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大麻使用與焦慮之因果關係：雙樣本孟德爾隨機化研究

Causality between Cannabis Use and Anxiety: A Two-Sample Mendelian Randomization Study

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A Two-Sample Mendelian Randomization Study

本論文係翁咏聖君(B09801009)在國立臺灣大學公共衛生學系完成之學士班學生論文，於民國 113 年 4 月 9 日承下列考試委員審查通過及口試及格，特此證明

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摘要

研究指出大麻使用是與焦慮有關的可改變行為因素，焦慮也可能反過來影響大麻的使用，但兩者之間的因果性仍有待商榷。我們利用最新公佈且可直接取得的全基因體關聯性研究匯總數據，對歐裔血統人群終生大麻使用、大麻使用障礙，以及焦慮之間的潛在因果關係執行了一項雙向、雙樣本孟德爾隨機化調查。藉由變異數倒數加權的相乘隨機效應模型，輔以基於中位數和眾數的估計、MR-Egger、MR-RAPS、MR-PRESSO 等穩健統計方法，我們在大麻使用障礙對焦慮症（OR_{IVW} = 1.088，95%信賴區間：1.018-1.163，p 值 = 0.013）、大麻使用障礙對社交恐懼症（OR_{IVW} = 1.290，95%信賴區間：1.062-1.567，p 值 = 0.010），以及焦慮症對終生大麻使用（OR_{IVW} = 1.166，95%信賴區間：1.007-1.351，p 值 = 0.040）的幾種測試中找到顯著的正向因果效應。我們的發現與既有流行病學證據相符，但整體而言，僅憑本研究似乎無法提供強而有力的證據來確認大麻使用對焦慮，或是焦慮對大麻使用之因果相關。部分結果與文獻不一致，可能源自於觀察性研究的干擾作用或反向因果，以及遺傳工具變項分析和臨床試驗或社區介入的差異。未來的工作需要擴大體學資料的豐富度及多樣性，探尋與大麻使用或焦慮在生物醫學意義上更為具體的位點，以提昇檢驗因果關聯的能力，並增進研究成果類推的適用性。最重要的是，對證據的混合比對，加上對因果謬誤的思辨，將更好地為物質使用議題及心理健康實踐指引方向。

關鍵詞：孟德爾隨機化、大麻、焦慮、因果推論、遺傳工具變項

Abstract

Research has suggested that cannabis use is a modifiable behavioral factor associated with anxiety, which in turn may influence cannabis use. However, the causality between cannabis use and anxiety is yet to be elucidated. We conducted a bidirectional, two-sample Mendelian randomization investigation leveraging the latest publicly available genome-wide association study summary statistics to explore potential causal relationships between lifetime cannabis use (LCU), cannabis use disorder (CUD), and anxiety disorders among individuals of European ancestry. Using the inverse-variance weighted (IVW) multiplicative random-effects model, with the median- and mode-based estimates, MR-Egger, MR-PRESSO, and other supplementary analyses, positive causal effects were found in several tests: CUD on anxiety disorders (OR_{IVW} = 1.088, 95% CI: 1.018-1.163, p-value = 0.013), CUD on social phobias (OR_{IVW} = 1.290, 95% CI: 1.062-1.567, p-value = 0.010), and anxiety disorders on LCU (OR_{IVW} = 1.166, 95% CI: 1.007-1.351, p-value = 0.040). Our findings align with existing epidemiological evidence, but on the whole, this study alone may not offer compelling evidence to confirm the causal associations between cannabis use and anxiety. Discrepant results with the literature may arise from confounding or reverse causation in observational studies, as well as differences between genetic instrumental

variable analysis and clinical trials or community interventions. To boost the power to detect causality and improve the generalizability of research findings, future efforts should be made for the richness and diversity of omics data. This includes identifying more specific genetic markers linked to cannabis use or anxiety with biomedical implications. Most importantly, evidence synthesis and triangulation, along with critical appraisal of causal fallacies, will provide better guidance for substance use issues and mental health practices.

Keywords: Mendelian randomization, cannabis, anxiety, causal inference, genetic instrumental variables

Table of Contents

List of Tables

List of Figures

List of Supplementary Tables

List of Supplementary Figures

Chapter 1 Introduction

1.1 Cannabis Use

Cannabis refers to preparations of the genus *Cannabis* [1]. Over 500 compounds have been reported from cannabis plants, with the most extensively studied cannabinoids being tetrahydrocannabinol (THC) and cannabidiol (CBD) [2]. These chemicals can modulate signal transduction via cannabinoid receptors and interact with the endocannabinoid system [3]. THC can produce euphoria and is more closely associated with dependence and addiction, while CBD is non-psychoactive, and some of its medicinal values have been documented [4]. Cannabis is the most commonly used drug worldwide. In 2021, approximately 219 million (95% CI: 158 million-276 million) individuals aged 15 to 64 had consumed cannabis around the globe, with prevalence rates varying by location but showing an overall growing trend [5]. In recent years, some countries have altered the legal status of cannabis products through legalization, decriminalization, or rescheduling [6]. Political, economic, and cultural globalization have shaped people's perceptions, attitudes, and behaviors toward cannabis, whether for medical or recreational purposes. Despite cannabis remaining illegal in Taiwan, its prevalence rate has not significantly decreased [7]. Furthermore, cannabis is associated with mental illness and substance use disorders [8-11] and is the main reason why

individuals seek treatment services in some geographical regions [5]. The global disease burden of cannabis use disorder has also escalated over the past 30 years [12, 13]. As a result, it is essential to clarify the harms and benefits of cannabis use and guide evidence-based decision-making.

1.2 Anxiety

Mental disorders involve impairments or disturbances in thinking, perception, emotion, behavior, and function [14, 15], which currently impact approximately one-eighth of the world's population [16]. In 2019, the disability-adjusted life years (DALYs) attributable to mental disorders ranked seventh among all categories globally and in Taiwan. In terms of the years lived with disability, it ranked second, slightly lower than musculoskeletal disorders [17]. Anxiety is often described as the natural response to stress or danger, characterized by a feeling of worry, fear, or uneasiness. Even so, excessive and persistent anxiety may develop into clinically diagnosed anxiety disorders, encompassing generalized anxiety disorder, social anxiety disorder (also termed social phobia), agoraphobia, specific phobia, panic disorder, and so on [18]. Different subtypes of pathological anxiety may coexist or be accompanied by physical and mental health conditions such as heart palpitations, depression, or sleep problems.

Anxiety disorders accounted for 22.9% (95% CI: 18.6%-27.5%) of global DALYs of 12 mental disorders in 2019, second only to depressive disorders at 37.4% (95% CI: 32.3%-43.0%). However, the age-standardized prevalence rate of anxiety disorders was 3779.5 per 100,000 population, ranking first among all mental disorders [17]. There has been no significant improvement in these situations across different regions, genders, and age groups since 1990. The incidence rate of anxiety disorders increased by 26% in the first year of the COVID-19 pandemic [19], emphasizing the urgency to allocate mental health resources and tackle global health emergencies. Given the universal value that "there is no health without mental health," anxiety warrants attention and concerted efforts to uncover its roots and offer professional support.

1.3 Causal Inference and Mendelian Randomization

Epidemiology is the cornerstone of health promotion and disease prevention, focusing on the distribution and determinants of health-related states, conditions, or events in specified populations to manage and control health issues [20]. Clarifying the associations between cannabis use and anxiety is essential for planning, implementing, and evaluating health programs. The relationships between cannabis use and anxiety have been studied through animal and laboratory experiments, expert opinions, and case

reports/series, which are generally considered to provide a lower level of scientific evidence. Findings from observational epidemiological research (e.g., cross-sectional, case-control, and cohort studies) remain inconclusive [21, 22], and the estimated health effects may be biased by confounding or reverse causation. A statistically significant correlation does not imply causation. Even if researchers have identified variables that may be common causes of cannabis use and anxiety and try to adjust them through stratified analysis, regression modeling, or propensity score matching, not all confounding factors are guaranteed to be accurately and precisely measured. Therefore, a more appropriate study design is required to examine potential evidence for causal associations between cannabis use and anxiety. Randomized controlled trials (RCTs) have been held up as the "gold standard" in empirical health science, ensuring comparability between the intervention and control groups. In other words, except for the exposure of interest, the other factors should be balanced on average across the subgroups. Nevertheless, the feasibility of RCTs may be constrained by ethical considerations, economic costs, and other limitations in the field. For example, it may be difficult to conduct an RCT in Taiwan to assess whether cannabis users are significantly more likely to be diagnosed with anxiety disorders compared to those who have never used cannabis within a specific timeframe, or to evaluate the effectiveness of

cannabis abstinence in alleviating anxiety signs and symptoms. These factors make it more challenging to comprehend the etiology of cannabis use and anxiety.

Research on instrumental variables (IVs) has expanded from econometrics to genetic epidemiology, biomedical statistics, and health information technology. Cannabis use and anxiety are complex traits, and single nucleotide polymorphisms (SNPs) associated with these phenotypes have been discovered through large-scale genome-wide association studies (GWAS). International consortia and multi-omics databases have fueled innovative applications of Mendelian randomization (MR), which uses genetic variants as IVs to make inferences about causal effects from observational data [23, 24]. This study approach draws on Mendel's Laws of Inheritance [25], which state that alleles located on different chromosomes are randomly separated and independently distributed to offspring during gamete formation in parents. Genetic variations are basically determined at conception and precede the occurrence of health events, so the exposure-outcome association is less likely to be reversed or confounded by environmental factors (e.g., experiences, lifestyles, or sociodemographic characteristics) from a population perspective. This feature makes MR analogous to a natural RCT, reinforcing causal inference in public health or clinical medicine in the

presence of residual confounding [26]. The objective of our study is to figure out whether genetically inferred cannabis use has a causal influence on anxiety and whether genetic liability to anxiety may causally affect cannabis use.

Chapter 2 Materials and Methods

2.1 Study Design

A two-sample MR investigation [27] was undertaken to elucidate the bidirectional causal relationships between cannabis use and anxiety. We referenced the *Strengthening the Reporting of Observational Studies in Epidemiology using Mendelian Randomization* (STROBE-MR) guidelines to scrutinize the plausibility, coherence, and rigor of our argumentation [28]. The key point of an MR study lies in determining whether the genetic variants act as valid IVs to provide unbiased causal estimates. To achieve this goal, the genetic variants must satisfy the following three assumptions:

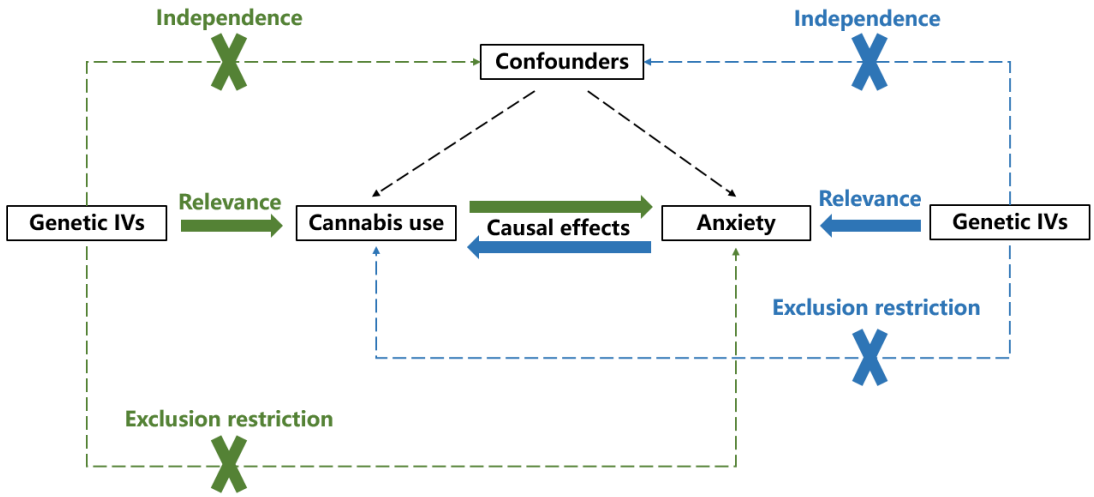
1. Relevance: The genetic variants have a strong association with the exposure.

2. Independence: The genetic variants are not related to any potential confounders.

3. Exclusion Restriction: The genetic variants affect the outcome only through the exposure.

A directed acyclic graph provides an intuitive understanding of statistical thinking and causal inference. As shown in Figure 1, it consists of a set of nodes and arrows. There are arrows from the genetic IVs to the exposure (relevance assumption), from the exposure to the outcome, and from the confounders to the exposure and the outcome. Ideally, the genetic variants are assumed to be independent of confounding factors (independence assumption), and there are no other paths from the genetic instruments to the outcome except through the exposure (exclusion restriction assumption). If the SNPs affect the outcome via alternative pathways other than the exposure, this is known as horizontal pleiotropy. Such a situation would violate the second or third assumption, thereby compromising the internal validity of MR analysis. As cannabis use and anxiety may be influenced by multiple genetic and environmental factors, integrating biomedical knowledge and statistical techniques to solve these problems has become the focus of our research.

Figure 1. Schematic diagram of the bidirectional, two-sample MR study



The green part illustrates the forward analysis, with cannabis use as the exposure and anxiety as the outcome, while the blue part depicts the reverse analysis, with anxiety as the exposure and cannabis use as the outcome. The associations of the genetic variants with the exposure and the outcome are derived from independent samples.

2.2 Data Sources

Genetic information on cannabis use and anxiety was extracted from the largest publicly available GWAS to date. SNP-exposure associations and SNP-outcome associations were estimated in non-overlapping samples from the same underlying population. Lifetime cannabis use (LCU) and cannabis use disorder (CUD) summarized data were queried for the analysis of cannabis use phenotype. The LCU sample was defined as self-reported cannabis use at least once in a lifetime and comprised 162,082 individuals (43,380 cases and 118,702 controls), with data from the International Cannabis Consortium (ICC) and the UK Biobank (UKB) [29]. CUD summary statistics

were derived from a meta-analysis of GWAS the Psychiatric Genomics Consortium (PGC), the Lundbeck Foundation Initiative for Integrative Psychiatric Research (iPSYCH), and deCODE Genetics [30]. Among 357,806 participants (14,080 cases and 343,726 controls), CUD cases were those who met the criteria for cannabis use disorder in the DSM-5, or cannabis abuse and cannabis dependence in the ICD-10, DSM-III-R, or DSM-IV.

GWAS data for anxiety disorders (AD) were obtained from the 10th release of the FinnGen Project to ensure sample independence and minimize population stratification [31]. 346,542 Finnish citizens (44,663 cases and 301,879 controls) were recruited, with cases primarily coded as F40-F48 (neurotic, stress-related, and somatoform disorders) according to the ICD-10. Summary statistics for various anxiety endpoints, such as generalized anxiety disorder (GAD), social phobias (SP), agoraphobia (AG), and panic disorder (PD), were also analyzed in our study for multiple comparisons. All GWAS for the univariable MR study collected data from individuals of European ancestry only, and samples were genotyped on different platforms. Further details, including covariate adjustment, quality control, and imputation, can be found in the original publications.

Table 1. Sample description

Trait	Category	Sample size (case/control)	Database	Criteria
Lifetime cannabis use (LCU)	Binary	162,082 (43,380/118,702)	ICC UKB	Self-report
Cannabis use disorder (CUD)		357,806 (14,080/343,726)	PGC iPSYCH deCODE	ICD-10 DSM-5 DSM-IV DSM-III-R
Anxiety disorders (AD)		346,542 (44,663/301,879)	FinnGen	ICD-10 ICD-9 ICD-8
Generalized anxiety disorder (GAD)		373,334 (5,280/368,054)		ICD-10 ICD-9
Social phobias (SP)		370,878 (2,824/368,054)		ICD-10 ICD-9
Agoraphobia (AG)		369,057 (1,003/368,054)		ICD-10 ICD-9
Panic disorder (PD)		373,422 (5,368/368,054)		ICD-10 ICD-9

2.3 Selection of Genetic Instrumental Variables

SNPs with p-values < 5×10^{-8} were initially filtered from the summary-level GWAS data, in accordance with the relevance assumption. The PLINK [32] clumping algorithm ($r^2 = 0.001$, window size = 10,000 kb) was then performed to make sure that IVs for the exposure were independent of each other. The European ancestry (EUR) reference panel from the 1000 Genomes Project was utilized to eliminate linkage disequilibrium (LD) and avoid redundant calculation of the contribution of any specific

variants that may be correlated [33]. Only 4 genome-wide significant SNPs were identified for LCU and 2 for CUD. Subsequently, we relaxed the threshold to $p < 5\times10^{-7}$ to increase the number of available variants. A similar approach was taken for GAD, SP, AG, and PD, with the p-value set at 5×10^{-6}, while also paying attention to horizontal pleiotropy. The variance explained by SNPs of an exposure phenotype was estimated using R^2. As a rule of thumb, a genetic predictor with an F-statistic < 10 would be preferentially removed to control for weak instrument bias [34, 35]. The formulas for R^2 and F-statistic are as follows:

$$R^2 = \frac{2{\beta_X}^2 \times EAF \times (1 - EAF)}{2{\beta_X}^2 \times EAF \times (1 - EAF) + 2N(SE(\beta_X))^2 \times EAF \times (1 - EAF)}$$

$$F = \frac{R^2 \times (N - 2)}{(1 - R^2)} = (\frac{\beta_X}{SE(\beta_X)})^2$$

where EAF is the effect allele frequency of the exposure variant, β_X is the point estimate of the SNP-exposure effect, $SE(\beta_X)$ is the standard error of β_X, and N is the sample size of the exposure dataset in which the SNP is present.

Concerning the independence assumption, we reviewed literature and web-based catalogs[1] to exclude genetic variants closely associated with the biologically plausible

[1] Accessed at PhenoScanner (http://www.phenoscanner.medschl.cam.ac.uk/), LDlink (https://ldlink.nih.gov/), NHGRI-EBI (https://www.ebi.ac.uk/gwas/), IEU OpenGWAS (https://gwas.mrcieu.ac.uk/), FinnGen R10 (https://r10.finngen.fi/), etc.

confounding factors for the putative causal pathway [36], including rs1154693 for LCU and rs11783093 for CUD [30, 37], as many of the published GWAS or MR research have noticed that these loci may be relevant to tobacco use. Subsequently, we retrieved eligible exposure variants from the outcome GWAS summary statistics. If information about the locus could not be found, an LD proxy for that variant was not sought. Caution was given to the consistency of DNA strand information. Before causal modeling, effect estimates of the exposure and the outcome were harmonized so that the SNP-exposure and SNP-outcome associations aligned correctly with the same reference allele. In cases where palindromic SNPs exist, we attempted to infer which allele may be the effect allele according to allele frequencies. If this was not feasible, these variants were dropped instead.

2.4 Statistical Analysis

Variants were chosen from multiple gene regions. The primary analytical method in this study was the inverse-variance weighted (IVW) multiplicative random-effects model [38]. Assuming all genetic variants are valid IVs, the IVW method gives the most efficient causal estimates by combining the Wald ratios from multiple genetic variants. Let there be J SNPs (G_j, j=1, 2, …, J), β_{X_j} is the effect size of genetic

association between G_j and the exposure X, β_{Y_j} is the effect size of genetic association between G_j and the outcome Y, and α_j reflects the horizontal pleiotropy. If every G_j is uncorrelated with each other and independent of α_j, and the weighted average of α_j does not differ from zero (i.e., there is no directional pleiotropy), then the regression model can be expressed as:

$$\hat{\beta}_{Y_j} = \theta\hat{\beta}_{X_j} + \varepsilon_j, \quad \varepsilon_j \sim N(0, \phi_M^2 \mathrm{SE}(\hat{\beta}_{Y_j})^2)$$

where θ represents the causal effect of the exposure on the outcome, and ϕ_M^2 denotes the overdispersion parameter indicating the heterogeneity of the variant-specific causal estimates.

In this study, cannabis use and anxiety traits were all categorized as binary variables. IVW estimated the causal parameters through weighted logistic regression of SNP-outcome associations to SNP-exposure associations, where the intercept was set to zero, and the slope was the log odds ratio. Given that the core MR assumptions may not have been entirely justified, a set of robust statistical methods has been proposed for polygenic MR in two-sample settings [39]. We performed additional analyses to evaluate the sensitivity of the results, incorporating at least one common method from each of the following categories: (1) consensus methods (e.g., median- or mode-based

estimates), (2) modeling methods (e.g., MR-Egger and MR-RAPS), and (3) outlier-robust methods (e.g., MR-PRESSO). These methods rely on weaker assumptions than the IVW estimation, and each of them has its advantages and concerns. If several approaches present highly concordant results, the evidence supporting or questioning a causal finding would likely be stronger.

The median-based estimate corresponds to the 50th percentile of the empirical density function of the unweighted (or inverse-variance weighted) Wald ratios [40]. When at least 50% of the genetic variants are valid instrumental variables (or at least 50% of the weight comes from valid genetic instruments), the simple median (or weighted median) method can provide a more valuable causal effect estimation. The mode-based estimate is determined by the mode of the empirical density function of the unweighted (or inverse-variance weighted) Wald ratios [41]. To comply with the consistency assumption, the simple mode (or weighted mode) method requires that the largest subset of instrumental variables estimating the same causal effect should be valid (in terms of quantity or weight). The mode- and median-based estimates are robust to outliers but sensitive to the addition or removal of genetic variants.

In contrast to the IVW estimate, MR-Egger only needs the "InSIDE" (instrument strength independent of direct effect) assumption to hold [42]. This means that the weighted correlation between SNP-exposure association and the pleiotropic effect of a variant must be zero. The Egger regression model allows for a non-zero intercept θ_0 to indicate unbalanced pleiotropy:

$$\hat{\beta}_{Y_j} = \theta_0 + \theta\hat{\beta}_{X_j} + \varepsilon_j, \quad \varepsilon_j \sim N(0, \mathrm{SE}(\hat{\beta}_{Y_j})^2)$$

Nonetheless, the MR-Egger is sensitive to violations of the InSIDE assumption, which is essentially implausible. The precision of Egger estimates is also prone to influential points and the variance between the genetic associations with the exposure. We calculated the I_{GX}^2 statistic to evaluate the magnitude of the violation of the "no measurement error" (NOME) assumption for MR-Egger [43]. An I_{GX}^2 below 0.9 may suggest the presence of regression dilution bias, making the estimation less valuable. Another modeling approach was MR using robust adjusted profile score (MR-RAPS). It accounts for the measurement error in SNP-exposure effects and is robust to systematic and idiosyncratic pleiotropy [44]. We used Turkey's loss function to enhance robustness against outliers. The pleiotropic effects are assumed to be normally distributed around zero with an unknown variance, independent of the genetic associations with the exposure. Under the assumption, the MR-RAPS estimates would be unbiased even

when numerous weak instruments exist.

The Mendelian randomization pleiotropy residual sum and outlier (MR-PRESSO) method was used with 10,000 bootstrap replications to identify and remove outliers based on the contribution of genetic variants to the variability in the causal estimates. MR-PRESSO is efficient with valid IVs and can be divided into three major parts [45]:

1. Global test: Compare the observed residual sum of squares (RSS_{obs}) of all genetic variants to the IVW regression with the expected residual sum of squares (RSS_{exp}) under normal distribution and the null hypothesis of no horizontal pleiotropy to assess overall pleiotropic effects.

2. Outlier test: Make a comparison between the RSS_{obs} and RSS_{exp} for each genetic variant to identify specific outliers that may indicate horizontal pleiotropy.

3. Distortion test: Remove outliers and test the significance of the difference in causal estimates before and after model correction.

We also performed leave-one-out analyses to detect outlier genetic variants. Each SNP was excluded from the combination of IVs one by one to determine if the results

were biased on account of a specific variant. Additionally, Cochran's Q for IVW and Rücker's Q' for MR-Egger served as measures of heterogeneity [46]. Low heterogeneity implies increased credibility of the MR estimates. We carefully interpret the causal effects estimated from generalized linear models in view of the non-collapsibility of odds ratio (OR). Accordingly, we prioritized the strength of evidence and the directionality of causation over the absolute size of estimates [47, 48]. For the association between cannabis use and AD, a two-tailed test with a p-value less than 0.05 was considered to indicate a statistical significance. When comparing causal relationships between cannabis use and other FinnGen anxiety subtypes (i.e., GAD, SP, AG, and PD), we applied the Bonferroni correction with a significance level set at 0.0125 (0.05 divided by 4). Data were analyzed using the TwoSampleMR (version 0.5.11), MR-PRESSO (version 1.0), and mr.raps (version 0.2) packages in the R environment (version 4.3.2).

Figure 2. Study flowchart

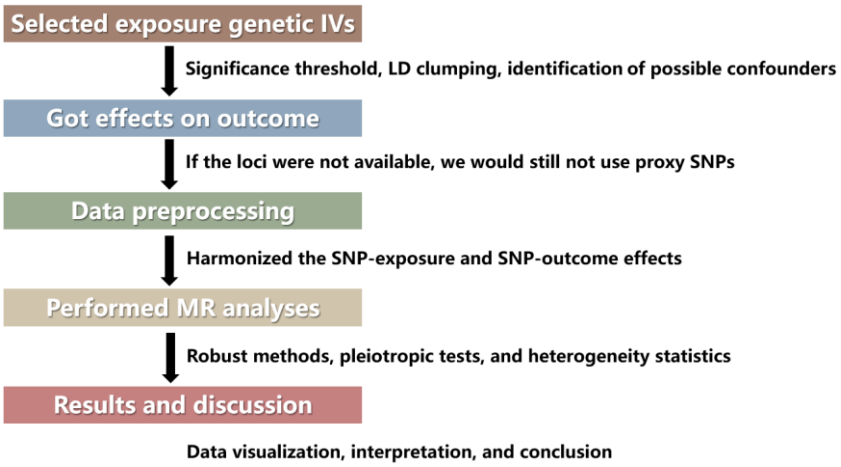


Table 2. Key analytical steps and choices of genetic instrumental variables

	LCU	CUD	AD
Original variants	11,534,876	8,851,634	21,304,791
Significant loci (p-value threshold)	954 (5×10^{-7})	106 (5×10^{-7})	557 (5×10^{-8})
After LD clumping	12	12	13
After searching for possible confounders (SNP removed)	11 (rs1154693)	11 (rs11783093)	13
After extracting outcome data (SNPs not available)	9 (rs353253, rs9435794)	11	11 (rs145274568, rs1480567, rs145281382)
After harmonization (non-inferable SNP)	8 (rs9578502)	10 (rs17514242)	9 (rs6874581, rs7981741)
Candidate IVs	rs9919557 rs17761723 rs10883796 rs1368740 rs1816793 rs4099556 rs75448266 rs9972422	rs7783012 rs11715758 rs1392816 rs1509513 rs17271123 rs553920 rs72818514 rs9787909 rs719012 rs719504	rs10092618 rs1480567 rs2071044 rs2397672 rs4619804 rs4955420 rs215890 rs77683334 rs7502307
GAD	**SP**	**AG**	**PD**
21,305,513	21,305,431	21,305,391	21,305,471
175 (5×10^{-6})	76 (5×10^{-6})	170 (5×10^{-6})	294 (5×10^{-6})
13	12	7	21
13	12	7	21

Palindromic SNPs include rs12031222 for GAD and rs2201195 for PD.

