# An Example of Two-sample Mendelian Randomization Study

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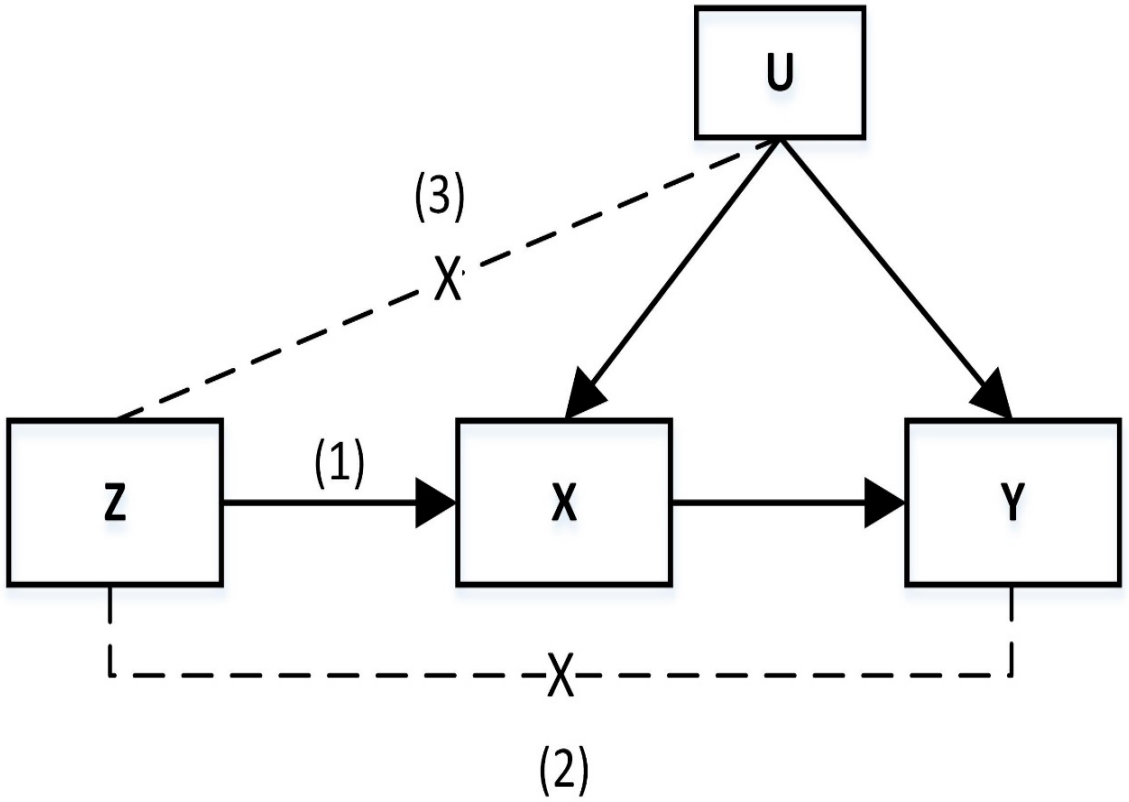
**Table 1.** Steps for MR analysis

| Step | Description |
|---|---|
| 1 | Define hypotheses for causal inference |
| 2 | Select genetic IV using GWAS or supporting information in the literature |
| 3 | Identify the MR assumptions for the selected IV |
| 4 | Perform an MR analysis |
| 5 | Interpret and discuss results |

GWAS = genome wide association studies; IV = instrumental variable; MR = Mendelian Randomization

# Causal diagram for a Mendelian randomization study.




**3 assumptions** that must be satisfied to obtain suitable results:

(1) The genetic variant **(Z)** is strongly associated with the exposure **(X)**
(2) The genetic variant (Z) is **independent of the outcome (Y)**, given the exposure and all confounders (measured and unmeasured) of the exposure-outcome association
(3) The genetic variant **(Z)** is independent of factors **(U)** (measured and unmeasured) that confound the exposure-outcome relationship

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ORIGINAL RESEARCH

# Assessment Causality in Associations Between Serum Uric Acid and Risk of Schizophrenia: A Two-Sample Bidirectional Mendelian Randomization Study

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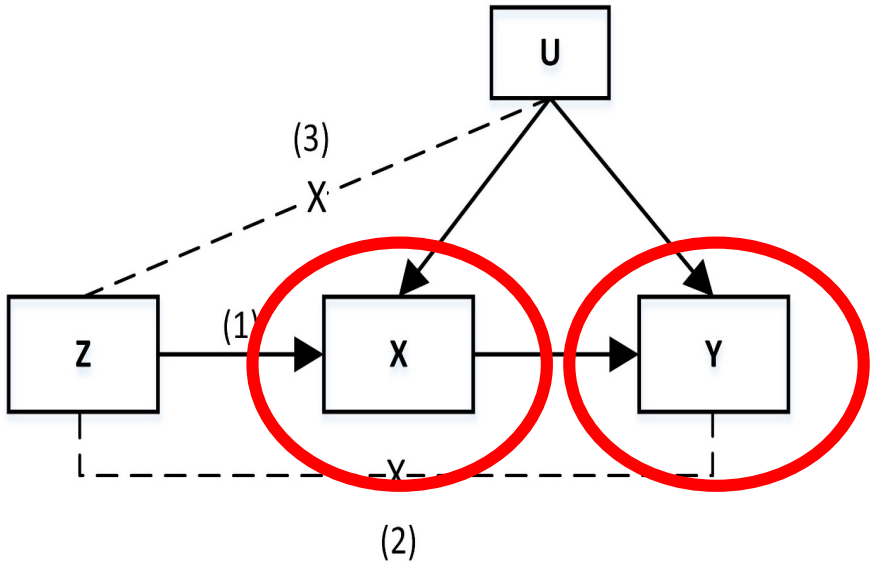
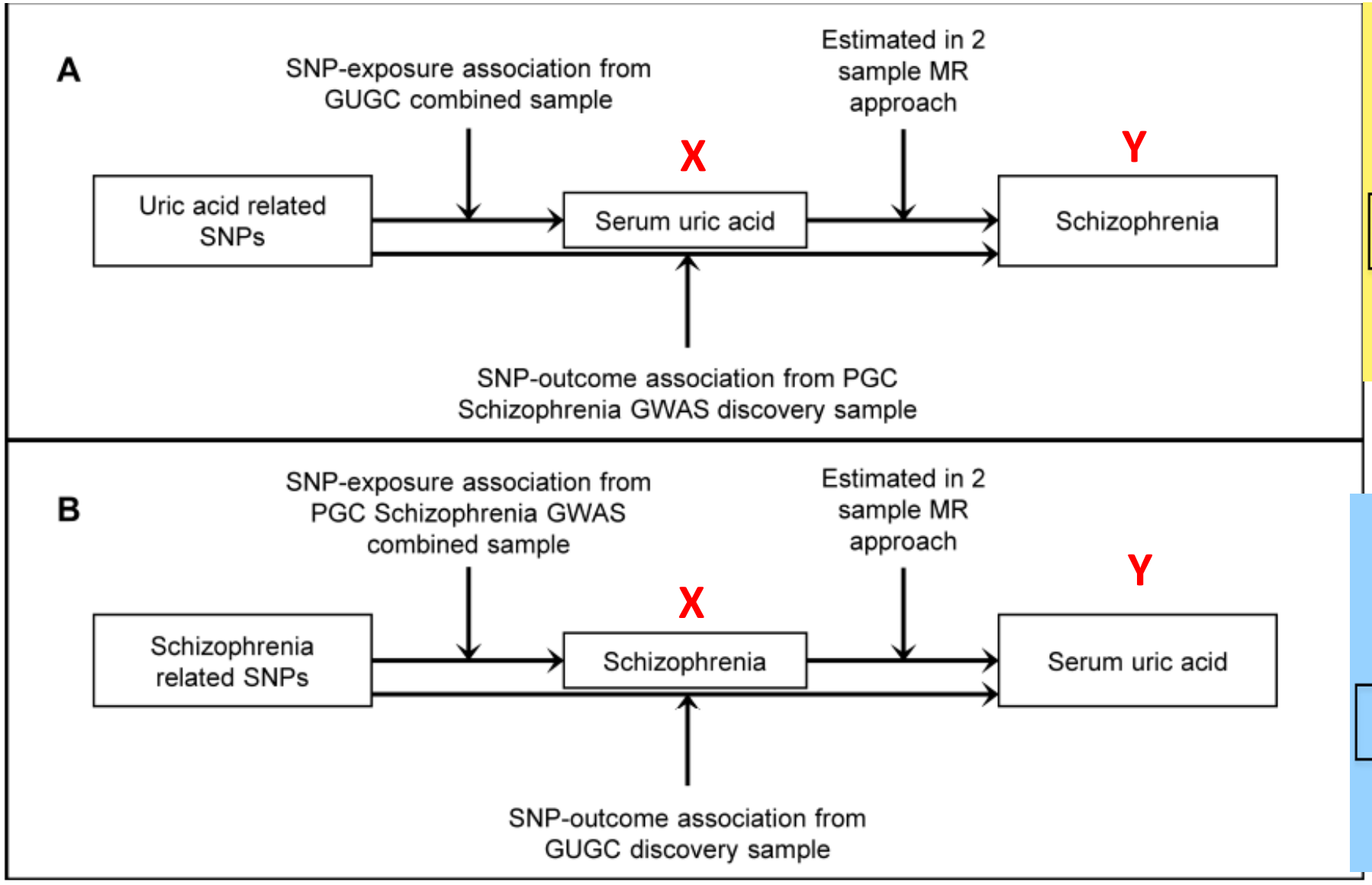
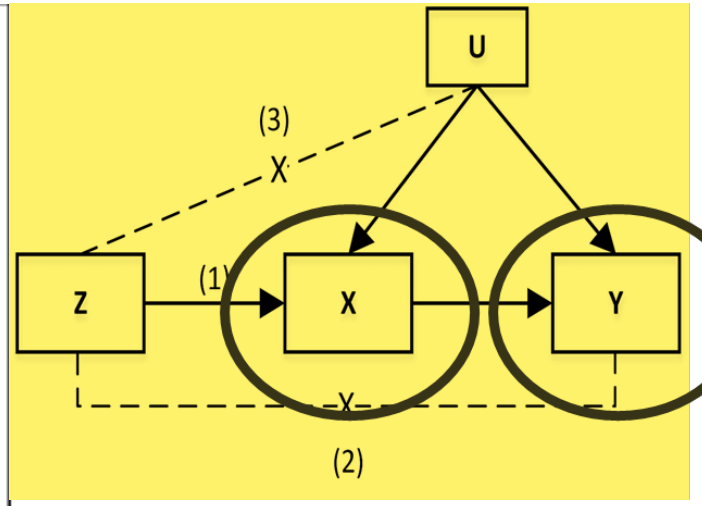
*Clinical Epidemiology*

