, BHF, and MRC.

Plasma urate and coronary heart disease: Mendelian randomisation analysis.

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Abstract

***Background*:** Higher circulating plasma urate concentration is associated with an increased risk of coronary heart disease (CHD), but the extent of any causative influence of urate on CHD risk is still unclear.

***Methods*:** We first conducted a fixed effects meta-analysis of the observational association of plasma urate and risk of CHD. We then used a conventional Mendelian randomisation (MR) approach to investigate the causal relevance using a genetic instrument based on 31 urate-associated single nucleotide polymorphisms (SNPs). To account for potential pleiotropic associations of certain SNPs with risk factors other than urate, we additionally conducted both a multivariable MR analysis in which the genetic associations of SNPs on systolic and diastolic blood pressure, high density lipoprotein-cholesterol and triglycerides were included as covariates, and MR-Egger to estimate a causal effect accounting for unmeasured pleiotropy. The analyses utilised data from 347 195 individuals in 134 studies, including 65 877 CHD cases.

***Findings*:** In meta-analysis of 17 prospective observational studies (166 486 individuals; 9 784 CHD events) a 1 standard deviation (SD) higher urate concentration was associated with an odds ratio (OR) for CHD of 1·07 (95% confidence interval [CI], 1·04, 1·10) after adjustment. The corresponding OR estimates from the conventional, multivariable adjusted, and Egger MR analysis (198 598 individuals; 65 877cases; 58 studies) were 1·18 (95%CI: 1·08, 1·29), 1·10 (95%CI, 1·00, 1.22), and 1.05 (95%CI: 0.92, 1.20) respectively, per 1-SD increment in plasma urate.

Interpretation: Conventional and multivariate MR analysis implicates a causal role for urate in the development of CHD, but these estimates may be inflated by hidden pleiotropy. MR-Egger, which has less statistical power, but accounts for hidden pleiotropy suggests the true effect of urate on CHD could include the null. These results may help investigators determine the priority of trials of urate lowering for CHD prevention as compared to other potential interventions.

Funding: The UCLEB consortium is supported by funding from NIHR, BHF, and MRC.

Introduction

Plasma urate is a circulating product of human purine metabolism synthesised from hypoxanthine and xanthine by the action of the enzyme xanthine oxidoreductase. With extreme elevations in urate concentration, monosodium urate crystals are deposited in the joints, soft tissue and renal parenchyma causing acute inflammatory arthropathy (gout), gouty tophi, and nephropathy, respectively.[1] While the causal role of higher circulating urate concentrations in gout has been demonstrated by Mendelian randomisation analysis,[2] (and urate lowering is the principal treatment), the role of urate in CHD has been under debate since the 19th century.[3]

Patients with established coronary heart disease (CHD) exhibit elevated levels of plasma urate compared with individuals free of disease. Furthermore, elevated plasma urate is associated with increased risk of incident CHD.[4]

Beneficial and deleterious actions of urate on the cardiovascular system are reported, making the role of urate in atherosclerosis unclear. Urate ions have potentially atheroprotective, free-radical scavenging properties and infusion of urate may correct endothelial dysfunction.[5] However, pro-atherogenic effects of urate have also been described, including induction of cellular oxidative stress leading to attenuated nitric oxide bioavailability, linked to platelet and endothelial cell activation, and vascular smooth muscle proliferation.[6]

A higher urate concentration is associated in population studies with several CHD risk factors including high blood pressure, elevated body mass index (BMI), type 2 diabetes, reduced HDL-cholesterol (HDL-C), and higher concentrations of triglycerides (TG) and LDL-cholesterol (LDL-C).[4] However, whether these variables confound, or mediate the association of urate with CHD is uncertain (Figure 1). Statistical adjustment for these variables in prospective observational studies attenuates the association of urate with CHD.[4] Whether residual confounding results in over-estimation or whether the effect is underestimated because some of the variables are mediators remains unknown.

Randomised trials provide some evidence that allopurinol (a urate lowering therapeutic) has beneficial effects on intermediate cardiovascular end-points including endothelial function, angina symptoms, blood pressure, left ventricular mass, and exercise capacity. Allopurinol acts through inhibition of xanthine oxidoreductasae which also reduces the generation of reactive oxygen species, which are formed as a by-product of the metabolism of xanthine and hypoxanthine to urate.[7,8] Therefore, it remains unclear whether any benefits of allopurinol on these end-points are due to urate lowering, inhibition of free-radical generation or both. Moreover, no trial, with any urate lowering agent has yet reported an effect on clinically relevant cardiovascular end-points, [9] although a trial of this type is ongoing (http://www.isrctn.com/ISRCTN32017426*).*

We estimated the extent of any causal relationship between urate and CHD risk using Mendelian randomisation (MR) [10]. MR exploits the random allocation of genetic variants from parents to offspring at gametogenesis, protecting genotype to phenotype associations from the usual sources of confounding seen in observational studies and from reverse causation. Providing certain assumptions are met, where a genetic variant (or variants) associate with both biomarker (e.g. urate) and with CHD risk in an Instrumental Variable (IV) regression, this supports a causal role for the biomarker in CHD.[10]

Although MR protects against many of the confounding factors that bedevil observational analysis, MR is potentially confounded by pleiotropy (the situation where variation in a gene associates with multiple phenotypes). Pleiotropy may be 'vertical'; the gene influences more than one point in the same causal pathway, or 'horizontal'; the gene influences more than one independent causal pathway. Whereas vertical pleiotropy does not breach the assumptions of MR, unmeasured horizontal pleiotropy can lead to entirely spurious conclusions about causality.

Two methods have been proposed to address horizontal pleiotropy, the first simply includes the effect of the instrument on the pleiotropic factor as a covariate in the MR analysis (termed multivariable MR, MVMR).[11] The second uses Egger regression to account for the more general

case where there is a net pleiotropic effect on the instrument from multiple unmeasured sources (termed MR-Egger).[12]

We selected a set of single nucleotide polymorphisms (SNPs) identified from genome wide association studies (GWAS) that associated with urate concentration. Using these SNPs we constructed a genetic instrument,[13] and conducted conventional MR (unadjusted for pleiotropy). To account for pleiotropy, we then conducted MVMR and MR-Egger.

Methods

We identified a range of datasets to address the research question, focusing on those with participants reported to be predominantly of European descent (see original references for detail).

Observational association between urate and CHD events and risk factors.

We used fixed effects meta-analysis of study summary estimates to update the observational study by Wheeler et al.[4] by the addition of 326 myocardial infarction/coronary revascularisation cases and 1618 controls from the British Women's Health and Heart Study (BWHHS), which was the only study available to the UCLEB consortium[14] with suitable data (that had not already contributed to the report by Wheeler). This gave a combined observational dataset of 17 studies, 166 486 individuals, and 9 784 CHD events in all. Analyses were conducted without adjustment for renal function.

To estimate the observational association between urate and several CHD risk factors, including body mass index (BMI), creatinine, blood pressure, glucose, HDL-C, LDL-C, total cholesterol (TC), and TG, we assimilated (by fixed effects meta-analysis) data from UCLEB with studies that contributed to the analysis by Wheeler *et al.*[14,4] (Table S1 (page2), Table S2 (page3)).

Development of a genetic instrument for urate.

To generate a genetic instrument for urate concentration, we searched for SNPs from the GWAS catalogue (http://www.genome.gov/gwastudies, accessed 18th Feb 2015) associated with urate

concentration. We identified 31 independent loci (R^2<0.3; separated by > 140kb) that had associations with urate at P<$5x10^{-7}$ (Table 1). Where the P-value was greater than $5x10^{-8}$, inclusion was only on the basis of a clear functional role in urate metabolism (this applied to only one SNP, rs164009, which has the GRAIL (Gene Relationships Across Implicated Loci)[15] identified gene PRPSAP1). In all cases the SNP association had been replicated in studies conducted mainly in populations of European ancestry and effect sizes were taken from published meta-analyses. For each locus we recorded the published effect size and the standard error (SE) for the lead SNP (SNP with strongest association in the largest dataset) . Where possible we collected effect estimates for the lead SNP, or a suitable proxy, from additional publications (Table S1 (page 2) - S3 (pages 2-5)) and combined the estimates for a SNP by fixed effects meta-analysis. Details of lead SNPs and putative genes are given in Table 1. We note that an almost identical set of loci was used as an instrument for urate with a reported R^2 of ~ 4.2% in the Rotterdam Study (n = 5791).[16]The 31 selected SNPs had been genotyped in the largest reported genetic association studies of CHD (CARDIoGRAMplusC4D, comprising C4D [Coronary Artery Disease consortium] and CARDIoGRAM [Coronary ARtery DIsease Genome wide Replication And Meta-analysis consortium]). Details of the original sources of information on SNP association with urate are given in Table S2 (page 3). Genotyping in the UCLEB studies was performed using the Illumina CardioMetabochip (Illumina, San Diego, CA, USA) and in the other consortia as described in the original publications.

We used a gene ontology (GO) enrichment analysis based on genes in closest proximity to the selected SNPs (AmiGO 2.1.4, http://amigo.geneontology.org/amigo/landing) to identify which GO terms were over-represented in this set of genes relative to a null hypothesis that the SNPs were selected independently of their published associations (p-values were obtained from the hypergeometric distribution).

Instrumental variable (IV) analysis of urate and CHD

Our conventional MR analysis was constrained (forced to pass through the origin) and weighted (by inverse variance of outcome effect estimate) linear regression of the coefficients for outcome/SNP on those for exposure/SNP to estimate the IV effect size. This equates to the

summary method proposed by Johnson,[17] and is the uni-variate case of the MVMR method for summarised data described by Burgess *et al.*[11]

To correct for observed pleiotropy we included regression coefficients for phenotypes exhibiting pleiotropy with the urate instrument as covariates the IV model. Summary level association statistics used in the analysis were obtained from the relevant publications or from the public domain data deposits from the relevant GWAS (Table S2 (page3)), incorporating additional non-overlapping data from UCLEB where available.

Data on coronary artery disease / myocardial infarction contributed by CARDIoGRAMplusC4D investigators were downloaded from www.CARDIOGRAMPLUSC4D.ORG.

Summary statistics for the association of each of the 31 urate-associated SNPs with glucose, BMI, type 2 diabetes, plasma lipids, and blood pressure were obtained, respectively, from MAGIC (Meta-Analyses of Glucose and Insulin-related traits Consortium), GIANT (Genetic Investigation of ANthropometric Traits), DIAGRAM (DIAbetes Genetics Replication And Meta-analysis), GLGC (Global Lipids Genetic Consortoum), and ICBP (International Consortium for Blood Pressure) GWAS consortia data (Table S2 (page 3)).

To test for unmeasured net pleiotropy, we used the recently published method of Bowden et al.[12] to test the hypothesis that the strength of the IV estimates of individual SNPs were symmetrically distributed around the point estimate. Symmetrical distribution indicates that pleiotropic effects, if present, are balanced and should not systematically bias the estimate of causal effect. To avoid the need to infer the standard error we re-sampled distributions of the summary statistics of the SNPs 100 000 times with replacement, recalculating the MR estimate each time. We report statistical significance and confidence intervals from this empirically derived distribution.

Consistency between associations of urate with CHD events in observational and instrumental variables analysis

We compared estimates for a 1-SD elevation in urate generated using the instrumental variables meta-analysis with the updated observational estimate of the urate-CHD association. CHD risk estimates in Wheeler *et al.*[4] were originally reported as comparisons of the top vs. bottom tertile of

the urate distribution. To derive the per-SD estimate from this range, we exploited the properties of the normal distribution in which the top and bottom tertiles are separated by 2·18 standard deviations; we checked that the distribution of urate in participant data in the UCLEB consortium was approximately normal (Figure S1 (page 12)).

Sensitivity analyses.

We examined the stability of the summary causal estimate by repeatedly (100 000 times) excluding 6 (~20%) SNPs from the instrument, chosen at random in each cycle, and collecting the resulting IV estimates. By noting the proportion of these 'sensitivity coefficients' that lie outside the confidence interval (CI) from the normal distribution of the estimate with complete data, we obtained an indication of sensitivity. That is, when more than 5% of the sensitivity coefficients were outside the CI there was evidence that the result was sensitive to SNP selection. We repeated the sensitivity analysis for an appropriate range of covariate models covering all phenotypes identified as potentially pleiotropic.

Power

We estimated the power of the analysis using the method of Brion *et al.*[18], as implemented at http://cnsgenomics.com/shiny/mRnd/. The origin and magnitude of the data used to generate the estimates are reported in Supplementary Tables 4 and 5. For these calculations, we interpreted fully-adjusted observational associations between urate and cardiovascular risk factors and events as the most realistic approximation to the causal effect of urate. We also estimated power retrospectively using the IV estimates and corresponding SEs for each method. In this case power was the complement of the false rejection rate with two-sided $\alpha = 0.05$.

These prospective estimates of power suggested we had 83% power to detect the same magnitude of association as for the observational association of urate and risk of CHD (Table S4 (page 5)). However, using the IV estimates from the different methods in a retrospective analysis we found that available power to detect the effect of urate on CHD was much lower than this (Table S5 (page 5), Figure S2 (page 13)) and that power from the MR-Egger analysis was notably lower

in power than the other MR methods.

Druggability of individual genes in the instrument

To identify drugs and research compounds targeting genes identified in this study, we queried the ChEMBL (Chemical database of the European molecular biology laboratory) database (release chembl_19).[19] To link genes to target identifiers in the ChEMBL database, we used the Ensembl Rest API and Uniprot web-services and thus obtained Uniprot accession keys representing the translated product of each gene queried.[20,21]

Drugs were retrieved from the Mechanism/Binding Annotation table, which provides manually curated compound-target associations for licensed drugs. Research compounds were retrieved from the Activities table, which stores measured compound-target interactions. Results were limited to measurements from binding or functional assays with an assigned pChEMBL value, where pChEMBL is defined as −log10(molar IC50, XC50, EC50, AC50, Ki, Kd or Potency). Assay targets were required to be identical or homologous to the submitted query.

Statistical analyses

Analyses were conducted in R (version 2.15.2) (http://www.R-project.org). Meta-analyses and Egger tests were conducted and forest and funnel plots drawn using the metafor() package.

Results

In meta-analysis of prospective cohort studies in which urate was quantified prior to incident CHD, plasma urate concentration was observationally associated with higher CHD risk: a 1-SD higher urate concentration was associated with an OR of CHD of 1·07 (95%CI: 1·04, 1·10; 9784 cases, 166 486 individuals from 17 studies; I^2=41%; fixed effects meta-analysis) after adjusting for age, gender and other variables (Figure S3 (page 14)). Urate concentration was also observationally associated with other established or putative risk factors for CHD including age, smoking status, BMI, blood pressure, total cholesterol, and triglycerides (Table 3).

Examining the individual SNPs in the instrument; in a meta-analysis of up to 68 studies including 145 000 individuals, each of the 31 SNPs selected for inclusion in the genetic instrument was associated with urate(Figure S4 (page 15)). From a set of 21 804 annotated genes, those in proximity to the 31 urate-associated genetic variants revealed significant functional enrichment for both urate and purine metabolism (Table S6 (page 6)).

We identified potential pleiotropic effects of a subset of the 31 SNPs. For example, in addition to associations with urate, SNPs within OVOL1/LTBP3, ATXN2/PTPN11 associated with SBP, DBP, HDL-C and TG (Systolic Blood Pressure, Diastolic Blood Pressure, High Density Lipo-protein Cholesterol, Triglycerides); NFAT5, INHCB/INHCE, BAZ1B/MLX1PL and GCKR associated with HDL-C and TG; SNPs within TMEM171, IGFR1, SLC17A1/SLC17A3, ABCG2 and VEGFA associated with HDL-C; and SNPs within BCAS3 and VEGFA associated with SBP and DBP (Figure S4 (page 15) - Figure S7 (page 17)). We subsequently considered adjustment for this observed pleiotropy by the inclusion of combinations of these phenotype effect estimates as covariates in our multivariate MR. Putative gene functions for these loci are given in Table S7 (page 7).

In combination, the 31 SNP urate instrument was associated with SBP, DBP, HDL-C and TG (each $P<0.05$) (Table 3, Figure S4 (page 15), Figure S6 (page 17)) indicating pleiotropy of the instrument. Data from ~145 000 individuals (68 studies) with information on genotype and urate concentration and 198 598 individuals (51 studies) with information on genotype and CHD (60 785 CHD events) contributed to the MR analysis of the association of plasma urate with CHD. The instrumental variable effect estimate derived from conventional MR was OR 1·18 (95%CI: 1·08, 1·29) (Figure 2) The Cochran Q test showed heterogeneity amongst the IV estimates from individual SNPs ($Q=47.67$, P-value = 0.02).

To examine the influence of the association of the 31 variants on SBP, DBP, HDL-C and TG on the MR estimate we included all combinations of SBP, DBP, TG and HDL-C as covariates in an MVMR by including the genetic association of SNPs with these covariates in the IV regression analysis (Figure 2, Table S8 (page 8)). MVMR yielded an OR for CHD of 1·10 (95%CI, 1·00, 1.22) per 1-SD change in urate (Table 4, Figure 3).

The Egger test indicated presence of unmeasured pleiotropy of the instrument (Egger test, P=0.01) (Figure S8 (page 18)). Using MR-Egger to account for this unmeasured pleotropy, we derived a causal estimate of OR 1.05 (95%CI: 0.92, 1.20) per 1-SD increase in urate. Observational estimates of the influence of urate concentration on all phenotypes studied are presented in Table 3, Figure 4 and Figure 5.

We assessed the sensitivity of the MR effect estimates to the exclusions of different combinations of 6 SNPs at random from the instrument and to the inclusion of different combinations of the covariates in a multivariable MR analysis. Our results show that the model containing all covariates was not overly influenced by SNP selection (data and explanation presented in Table S8 (page 8) and Figure S9 (page 19)). We noted that models containing combinations of SBP, DBP and HDL-C appeared insensitive to SNP selection, however the unadjusted (conventional MR) model and the model with TG alone gave higher effect estimates and a larger proportion of those estimates were outside the 95% CI of the corresponding model fitted over effect estimates from all 31 SNPs. MR-Egger analysis proved insensitive to SNP selection with only 3.8% of estimates lying outside the 95% confidence interval for MR-Egger regression estimates with all SNPs included. One gene in the instrument (SLC22A11) encoded a target for probenecid, a drug previously used to lower urate concentration (Table S7 (page 7)). An IV analysis based solely on the rs2078267 at the SLC22A11 locus yielded an OR for CHD of 1·19 per 1-SD increment in urate; 95%CI 0·75, 1·78. Other genes represented in the instrument (such as VEGFA, IGF1R, ABGG2 and GCKR) were the target of licensed or late-phase therapeutic agents for angiogenesis therapeutics, or growth hormone, or were associated with compounds at much earlier stages of development.[22]

Discussion

We investigated a potential causal role for plasma urate in the development of CHD using 31 SNPs identified from GWAS and utilizing several complementary MR approaches (see Putting research in context, panel). The well-powered, but potentially biased, conventional MR analysis suggested a causal influence of urate on CHD. However, the 31-SNP genetic instrument exhibited pleiotropic associations with several cardiovascular risk factors (including SBP and TG) that could bias this effect estimate. Multivariate MR regression analysis that adjusted for the associations of the genetic instrument with measured confounders yielded a causal estimate that was consistent with the results of both observational, and the conventional MR analysis. However the confidence intervals for the causal effect derived from multivariate MR were wider and included the null. While multivariate MR accounts for measured pleiotropy, as for conventional observational epidemiology, it cannot negate the effects of unmeasured or unknown confounding. Therefore the recently developed MR-Egger analysis was used, which reduces inflation of a causal effect estimate due to both measured and unmeasured net pleiotropy, at the cost of lower power. While MR-Egger confirmed the presence of unmeasured net pleiotropy, it was again directionally consistent with the other two approaches, albeit of smaller magnitude and even wider confidence limits (which as for multivariate MR, again included the null). Taken together, the most conservative conclusion from the data is that plasma urate exhibits a modest, if any, causal influence on risk of CHD.

The principal assumptions of MR are that (i) the genetic variant strongly associates with the exposure; (ii) the genetic instrument associates exclusively with the risk factor of interest (and not with any confounders of the risk factor – disease outcome association), and, (iii) the effect of the instrument on disease outcome is mediated exclusively through the risk factor of interest.[10] In this study we show a strong association of the genetic instrument with plasma urate, but we also show our instrument associated with potential confounders however, we were able to deploy recently developed methods to account for this. Specifically, the genetic instrument showed association with HDL-C, TG, SBP, and DBP, which could be due to horizontal or vertical pleiotropy between some of the SNPs included and these phenotypes. While it is difficult to tease horizontal from vertical pleiotropy, that horizontal pleiotropy is the explanation for these findings is supported by

the observation that the MR association with CHD generally persisted even after the associations of SNPs with SBP, DBP, HDL-C, and TG were added to the model in multivariate MR (Table S8 (page 8)).

Our finding adds to prior MR studies of urate that investigated ~70,000 participants with over 7000 CHD cases from the Copenhagen General Population and Copenhagen City Heart Study that found no evidence for a causal effect of urate on CHD.[24] However, the prior study was based on a single urate associated variant in SLC2A9 (rs7442295) and although the sample size was relatively large, it included only one ninth of the CHD cases incorporated in the present analysis. Consistent with this report, in our much larger analysis, SLC2A9 was not associated with CHD. Kleber et al.,[25] recently identified a causal effect of uric acid on cardiovascular death and sudden cardiac death, in a dataset of 3315 patients hospitalized for angiography, however this is a different outcome to CHD. Two prior studies implicated a causal effect of urate on blood pressure: one that used only a single SNP in SLC2A9 (rs16890979),[26] and another that used a 30-SNP score, but based in only 5791 participants.[16]

The strengths and limitations of the present analysis are worthy of note. Strengths include: (i) the incorporation of multiple urate-associated SNPs identified from GWAS to generate a genetic instrument with greater power than any single variant in isolation; (ii) use of two-sample MR methodologies that facilitate incorporation of summary effect estimates from very large, publicly available GWAS datasets (such as CARDIoGRAMplusC4D and DIAGRAM) to bolster power several-fold; and, (iii) emerging approaches to MR that allow statistical adjustment for measured confounders and adjustment for unbalanced net pleiotropy (namely multivariable MR and MR-Egger, respectively).

Limitations include much of the data arising from case-control studies participating in discovery genetics consortium, where CHD cases are recruited after presentation with an acute coronary syndrome, which is contingent on survival. It is therefore possible that findings we report are

influenced by survival advantage. However, the association of urate with CHD risk in prospective cohort studies (where urate was measured prior to CHD events) argues against survivorship bias. The mechanism by which some of the variants in our instrument influence urate concentration is not clear. However, understanding precise mechanisms is not a prerequisite for MR, and in any regard, seven of the 31 genes regulate urate or purine metabolism. Finally, the observational association of plasma urate with CHD may be biased towards the null due to regression dilution bias, however, repeated measures of plasma urate were unavailable.

Our study was designed to evaluate the causal role of uric acid in CHD risk, not the safety and efficacy of reducing uric through any particular therapeutic target. Randomised intervention trials will be required to test whether individual urate-lowering drugs might be effective for CHD prevention with an acceptable safety profile. Allopurinol and febuxostat that target xanthine oxidoreductase, as well as probenicid and sulfinpyrazone that inhibit renal urate reabsorption, might be considered. Although variants in genes encoding drug targets of the latter two therapeutics were included in the genetic instrument (together with GCKR which is a target for drug development for different reasons),[22] given the imprecision around the causal estimates for individual SNPs (together with estimates derived from multivariate and MR-Egger), the efficacy (or safety) of using one of these drugs for the prevention of CHD remains uncertain. Further genetic analyses focusing on SNPs in genes encoding the targets of urate-lowering drugs (e.g. SNPs in XDH encoding xanthine oxidoreductase, the target of allopurinol should these be demonstrated to associate with urate concentration), using a range of clinical outcomes, including but extending beyond CHD, would be required to address this distinct question as has been done for other potential therapeutic targets[27,28]. Our study was also designed to inform on any potential causal role of plasma urate in the onset rather than the progression or outcome from CHD. Different datasets would be required to address the separate question of the effect of lowering plasma urate on outcome following a diagnosis of CHD, such as that which has been assembled by the Genetics of Subsequent Coronary Heart Disease (GENIUS-CHD) consortium. We note, however, that a phase III randomised clinical trial of allopurinol (600mg daily) plus standard care vs. standard care alone in patients with established CHD designed to evaluate an effect on risk of CHD, stroke and

cardiovascular death is ongoing (ALL-HEART; http://allheartstudy.org/).

In summary, genetic evidence based on conventional and novel MR approaches suggest a modest, if any, causal effect of plasma urate concentration in the development of CHD. The findings may help investigators judge the relative priority of plasma urate, as against other risk factors, as a therapeutic target for the prevention of CHD.

Author Contributions:

Contibuted to design, executed the analysis, interpreted the findings, and wrote and revised the first and subsequent drafts of the manuscript: Jon White, Reecha Sofat, Gibran Hermani, Claudia Langenberg, Felix Kruger, Ruth Lovering, Mika Kivimaki, Tom Gaunt, George Davey Smith, John Whittaker, Frank Dudbridge, Juan Pablo Casas, Michael Holmes, Aroon Hingorani

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Table 1. SNPs used to construct the genetic instrument for plasma urate[a].

Index	SNP	CHR	BP	GENE (nearest/GRAIL)	Allele	Meta-analysis beta	Meta-analysis SE	N	S	Source Data
1	rs1471633	1	144435096	PDZK1/PDZK1	A	0·0568	0·0050	116404	54	Köttgen[29] and UCLEB [14]
2	rs1260326	2	27584444	GCKR/GCKR	T	0·0693	0·0049	117293	54	Köttgen[29] and UCLEB [14]
3	rs12498742	4	9553150	SLC2A9/SLC2A9	A	0·3600	0·0051	145110	68	Köttgen[29], UCLEB [14] Kolz[30]
4	rs2231142	4	89271347	ABCG2/ABCG2	T	0·1896	0·0077	140915	68	Köttgen[29], UCLEB [14] and Kolz[30]
5	rs675209	6	7047083	RREB1/RREB1	T	0·0556	0·0059	117293	54	Köttgen[29] and UCLEB *[14]*
6	rs1165151	6	25929595	SLC17A1/SLC17A3	T	-0·0779	0·0042	145201	68	Köttgen[29], UCLEB [14] and Kolz[30]
7	rs1171614	10	61139544	SLC16A9/SLC16A9	T	-0·0790	0·0070	110000	49	Köttgen[29]
8	rs2078267	11	64090690	SLC22A11/SLC22A11	T	-0·0732	0·0058	117293	54	Köttgen[29] and UCLEB [14]
9	rs478607	11	64234639	NRXN2/SLC22A12	A	-0·0264	0·0056	137967	49	Köttgen[29]
10	rs3741414	12	56130316	INHBC/INHBE	T	-0·0649	0·0068	117293	54	Köttgen[29] and UCLEB [14]
11	rs11264341	1	153418117	TRIM46/PKLR	T	-0·0500	0·0060	110000	49	Köttgen[29]
12	rs17050272	2	121022910	INHBB/INHBB	A	0·0350	0·0060	110000	49	Köttgen[29]
13	rs6770152	3	53075254	SFMBT1/MUSTN1	T	-0·0440	0·0050	110000	49	Köttgen[29]
14	rs17632159	5	72467238	TMEM171/TMEM171	C	-0·0390	0·0060	110000	49	Köttgen[29]
15	rs729761	6	43912549	VEGFA/VEGFA	T	-0·0470	0·0060	110000	49	Köttgen[29]
16	rs1178977	7	72494985	BAZ1B/MLXIPL	A	0·0470	0·0070	110000	49	Köttgen[29]
17	rs10480300	7	151036938	PRKAG2/PRKAG2	T	0·0350	0·0060	110000	49	Köttgen[29]
18	rs2941484	8	76641323	HNF4G/HNF4G	T	0·0440	0·0050	110000	49	Köttgen[29]
19	rs10821905	10	52316099	A1CF/ASAH2	A	0·0570	0·0070	110000	49	Köttgen[29]
20	rs642803	11	65317196	OVOL1/LTBP3	T	-0·0360	0·0050	110000	49	Köttgen[29]
21	rs653178	12	110492139	ATXN2/PTPN11	T	-0·0350	0·0050	110000	49	Köttgen[29]
22	rs1394125	15	73946038	UBE2Q2/NRG4	A	0·0430	0·0060	110000	49	Köttgen[29]
23	rs6598541	15	97088658	IGF1R/IGF1R	A	0·0430	0·0060	110000	49	Köttgen[29]
24	rs7193778	16	68121391	NFAT5/NFAT5	T	-0·0460	0·0080	110000	49	Köttgen[29]
25	rs7188445	16	78292488	MAF/MAF	A	-0·0320	0·0050	110000	49	Köttgen[29]

[a] Units are SD uric acid per copy of effect allele using a population SD for uric acid of 90·7 μmol/L (=1·5 mg/dl) reported by CHARGE. (Yang et al.).[2]

26	rs7224610	17	50719787	HLF/HLF	A	-0·0420	0·0050	110000	49	Köttgen[29]
27	rs742132	6	25715550	LRRC16A/LRRC16A	A	0·0540	0·0092	27923	14	Kolz[30]
28	rs2307394	2	148432898	ORC4L/ACVR2A	T	-0·0290	0·0050	110000	49	Köttgen[29]
29	rs17786744	8	23832951	STC1/STC1	A	-0·0290	0·0050	110000	49	Köttgen[29]
30	rs2079742	17	56820479	BCAS3/C17orf82	T	0·0430	0·0080	110000	49	Köttgen[29]
31	rs164009	17	71795264	QRICH2/PRPSAP1	A	0·028	0·005	110000	49	Köttgen[29]

Table 2. Observational associations of plasma urate with cardiovascular risk factors.

Variable	Studies *	N	Difference in risk factor for a 1-SD higher plasma urate	Lower and upper 95%CI	P-value
HDL-C (mmol/l)	4	22 669	-0·08	-0.087, -0.065	<0.0001
LDL-C (mmol/l)	2	19 195	0·07	-0.019, 0.163	0.121
Total cholesterol (mmol/l)	5	68 446	0·14	0.07, 0.213	0.0001
Triglycerides (mmol/l)	3	25 606	0·31	0.216, 0.393	<0.0001
Fasting glucose (mmol/l)	3	14 571	-0·08	-0.23, 0.066	0.276
Creatinine (mg/l)	2	6 696	4·43	1.235, 7.634	0.0066
BMI (kg/m^2)	7	84 419	1·29	0.879, 1.694	<0.0001
SBP (mmHg)	7	84 419	3·31	2.498, 4.128	<0.0001
DBP (mmHg)	4	19 033	1·95	0.926, 2.977	0.0002
Age (yrs)	3	5 713	0·21	0.045, 0.383	0.013
eGFR (ml/min/1·73m^2)	2	4 393	-4·59	-4.905, -4.269	<0.0001
Binary trait	**Studies**	**N/cases**	**Odds ratio per SD increase in plasma urate**	**Lower and upper 95%CI**	**P-value**
Sex (F vs M)	3	5 713/1 975	0·80	0.746, 0.865	<0.0001
Smoking (ever vs never)	2	4 293/2 678	1·11	1.041, 1.185	0.0015
Diabetes	2	4 394/517	1·07	0·976, 1·162	0.157

* Sources of data are given in S2

Table 3. Association of the 31 SNP urate instrument with cardiovascular traits.

Cardio-vascular trait*	Difference in risk factor per inverse variance weighted allele.	95%CI
HDL-C (mmol/L)	-0.0079	-0.0096, -0.0062
LDL-C (mmol/L)	-0.0014	-0.0032, 0.0005
TC (mmol/L)	0.0003	-0.0015, 0.0021
TG (mmol/L)	0.0142	0.0125, 0.0158
SBP (mm Hg)	0.0045	0.0026, 0.0064
DBP (mm Hg)	0.0054	0.0033, 0.0074
Fasting Glucose (mmol/L)	-0.0010	-0.0026, 0.0006
BMI (kg/m^2)	-0.0003	-0.0008, 0.0002
Diabetes (OR)	0.9991	0.992, 1.0064

* See Table 4 for numbers of individuals and studies

Table 4. Causal analysis of urate on risk of CHD derived from MR analysis.