For the other information, please check out Table S4 to Table S7 in the Appendices.

Chapter 3 Results

3.1 Causality between Lifetime Cannabis Use and Anxiety Disorders

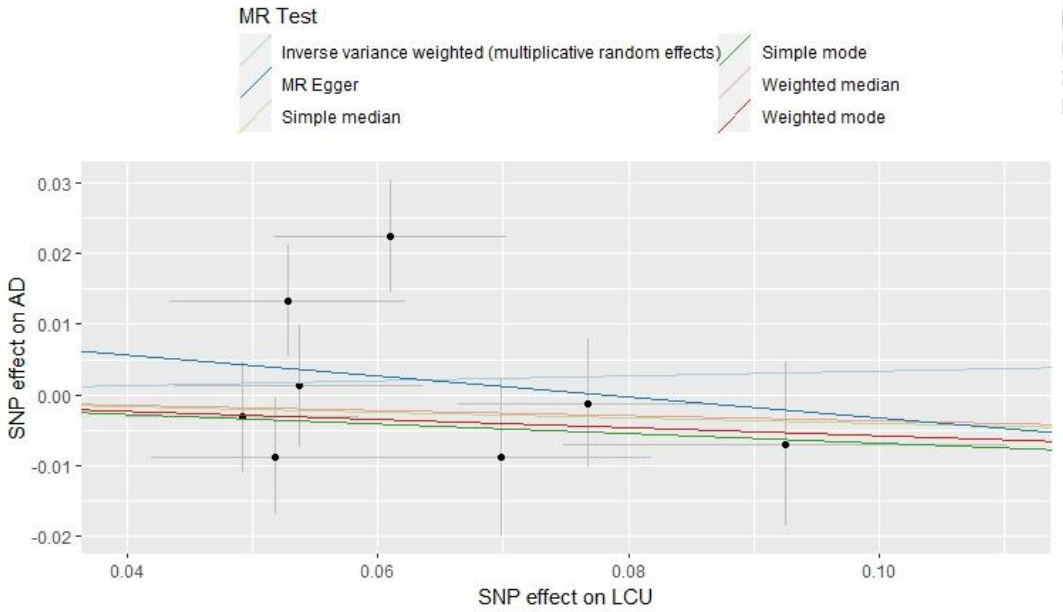
We did not find evidence of a causal effect of the genetic liability to LCU on AD (Table 3; Figure 3-1 to Figure 3-4). In the reverse analysis, there was weak evidence suggesting that the genetic predisposition to AD may causally influence LCU (OR_{IVW} = 1.166, 95% CI: 1.007-1.351, p-value < 0.05; $OR_{MR-RAPS}$ = 1.171, 95% CI: 1.012-1.356, p-value < 0.05; Table 3). Statistical tests of MR-Egger intercept, Cochran's Q, and Rücker's Q' indicated that the results were less likely to be affected by horizontal pleiotropy or heterogeneity, and the causation may not be driven by a single variant as excluding any SNP did not induce drastic changes in the causal estimates (Figure 3-8). In addition, MR-PRESSO could not provide evidence of outliers (global test p-value > 0.05). On the other hand, genetic variants from other datasets did not provide sufficient evidence to assess the bidirectional causal links between LCU and other anxiety traits (Table S8-1, Table S9 to Table S11; Figure S1-1 to Figure S4-8).

Table 3. MR analysis for the causality between LCU and AD

	LCU → AD		AD → LCU	
Method	**SNPs**	**OR [95% CI] (p-value)**	**SNPs**	**OR [95% CI] (p-value)**
IVW	8	1.035 [0.907-1.181] (0.611)	9	**1.166 [1.007-1.351] (0.040)**
Simple median		0.961 [0.841-1.099] (0.583)		1.071 [0.867-1.324] (0.524)
Weighted median		0.964 [0.842-1.103] (0.603)		1.151 [0.945-1.401] (0.162)
Simple mode		0.934 [0.769-1.134] (0.521)		1.168 [0.887-1.536] (0.301)
Weighted mode		0.944 [0.778-1.147] (0.556)		1.172 [0.894-1.536] (0.284)
MR-Egger		0.862 [0.433-1.717] (0.688)		1.666 [0.756-3.668] (0.246)
MR-RAPS		1.049 [0.780-1.410] (0.753)		**1.171 [1.012-1.356] (0.034)**
Egger regression intercept pleiotropy test p-value		0.614		0.394
Cochran's Q (heterogeneity test p-value)		12.563 (0.084)		8.657 (0.372)
Rücker's Q' (heterogeneity test p-value)		11.999 (0.062)		7.757 (0.355)
MR-PRESSO global test p-value		0.096		0.082

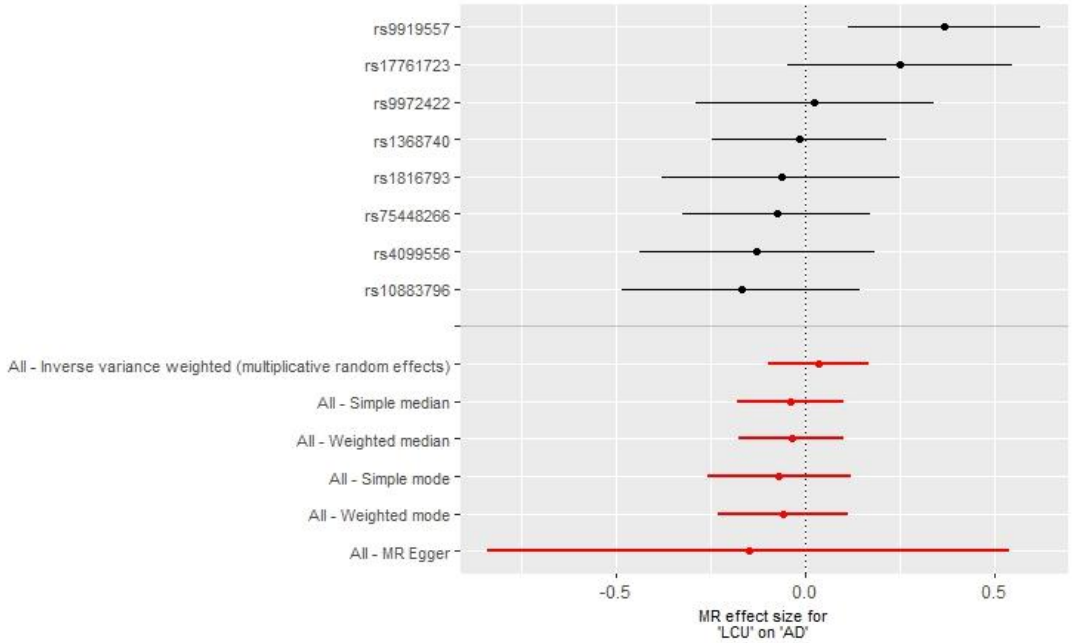
The table displays MR estimates for each method, representing the causal effects of the exposure on the outcome.

Figure 3-1. Scatter plot for LCU as the exposure and AD as the outcome



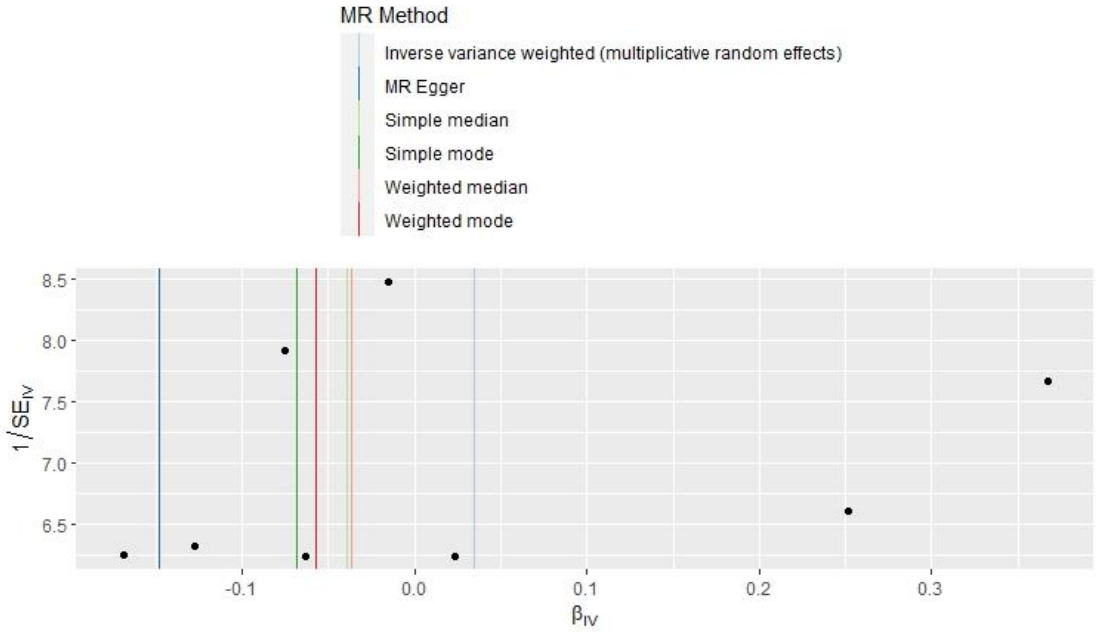
SNP effects on the outcome are plotted against SNP effects on the exposure. All SNPs with negative effects on the exposure are shown to be positive, with the sign of the effect on the outcome flipped. The slope of the line represents the causal association, and each method has a different line.

Figure 3-2. Forest plot for LCU as the exposure and AD as the outcome



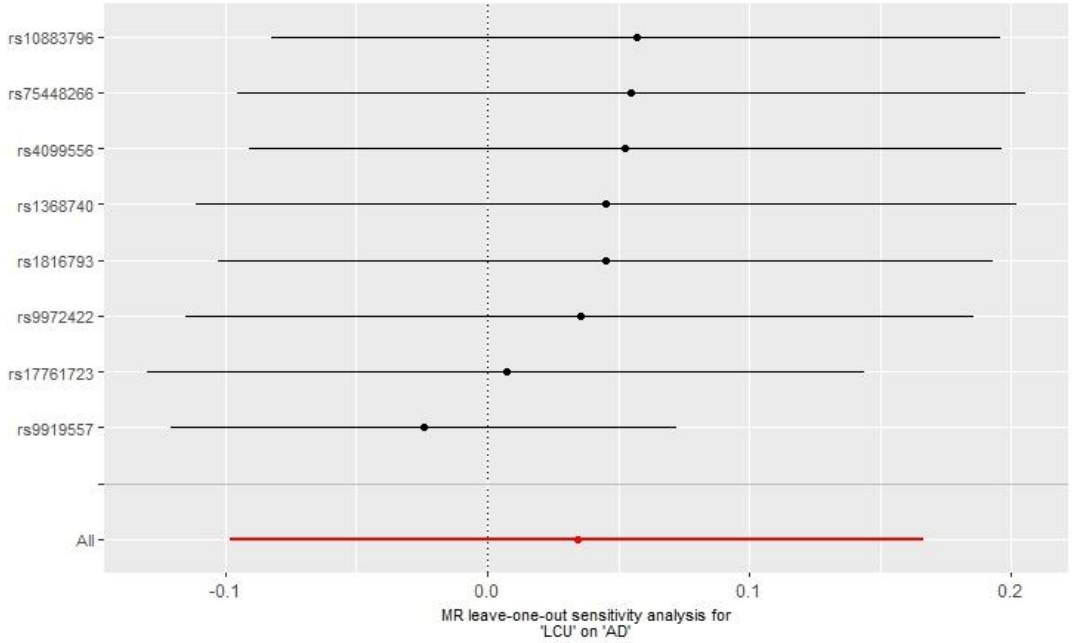
The causal effect of exposure on the outcome is estimated individually for each SNP using the Wald ratio and is depicted in a forest plot. The MR estimates using all SNPs with various methods are also presented.

Figure 3-3. Funnel plot for LCU as the exposure and AD as the outcome



Heterogeneity is assessed using the funnel plot. Less precise estimates (lower values on the y-axis) converge as they become more precise. A larger spread demonstrates greater heterogeneity, which may stem from horizontal pleiotropy. Asymmetry in the funnel plot indicates directional pleiotropy, which can bias a variety of MR methods. Results from the pleiotropy test in the Egger regression can also guard against this.

Figure 3-4. Leave-one-out plot for LCU as the exposure and AD as the outcome



Leave-one-out sensitivity analysis is performed to ascertain if an association is disproportionately

influenced by a single SNP. Each black point in the plot represents the MR analysis (using IVW) excluding that particular SNP. The overall analysis including all SNPs is also displayed for comparison.

Figure 3-5. Scatter plot for AD as the exposure and LCU as the outcome

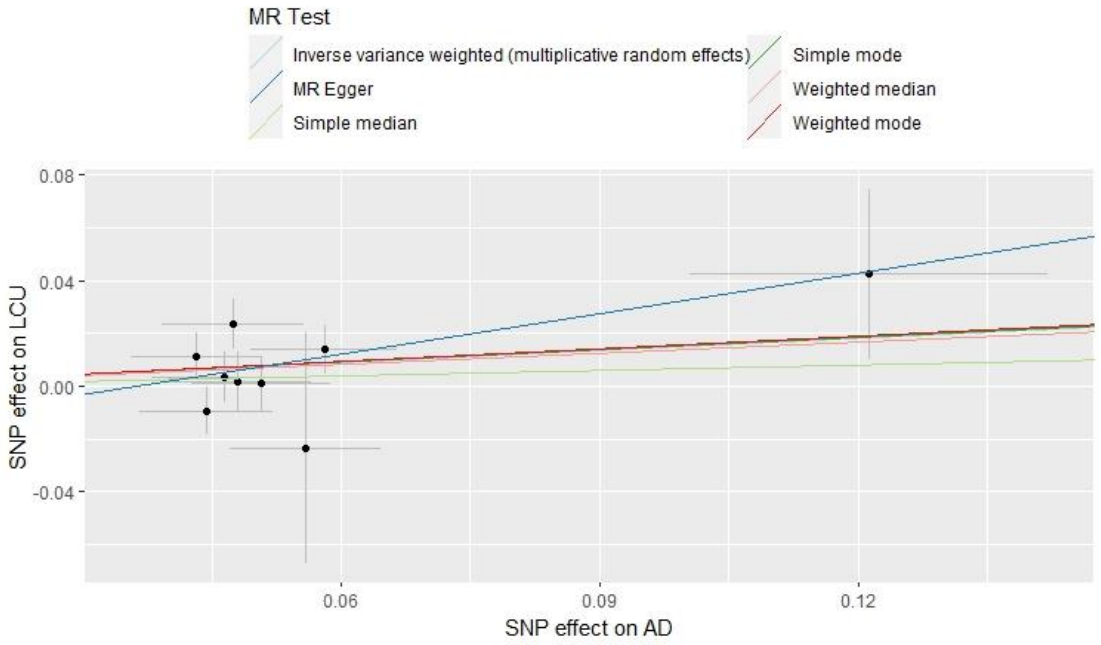


Figure 3-6. Forest plot for AD as the exposure and LCU as the outcome

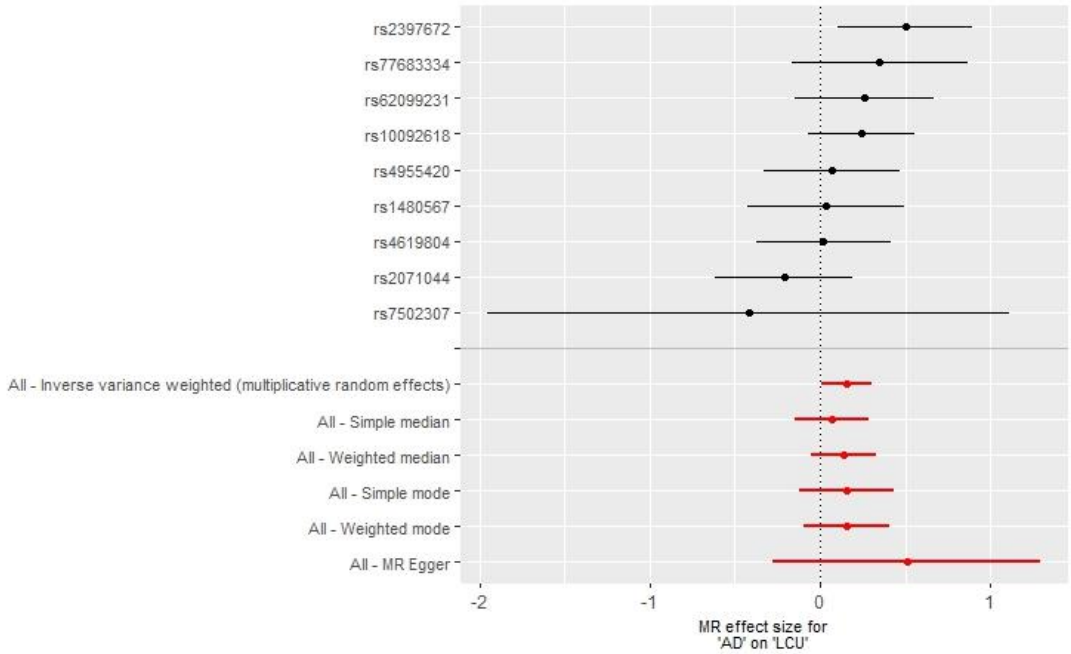


Figure 3-7. Funnel plot for AD as the exposure and LCU as the outcome

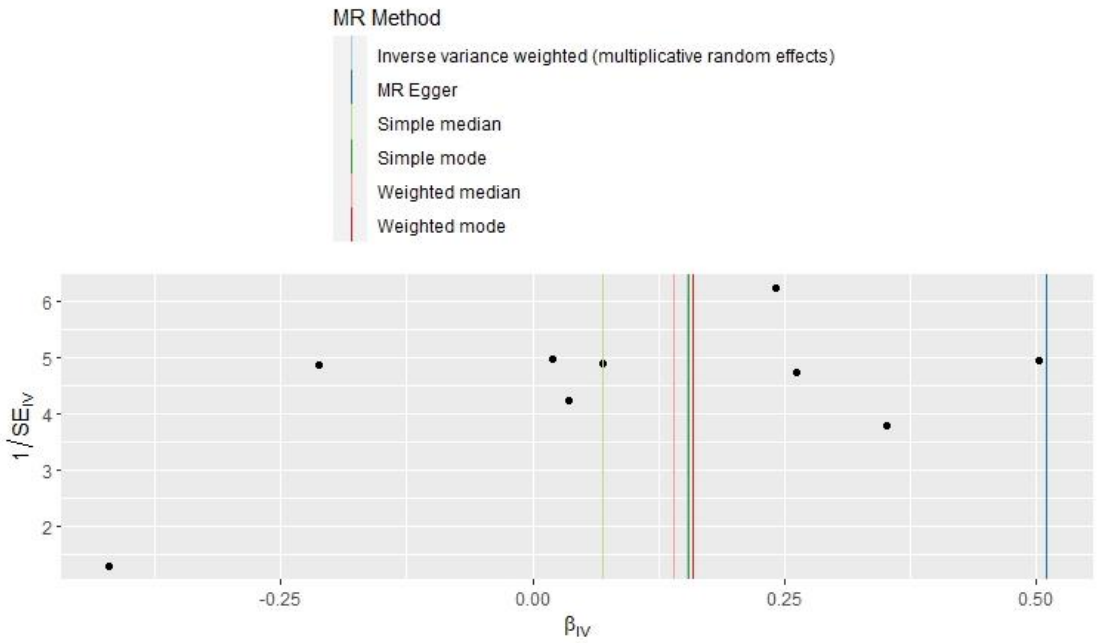
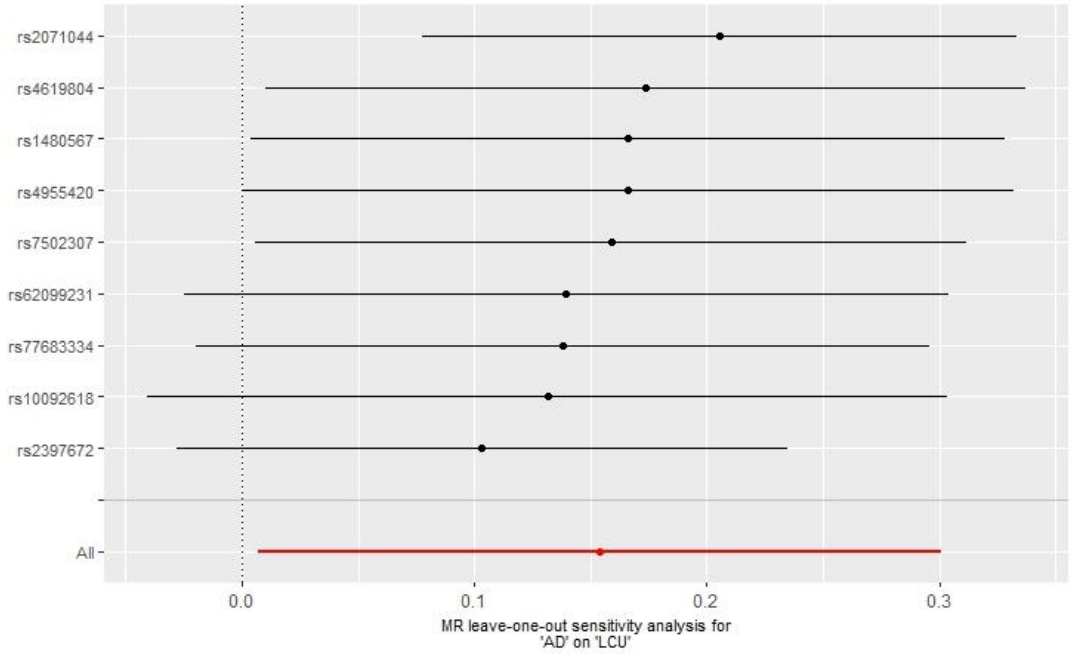


Figure 3-8. Leave-one-out plot for AD as the exposure and LCU as the outcome



3.2 Causality between Cannabis Use Disorder and Anxiety Disorders

The IVW, simple median, and weighted median methods suggested that genetic vulnerability to CUD may increase the risk of AD (Table 4). The Rücker's Q' test (p-value < 0.05) and funnel plot (Figure 4-3) implied heterogeneity in the causal effects, but the MR-Egger intercept did not significantly differ from zero (p-value > 0.05), thus failing to confirm the presence of directional pleiotropy. Leave-one-out analysis demonstrated that no specific SNP drove this causal connection (Figure 4-4), and MR-PRESSO could not provide additional evidence of outliers (global test p-value > 0.05). For the causal associations between CUD and other anxiety subtypes, we did not find supporting evidence except for the IVW and MR-RAPS estimates for CUD on SP (OR_{IVW} = 1.290, 95% CI: 1.062-1.567, p-value < 0.0125; $OR_{MR-RAPS}$ = 1.301, 95% CI: 1.078-1.570, p-value < 0.0125; Table S9; Figure S6-1 to Figure S6-4). There were no statistically significant causal effects identified in the reverse analysis (Table S8-1 to Table S10; Figure S5-1 to Figure S8-8), other than the MR-RAPS estimate for AD on CUD (Table 4).