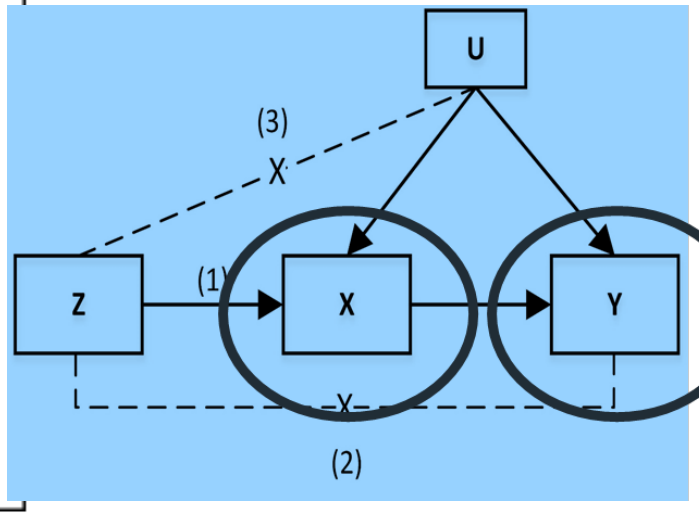
# 1 Define hypotheses for causal inference

- Aimed of  to assess whether the relationships between **serum UA levels** and **schizophrenia** are causal and to determine the direction of the association.




A
SNP-exposure association from GUGC combined sample
Estimated in 2 sample MR approach
X
Y
Uric acid related SNPs
Serum uric acid
Schizophrenia
SNP-outcome association from PGC Schizophrenia GWAS discovery sample
B
SNP-exposure association from PGC Schizophrenia GWAS combined sample
Estimated in 2 sample MR approach
X
Y
Schizophrenia related SNPs
Schizophrenia
Serum uric acid
SNP-outcome association from GUGC discovery sample

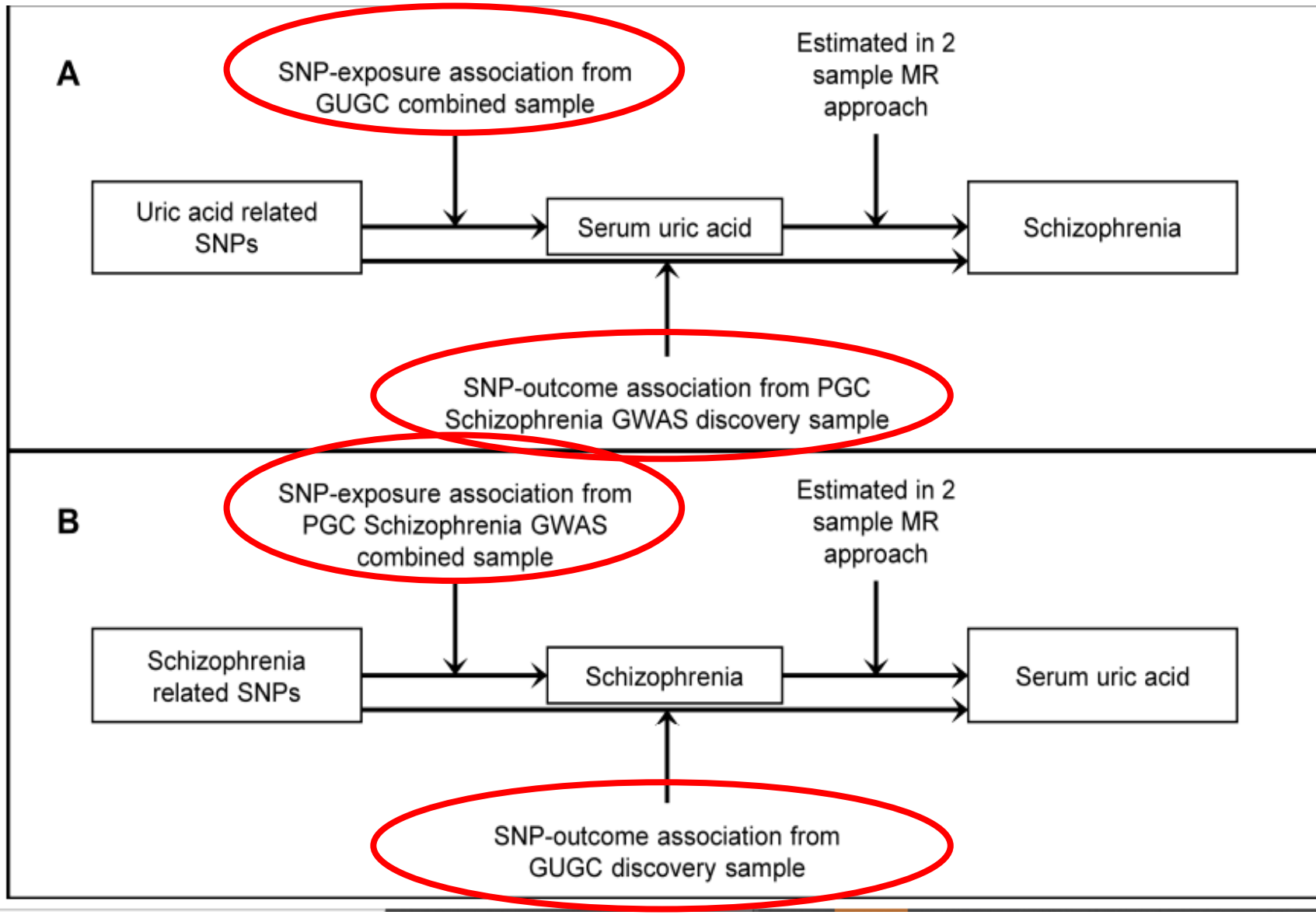

U
(3)
X
Z
(1)
X
Y
(2)