			31 SNP Instrument		Multivariate regression estimate***		MR-Egger	
Outcome	Studies	N (cases)	Beta* (SD of change in outcome per SD change in urate).	95%CI	Beta* (SD of change in outcome per SD change in urate).	95%CI	Beta* (SD of change in outcome per SD change in urate).	95%CI
CHD**	58	206822 (65877)	1·1766	1·0763, 1·2861	1.1013	0.996, 1.2178	1.0488	0.9191, 1.1968

* Beta is the regression coefficient for the trait on the urate instrument.
** odds ratio.
*** Covariates are DBP, SBP, TG and HDL. Note that the regression model precludes meaningful consideration of DBP, TG and HDL as outcomes.

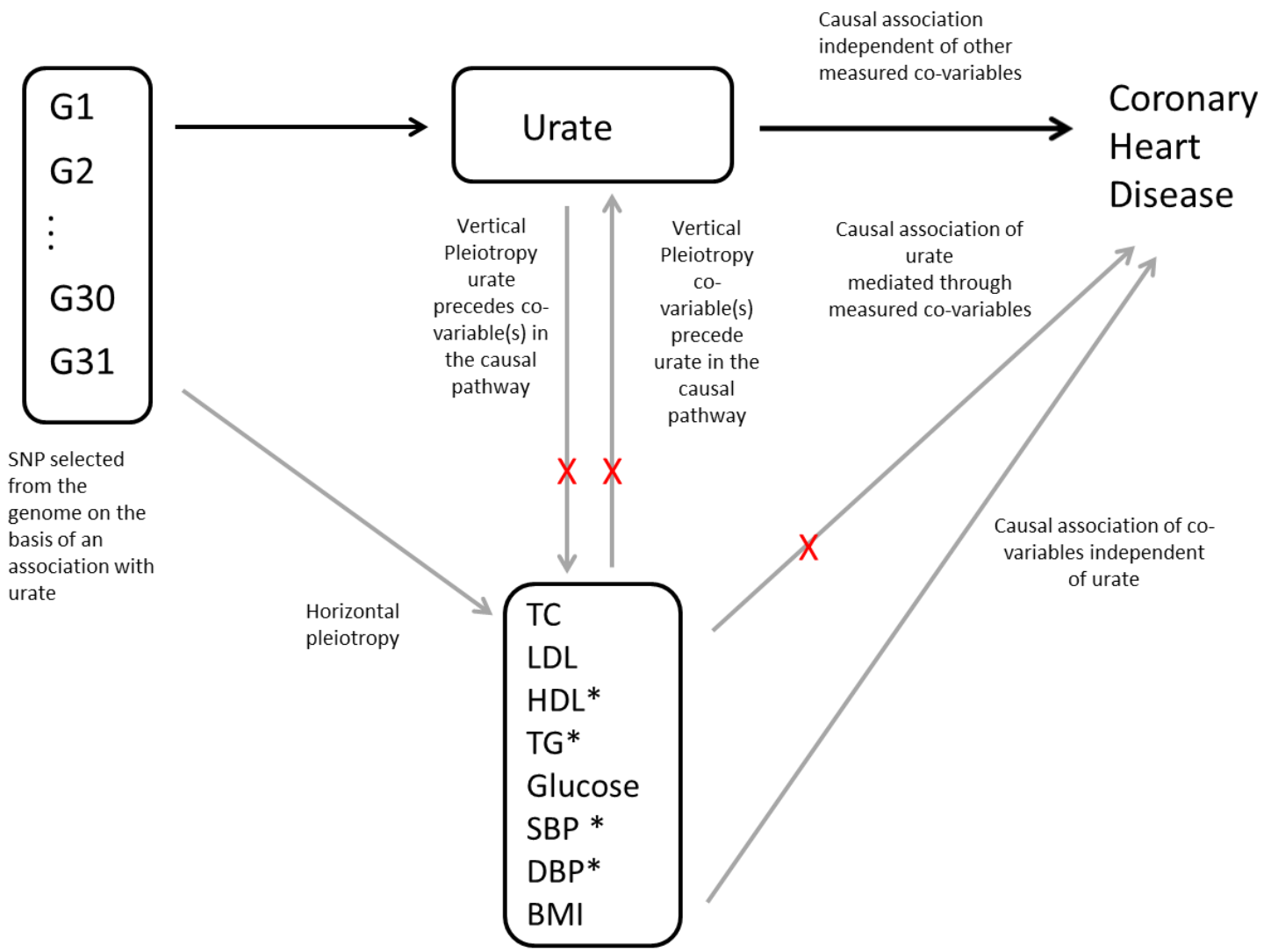

Figure 1. Conceptual framework for the Mendelian randomisation analysis of urate and CHD.

G1-31 are genes containing urate variants which together form the multilocus instrument for urate. Horizontal pleiotropy occurs when the instrument associates with traits other than urate which become confounders if also associated with CHD. Vertical pleiotropy occurs if their level is influenced by urate, and does not invalidate MR analysis Multivariable MR including DBP, SBP, HDL and TG as covariates was used to account for possible horizontal pleiotropy arising from association of the instrument with these variables. The effect of the adjustment is to block the paths indicated with red crosses. MR-Egger analysis was used to account for unknown or unmeasured, pleiotropic confounders. (see text for details).

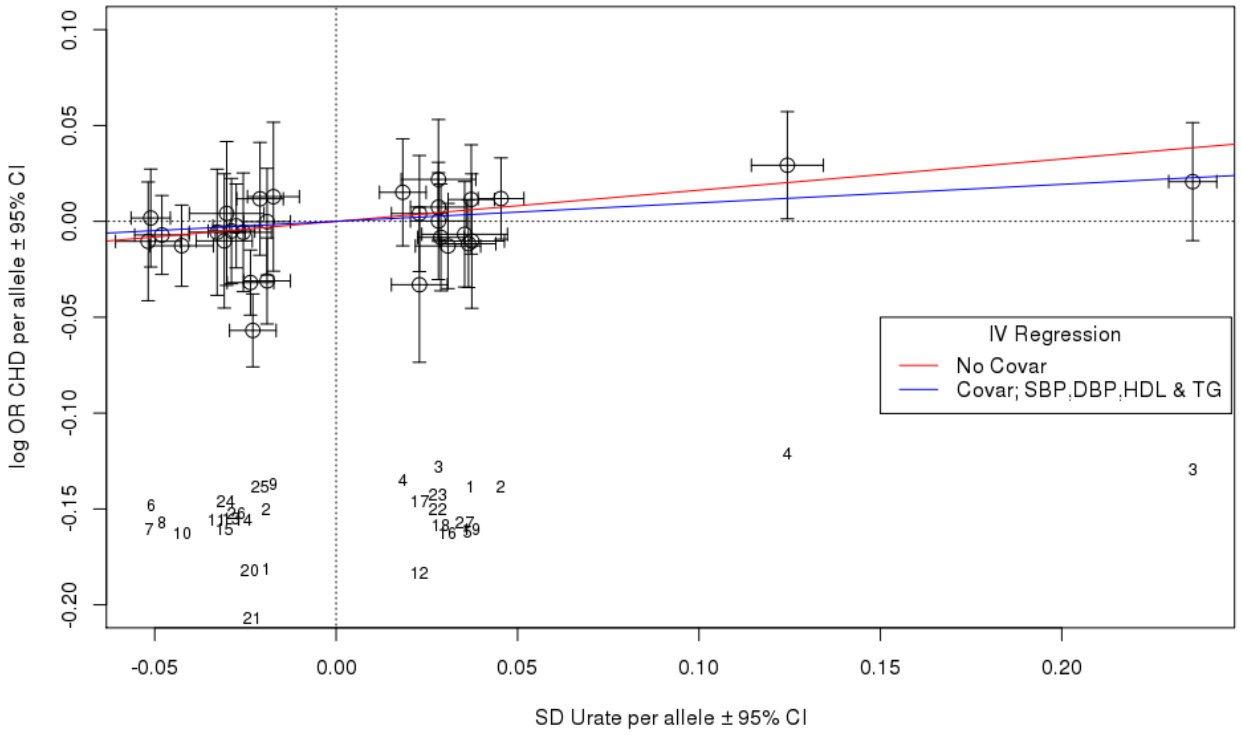

Figure 2. Association of individual SNPs with urate and CHD risk. Estimates are derived from meta-analysis over multiple studies (Table S2 (page3)). Bars represent 95% CI. The numbers below the main figure correspond to the index column in Table 1 to allow cross-referencing. The slopes of the lines are IV regression estimates of the effect of urate on CHD risk with (blue) and without (red) SBP, DBP, HDL and TG as covariates.

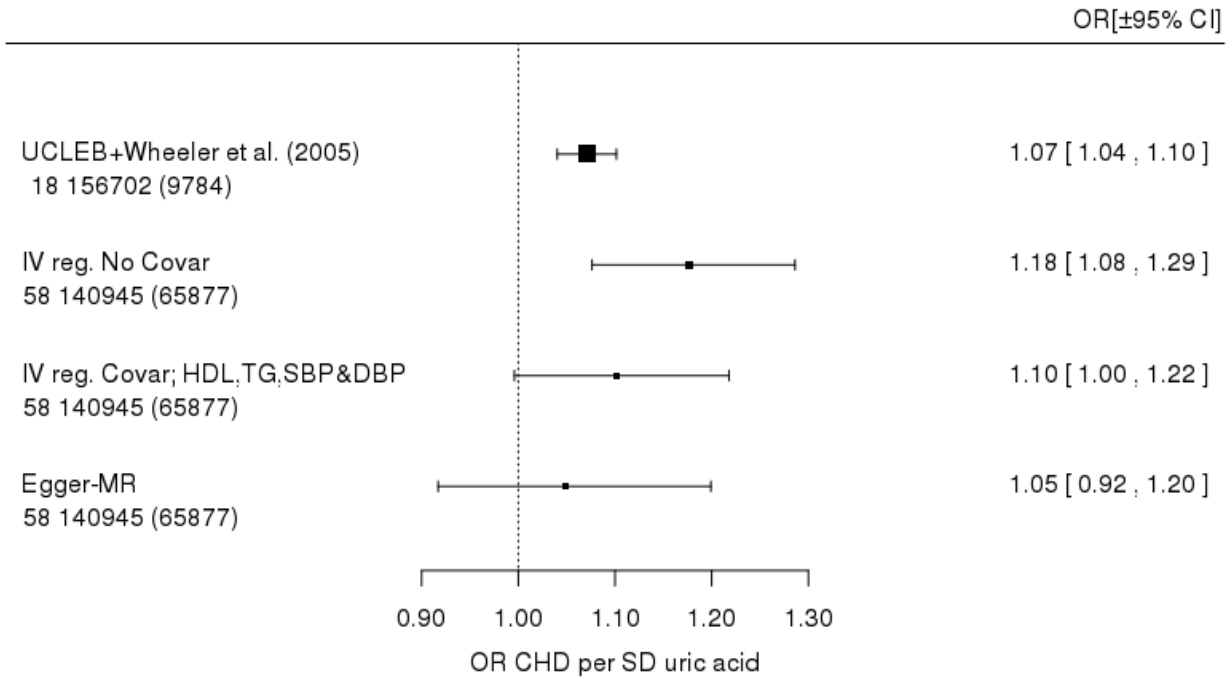

Figure 3. Observational and estimated causal effects of urate with risk of CHD. Values represent a per-1 SD increase in urate. Numbers below data description are No. studies, controls, and (cases).

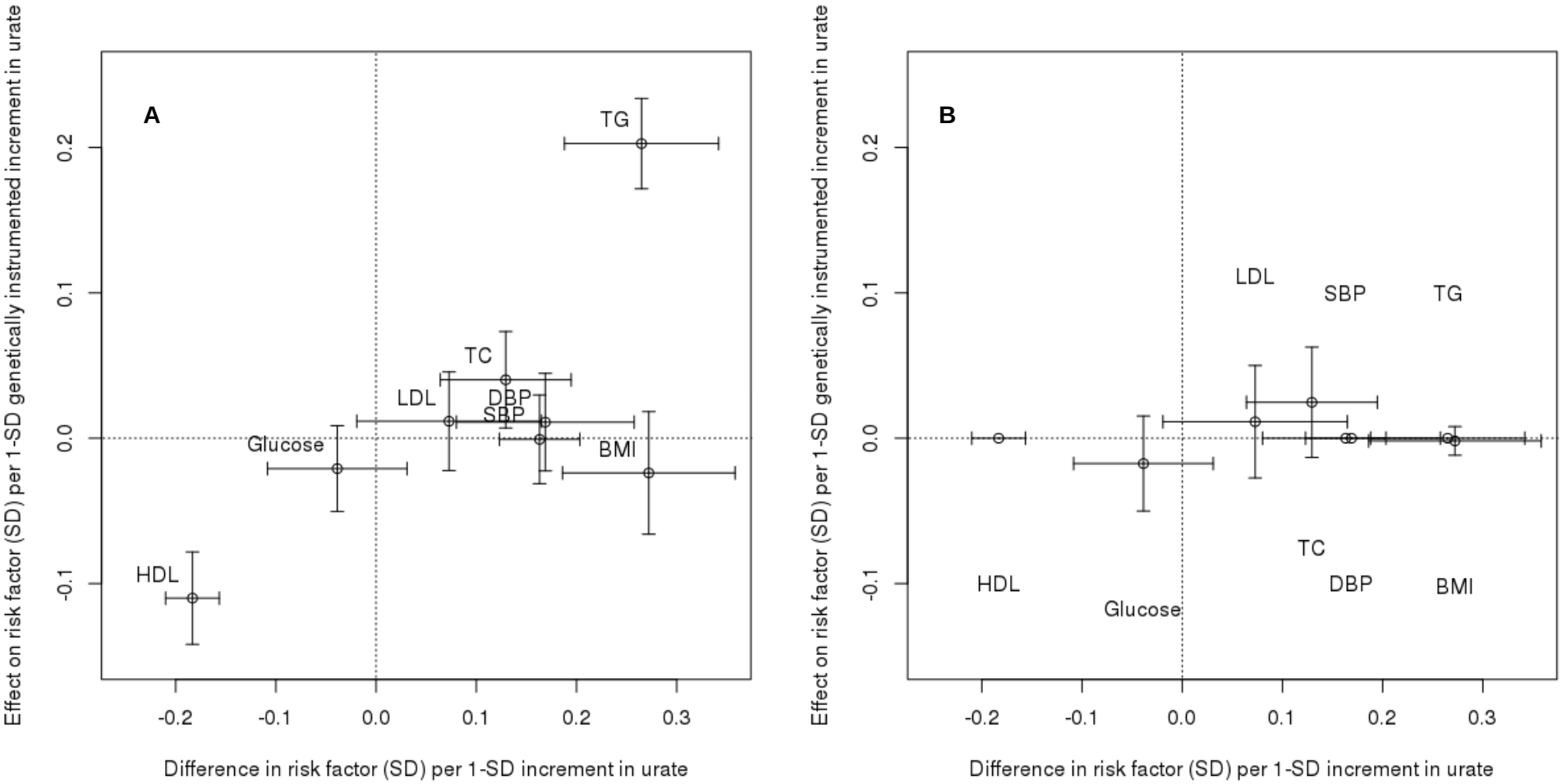

Figure 4. Comparison of observational and genetically instrumented associations between urate and several cardiovascular risk factors. (A) The genetically instrumented effect of urate without accounting for pleiotropic associations; and (B) the genetically instrumented effect with DBP, SBP, HDL and TG included as covariates in a multivariable MR analysis.

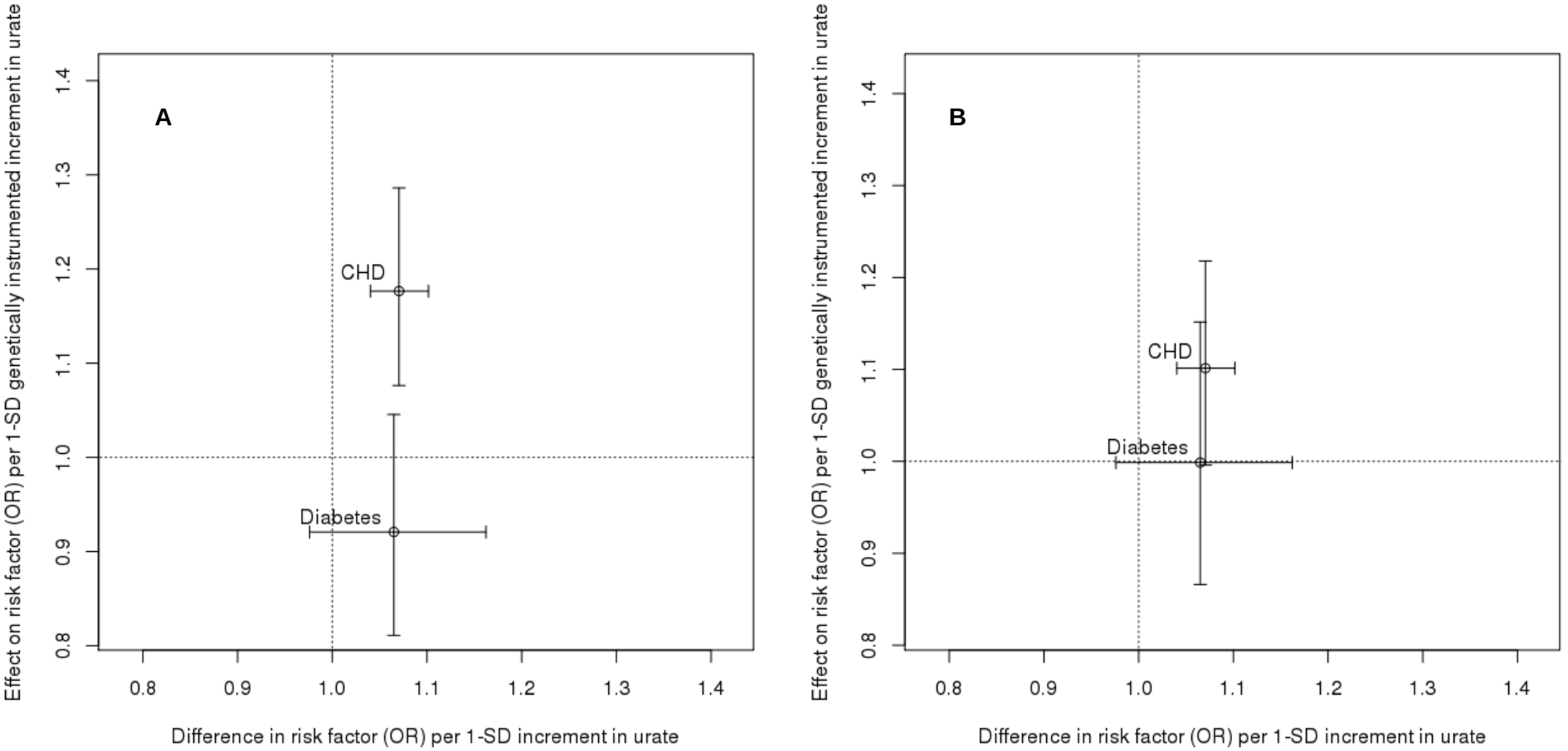

Figure 5. Observational association between binary traits and urate against Instrumental Variable association for (A) The 31-SNP instrument without covariates and (B) the 31-SNP instrument with DBP, SBP, HDL and TG as covariates.

References

1 Terkeltaub R.A. (2010) Update on gout: new therapeutic strategies and options. Nature Reviews Rheumatology 6:30-38

2 Yang Q, Köttgen A, Dehghan A, et al. 2010. Multiple Genetic Loci Influence Serum Urate and Their Relationship with Gout and Cardiovascular Disease Risk Factors Circ Cardiovasc Genet. 3(6): 523–530.

3 Davis N, (1897). The cardiovascular and renal relations and manifestations of gout. JAMA; 29: 261-262

4 Wheeler, JG, Juzwishin, DM, Eriksdottir, G, Gudnason, V, and Danesh J, (2005) Serum Uric Acid and Coronary Heart Disease in 9458 Incident Cases and 155084 Controls: Prospective Study and Meta-Analysis. PLoS Med. 2: 236–243.

5 Waring WS, McKnight JA, Webb DJ, Maxwell SRJ (2006) Uric Acid Restores Endothelial Function in Patients With Type 1 Diabetes and Regular Smokers. Diabetes 55:3127–3132

6 Cory DB, Eslami P, Yamamoto K, Nyby MD, Makino H, and Tuck ML, (2008). Uric acid stimulates vascular smooth muscle cell proliferation and oxidative stress via the vascular renin-angiotensin system. Journal of Hypertension; 26: 269–275.

7 Landmesser U, and Drexler H, (2002) Editorial: Allopurinol and Endothelial Function in heart Failure. Circulation. 106:173–175.

8 Struthers A and, Shearer F, (2012) Editorial. Allopurinol: novel indications in cardiovascular disease. Doi:10.1136/heartjnl-2012-302249

9 Sone PH, (2011). A New anit-ischemic rol for an old drug. JACC. 58:829–830.

10 Lawlor DA Harbrod RM, Sterne JAC, Timpson N, and Smith GD, (2008) Mendelian Randomisation: Using genes as instruments for making causal inferences in epidemiology. Statistics in Medicine. 27:1133-1163

11 Burgess, S. Dudbridge, F. and Thompson, S.G. (2015) Multivariable Mendelian Randomization: The Use of Pleiotropic Genetic Variants to Estimate Causal Effects. Am. J. Epidemiol. 181: 290-291

12 Bowden J, Smith, GD, and Burgess, S (2015) Mendelian randomization with invalid instruments: effect estimation and bias detection through Egger regression. Int. J. Epidemiology. doi: 10.1093/ije/dyv080

13 Palmer TM, Lawlor DA, Harbord RM, et al. (2011). Using multiple genetic varints as instrumental variables for modifiable risk factors. Stat. Met. in Med. Res. 21:223-242.

14 Shah T, Engmann J, Dale C, et al. (2013) Population Genomics of Cardiometabolic Traits: Design of the University College London-London School of Hygiene and Tropical Medicine-Edinburgh-Bristol (UCLEB) Consortium. PLoS ONE 8(8): e71345. doi:10.1371/journal.pone.0071345

14 Shah T, Engmann J, Dale C, et al. (2013) Population Genomics of Cardiometabolic Traits: Design of the University College London-London School of Hygiene and Tropical Medicine-Edinburgh-Bristol (UCLEB) Consortium. PLoS ONE 8(8): e71345. doi:10.1371/journal.pone.0071345

16 Sedaghat S, Pazoki R, Uitterlinden AG, Hofman A, Stricker BH, Ikram MA, Franco OH, and Dehghan A. (2014). Association of uric acid genetic risk score with blood pressure: the Rotterdam study. Hypertension. 64:1061-6. doi: 10.1161/HYPERTENSIONAHA.114.03757.

17 Johnson T, (2012) Efficient Calculation for Multi-SNP Genetic Risk Scores. http://cran.r-project.org/web/packages/gtx/vignettes/ashg2012.pdf

18 Brion M-J. A, Shakhbazov K and, Visscher PM (2013) Calculating statistical power in Mendelian randomization studies International Journal of Epidemiology 42: 1497–1501

19 Bento AP, Gaulton A, Hersey A, Bellis LJ, Chambers J, Davies M, et al. (2014) The ChEMBL bioactivity database: an update. Nucleic acids research. 42:D1083–90. doi:10.1093/nar/gkt1031

20 Yates A, Beal K, Keenan S, McLaren W, Pignatelli M, Ritchie GRS, et al. (2014) The Ensembl REST API: Ensembl Data for Any Language. Bioinformatics.;btu613. doi:10.1093/bioinformatics/btu613

21 Jain E, Bairoch A, Duvaud S, Phan I, Redaschi N, Suzek BE, et al. Infrastructure for the life sciences: design and implementation of the UniProt website. BMC bioinformatics. 009;10:136. doi:10.1186/1471-2105-10-136

22 Ashton KS, Andrews KL, Bryan MC, Chen J, Chen K, et al. (2014) Small Molecule Disruptors of the Glucokinase–Glucokinase Regulatory Protein Interaction: 1. Discovery of a Novel Tool Compound for in Vivo Proof-of-Concept. J Med Chem 57: 309–324. doi:10.1021/jm4016735.

23 C Reactive Protein Coronary Heart Disease Genetics Collaboration (CCGC) (2011) Association between C reactive protein and coronary heart disease: mendelian randomisation analysis based on individual participant data . BMJ 2011; 342 doi: tp://dx.doi.org/10.1136/bmj.d548

24 Palmer TM, Nordestgaard BG, Benn M, et al. (2013). Association of plasma uric acid with ischaemic heart disease and blood pressure: Mendelian randomisation analysis of two large cohorts. BMJ. 347:doi: 10.1136/bmj.f4262

25 Kleber, M.E., Delgado, G., Grammer, T.B., et al. (2015). Uric acid and cardiovascular events: a Mendelian randomisation study. J. Am. Soc. Nephrology. 26. doi: 10.1681/ASN.2014070660

26 Parsa A, Brown E, Weir MR, Fink JC, Shuldiner AR, Mitchell BD, and McArdle PF. (2012) Genotype-based changes in serum uric acid affect blood pressure. Kidney Int. 81:502-7. doi: 10.1038/ki.2011.414.

27 Interleukin-6 Receptor Mendelian Randomisation Analysis (IL6R MR) Consortium, Daniel I Swerdlow, Michael V Holmes, Karoline B Kuchenbaecker et al. (2012) The interleukin-6 receptor as a target for prevention of coronary heart disease: a mendelian randomisation analysis. Lancet. 379:1214-24. doi: 10.1016/S0140-6736(12)60110-X.

28 Holmes MV, Simon T, Exeter HJ, Folkersen L, et al. (2013) Secretory phospholipase A(2)-IIA and cardiovascular disease: a mendelian randomization study. J Am Coll Cardiol. 62:1966-76. doi: 10.1016/j.jacc.2013.06.044.

29 Köttgen A, Albrecht E, Teumer A, et al. (2013).Genome-wide association analyses identify 18 new loci associated with serum urate concentrations. Nature genetics;45(2):145–54

30 Kolz M, Johnson T, Sanna S, et al. (2009). Meta-analysis of 28,141 individuals identifies common variants within five new loci that influence uric acid concentrations.PLoS Genetics; Jun;5(6):e1000504

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*Manuscript with revisions highlighted

Plasma urate and coronary heart disease: Mendelian randomisation analysis.

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Abstract

***Background*:** Higher circulating plasma urate concentration is associated with an increased risk of coronary heart disease (CHD), but the extent of any causative influence of urate on CHD risk is still unclear.

***Methods*:** XX We first conducted a fixed effects meta-analysis of the observational association of plasma urate and risk of CHD. We then used a conventional Mendelian randomisation (MR) approach~~analysis~~ to investigate the causal relevance ~~of plasma urate for CHD~~ using a genetic instrument based on 31 urate-associated single nucleotide polymorphisms (SNPs). To~~In addition to a conventional Mendelian randomization analysis, tT~~o account for potential pleiotropic associations of certain SNPs with risk factors other than urate, we additionally conducted~~used~~ both a multivariable MR analysis~~approach~~ in which the genetic associations of SNPs on systolic and diastolic blood pressure, high density lipoprotein-cholesterol and triglycerides were included as covariates, and MR-Egger~~a recently developed adaption of Egger regression~~ to estimate a causal effect accounting for unmeasured pleiotropy. The analyses utilised data from ~~up to 134 studies and~~ 347 195 individuals in 134 studies, including 65 877 CHD cases.

***Findings*:** In meta-analysis of 17 prospective observational studies (166 486 individuals; 9 784 CHD events) a 1 standard deviation (SD) higher urate concentration was associated with an odds ratio (OR) for CHD of 1·07 (95% confidence interval [CI], 1·04, 1·10) after adjustment. The corresponding OR estimates from the conventional,~~an unadjusted, a~~ multivariable adjusted, and ~~an~~ Egger~~ regression~~ MR analysis (198 598 individuals; 65 877cases; 58 studies) were 1·18 (95%CI: 1·08, 1·29), 1·10 (95%CI, 1·00, 1.22), and 1.05 (95%CI: 0.92, 1.20) respectively, per 1-SD increment in plasma urate.

Interpretation: Conventional~~Unadjusted~~ and multivariate MR analysis implicates a causal role for urate in the development of CHD, but these estimates may be inflated by hidden pleiotropy. MR-Egger~~ MR~~, which has less statistical power, but accounts for hidden pleiotropy suggests the true effect of urate on CHD could include the null. These results may help investigators determine the priority of trials of urate lowering for CHD prevention as compared to other potential interventions.

Funding: The UCLEB consortium is supported by funding from NIHR, BHF, and MRC.Funding

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Introduction

Plasma urate is a circulating product of human purine metabolism synthesised from hypoxanthine and xanthine by the action of the enzyme xanthine oxidoreductase. ~~Urate is excreted into the renal tubule through cell surface transporters. Both the synthesis of urate and its excretion are targets of current drug therapies designed to reduce the concentration of plasma urate.~~ With extreme elevations in urate concentration, monosodium urate crystals are deposited in the joints, soft tissue and renal parenchyma causing ~~an~~ acute inflammatory arthropathy (gout), ~~in soft tissues causing~~ gouty tophi, and ~~in the renal parenchyma causing urate~~ nephropathy, respectively.[1] While the causal role of higher circulating urate concentrations in gout has been demonstrated by Mendelian randomisation analysis,[2] (and urate lowering is the principal treatment), the role of urate in CHD has been under debate since the 19th century.[3] ~~[W1, W2]~~

Patients~~More modest elevations of urate concentration are observed in patients~~ with established coronary heart disease (CHD) exhibit elevated levels of plasma urate)~~[b]~~ compared with individuals free of~~from~~ disease. Furthermore, elevated plasma~~,[1]~~ ~~and a higher circulating~~ urate ~~concentration (within the usual range)~~ is associated with increased~~a higher future~~ risk of incident CHD.[4] ~~events among initially healthy individuals from the general population.[2] CHD risk is also higher among patients with a diagnosis of gout.[W3,W4] While the causal role of higher circulating urate concentrations in gout has been demonstrated by Mendelian randomisation analysis,[W5] and urate lowering is the principal treatment, the role of urate in CHD has been under debate since the 19th century.[W6]~~

Beneficial ~~Both beneficial~~ and deleterious actions of urate on the cardiovascular system are reported, making~~have been described, leading to controversy on~~ the role of urate in atherosclerosis unclear. Urate ions have potentially atheroprotective, free-radical scavenging properties and infusion of urate may correct endothelial dysfunction.[5] However, ~~(a reversible early~~

~~[b] See table 1 for a list of all abbreviations~~

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~~feature of atherosclerosis) in patients with type I diabetes and in smokers.[3–7] However, potentially~~ pro-atherogenic effects of urate have also been described, including induction of cellular oxidative stress leading to attenuated nitric oxide bioavailability, ~~which has been~~ linked to platelet and endothelial cell activation, and vascular smooth muscle proliferation.[6]

~~[8,9] A further possible explanation for the urate-CHD association is that elevated urate concentration marks the presence of other risk factors for atherosclerosis without itself playing any causal (or protective) role.~~ A higher urate concentration is associated in population studies with several ~~established or putative~~ CHD risk factors including high blood pressure, elevated body mass index (BMI), type 2 diabetes, reduced HDL-cholesterol (HDL-C), and higher concentrations of ~~circulating~~ triglycerides (TG) and LDL-cholesterol (LDL-C).[4,2] However, whether these variables confound, or mediate the association of urate with CHD is uncertain (Figure 1).~~.~~ Statistical adjustment for ~~these~~the same variables in prospective observational studies attenuates the association of urate with CHD.[4] Whether residual confounding results in over-estimation or whether the effect is~~CHD[2],~~ ~~but incomplete adjustment due to unavoidable measurement error, or a failure to account for all confounders, means any true causal association could still be overestimated. Conversely, if one or more of these variables mediated the association of urate with CHD, statistical adjustment may be inappropriate and the true causal association~~ underestimated because some of the variables are mediators remains unknown. ~~.~~

Randomised trials provide some evidence that allopurinol (a~~have evaluated the effects of the~~ urate lowering therapeutic) has beneficial effects~~agent allopurinol~~ on intermediate cardiovascular end-points including endothelial function, angina symptoms, blood pressure, left ventricular mass, and exercise capacity. ~~in patients with stable CHD or heart failure suggesting that allopurinol offers additional benefit to optimally treated CHD patients. There is some indication that this is due to improved endothelial function.[W7,W11]~~ Allopurinol acts through inhibition of xanthine oxidoreductasae which also reduces the generation of reactive oxygen species, which are formed as a by-product of the metabolism of xanthine and hypoxanthine to urate.[7,8,W12,W13] Therefore, it remains unclear whether any benefits ~~potentially beneficial effect~~ of allopurinol on these end-points are~~is~~ due to

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urate lowering, inhibition of free-radical generation or both. Moreover, no trial, with anyallopurinol or other urate lowering agentagents has yet reported anthe effect of urate lowering on clinically relevant cardiovascular end-points,[9,10] although a trial of this type is ongoing (http://www.isrctn.com/ISRCTN32017426).[11]

We estimated the extent of any causal relationship between urate and CHD risk using Mendelian randomisation (MR) [10,12,13,W14]. MR exploits the random allocation of genetic variants from parents to offspring at gametogenesis, protecting genotype to phenotype associations from the usual sources of confounding seen in observational studies and from reverse causation. Providing certain assumptions are met, whereWhere a genetic variant (or variants) associateis associated with both a biomarker (e.g.such as urate) and with CHD risk in an Instrumental Variable (IV) regression, this supports a causal role for the biomarker in CHD.[10], providing certain assumptions are met. The approach has previously been used to evaluate the causal influence of urate on blood pressure and CHD[W15], as well as type II diabetes[W16], adiposity[W17], and triglycerides[W18] mainly using single SNPs as instruments. However, multiple genetic variants in combination may be more appropriate for MR to assess the causal relevance of non-protein traits (such as plasma urate), as these traits are not encoded by any one locus in isolation and the proportion of variation of the exposure explained by the genetic instrument utilising SNPs from multiple loci may increase power.