Table 4. MR analysis for the causality between CUD and AD

	CUD → AD		AD → CUD	
Method	**SNPs**	**OR [95% CI] (p-value)**	**SNPs**	**OR [95% CI] (p-value)**
IVW	10	**1.088 [1.018-1.163] (0.013)**	9	1.356 [0.961-1.912] (0.083)
Simple median		**1.086 [1.009-1.170] (0.029)**		1.268 [0.870-1.848] (0.216)
Weighted median		**1.085 [1.007-1.168] (0.033)**		1.258 [0.865-1.830] (0.229)
Simple mode		1.073 [0.961-1.199] (0.241)		1.589 [0.853-2.959] (0.183)
Weighted mode		1.070 [0.968-1.184] (0.218)		1.494 [0.861-2.593] (0.191)
MR-Egger		1.090 [0.707-1.682] (0.706)		0.394 [0.063-2.456] (0.352)
MR-RAPS		**1.093 [1.036-1.152] (0.001)**		**1.375 [1.060-1.783] (0.017)**
Egger regression intercept pleiotropy test p-value		0.992		0.220
Cochran's Q (heterogeneity test p-value)		15.730 (0.073)		14.690 (0.065)
Rücker's Q' (heterogeneity test p-value)		**15.730 (0.046)**		11.672 (0.112)
MR-PRESSO global test p-value		0.089		0.077

Figure 4-1. Scatter plot for CUD as the exposure and AD as the outcome

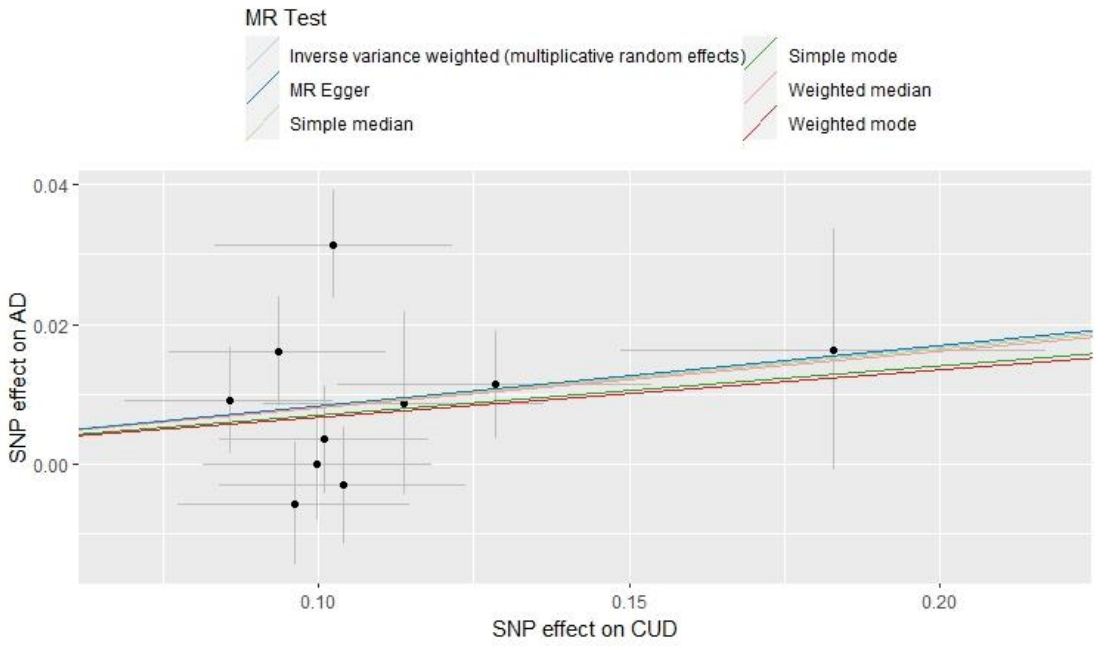


Figure 4-2. Forest plot for CUD as the exposure and AD as the outcome

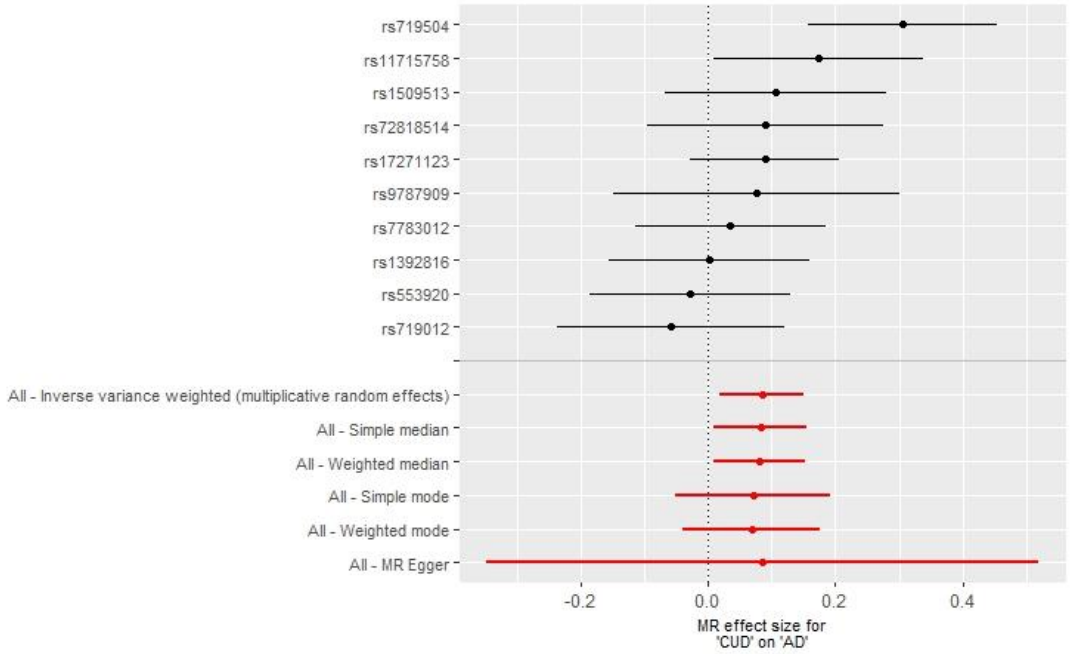


Figure 4-3. Funnel plot for CUD as the exposure and AD as the outcome

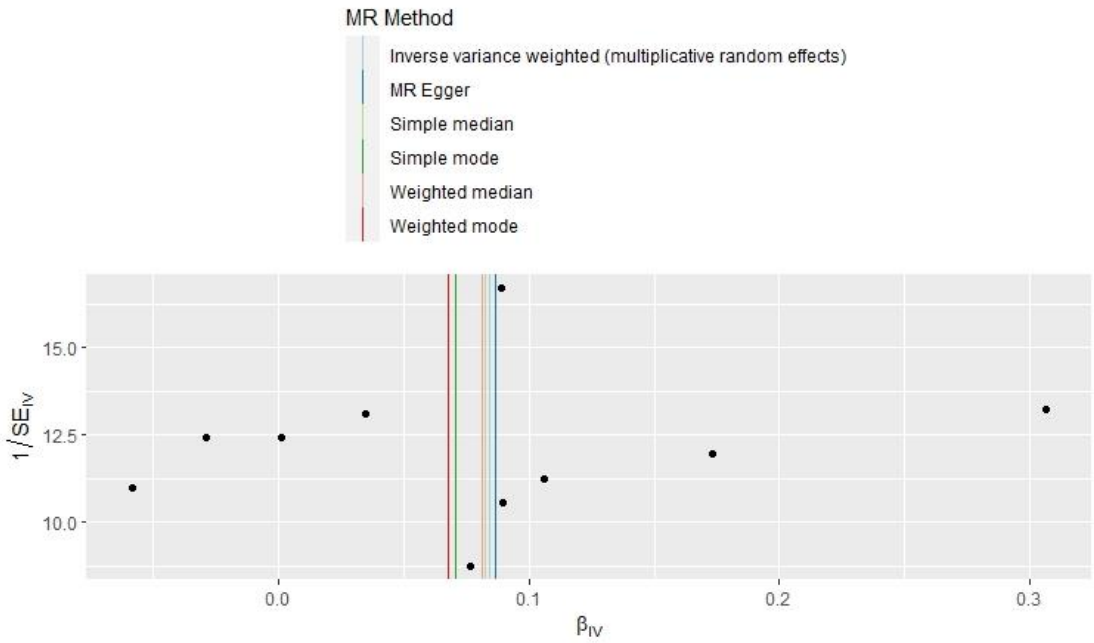


Figure 4-4. Leave-one-out plot for CUD as the exposure and AD as the outcome

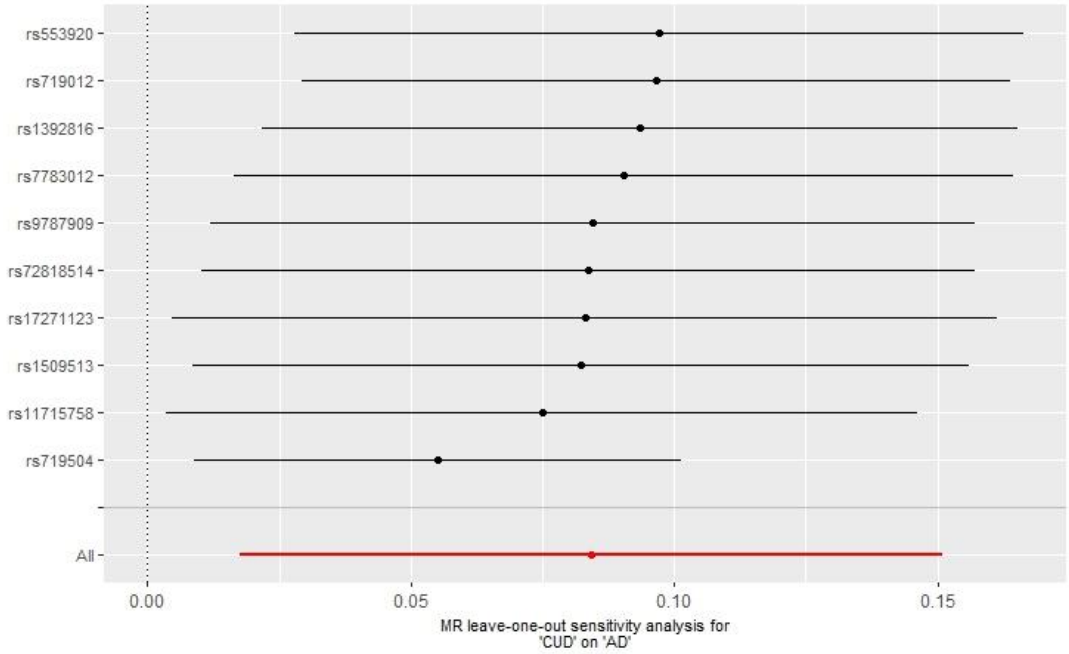


Figure 4-5. Scatter plot for AD as the exposure and CUD as the outcome

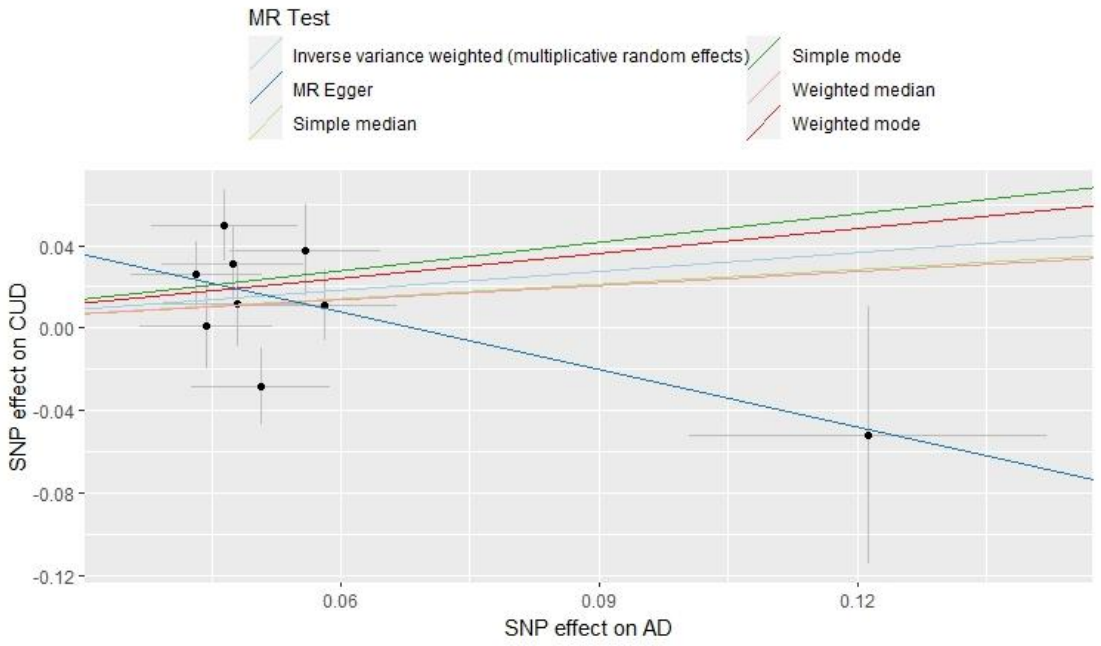


Figure 4-6. Forest plot for AD as the exposure and CUD as the outcome

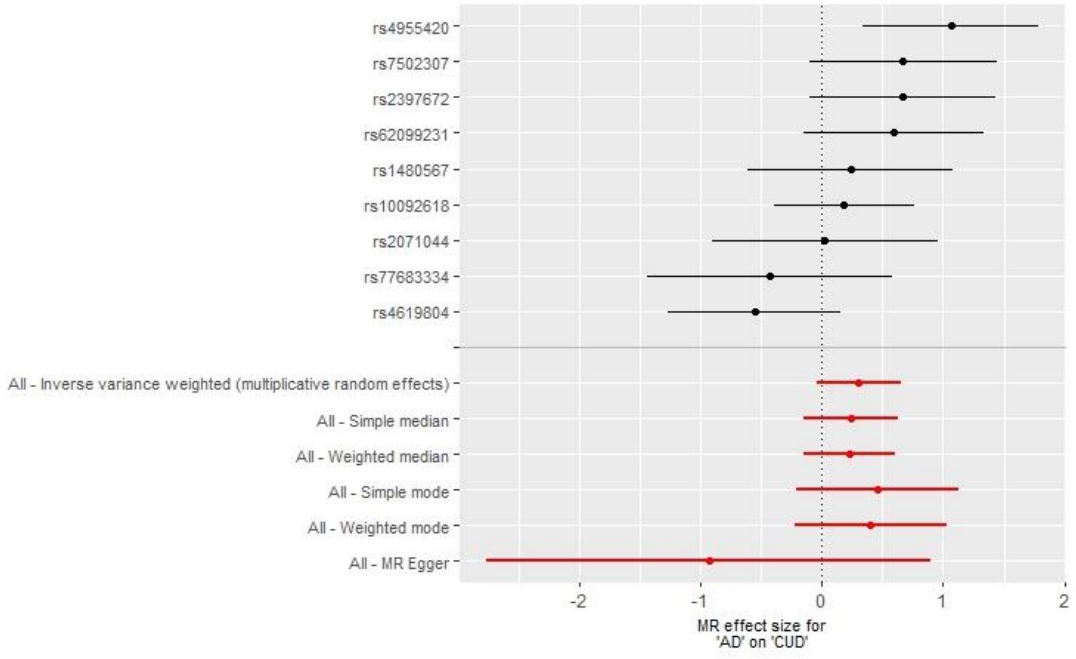


Figure 4-7. Funnel plot for AD as the exposure and CUD as the outcome

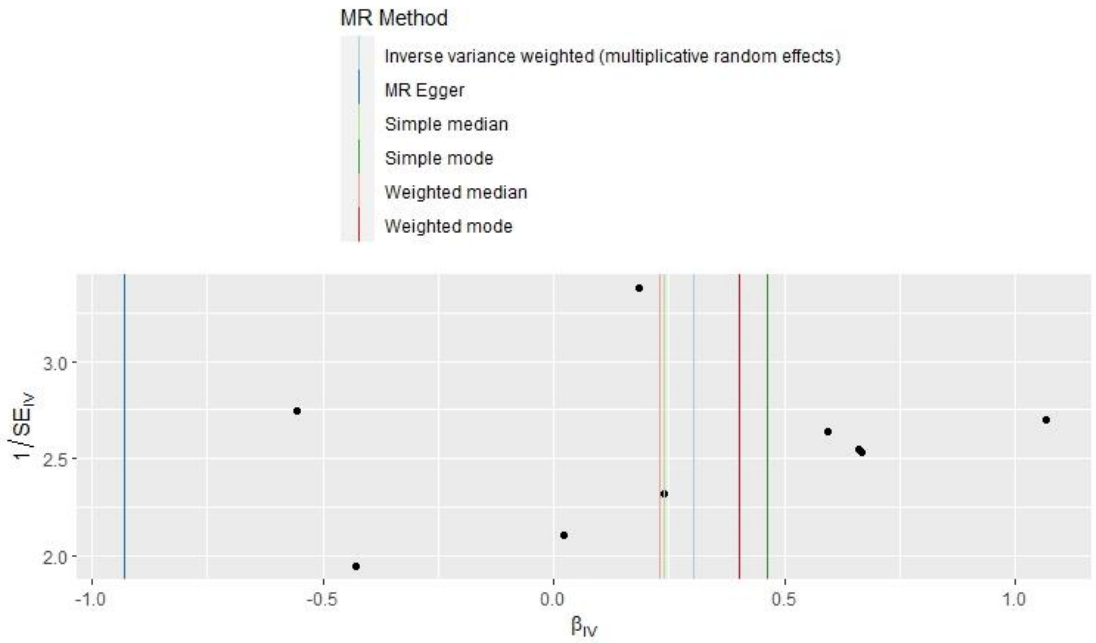
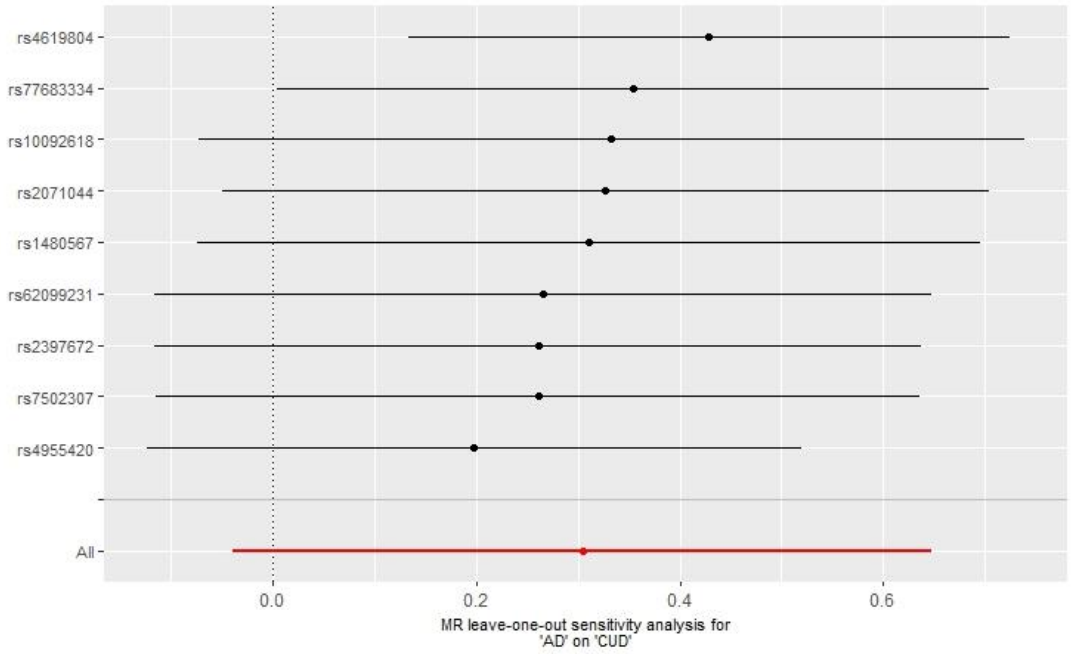


Figure 4-8. Leave-one-out plot for AD as the exposure and CUD as the outcome



Chapter 4 Discussion

To sum up, statistically significant positive causal effects were observed in several tests: CUD on AD (OR_{IVW} = 1.088, 95% CI: 1.018-1.163, p-value = 0.013), CUD on SP (OR_{IVW} = 1.290, 95% CI: 1.062-1.567, p-value = 0.010), and AD on LCU (OR_{IVW} = 1.166, 95% CI: 1.007-1.351, p-value = 0.040). These findings imply that cannabis use disorder may be a causal risk factor for anxiety disorders (social phobias in particular), while the use of cannabis may be a consequence of anxiety disorders, supporting the self-medication theory [22].

4.1 Literature Review

Cannabis use may have short- and long-term effects on anxiety [49]. In the current versions of ICD and DSM, anxiety is one of the diagnostic criteria for acute cannabis intoxication and cannabis withdrawal [14, 15]. A meta-analysis of 31 studies in the general population revealed a positive association between anxiety and LCU (OR = 1.24, 95% CI: 1.06-1.45) and CUD (OR = 1.68, 95% CI: 1.23-2.31) [50]. This may suggest that anxiety is a risk factor for LCU or CUD. We should note that anxiety does not necessarily lead to cannabis initiation, nor does it result in more frequent use of cannabis or CUD, and vice versa if we claim that LCU or CUD are the causes of anxiety

[22]. Five longitudinal studies from the above systematic review found a positive correlation between cannabis use at baseline and anxiety at follow-up after adjusting for possible confounders such as sociodemographic factors, prior anxiety disorder diagnosis, alcohol and tobacco use, and psychiatric comorbidity at age 15 (OR = 1.28, 95% CI: 1.06-1.54). While cannabis use may be associated with an increased likelihood of anxiety (OR = 1.25, 95% CI: 1.01-1.54) [51], another meta-analytical study has yielded a weaker effect size (OR = 1.15, 95% CI: 1.03-1.29), which decreased and did not reach significance when the analysis was restricted to high-quality studies, adjusted for publication bias, or dichotomized (i.e., cannabis use/non-use and anxiety diagnosis), suggesting that cannabis use may only be a minor risk factor for the development of elevated anxiety symptoms in the general population [52]. Cannabis use and anxiety are more prevalent among individuals in adolescence and early adulthood. There is also a small, statistically significant positive correlation between cannabis problems and SP evidenced by a meta-analysis ($r = 0.197$, p-value < 0.001) [53]. Other studies targeted at this age group have also estimated the associations but have reported mixed results [54-61].

Anxiety disorders have been found to co-occur with LCU and CUD [4].

Prospective cohort studies based on the U.S. National Epidemiologic Survey on Alcohol and Related Conditions have shown that past-year cannabis use or frequency of cannabis use at baseline was not significantly associated with the incidence of GAD (OR = 1.0, 95% CI: 0.7-1.4), SP (OR = 1.2, 95% CI: 0.8-1.8), or PD (OR = 0.8, 95% CI: 0.8-1.2) at the second wave of the survey after adjusting for covariates, with age appearing to moderate the relationship [62, 63]. CUD among younger adults and daily (or almost daily) cannabis use among older adults at baseline were associated with a higher incidence of SP three years later (adjusted OR = 2.45, 95% CI: 1.19-5.06; adjusted OR = 2.83, 95% CI: 1.26-6.35) [63]. Regular cannabis use (at least once a week) may specifically predict the onset of PD with SP (OR = 1.89, 95% CI = 1.54-2.32) and AG (OR = 1.56, 95% CI = 1.11-2.19) [64]. From research based on different cohorts, cannabis use in combination with tobacco and alcohol intake may increase the risk of GAD [65, 66], and a meta-analysis of nationally representative samples confirmed the high co-occurrence of CUD with GAD and major depressive disorder [67]. A longitudinal study in the Netherlands found bidirectional mediation among cannabis use, anxiety/depressive symptoms, and psychotic experiences [68]. Still, the causal role of cannabis use or anxiety remains unclear [69-71]. It depends on how the researchers conceptualized and operationalized the phenomenon, how they quantified

and qualified the variables, as well as how they analyzed and interpreted the data.

Preclinical studies have proposed molecular targets of CBD and epigenetic components [72]. Small-scale parallel and crossover RCTs have shown that the use of CBD by patients with social phobia is associated with a greater improvement in the anxiety factor of the visual analogue mood scales [73]. Limited literature suggests that synthetic cannabinoids such as dronabinol, nabilone, and nabiximols may have short-term benefits for anxiety states reported by patients with psychoneurotic anxiety, chronic pain, or multiple sclerosis compared to a placebo [74]. Anxiety is a common reason for self-reported cannabis use [75], but it has also been listed as an approved indication for medical cannabis in some places [76]. In spite of this, an RCT in the United States found that patients who immediately held a medical marijuana card and were primarily concerned about anxiety or depressive symptoms did not experience significant improvement in their conditions. Instead, a higher incidence rate and severity of CUD were observed [77]. A secondary analysis of another RCT indicated that reducing cannabis use among patients with CUD may be linked to improvements in anxiety, depression, and sleep quality [78]. There is still no substantial high-quality international research to prove that cannabis preparations are beneficial for treating

anxiety. The concentration and potency of cannabinoids, as well as their biological functions in mood regulation, stress response, and reward circuitry in the nervous and endocrine systems, may explain some of the bidirectional and biphasic properties of cannabis use and anxiety. Epidemiological studies have pointed out that anxiogenic effects are mainly attributed to THC (especially at higher doses) [79], whereas CBD may be associated with anxiolytic effects [80]. The endocannabinoid system (ECS) regulates the release of neurotransmitters like dopamine, γ-aminobutyric acid, glutamate, serotonin, and norepinephrine [4]. ECS also participates in the activation and inhibition of the hypothalamic-pituitary-adrenal axis [81], which may explain some of the bidirectional and biphasic relationships between cannabis use and anxiety.

In addition to shared neurobiological and social-environmental factors, there may also be an overlapping genetic basis between cannabis use and anxiety, which can be further modified by epigenetic inheritance. Estimated heritability from twin studies suggests that LCU is about 40% to 50%, whereas CUD is about 50% to 70% [82]. Anxiety disorders are determined by genetic factors to about 20% to 60% in general [83], but the heritability of different types of anxiety disorders varies, with GAD being about 35%, and SP, AG, or PD being around 50% [18]. Aside from LCU and CUD,

genetic variants associated with the age of first cannabis use [84] and cannabis dependence [85, 86] have also been identified. For European ancestry samples with different anxiety diagnostic criteria, the Anxiety Neurogenetics Study (ANGST) [87] and the iPSYCH [88] each reported only one genome-wide significant locus, while the Million Veteran Program [89] and the UKB [83] each reported five genome-wide significant loci.

The gene expression of C*ADM2* may play a fundamental role in LCU, and the risk variant was found to be associated with reduced anxiety and neuroticism, increased risk-taking behavior, and a carefree personality [29]. The locus rs6462203 was identified from the iPSYCH GWAS of anxiety and stress-related disorders, and its nearest gene region *SDK1* is also where the LCU genetic hit (rs10085617) was mapped [29, 88]. According to a phenome-wide association study using the BioVU Biobank, the polygenic risk score (PRS) for CUD was positively correlated with anxiety disorders at significant levels, but the effect was attenuated when the analysis was conditioned on smoking initiation, and there was a null result after adjusting for tobacco use disorder [30]. Using MVP data primarily based on GAD-2 scores to perform LD score regression, a positive genetic correlation between anxiety disorders and cannabis

initiation or CUD was revealed [90]. Additionally, the polymorphism of the serotonin transporter-linked promoter region (5-HTTLPR) gene may moderate the association between adolescent cannabis use and anxiety [59]. Whether the catechol-O-methyltransferase (COMT) Val158Met polymorphism is involved in the gene-environment interactions necessitates additional investigations [91]. In brief, underlying mechanisms may contribute to the genetic pleiotropy on the causal pathway between cannabis use and anxiety. In-depth "post-GWAS" analyses such as genomic structural equation modeling, latent causal variables, and functional enrichment analysis, can also shed light on the sophisticated genetic architecture of cannabis use and anxiety [92].

4.2 Strengths and Limitations

To the best of our knowledge, this study may be the first MR investigation to delve into causal links between cannabis use and anxiety. We harnessed human genome informatics to address confounding or reverse causation in conventional epidemiological studies and tried to overcome the obstacles of clinical trials by employing large sample sizes, robust statistical methods, and concise data visualization. Breakthroughs in biotechnology (e.g., DNA microarray and next-generation sequencing), statistical genetics, and open-access resources, have made it easier, faster,

and more transparent to conduct or replicate two-sample MR studies. Even though the approach has been put in practice to infer cause-and-effect relationships between cannabis use and a wide range of health topics, there is a gap in the literature regarding the causal nexus between cannabis use and anxiety, which our study aims to bridge.