U
(3)
X
Z
(1)
X
Y
(2)

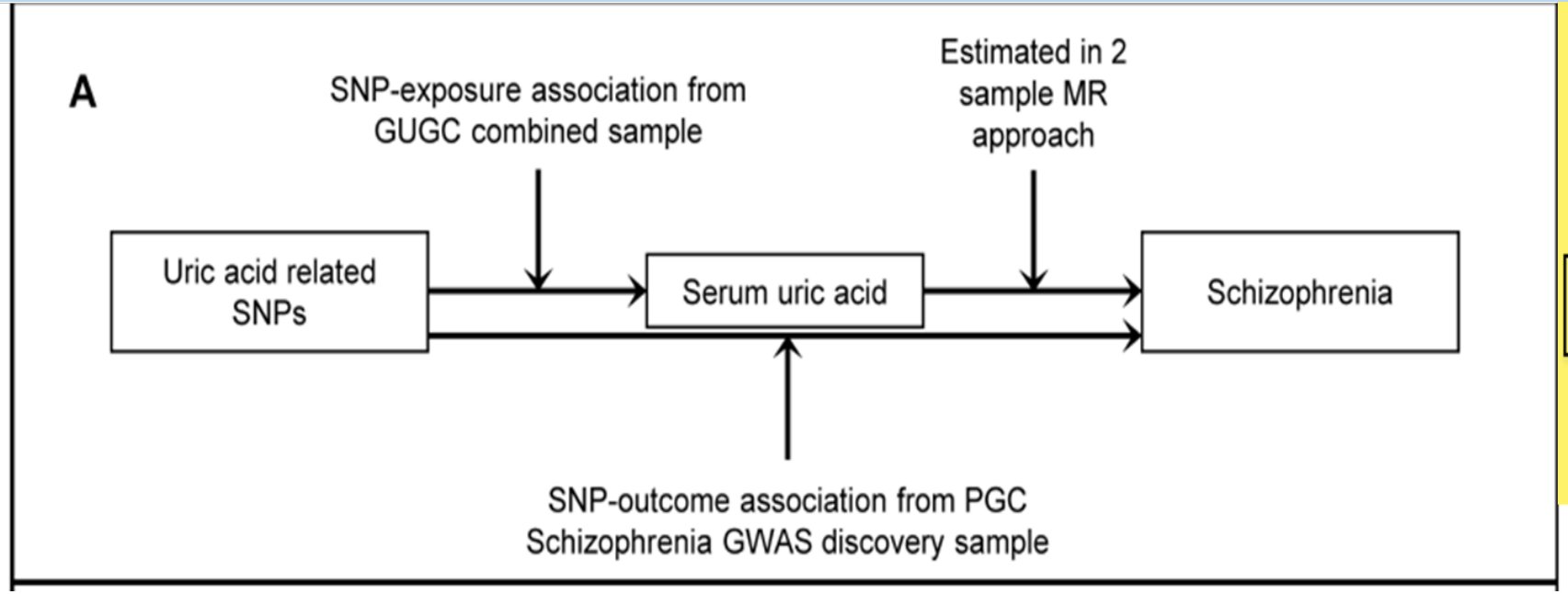
# 2 Select genetic instrumental variable (IV) using GWAS or supporting information in the literature

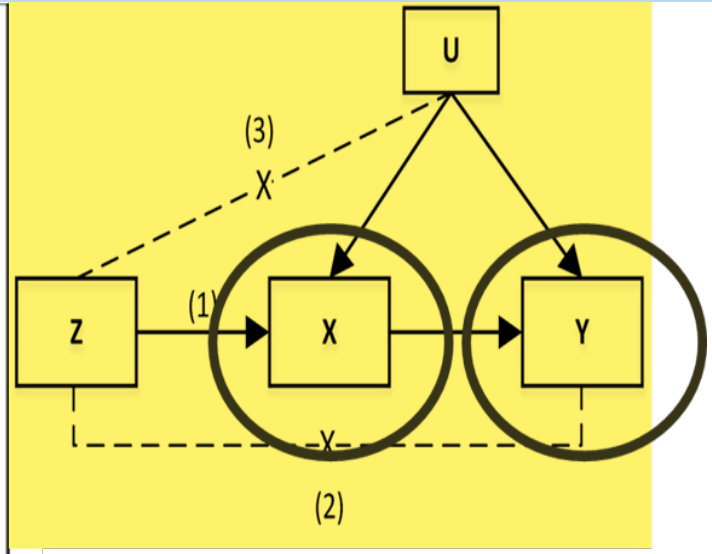
- **Utilizing the summary data from genome-wide association studies (GWAS) within the Global Urate Genetics Consortium (GUGC) and the Psychiatric Genomics Consortium.**

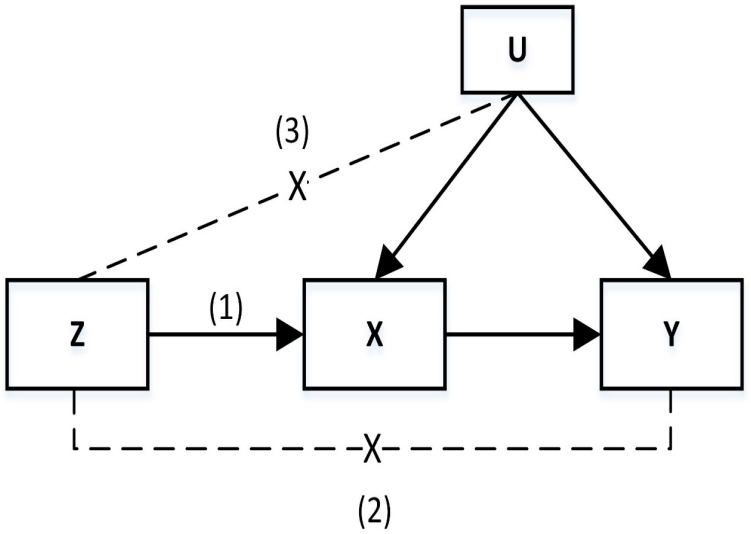
**SNP,** single nucleotide polymorphism;
**GUGC,** Global Urate Genetics Consortium;
**PGC,** Psychiatric Genomics Consortium;
**MR,** Mendelian randomization.
**GWAS:** genome-wide association studies