Although MR protects against many of the confounding factors that bedevil observational analysis, MR is potentially confounded by pleiotropy (the situation where variation in a gene associates with multiple phenotypes). Pleiotropy may be 'vertical'; the gene influences more than one point in the same causal pathway, or 'horizontal'; the gene influences more than one independent causal pathway. Whereas vertical pleiotropy does not breach the assumptions of MR, unmeasured horizontal pleiotropy can lead to entirely spurious conclusions about causality.

Two methods have been proposed to address horizontal pleiotropy, the first simply includes the effect of the instrument on the pleiotropic factor as a covariate in the MR analysis (termed multivariable MR, MVMR).[11] The second uses Egger regression to account for the more general

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case where there is a net pleiotropic effect on the instrument from multiple unmeasured sources (termed MR-Egger).[12]

We~~The principle assumptions of MR are that (i) the genetic variant strongly associates with the exposure; (ii) the genetic instrument associates exclusively with the risk factor of interest (and not with any confounders of the risk factor – disease outcome association), and, (iii) the effect of the instrument on disease outcome is mediated exclusively through the risk factor of interest.[13] Such assumptions are sometimes met by SNPs in genes encoding a risk factor of interest (e.g. C-reactive protein or fibrinogen[W19,W20]). However, when a multi-SNP genetic instrument is used, it may exhibit associations with other risk factors. Such non-exclusive associations can arise due to "vertical pleiotropy", due to effects on the downstream (vertical) pathway to disease distal to the risk factor of interest. This does not violate the assumptions of MR; rather it provides insight on potential mediators of the effect. However, non-exclusive associations can also arise due to "horizontal pleiotropy", whereby the selected gene variant associates with the disease outcome through traits proximal to the risk factor of interest. Such horizontal pleiotropic effects can be brought about through a number of mechanisms including alternative splicing of transcribed mRNA leading to more than one protein being encoded by a single gene, or through a single encoded protein having diverse functions such that risk factors on more than one pathway are altered. Horizontal pleiotropy violates the assumptions of MR analysis as originally conceived.~~

~~In theory, associations that arise because of horizontal pleiotropy at one locus in a multiple variant genetic instrument should be independent of horizontal pleiotropic effects at other loci, such that the unsystematic horizontal pleiotropic associations should be diluted relative to associations with the trait of interest.[13] However, in practice, residual non-exclusive associations may remain.[15] Multivariable MR analysis was developed in an attempt to address this. In this method pleiotropic associations among a pre-specified set of risk factors thought to confound the causal association of the risk factor and outcome are accounted for in the instrumental variable analysis, to estimate the independent causal effect of the risk factor of interest.[16,17] However, this approach can only address pleiotropy that can be quantified among the measured set of risk factors and assumes all pleiotropy is horizontal. It has also been suggested that this method may, in some circumstances,~~

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~~induce a signal by removing a known pleiotropic influence without removing an unobserved but balancing influence (so-called "collider bias"). Bowden et al. recently reported a method to quantify the presence of "hidden pleiotropy" due to association of an instrument with unmeasured risk factors. This is an adaptation of a form of Egger regression (originally conceived as a method to quantify detect small study bias in meta-analysis of treatment trials) for the MR setting to estimate the causal effect of a risk factor on the outcome of interest while accounting for unmeasured horizontal pleiotropy[14].~~

~~Plasma urate concentration is a heritable trait.[W21] In this study, we~~ selected a set of single nucleotide polymorphisms (SNPs) identified from genome wide association studies (GWAS) that associated~~were identified for an association~~ with urate concentration. Using these SNPs we constructed a genetic instrument,[13], and ~~examined them individually for consistency in the direction and magnitude of their associations with urate and CHD. Next, we~~ conducted conventional MR (unadjusted for pleiotropy). To account for pleiotropy, we ~~(unadjusted) MR analyses using genetic instruments composed of multiple urate-associated SNPs to investigate the causal effect of urate on CHD. We~~ then conducted MVMR~~a multivariate MR (adjusted for potential horizontal and vertical pleiotropy)~~ and MR-Egger ~~(to adjust for non-measured pleiotropy).[18]~~

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Methods

We identified a range of datasets to address~~serve the needs of~~ the research question, focusing on those with~~that included~~ participants reported to be predominantly~~mainly~~ of European descent (see original references for detail).~~.~~

Observational association between urate and CHD events and risk factors.

We used fixed effects meta-analysis of study summary estimates to update ~~Two of~~ the observational study ~~studies (Edinburgh Artery Study and British Regional Heart Study) that contributed to the meta-analysis of studies examining the observational association of urate with CHD risk~~ by Wheeler et al.[4] by ,[2] ~~also participated in the UCLEB consortium.[W22]~~

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~~We therefore updated this meta-analysis with~~ the addition of ~~new data from another UCLEB study, the British Women's Heart and Health Survey, contributing another~~ 326 myocardial infarction/coronary revascularisation cases and 1618 controls from the British Women's Health and Heart Study (BWHHS), which was the only study available to the UCLEB consortium[14] with suitable data (that had not already contributed to the report by Wheeler). This gave~~, providing~~ a combined observational dataset of 17 studies, 166 486 individuals, and 9 784 CHD events in all. Analyses were conducted without adjustment for renal function.

~~For studies in the UCLEB consortium, CHD cases were defined as individuals who had experienced a myocardial infarction and/or undergone coronary revascularisation.~~ To estimate the observational association between urate and several ~~established and putative~~ CHD risk factors, including body mass index (BMI), creatinine, blood pressure, glucose, HDL-C, LDL-C, total cholesterol (TC),~~,~~ and TG, we assimilated (by fixed effects meta-analysis) data~~information~~ from UCLEB with studies that contributed to the analysis by Wheeler *et al.*[14,4] (~~[2,W23 W29] where data were reported (Supplementary~~ Table S1 (page2),~~2, Supplementary~~ Table S2 (page3)).~~3).~~

Development of a ~~urate~~ genetic instrument for urate~~Mendelian randomisation analysis~~.

To generate a genetic instrument for urate concentration, we searched for SNPs from the GWAS~~genome-wide association study (GWAS)~~ catalogue (http://www.genome.gov/gwastudies, accessed 18th Feb 2015) associated with urate concentration. We identified 31 independent loci (R^2<0.3; separated by > 140kb) that had ~~replicated~~ associations with urate at P<5x10^{-7} (Table 1~~2~~). Where the P-value was greater than 5x10^{-8}, inclusion was only on the basis of a clear functional role in urate metabolism (this applied to only one SNP, rs164009, which has the GRAIL (Gene Relationships Across Implicated Loci)[15~~W29~~] identified gene PRPSAP1). ~~No SNPs were excluded.~~ In all cases the SNP association had been replicated in studies conducted mainly in populations of European ancestry and effect sizes were taken from published meta-analyses~~analysis~~. For each locus we recorded the published effect size and the standard error (SE) for the ~~identified a~~ lead SNP (~~which was the~~ SNP ~~defined as the one~~ with ~~the~~ strongest association in the largest dataset)

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. We recorded its published effect size and the standard error (SE). Where possible we collected effect estimates for the lead SNP, or a suitable proxy, from additional publications (Supplementary Table S1 (page 2) - S3 (pages 2-5)) and Supplementary Table 3) and combined the estimates for a SNP by fixed effects meta-analysis. Details of The genomic position for the lead SNPs and the putative genes are given in Table 1.(nearest gene and GRAIL gene) influencing urate concentration is provided in Table 2. No pair of SNPs was separated by less than 140kb. We note that an almost identical set of loci was used by Sedaghat et al.[19] as an instrument for urate with a reported R^2 and that they report an R^2 for the instrument of ~ 4.2% in the Rotterdam Study (n = 5791).[16] The

A gene ontology (GO) enrichment analysis based on genes in closest proximity to the selected SNPs (AmiGO 2.1.4, http://amigo.geneontology.org/amigo/landing) was undertaken to identify which GO terms were over-represented in this set of genes relative to a null hypothesis that the SNPs were selected independently of their published associations (p-values were obtained from the hypergeometric distribution).

The 31 selected SNPs had beenwere genotyped in the largest reported genetic association studies of CHD (CARDIoGRAMplusC4D, comprising C4D [Coronary Artery Disease consortium] and CARDIoGRAM [Coronary ARtery DIsease Genome wide Replication And Meta-analysis consortium]). Details).[W30–W32] Details of the original sources of information on SNP association with urate are given in Supplementary Table S2 (page 3). Genotyping in the UCLEB studies was performed using the Illumina CardioMetabochip (Illumina, San Diego, CA, USA) and in the other consortia as described in the original publications.[W22, W30–W34]

We used a gene ontology (GO) enrichment analysis based on genes in closest proximity to the selected SNPs (AmiGO 2.1.4, http://amigo.geneontology.org/amigo/landing) to identify which GO terms were over-represented in this set of genes relative to a null hypothesis that the SNPs were selected independently of their published associations (p-values were obtained from the hypergeometric distribution).

Instrumental variable (IV) analysis of urate and CHD

Our conventional MR analysis was constrained (forced to pass through We used the origin) and regression method for summarised data of Burgess *et al.*[17] in which a weighted (by inverse

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variance of outcome effect estimate) linear regression of the coefficients for outcome/SNP on those for exposure/SNP ~~is used~~ to estimate the IV effect size. This equates to the ~~The weights used are the inverse variances of the estimated outcome/SNP regression coefficients. Without covariates this method equates to a conventional MR on~~ summary method~~data as~~ proposed by Johnson,[17] and is the uni-variate case of the MVMR method for summarised data described by Burgess *et al.*[11]

To correct.[21] ~~Crucially, the Burgess approach allows the inclusion of regression coefficients~~ for observed pleiotropy we included regression coefficients for ~~other~~ phenotypes exhibiting pleiotropy~~in the IV model thus facilitating adjustment for pleiotropy without removing individually pleiotropic SNPs. Regression coefficients (and the corresponding SE) for the association of each of the 31 SNPs~~ with the urate instrument as covariates the IV model. ~~and with potential confounders and mediators were incorporated as the independent variables and the corresponding log odds ratio (OR) for CHD as the dependent variable.~~ Summary level association statistics used in the analysis were obtained from the relevant publications or from the public domain data deposits from the relevant GWAS (~~Supplementary~~ Table S2 (page3)),~~3),~~ incorporating additional non-overlapping data from UCLEB where available.

Data on coronary artery disease / myocardial infarction contributed by CARDIoGRAMplusC4D investigators were downloaded from www.CARDIOGRAMPLUSC4D.ORG. ~~www.CARDIOGRAMPLUSC4D.ORG.~~

Summary statistics for the association of each of the 31 urate-associated SNPs with glucose, BMI, type 2 diabetes, plasma lipids, and blood pressure ~~these traits~~ were obtained, respectively, from MAGIC (Meta-Analyses of Glucose and Insulin-related traits Consortium), GIANT (Genetic Investigation of ANthropometric Traits), DIAGRAM (DIAbetes Genetics Replication And Meta-analysis), GLGC (Global Lipids Genetic Consortoum), and ICBP (International Consortium for Blood Pressure) GWAS consortia data (Table S2 (page 3)).~~[22-26]~~

To test for unmeasured net~~hidden~~ pleiotropy, we ~~tested the effect estimates for the individual SNPs for heterogeneity (Cochran Q test) as this could indicate disproportionate influence from certain loci, which might be due to pleiotropy. The weighted regression method used in the multivariate~~

Comment [SEL16]: In the revised manuscript, the next passage was moved to appendix, which meant that neither the paper nor the appendix provided a clear picture of the Methods used. In the end, we think it's more straightforward to leave the Methods here in the main paper.

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~~MR allows the inclusion as covariates of effect estimates for the SNPs in the instrument on known pleiotropic phenotypes thus removing their confounding effect.[17] However, there remains the possibility of unmeasured pleiotropy. We~~ used the recently published method of Bowden et al.[12~~14~~] to test the hypothesis that the strength of the IV estimates of individual SNPs were symmetrically distributed around the point estimate. Symmetrical distribution indicates that pleiotropic effects, if present, are balanced and should not systematically bias the estimate of~~summary~~ causal effect.

~~Bowden et al.[14] also show that an unconstrained regression of the estimated SNP effects on CHD on their effects on plasma urate weighted with the inverse variance of the SNP effect estimate for CHD should give an unbiased estimate of the causal effect in the presence of hidden pleiotropy. However, there is currently some debate as to how best to determine the standard error of this estimate.~~ To avoid the need to infer the standard error we ~~performed 100,000 bootstrap estimates of the Egger-MR slope co-efficient (~~re-sampled~~sampling the~~ distributions of the summary statistics of~~for~~ the SNPs 100 000 times with replacement, recalculating~~in~~ the MR estimate each time. We~~instrument) and~~ report statistical significance and confidence intervals from this empirically derived distribution.

Consistency between associations of urate with CHD events in observational and instrumental variables analysis

We compared estimates for a 1-SD elevation in urate generated using the instrumental variables meta-analysis with the updated observational estimate of the urate-CHD association. CHD risk estimates in Wheeler *et al.*[42] were originally reported as comparisons of the top vs. bottom tertile of the urate distribution. To derive the per-SD estimate from this range, we exploited the properties of the normal distribution in which the top and bottom tertiles are separated by 2·18 standard deviations; we checked that the distribution of urate in participant data in the UCLEB consortium was approximately normal (~~Supplementary~~ Figure S1 (page 12)).~~1).~~[27]

Sensitivity analyses.

We examined the stability of the summary causal estimate by repeatedly (100 000 times) excluding

6 (~20%) SNPs from the instrument, chosen at random in each cycle, and collecting the resulting IV estimates. By noting the proportion of these 'sensitivity coefficients' that lie outside the confidence interval (CI) from the assumed normal distribution of the estimate with complete data, we obtained an indication of sensitivity. That is, when more than 5% of the sensitivity coefficients were outside the CI there was evidence that the result was sensitive to SNP selection. We repeated the sensitivity analysis for an appropriate range of covariate models covering all phenotypes identified as potentially pleiotropic.

Power

Comment [SEL17]: The Results section also contains a section on Power – this should be moved up and integrated here, and the power of the study satted here.

We estimated the power of the analysis using the method of Brion *et al.*[18,28], as implemented at http://cnsgenomics.com/shiny/mRnd/. The origin and magnitude of the data used to generate the estimates are reported in Supplementary TablesTable 4 and 5.Supplementary Table 5. For these calculations, we interpreted fully-adjusted observational associations between urate and cardiovascular risk factors and events as the most realistic approximation to the causal effect of urate. We also estimated power retrospectively using the IV estimates and corresponding SEs for each method. In this case power was the complement of the false rejection rate with two-sided α =0.05.

These prospective estimates of power suggested we had 83% power to detect the same magnitude of association as for the observational association of urate and risk of CHD (Table S4 (page 5)). However, using the IV estimates from the different methods in a retrospective analysis we found that available power to detect the effect of urate on CHD was much lower than this (Table S5 (page 5), Figure S2 (page 13)) and that power from the MR-Egger analysis was notably lower in power than the other MR methods.

Druggability of individual genes in the instrument

To identify drugs and research compounds targeting genes identified in this study, we queried the ChEMBL (Chemical database of the European molecular biology laboratory) database (release chembl_19).[19,29] To link genes to target identifiers in the ChEMBL database, we used the Ensembl

Rest API and Uniprot web-services and thus obtained Uniprot accession keys representing the translated product of each gene queried.[20,21,W35,W36]

Drugs were retrieved from the Mechanism/Binding Annotation table, which provides manually curated compound-target associations for licensed drugs. Research compounds were retrieved from the Activities table, which stores measured compound-target interactions. Results were limited to measurements from binding or functional assays with an assigned pChEMBL value, where pChEMBL is defined as −log10(molar IC50, XC50, EC50, AC50, Ki, Kd or Potency). Assay targets were required to be identical or homologous to the submitted query.

Statistical analyses

Analyses were conducted in R (version 2.15.2) ().[W37] http://www.R-project.org). Meta-analyses and Egger tests were conducted and forest and funnel plots drawn using the metafor()[W38] package.

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Results

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~~*Association of plasma urate with CHD events*~~

In meta-analysis of prospective cohort studies in which urate was quantified prior to incident CHD, plasma urate concentration was observationally associated with higher CHD risk: a 1-SD higher urate concentration was associated with an OR of CHD of 1·07 (95%CI: 1·04, 1·10; 9784 cases, 166 486 individuals from 17 studies; I^2=41%; fixed effects meta-analysis) after adjusting for age, gender and other variables (Figure S3 (page 2)~~. A random effects meta-analysis generated a similar summary estimate (OR 1.09; 95%CI: 1.04, 1.14)).~~ Figure 2).~~ Urate concentration was also observationally~~positively~~ associated with other established or putative risk factors for CHD including age, smoking status, BMI, blood pressure, total cholesterol, and triglycerides (Table 3).

Comment [SEL19]: Our understanding is that the main findings of the paper pertain to the MR analyses; thus, unless these results are markedly different than other published meta-analyses, we request that you move figure 2 to the appendix.

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Examining the ~~*Association of*~~ individual SNPs in the instrument; in~~*with urate and other biomarkers and risk factors*~~

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~~In~~ a meta-analysis of up to 68 studies including 145 000 individuals, ~~with measures of urate concentration (Supplementary Figure 3),~~ each of the 31 SNPs selected for inclusion in the genetic instrument was ~~individually~~ associated with urate (Figure S4 (page 15)). ~~.~~ From a set of 21 804 annotated genes, those in proximity to the 31 urate-associated genetic variants revealed significant functional enrichment for both urate and purine metabolism (~~Supplementary~~ Table S6 (page 6)). ~~)~~.

We identified potential pleiotropic effects of a subset of the 31 SNPs. For example, in addition to associations with urate, SNPs within OVOL1/LTBP3, ATXN2/PTPN11 associated with SBP, DBP, HDL-C and TG (Systolic Blood Pressure, Diastolic Blood Pressure, High Density Lipo-protein Cholesterol, Triglycerides); ~~-~~ NFAT5, INHCB/INHCE, BAZ1B/MLX1PL and GCKR associated with HDL-C and TG; SNPs within TMEM171, IGFR1, SLC17A1/SLC17A3, ABCG2 and VEGFA ~~-~~ associated with HDL-C; and SNPs within BCAS3 and VEGFA associated with SBP and DBP (~~Supplementary~~ Figure S4 (page 15) ~~-2 - Supplementary~~ Figure S7 (page 17)). ~~5).~~ We subsequently considered adjustment for this observed pleiotropy by the inclusion of combinations of these phenotype effect estimates as covariates in our multivariate MR. Putative gene functions for these loci are given in ~~Supplementary~~ Table S7 (page 7). ~~.~~

In combination, the 31 SNP urate instrument was associated with SBP, DBP, HDL-C and TG (each P<0.05) (Table 3, Figure S4 (page 15), Figure S6 (page 17)) indicating pleiotropy of the instrument.

~~*Estimated causal relationship of urate and risk of CHD*~~

~~The relationship between the association of SNPs included in the instrument with plasma urate concentration and their association with risk of CHD is shown in Figure 3.~~ Data from ~145 000 individuals (68 studies) with information on genotype and urate concentration and 198 598 individuals (51 studies) with information on genotype and CHD (60 785 CHD events) contributed to the MR ~~instrumental variable~~ analysis of the association of plasma urate with CHD. ~~-~~The instrumental variable effect estimate derived from conventional MR was ~~(two-stage) MR for a 1-SD increment in urate on CHD risk, based on the 31-SNP instrument was an~~ OR 1·18 (95%CI: 1·08, 1·29) (Figure 2) ~~), larger in magnitude than~~ The Cochran Q test showed heterogeneity amongst the IV estimates from individual SNPs (Q=47.67, P-value = 0.02).

To examine the influence of the association of the 31 variants on SBP, DBP, HDL-C and TG on the MRobservational estimate (Figure 4)

The we included 31 SNP urate instrument was also associated with SBP, DBP, HDL-C and TG (each P<0.05) (Table 4, Supplementary Figure 2, Supplementary Figure 4) indicating potential vertical or horizontal pleiotropy of the instrument. To examine this further we repeated the MR analysis including all combinations of SBP, DBP, TG and HDL-C as covariates in an MVMRa multivariate MR by including the genetic association of SNPs with these covariates in the IV regression analysis (Figure 2, Supplementary Table S8 (page 8)). MVMR). These analysis yielded an OR for CHD of 1per 1-SD increment in urate of OR 1·10 (95%CI, 1·00, 1.22) per 1-SD change in urate (Table 45, Figure 3).

The 4). Finally, we obtained an Egger test indicated presence of unmeasured pleiotropy of the instrument (Egger test, P=0.01) (Figure S8 (page 18)). Using MR-Egger to account for this unmeasured pleotropy, we derived a causal estimate of OR 1.05 (95%CI: 0.92, 1.20) for the OR per 1-SD increase in urate. Observational and IV estimates of thecausal influence of urate concentration on all phenotypes studied are presented in Table 3,Figure 5 and Figure 4 and Figure 56.

Test of differential pleiotropy of the SNPs used in the genetic instrument

The Cochran Q test showed heterogeneity amongst the IV estimates from individual SNPs (Q=47.67, P value = 0.02). The Egger test indicated presence of unmeasured pleiotropy of the instrument (Egger test, P=0.01) (Supplementary Figure 6).

Sensitivity of the instrument.

We assessed the sensitivity of the MR effect estimates to the exclusions of different combinations of 6 SNPs at random from the instrument and to the inclusion of different combinations of the covariates in a multivariable MR analysis. Our results show that the model containing all covariates was not overly influenced by SNP selection (data and explanation presented in Supplementary Table S8 (page 8) and Supplementary Figure S9 (page 19)).7). We noted that

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models containing combinations of SBP, DBP and HDL-C appeared insensitive to SNP selection, however the unadjusted (conventional MR) model and the model with TG alone gave higher effect estimates and a larger proportion of those estimates were outside the 95% CI of the corresponding model fitted over effect estimates from all 31 SNPs. MR-Egger analysis proved insensitive to SNP selection with only 3.8% of estimates lying outside the 95% confidence interval for MR-Egger regression estimates with all SNPs included.

Druggability of individual genes in the instrument

One gene in the instrument (SLC22A11) encoded a target for probenecid, a drug previously used to lower urate concentration (Supplementary Table S7 (page 7)).) An IV analysis based solely on the rs2078267 at the SLC22A11 locus yielded an OR for CHD of 1·19 per 1-SD increment in urate; 95%CI 0·75, 1·78. Other genes represented in the instrument (such as VEGFA, IGF1R, ABGG2 and GCKR) were the target of licensed or late-phase therapeutic agents for angiogenesis therapeutics, or growth hormone, or were associated with compounds at much earlier stages of development.[22,30]

Power Calculations

Prospective estimates of power using the 31 SNP instrument suggested we had 83% power to detect the same magnitude of association as for the observational association of urate and risk of CHD (Supplementary Table 4). The instrumental variable analyses of urate and lipid fractions, blood pressure, and diabetes each had >99% power (Supplementary Table 5). For fasting glucose, power was lower (48%). All power estimates are expressed based on α= 0·05.

However, using the IV estimates from the different methods in a retrospective analysis we found that available power to detect the effect of urate on CHD was much lower than this (Supplementary Figure *8*) and that power from the MR-Egger analysis was notably lower in power than the other MR methods.

Comment [SEL20]: This paragraph should be moved up and integrated into the Methods section

Discussion

We investigated~~The analyses in this report were designed to investigate~~ a potential causal role for plasma urate in the development of CHD using 31 SNPs identified from GWAS and utilizing several complementary MR approaches (see Putting research in context, panel).~~.~~ The well-powered, but potentially biased, conventional~~2 stage least squares instrumental variable~~ MR analysis suggested a causal influence of~~role for~~ urate on~~in the development of~~ CHD. However, the 31-SNP genetic instrument exhibited pleiotropic associations with several cardiovascular risk factors (including SBP~~, DBP, HDL-C~~ and TG)~~TGs~~ that could bias this effect~~be horizontal in nature, possibly providing an inflated~~ estimate. Multivariate MR~~ of the true causal effect. On the assumption that all of these associations are due to horizontal pleiotropy, we conducted a multivariate IV~~ regression analysis that adjusted for the~~incorporating~~ associations of the genetic instrument~~same SNPs~~ with measured confounders~~HDL-C, TG, SBP and DBP as covariates. This analysis~~ yielded a causal estimate ~~for the effect of plasma urate with CHD risk~~ that was consistent with the results of both ~~adjusted~~ observational ~~estimate~~, and ~~with~~ the conventional~~estimate from the standard~~ MR analysis. However the confidence intervals for~~, however~~ the causal effect derived from multivariate MR~~95%CI~~ were wider and included the null. While ~~The~~ multivariate MR accounts for measured pleiotropy, as for conventional observational epidemiology, it~~observed horizontal pleiotropy among the set of measured risk factors. However the method~~ cannot negate the effects of unmeasured or unknown confounding~~pleiotropy~~. Therefore the recently developed MR-Egger analysis was used~~undertaken~~, which reduces ~~the risk of~~ inflation of a causal effect estimate due to both measured and unmeasured net~~instrument~~ pleiotropy, at~~but which is less well powered than~~ the cost of lower power. While~~other two approaches.~~ MR-Egger confirmed the presence of unmeasured net pleiotropy, it~~pleiotropy and likely over-estimation of the causal effect by the first two methods. Although the point estimate from the MR-Egger analysis~~ was again directionally consistent ~~in direction~~ with the other two approaches, albeit of~~it was much~~ smaller ~~in~~ magnitude~~,~~ and even~~the~~ wider confidence limits (which as for multivariate MR, again included the null)~~.~~ Taken together, the most conservative conclusion from the data is that plasma urate exhibits a modest, if any, causal influence on~~association with~~ risk of CHD.

Comment [SEL21]: We were happy with the changes to the Discussion, so please go ahead with what you had done. However, we thought that some of the Introductory comments on the different strengths/uses of the different MR approaches would be better placed here.

The principal assumptions of MR are that (i) the genetic variant strongly associates with the exposure; (ii) the genetic ~~Instrumental variable regression based on the 31-SNP~~ instrument associates exclusively with the risk factor of interest (and not with ~~provided no evidence for a causal association of urate with systolic or diastolic blood pressure, BMI, or diabetes risk (Table 4), suggesting that these variables are unlikely to mediate~~ any confounders of the risk factor – disease outcome association), and, (iii) the effect of the~~causal effect of urate on CHD. Interestingly, the same~~ instrument on disease outcome is mediated exclusively through the risk factor of interest.[10] In this study we show a strong association of the genetic instrument with plasma urate, but we also show our instrument ~~was~~ associated with potential confounders however, we were able to deploy recently developed methods to account for this. Specifically, the genetic instrument showed association with HDL-C, TG, SBP, and DBP, which could~~SBP, DBP, TG and HDL-C. This may~~ be due to horizontal or vertical pleiotropy between some of the SNPs included and these ~~three~~ phenotypes.~~,~~ While it is difficult to tease horizontal from vertical pleiotropy, that horizontal pleiotropy is the explanation for these findings is supported by the observation that the MR~~IV~~ association with CHD generally persisted even after the associations of SNPs with SBP, DBP, HDL-C, and TG were added to the model in multivariate MR (Table S8 (page 8)). ~~The apparent contradiction is a result of the weighting of individual SNPs in the model by the magnitude of their effect on urate since the primary purpose of the instrument in the MR analysis is to predict the genetic variation of urate and, consequently, the effect of urate on CHD risk.~~

Our finding adds to~~is at odds with a~~ prior MR studies of urate~~study~~ that investigated ~70,000 participants with over 7000 CHD cases from the Copenhagen General Population and Copenhagen City Heart Study that found no evidence for a causal effect~~association~~ of urate on~~with~~ CHD.[24~~W15~~] However, the prior study was based on a single urate associated variant in SLC2A9 (rs7442295) and although the sample size was relatively large, it included only one ninth of the CHD cases incorporated in the present analysis. Consistent with this report,~~Even~~ in our much larger analysis, SLC2A9 was not ~~significantly~~ associated with CHD. Kleber et al.,[25] recently identified ~~, highlighting the need to use multiple variants in combination to obtain sufficient~~

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~~statistical power to make causal deductions. Our findings of a lack of~~ a causal effect of uric acid on cardiovascular death~~urate with BMI~~ and sudden cardiac death, in a dataset of 3315 patients hospitalized for angiography, however this~~type 2 diabetes risk~~ is a different outcome to CHD. Two~~in keeping with~~ prior ~~MR~~ studies~~. One prior MR analysis~~ implicated a causal effect of~~association between~~ urate on~~and~~ blood pressure~~: one that used but this analysis was also based on~~ only a single ~~uric-associated~~ SNP in ~~the~~ SLC2A9 ~~gene~~ (rs16890979),[26] and another that used a 30-SNP ~~).[32] A further MR analysis based on a thirty variant gene~~ score, but ~~provided evidence for a causal association of urate with blood pressure, but this analysis was~~ based in only 5791 participants.[16~~19~~]~~.~~

The strengths and limitations of the present analysis are worthy of note~~discussion~~. Strengths include: (i) the incorporation of multiple urate-associated SNPs identified from GWAS to generate a genetic instrument with greater power than any single variant in isolation;~~,~~ (ii) use~~utilisation~~ of two-sample MR methodologies that facilitate incorporation~~allow the use~~ of summary effect estimates from very large, publicly available GWAS datasets (such as CARDIoGRAMplusC4D and DIAGRAM) to bolster power several-fold; and, (iii) emerging approaches to MR that allow statistical adjustment for measured confounders and adjustment for unbalanced net pleiotropy (namely~~; (iii) the use of~~ multivariable MR and MR-Egger, respectively)~~.to address the possibility of horizontal pleiotropy, and (iv) an estimate of the causal effect which is independent of unmeasured horizontal pleiotropy using Egger-MR.~~

Limitations include much of the data arising~~being~~ from case-control studies participating in discovery genetics ~~the CARDIoGRAMplusC4D~~ consortium, where CHD~~. Many such~~ cases are~~would have been~~ recruited after presentation with an acute coronary syndrome, which is contingent on survival. It is therefore possible~~, so there remains a possibility~~ that findings we report~~of the instrumental variable analysis~~ are influenced~~affected~~ by survival advantage. However, the association of urate with CHD risk in prospective cohort studies (where urate was measured prior to ~~the occurrence of~~ CHD events) argues against survivorship bias. The mechanism~~mechanisms~~ by which some of the variants in our instrument influence urate concentration is not clear. However, understanding precise mechanisms is not a prerequisite for

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MR, and in any regard, seven of the 31~~7 of the loci from which SNPs in the instrument were contain~~ genes regulate~~involved in~~ urate or purine metabolism. Finally, the observational association of plasma urate with CHD may be biased towards the null due to regression dilution bias, however, ~~we did not have~~ repeated measures of plasma urate were unavailable.~~to quantify this.~~

Our study was designed to evaluate the causal role of uric acid in CHD risk, not the safety and efficacy of reducing uric through any particular therapeutic target. Randomised intervention trials will be required to test whether individual urate-~~drugs, which are or have been used for urate~~ lowering drugs~~for the treatment or prevention of gout~~ might be effective for CHD prevention with an acceptable safety profile. Allopurinol and febuxostat that target xanthine oxidoreductase, as well as probenicid and sulfinpyrazone that inhibit renal urate reabsorption, might be considered. Although variants in genes encoding drug targets of the latter two therapeutics were included in the genetic instrument (together with GCKR which is a target for drug development for different reasons),[22]~~,[30],~~ given the imprecision around the causal estimates for individual SNPs (together with estimates derived from multivariate and MR-Egger),~~,~~ the efficacy (or safety) of using one of these drugs for the prevention of CHD remains uncertain.~~ from this analysis alone. We are also unable to comment on the likely balance between the safety and efficacy of any such approach.~~ Further genetic analyses focusing on SNPs in genes encoding the targets of urate-lowering drugs (e.g. SNPs in XDH encoding xanthine oxidoreductase, the target of allopurinol should these be demonstrated to associate with urate concentration), using a range of clinical outcomes, including but extending beyond CHD, would be required to address this distinct question as has been done for other potential therapeutic targets[27,28].~~targets [33,34].~~ Our study was also designed to inform on any potential causal role of plasma urate in the onset rather than the progression or outcome from CHD. Different datasets would be required to address the separate question of the effect of lowering plasma urate on outcome following a diagnosis of CHD, such as that which has been assembled by the Genetics of Subsequent Coronary Heart Disease (GENIUS-CHD) consortium. We note, however, that a phase III randomised clinical trial of allopurinol (600mg daily) plus standard care vs. standard care alone in patients with established CHD designed to evaluate an

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effect on risk of CHD, stroke and cardiovascular death is ongoing (ALL-HEART; http://allheartstudy.org/).