There is no single right way to carry out MR research. Reflection on our study helps in making causal assumptions and promoting methodological innovations carefully. First of all, existing GWAS of cannabis use or anxiety have identified a relatively small number of significant loci, explaining only a small proportion of the phenotypic variance. We set a lenient significance threshold to select more exposure variants (except for AD). There were no F-statistics less than 10, but the statistical power to detect causal effects may still be suboptimal, resulting in non-significant estimates. Care should also be taken regarding the "winner's curse" [93], where the strongest novel loci discovered by a single GWAS from the FinnGen may overestimate SNP-exposure associations. In our analysis, genetic variants for cannabis use and anxiety were sourced from separate datasets that included only individuals of European ancestry, reducing the chance of population stratification and sample overlap, but the findings may not be applicable to other ethnicities. Owing to the lack of individual-level

raw data, we were unable to merge multiple instrumental variables into a single allele score using PRS or to perform subgroup analyses stratified by age, gender, and other general factors.

Secondly, the operational definitions of the exposure and the outcome may vary among different descriptive or analytical studies. The GWAS measured cannabis use and anxiety phenotypes as binary variables, but the dichotomization per se may oversimplify the continuous distribution of risks in reality. For example, information on non-pathological anxiety of varying severity, as well as the onset time or progression of several anxiety disorders, could not be captured. The scenarios of "having used cannabis only once in the past" and "using cannabis frequently so far" cannot be distinguished by the self-reported LCU questionnaire, but the former may be expected to have a negligible direct effect on anxiety disorders. Apart from the frequency of cannabis use, other precise details were also unknown, including the timing (e.g., age at first cannabis use, duration of cannabis use), content (e.g., dose and potency of cannabinoids, THC/CBD ratio), type (e.g., raw cannabis, cannabis-based products, purified cannabinoids with marketing authorization), route of administration (e.g., smoke inhalation, digestion of edibles, transdermal delivery), pattern (e.g., concurrent

use of other substances), and purpose (e.g., medical, non-medical use), which are particularly important when looking for temporality, biological gradient (dose-response relationships), specificity, and other clues for logical reasoning [94]. The genetic IVs in this study can be viewed as indirect indicators of behaviors or mental states, rather than protein, metabolite, and other molecular biomarkers that may have a more specific biomedical meaning. Moreover, the legality of cannabis, as well as people's expectations regarding substance use and psychosocial disability, may also influence the completeness and correctness of recall or reporting, introducing information bias. Given these restrictions, future directions for advanced MR studies will involve diversifying databases (e.g., meta-analytical and multi-ancestry GWAS) and optimizing statistical models and intelligent computing (e.g., using high-throughput algorithms and machine learning skills).

In many epidemiological contexts, causal claims are not always deterministic but rather stochastic or probabilistic. For instance, participants in the anxiety GWAS may have a genetic susceptibility to cannabis use but have never been exposed to cannabis or developed CUD. Similarly, individuals in the cannabis use GWAS may carry risk alleles for anxiety but have not reported any forms of anxiety or been diagnosed with

anxiety disorders. Under certain assumptions, the MR estimates indicate the average causal effects of the genetic predisposition to lifelong exposure on the outcome among our study participants and may not be equivalent to the actual causal effects of interventions in the general population. We must bear in mind that not merely genetic underpinning but also the environment can play a crucial role. Cannabis use and anxiety are inextricably intertwined with physical, mental, social, and spiritual factors, and the interactions between them may vary throughout a person's life course. The absence of adequate genetic evidence in our study does not completely negate the causality between these traits.

Last but not least, the pitfalls of horizontal pleiotropy could not be completely ruled out, although we have introduced a series of robust statistical methods and SNP lookup tools. Extensions to basic MR paradigms such as multivariable MR [95], network MR [96], factorial MR [97], nonlinear MR, or meta-analysis of MR, are potential avenues for further exploration and refinement of our approach. Other situations that do not adhere to the assumption of random distribution of genetic variation in a population include assortative mating (e.g., cannabis users may select partners who also use cannabis) and dynastic effects (e.g., parents who use cannabis

may influence their children's cannabis use habits), which can be examined using sibling or within-family study designs [98]. Most importantly, real-world evidence from systems biology, multiomics, and social science should be synthesized and triangulated to gain insights into the causality between cannabis use and anxiety [99].

Chapter 5 Conclusion

While there is statistical genetic evidence suggestive of positive causal relationships between cannabis use and anxiety, the nature of causality should be cross-validated in well-designed observational and experimental studies. This will help maintain vigilance over potential threats of cannabis and encourage the development of regulatory frameworks and scientific applications. Cannabis use and anxiety are contemporary health issues that require collaborative endeavors across disciplines, sectors, and borders. Based on our findings, we recommend prioritizing research, education, and services for individuals with cannabis use disorder to prevent anxiety disorders, and for persons with anxiety to avoid cannabis consumption. Besides Mendelian randomization, various causal models and health data analytics are also an integral part of precision health. A deeper comprehension of disease etiology and natural history will lay the groundwork for preventive medicine strategies to mitigate the adverse mental health and social consequences of cannabis use.

Chapter 6 Additional Information

6.1 Data Availability

The GWAS summary statistics are available for download at the following links:

1. Lifetime cannabis use: https://www.ru.nl/bsi/research/group-pages/substance-use-addiction-food-saf/vm-saf/genetics/international-cannabis-consortium-icc/

2. Cannabis use disorder: https://www.med.unc.edu/pgc/download-results/

3. Anxiety disorders: https://finngen.gitbook.io/documentation/data-download/

The R software can be installed from https://cran.r-project.org/. For information about the TwoSampleMR, MR-PRESSO, and mr.raps packages, please refer to https://github.com/MRCIEU/TwoSampleMR, https://github.com/rondolab/MR-PRESSO, and https://github.com/qingyuanzhao/mr.raps.

6.2 Ethics Declarations

We conducted secondary data analysis using publicly available, de-identified GWAS summary statistics. All the data were obtained from studies that received informed consent from participants and passed research ethics reviews. Throughout the

study, we adhered to academic integrity and the ethical principles outlined in the World Medical Association's Declaration of Taipei regarding health databases, big data, and biobanks [100]. No potential conflict of interest relevant to this article was disclosed.

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Chapter 8 Appendices

Table S1. Information on LCU genetic variants

LCU → AD (or GAD, SP, AG, PD) Significance threshold: $p < 5\times10^{-7}$, $I^2_{GX} = 0.304$					
SNP	**EA** / **OA** **EAF**	β_X **[SE(β_X)]** **(p-value)**	**N**	**R^2**	**F**
rs9919557	T / C 0.613	-0.061 [0.009] (6.534×10^{-11})	162,082	2.653×10^{-4}	43.022
rs17761723	T / C 0.345	0.053 [0.009] (2.153×10^{-8})	162,082	1.946×10^{-4}	31.551
rs10883796	A / G 0.294	0.052 [0.010] (1.248×10^{-7})	162,082	1.723×10^{-4}	27.938
rs1368740	A / G 0.751	-0.077 [0.010] (1.338×10^{-13})	162,082	3.363×10^{-4}	54.532
rs1816793	T / C 0.369	0.049 [0.009] (1.248×10^{-7})	162,082	1.726×10^{-4}	27.987
rs4099556	A / G 0.824	0.070 [0.012] (5.908×10^{-9})	162,082	2.093×10^{-4}	33.930
rs75448266	A / C 0.914	-0.093 [0.018] (1.637×10^{-7})	162,082	1.685×10^{-4}	27.311
rs9972422	A / G 0.710	-0.054 [0.010] (6.153×10^{-8})	162,082	1.815×10^{-4}	29.422

SNP = single nucleotide polymorphism, EA = effect allele, OA = other allele, EAF = effect allele frequency, β_X = point estimate of SNP-exposure effect size, SE(β_X) = standard error of β_X, N = sample size of the exposure dataset. rs9919557 in *NCAM1* on chromosome 11 and rs17761723 in *SMG6* on chromosome 17 are the lead SNPs reported in the original GWAS by Pasman et al.

Table S2. Information on CUD genetic variants

CUD → AD (or GAD, SP, AG, PD) Significance threshold: $p < 5\times10^{-7}$, $I^2_{GX} = 0$					
SNP	**EA** / **OA** **EAF**	**β_X [SE(β_X)]** **(p-value)**	**N**	**R^2**	**F**
rs7783012	A / G N/A	0.101 [0.017] (1.835×10^{-9})	355,548	1.012×10^{-4}	36.143
rs11715758	A / G N/A	-0.094 [0.018] (8.911×10^{-8})	355,885	8.021×10^{-5}	28.546
rs1392816	T / C N/A	-0.100 [0.018] (6.140×10^{-8})	351,174	8.377×10^{-5}	29.419
rs1509513	A / G N/A	0.086 [0.017] (3.179×10^{-7})	356,900	7.291×10^{-5}	26.022
rs17271123	T / G N/A	0.128 [0.025] (3.543×10^{-7})	291,017	8.920×10^{-5}	25.961
rs553920	T / C N/A	0.104 [0.020] (1.597×10^{-7})	353,969	7.794×10^{-5}	27.589
rs72818514	T / C N/A	-0.183 [0.034] (9.331×10^{-8})	355548	8.035×10^{-5}	28.569
rs9787909	A / C N/A	0.114 [0.023] (4.522×10^{-7})	355548	7.204×10^{-5}	25.536
rs719012	T / C N/A	0.096 [0.019] (2.866×10^{-7})	356,928	7.399×10^{-5}	26.410
rs719504	A / G N/A	0.103 [0.019] (8.996×10^{-8})	350,403	8.133×10^{-5}	28.500

SNP = single nucleotide polymorphism, EA = effect allele, OA = other allele, EAF = effect allele frequency, β_X = point estimate of SNP-exposure effect size, SE(β_X) = standard error of β_X, N = sample size of the exposure dataset. EAF is not provided in the original GWAS summary statistics. rs77830123 in *FOXP2* on chromosome 7 is the lead SNP reported in the original GWAS by Johnson et al.

Table S3. Information on AD genetic variants

AD → LCU (or CUD) Significance threshold: $p < 5\times10^{-8}$, $I^2_{GX} = 0.459$					
SNP	**EA** / **OA** **EAF**	β_X **[SE(β_X)]** **(p-value)**	**N**	**R^2**	**F**
rs10092618	A / G 0.709	0.058 [0.008] (8.074×10^{-12})	346,542	1.348×10^{-4}	46.748
rs1480567	C / T 0.262	0.048 [0.009] (2.458×10^{-8})	346,542	8.972×10^{-5}	31.094
rs2071044	T / C 0.476	-0.044 [0.008] (6.690×10^{-9})	346,542	9.701×10^{-5}	33.623
rs2397672	A / G 0.689	0.047 [0.008] (9.507×10^{-9})	346,542	9.504×10^{-5}	32.939
rs4619804	C / A 0.661	0.051 [0.008] (3.367×10^{-10})	346,542	1.138×10^{-4}	39.449
rs4955420	C / T 0.724	-0.046 [0.009] (4.578×10^{-8})	346,542	8.624×10^{-5}	29.888
rs215890	A / G 0.459	0.043 [0.008] (1.564×10^{-8})	346,542	9.225×10^{-5}	31.972
rs7502307	G / C 0.252	0.056 [0.009] (1.670×10^{-10})	346,542	1.178×10^{-4}	40.819
rs77683334	T / C 0.037	-0.121 [0.021] (5.058×10^{-9})	346,542	9.858×10^{-5}	34.167

SNP = single nucleotide polymorphism, EA = effect allele, OA = other allele, EAF = effect allele frequency, β_X = point estimate of SNP-exposure effect size, SE(β_X) = standard error of β_X, N = sample size of the exposure dataset.

Table S4. Information on GAD genetic variants

GAD → LCU Significance threshold: $p < 5\times10^{-6}$, $I^2_{GX} = 0.513$					
SNP	**EA** / **OA** **EAF**	β_X [SE(β_X)] **(p-value)**	**N**	**R^2**	**F**
rs10850270	C / T 0.532	0.099 [0.020] (7.274×10^{-7})	373,334	6.602×10^{-5}	24.541
rs1186396	A / G 0.403	0.093 [0.020] (3.519×10^{-6})	373,334	5.771×10^{-5}	21.510
rs12742734	G / T 0.502	-0.099 [0.020] (5.905×10^{-7})	373,334	6.656×10^{-5}	24.943
rs1947216	A / G 0.299	0.101 [0.022] (2.831×10^{-6})	373,334	5.876×10^{-5}	21.928
rs2191309	A / C 0.063	0.200 [0.039] (2.488×10^{-7})	373,334	7.125×10^{-5}	26.611
rs330794	G / A 0.786	0.132 [0.025] (9.761×10^{-8})	373,334	7.649×10^{-5}	28.421
rs7580625	A / G 0.190	0.117 [0.025] (2.041×10^{-6})	373,334	6.051×10^{-5}	22.556
rs79314110	T / C 0.030	0.249 [0.054] (4.833×10^{-6})	373,334	5.591×10^{-5}	20.902
rs79669173	T / C 0.055	-0.226 [0.046] (1.045×10^{-6})	373,334	6.399×10^{-5}	23.842
rs8076244	T / C 0.103	0.154 [0.032] (1.107×10^{-6})	373,334	6.377×10^{-5}	23.732
rs883299	G / A 0.600	0.098 [0.020] (1.831×10^{-6})	373,334	6.071×10^{-5}	22.765
rs890210	T / C 0.315	-0.103 [0.022] (1.737×10^{-6})	373,334	6.138×10^{-5}	22.866

SNP = single nucleotide polymorphism, EA = effect allele, OA = other allele, EAF = effect allele frequency, β_X = point estimate of SNP-exposure effect size, SE(β_X) = standard error of β_X, N = sample size of the exposure dataset. rs79669173 is not present when CUD is the outcome.

Table S5. Information on SP genetic variants

SP → LCU Significance threshold: $p < 5\times10^{-6}$, $I^2_{GX} = 0.764$					
SNP	**EA** / **OA** **EAF**	β_X [SE(β_X)] **(p-value)**	**N**	**R^2**	**F**
rs112089458	A / G 0.144	0.192 [0.037] (1.745×10^{-7})	370,878	7.360×10^{-5}	27.297
rs117999033	T / C 0.004	-1.800 [0.388] (3.447×10^{-6})	370,878	5.810×10^{-5}	21.550
rs11818271	G / A 0.077	-0.260 [0.055] (2.684×10^{-6})	370,878	5.940×10^{-5}	22.030
rs12495332	T / C 0.245	-0.150 [0.032] (3.132×10^{-6})	370,878	5.860×10^{-4}	21.734
rs12797711	T / C 0.189	0.156 [0.033] (3.105×10^{-6})	370,878	5.864×10^{-5}	21.751
rs149823833	G / A 0.031	-0.418 [0.088] (2.327×10^{-6})	370,878	6.014×10^{-5}	22.304
rs1844754	A / G 0.914	0.238 [0.051] (3.429×10^{-6})	370,878	5.813×10^{-5}	21.560
rs2523455	G / A 0.760	0.167 [0.032] (2.762×10^{-7})	370,878	7.120×10^{-5}	26.409
rs72740121	G / A 0.068	0.240 [0.051] (2.444×10^{-6})	370,878	5.988×10^{-5}	22.210
rs74648610	A / G 0.050	0.281 [0.057] (6.394×10^{-7})	370,878	6.684×10^{-5}	24.790
rs77610227	A / G 0.301	-0.139 [0.030] (4.563×10^{-6})	370,878	5.665×10^{-5}	21.013
rs80216038	T / C 0.016	-0.622 [0.131] (2.133×10^{-6})	370,878	6.059×10^{-5}	22.471

SNP = single nucleotide polymorphism, EA = effect allele, OA = other allele, EAF = effect allele frequency, β_X = point estimate of SNP-exposure effect size, SE(β_X) = standard error of β_X, N = sample size of the exposure dataset. rs117999033 and rs74648610 are not present when CUD is the outcome.

Table S6. Information on AG genetic variants

AG → LCU Significance threshold: $p < 5\times10^{-6}$, $I^2_{GX} = 0.679$					
SNP	**EA** / **OA** **EAF**	β_X **[SE(β_X)]** **(p-value)**	**N**	**R^2**	**F**
rs12620614	T / A 0.715	0.252 [0.050] (6.661×10^{-7})	369,057	6.695×10^{-5}	24.711
rs145959102	C / T 0.001	1.746 [0.372] (2.658×10^{-6})	369,057	5.974×10^{-5}	22.049
rs28397779	G / T 0.087	0.342 [0.073] (2.771×10^{-6})	369,057	5.952×10^{-5}	21.969
rs34686714	A / C 0.274	0.229 [0.049] (2.516×10^{-6})	369,057	6.003×10^{-4}	22.154
rs36764	T / G 0.189	0.256 [0.055] (2.752×10^{-6})	369,057	5.956×10^{-5}	21.982
rs6554147	G / T 0.468	0.239 [0.045] (1.139×10^{-7})	369,057	7.619×10^{-5}	28.122
rs7959861	G / A 0.355	0.213 [0.046] (3.557×10^{-6})	369,057	5.823×10^{-5}	21.490

SNP = single nucleotide polymorphism, EA = effect allele, OA = other allele, EAF = effect allele frequency, β_X = point estimate of SNP-exposure effect size, SE(β_X) = standard error of β_X, N = sample size of the exposure dataset. rs145959102 is not present when CUD is the outcome.

Table S7. Information on PD genetic variants

PD → LCU Significance threshold: $p < 5\times10^{-6}$, $I^2_{GX} = 0.662$					
SNP	**EA** / **OA** **EAF**	β_X [SE(β_X)] **(p-value)**	**N**	**R^2**	**F**
rs10958994	C / A 0.122	0.141 [0.029] (1.109×10^{-6})	373,422	6.330×10^{-5}	23.729
rs11927622	G / C 0.1340	-0.140 [0.030] (3.383×10^{-6})	373,422	5.832×10^{-5}	21.586
rs142145142	A / G 0.0810	0.169 [0.035] (1.060×10^{-6})	373,422	6.243×10^{-5}	23.817
rs150203643	G / A 0.054	-0.215 [0.046] (3.271×10^{-6})	373,422	5.850×10^{-5}	21.651
rs1873048	T / C 0.532	-0.100 [0.020] (3.484×10^{-7})	373,422	6.694×10^{-5}	25.961
rs2049634	C / T 0.832	0.130 [0.027] (1.507×10^{-6})	373,422	6.208×10^{-5}	23.140
rs532928	C / T 0.776	-0.120 [0.023] (1.978×10^{-7})	373,422	7.289×10^{-5}	27.055
rs6504568	T / C 0.733	-0.104 [0.022] (2.381×10^{-6})	373,422	5.984×10^{-5}	22.260
rs6550617	G / A 0.655	0.096 [0.021] (4.272×10^{-6})	373,422	5.596×10^{-5}	21.139
rs6557329	G / A 0.261	0.101 [0.022] (4.208×10^{-6})	373,422	5.644×10^{-5}	21.168
rs668375	C / T 0.107	-0.157 [0.033] (2.090×10^{-6})	373,422	6.061×10^{-5}	22.511
rs74107812	A / G 0.039	-0.260 [0.055] (2.058×10^{-6})	373,422	5.984×10^{-5}	22.540
rs74611085	A / G 0.030	-0.300 [0.063] (1.666×10^{-6})	373,422	6.072×10^{-5}	22.946
rs7696788	G / C 0.0087	-0.590 [0.128] (4.268×10^{-6})	373,422	5.689×10^{-5}	21.141
rs77027797	G / A 0.039	0.224 [0.048] (2.834×10^{-6})	373,422	5.832×10^{-5}	21.925

rs77094699	T	C	0.152 [0.033]	373,422	5.681×10^{-5}	20.854
	0.090		(4.957×10^{-6})			
rs9557771	C	T	0.091 [0.020]	373,422	5.544×10^{-5}	20.844
	0.090		(4.983×10^{-6})			

SNP = single nucleotide polymorphism, EA = effect allele, OA = other allele, EAF = effect allele frequency, β_X = point estimate of SNP-exposure effect size, SE(β_X) = standard error of β_X, N = sample size of the exposure dataset. When CUD is the outcome, rs11927622, rs142145142, and rs7696788 are not present, but rs181228863 can be found:

rs181228863	G	A	0.230 [0.047]	373,422	6.490×10^{-5}	24.267
	0.041		(8.386×10^{-7})			

Table S8-1. MR analysis for GAD as the variable of interest

	LCU → GAD		GAD → LCU	
Methods	**SNPs**	**OR [95% CI] (p-value)**	**SNPs**	**OR [95% CI] (p-value)**
IVW	8	0.981 [0.677-1.421] (0.918)	12	0.972 [0.917-1.031] (0.351)
Simple median		1.018 [0.716-1.446] (0.921)		0.990 [0.913-1.073] (0.807)
Weighted median		0.955 [0.656-1.392] (0.812)		0.963 [0.890-1.042] (0.343)
Simple mode		1.141 [0.640-2.034] (0.668)		0.976 [0.844-1.128] (0.750)
Weighted mode		0.932 [0.590-1.473] (0.772)		0.931 [0.825-1.049] (0.266)
MR-Egger		0.939 [0.130-6.800] (0.952)		0.923 [0.705-1.210] (0.576)
MR-RAPS		0.980 [0.753-1.274] (0.878)		0.971 [0.916-1.030] (0.328)
Egger regression intercept (pleiotropy test p-value)		0.967		0.709
Cochran's Q (heterogeneity test p-value)		**14.506 (0.043)**		12.038 (0.361)
Rücker's Q' (heterogeneity test p-value)		**14.502 (0.025)**		11.863 (0.294)
MR-PRESSO global test p-value		0.058		0.370
	CUD → GAD		**GAD → CUD**	
Methods	**SNPs**	**OR [95% CI] (p-value)**	**SNPs**	**OR [95% CI] (p-value)**
IVW	10	0.970 [0.860-1.093] (0.614)	11	1.073 [0.911-1.263] (0.399)
Simple median		0.995 [0.841-1.177] (0.955)		1.074 [0.909-1.270] (0.402)
Weighted median		0.980 [0.820-1.171] (0.821)		1.121 [0.949-1.324] (0.179)
Simple mode		1.011 [0.769-1.328] (0.941)		1.206 [0.957-1.519] (0.143)
Weighted mode		0.989 [0.784-1.248] (0.927)		1.178 [0.948-1.466] (0.171)
MR-Egger		1.228 [0.546-2.765] (0.633)		0.668 [0.297-1.503] (0.355)
MR-RAPS		0.969 [0.844-1.112] (0.653)		1.079 [0.968-1.203] (0.169)
Egger regression intercept pleiotropy test p-value		0.579		0.273
Cochran's Q (heterogeneity test p-value)		7.432 (0.592)		**23.677 (0.009)**
Rücker's Q' (heterogeneity test p-value)		7.097 (0.526)		**20.561 (0.015)**
MR-PRESSO global test p-value		0.614		**0.013**

In the MR analysis with GAD as the exposure and CUD as the outcome, the MR-PRESSO global test

shows statistical significance, and the outlier test further identifies rs2191309 as a potential outlier to be removed (p-value = 0.015). After excluding this SNP, the distortion test does not reveal any evidence of a significant difference compared to the original IVW estimate (p-value = 0.635).