# 3 Identify the Mendelian Randomization (MR) assumptions for the selected IV



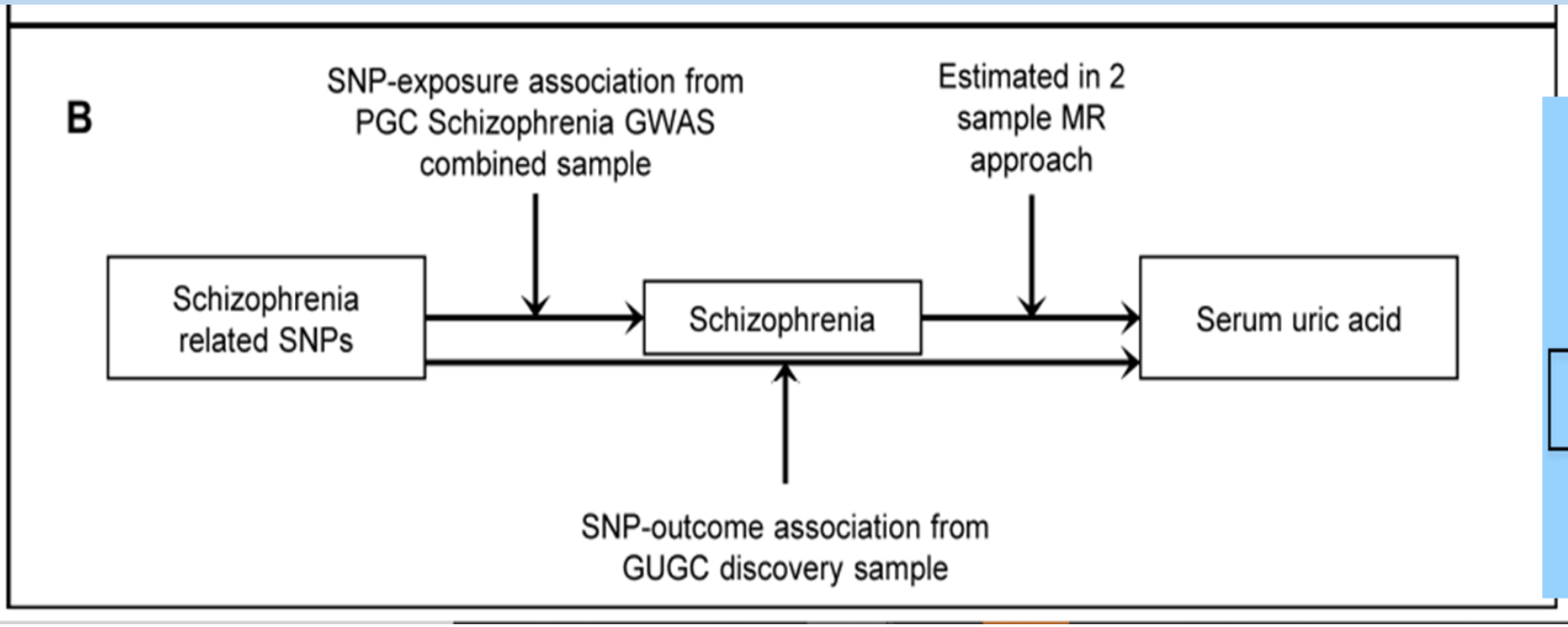

**3 assumptions** that must be satisfied to obtain suitable results:

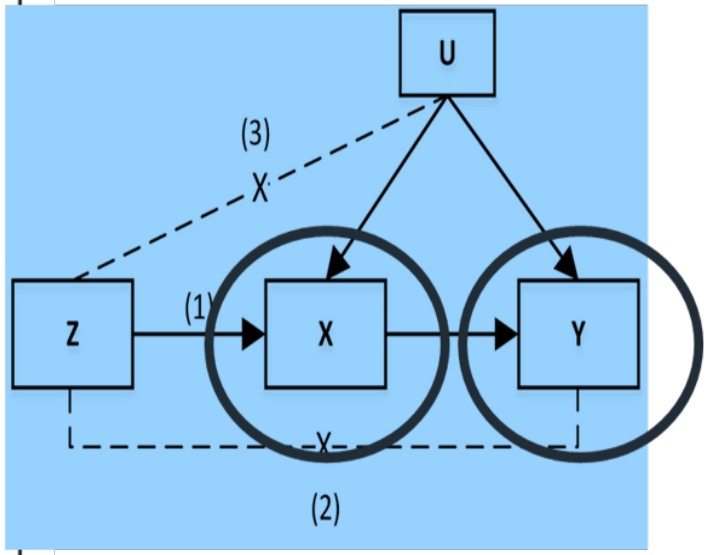
**(1)** **Uric acid related SNP is strongly associated** with the **serum uric acid**

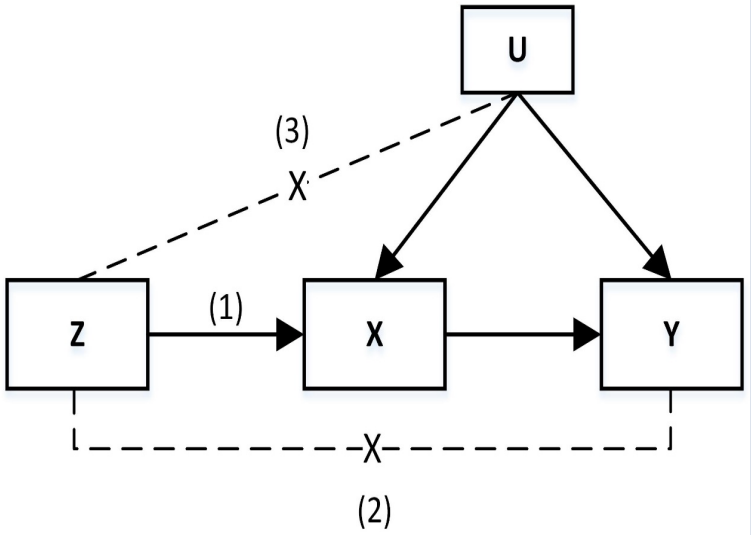
**(2)** **Uric acid related SNP** is **independent of the Schizophrenia ,** given the exposure and all confounders (measured and unmeasured) of the exposure-outcome association

**(3)** **Uric acid related SNP** is independent of **Smoking** (measured and unmeasured) that confound the exposure-outcome relationship

# 3 Identify the Mendelian Randomization (MR) assumptions for the selected IV




**3 assumptions** that must be satisfied to obtain suitable results:

**(1)** **Schizophrenia related SNP is <u>strongly associated</u>** with the Schizophrenia

**(2)** **Schizophrenia related SNP** is **<u>independent</u> of the Serum Uric acid,** given the exposure and all confounders (measured and unmeasured) of the exposure-outcome association

**(3)** **Schizophrenia related SNP** is independent of **<u>Smoking</u>** (measured and unmeasured) that confound the exposure-outcome relationship

# 4 Perform an MR analysis

Three MR methods were used in two sets of two-sample MR analyses:

**(1) Inverse variance weighted (IVW) method**

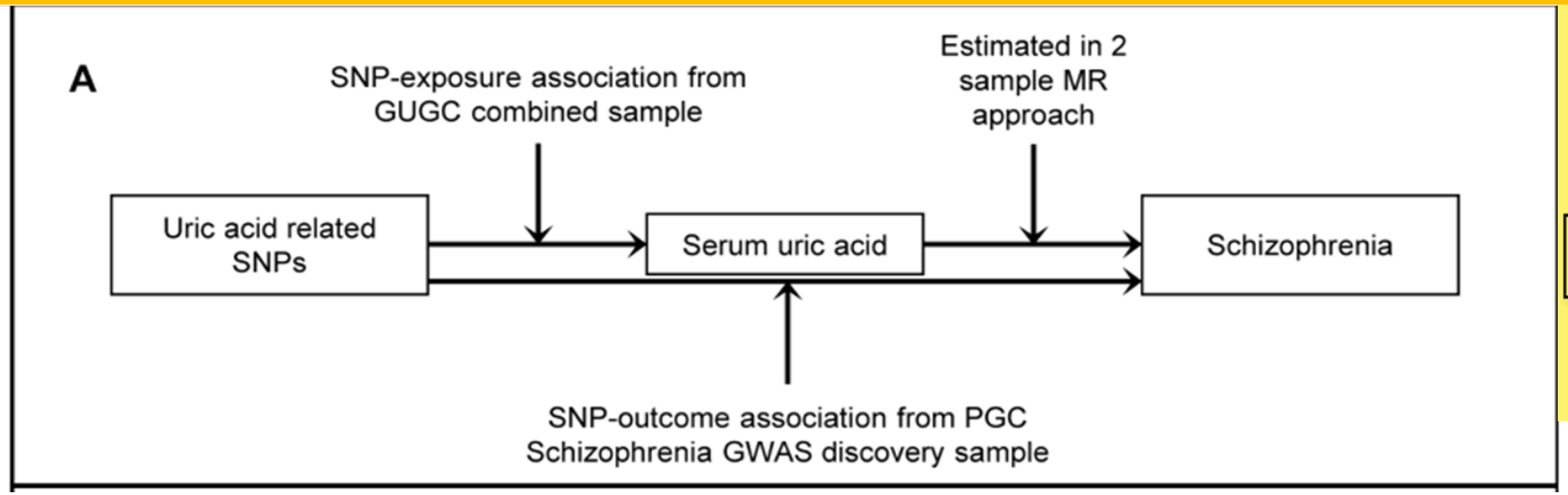
which the **SNP–outcome estimation** is **regressed** on the SNP–exposure estimation.

This approach **assumes that all SNPs are valid instruments**; thus, the overall bias is zero;

(2) **MR Egger regression analysis**,
whose **slope represents the causal effect estimate**, is robust to invalid instruments against directional pleiotropy;

(3) **Weighted median approach**, which assesses consistently regardless of whether up to half of the weight comes from invalid instruments.