In summary, genetic evidence based on conventional and novel MR approaches ~~multivariate MR analysis~~ suggest a modest, if any, causal effect of plasma urate concentration in the development of CHD. The findings may help investigators judge the relative priority of plasma urate, as against other risk factors, as a therapeutic target for the prevention of CHD.

Author Contributions:

Contibuted to design, executed the analysis, interpreted the findings, and wrote and revised the first and subsequent drafts of the manuscript: Jon White, Reecha Sofat, Gibran Hermani, Claudia Langenberg, Felix Kruger, Ruth Lovering, Mika Kivimaki, Tom Gaunt, George Davey Smith, John Whittaker, Frank Dudbridge, Juan Pablo Casas, Michael Holmes, Aroon Hingorani

Established and coordinated the consortium of studies: Tom Palmer, Shah Ebrahim, Debbie A Lawlor, Philippa J Talmud, Steve E. Humphries, Christine Power, Elina Hypponen, Marcus Richards, Rebecca Hardy, Diana Kuh, Nicholas Wareham, George Davey-Smith, Yoav Ben-Shlomo, Ian N. Day, Peter Whincup, Richard Morris, Mark W. J. Strachan, Jacqueline Price, Meena Kumari, Mika Kivimaki, Juan P Casas, Aroon Hingorani

Contributed to data collection and / or preparation for the analysis: Tina Shah, Jorgen Engmann, Sonia Shah, Claudia Giambarolomei, Daniel I Swerdlow, Stela McLachan, Delilah Zabaneh, Alana Cavadino, Barbara Jefferis, Andrew Wong, Antoinette Amuzu, Ken Ong, Tom Gaunt, Helen Warren, Teri-Louise Davies, Jackie Cooper, Elina Hypponen, Shah Ebrahim, Debbie A. Lawlor, Philippa J. Talmud, Steve E. Humphries, Christine Power, Elina Hypponen, Marcus Richards, Rebecca Hardy, Diana Kuh, Nicholas Wareham, George Davey-Smith, Yoav Ben-Shlomo, Ian N. Day, Peter Whincup, Richard Morris, Mark W. J. Strachan, Jacqueline Price, Meena Kumari, Mika Kivimaki, Vincent Plagnol, Diana Kuh.

Critical reading and contribution to revisions of the draft manuscript: All authors.

Declaration of interests:

The role of the sponsors in the study design: None
The role of the sponsors in the collection, analysis, or interpretation of the data: None.

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Comment [SEL23]: Please add a statement to the main paper (we could not find this in the revised MS): this should be in line with information provided on author ICMJE forms

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The role of the sponsors in the writing of the report: None
Those who had access to the raw data (by author initials): JW
The corresponding author had full access to all of the data and the final responsibility to submit for publication. Funding sources had no involvement in the preparation of the manuscript.

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Table 1. Table of Acronyms

Acronym	Meaning
C4D	Coronary Artery Disease consortium
CARDIoGRAM	Coronary ARtery DIsease Genome wide Replication and Meta-analysis consortium
CHD	Coronary heart disease.
ChEMBL	Chemical data base of the European molecular biology laboratory
CI	Confidence interval (95% unless otherwise stated)
DBP	Diastolic blood pressure
DIAGRAM	DIAbetes Genetics Replication And Meta-analysis
DNA	Deoxyribonucleic acid
GIANT	Genetic Investigation of ANthropometric Traits
GLGC	Global Lipids Genetic Consortium
GO	Gene ontology
GRAIL	Gene Relationships Across Implicated Loci
GWAS	Genome-wide association study
HDL-C	Serum High density lipoprotein cholesterol
ICBP	International Consortium for Blood Pressure
IV	Instrumental variable
LDL-C	Serum Low density lipoprotein cholesterol
MAGIC	Meta-Analyses of Glucose and Insulin-related traits Consortium
MR	Mendelian randomisation
MVMR	Multi-variable Mendelian randomisation
OR	Odds ratio
SBP	Systolic blood pressure
SD	Standard deviation
SE	Standard error
SNP	Single nucleotide polymorphism
TC	Serum total cholesterol
TG	Serum triglycerides
UCLEB	University College London School Edinburgh Bristol consortium

Comment [SEL24]: As agreed, this table to be removed and abbreviations defined on first use

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Table 2. SNPs used to construct the genetic instrument for plasma urate[a].

Index	SNP	CHR	BP	GENE (nearest/GRAIL)	Allele	Meta-analysis Met_beta	Meta-analysis SEMet_se	N	S	Source Data
1	rs1471633	1	144435096	PDZK1/PDZK1	A	0·0568	0·0050	116404	54	Köttgen[29]Köttgen[W33] and UCLEB [14W]22
2	rs1260326	2	27584444	GCKR/GCKR	T	0·0693	0·0049	117293	54	Köttgen[29]Köttgen[W33] and UCLEB [14W]22
3	rs12498742	4	9553150	SLC2A9/SLC2A9	A	0·3600	0·0051	145110	68	Köttgen[29]Köttgen[W33], UCLEB [14] Kolz[30W]22d Kolz[W33]
4	rs2231142	4	89271347	ABCG2/ABCG2	T	0·1896	0·0077	140915	68	Köttgen[29]Köttgen[W33], UCLEB [14W]22 and Kolz[30]Kolz[W33]
5	rs675209	6	7047083	RREB1/RREB1	T	0·0556	0·0059	117293	54	Köttgen[29]Köttgen[W33] and UCLEB [14W22]
6	rs1165151	6	25929595	SLC17A1/SLC17A3	T	-0·0779	0·0042	145201	68	Köttgen[29]Köttgen[W33], UCLEB [14W]22 and Kolz[30]Kolz[W33]
7	rs1171614	10	61139544	SLC16A9/SLC16A9	T	-0·0790	0·0070	110000	49	Köttgen[29]Köttgen[W33]
8	rs2078267	11	64090690	SLC22A11/SLC22A11	T	-0·0732	0·0058	117293	54	Köttgen[29]Köttgen[W33] and UCLEB [14W]22
9	rs478607	11	64234639	NRXN2/SLC22A12	A	-0·0264	0·0056	137967	49	Köttgen[29]Köttgen[W33]
10	rs3741414	12	56130316	INHBC/INHBE	T	-0·0649	0·0068	117293	54	Köttgen[29]Köttgen[W33] and UCLEB [14W]22
11	rs11264341	1	153418117	TRIM46/PKLR	T	-0·0500	0·0060	110000	49	Köttgen[29]Köttgen[W33]
12	rs17050272	2	121022910	INHBB/INHBB	A	0·0350	0·0060	110000	49	Köttgen[29]Köttgen[W33]
13	rs6770152	3	53075254	SFMBT1/MUSTN1	T	-0·0440	0·0050	110000	49	Köttgen[29]Köttgen[W33]
14	rs17632159	5	72467238	TMEM171/TMEM171	C	-0·0390	0·0060	110000	49	Köttgen[29]Köttgen[W33]
15	rs729761	6	43912549	VEGFA/VEGFA	T	-0·0470	0·0060	110000	49	Köttgen[29]Köttgen[W33]
16	rs1178977	7	72494985	BAZ1B/MLXIPL	A	0·0470	0·0070	110000	49	Köttgen[29]Köttgen[W33]
17	rs10480300	7	151036938	PRKAG2/PRKAG2	T	0·0350	0·0060	110000	49	Köttgen[29]Köttgen[W33]
18	rs2941484	8	76641323	HNF4G/HNF4G	T	0·0440	0·0050	110000	49	Köttgen[29]Köttgen[W33]
19	rs10821905	10	52316099	A1CF/ASAH2	A	0·0570	0·0070	110000	49	Köttgen[29]Köttgen[W33]
20	rs642803	11	65317196	OVOL1/LTBP3	T	-0·0360	0·0050	110000	49	Köttgen[29]Köttgen[W33]
21	rs653178	12	110492139	ATXN2/PTPN11	T	-0·0350	0·0050	110000	49	Köttgen[29]Köttgen[W33]
22	rs1394125	15	73946038	UBE2Q2/NRG4	A	0·0430	0·0060	110000	49	Köttgen[29]Köttgen[W33]
23	rs6598541	15	97088658	IGF1R/IGF1R	A	0·0430	0·0060	110000	49	Köttgen[29]Köttgen[W33]
24	rs7193778	16	68121391	NFAT5/NFAT5	T	-0·0460	0·0080	110000	49	Köttgen[29]Köttgen[W33]
25	rs7188445	16	78292488	MAF/MAF	A	-0·0320	0·0050	110000	49	Köttgen[29]Köttgen[W33]

[a] Units are SD uric acid per copy of effect allele using a population SD for uric acid of 90·7 µmol/L (=1·5 mg/dl) reported by CHARGE. (Yang et al.).[2] [W5]

Comment [SEL25]: As agreed, this table to be moved to appendix

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26	rs7224610	17	50719787	HLF/HLF	A	-0·0420	0·0050	110000	49	Köttgen[29] ~~Köttgen~~[w33]
27	rs742132	6	25715550	LRRC16A/LRRC16A	A	0·0540	0·0092	27923	14	Kolz[30] ~~Kolz~~[w33]
28	rs2307394	2	148432898	ORC4L/ACVR2A	T	-0·0290	0·0050	110000	49	Köttgen[29] ~~Köttgen~~[w33]
29	rs17786744	8	23832951	STC1/STC1	A	-0·0290	0·0050	110000	49	Köttgen[29] ~~Köttgen~~[w33]
30	rs2079742	17	56820479	BCAS3/C17orf82	T	0·0430	0·0080	110000	49	Köttgen[29] ~~Köttgen~~[w33]
31	rs164009	17	71795264	QRICH2/PRPSAP1	A	0·028	0·005	110000	49	Köttgen[29] ~~Köttgen~~[w33]

Table 23. Observational associations of plasma urate with cardiovascular risk factors.

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Variable	Studies *	N	Difference in risk factor for a 1-SD higher plasma urate	Lower and upper 95%CI	P-value
HDL-C (mmol/l)	4	22 669	-0·08	-0.087, -0.065	<0.0001
LDL-C (mmol/l)	2	19 195	0·07	-0.019, 0.163	0.121
Total cholesterol (mmol/l)	5	68 446	0·14	0.07, 0.213	0.0001
Triglycerides (mmol/l)	3	25 606	0·31	0.216, 0.393	<0.0001
Fasting glucose (mmol/l)	3	14 571	-0·08	-0.23, 0.066	0.276
Creatinine (mg/l)	2	6 696	4·43	1.235, 7.634	0.0066
BMI (kg/m^2)	7	84 419	1·29	0.879, 1.694	<0.0001
SBP (mmHg)	7	84 419	3·31	2.498, 4.128	<0.0001
DBP (mmHg)	4	19 033	1·95	0.926, 2.977	0.0002
Age (yrs)	3	5 713	0·21	0.045, 0.383	0.013
eGFR (ml/min/1·73m^2)	2	4 393	-4·59	-4.905, -4.269	<0.0001
Binary trait	**Studies**	**N/cases**	**Odds ratio per SD increase in plasma urate**	**Lower and upper 95%CI**	**P-value**
Sex (F vs M)	3	5 713/1 975	0·80	0.746, 0.865	<0.0001
Smoking (ever vs never)	2	4 293/2 678	1·11	1.041, 1.185	0.0015
Diabetes	2	4 394/517	1·07	0·976, 1·162	0.157

* Sources of data are given in S2Supplementary Table 2

Table 34. Association of the 31 SNP urate instrument with cardiovascular traits.

Cardio-vascular trait*	Difference in risk factor per inverse variance weighted allele.	95%CI
HDL-C (mmol/L)	-0.0079	-0.0096, -0.0062
LDL-C (mmol/L)	-0.0014	-0.0032, 0.0005
TC (mmol/L)	0.0003	-0.0015, 0.0021
TG (mmol/L)	0.0142	0.0125, 0.0158
SBP (mm Hg)	0.0045	0.0026, 0.0064
DBP (mm Hg)	0.0054	0.0033, 0.0074
Fasting Glucose (mmol/L)	-0.0010	-0.0026, 0.0006
BMI (kg/m^2 ~~.m^{-2}~~)	-0.0003	-0.0008, 0.0002
Diabetes (OR)	0.9991	0.992, 1.0064

* See Table 45 for numbers of individuals and studies

Table 45. Causal analysis of urate on risk of CHD ~~and other risk factors~~ derived from MR~~instrumental variable~~ analysis.

			31 SNP Instrument		Multivariate regression estimate***		MR-Egger	
Outcome	Studies	N (cases)	Beta* (SD of change in outcome per SD change in urate).	95%CI	Beta* (SD of change in outcome per SD change in urate).	95%CI	Beta* (SD of change in outcome per SD change in urate).	95%CI
CHD**	58	206822 (65877)	1·1766	1·0763, 1·2861	1.1013	0.996, 1.2178	1.0488	0.9191, 1.1968
~~HDL-C~~	~~69~~	~~198106~~	~~-0·1100~~	~~-0·1418, -0·0783~~	~~0~~	~~0, 0~~	~~-0.0045~~	~~-0.0518, 0.0428~~
~~LDL-C~~	~~69~~	~~183914~~	~~0·0117~~	~~-0·0222, 0·0456~~	~~0.0113~~	~~-0.0274, 0.05~~	~~0.0626~~	~~0.0123, 0.113~~
~~TC~~	~~69~~	~~198299~~	~~0·0402~~	~~0·007, 0·0735~~	~~0.0247~~	~~-0.0132, 0.0627~~	~~0.0766~~	~~0.0258, 0.1275~~
~~TG~~	~~69~~	~~188681~~	~~0·2027~~	~~0·1717, 0·2338~~	~~0~~	~~0, 0~~	~~0.0226~~	~~-0.0348, 0.08~~
~~SBP~~	~~37~~	~~80919~~	~~-0·0008~~	~~-0·0313, 0·0297~~	~~0~~	~~0, 0~~	~~-0.0518~~	~~-0.1003, -0.0033~~
~~DBP~~	~~37~~	~~80917~~	~~0·0111~~	~~-0·0225, 0·0446~~	~~0~~	~~0, 0~~	~~-0.026~~	~~-0.0794, 0.0274~~
~~Fasting Glucose~~	~~28~~	~~142304~~	~~-0·0209~~	~~-0·0504, 0·0086~~	~~-0.0174~~	~~-0.0501, 0.0153~~	~~-0.0149~~	~~-0.0575, 0.0277~~
~~BMI~~	~~64~~	~~139020~~	~~-0·0239~~	~~-0·066, 0·0183~~	~~-0.0019~~	~~-0.0117, 0.008~~	~~-0.0025~~	~~-0.0151, 0.0101~~
~~Diabetes**~~	~~20~~	~~69033 (12171)~~	~~0·9208~~	~~0·8109, 1·0455~~	~~0.9987~~	~~0.8661, 1.1516~~	~~0.8689~~	~~0.7266, 1.0391~~

* Beta is the regression coefficient for the trait on the urate instrument.
** odds ratio.
*** Covariates are DBP, SBP, TG and HDL. Note that the regression model precludes meaningful consideration of DBP, TG and HDL as outcomes.

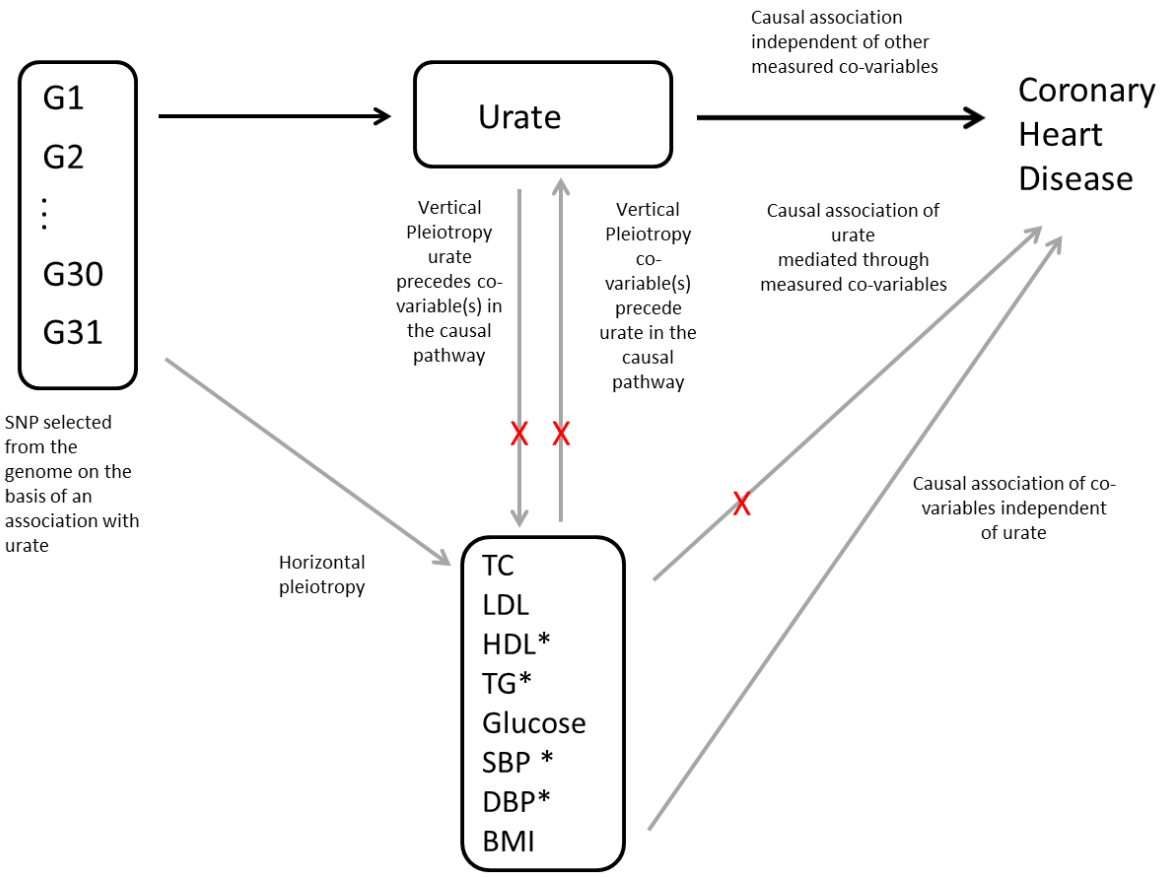
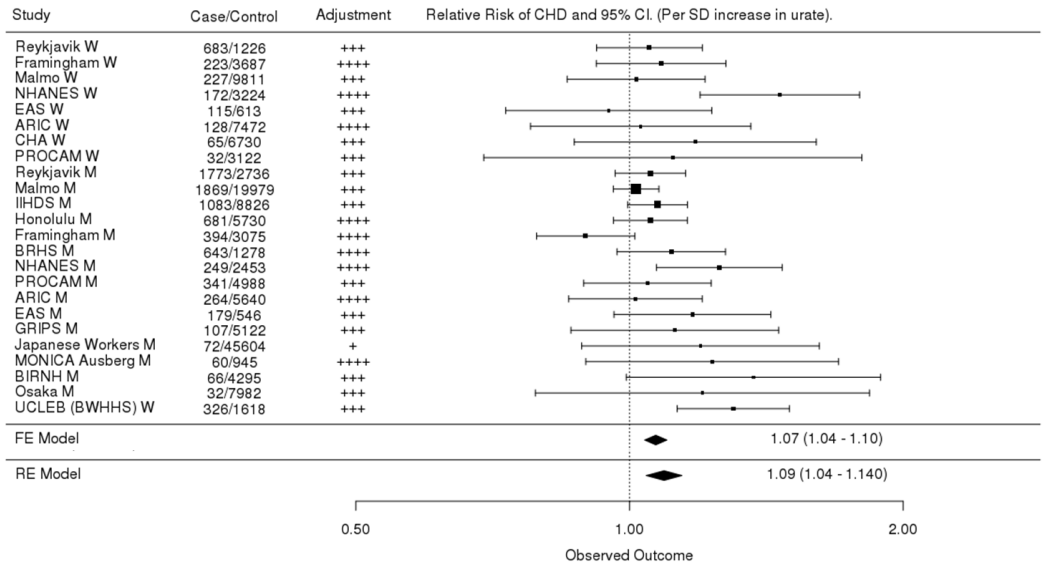


Figure 1. Conceptual framework for the Mendelian randomisation analysis of urate and CHD.

G1-31 are genes containing urate variants which together form the multilocus instrument for urate. Horizontal pleiotropy occurs when the instrument associates with traits other than urate which become confounders if also associated with CHD. Vertical pleiotropy occurs if their level is influenced by urate, and does not invalidate MR analysis Multivariable MR including DBP, SBP, HDL and TG as covariates was used to account for possible horizontal pleiotropy arising from association of the instrument with these variables. The effect of the adjustment is to block the paths indicated with red crosses. ~~Egger~~ MR-Egger analysis was used to account for unknown or unmeasured, pleiotropic confounders. (see text for details).



~~**Figure 2. Observational association between urate concentration and relative risk of CHD in 17 prospective population-based cohorts.** Summary estimates obtained by fixed-effects (FE) and random effects (RE) meta-analysis are presented. Adjustment: + age and sex; +++ age, sex, smoking and some additional risk factors, ++++ as +++ with adjustment for pre-existing CHD. Apart from UCLEB (BWHHS) the data were obtained from Wheeler *et al.* 2005, the order of studies mirrors that publication.. (Size of point markers is proportional to the inverse variance). W = Women, M = Men.~~

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Comment [SEL26]: As noted earlier, we request you move this figure to the appendix. Please use the updated version of this figure that shows the RR for each individual study. Please also give a reference number for each line (with a separate list of references in appendix) so identity of the individual studies is more clear.

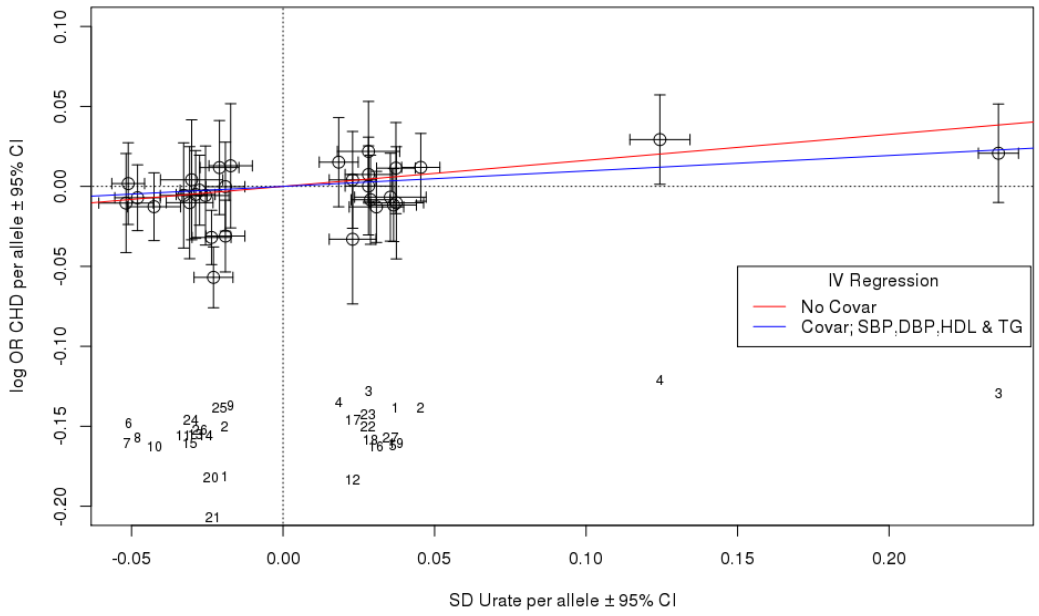

Figure 2~~3~~. Association of individual SNPs with urate and CHD risk. Estimates are derived from meta-analysis over multiple studies (~~Supplementary~~ Table S2 (page3)).~~3).~~ Bars represent 95% CI. The numbers below the main figure correspond to the index column in Table 1~~2~~ to allow cross-referencing. The slopes of the lines are IV regression estimates of the effect of urate on CHD risk with (blue) and without (red) SBP, DBP, HDL and TG as covariates.

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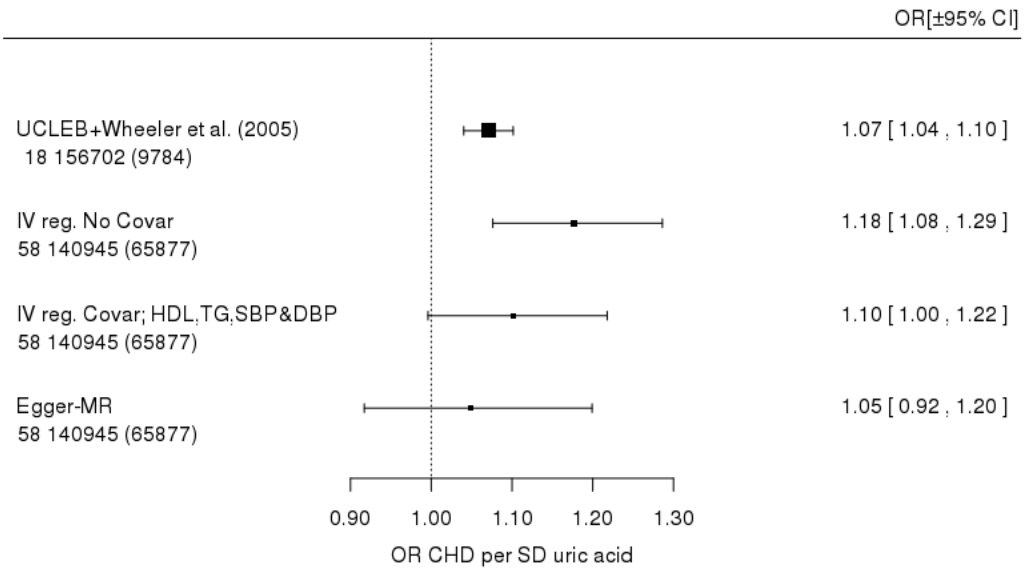

Figure 34. Observational and estimated causal effectsassociations of urate with risk of CHD. Values represent a per-1 SD increase in urate. Numbers below data description are No. studies, controls, and (cases).

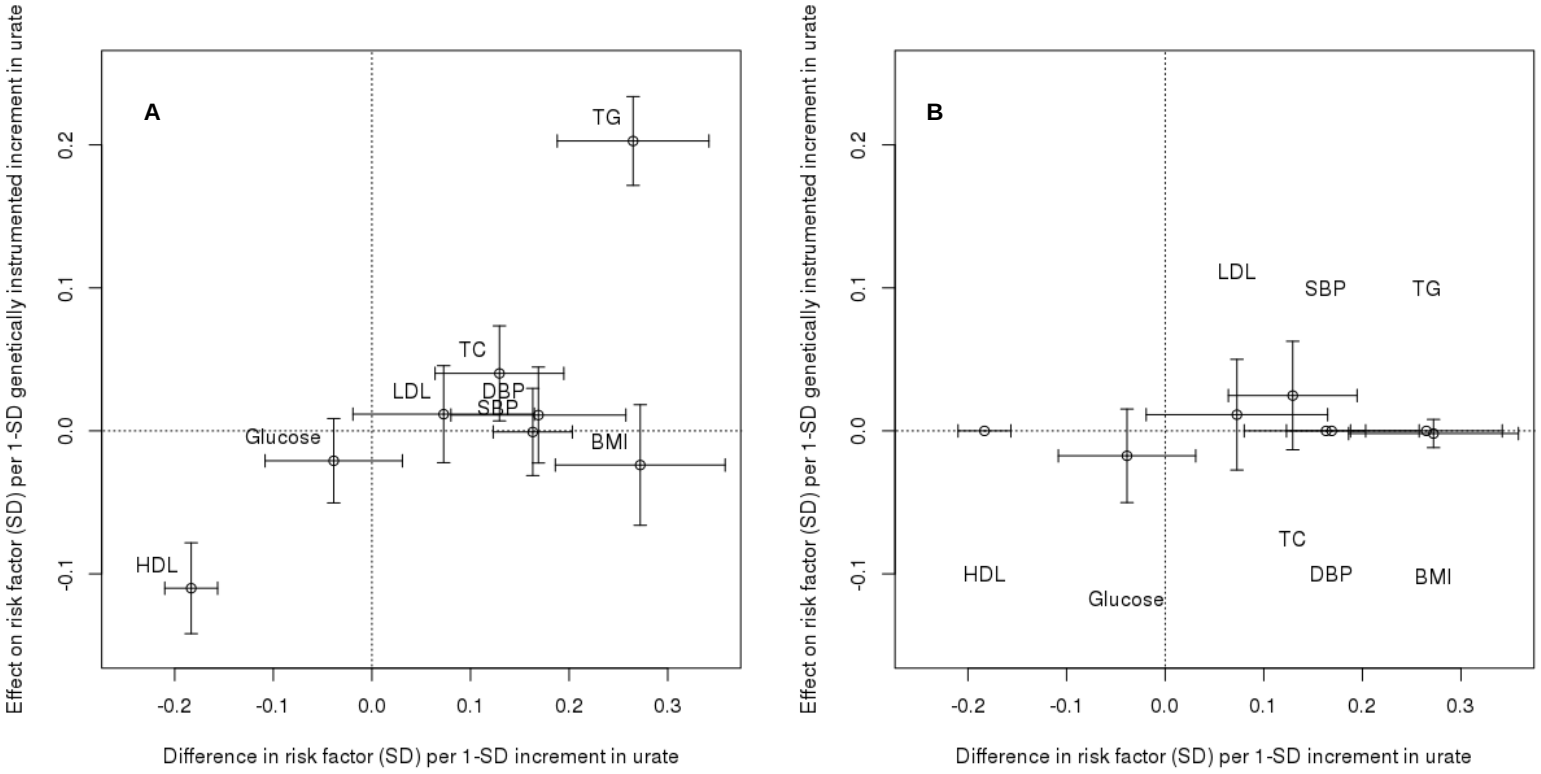

Figure 45. Comparison of observational and genetically instrumented associations between urate and several cardiovascular risk factors. (A) The genetically instrumented effect of urate without accounting for pleiotropic associations; and (B) the genetically instrumented effect with DBP, SBP, HDL and TG included as covariates in a multivariable MR analysis.