Table S8-2. MR analysis for GAD as the exposure and CUD as the outcome

(After removing the outlier rs2191309 identified by MR-PRESSO)

GAD → CUD		
Method	**SNPs**	**OR [95% CI] (p-value)**
IVW	10	1.121 [0.976-1.288] (0.106)
Simple median		1.113 [0.956-1.297] (0.168)
Weighted median		1.138 [0.965-1.342] (0.123)
Simple mode		1.213 [0.953-1.544] (0.152)
Weighted mode		1.181 [0.944-1.477] (0.179)
MR-Egger		1.102 [0.453-2.679] (0.836)
MR-RAPS		1.129 [1.006-1.266] (0.038)
Egger regression intercept pleiotropy test p-value		0.970
Cochran's Q (heterogeneity test p-value)		14.309 (0.112)
Rücker's Q' (heterogeneity test p-value)		14.306 (0.074)
MR-PRESSO global test p-value		0.142

Table S9. MR analysis for SP as the variable of interest

	LCU → SP		SP → LCU	
Method	**SNPs**	**OR [95% CI] (p-value)**	**SNPs**	**OR [95% CI] (p-value)**
IVW	8	1.033 [0.798-1.339] (0.804)	12	1.005 [0.967-1.044] (0.815)
Simple median		1.068 [0.696-1.639] (0.764)		0.995 [0.943-1.051] (0.867)
Weighted median		1.097 [0.697-1.726] (0.690)		0.989 [0.943-1.037] (0.637)
Simple mode		1.298 [0.632-2.668] (0.501)		0.991 [0.933-1.052] (0.761)
Weighted mode		1.255 [0.629-2.503] (0.540)		0.987 [0.944-1.031] (0.561)
MR-Egger		1.815 [0.318-10.364] (0.527)		0.980 [0.925-1.038] (0.506)
MR-RAPS		1.034 [0.717-1.491] (0.859)		1.005 [0.970-1.040] (0.788)
Egger regression intercept pleiotropy test p-value		0.542		0.290
Cochran's Q (heterogeneity test p-value)		3.787 (0.804)		14.607 (0.201)
Rücker's Q' (heterogeneity test p-value)		3.368 (0.761)		12.986 (0.224)
MR-PRESSO global test p-value		0.821		0.260
	CUD → SP		**SP → CUD**	
Method	**SNPs**	**OR [95% CI] (p-value)**	**SNPs**	**OR [95% CI] (p-value)**
IVW	10	**1.290 [1.062-1.567] (0.010)**	10	0.997 [0.918-1.083] (0.942)
Simple median		1.219 [0.955-1.555] (0.112)		1.056 [0.942-1.183] (0.351)
Weighted median		1.290 [1.006-1.654] (0.044)		0.992 [0.886-1.110] (0.883)
Simple mode		1.190 [0.755-1.875] (0.474)		1.112 [0.905-1.365] (0.339)
Weighted mode		1.312 [0.869-1.980] (0.228)		0.938 [0.794-1.108] (0.470)
MR-Egger		3.609 [1.195-10.895] (0.052)		0.887 [0.732-1.075] (0.256)
MR-RAPS		**1.301 [1.078-1.570] (0.006)**		0.996 [0.918-1.083] (0.940)
Egger regression intercept pleiotropy test p-value		0.102		0.227
Cochran's Q (heterogeneity test p-value)		10.579 (0.306)		9.810 (0.366)
Rücker's Q' (heterogeneity test p-value)		7.160 (0.519)		8.078 (0.426)
MR-PRESSO global test p-value		0.325		0.365

Table S10. MR analysis for AG as the variable of interest

	LCU → AG		AG → LCU	
Method	**SNPs**	**OR [95% CI] (p-value)**	**SNPs**	**OR [95% CI] (p-value)**
IVW	8	0.648 [0.316-1.327] (0.235)	7	0.977 [0.953-1.002] (0.074)
Simple median		0.680 [0.311-1.490] (0.336)		0.976 [0.927-1.028] (0.365)
Weighted median		0.678 [0.300-1.536] (0.352)		0.985 [0.941-1.031] (0.517)
Simple mode		0.720 [0.203-2.549] (0.626)		0.985 [0.937-1.037] (0.590)
Weighted mode		0.704 [0.212-2.346] (0.586)		0.986 [0.941-1.033] (0.571)
MR-Egger		14.458 [0.802-260.689] (0.120)		0.983 [0.899-1.074] (0.716)
MR-RAPS		0.634 [0.347-1.156] (0.137)		0.977 [0.939-1.017] (0.252)
Egger regression intercept pleiotropy test p-value		0.075		0.897
Cochran's Q (heterogeneity test p-value)		10.520 (0.161)		2.809 (0.832)
Rücker's Q' (heterogeneity test p-value)		5.902 (0.434)		2.791 (0.732)
MR-PRESSO global test p-value		0.184		0.895
	CUD → AG		**AG → CUD**	
Method	**SNPs**	**OR [95% CI] (p-value)**	**SNPs**	**OR [95% CI] (p-value)**
IVW	10	1.296 [0.948-1.771] (0.105)	6	0.950 [0.880-1.025] (0.186)
Simple median		1.315 [0.879-1.968] (0.183)		0.961 [0.882-1.048] (0.369)
Weighted median		1.093 [0.737-1.620] (0.659)		0.983 [0.896-1.078] (0.709)
Simple mode		1.110 [0.579-2.129] (0.760)		1.000 [0.859-1.164] (0.999)
Weighted mode		0.956 [0.516-1.772] (0.890)		0.999 [0.872-1.144] (0.987)
MR-Egger		0.924 [0.123-6.940] (0.941)		0.921 [0.449-1.888] (0.833)
MR-RAPS		1.310 [0.958-1.792] (0.090)		0.948 [0.881-1.019] (0.147)
Egger regression intercept pleiotropy test p-value		0.748		0.936
Cochran's Q (heterogeneity test p-value)		9.867 (0.361)		6.182 (0.289)
Rücker's Q' (heterogeneity test p-value)		9.732 (0.284)		6.171 (0.187)
MR-PRESSO global test p-value		0.374		0.341

Table S11. MR analysis for PD as the variable of interest

	LCU → PD		PD → LCU	
Method	**SNPs**	**OR [95% CI] (p-value)**	**SNPs**	**OR [95% CI] (p-value)**
IVW	8	1.010 [0.775-1.315] (0.942)	17	1.026 [0.985-1.068] (0.213)
Simple median		1.044 [0.740-1.473] (0.807)		1.016 [0.948-1.088] (0.657)
Weighted median		1.059 [0.757-1.482] (0.737)		1.017 [0.954-1.085] (0.599)
Simple mode		1.063 [0.602-1.875] (0.840)		1.005 [0.909-1.110] (0.927)
Weighted mode		1.073 [0.618-1.863] (0.809)		1.010 [0.913-1.116] (0.851)
MR-Egger		1.649 [0.427-6.372] (0.495)		0.930 [0.826-1.047] (0.250)
MR-RAPS		1.010 [0.776-1.315] (0.940)		1.027 [0.590-1.788] (0.283)
Egger regression intercept pleiotropy test p-value		0.495		0.099
Cochran's Q (heterogeneity test p-value)		7.496 (0.379)		12.181 (0.731)
Rücker's Q' (heterogeneity test p-value)		6.890 (0.331)		9.084 (0.873)
MR-PRESSO global test p-value		0.392		0.708
	CUD → PD		**PD → CUD**	
Method	**SNPs**	**OR [95% CI] (p-value)**	**SNPs**	**OR [95% CI] (p-value)**
IVW	10	1.103 [0.916-1.328] (0.303)	15	1.074 [0.956-1.206] (0.230)
Simple median		0.967 [0.792-1.182] (0.746)		1.119 [0.974-1.285] (0.113)
Weighted median		0.968 [0.792-1.184] (0.753)		1.106 [0.972-1.259] (0.126)
Simple mode		0.921 [0.612-1.387] (0.703)		1.208 [0.960-1.519] (0.129)
Weighted mode		0.940 [0.651-1.357] (0.750)		1.174 [0.930-1.480] (0.198)
MR-Egger		1.287 [0.387-4.280] (0.691)		1.079 [0.763-1.526] (0.675)
MR-RAPS		1.110 [0.971-1.268] (0.127)		1.079 [0.983-1.185] (0.111)
Egger regression intercept pleiotropy test p-value		0.805		0.977
Cochran's Q (heterogeneity test p-value)		**18.199 (0.033)**		23.372 (0.054)
Rücker's Q' (heterogeneity test p-value)		**18.051 (0.021)**		**23.370 (0.037)**
MR-PRESSO global test p-value		**0.040**		0.062

In the MR analysis using CUD as the exposure and PD as the outcome, the MR-PRESSO global test

reach statistical significance, but the outlier test does not detect any outliers (p-value > 0.05).