# 4 Perform an MR analysis



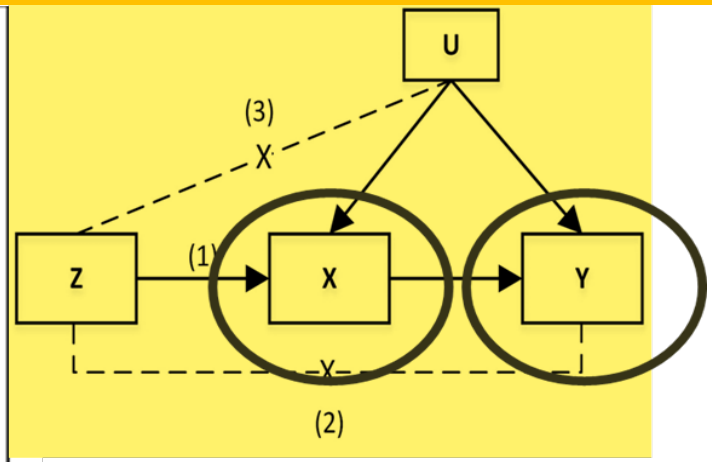
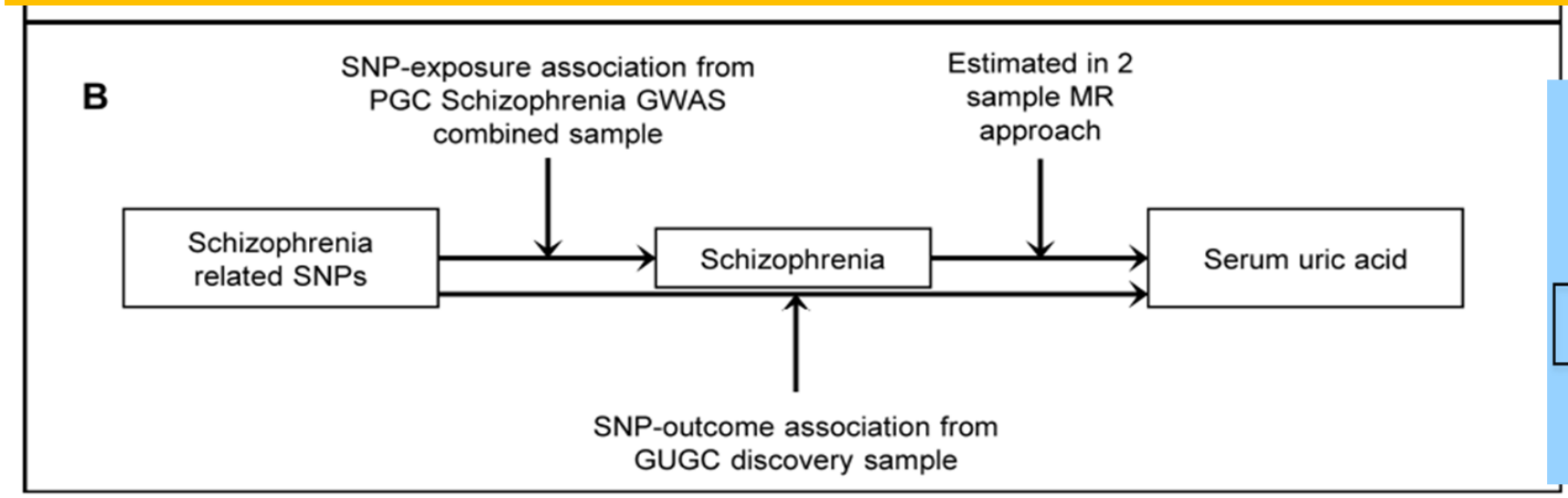




**Table 1** Results of Two-Sample Bidirectional MR Analyses on the Causal Effects Between Serum UA and Schizophrenia

| MR Method | Number of SNPs | Beta (95% CI) | SE | OR (95% CI) | P-value |
|---|---|---|---|---|---|
| Serum UA to schizophrenia[a] | | | | | |
| Inverse variance weighted | 26 | −0.060 (−0.144, 0.024) | 0.043 | 0.942 (0.866, 1.025) | 0.163 |
| MR Egger | 26 | −0.037 (−0.158, 0.084) | 0.062 | 0.964 (0.854, 1.087) | 0.555 |
| Weighted median | 26 | −0.035 (−0.097, 0.026) | 0.031 | 0.965 (0.908, 1.026) | 0.260 |
| Serum UA to schizophrenia[b] | | | | | |
| Inverse variance weighted | 21 | −0.038 (−0.097, 0.020) | 0.030 | 0.962 (0.908, 1.020) | 0.195 |
| MR Egger | 21 | −0.029 (−0.110, 0.053) | 0.042 | 0.972 (0.895, 1.054) | 0.496 |
| Weighted median | 21 | −0.033 (−0.096, 0.030) | 0.032 | 0.968 (0.908, 1.031) | 0.307 |

# 4 Perform an MR analysis



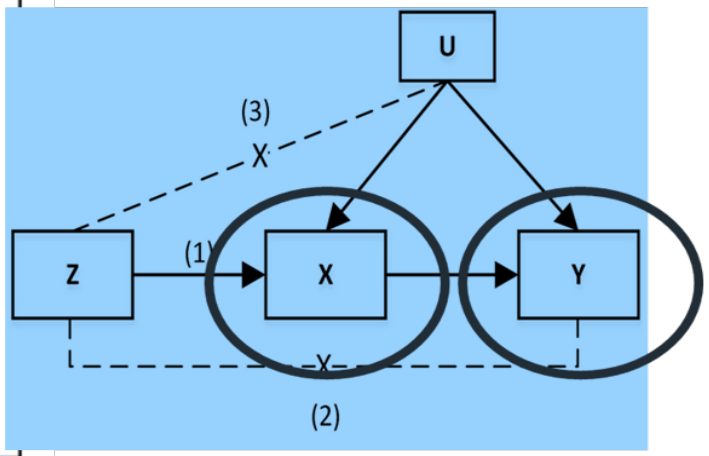




**Table 1** Results of Two-Sample Bidirectional MR Analyses on the Causal Effects Between Serum UA and Schizophrenia

| MR Method | Number of SNPs | Beta (95% CI) | SE | OR (95% CI) | P-value |
|---|---|---|---|---|---|
| Schizophrenia to serum UA[c] | | | | | |
| Inverse variance weighted | 45 | −0.045 (−0.073, −0.017) | 0.014 | 0.956 (0.929, 0.983) | 0.002* |
| MR Egger | 45 | 0.143 (−0.052, 0.337) | 0.099 | 1.153 (0.949, 1.401) | 0.159 |
| Weighted median | 45 | −0.038 (−0.073, −0.002) | 0.018 | 0.963 (0.930, 0.998) | 0.036* |
| Schizophrenia to serum UA[d] | | | | | |
| Inverse variance weighted | 44 | −0.039 (−0.065, −0.013) | 0.013 | 0.962 (0.937, 0.987) | 0.003* |
| MR Egger | 44 | 0.134 (−0.044, 0.311) | 0.091 | 1.143 (0.957, 1.365) | 0.147 |
| Weighted median | 44 | −0.036 (−0.071, −0.001) | 0.018 | 0.964 (0.931, 0.999) | 0.043* |