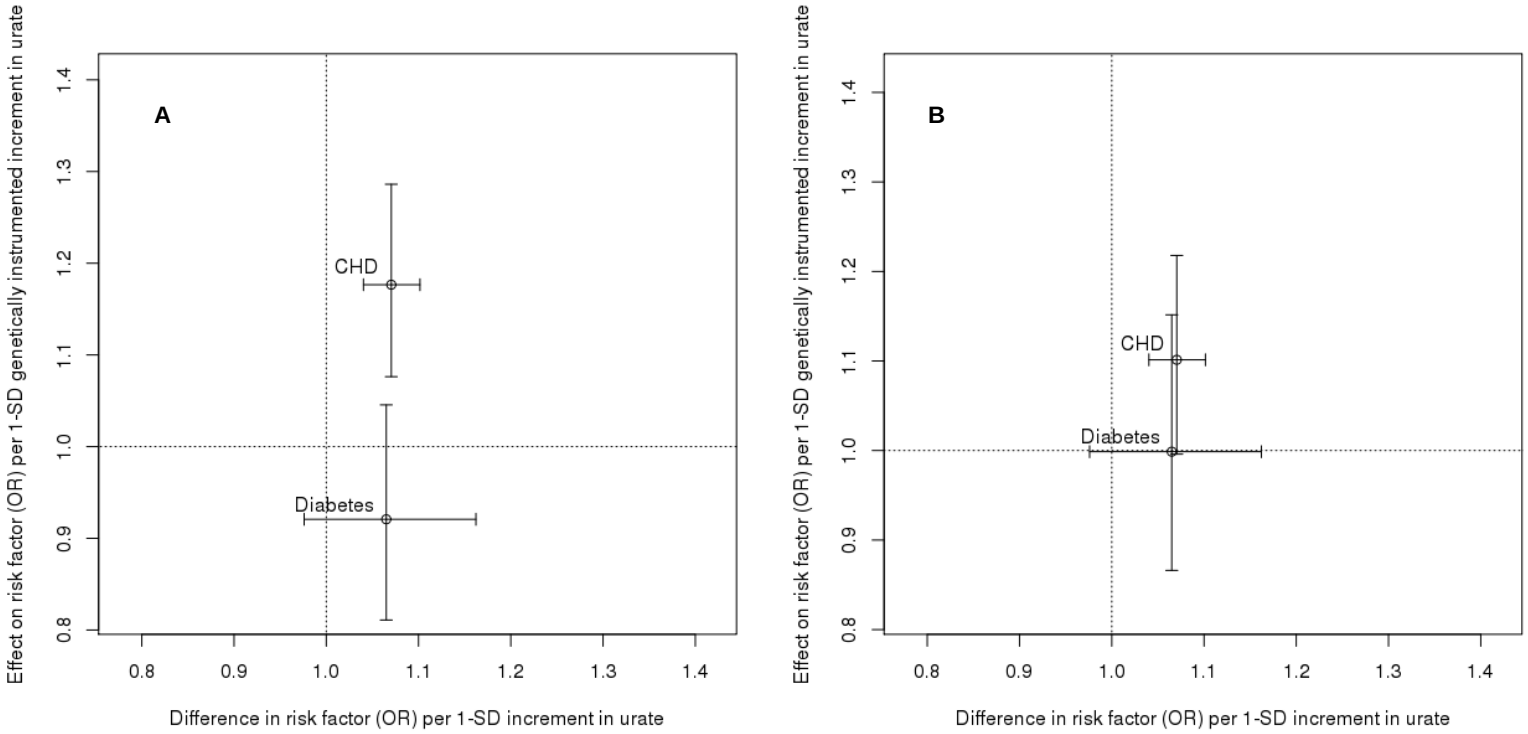

Figure 5~~6~~. Observational association between binary traits and urate against Instrumental Variable association for (A) The 31-SNP instrument without covariates and (B) the 31-SNP instrument with DBP, SBP, HDL and TG as covariates.

References

1 Feig DI, Kang DH, and Johnson RJ, (2008a). Uric acid and Cardiovascular Risk. N.Engl.J.Med.; 359:1811-1821.

2 Wheeler, JG, Juzwishin, DM, Eriksdottir, G, Gudnason, V, and Danesh J, (2005) Serum Uric Acid and Coronary Heart Disease in 9458 Incident Cases and 155084 Controls: Prospective Study and Meta-Analysis. PLoS Med. 2: 236-243.

3 Regoli F and Winston GW (1999) Quantification of total oxidant scavenging capacity of antioxidants for peroxynitrite, peroxyl radicals, and hydroxyl radicals. Toxicol Appl Pharmacol 156:96–105.

4 Wayner, D. D. M., Burton, G. W., Ingold, K. U., Barclay,L. R. C., and Locke, S. *J.* (1987) The relative contributions of vitamin E,urate, ascorbate and proteins to the total peroxyl radical-trapping antioxidant activity of human blood plasma. Biochim.Biophys. Acta 924:408-419.

5 Teng Ru-J, Ye Y-Zu, Parks DA, and Beckman JS (2002) Urate produced during hypoxia protects heart proteins from peroxynitrite-mediated protein nitration. Free Radic Biol Med 33:1243–1249

6 Ames BN, Cathcart R, Schwiers E, and Hochstein P. (1981)Uric acid provides an antioxidant defense in humans against oxidant- and radical-caused aging and cancer: a hypothesis. Proc Natl. Acad. Sci. USA. 78:6858-62.

7 Waring WS, McKnight JA, Webb DJ, Maxwell SRJ (2006) Uric Acid Restores Endothelial Function in Patients With Type 1 Diabetes and Regular Smokers. Diabetes 55:3127-3132

8 Cory DB, Eslami P, Yamamoto K, Nyby MD, Makino H, and Tuck ML, (2008). Uric acid stimulates vascular smooth muscle cell proliferation and oxidative stress via the vascular renin-angiotensin system. Journal of Hypertension; 26: 269-275.

9 Loscalzo J, (2001). Nitric oxide insufficiency, platelet activation and arterial thrombosis. Circ. Res; 88: 756-762.

10 Sone PH, (2011). A New anit ischemic rol for an old drug. JACC. 58:829-830.

11 ISRCTN Registry. Allopurinol and cardiovascular outcomes in patients with ischemic heart disease (IHD). http://www.isrctn.com/ISRCTN32017426

12 Hingorani A, and Humphries S, 2005. Nature's Randomised Trials. Lancet 366: 1906-1908.

13 Lawlor DA Harbrod RM, Sterne JAC, Timpson N, and Smith GD, (2008) Mendelian Randomisation: Using genes as instruments for making causal inferences in epidemiology. Statistics in Medicine. 27:1133-1163

14 Bowden, J., Smith, GD. and Burges, S. *(2015) Mendelian randomization with invalid instruments: effect estimation and bias detection through Egger regression. Int. J. Epidemiology.* In Press. Prepublication copy from: http://www.mrc-bsu.cam.ac.uk/wp-content/uploads/IJEpaperFinal.pdf

15 Smith, G.D. and Hemani, G. 2014. Mendelian randomization: genetic anchors for causal inference in epidemiological studies. Hum. Mol. Genet. 23 (R1): R89-R98.

16 Burgess S, Freitag DF, Khan H, Gorman DN, and Thompson SG, (2014) Using Multivariable Mendelian Randomization to Disentangle the Causal Effects of Lipid Fractions. PLOSone. DOI: 10.1371/journal.pone.0108891

17 Burgess, S. Dudbridge, F. and Thompson, S.G. (2015) Multivariable Mendelian Randomization: The Use of Pleiotropic Genetic Variants to Estimate Causal Effects. Am. J. Epidemiol. 181: 290-291

18 Palmer TM, Lawlor DA, Harbord RM, et al. (2011). Using multiple genetic varints as instrumental variables for modifiable risk factors. Stat. Met. in Med. Res. 21:223-242.

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19. Sedaghat S, Pazoki R, Uitterlinden AG, Hofman A, Stricker BH, Ikram MA, Franco OH, and Dehghan A. (2014). Association of uric acid genetic risk score with blood pressure: the Rotterdam study. Hypertension. 64:1061-6. doi: 10.1161/HYPERTENSIONAHA.114.03757.

20. Voight BF, Peloso GM, Orho-Melander M, et al. (2012). Plasma HDL cholesterol and risk of myocardial infarction: a mendelian randomisation study. Lancet. 380: 572–80

21. Johnson T, (2012) Efficient Calculation for Multi-SNP Genetic Risk Scores. http://cran.r-project.org/web/packages/gtx/vignettes/ashg2012.pdf

22. Morris AP, Voight BF, Teslovich TM, *et al.* DIAbetes Genetics Replication And Meta-analysis (DIAGRAM) Consortium. (2012) Large-scale association analysis provides insights into the genetic architecture and pathophysiology of type 2 diabetes. Nat Genet. 44:981–90

23. Global Lipids Genetics Consortium 2013. Discovery and refinement of loci associated with lipid levels Nature Genetics 45, 1274–1283.

24. Speliotes EK, Willer CJ, Berndt SI, *et al.* (2010). Association analyses of 249,796 individuals reveal 18 new loci associated with body mass index. Nat Genet 42, 937-948.

25. Dupuis J, Langenberg C, Prokopenko I, *et al.* (2010) New genetic loci implicated in fasting glucose homeostasis and their impact on type 2 diabetes risk. Nature Genetics. 42. 105-116.

26. ICBP, Ehret GB, Munroe PB, Rice KM, *et al.* (2011). Genetic variants in novel pathways influence blood pressure and cardiovascular disease risk. Nature. 478. 103–109.

27. Danesh, J, Collins R, Appleby P and, Peto R (1998). Association of Fibrinogen, C-reactive Protein, Albumin, or Leucocyte Count With Coronary Heart Disease. JAMA. 279:1477–1482

28. Brion M-J. A, Shakhbazov K and, Visscher PM (2013) Calculating statistical power in Mendelian randomization studies International Journal of Epidemiology 42: 1497–1501.

29. Bento AP, Gaulton A, Hersey A, Bellis LJ, Chambers J, Davies M, et al. (2014) The ChEMBL bioactivity database: an update. Nucleic acids research. 42:D1083–90. doi:10.1093/nar/gkt1031

30. Ashton KS, Andrews KL, Bryan MC, Chen J, Chen K, et al. (2014) Small Molecule Disruptors of the Glucokinase–Glucokinase Regulatory Protein Interaction: 1. Discovery of a Novel Tool Compound for in Vivo Proof of Concept. J Med Chem 57: 309–324. doi:10.1021/jm4016735.

31. Kloker, M.E., Delgado, C., Grammer, T.B., et al. (2015). Uric acid and cardiovascular events: a Mendelian randomisation study. J. Am. Soc. Nephrology. 26. doi: 10.1681/ASN.2014070660

32. Parsa A, Brown E, Weir MR, Fink JC, Shuldiner AR, Mitchell BD, and McArdle PF. (2012) Genotype-based changes in serum uric acid affect blood pressure. Kidney Int. 81:502–7. doi: 10.1038/ki.2011.414.

33. Interleukin-6 Receptor Mendelian Randomisation Analysis (IL6R MR) Consortium, Daniel I. Swerdlow, Michael V Holmes, Karoline B Kuchenbaecker et al. (2012) The interleukin-6 receptor as a target for prevention of coronary heart disease: a mendelian randomisation analysis. Lancet. 379:1214-24. doi: 10.1016/S0140-6736(12)60110-X.

34. Holmes MV, Simon T, Exeter HJ, Folkersen L, et al. (2013) Secretory phospholipase A(2)-IIA and cardiovascular disease: a mendelian randomization study. J Am Coll Cardiol. 62:1966-76. doi: 10.1016/j.jacc.2013.06.044.

1 Terkeltaub R.A. (2010)

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~~Funding~~**Acknowledgements**Update on gout: new therapeutic strategies and options. Nature Reviews Rheumatology 6:30-38

2 Yang Q, Köttgen A, Dehghan A, et al. 2010. Multiple Genetic Loci Influence Serum Urate and Their Relationship with Gout and Cardiovascular Disease Risk Factors Circ Cardiovasc Genet. 3(6): 523–530.

3 Davis N, (1897). The cardiovascular and renal relations and manifestations of gout. JAMA; 29: 261-262

4 Wheeler, JG, Juzwishin, DM, Eriksdottir, G, Gudnason, V, and Danesh J, (2005) Serum Uric Acid and Coronary Heart Disease in 9458 Incident Cases and 155084 Controls: Prospective Study and Meta-Analysis. PLoS Med. 2: 236–243.

5 Waring WS, McKnight JA, Webb DJ, Maxwell SRJ (2006) Uric Acid Restores Endothelial Function in Patients With Type 1 Diabetes and Regular Smokers. Diabetes 55:3127–3132

6 Cory DB, Eslami P, Yamamoto K, Nyby MD, Makino H, and Tuck ML, (2008). Uric acid stimulates vascular smooth muscle cell proliferation and oxidative stress via the vascular renin-angiotensin system. Journal of Hypertension; 26: 269–275.

7 Landmesser U, and Drexler H, (2002) Editorial: Allopurinol and Endothelial Function in heart Failure. Circulation. 106:173–175.

8 Struthers A and, Shearer F, (2012) Editorial. Allopurinol: novel indications in cardiovascular disease. Doi:10.1136/heartjnl-2012-302249

9 Sone PH, (2011). A New anit-ischemic rol for an old drug. JACC. 58:829–830.

10 Lawlor DA Harbrod RM, Sterne JAC, Timpson N, and Smith GD, (2008) Mendelian Randomisation: Using genes as instruments for making causal inferences in epidemiology. Statistics in Medicine. 27:1133-1163

11 Burgess, S, Dudbridge, F, and Thompson, S.G. (2015) Multivariable Mendelian Randomization: The Use of Pleiotropic Genetic Variants to Estimate Causal Effects. Am. J. Epidemiol. 181: 290-291

12 Bowden J, Smith, GD, and Burgess, S (2015) Mendelian randomization with invalid instruments: effect estimation and bias detection through Egger regression. Int. J. Epidemiology. doi: 10.1093/ije/dyv080

13 Palmer TM, Lawlor DA, Harbord RM, et al. (2011). Using multiple genetic varints as instrumental variables for modifiable risk factors. Stat. Met. in Med. Res. 21:223-242.

14 Shah T, Engmann J, Dale C, et al. (2013) Population Genomics of Cardiometabolic Traits: Design of the University College London-London School of Hygiene and Tropical Medicine-Edinburgh-Bristol (UCLEB) Consortium. PLoS ONE 8(8): e71345. doi:10.1371/journal.pone.0071345

14 Shah T, Engmann J, Dale C, et al. (2013) Population Genomics of Cardiometabolic Traits: Design of the University College London-London School of Hygiene and Tropical Medicine-Edinburgh-Bristol (UCLEB) Consortium. PLoS ONE 8(8): e71345. doi:10.1371/journal.pone.0071345

16 Sedaghat S, Pazoki R, Uitterlinden AG, Hofman A, Stricker BH, Ikram MA, Franco OH, and Dehghan A, (2014). Association of uric acid genetic risk score with blood pressure: the Rotterdam study. Hypertension. 64:1061-6. doi: 10.1161/HYPERTENSIONAHA.114.03757.

17 Johnson T, (2012) Efficient Calculation for Multi-SNP Genetic Risk Scores. http://cran.r-project.org/web/packages/gtx/vignettes/ashg2012.pdf

18 Brion M-J. A, Shakhbazov K and, Visscher PM (2013) Calculating statistical power in Mendelian randomization studies International Journal of Epidemiology 42: 1497–1501

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19 Bento AP, Gaulton A, Hersey A, Bellis LJ, Chambers J, Davies M, et al. (2014) The ChEMBL bioactivity database: an update. Nucleic acids research. 42:D1083–90. doi:10.1093/nar/gkt1031

20 Yates A, Beal K, Keenan S, McLaren W, Pignatelli M, Ritchie GRS, et al. (2014) The Ensembl REST API: Ensembl Data for Any Language. Bioinformatics.:btu613. doi:10.1093/bioinformatics/btu613

21 Jain E, Bairoch A, Duvaud S, Phan I, Redaschi N, Suzek BE, et al. Infrastructure for the life sciences: design and implementation of the UniProt website. BMC bioinformatics. 009;10:136. doi:10.1186/1471-2105-10-136

22 Ashton KS, Andrews KL, Bryan MC, Chen J, Chen K, et al. (2014) Small Molecule Disruptors of the Glucokinase–Glucokinase Regulatory Protein Interaction: 1. Discovery of a Novel Tool Compound for in Vivo Proof-of-Concept. J Med Chem 57: 309–324. doi:10.1021/jm4016735.

23 C Reactive Protein Coronary Heart Disease Genetics Collaboration (CCGC) (2011) Association between C reactive protein and coronary heart disease: mendelian randomisation analysis based on individual participant data . BMJ 2011; 342 doi: tp://dx.doi.org/10.1136/bmj.d548

24 Palmer TM, Nordestgaard BG, Benn M, et al. (2013). Association of plasma uric acid with ischaemic heart disease and blood pressure: Mendelian randomisation analysis of two large cohorts. BMJ. 347:doi: 10.1136/bmj.f4262

25 Kleber, M.E., Delgado, G., Grammer, T.B., et al. (2015). Uric acid and cardiovascular events: a Mendelian randomisation study. J. Am. Soc. Nephrology. 26. doi: 10.1681/ASN.2014070660

26 Parsa A, Brown E, Weir MR, Fink JC, Shuldiner AR, Mitchell BD, and McArdle PF. (2012) Genotype-based changes in serum uric acid affect blood pressure. Kidney Int. 81:502-7. doi: 10.1038/ki.2011.414.

27 Interleukin-6 Receptor Mendelian Randomisation Analysis (IL6R MR) Consortium, Daniel I Swerdlow, Michael V Holmes, Karoline B Kuchenbaecker et al. (2012) The interleukin-6 receptor as a target for prevention of coronary heart disease: a mendelian randomisation analysis. Lancet. 379:1214-24. doi: 10.1016/S0140-6736(12)60110-X.

28 Holmes MV, Simon T, Exeter HJ, Folkersen L, et al. (2013) Secretory phospholipase A(2)-IIA and cardiovascular disease: a mendelian randomization study. J Am Coll Cardiol. 62:1966-76. doi: 10.1016/j.jacc.2013.06.044.

29 Köttgen A, Albrecht E, Teumer A, et al. (2013).Genome-wide association analyses identify 18 new loci associated with serum urate concentrations. Nature genetics;45(2):145–54.

30 Kolz M, Johnson T, Sanna S, et al. (2009). Meta-analysis of 28,141 individuals identifies common variants within five new loci that influence uric acid concentrations.PLoS Genetics; Jun;5(6):e1000504

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Catherine T. MacArthur Foundation Research Networks on Successful Midlife Development and Socio-economic Status and Health. Samples from the ELSA DNA Repository (EDNAR), received support under a grant (AG1764406S1) awarded by the National Institute on Ageing (NIA). ELSA was developed by a team of researchers based at the National Centre for Social Research, University College London and the Institute of Fiscal Studies. The data were collected by the National Centre for Social Research. MRC NSHD is funded by the UK Medical Research Council. DNA collection of the 1958BC was funded by the UK Medical Research Council (G0000934) and the Wellcome Trust (Grant 068545/Z/02). Genotyping was supported by a contract from the European Commission Framework Programme 6 (018996) and grants from the French Ministry of Research. BWHHS is supported by funding from the British Heart Foundation and the Department of Health Policy Research Programme (England). EAS is funded by the British Heart Foundation (Programme Grant RG/98002), with Metabochip genotyping funded by a project grant from the Chief Scientist Office of Scotland (Project Grant CZB/4/672). AAAT was funded by the British Heart Foundation (Programme Grant RG/97006), the Wellcome Trust (Project Grant 057762), the Chief Scientist Office of Scotland (Project Grant K/OPR/2/2/D320), Chest Heart and Stroke Scotland (Project Grant Res03/A75) and Bayer plc (Unrestricted Investigator Led Grant). Research clinics were held at the Wellcome Trust Clinical Research Facility in Edinburgh. ET2DS is funded by the Medical Research Council (Project Grant G0500877), the Chief Scientist Office of Scottish (Programme Support Grant CZQ/1/38), Pfizer plc (Unrestricted Investigator Led Grant) and Diabetes UK (Clinical Research Fellowship 10/0003985). Research clinics were held at the Wellcome Trust Clinical Research Facility and Princess Alexandra Eye Pavilion in Edinburgh. EHDPS was funded by the Medical Research Council and by the Chief Scientist Office of Scotland (Project Grant CZB/4/672). DNA standardisation was conducted at the Genetics Core of the Wellcome Trust Clinical Research Facility in Edinburgh. CaPS was funded by the Medical Research Council and undertaken by the former MRC Epidemiology Unit (South Wales). The DNA bank was established with funding from a MRC project grant. The data archive is maintained by the University of Bristol. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Supplementary Table 1. Proxy SNPs used in the Instrument.

Index	Lead SNP	Proxy SNP	R^2
3	rs12498742	rs734553	0.89
6	rs1165151	rs1183201	1
9	rs478607	rs505802	0.44

Supplementary Table 2 Sources of data for estimate of observational association with urate.

Data Source	SBP	DBP	TC	TG	HDL-C	LDL-C	Creatinine	BMI	Glucose
UCLEB consortium	5691	5691	5691	5691	5691	5691	5691	5691	5691
Liese *et al.*1999.[W26]	1005	1005	1005	-	1005	-	1005	1005	-
Puddu *et al.* 2001.[W28]	2469	-	-	-	2469	-	-	2469	2469
Fang *et al.* 2000.[W24]	5926	5926	5926	-	-	-	-	5926	-
Medalie *et al.* 1973.[W23]	6411	6411	6411	6411	-	-	-	6411	6411
Moriarity *et al.* 2000.[W27]	13504	-	-	13504	13504	13504	-	13504	-
Tomita *et al.*2000.[W25]	49413	-	49413	-	-	-	-	49413	-

Note: Estimates reported for smoking, age, sex, eGFR and, diabetes were made in UCLEB consortium data only.

Supplementary Table 3. Sources of regression statistics used in this study.

Association (A with B)	Data	N individuals	N studies
Observational Association			
Urate – lipid phenotypes	Leise *et al.* [W26] Puddu *et al.* [W28] Medalie *et al.* [W23] Moriarty *et al.* [W27] UCLEB [W20]	1 005 2 469 6 411 13 504 5 691	
Urate – BMI	Leise *et al.* [W26] Puddu *et al.* [W28] Medalie *et al.* [W23] Moriarty *et al.* [W27] Fang *et al.* [W24] Tomita *et al.* [W25] UCLEB [W22]	1 005 2 469 6 411 13 504 5 926 49 413 5 691	
Urate – T2D			
Urate – fasting glucose	Leise *et al.* [W26] Puddu *et al.* [W28] Moriarty *et al.* [W27] UCLEB [W20]	1 005 2 469 13 504 5 691	
Urate - BP	Leise *et al.* [W26] Puddu *et al.* [W28] Medalie *et al.* [W23] Moriarty *et al.* [W27] Fang *et al.* [W24] Tomita *et al.* [W25] UCLEB [W20]	1 005 2 469 6 411 13 504 5 926 49 413 5 691	
Urate - CHD	Wheeler *et al.* [2] UCLEB [W20]	174 326(9 458 cases) 1 944 (326 cases)	17 1
Genetic Association			
SNP - urate	Köttgen *et al.* [W33] Kolz *et al.* [W33] UCLEB [W20]	110 347 27 817 7 151	48 14 3
SNP – CHD	CARDIoGRAM [W30] **and/or** C4D [W31] **or** CARDIoGRAM plus C4D [W32] **with** UCLEB [W20]	78 856 (19 368 cases) 30 393 (15 357 cases) 186 203 (60 785 cases) 12 395 (2 131 cases)	37 14 48 7
SNP – lipid phenotypes	GLGC [23] UCLEB [W20]	187 190 9 431	64 4
SNP – T2D	DIAGRAM [22] UCLEB [W20]	69 033 (12 717 cases) 15 605 (2 643 cases)	12 8
SNP – fasting glucose	MAGIC UCLEB [W20]	46 186 11 211	21 7
SNP - BP	ICBP [26] UCLEB [W20]	69 590 20 077	29 8
SNP - BMI	GIANT [24]	127 600	64
Confounding associations			
TC - CHD	Liese et al, UCLEB, Fang *et al.* Tomita et al, Medalie *et al.* [W23-W26, W20]	64 446	5
HDLc - CHD	Liese et al, UCLEB, Puddu *et al.*, Morarty *et al.* [W26- W28, W22]	22 669	4
LDLc - CHD	UCLEB, Morarty *et al.* [W27, W20]	19 195	2
TG - CHD	Morarty et al, UCLEB, Medalie *et al.* [W23,W27, W20]	25 606	3
BMI - CHD	Fang et al, Morarty *et al.*, Puddu *et al.*, Liese *et al.*, UCLEB, Tomita *et al.*, Medalie *et al.* [W23-W28, W22]	84 419	7
Fasting glucose - CHD	Puddu et al., UCLEB, Medalie et al. [W23,W28]	15 471	3

	[W22]		
SBP - CHD	Fang *et al.*, Morarty *et al.*, Puddu *et al.*, Liese *et al.*, UCLEB, Tomita *et al.* Medalie *et al.* [W23-W28, W20]	84 419	7
DPB - CHD	Liese *et al.*, UCLEB, Fang *et al.* Tomita *et al.*, Medalie *et al.* [W23-W26, W20]	19 033	4

Supplementary Table 4. Power (two-sided α=0·05) for IV regression of the binary outcomes.

Outcome	Proportion cases	Observational OR (per SD urate)	R^2 of instrument	N required for 80% power	Actual n	Power at actual n
CHD	0·317	1·07	0·042	183868	198598	0.83
T2D	0·175	1·32	0·042	13910	84638	1

Supplementary Table 5. Power (α=0·05) for IV regression of the continuous outcomes.

Outcome (units)	$\beta_{yx}(\text{true})$[a]	R^2	N required for 80% power	Actual n	Power at actual n
LDL-C (mmol/L)	0·073	0·042	34882	196621	1
HDL-C (mmol/L)	-0·183	0·042	5394	196621	1
TC (mmol/L)	0·129	0·042	11044	196621	1
TG (mmol/L)	0·265	0·042	2475	196621	1
SBP (mmHg)	0.163	0·042	6847	89667	1
DBP (mmHg)	0.169	0·042	6357	89667	1
Fasting Glucose (mmol/L)	-0·039	0·042	122697	57397	0·48

Supplementary Table 6. Gene Ontology Enrichment Analysis.

Term (GO reference)	Background frequency	Sample frequency	Bonferroni corrected P-value
urate metabolic process (GO:0046415)	13	7	3.96E-13
purine-containing compound metabolic process (GO:0072521)	311	8	5.57E-05
heterocycle metabolic process (GO:0046483)	4328	19	1.84E-03
cellular aromatic compound metabolic process (GO:0006725)	4332	19	1.87E-03
organic cyclic compound metabolic process (GO:1901360)	4571	19	4.51E-03
cellular nitrogen compound metabolic process (GO:0034641)	4598	19	4.97E-03
nitrogen compound metabolic process (GO:0006807)	5014	19	2.01E-02

[a] See Supplementary Table 3~~Supplementary Table 3~~ for sources of data used to estimate the regression coefficient. Units are SD/SD.

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Supplementary Table 7. Function and druggability of genes represented in the multiple-instrument.

SNP	CHR	GENE (nearest/GRAIL)	Drugs	Gene function (from; http://www.genecards.org/)
rs1471633	1	PDZK1/PDZK1	None	PDZK1: This gene encodes a PDZ domain-containing scaffolding protein. PDZ domain-containing molecules bind to and mediate the subcellular localization of target proteins. The encoded protein mediates the localization of cell surface proteins and plays a critical role in cholesterol metabolism by regulating the HDL receptor, scavenger receptor class B type 1. Single nucleotide polymorphisms in this gene may be associated with metabolic syndrome, and overexpression of this gene may play a role in drug resistance of multiple myeloma. Pseudogenes of this gene are located on the long arm of chromosome 1. Alternatively spliced transcript variants encoding multiple isoforms have been observed for this gene.
rs1260326	2	GCKR/GCKR	In development. 61	This gene encodes a protein belonging to the GCKR subfamily of the SIS (Sugar ISomerase) family of proteins. The gene product is a regulatory protein that inhibits glucokinase in liver and pancreatic islet cells by binding non-covalently to form an inactive complex with the enzyme. This gene is considered a susceptibility gene candidate for a form of maturity-onset diabetes of the young (MODY).
rs12498742	4	SLC2A9/SLC2A9	None	This gene encodes a member of the SLC2A facilitative glucose transporter family. Members of this family play a significant role in maintaining glucose homeostasis. The encoded protein may play a role in the development and survival of chondrocytes in cartilage matrices. Two transcript variants encoding distinct isoforms have been identified for this gene.
rs2231142	4	ABCG2/ABCG2		The membrane-associated protein encoded by this gene is included in the superfamily of ATP-binding cassette (ABC) transporters. ABC proteins transport various molecules across extra- and intra-cellular membranes. ABC genes are divided into seven distinct subfamilies (ABC1, MDR/TAP, MRP, ALD, OABP, GCN20, White). This protein is a member of the White subfamily. Alternatively referred to as a breast cancer resistance protein, this protein functions as a xenobiotic transporter which may play a major role in multi-drug resistance. It likely serves as a cellular defense mechanism in response to mitoxantrone and anthracycline exposure. Significant expression of this protein has been observed in the placenta, which may suggest a potential role for this molecule in placenta tissue. Multiple transcript variants encoding different isoforms have been found for this gene.
rs675209	6	RREB1/RREB1	None	RREB1: The protein encoded by this gene is a zinc finger transcription factor that binds to RAS-responsive elements (RREs) of gene promoters. It has been shown that the calcitonin gene promoter contains an RRE and that the encoded protein binds there and increases expression of calcitonin, which may be involved in Ras/Raf-mediated cell differentiation. Multiple transcript variants encoding several different isoforms have been found for this gene. LY86: May cooperate with CD180 and TLR4 to mediate the innate immune response to bacterial lipopolysaccharide (LPS) and cytokine production. Important for efficient CD180 cell surface expression (By similarity)
rs1165151	6	SLC17A1/SLC17A3	None	SLC17A1 (solute carrier family 17 (organic anion transporter), member 1) is a protein-coding gene. Diseases associated with SLC17A1 include cardiovascular disease risk factor. GO annotations related to this gene include sodium-dependent phosphate transmembrane transporter activity and symporter activity. An important paralog of this gene is SLC17A7.
rs1171614	10	SLC16A9/SLC16A9	None	SLC16A9 (solute carrier family 16, member 9) is a protein-coding gene. GO annotations related to this gene include symporter activity. An important paralog of this gene is SLC16A4.