Figure S1-1. Scatter plot for LCU as the exposure and GAD as the outcome

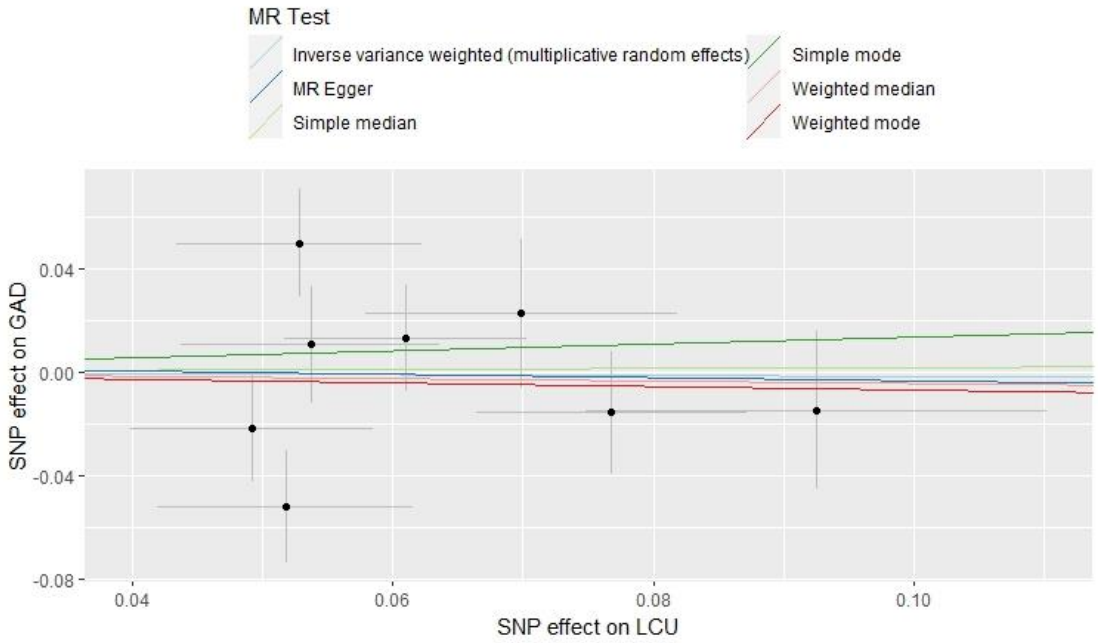

Figure S1-2. Forest plot for LCU as the exposure and GAD as the outcome

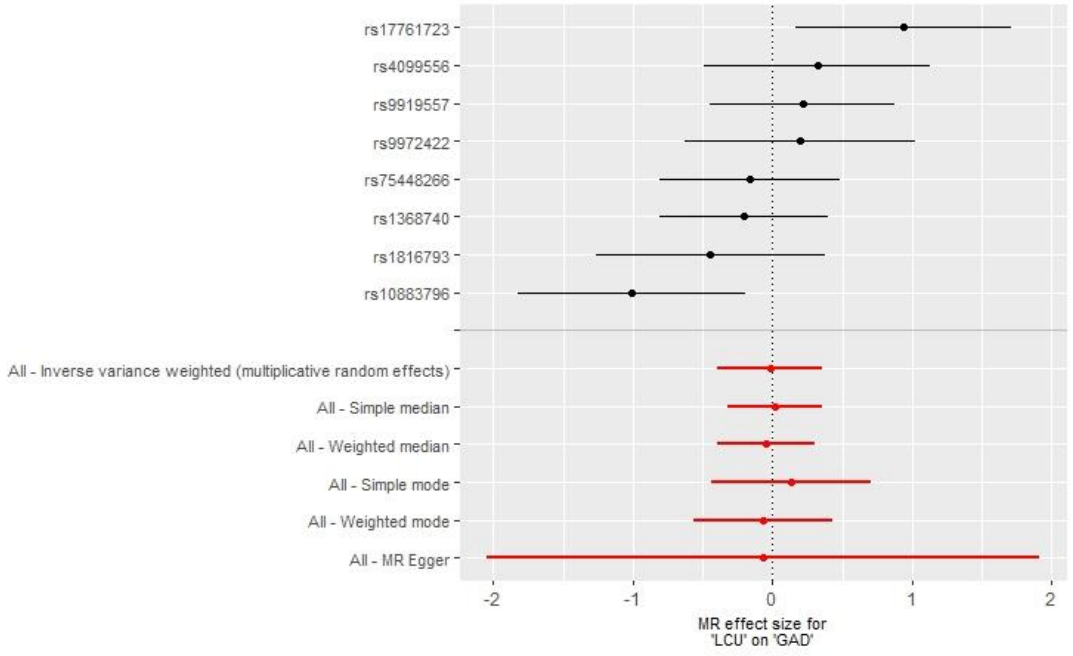

Figure S1-3. Funnel plot for LCU as the exposure and GAD as the outcome

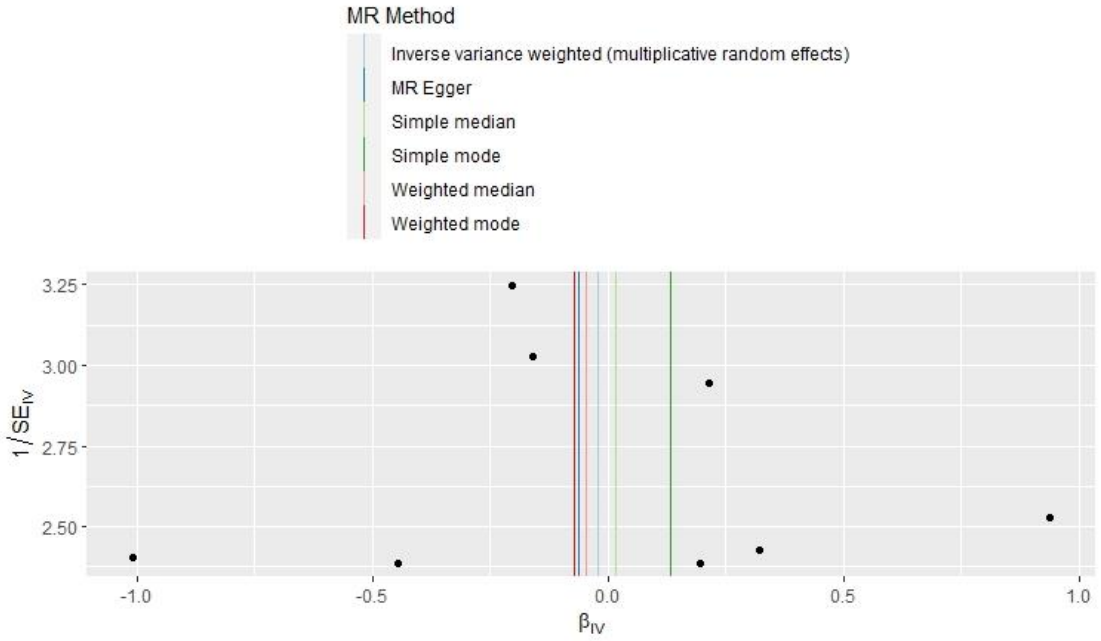

Figure S1-4. Leave-one-out plot for LCU as the exposure and GAD as the outcome

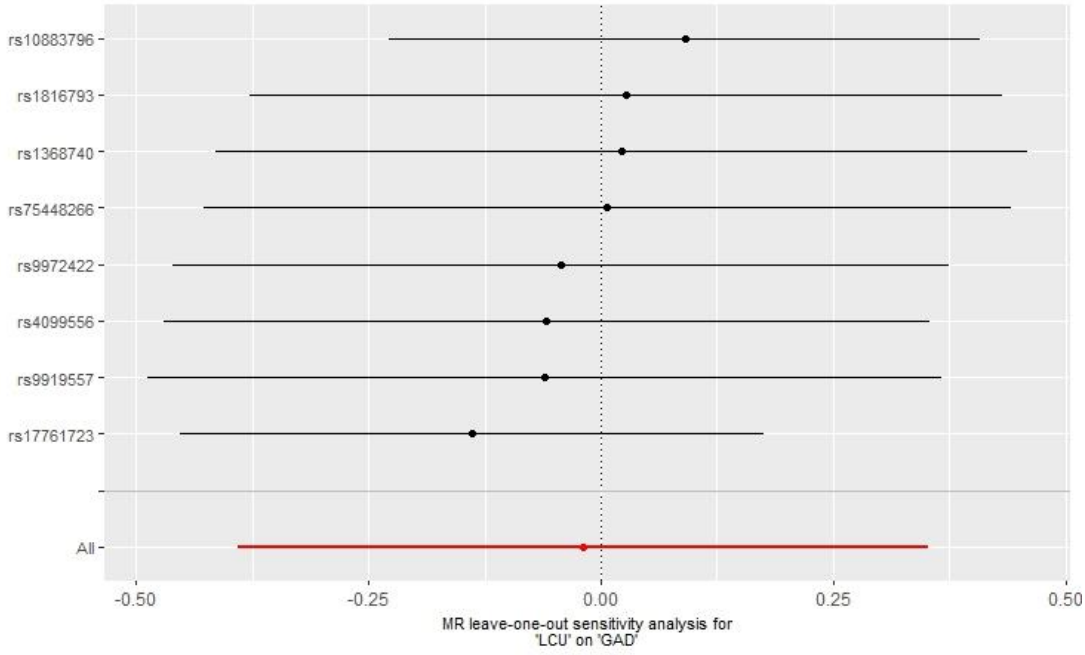

Figure S1-5. Scatter plot for GAD as the exposure and LCU as the outcome

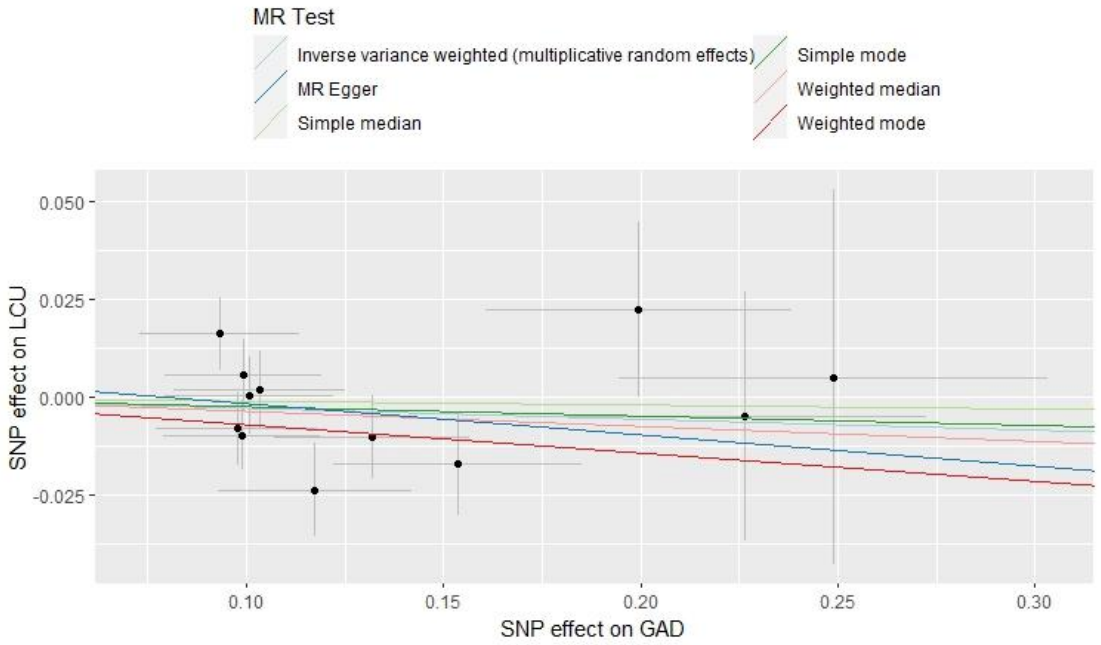

Figure S1-6. Forest plot for GAD as the exposure and LCU as the outcome

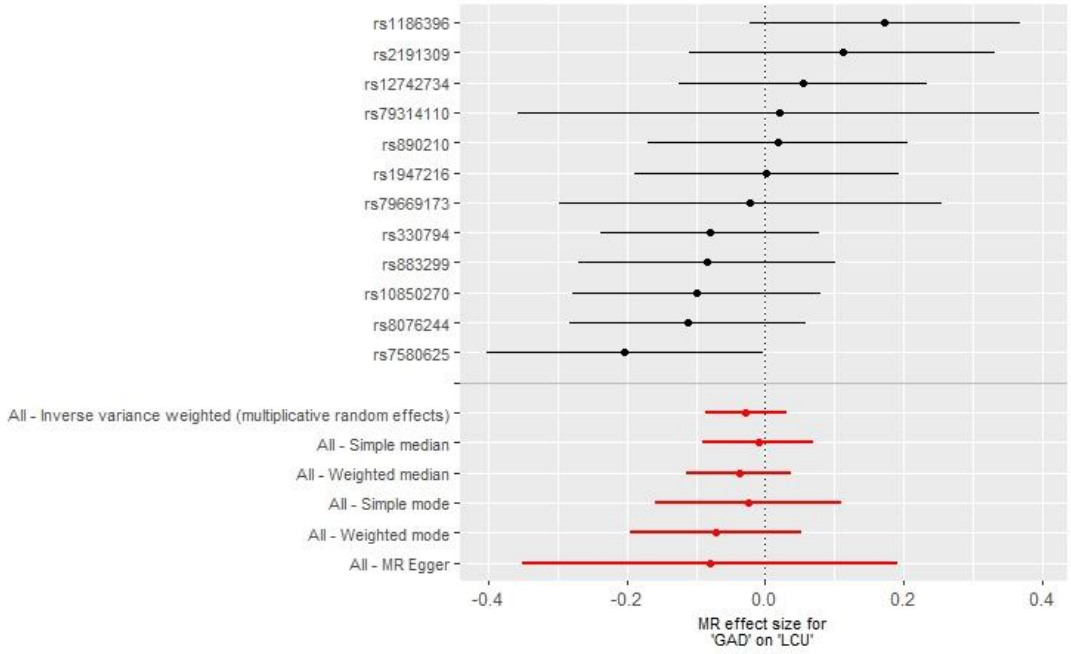

Figure S1-7. Funnel plot for GAD as the exposure and LCU as the outcome

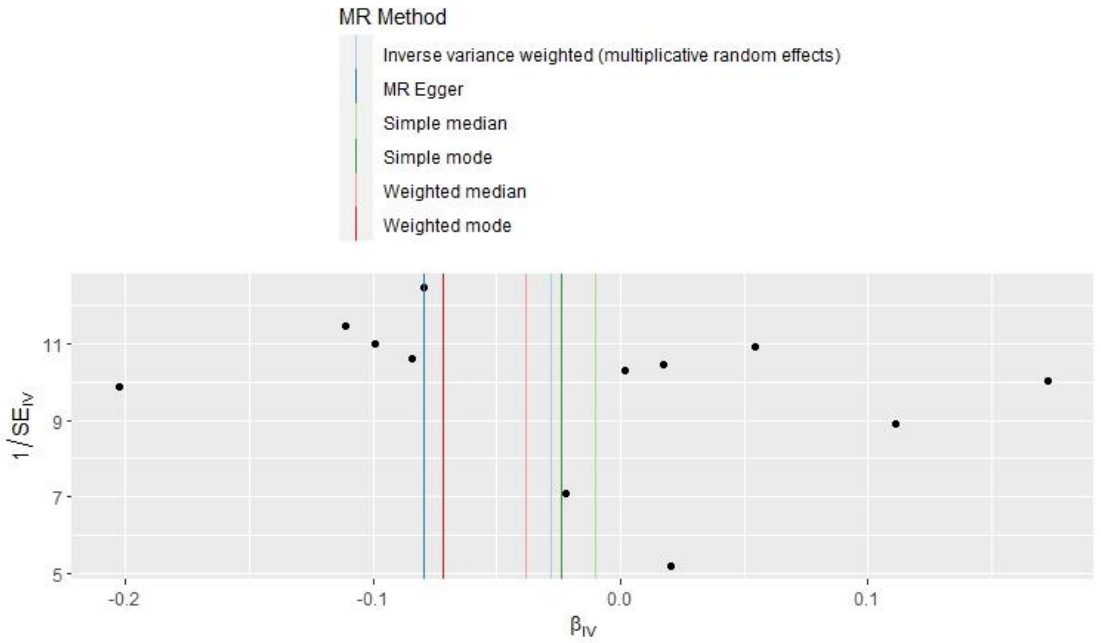

Figure S1-8. Leave-one-out plot for GAD as the exposure and LCU as the outcome

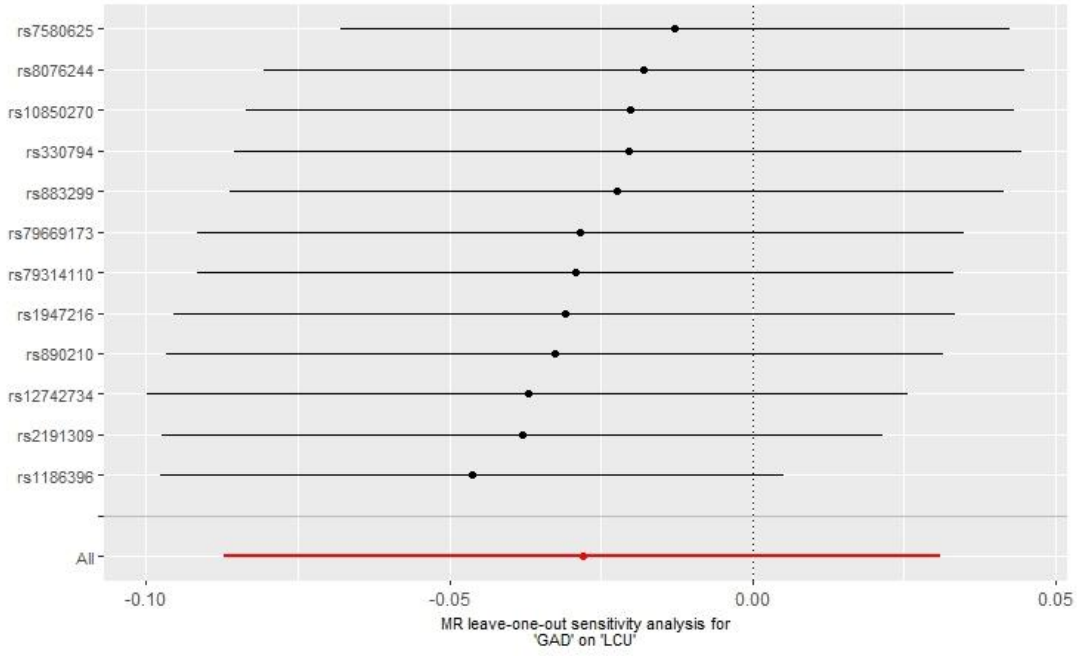

Figure S2-1. Scatter plot for LCU as the exposure and SP as the outcome

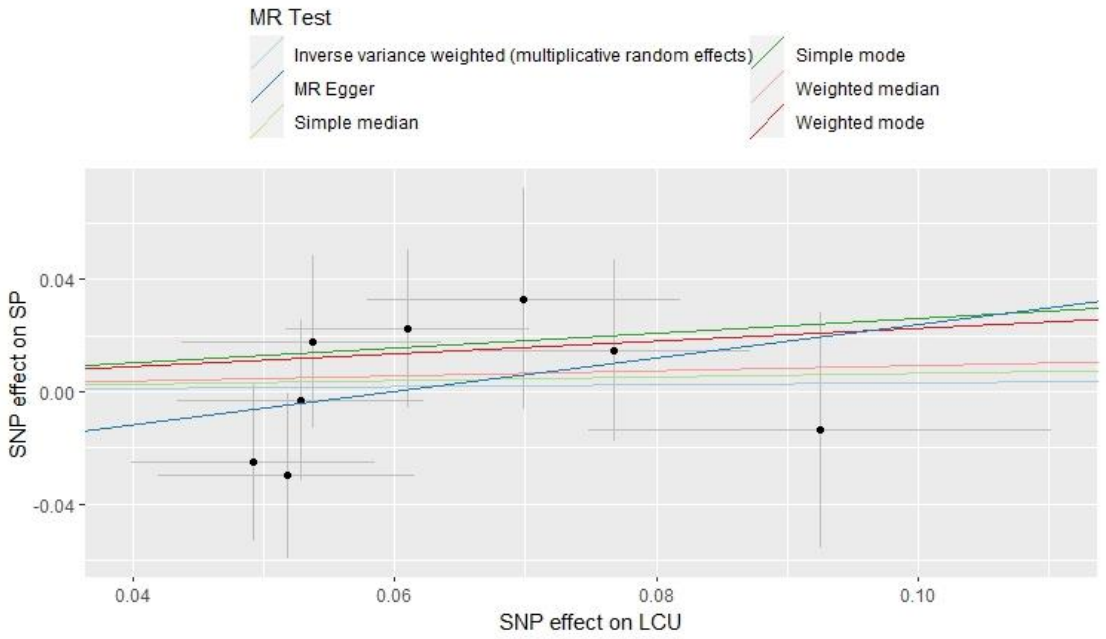

Figure S2-2. Forest plot for LCU as the exposure and SP as the outcome

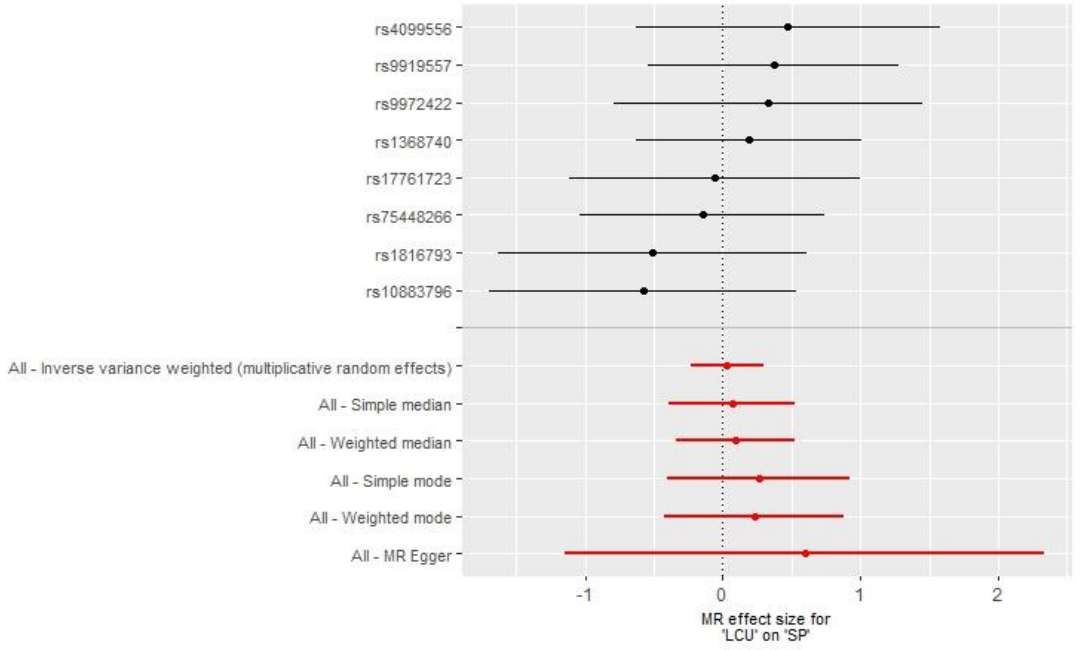

Figure S2-3. Funnel plot for LCU as the exposure and SP as the outcome

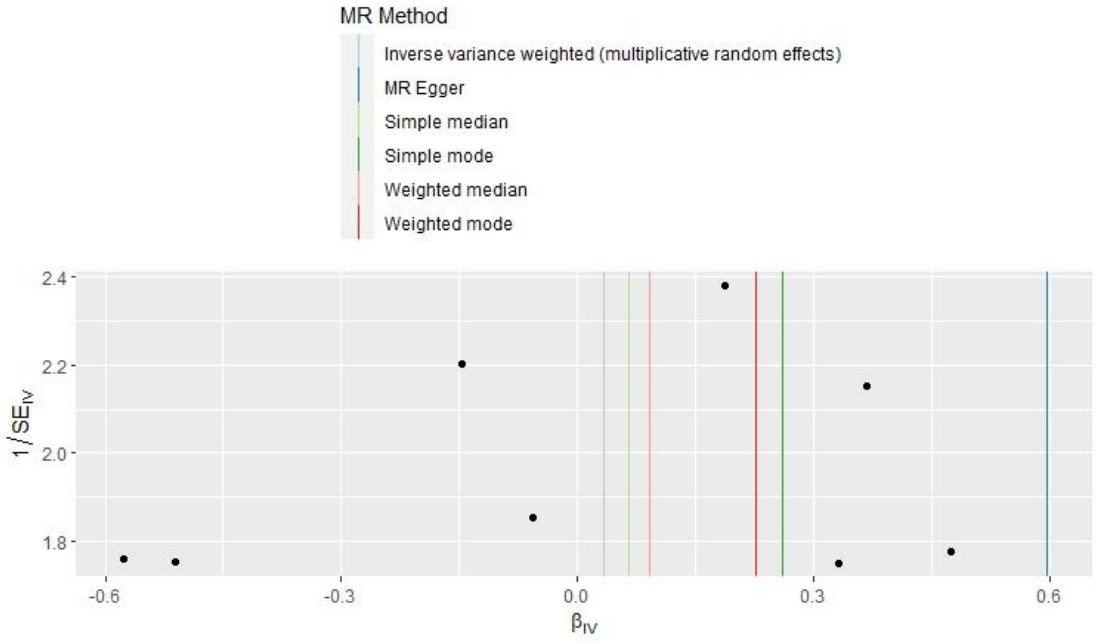

Figure S2-4. Leave-one-out plot for LCU as the exposure and SP as the outcome

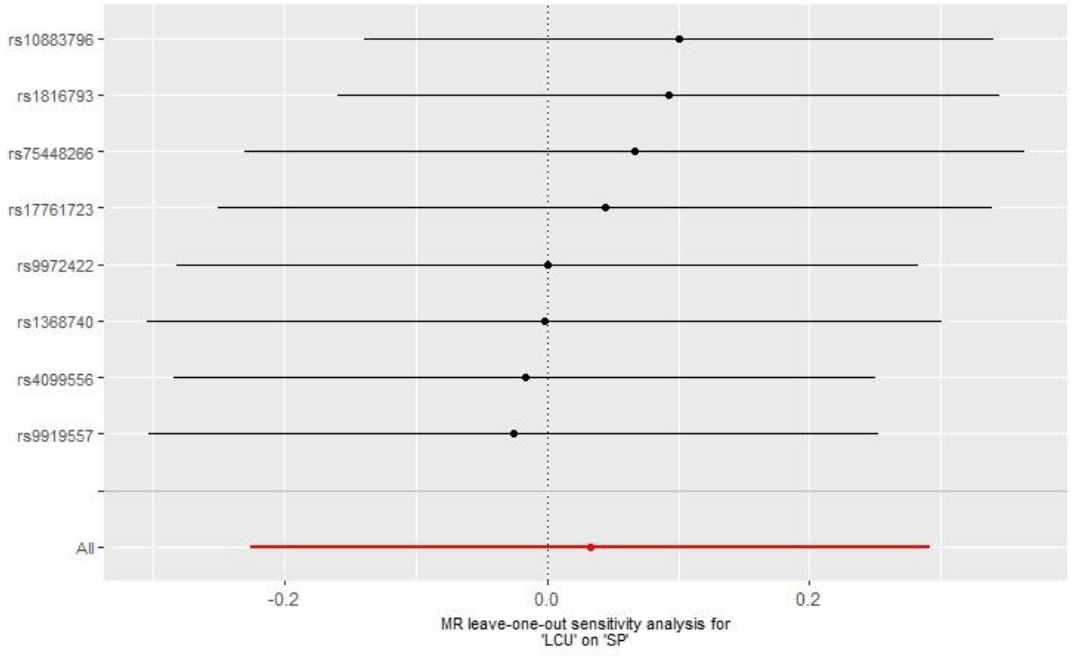

Figure S2-5. Scatter plot for SP as the exposure and LCU as the outcome

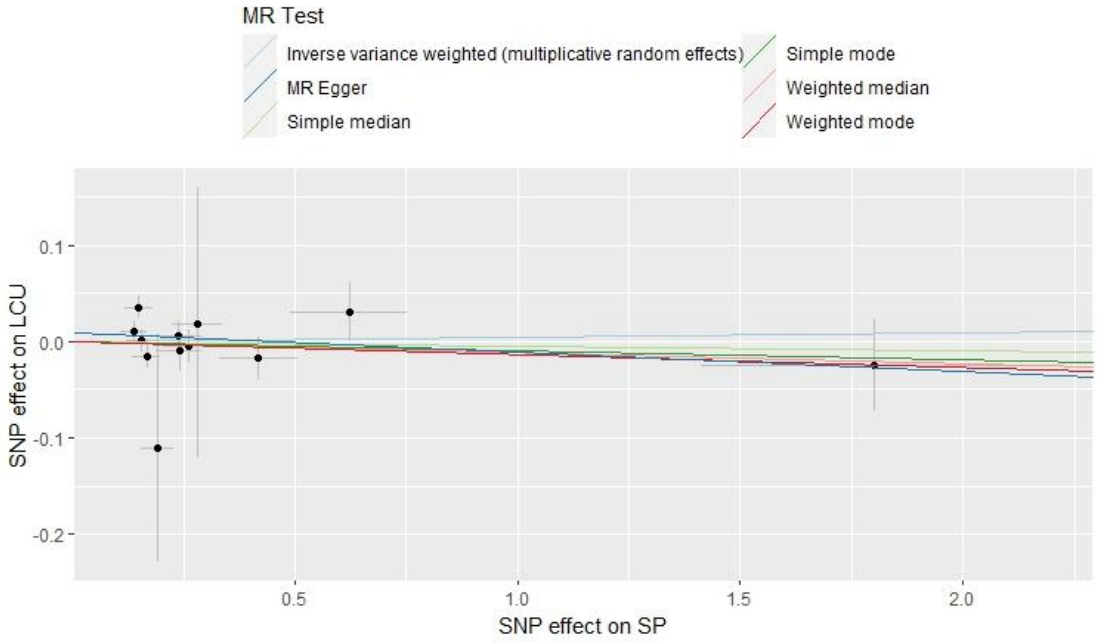

Figure S2-6. Forest plot for SP as the exposure and LCU as the outcome

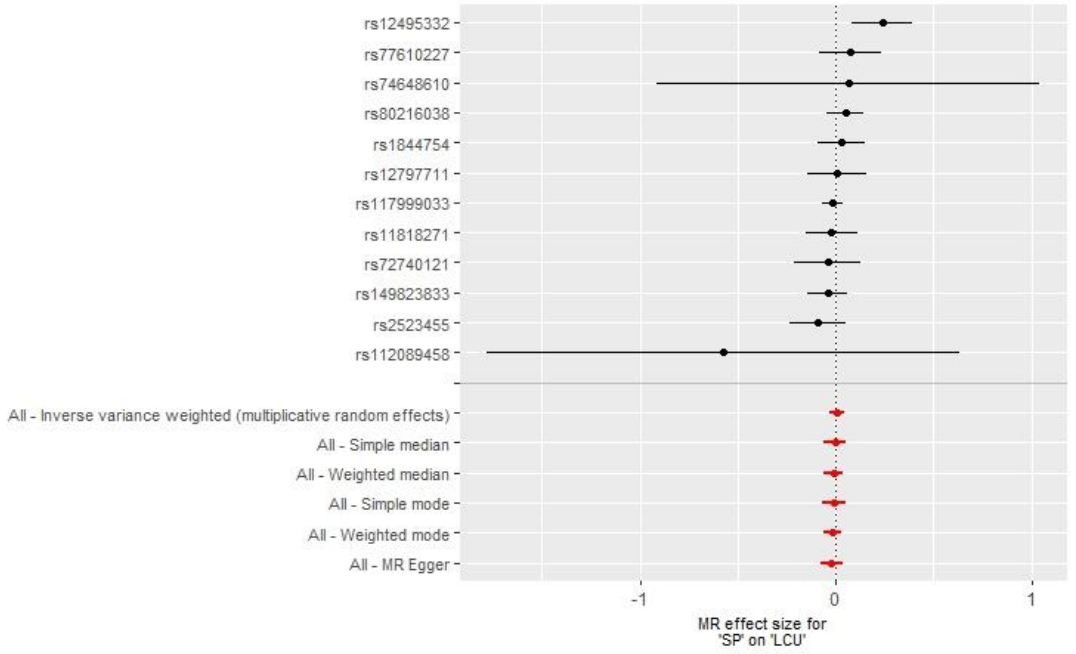

Figure S2-7. Funnel plot for SP as the exposure and LCU as the outcome

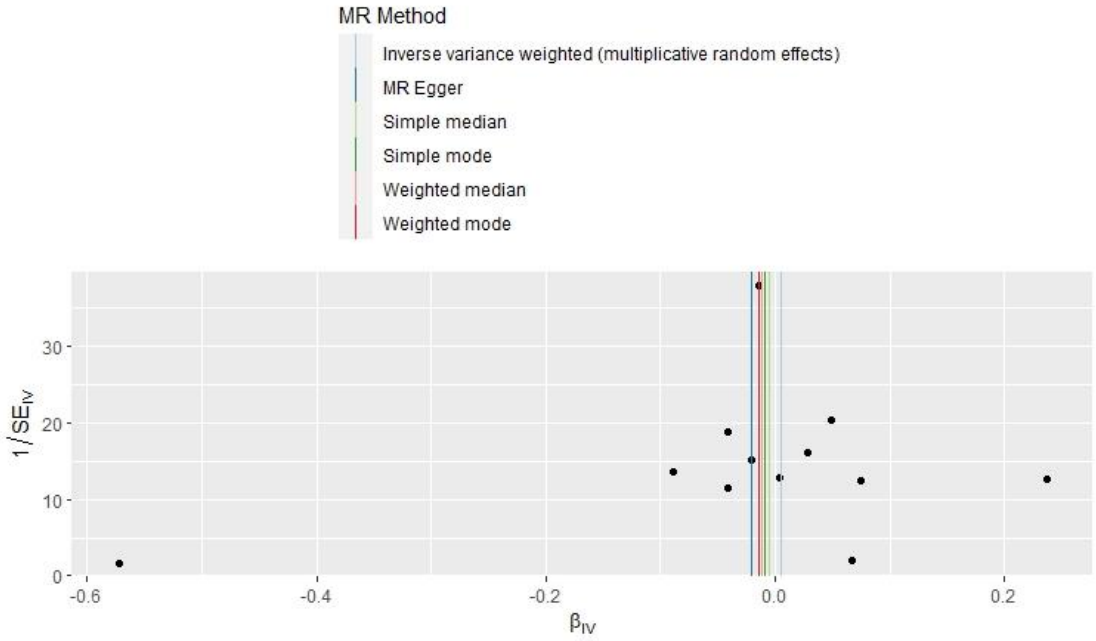