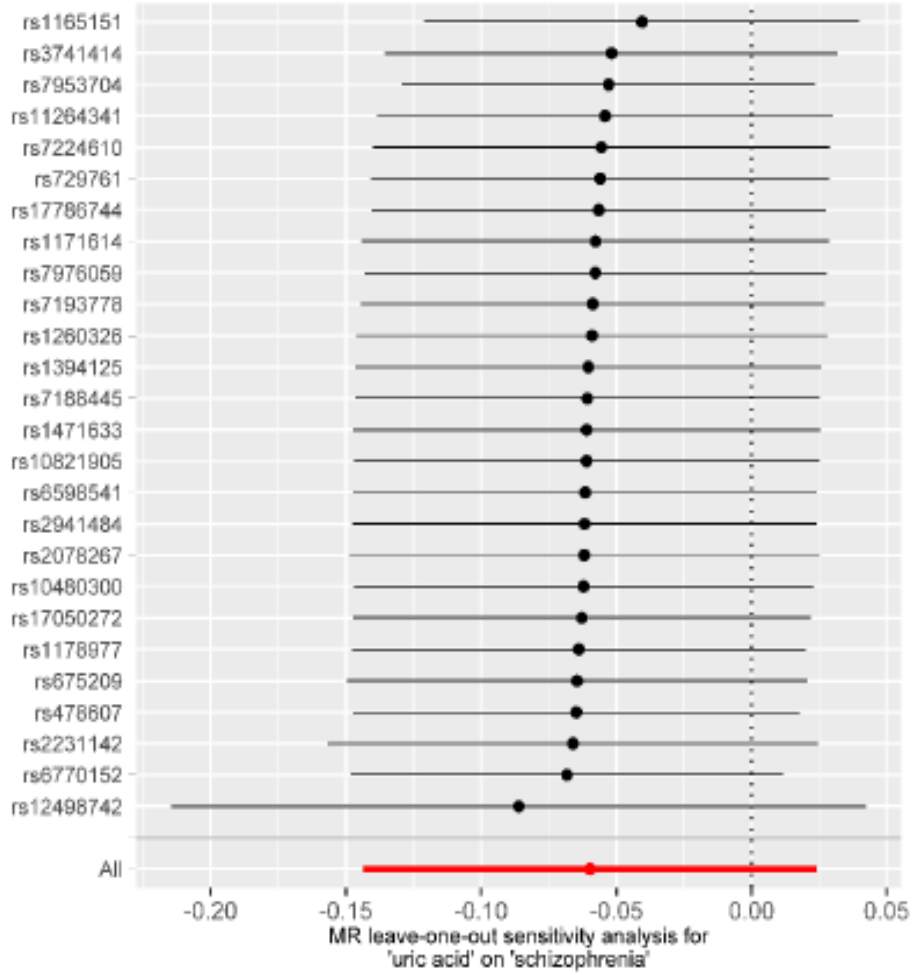



**Figure S1** Leave-one-out analysis depicting uric acid (UA) (before removal of the 5 single nucleotide polymorphisms (SNPs) with potential pleiotropic effects)-to-schizophrenia Mendelian randomization (MR) results (inverse variance weighted (IVW) method) after excluding each of the genetic variants from the analysis one at a time. We could determine whether the overall effect is driven by one specific genetic variant through this analysis.

**Abbreviation:** MR, Mendelian randomization.

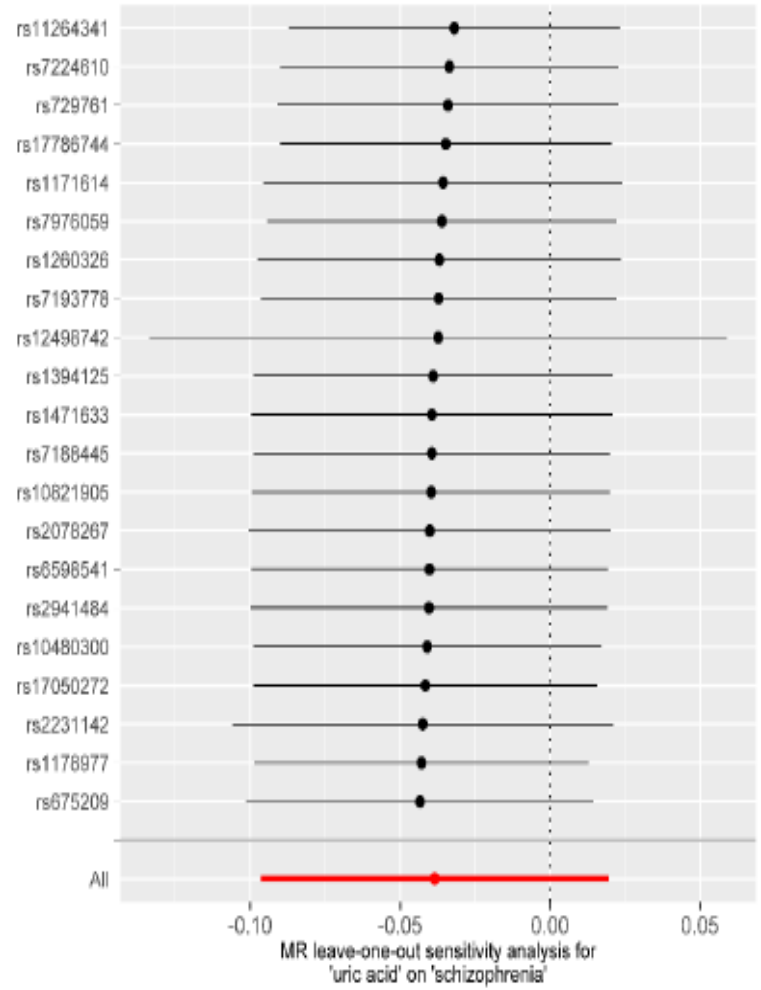



**Figure S2** Leave-one-out analysis depicting serum UA (after removal of the 5 SNPs with potential pleiotropic effects)-to-schizophrenia MR results (IVW method) after excluding each of the genetic variants from the analysis one at a time. We could determine whether the overall effect is driven by one specific genetic variant through this analysis.

**Abbreviation:** MR, Mendelian randomization.

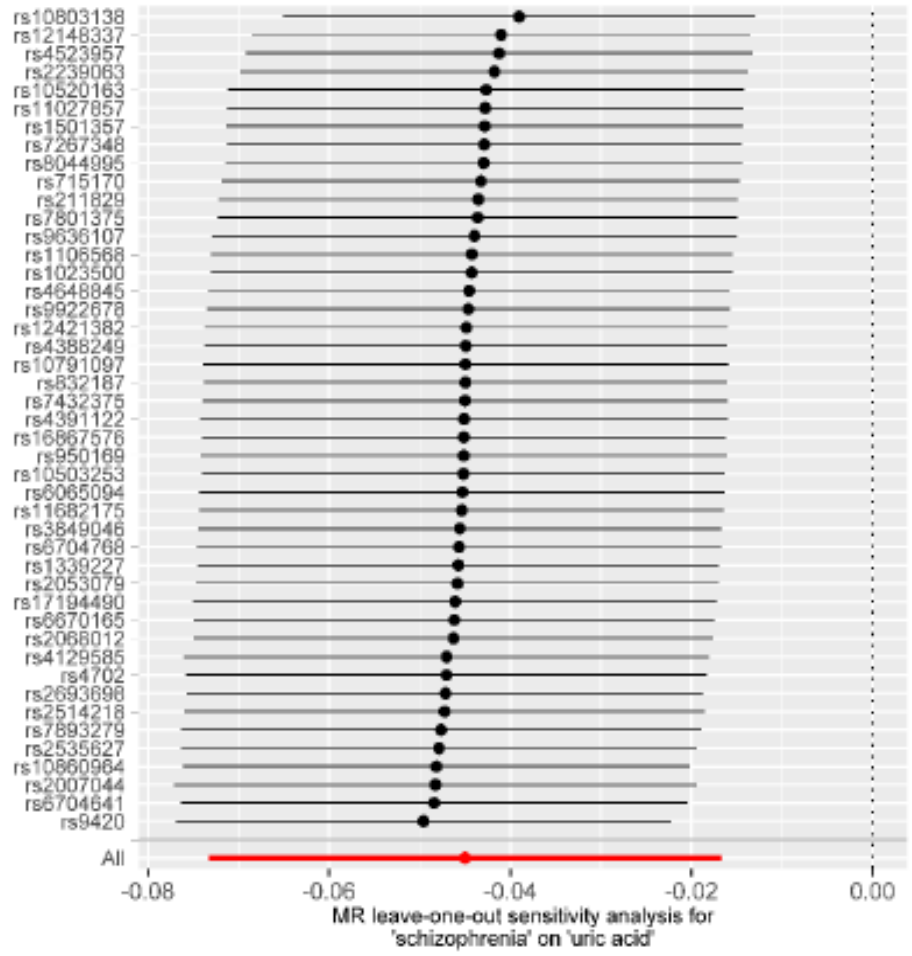



**Figure S3** Leave-one-out analysis depicting schizophrenia (before removal of the SNP with potential pleiotropic effects)-to-serum UA MR results (IVW method) after excluding each of the genetic variants from the analysis one at a time. We could determine whether the overall effect is driven by one specific genetic variant through this analysis.