rs2078267	11	SLC22A11/SLC22A11	Probenecid	SLC22A11 (solute carrier family 22 (organic anion/urate transporter), member 11) is a protein-coding gene. Diseases associated with SLC22A11 include cardiovascular disease risk factor. GO annotations related to this gene include inorganic anion exchanger activity and sodium-independent organic anion transmembrane transporter activity. An important paralog of this gene is SLC22A5.
rs478607	11	NRXN2/SLC22A12	Sulfinpyrazone	SLC22A12 (solute carrier family 22 (organic anion/urate transporter), member 12) is a protein-coding gene. Diseases associated with SLC22A12 include renal hypouricemia 1, and renal hypouricemia. GO annotations related to this gene include PDZ domain binding and urate transmembrane transporter activity. An important paralog of this gene is SLC22A11.
rs3741414	12	INHBC/INHBE	None	INHBC (inhibin, beta C) is a protein-coding gene. Diseases associated with INHBC include gastric diffuse adenocarcinoma, and endometrial adenocarcinoma. GO annotations related to this gene include growth factor activity and transforming growth factor beta receptor binding. An important paralog of this gene is GDF11. / INHBE: (inhibin, beta E) is a protein-coding gene. Diseases associated with INHBE include endometrial adenocarcinoma, and germ cell tumors. GO annotations related to this gene include growth factor activity and hormone activity. An important paralog of this gene is GDF11.
rs11264341	1	TRIM46/PKLR	None/compounds in development	TRIM46: Protein coding. Paaralog is TRIM13 which is associated with leukemia. PKLR: The protein encoded by this gene is a pyruvate kinase that catalyzes the transphosphorylation of phohsphoenolpyruvate into pyruvate and ATP, which is the rate-limiting step of glycolysis. Associated with hemolytic anemia.
rs17050272	2	INHBB/INHBB	None	INHBB: A protein-coding gene. Diseases associated with INHBB include varicocele, and ectopic pregnancy. GO annotations related to this gene include growth factor activity and protein homodimerization activity. An important paralog of this gene is GDF11.
rs6770152	3	SFMBT1/MUSTN1	None/None	SFMBT1: (Scm-like with four mbt domains 1) is a protein-coding gene. Diseases associated with SFMBT1 include normal pressure hydrocephalus, and acute poststreptococcal glomerulonephritis. GO annotations related to this gene include histone binding and transcription corepressor activity. An important paralog of this gene is L3MBTL1./MUSTN1: May be involved in the development and regeneration of the musculoskeletal system (By similarity)
rs17632159	5	TMEM171/TMEM171	None	Transmembrane protein.
rs729761	6	VEGFA/VEGFA	Pegaptanib Sodium(Top), Ranibizumab(Top), Aflibercept(Top), Bevacizumab(Can)	Growth factor active in angiogenesis, vasculogenesis and endothelial cell growth. Induces endothelial cell proliferation, promotes cell migration, inhibits apoptosis and induces permeabilization of blood vessels. Binds to the FLT1/VEGFR1 and KDR/VEGFR2 receptors, heparan sulfate and heparin. NRP1/Neuropilin-1 binds isoforms VEGF-165 and VEGF-145. Isoform VEGF165B binds to KDR but does not activate downstream signaling pathways, does not activate angiogenesis and inhibits tumor growth.
rs1178977	7	BAZ1B/MLXIPL	None/None	BAZ1B: (bromodomain adjacent to zinc finger domain, 1B) is a protein-coding gene. Diseases associated with BAZ1B include williams-beuren syndrome, and williams syndrome. GO annotations related to this gene include chromatin binding and non-membrane spanning protein tyrosine kinase activity. An important paralog of this gene is BAZ1A./MLXIPL: This gene encodes a basic helix-loop-helix leucine zipper transcription factor of the Myc/Max/Mad superfamily. This protein forms a heterodimeric complex and binds and activates, in a glucose-dependent manner, carbohydrate response element (ChoRE) motifs in the promoters of triglyceride synthesis genes. The gene is deleted in Williams-Beuren syndrome, a multisystem developmental disorder caused by the deletion of contiguous genes

				at chromosome 7q11.23.
rs10480300	7	PRKAG2/PRKAG2	None	PRKAG2 (protein kinase, AMP-activated, gamma 2 non-catalytic subunit) is a protein-coding gene. Diseases associated with PRKAG2 include cardiomyopathy, familial hypertrophic 6, and wolff-parkinson-white syndrome. GO annotations related to this gene include protein kinase binding and cAMP-dependent protein kinase regulator activity. An important paralog of this gene is PRKAG1
rs2941484	8	HNF4G/HNF4G	None	HNF4G (hepatocyte nuclear factor 4, gamma) is a protein-coding gene. GO annotations related to this gene include steroid hormone receptor activity and sequence-specific DNA binding transcription factor activity. An important paralog of this gene is RXRA.
rs10821905	10	A1CF/ASAH2	None	A1CF: Essential component of the apolipoprotein B mRNA editing enzyme complex which is responsible for the postranscriptional editing of a CAA codon for Gln to a UAA codon for stop in APOB mRNA. Binds to APOB mRNA and is probably responsible for docking the catalytic subunit, APOBEC1, to the mRNA to allow it to deaminate its target cytosine. The complex also protects the edited APOB mRNA from nonsense-mediated decay/ASAH2: Hydrolyzes the sphingolipid ceramide into sphingosine and free fatty acid at an optimal pH of 6.5-8.5. Acts as a key regulator of sphingolipid signaling metabolites by generating sphingosine at the cell surface. Acts as a repressor of apoptosis both by reducing C16-ceramide, thereby preventing ceramide-induced apoptosis, and generating sphingosine, a precursor of the antiapoptotic factor sphingosine 1-phosphate. Probably involved in the digestion of dietary sphingolipids in intestine by acting as a key enzyme for the catabolism of dietary sphingolipids and regulating the levels of bioactive sphingolipid metabolites in the intestinal tract.
rs642803	11	OVOL1/LTBP3	None	OVOL1: Putative transcription factor. Involved in hair formation and spermatogenesis. May function in the differentiation and/or maintenance of the urogenital system (By similarity)/LTBP3: May be involved in the assembly, secretion and targeting of TGFB1 to sites at which it is stored and/or activated. May play critical roles in controlling and directing the activity of TGFB1. May have a structural role in the extra cellular matrix (ECM)
rs653178	12	ATXN2/PTPN11	None/Enoxolone	ATXN2: Involved in EGFR trafficking, acting as negative regulator of endocytic EGFR internalization at the plasma membrane./PTPN11: Acts downstream of various receptor and cytoplasmic protein tyrosine kinases to participate in the signal transduction from the cell surface to the nucleus. Dephosphorylates ROCK2 at Tyr-722 resulting in stimulatation of its RhoA binding activity.
rs1394125	15	UBE2Q2/NRG4	None	UBE2Q2: Accepts ubiquitin from the E1 complex and catalyzes its covalent attachment to other proteins. In vitro catalyzes 'Lys-48'-linked polyubiquitination/ NRG4: Low affinity ligand for the ERBB4 tyrosine kinase receptor. Concomitantly recruits ERBB1 and ERBB2 coreceptors, resulting in ligand-stimulated tyrosine phosphorylation and activation of the ERBB receptors. Does not bind to the ERBB1, ERBB2 and ERBB3 receptors (By similarity)
rs6598541	15	IGF1R/IGF1R	Mecasermin Rinfabate (Igf), Mecasermin(Igf)	IGF1R: IGF1R (insulin-like growth factor 1 receptor) is a protein-coding gene. Diseases associated with IGF1R include insulin-like growth factor 1 resistance to, and insulin-like growth factor i deficiency. GO annotations related to this gene include insulin receptor binding and identical protein binding. An important paralog of this gene is ROR1.
rs7193778	16	NFAT5/NFAT5	None	Transcription factor involved in the transcriptional regulation of osmoprotective and inflammatory genes. Regulates hypertonicity-induced cellular accumulation of osmolytes
rs7188445	16	MAF/MAF	None	MAF (v-maf avian musculoaponeurotic fibrosarcoma oncogene homolog) is a protein-coding gene. Diseases associated with MAF include nephrogenic adenofibroma, and plasma cell leukemia. GO annotations related to this gene include sequence-specific DNA

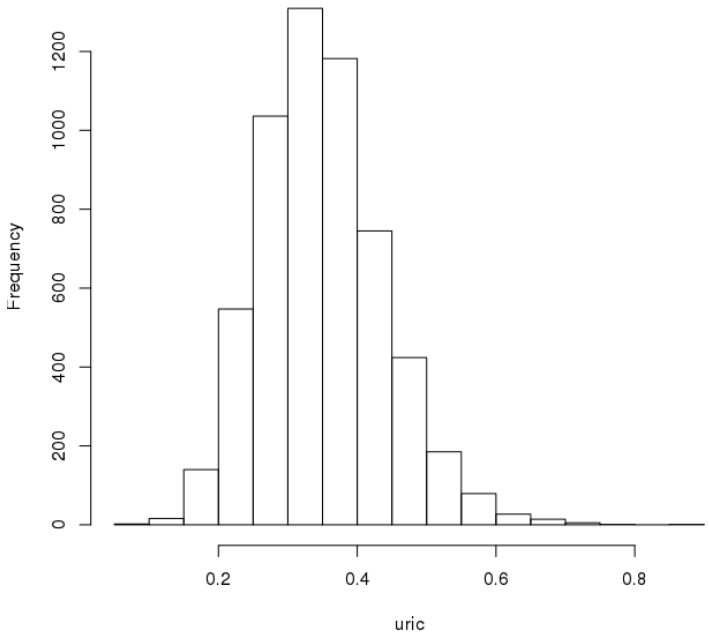
				binding and sequence-specific DNA binding transcription factor activity. An important paralog of this gene is NRL.
rs7224610	17	HLF/HLF	None	HLF (hepatic leukemia factor) is a protein-coding gene. Diseases associated with HLF include leukemia, acute lymphoblastic 3, and acute lymphoblastic leukemia. GO annotations related to this gene include double-stranded DNA binding and sequence-specific DNA binding transcription factor activity. An important paralog of this gene is DBP.
rs742132	6	LRRC16A/LRRC16A	None	LRRC16A (leucine rich repeat containing 16A) is a protein-coding gene. Diseases associated with LRRC16A include acute urate nephropathy. An important paralog of this gene is LRRC16B.
rs2307394	2	ORC4L/ACVR2A	None/Dasatinib,Lestaurtinib,Alvocidib	ORC4 (origin recognition complex, subunit 4) is a protein-coding gene. Diseases associated with ORC4 include meier-gorlin syndrome 2, and meier-gorlin syndrome. GO annotations related to this gene include DNA replication origin binding and nucleotide binding./ACVR2A (activin A receptor, type IIA) is a protein-coding gene. Diseases associated with ACVR2A include multiple synostoses syndrome. GO annotations related to this gene include PDZ domain binding and growth factor binding. An important paralog of this gene is ACVR1C.
rs17786744	8	STC1/STC1	None	STC1 (stanniocalcin 1) is a protein-coding gene. Diseases associated with STC1 include pheochromocytoma, and fibrosarcoma. GO annotations related to this gene include hormone activity. An important paralog of this gene is STC2. The protein may play a role in the regulation of renal and intestinal calcium and phosphate transport, cell metabolism, or cellular calcium/phosphate homeostasis.
rs2079742	17	BCAS3/C17orf82	None	BCAS3 (breast carcinoma amplified sequence 3) is a protein-coding gene. Diseases associated with BCAS3 include breast cancer./C17orf82 (chromosome 17 open reading frame 82) is a protein-coding gene.
rs164009	17	QRICH2/PRPSAP1	None	QRICH2 (glutamine rich 2) is a protein-coding gene./ PRPSAP1 (phosphoribosyl pyrophosphate synthetase-associated protein 1) is a protein-coding gene. GO annotations related to this gene include enzyme inhibitor activity and magnesium ion binding. An important paralog of this gene is PRPS1.

Supplementary Table 8. Sensitivity tests with different covariate models.

Outcome/Exposure	Covariates	Point estimate: IV (OR) (95% CI) from MVMR with specified model; full data.	Mean (median) estimate from sensitivity test in which the model was fitted 100,000 times removing 6 SNPs at random from the data in each cycle.	95% range of estimates from sensitivity test.	% of estimates from the sensitivity test which lie outside the confidence interval of the IV regression.
CHD/Urate	-	1.177 (1.076, 1.286)	1.184 (1.176)	1.122, 1.299	4.85
CHD/Urate	HDL	1.094 (0.991, 1.208)	1.096 (1.094)	1.044, 1.168	0.34
CHD/Urate	TG	1.173 (1.068, 1.289)	1.18 (1.169)	1.111, 1.314	6.43*
CHD/Urate	DBP	1.097 (1, 1.202)	1.098 (1.103)	1.022, 1.166	0.92
CHD/Urate	SBP	1.121 (1.024, 1.227)	1.118 (1.128)	1.008, 1.176	2.89
CHD/Urate	SBP+HDL	1.111 (1.006, 1.227)	1.108 (1.119)	0.996, 1.171	2.77
CHD/Urate	SBP+TG	1.136 (1.033, 1.249)	1.134 (1.141)	1.031, 1.216	2.83
CHD/Urate	SBP+DBP	1.101 (1.003, 1.208)	1.101 (1.108)	1.013, 1.166	2.12
CHD/Urate	HDL+TG	1.102 (0.999, 1.217)	1.103 (1.099)	1.045, 1.195	0.75
CHD/Urate	HDL+DBP	1.09 (0.987, 1.203)	1.091 (1.095)	1.018, 1.165	1.04
CHD/Urate	TG+DBP	1.107 (1.005, 1.218)	1.107 (1.111)	1.028, 1.2	1.53
CHD/Urate	TG+DBP+HDL	1.094 (0.991, 1.208)	1.092 (1.095)	1.016, 1.18	1.34
CHD/Urate	SBP+HDL+DBP	1.095 (0.991, 1.211)	1.095 (1.101)	1.006, 1.169	2.04
CHD/Urate	SBP+TG+DBP	1.114 (1.011, 1.228)	1.112 (1.118)	1.023, 1.202	2.13
CHD/Urate	SBP+HDL+TG	1.116 (1.011, 1.232)	1.111 (1.118)	1.017, 1.19	2.42
CHD/Urate	SBP+HDL+TG+DBP	1.101 (0.996, 1.218)	1.096 (1.101)	1.013, 1.185	1.89
CHD/Urate	MR Egger method	1.049 (0.918, 1.200)	1.035 (1.045)	0.699, 1.134	3.81

* Distribution of sensitivity test does not fit within the assumed normal distribution of the point estimate in full data for the model. This indicates that the model is sensitive to SNP selection and the confidence interval on the point estimate is anti-conservative. Conversely if the value is less than 5% it suggests the model is insensitive to SNP selection and the interval on the point estimate is likely to be conservative.

Supplementary Figure 1. The distribution of uric acid data in the UCLEB consortium data.



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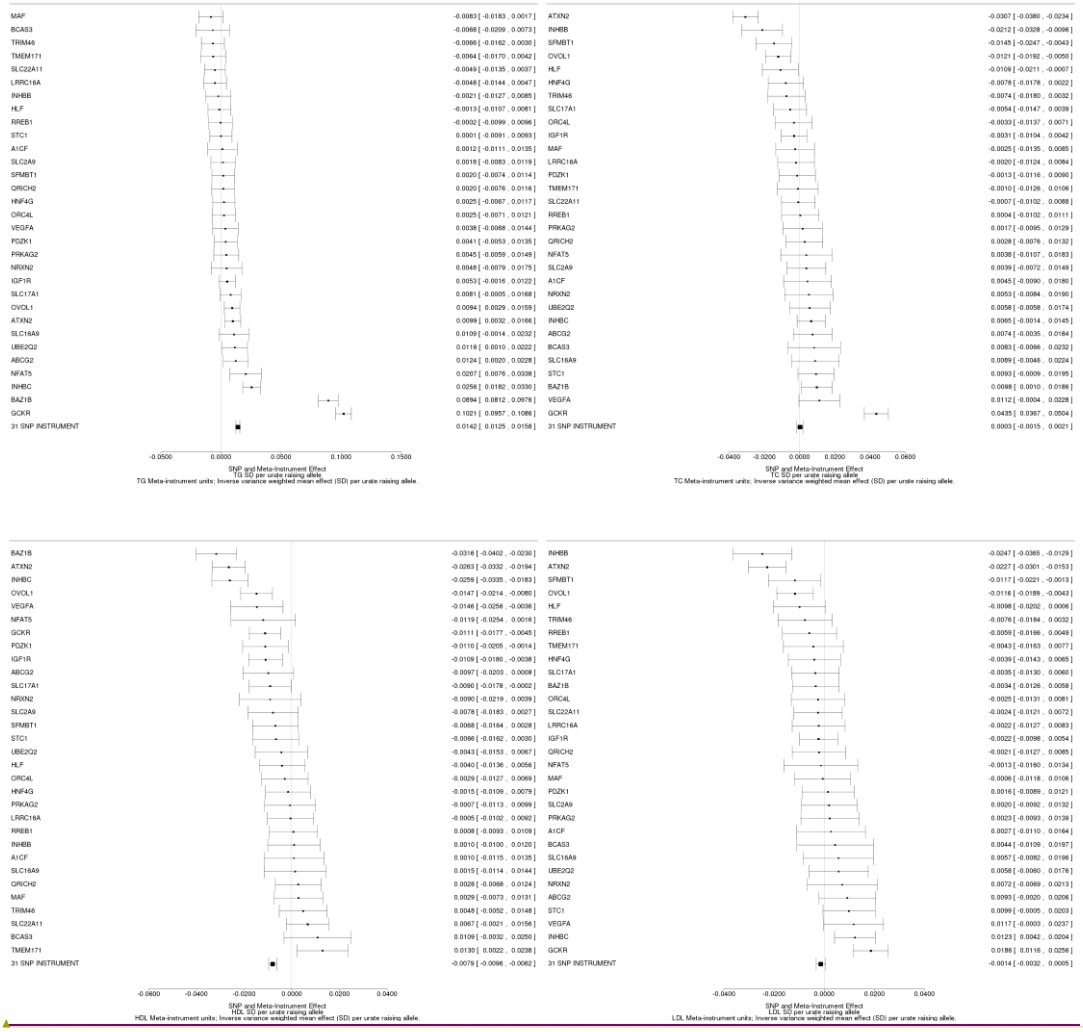

Supplementary Figure 2. The association of individual SNPs and the 31 SNP instrument for urate with continuous phenotypes. (Error bars are 95%CI, SNP order is by magnitude of effect within a phenotype, and all effects are with respect to the urate raising allele). Significant association in the 31 SNP instrument is indicative of pleiotropy.

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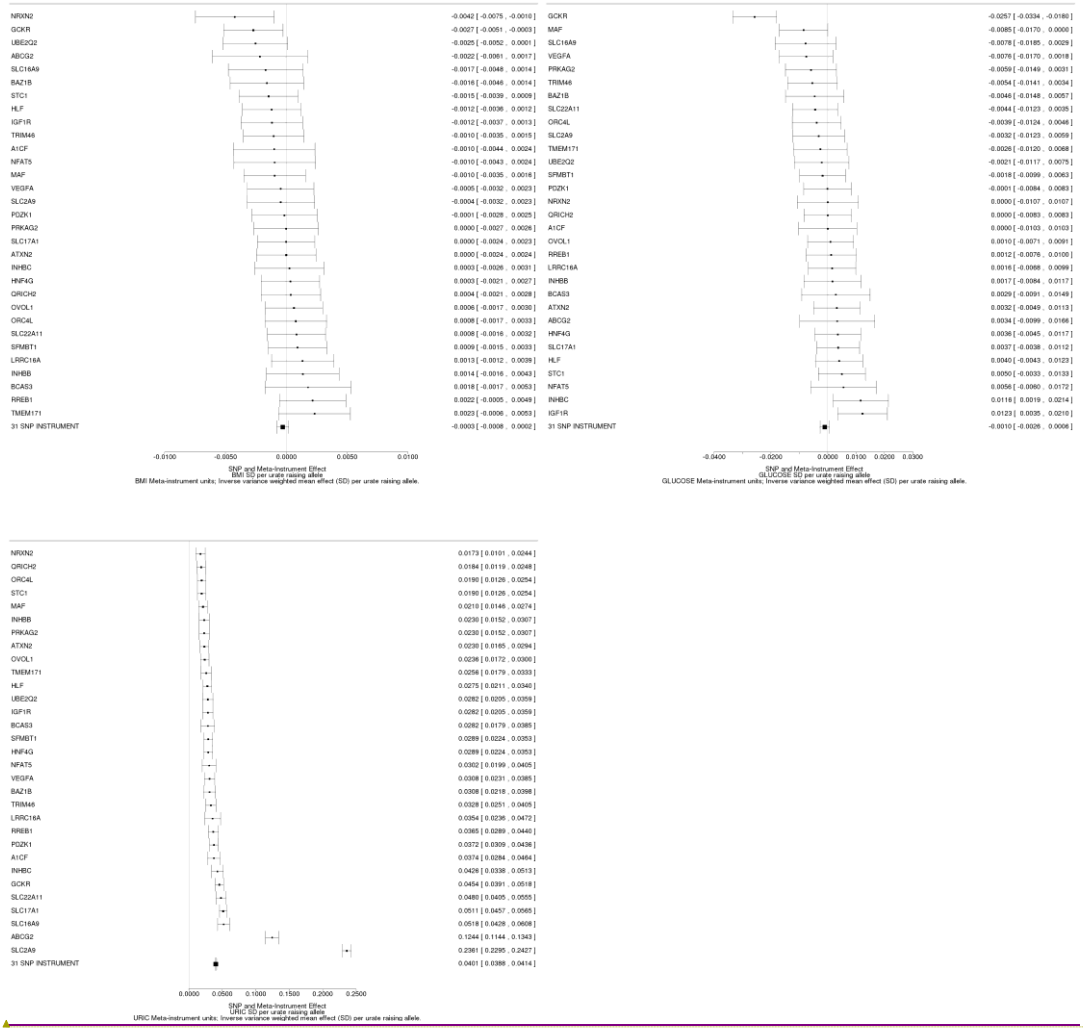

Supplementary Figure 3. The association of individual SNPs and the 31 SNP instrument for urate with continuous phenotypes. (Error bars are 95%CI, SNP order is by magnitude of effect within a phenotype, and all effects are with respect to the urate raising allele). Significant association in the 31 SNP instrument is indicative of pleiotropy.

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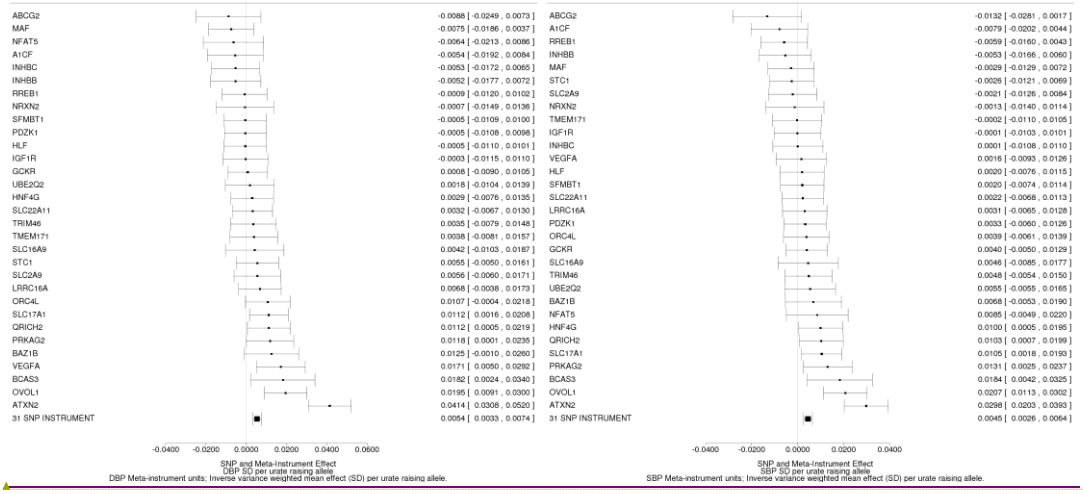
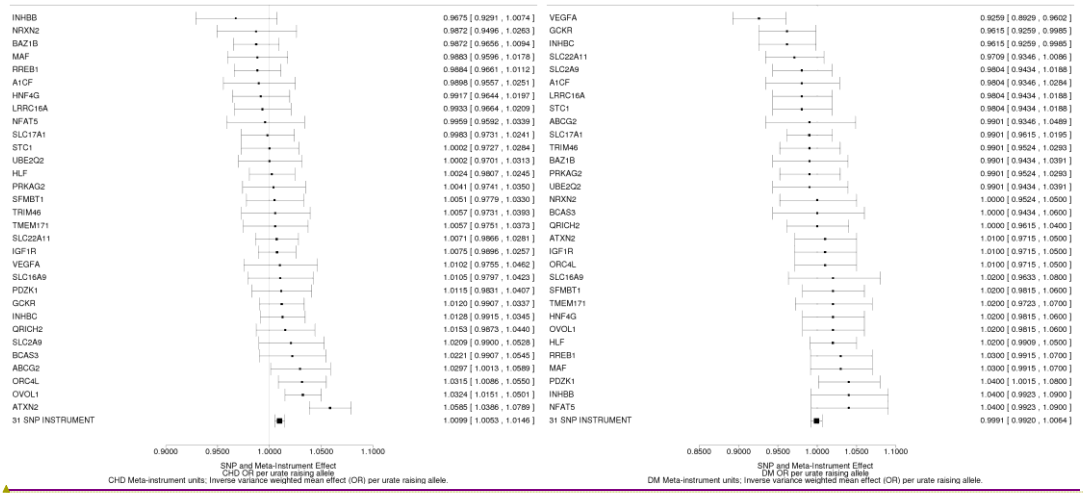


Supplementary Figure 4. The association of the individual SNPs and the 31 SNP instrument for urate with blood pressure. (Error bars are 95%CI, SNP order is by magnitude of effect within a phenotype, and all effects are with respect to the urate raising allele). Significant association in the 31 SNP instrument is indicative of pleiotropy.



Supplementary Figure 5. The association of the individual SNPs and the 31 SNP instrument for urate with binary phenotypes. (Error bars are 95%CI, SNP order is by magnitude of effect within a phenotype, and all effects are with respect to the urate raising allele). Significant association in the 31 SNP instrument is indicative of pleiotropy.

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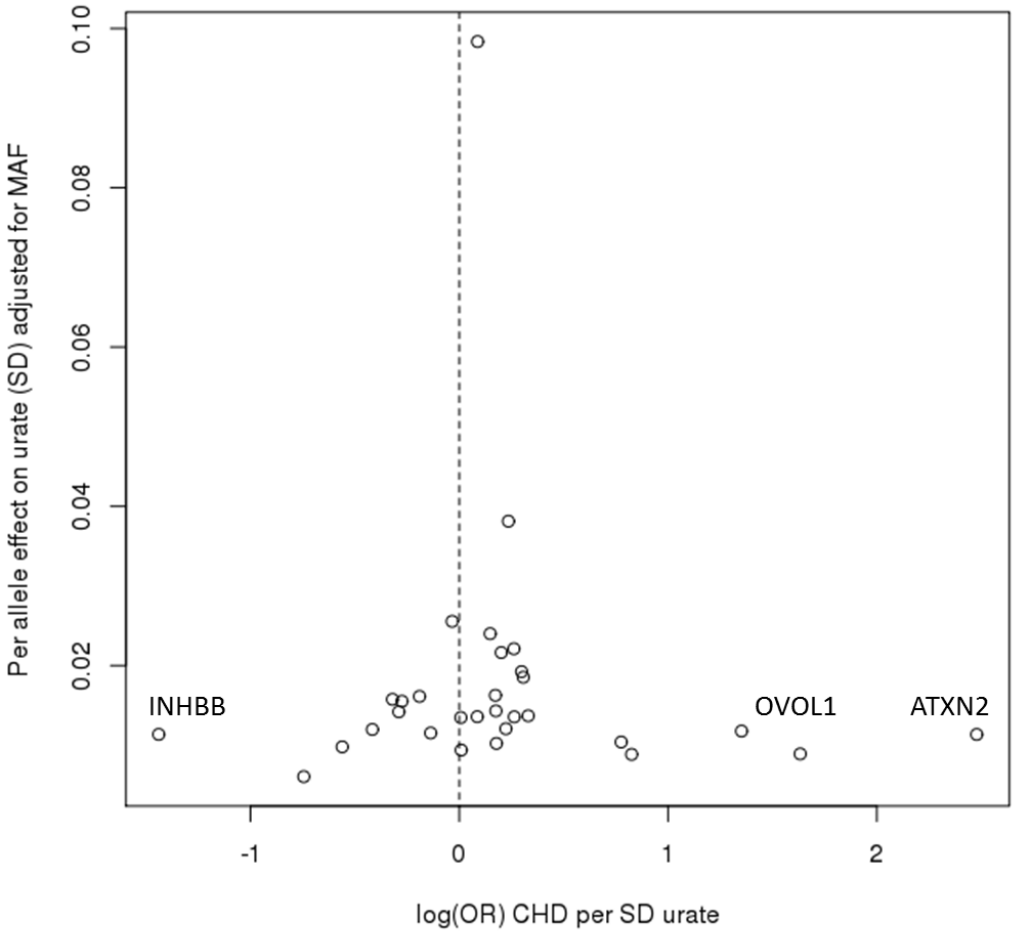

Supplementary Figure 6. Funnel plot of individual IV beta estimates for SNPs in the instrument. The distribution about the point estimate is asymmetric suggesting there is an unmeasured net pleiotropic effect on the instrument. (Egger test for funnel plot symmetry P.value = 0.011).

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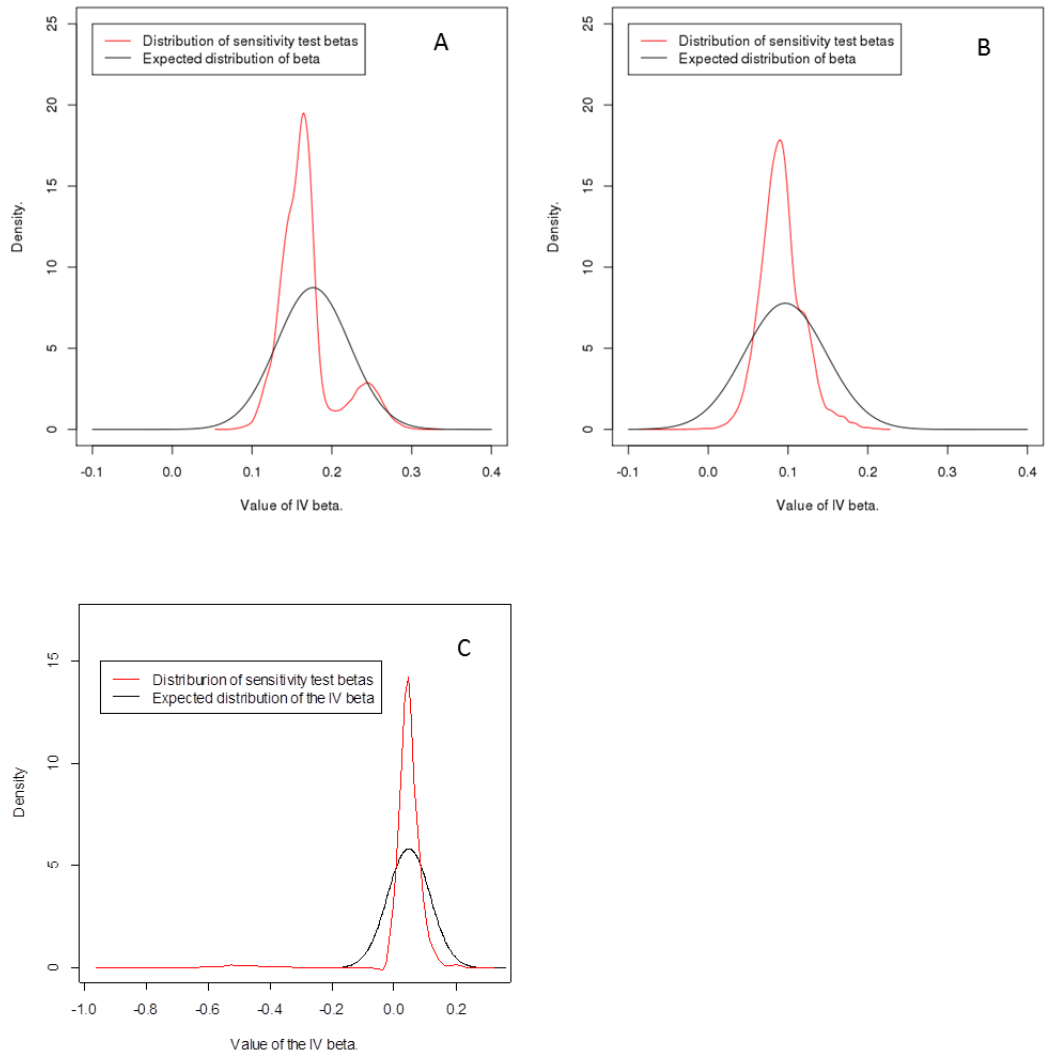

Supplementary Figure 7. Sensitivity test. The assumed normal distribution (black) of the point estimate of the IV beta using the 31 SNP instrument with (B) and without (A) covariates. Similar for MR Egger regression(C). In each case the red curve is the empirical distribution of the IV beta estimated in 100 000 25 SNP instruments obtained by repeatedly excluding 6 SNPs at random.

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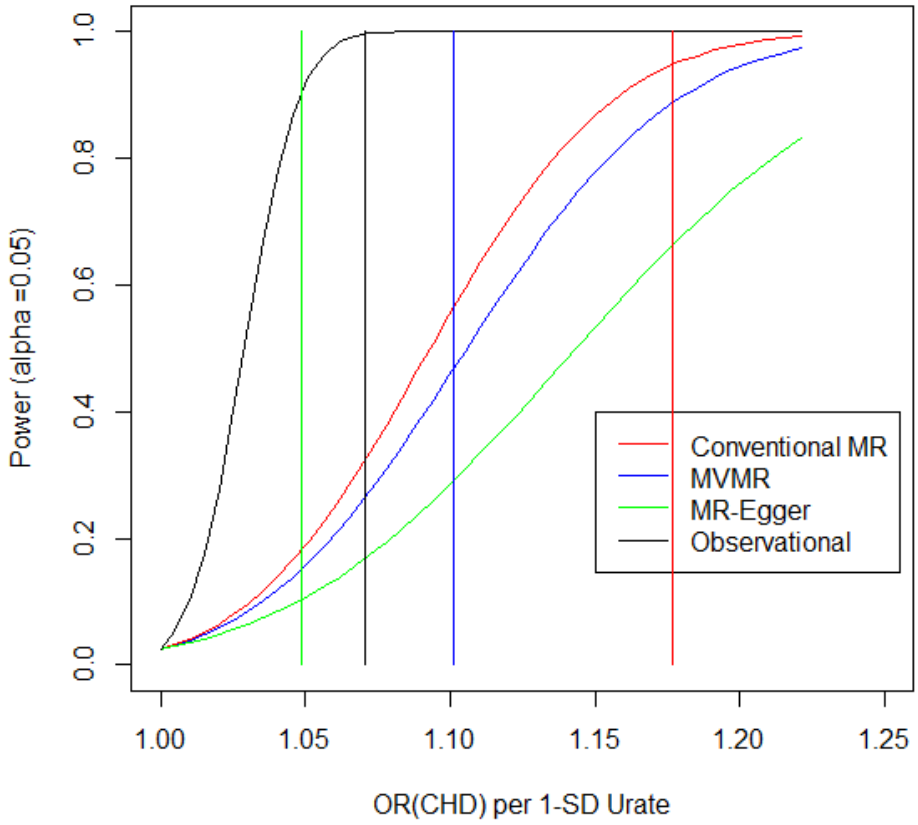

Supplementary Figure 8. Power curves derived from analytical outcomes. The vertical lines represent the effect, estimated by each method, of urate on CHD risk, colour coded as legend.