Figure S2-8. Leave-one-out plot for SP as the exposure and LCU as the outcome

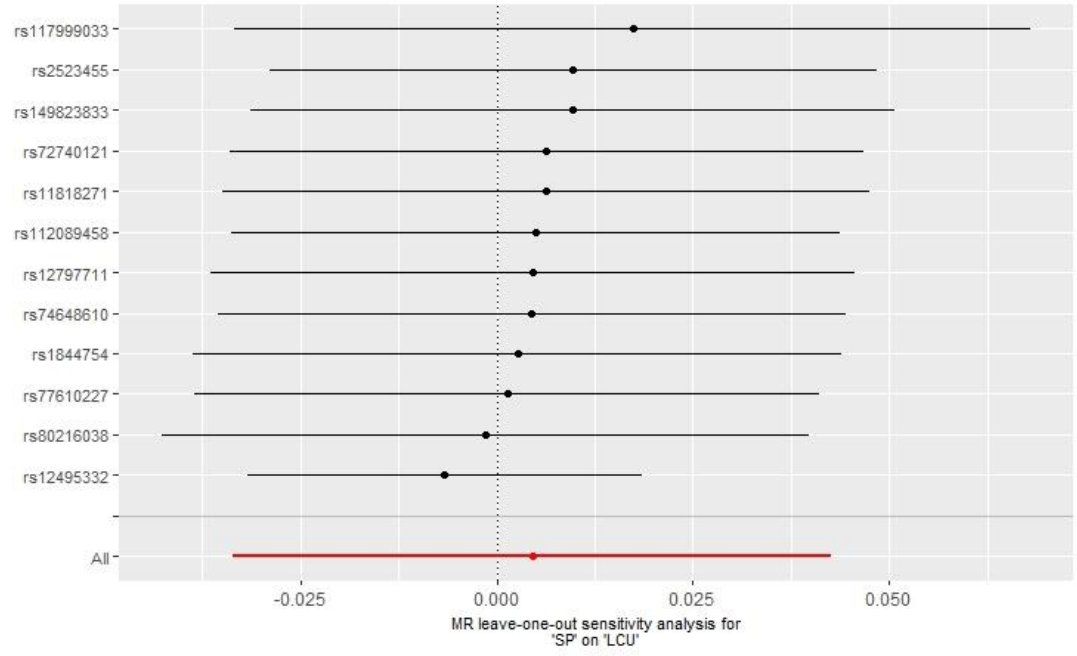

Figure S3-1. Scatter plot for LCU as the exposure and AG as the outcome

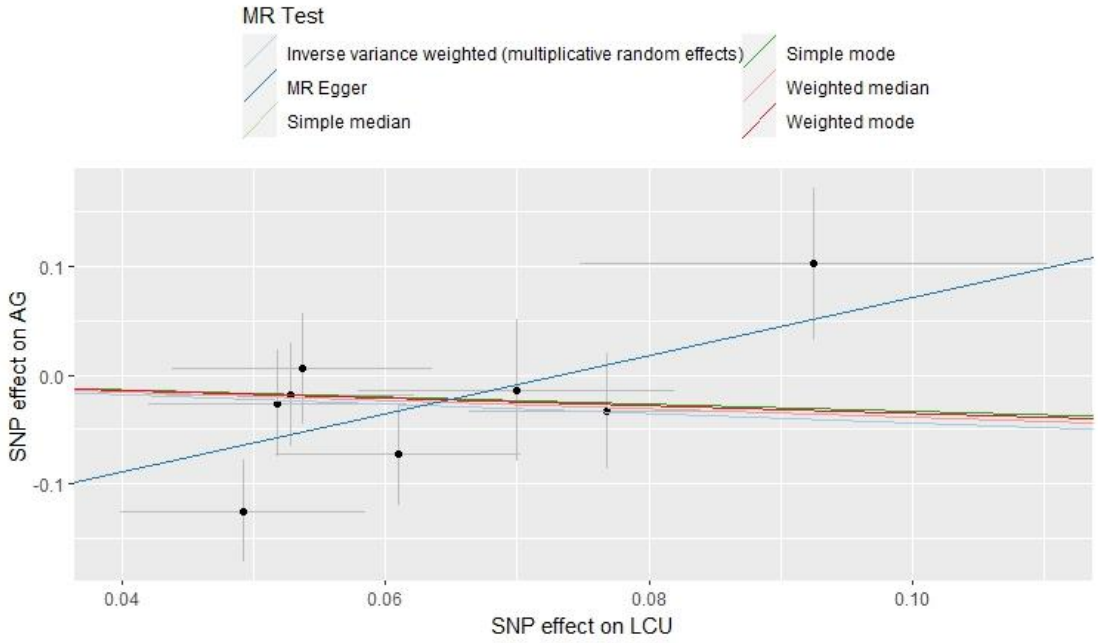

Figure S3-2. Forest plot for LCU as the exposure and AG as the outcome

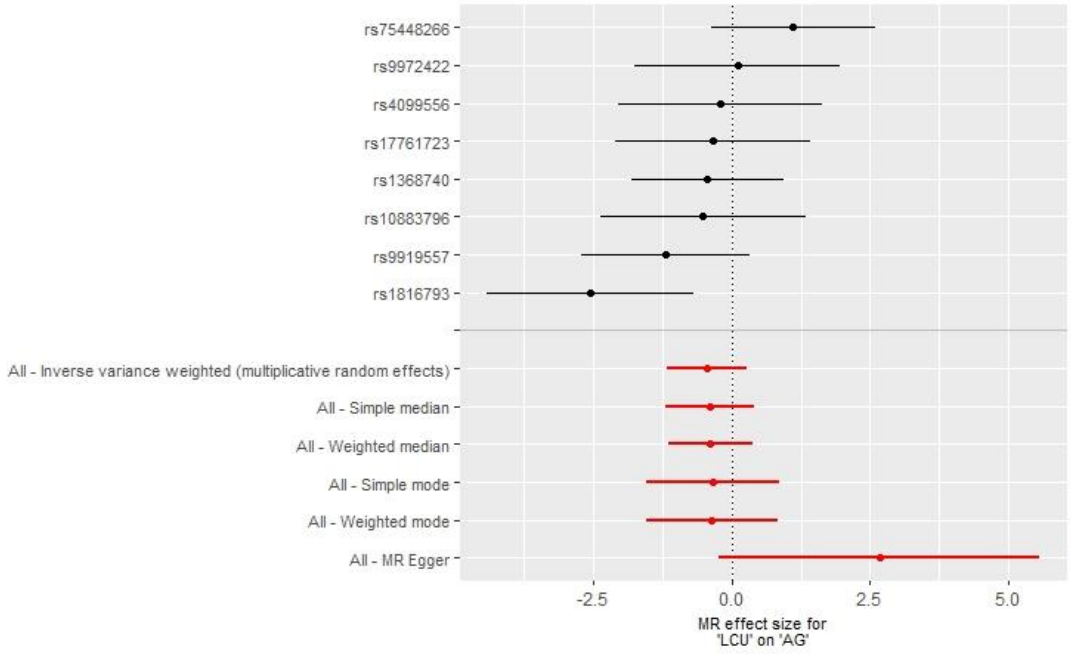

Figure S3-3. Funnel plot for LCU as the exposure and AG as the outcome

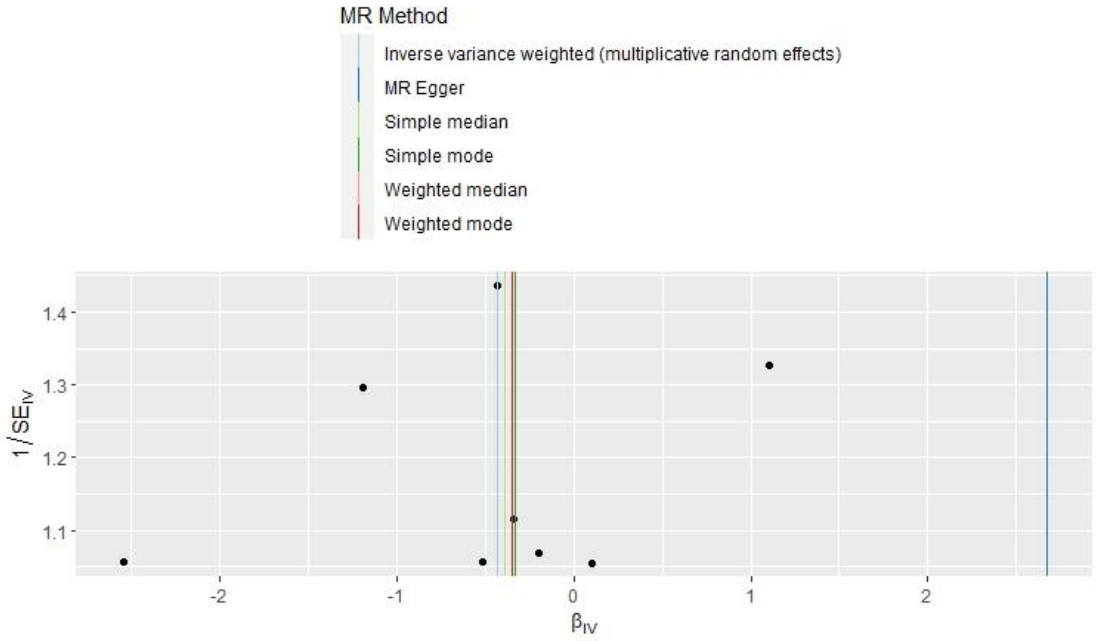

Figure S3-4. Leave-one-out plot for LCU as the exposure and AG as the outcome

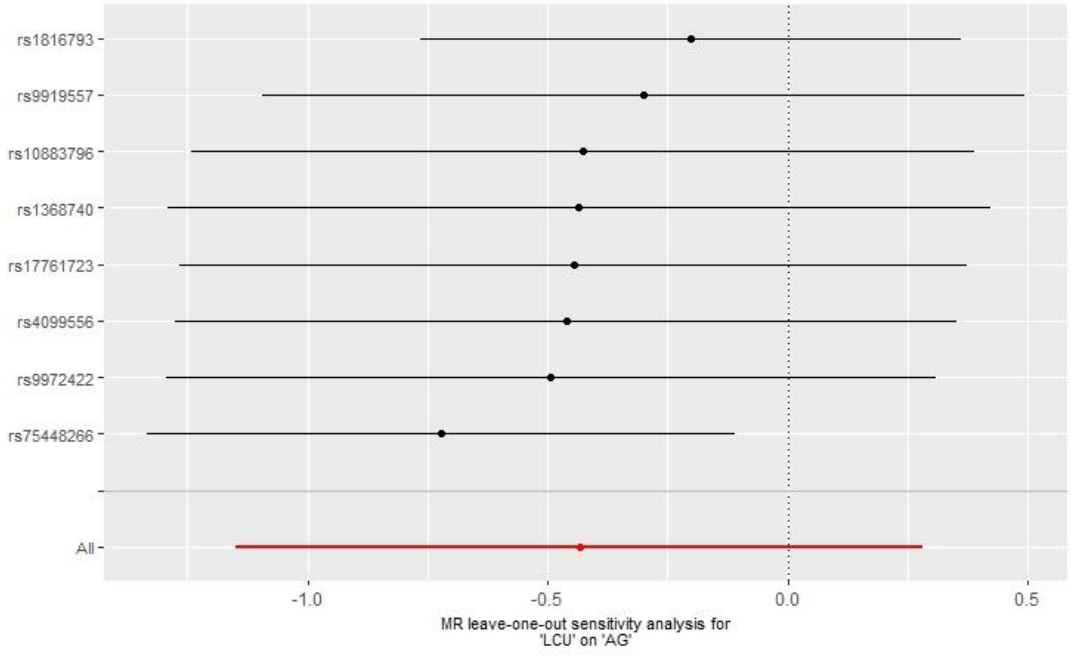

Figure S3-5. Scatter plot for AG as the exposure and LCU as the outcome

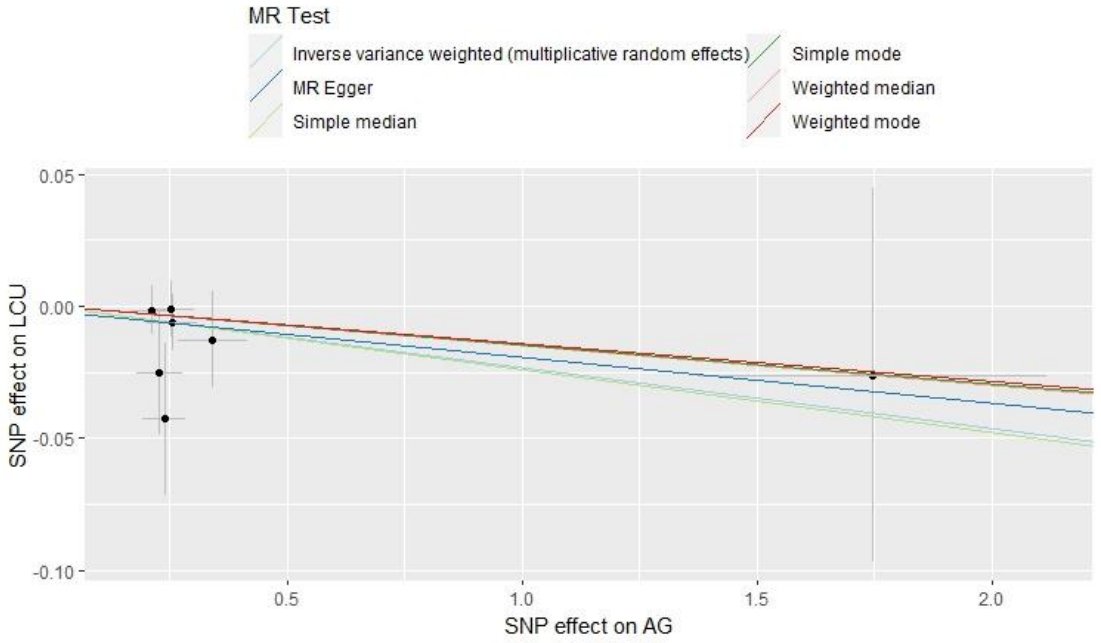

Figure S3-6. Forest plot for AG as the exposure and LCU as the outcome

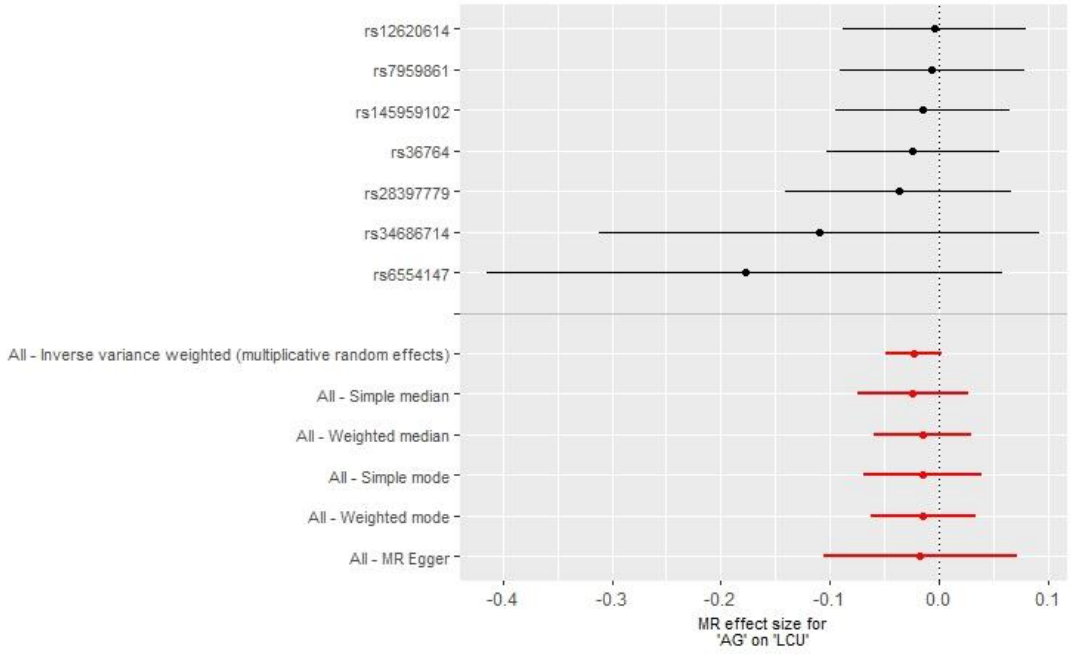

Figure S3-7. Funnel plot for AG as the exposure and LCU as the outcome

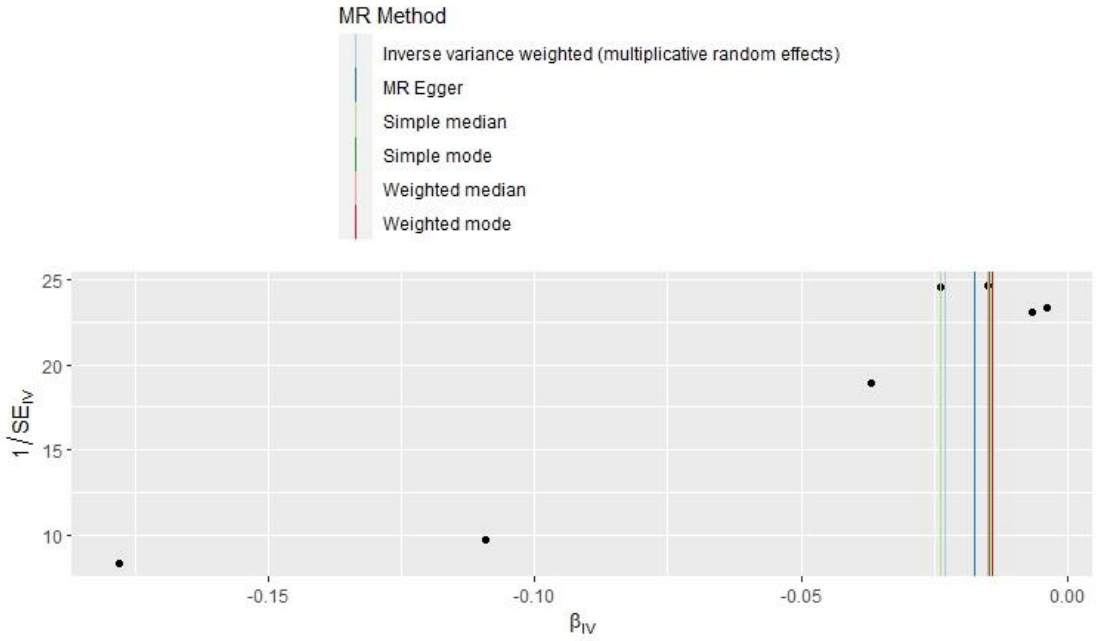

Figure S3-8. Leave-one-out plot for AG as the exposure and LCU as the outcome

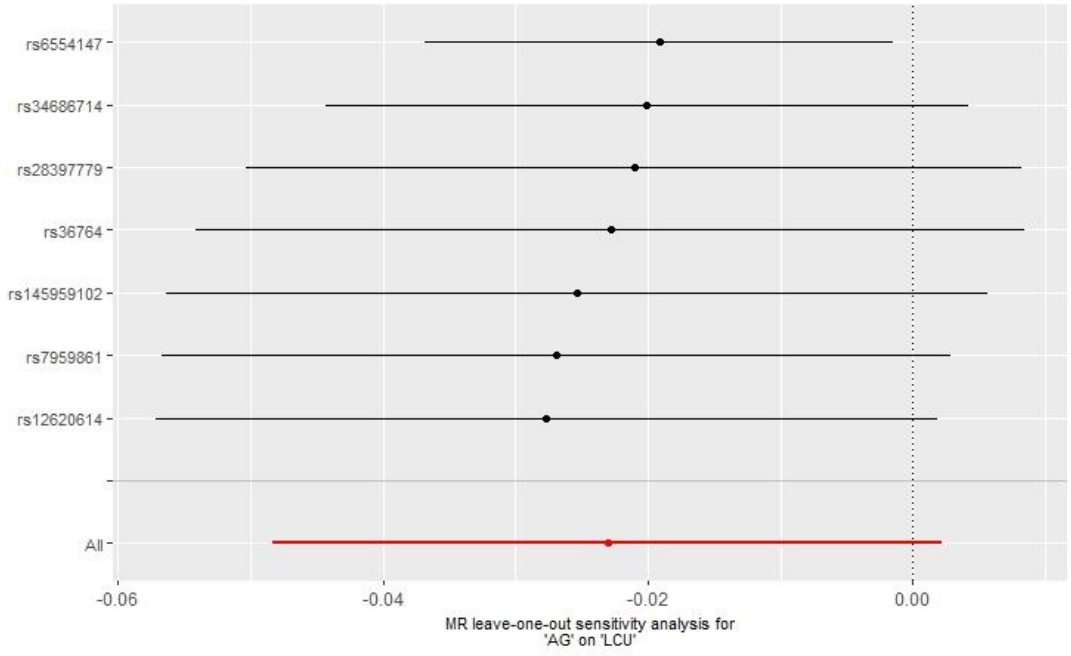

Figure S4-1. Scatter plot for LCU as the exposure and PD as the outcome

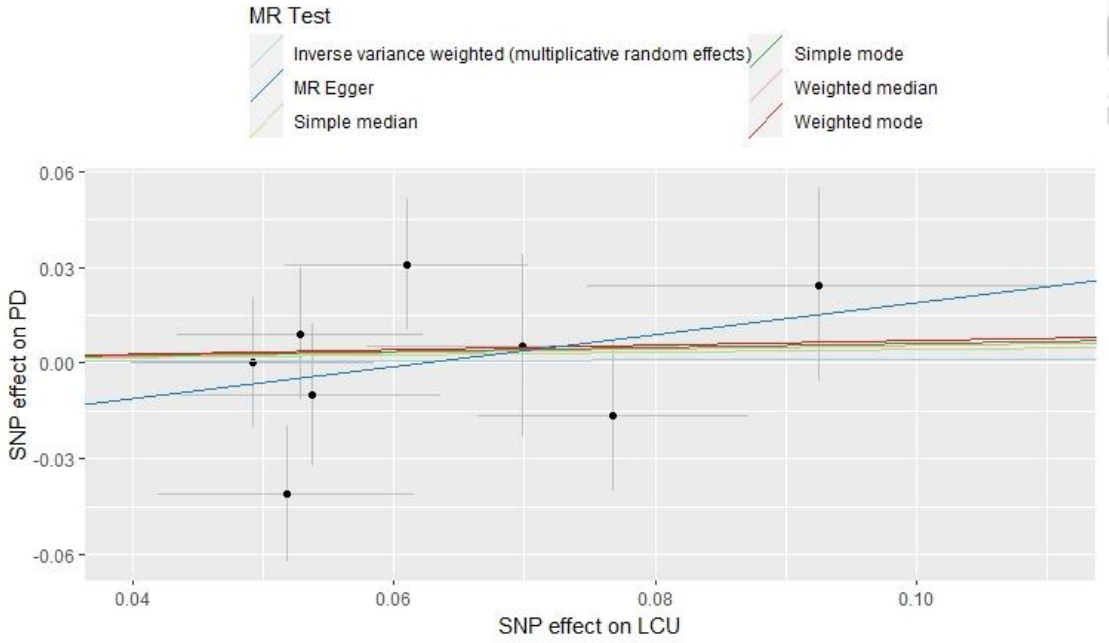

Figure S4-2. Forest plot for LCU as the exposure and PD as the outcome

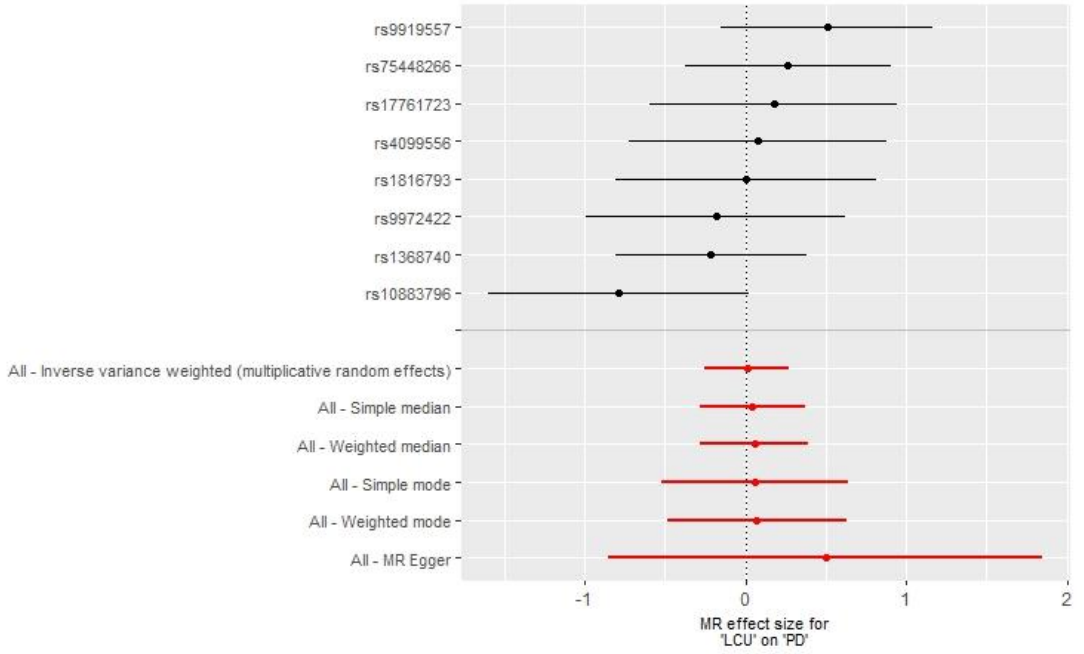

Figure S4-3. Funnel plot for LCU as the exposure and PD as the outcome

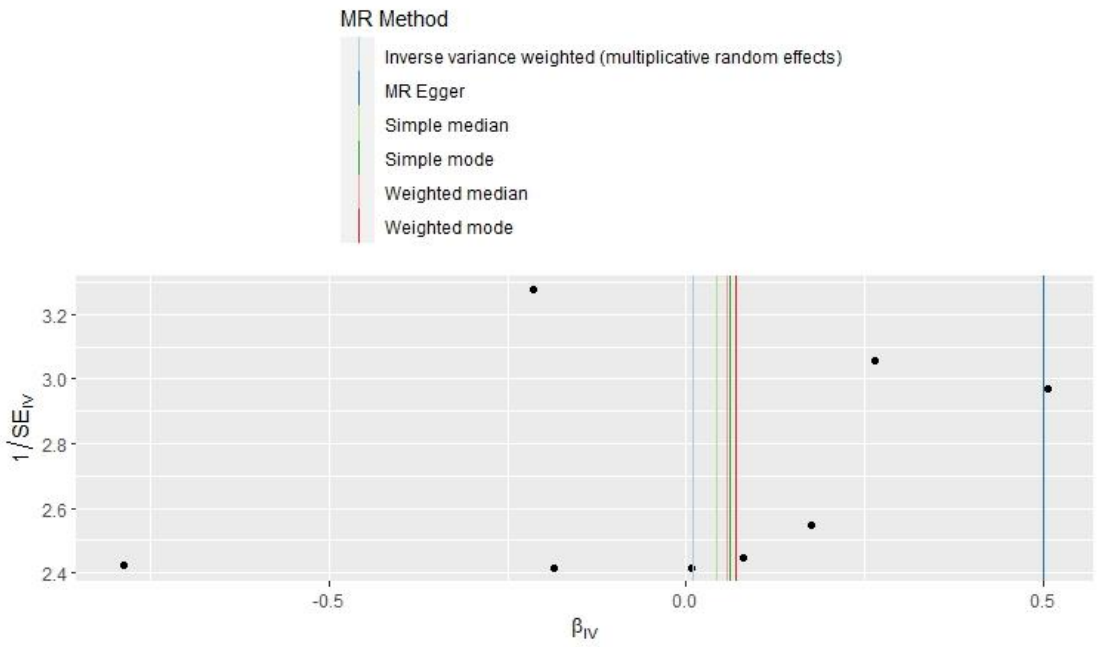

Figure S4-4. Leave-one-out plot for LCU as the exposure and PD as the outcome

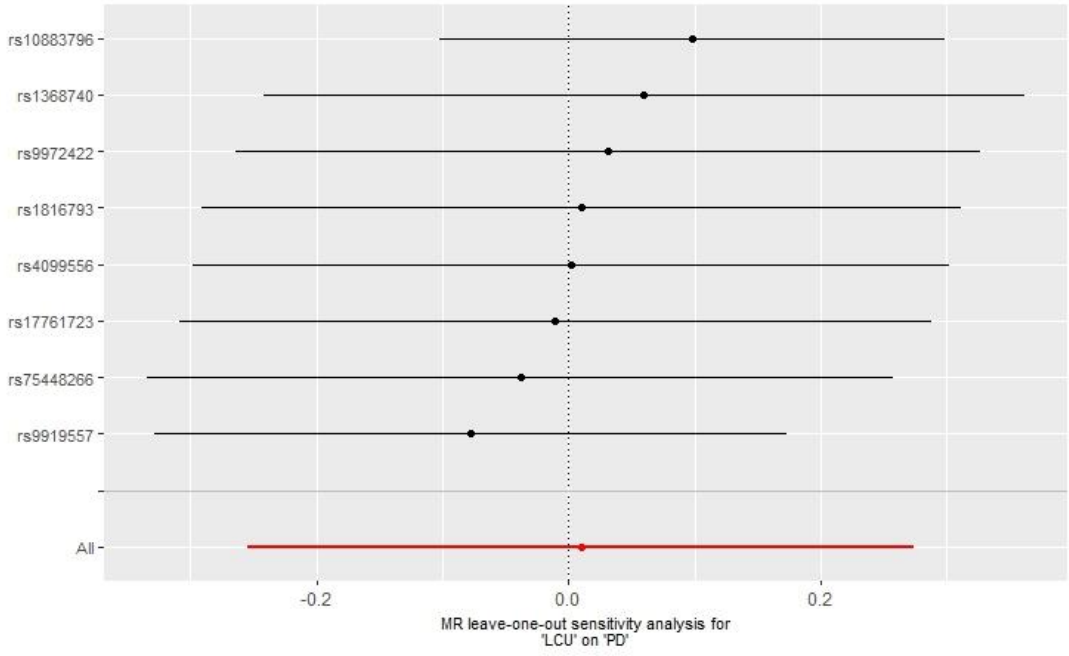

Figure S4-5. Scatter plot for PD as the exposure and LCU as the outcome

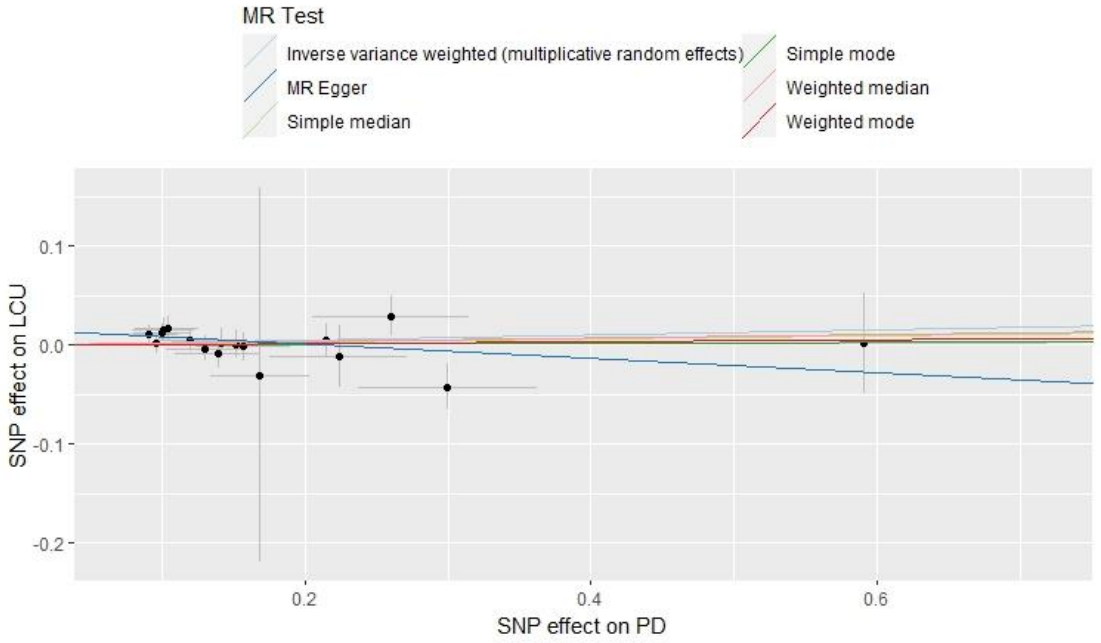

Figure S4-6. Forest plot for PD as the exposure and LCU as the outcome

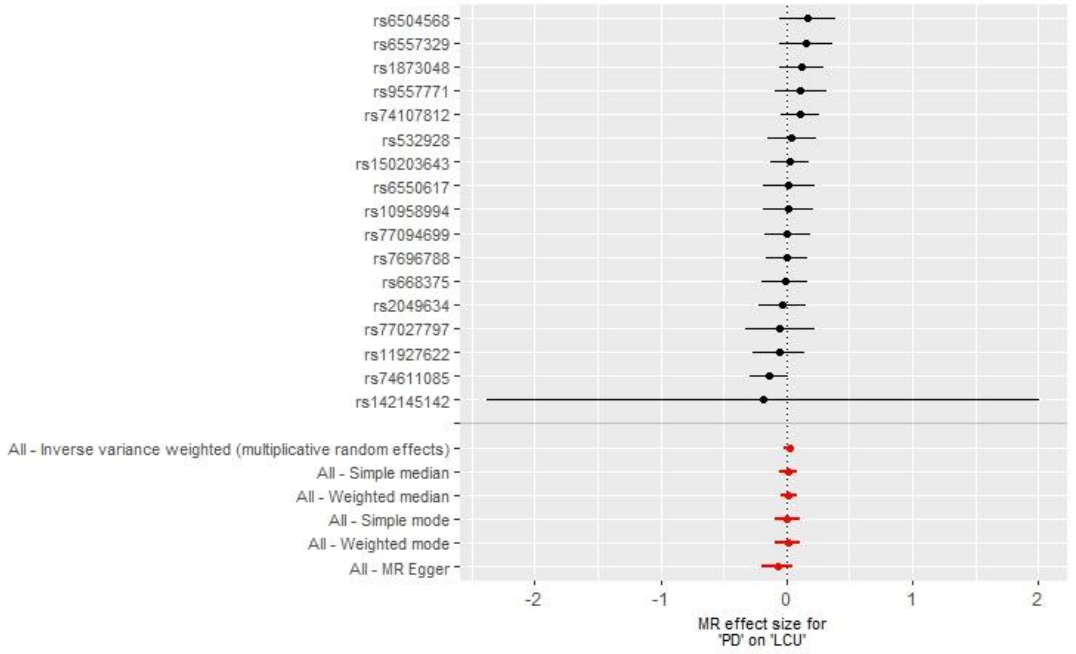

Figure S4-7. Funnel plot for PD as the exposure and LCU as the outcome