**Abbreviation:** MR, Mendelian randomization.

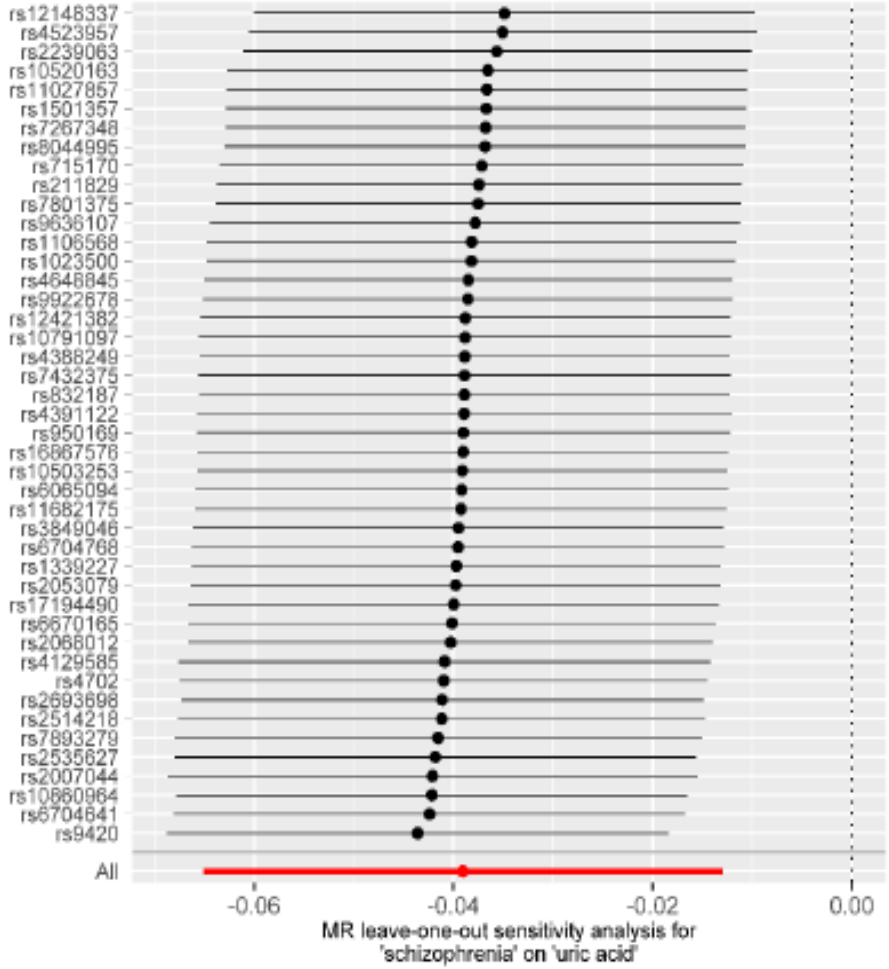
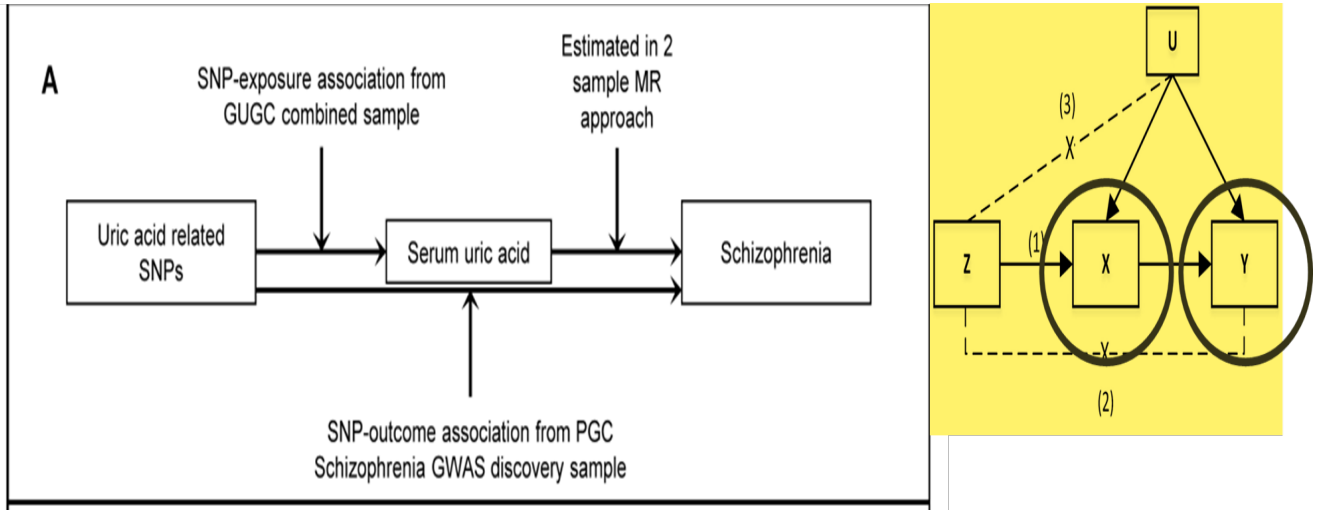



**Figure S4** Leave-one-out analysis depicting schizophrenia (after removal of the SNP with potential pleiotropic effects)-to-serum UA MR results (IVW method) after excluding each of the genetic variants from the analysis one at a time. We could determine whether the overall effect is driven by one specific genetic variant through this analysis.

**Abbreviation:** MR, Mendelian randomization.




# $GRS_{UA}$ and Schizophrenia,

**The $GRS_{UA}$ revealed no causal effect of serum UA on schizophrenia risk (per 10-s% increment in UA, OR: 0.963, 95% CI: 0.913–1.015, P = 0.154)**

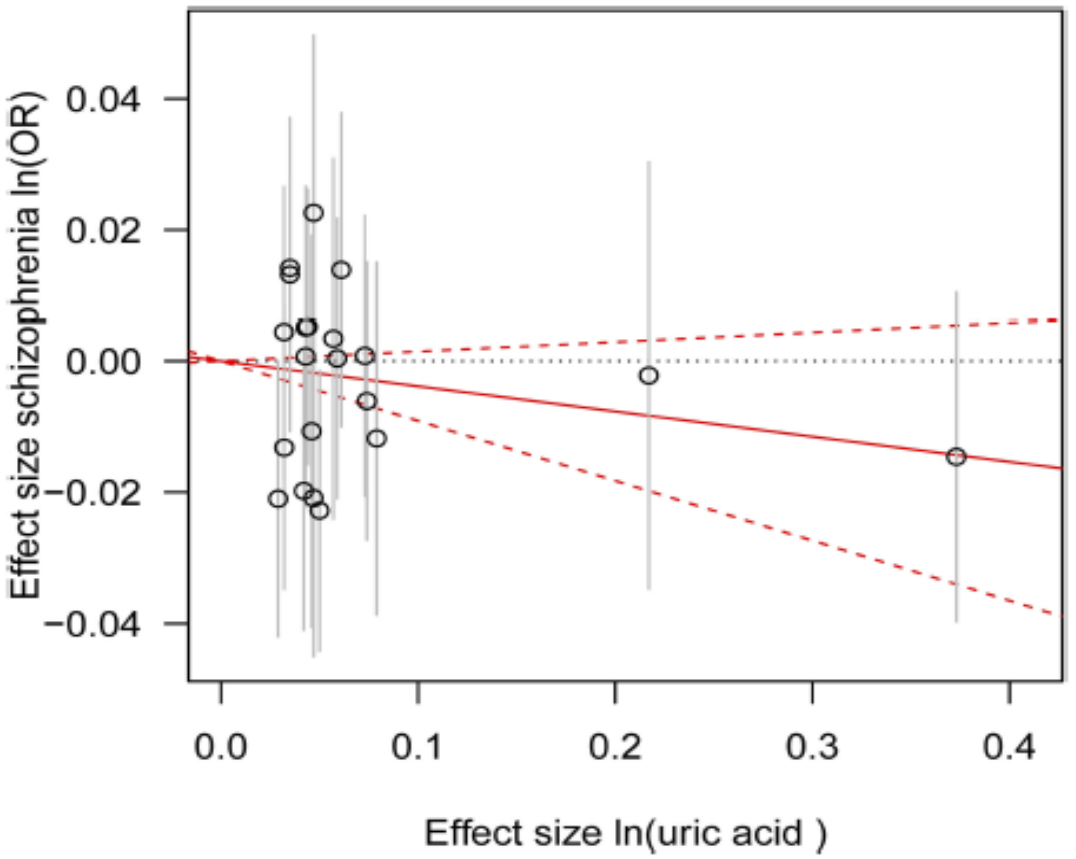
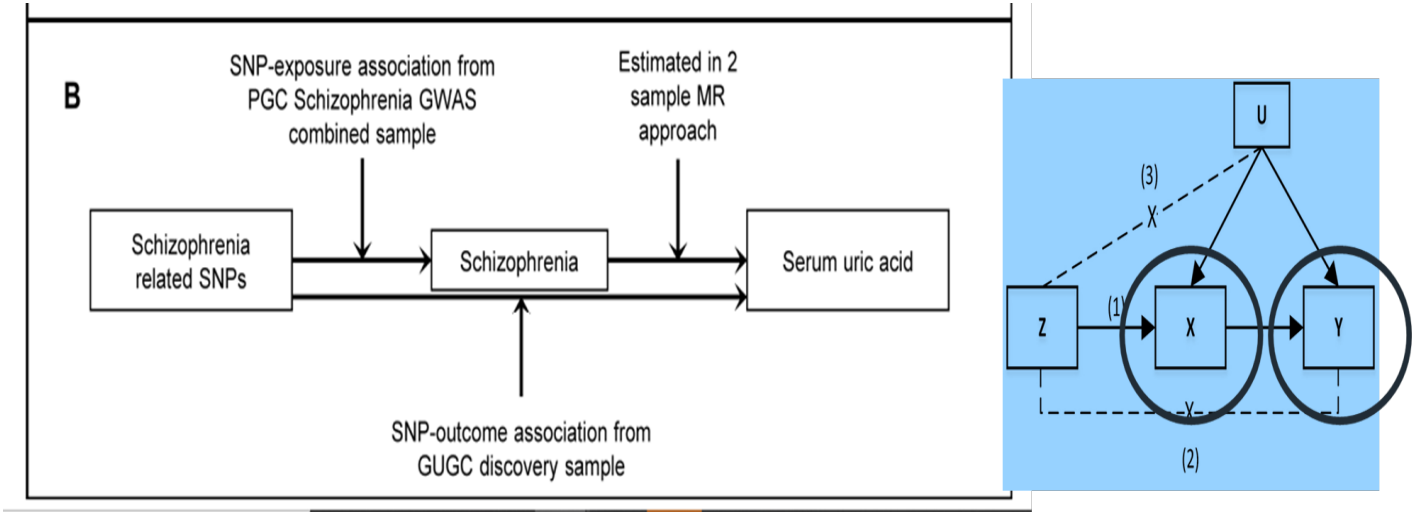