Supplementary Appendix 1. Contributors to the ICBP

Ehret GB, Munroe PB, Rice KM, Bochud M, Johnson AD, Chasman DI, Smith AV, Tobin MD, Verwoert GC, Hwang SJ, Pihur V, Vollenweider P, O'Reilly PF, Amin N, Bragg-Gresham JL, Teumer A, Glazer NL, Launer L, Zhao JH, Aulchenko Y, Heath S, Sõber S, Parsa A, Luan J, Arora P, Dehghan A, Zhang F, Lucas G, Hicks AA, Jackson AU, Peden JF, Tanaka T, Wild SH, Rudan I, Igl W, Milaneschi Y, Parker AN, Fava C, Chambers JC, Fox ER, Kumari M, Go MJ, van der Harst P, Kao WH, Sjögren M, Vinay DG, Alexander M, Tabara Y, Shaw-Hawkins S, Whincup PH, Liu Y, Shi G, Kuusisto J, Tayo B, Seielstad M, Sim X, Nguyen KD, Lehtimäki T, Matullo G, Wu Y, Gaunt TR, Onland-Moret NC, Cooper MN, Platou CG, Org E, Hardy R, Dahgam S, Palmen J, Vitart V, Braund PS, Kuznetsova T, Uiterwaal CS, Adeyemo A, Palmas W, Campbell H, Ludwig B, Tomaszewski M, Tzoulaki I, Palmer ND, CARDIoGRAM consortium, CKDGen Consortium, KidneyGen Consortium, EchoGen consortium, CHARGE-HF consortium, Aspelund T, Garcia M, Chang YP, O'Connell JR,

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Web References.

W 1 Terkeltaub RA. (2003) Clinical practice. Gout. N Engl J Med, 349:1647–55

W 2 Terkeltaub R.A. (2010) ~~Update on gout: new therapeutic strategies and options. Nature Reviews Rheumatology, 6:30-38~~

~~W 3 Teng GG, Ang LW, Saag KG, Yu MC, Yuan JM, Koh WP. (2012) Mortality due to coronary heart disease and kidney disease among middle-aged and elderly men and women with gout in the Singapore Chinese Health Study. Ann Rheum Dis. 71:924-8~~

~~W 4 Clarson LE, Hider SL, Belcher J, Heneghan C, Roddy E, Mallen CD. (2015) Increased risk of vascular disease associated with gout: a retrospective, matched cohort study in the UK clinical practice research datalink. Ann Rheum Dis. 74:642-7.~~

~~W 5 Yang Q, Köttgen A, Dehghan A, et al. 2010. Multiple Genetic Loci Influence Serum Urate and Their Relationship with Gout and Cardiovascular Disease Risk Factors Circ Cardiovasc Genet. 3(6): 523–530.~~

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W 6 Davis N, (1897). The cardiovascular and renal relations and manifestations of gout. JAMA; 29: 261-262.

W 7 Rajendra NS, Irel,and S, George J, Belch JJF, Lang CC, and Struthers AD, (2011). Mechanistic Insights Into the Therapeutic Use of High-Dose Allopurinol in Angina Pectoris. Journal of the American College of Cardiology. 58:821–828

W 8 Noman A, Ang DS, Ogston S, Lang CC,, and Struthers AD, (2010) Effect of high-dose allopurinol on exercise in patients with chronic stable angina: a randomised, placebo controlled crossover trial. Lancet. 375:2161–2167.

W 9 Kanbay M, Huddam B, Azak, A, et al. (2011). A Randomized Study of Allopurinol on Endothelial Function and Estimated Glomular Filtration Rate in Asymptomatic Hyperuricemic Subjects with Normal Renal Function. Clin. J. Am. Soc. Nephrol. 6: 1887–1894.

W 10 Rekhraj S, Gandy SJ, Szwejkowski BR, et al. (2013). High-Dose Allopurinol Reduces Left Ventricular Mass in Patients With Ischemic Heart Disease. Journal of the American College of Cardiology. Vol. 61:927–932

W 11 Feig DI, Soletsky B and Johnson, RJ, (2008b). Effect of allopurinol on the blood pressure of adolecents with newly diagnosed essential hypertension. JAMA; 300:924–932.

W 12 Landmesser U, and Drexler H, (2002) Editorial: Allopurinol and Endothelial Function in heart Failure. Circulation. 106:173–175.

W 13 Struthers A and, Shearer F, (2012) Editorial. Allopurinol: novel indications in cardiovascular disease. Doi:10.1136/heartjnl-2012-302249

W 14 Greenland S, (2000). An introduction to instrumental variables for epidemiologists. International Journal of Epidemiology. 29: :722–729

W 15 Palmer TM, Nordestgaard BG, Benn M, et al. (2013). Association of plasma uric acid with ischaemic heart disease and blood pressure: Mendelian randomisation analysis of two large cohorts. BMJ. 347:doi: 10.1136/bmj.f4262.

W 16 Pfister R, Barnes D, Luben R, Forouhi NG, Bochud M, Khaw KT, Wareham NJ, Langenberg C. (2011). No evidence for a causal link between uric acid and type 2 diabetes: a Mendelian randomisation approach. Diabetologia. 54:2561-9. doi: 10.1007/s00125-011-2235-0.

W 17 Lyngdoh T, Vuistiner P, Marques-Vidal P, Rousson V, Waeber G, Vollenweider P, and Bochud M. (2012) Serum uric acid and adiposity: deciphering causality using a bidirectional Mendelian randomization approach. PLoS One. 2012;7(6):e39321. doi: 10.1371/journal.pone.0039321.

W 18 Rasheed H, Hughes K, Flynn TJ, and Merriman TR. (2014). Mendelian randomization provides no evidence for a causal role of serum urate in increasing serum triglyceride levels. Circ Cardiovasc Genet. 7:830-7.

W 19 C-Reactive Protein Coronary Heart Disease Genetics Collaboration (CCGC) (2011) Association between C reactive protein and coronary heart disease: mendelian randomisation analysis based on individual participant data . BMJ 2011; 342 doi: tp://dx.doi.org/10.1136/bmj.d548.

W 20 Ken-Dror, G., Humphries, S.E., Kumari, M., Kivimaki, M., and Drenos, F. (2012) A genetic instrument for Mendelian randomization of fibrinogen Eur J Epidemiol. 27: 267–279.

W 21 Krishnan E, Lessov-Schlaggar CN, Krasnow RE, and Swan GE. (2012) Nature versus nurture in gout: a twin study. Am J Med. 125:499-504.

W 22 Shah T, Engmann J, Dale C, et al. (2013) Population Genomics of Cardiometabolic Traits: Design of the University College London-London School of Hygiene and Tropical Medicine

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Edinburgh-Bristol (UCLEB) Consortium. PLoS ONE 8(8): e71345. doi:10.1371/journal.pone.0071345

W 23 Medalie JH, Kahn HA, Neufeld HN, Riss E, and Goldbourt U (1973) Five-year myocardial infarction incidence. II. Association of single variables to age and birthplace. J Chronic Dis 26: 325–349.

W 24 Fang J, Alderman MH, (2000) Serum uric acid and cardiovascular mortality the NHANES I epidemiological follow-up study, 1971–1992. National Health and Nutrition Examination Survey. JAMA 283: 2404–2410.

W 25 Tomita M, Mizuno S, Yamanaka H, et al. (2000) Does hyperuricemia affect mortality? A prospective cohort study of Japanese male workers. J Epidemiol 10: 403–409.

W 26 Liese AD, Hense HW, Lowel H, et al. (1999) Association of serum uric acid with all-cause and cardiovascular disease mortality and incident myocardial infarction in the MONICA Augsburg cohort. World Health Organization Monitoring Trends and Determinants in Cardiovascular Diseases. Epidemiology 10: 391–397.

W 27 Moriarity JT, Folsom AR, Iribarren C, Nieto FJ, and Rosamond WD, (2000) Serum uric acid and risk of coronary heart disease: Atherosclerosis Risk in Communities (ARIC) Study. Ann Epidemiol 10: 136–143.

W 28 Puddu PE, Lanti M, Menotti A, et al. (2001) Serum uric acid for short-term prediction of cardiovascular disease incidence in the Gubbio population study. Acta Cardiol 56: 243–251.

W 29 Raychaudhuri, S., Plenge, R.M., Rossin, E.J., Ng, A.C.Y., International Schizophrenia Consortium, Purcell, S.M., Sklar, P., Scolnick, E.M., Xavier, R.J., Altshuler, D., and Daly, M.J. Identifying Relationships Among Genomic Disease Regions: Predicting Genes at Pathogenic SNP Associations and Rare Deletions. PLOS Genetics, 2009. 5(6):e1000534.. doi: 10.1161/HYPERTENSIONAHA.114.03757.

W 30 Schunkert H, König IR, Kathiresan S, et al. (2011) Large-scale association analysis identifies 13 new susceptibility loci for coronary artery disease. Nat Genet. 43: 333–338

W 31 Coronary Artery Disease (C4D) Genetics Consortium (Writing Committee: Peden JF, Hopewell JC, Saleheen D, et al.). (2011). A genome-wide association study in Europeans and South Asians identifies five new loci for coronary artery disease. Nat Genet. 43: 339–344

W 32 CARDIoGRAMplusC4D Consortium, Deloukas P, Kanoni S, Willenborg C, et al. (2013). Large-scale association analysis identifies new risk loci for coronary artery disease. Nat Genet 45:25–33

W 33 Köttgen A, Albrecht E, Teumer A, et al. (2013).Genome-wide association analyses identify 18 new loci associated with serum urate concentrations. Nature genetics;45(2):145–54.

W 34 Kolz M, Johnson T, Sanna S, et al. (2009). Meta-analysis of 28,141 individuals identifies common variants within five new loci that influence uric acid concentrations.PLoS Genetics; Jun;5(6):e1000504

W 35 Yates A, Beal K, Keenan S, McLaren W, Pignatelli M, Ritchie GRS, et al. (2014) The Ensembl REST API: Ensembl Data for Any Language. Bioinformatics.;btu613. doi:10.1093/bioinformatics/btu613.

W 36 Jain E, Bairoch A, Duvaud S, Phan I, Redaschi N, Suzek BE, et al. Infrastructure for the life sciences: design and implementation of the UniProt website. BMC bioinformatics. 009;10:136. doi:10.1186/1471-2105-10-136.

W 37 R Core Team (2013). R: A Language and Environment for Statistical Computing, Vienna, Austria. *http://www.R-project.org*

W 38 http://cran.r-project.org/web/packages/metafor/index.html

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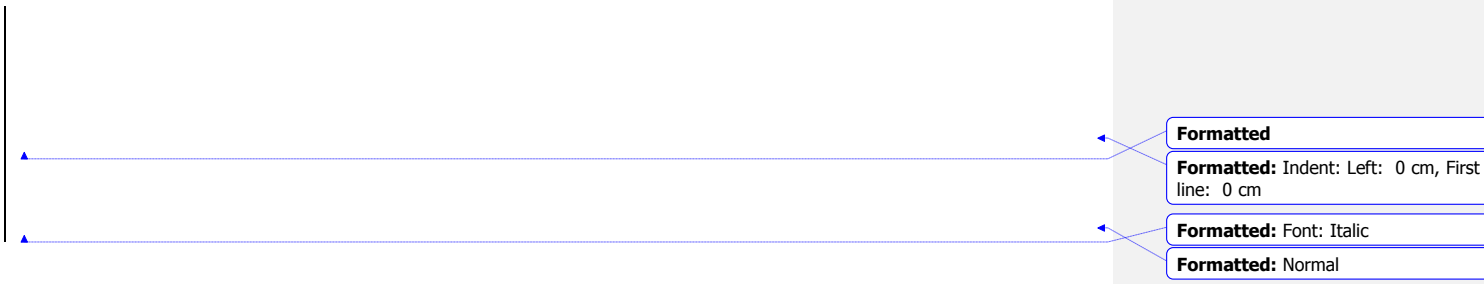
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***Reply to Reviewers Comments**

Response to Reviewers:

This response relates to specific editorial suggestions made by the editor. The suggestions were in the form of comments in the manuscript. We have tried to address all the points by re-editing the manuscript and retained the comments in the version showing the corresponding tracked changes. This seemed the best way to record the re-editing process.

Regards,

J. White

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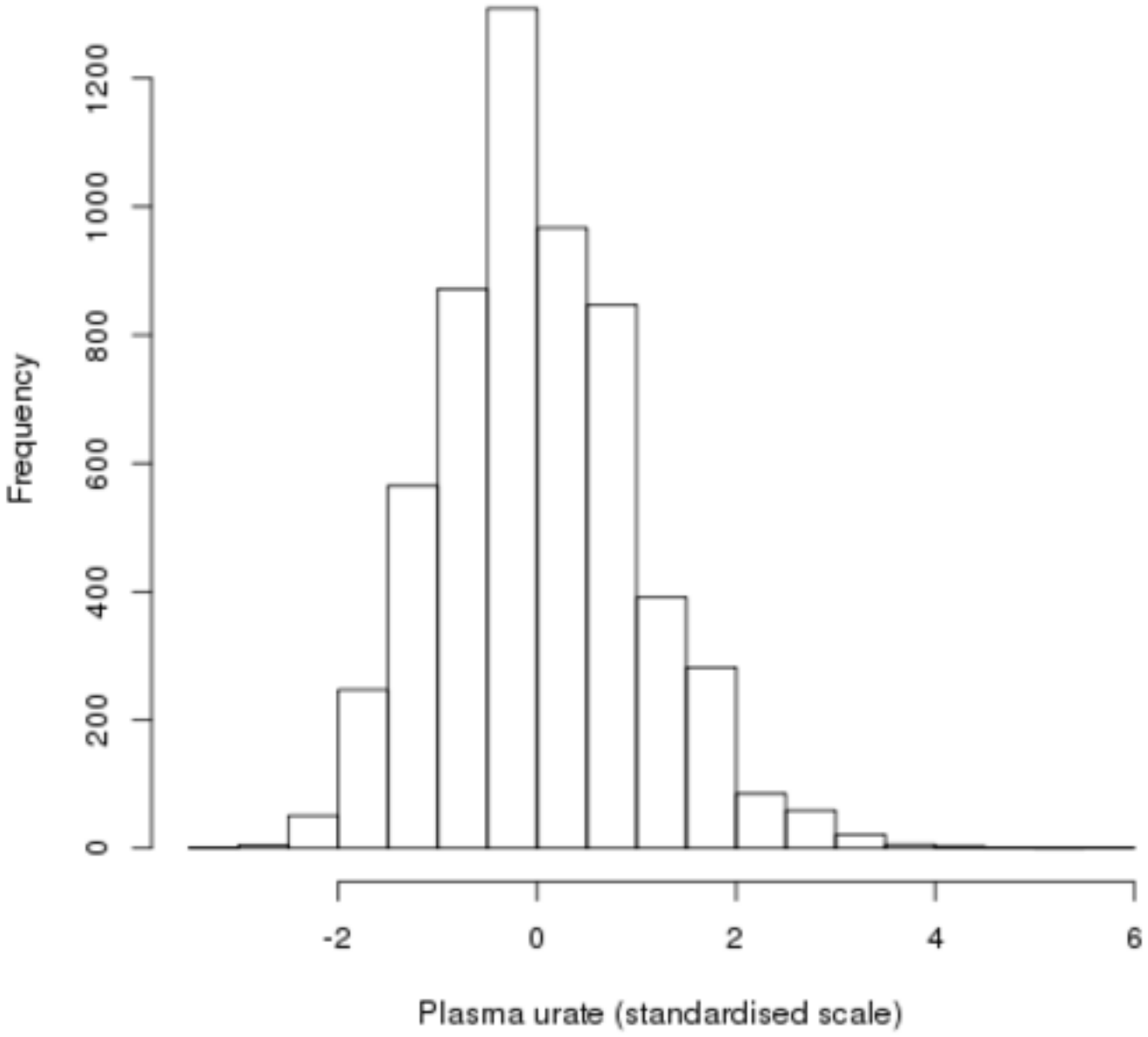

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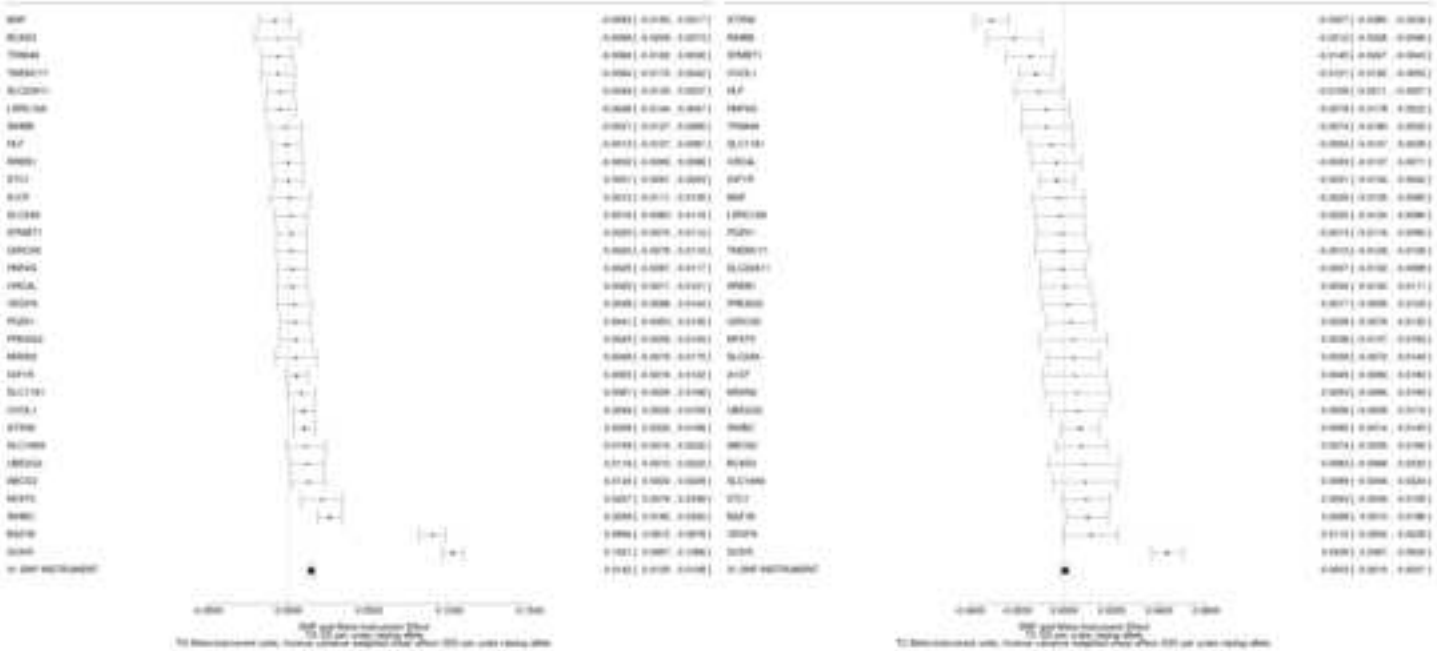

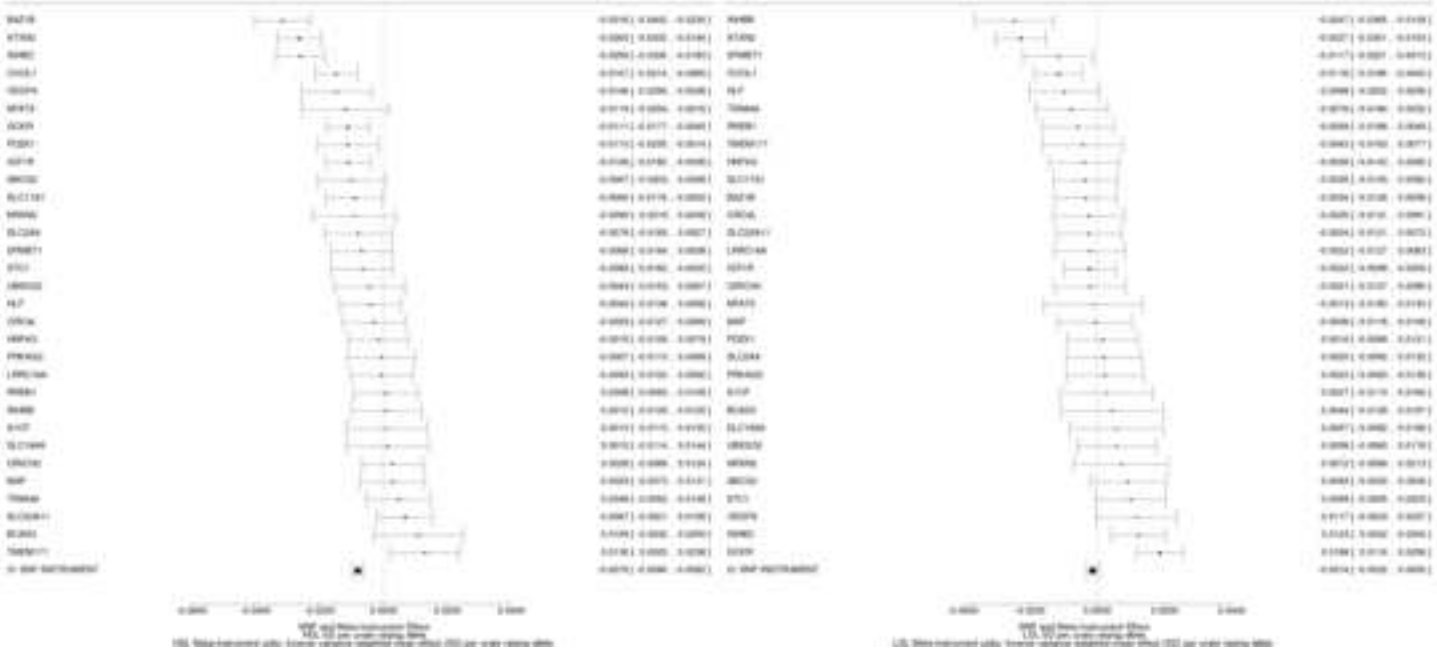

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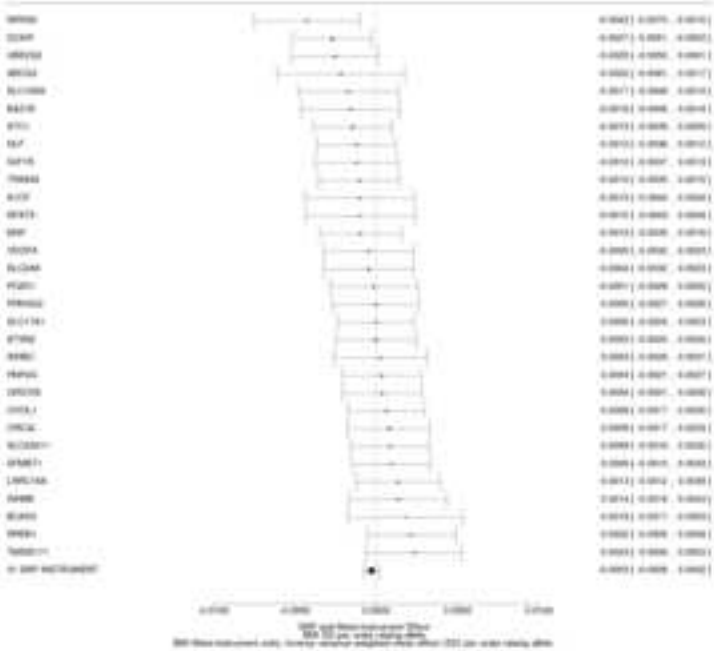

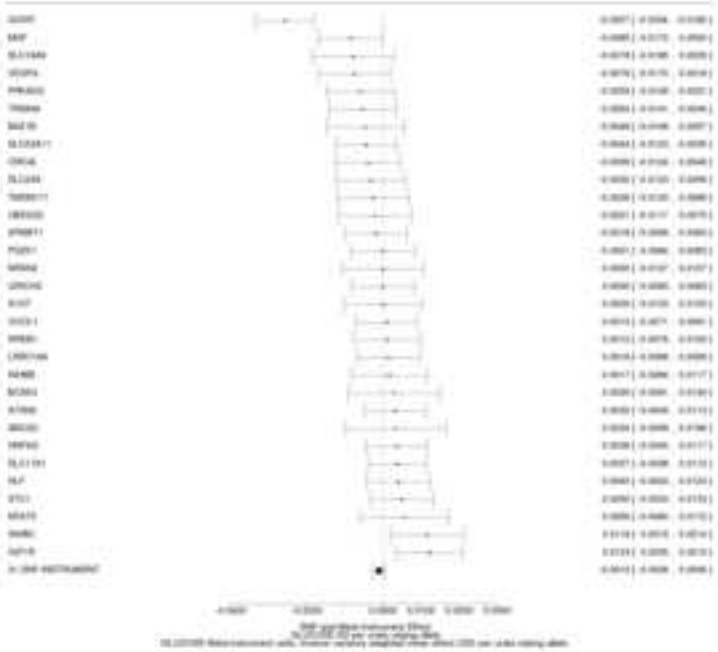

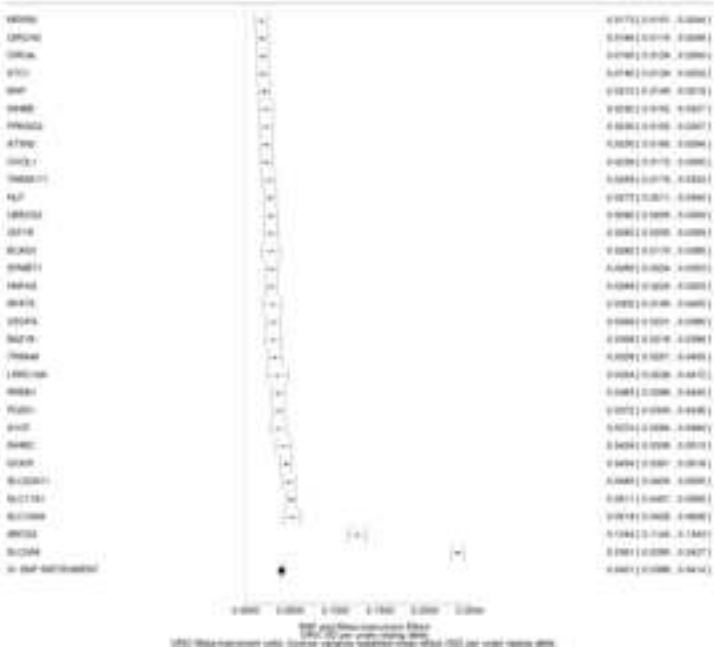

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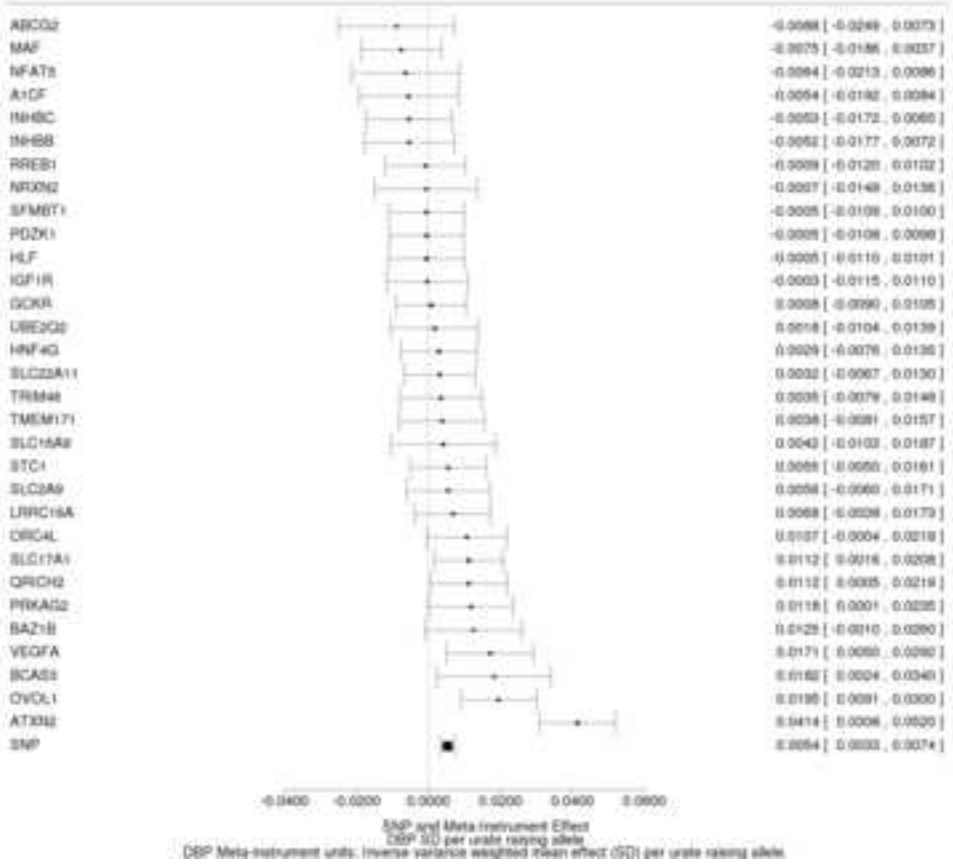

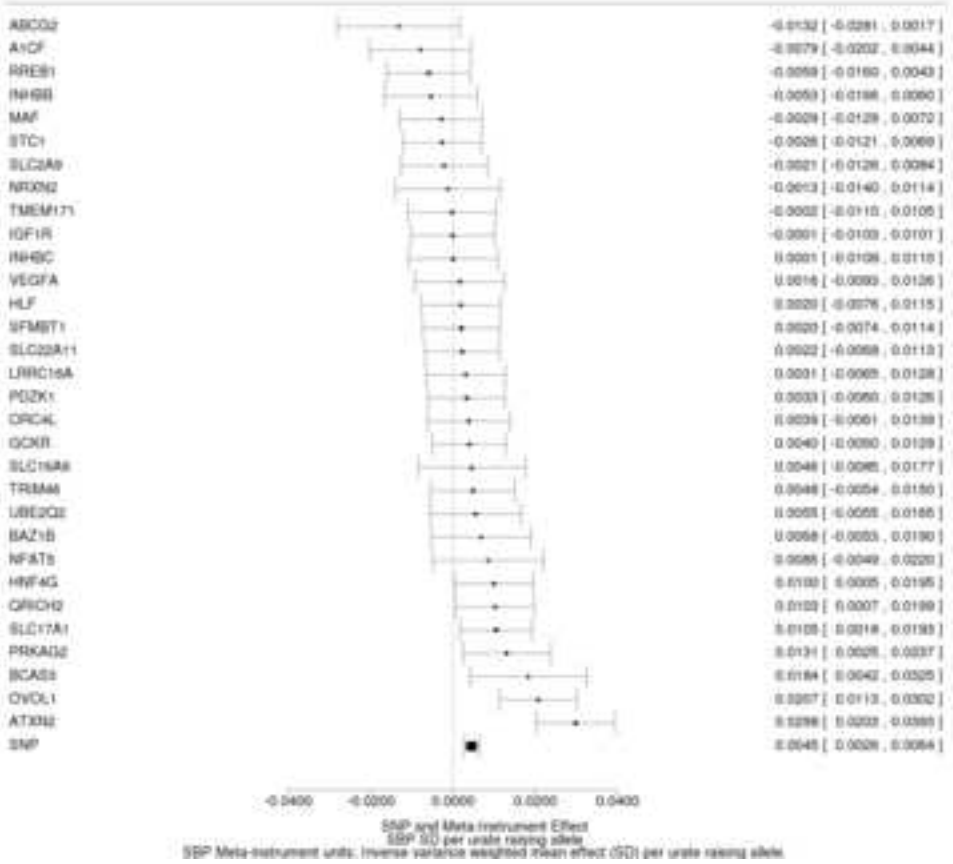

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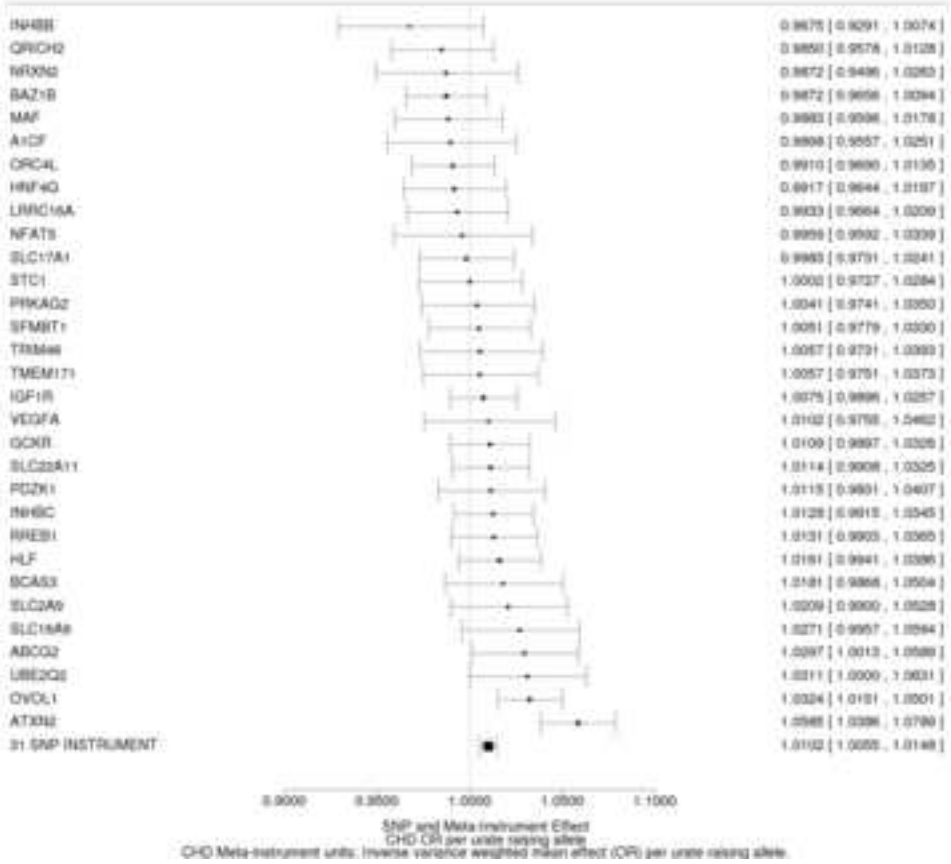

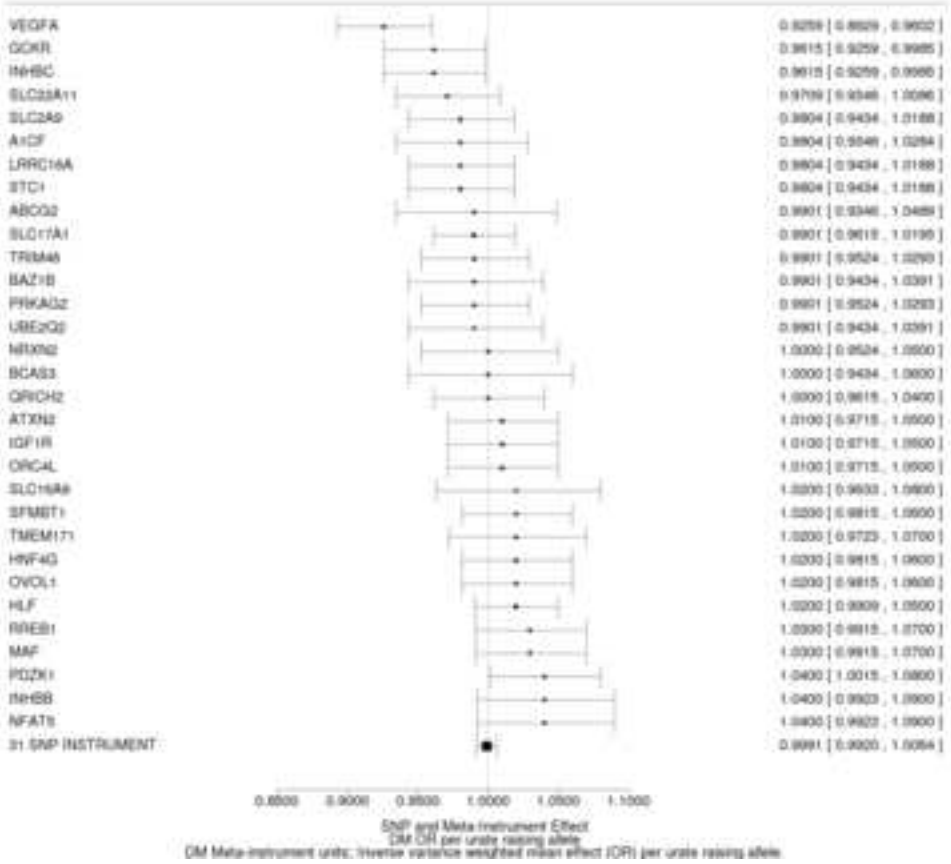

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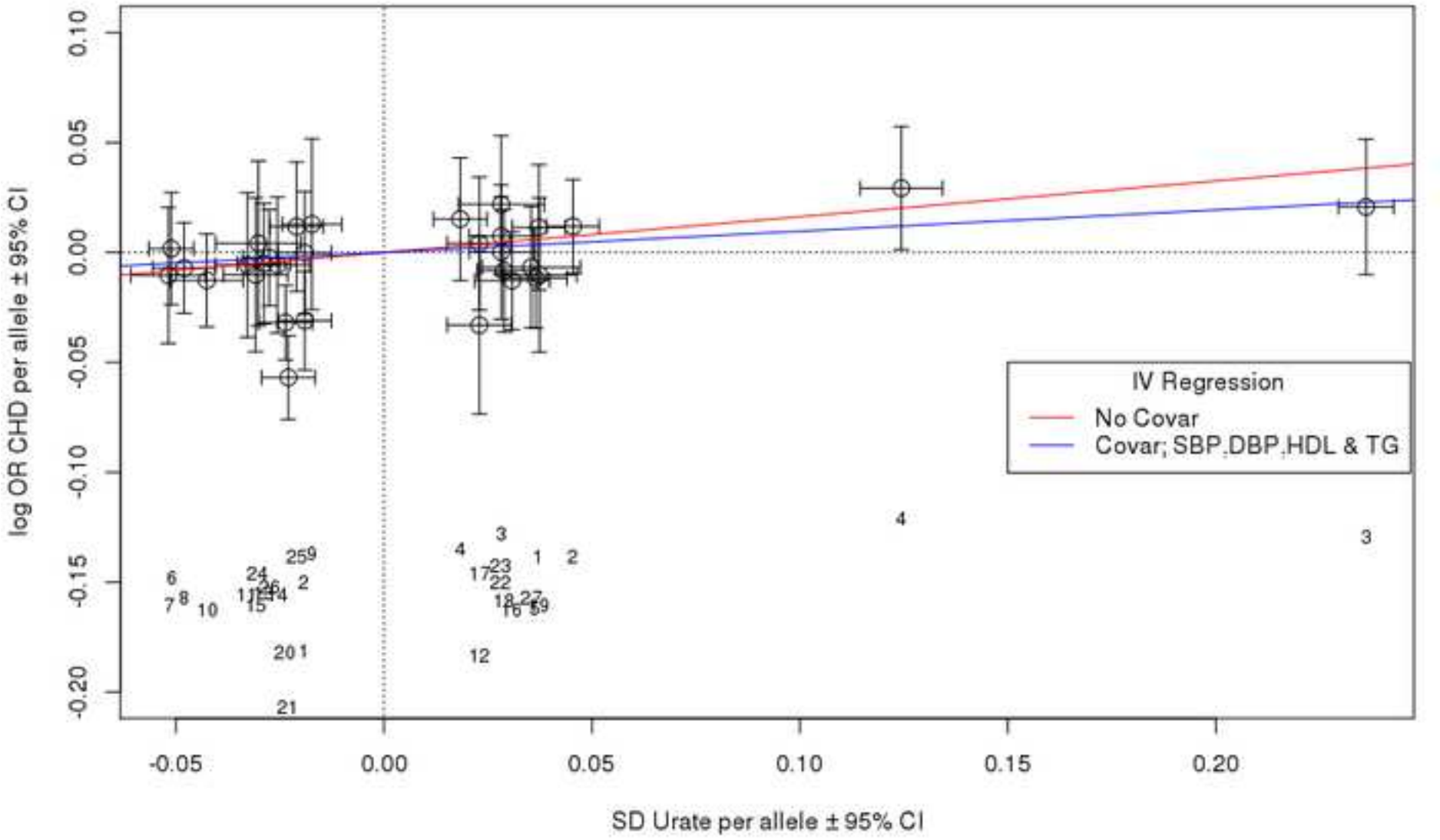

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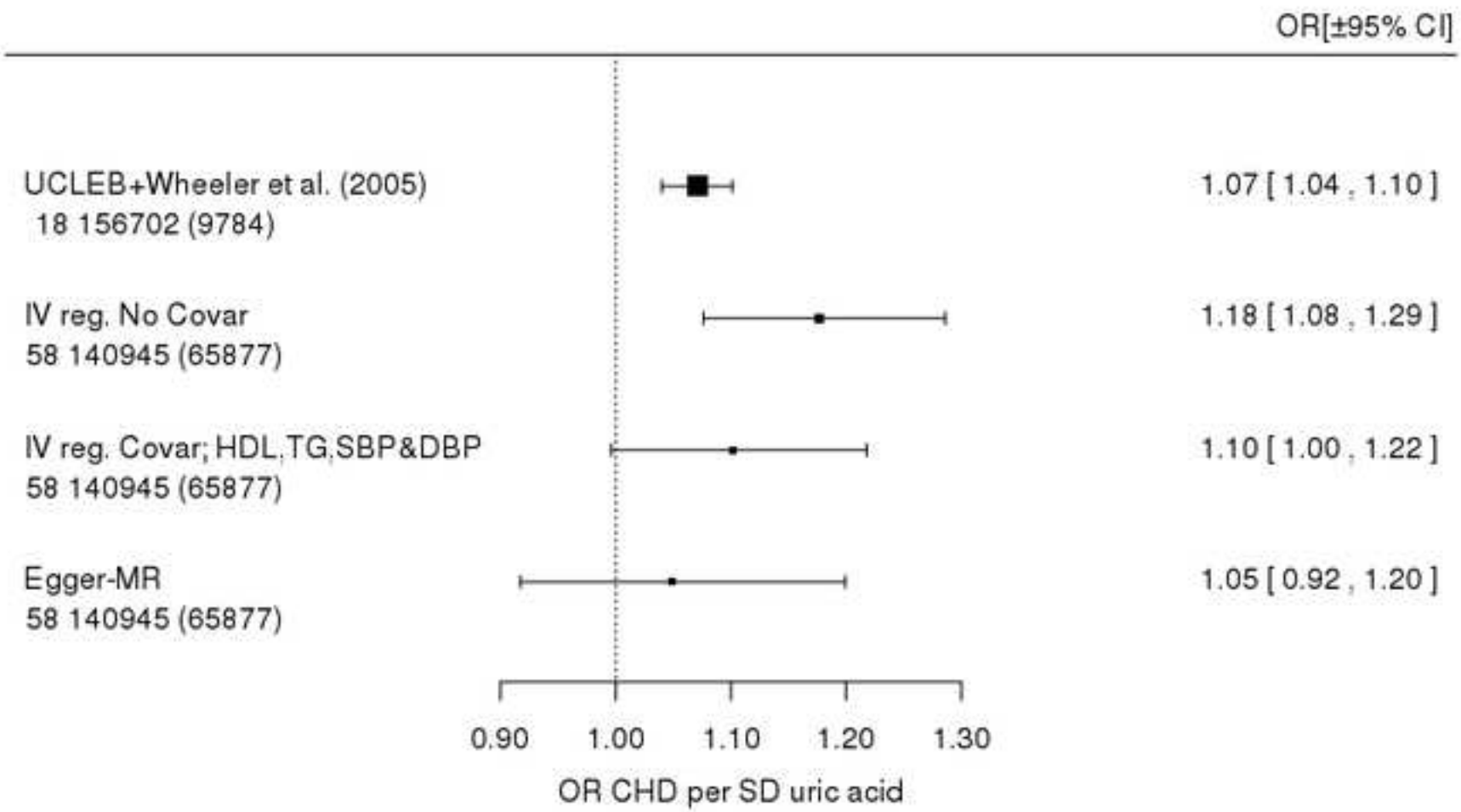

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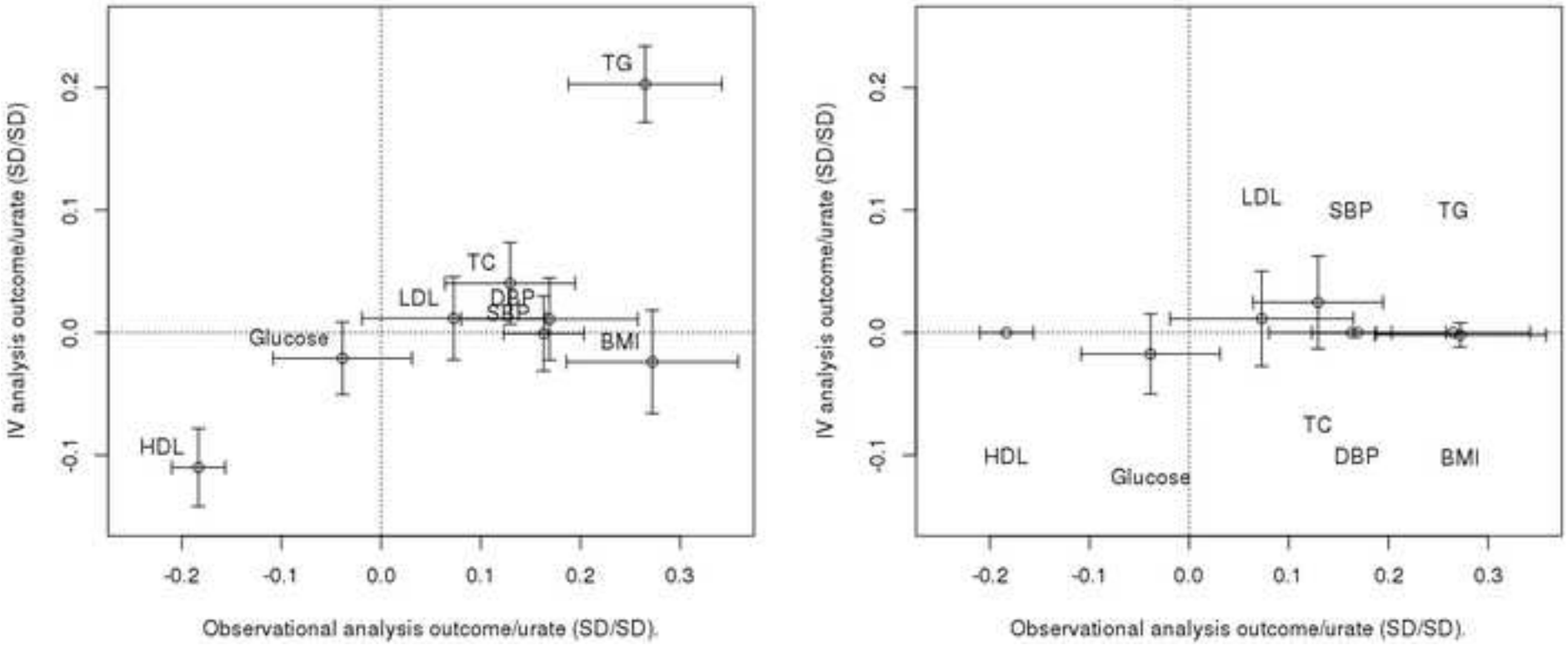

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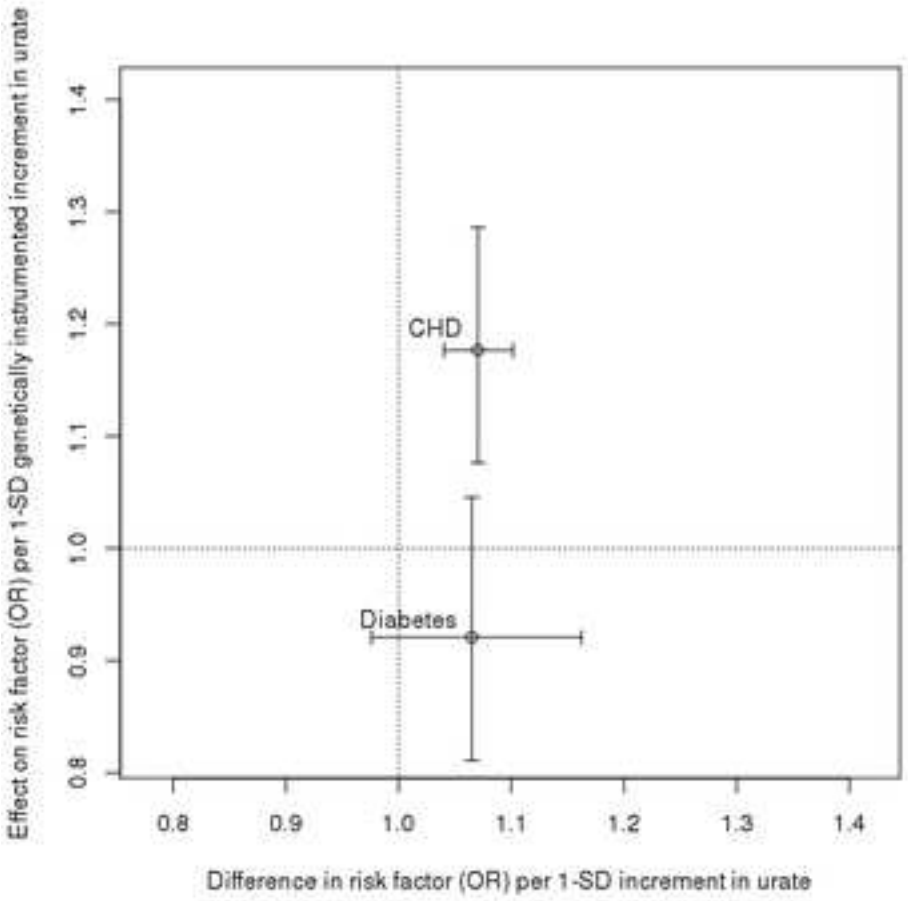

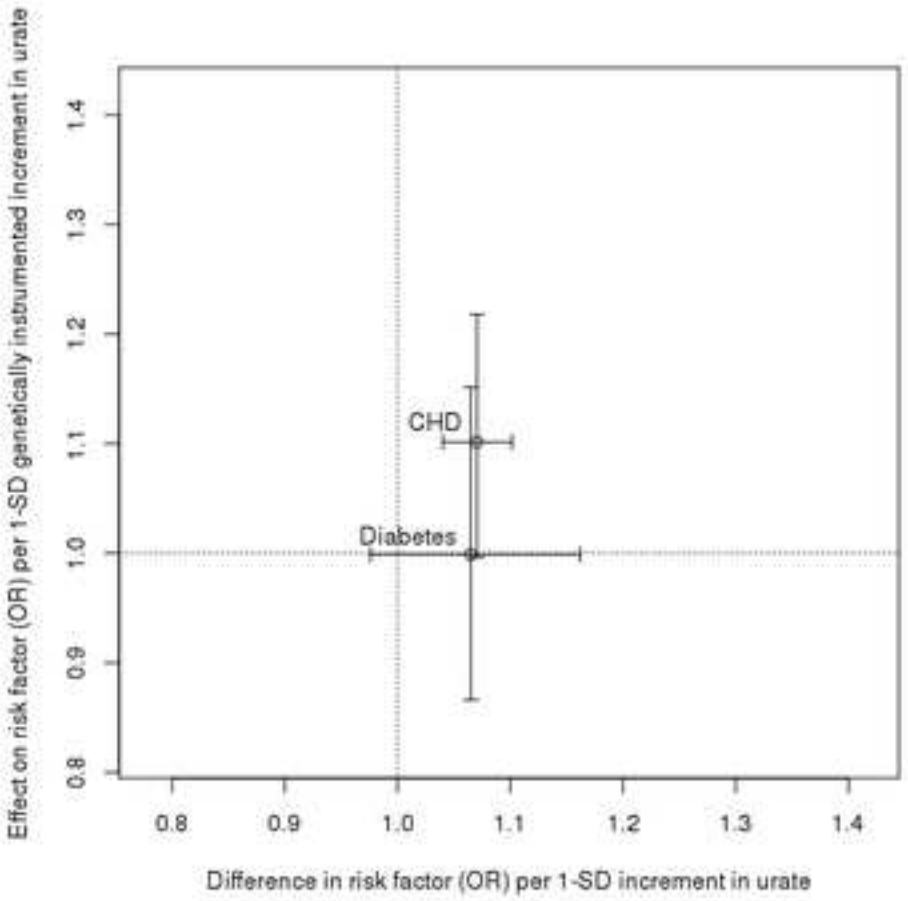

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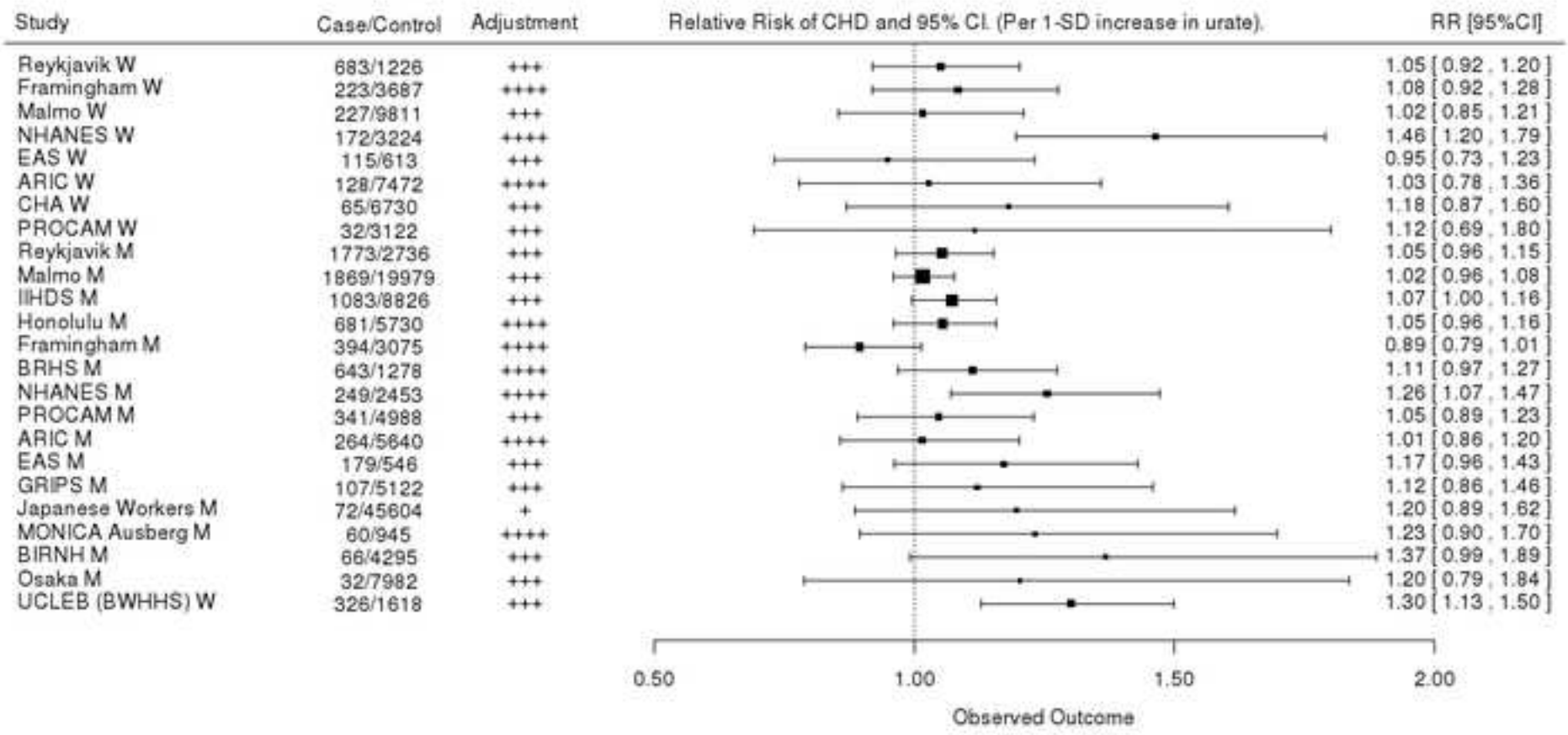

Figure

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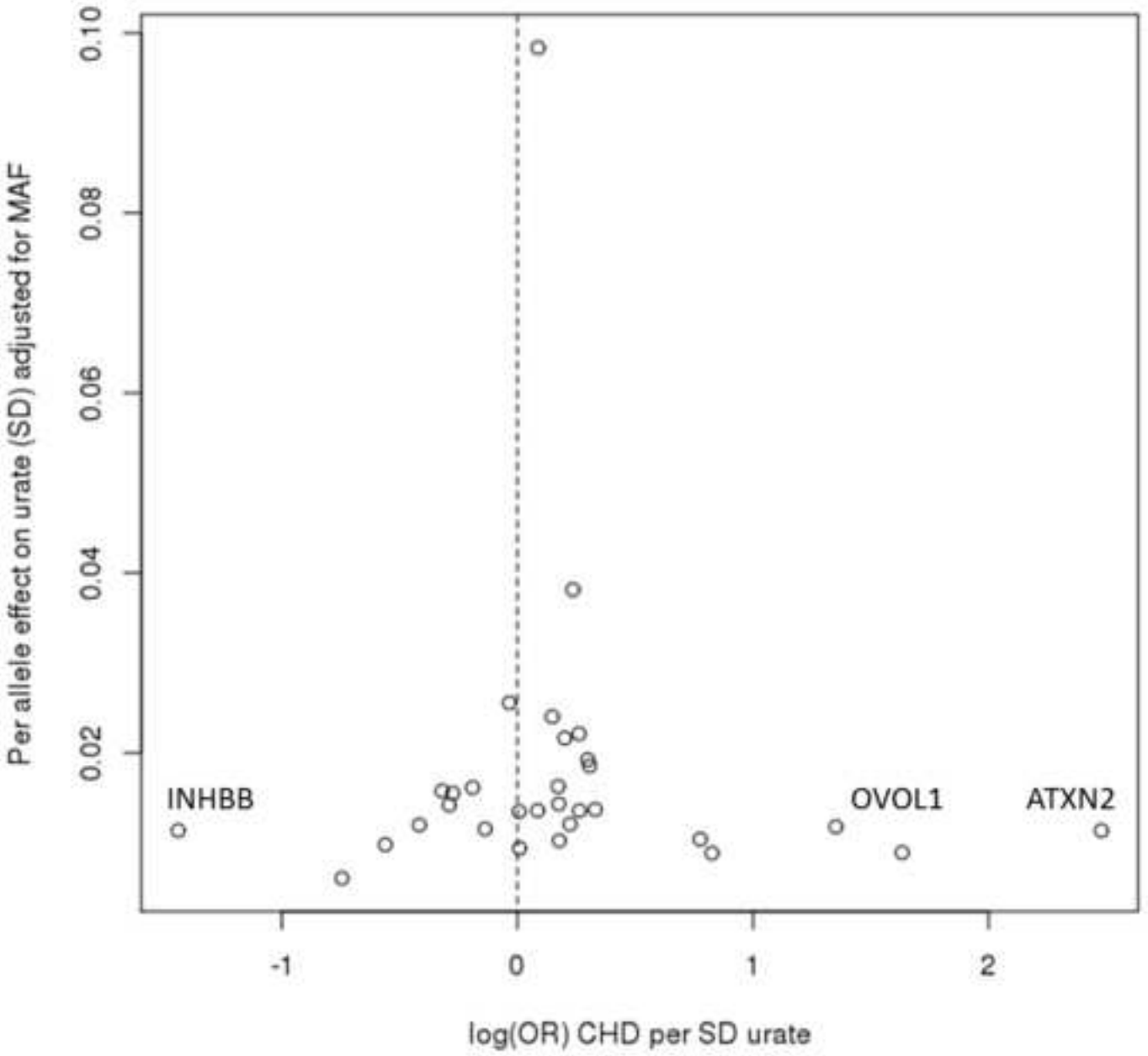

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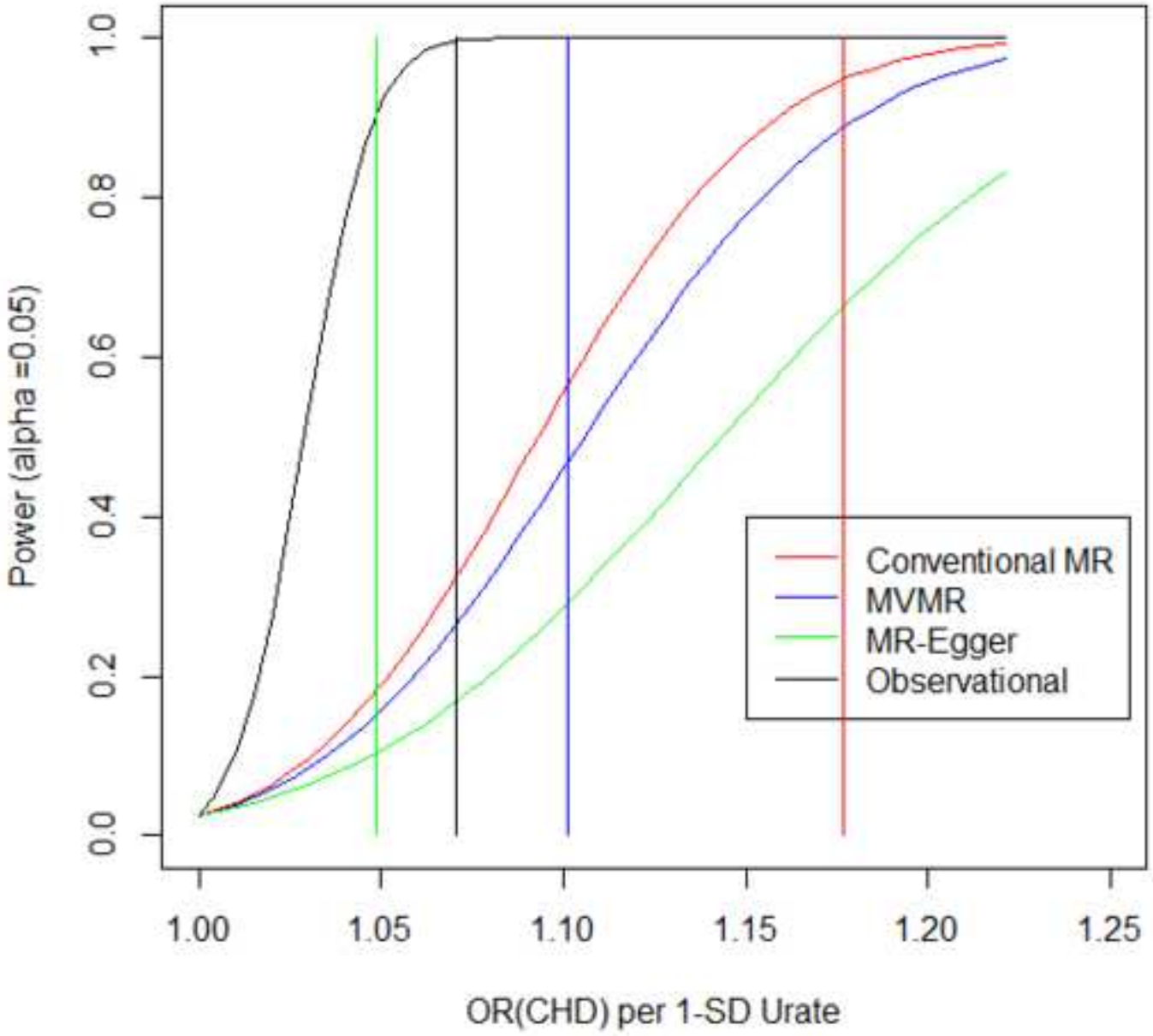

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