**Figure 2** Genetic risk score $GRS_{UA}$ for schizophrenia.
**Notes:** The estimated effects on schizophrenia risk (vertical axis) are plotted against the estimated effects on serum UA (horizontal axis). The 95% confidence interval (CI) for each individual UA-associated SNP is shown by vertical grey lines. The estimate of causal effect of serum UA levels on schizophrenia risk is shown by a red solid line with gradient, and 95% CI is denoted by red dashed lines.
**Abbreviation:** OR, odds ratio.




# $GRS_{SCZ}$ and Serum UA

The $GRS_{SCZ}$ showed a significant effect of schizophrenia on serum UA (per 10-s% increase in schizophrenia risk, beta: −0.039, SE: 0.013, P = 0.002)

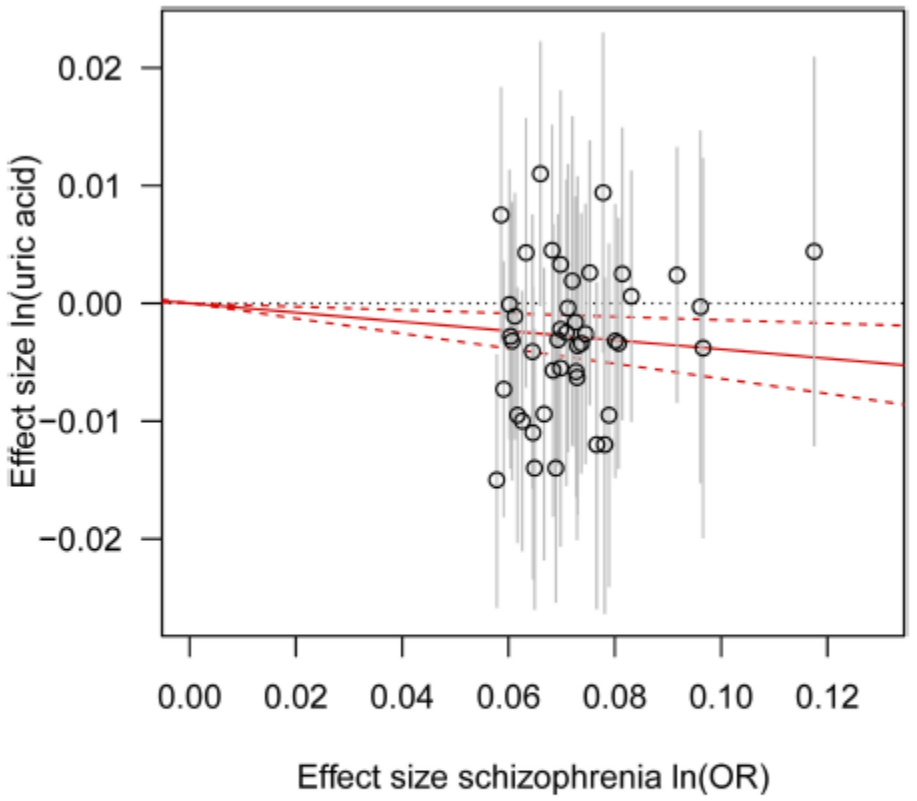



**Figure 3** Genetic risk score $GRS_{SCZ}$ for serum UA.
**Notes:** The estimated effects on serum UA (vertical axis) are plotted against estimated effects on schizophrenia risk (horizontal axis). The 95% confidence interval (CI) for each individual schizophrenia-associated SNP is shown by vertical grey lines. The estimate of causal effect of schizophrenia risk on serum UA levels is shown by a red solid line with gradient, and 95% CI is denoted by red dashed lines.
**Abbreviation:** OR, odds ratio.

# Heterogeneity

**Table 3** Cochran's Heterogeneity Statistic and MR Egger Intercept, Indicating Horizontal Pleiotropy for Two-Sample Bidirectional MR Analyses Between Serum UA and Schizophrenia

| Exposure | Outcome | Number of SNPs | Cochran's Heterogeneity Statistic (IVW) | | Cochran's Heterogeneity Statistic (MR Egger) | | MR EGGER | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | Q | P-value | Q | P-value | Intercept | SE[e] | P-value |
| Serum UA | Schizophrenia | 26[a] | 69.491 | 4.58E-06 | 68.708 | 3.42E-06 | −0.003 | 0.006 | 0.606 |
| Serum UA | Schizophrenia | 21[b] | 24.191 | 0.234 | 24.051 | 0.194 | −0.001 | 0.004 | 0.742 |
| Schizophrenia | Serum UA | 45[c] | 57.803 | 0.079 | 53.302 | 0.135 | −0.014 | 0.007 | 0.063 |
| Schizophrenia | Serum UA | 44[d] | 46.941 | 0.314 | 43.120 | 0.423 | −0.013 | 0.006 | 0.060 |

**Notes:** [a]Serum UA to schizophrenia MR before removal of the five SNPs with potential pleiotropic effects. [b]Serum UA to schizophrenia MR after removal of the five SNPs with potential pleiotropic effects. [c]Schizophrenia to serum UA MR before removal of the SNP with potential pleiotropic effects. [d]Schizophrenia to serum UA MR after removal of the SNP with potential pleiotropic effects. [e]SE of the intercept.

**Abbreviations:** MR, Mendelian randomization; UA, uric acid; SNP, single nucleotide polymorphism; IVW, inverse variance weighted; SE, standard error.

# 5 Interpret and discuss the results

- Three **MR methods** provided
  - **No causal relationship** between **serum UA and schizophrenia**.
  - Furthermore, GRS approach showed similar results in the three MR methods after adjustment for heterogeneity.

  - **Having causal effect of schizophrenia risk on serum UA** after adjustment for heterogeneity (per 10-symmetric percentage increase in schizophrenia risk, beta: −0.039, standard error (SE): 0.013, P = 0.003; beta: −0.036, SE: 0.018, P = 0.043; beta: −0.039, SE: 0.013, P = 0.002; respectively).

  - The heterogeneity **(Q test)** and sensitivity tests suggested **no strong evidence of bias due to pleiotropy**.

*Thank you for your attention.*