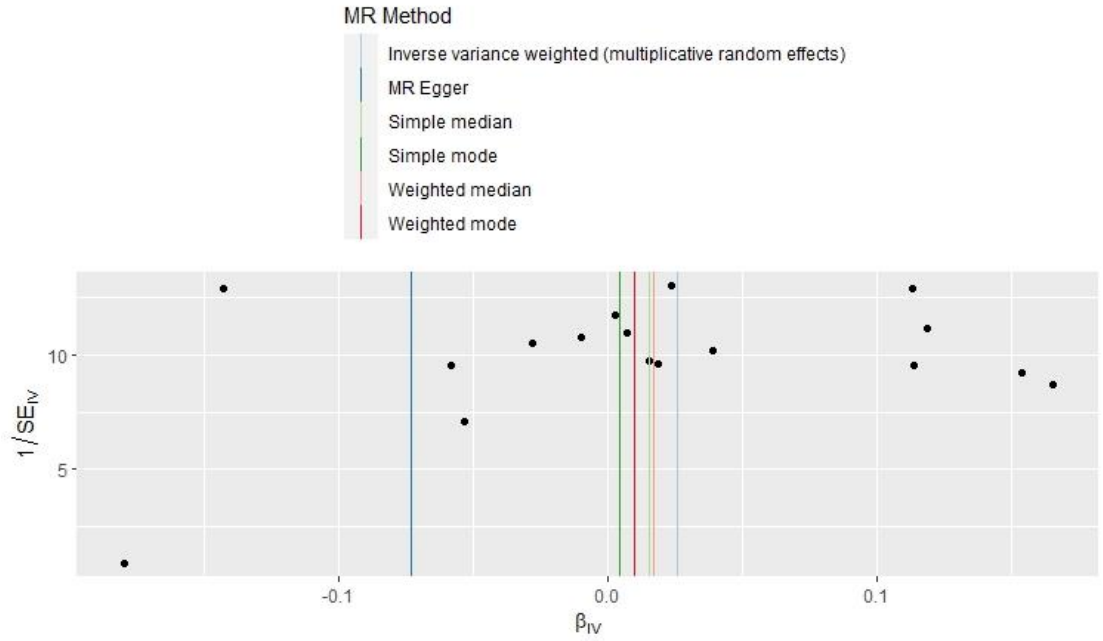

Figure S4-8. Leave-one-out plot for PD as the exposure and LCU as the outcome

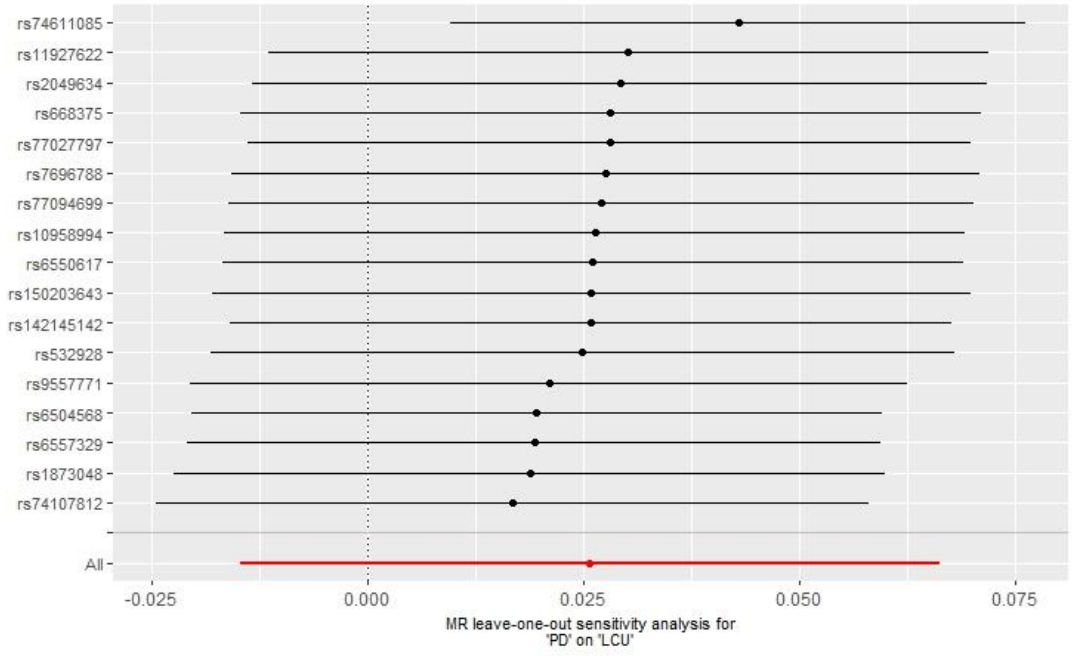

Figure S5-1. Scatter plot for CUD as the exposure and GAD as the outcome

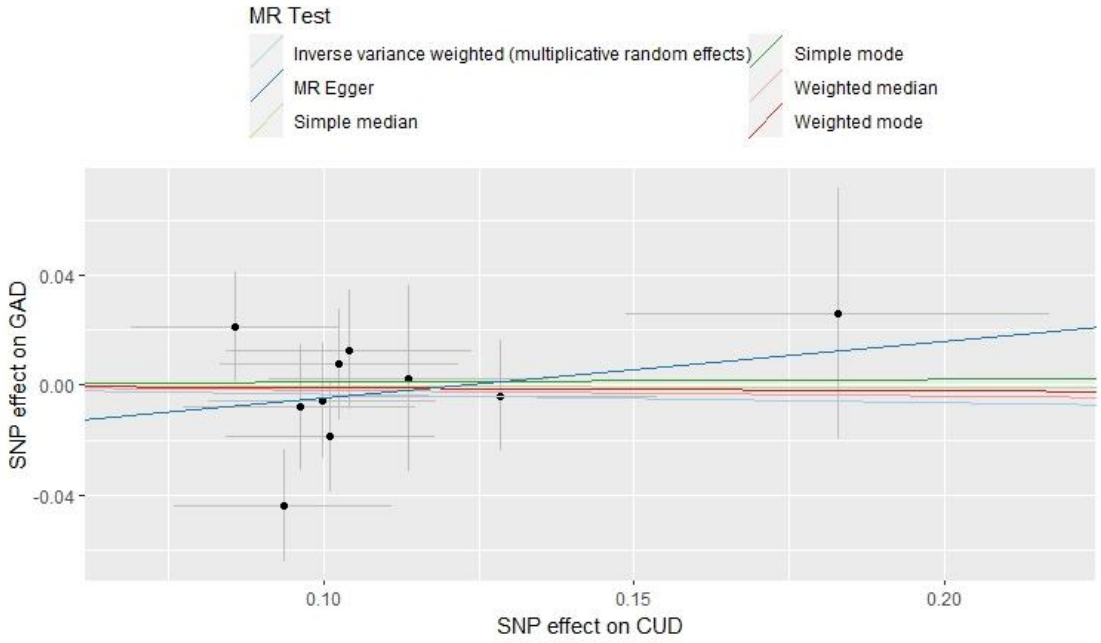

Figure S5-2. Forest plot for CUD as the exposure and GAD as the outcome

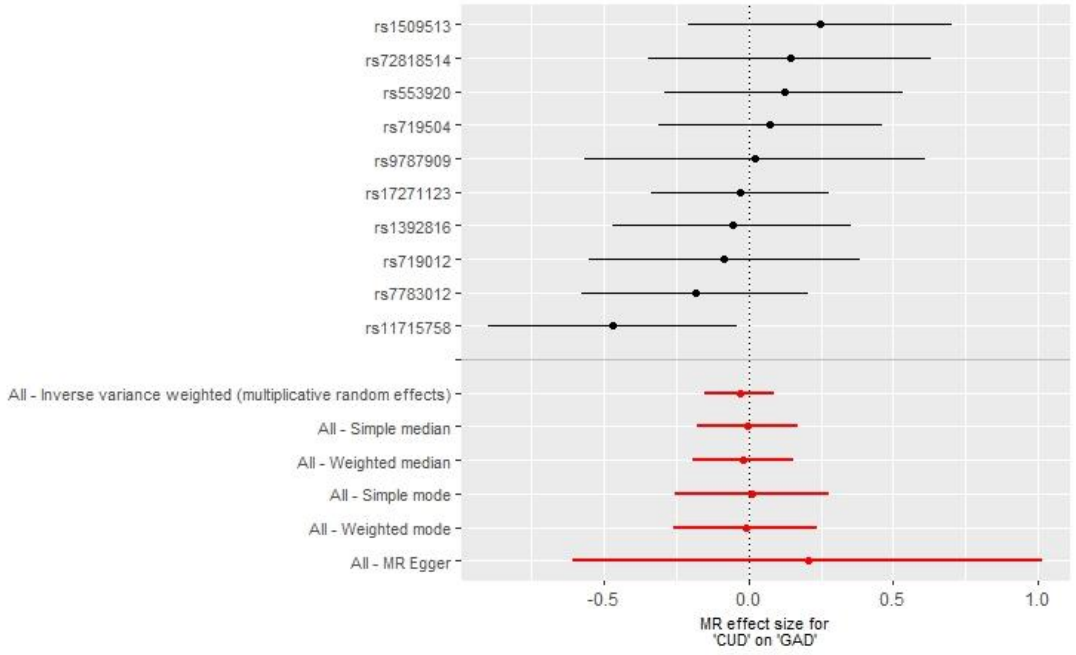

Figure S5-3. Funnel plot for CUD as the exposure and GAD as the outcome

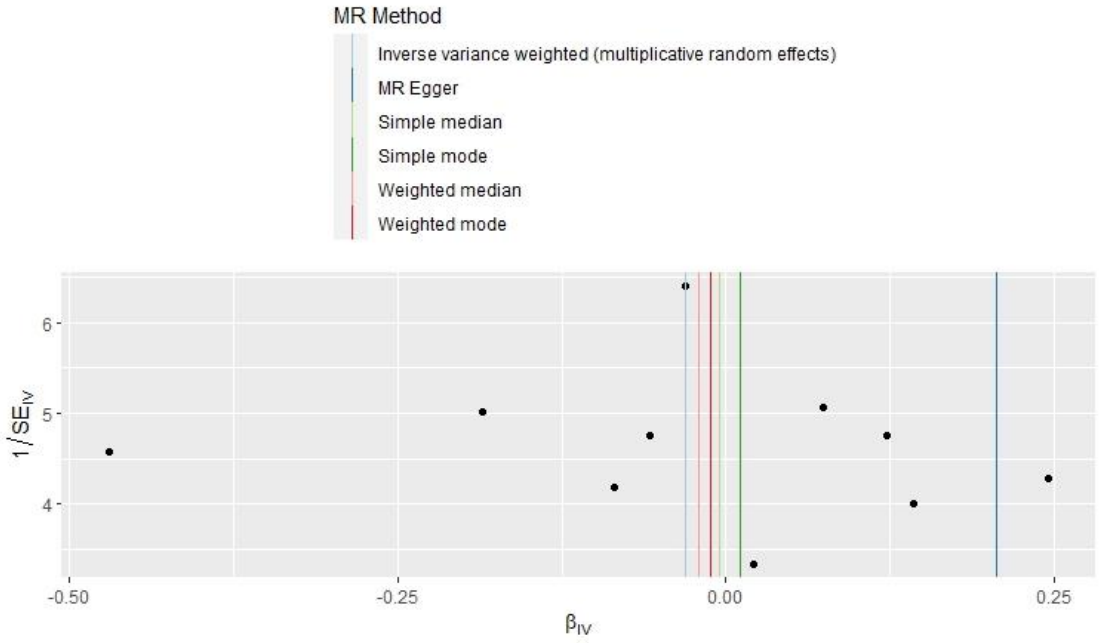

Figure S5-4. Leave-one-out plot for CUD as the exposure and GAD as the outcome

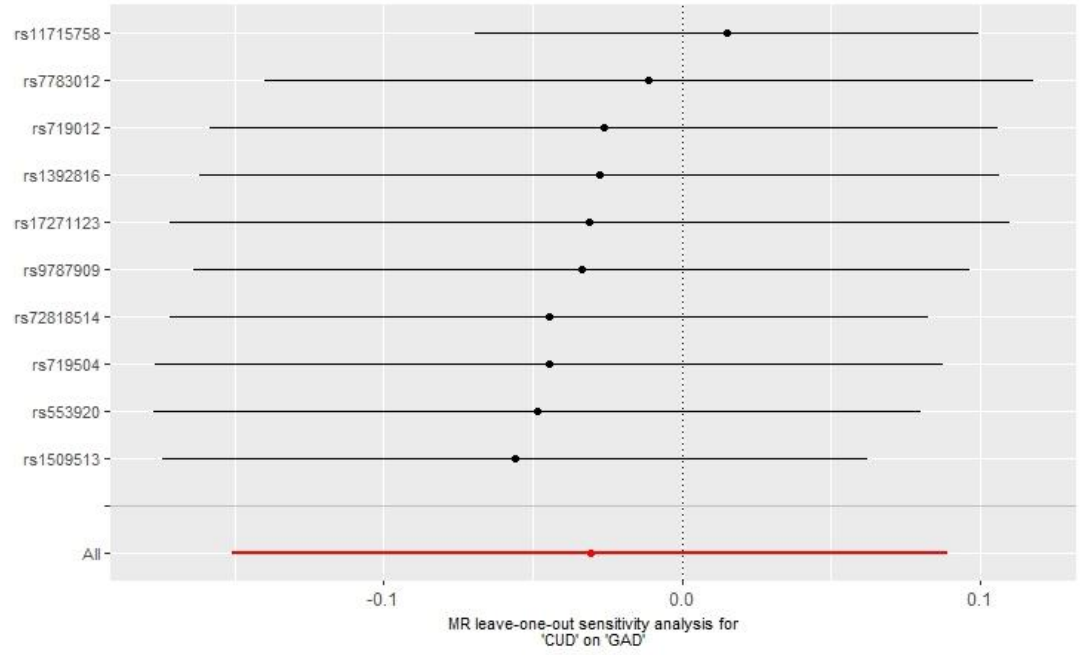

Figure S5-5. Scatter plot for GAD as the exposure and CUD as the outcome

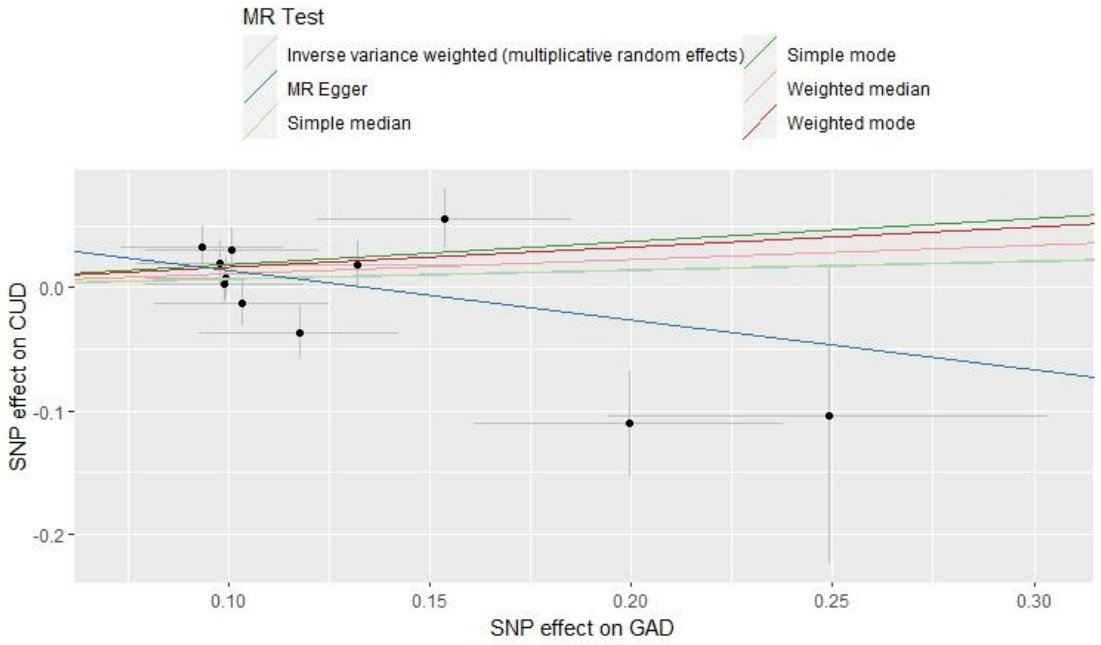

Figure S5-6. Forest plot for GAD as the exposure and CUD as the outcome

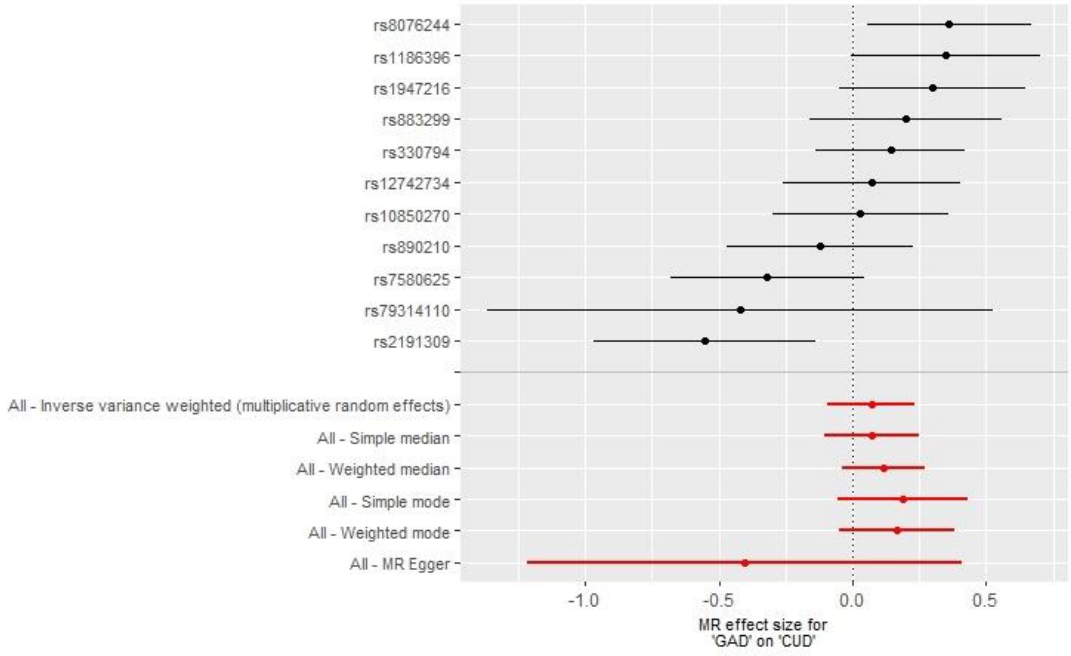

Figure S5-7. Funnel plot for GAD as the exposure and CUD as the outcome

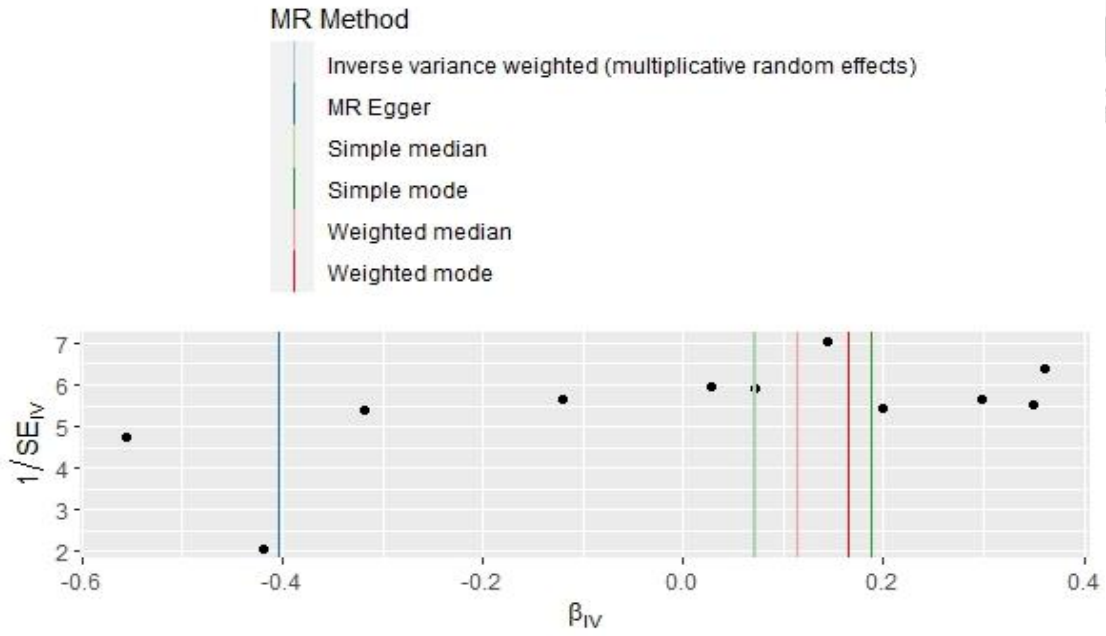

Figure S5-8. Leave-one-out plot for GAD as the exposure and CUD as the outcome

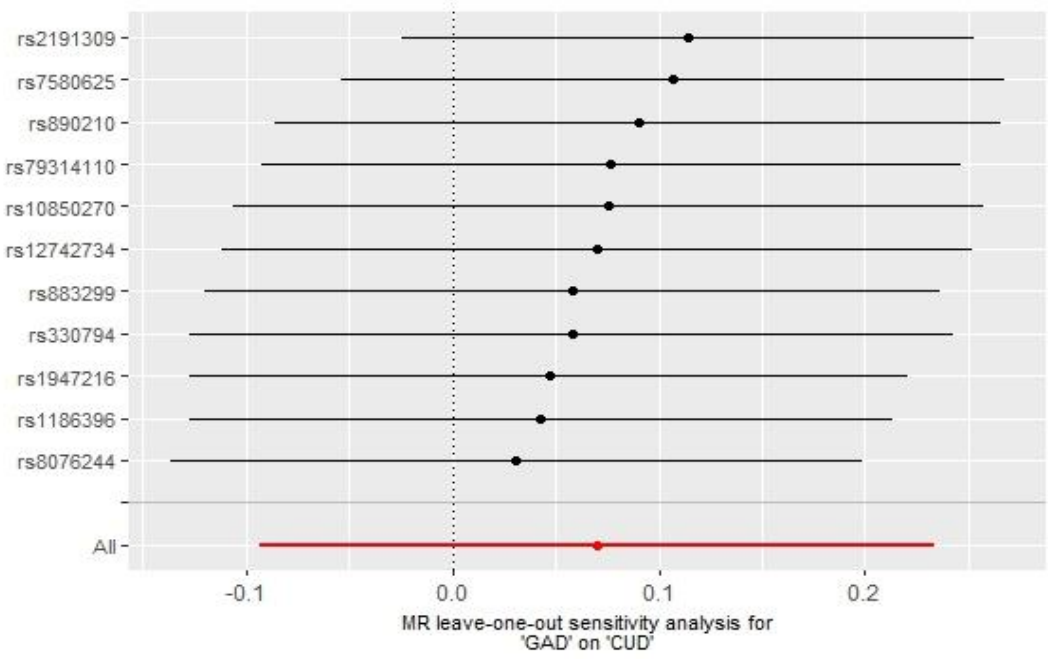

Figure S5-9. Scatter plot for GAD as the exposure and CUD as the outcome

(After removing the outlier rs2191309 identified by MR-PRESSO)

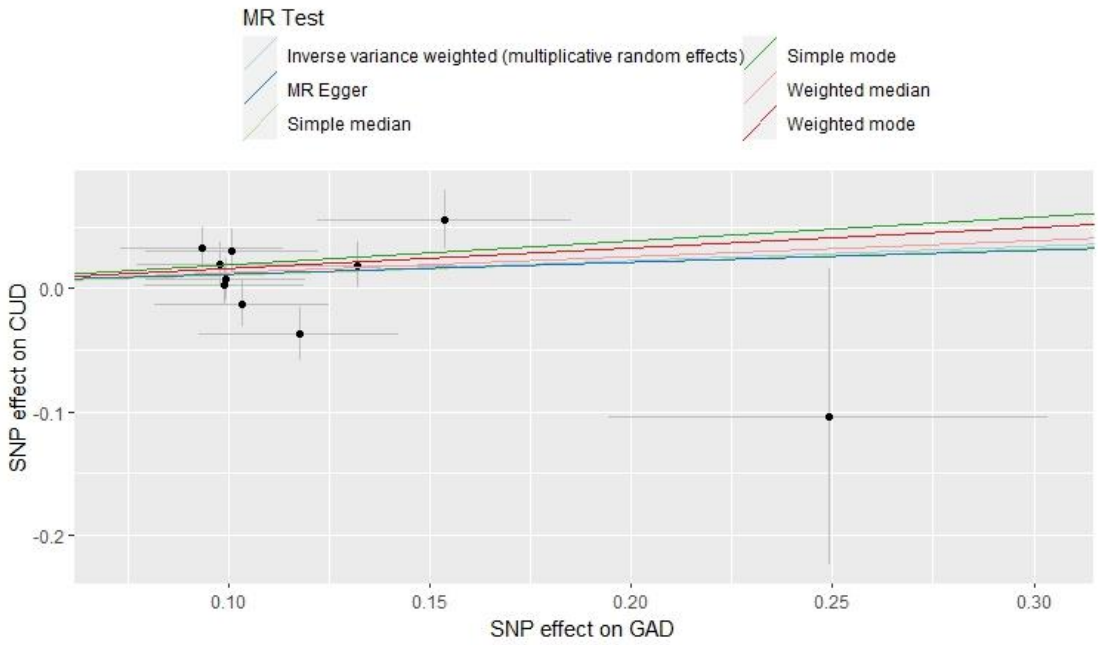

Figure S5-10. Forest plot for GAD as the exposure and CUD as the outcome

(After removing the outlier rs2191309 identified by MR-PRESSO)

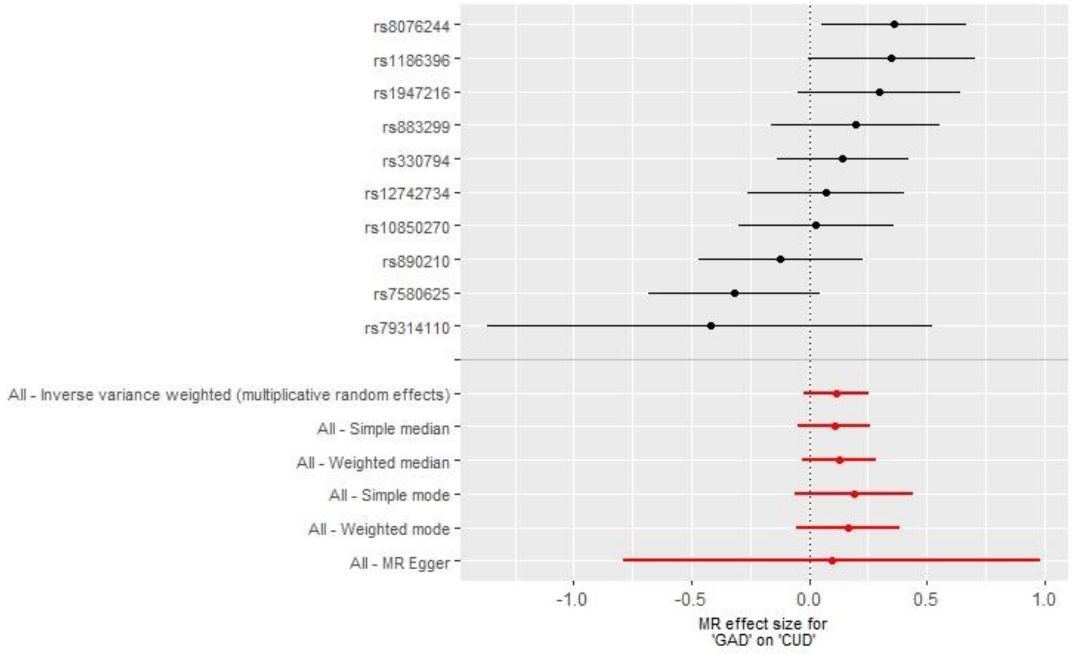

Figure S5-11. Funnel plot for GAD as the exposure and CUD as the outcome

(After removing the outlier rs2191309 identified by MR-PRESSO)

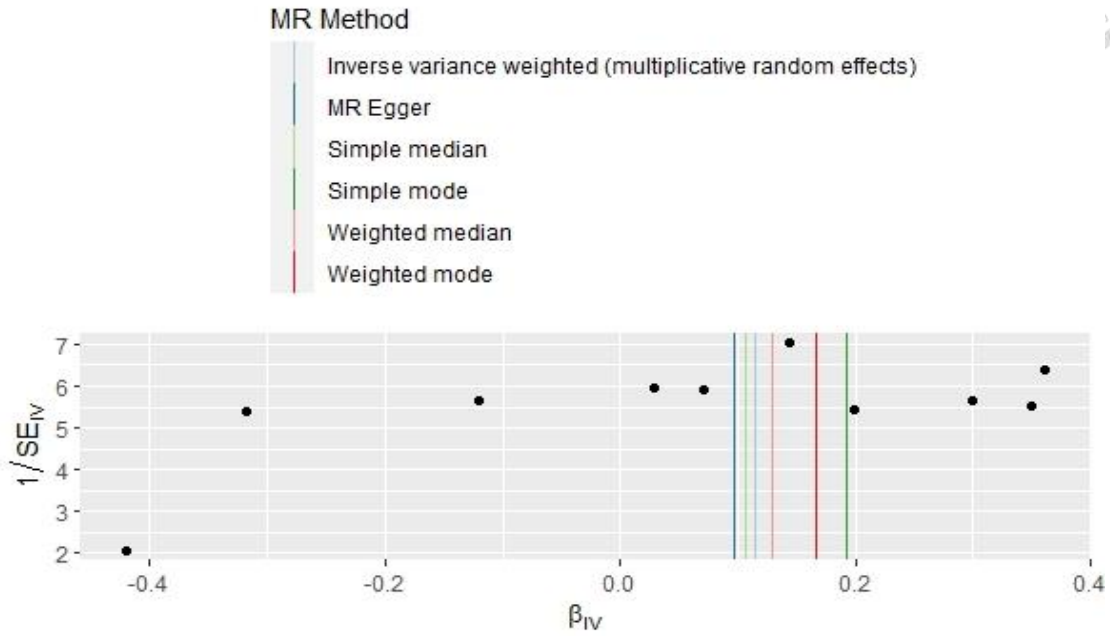

Figure S5-12. Leave-one-out plot for GAD as the exposure and CUD as the outcome

(After removing the outlier rs2191309 identified by MR-PRESSO)

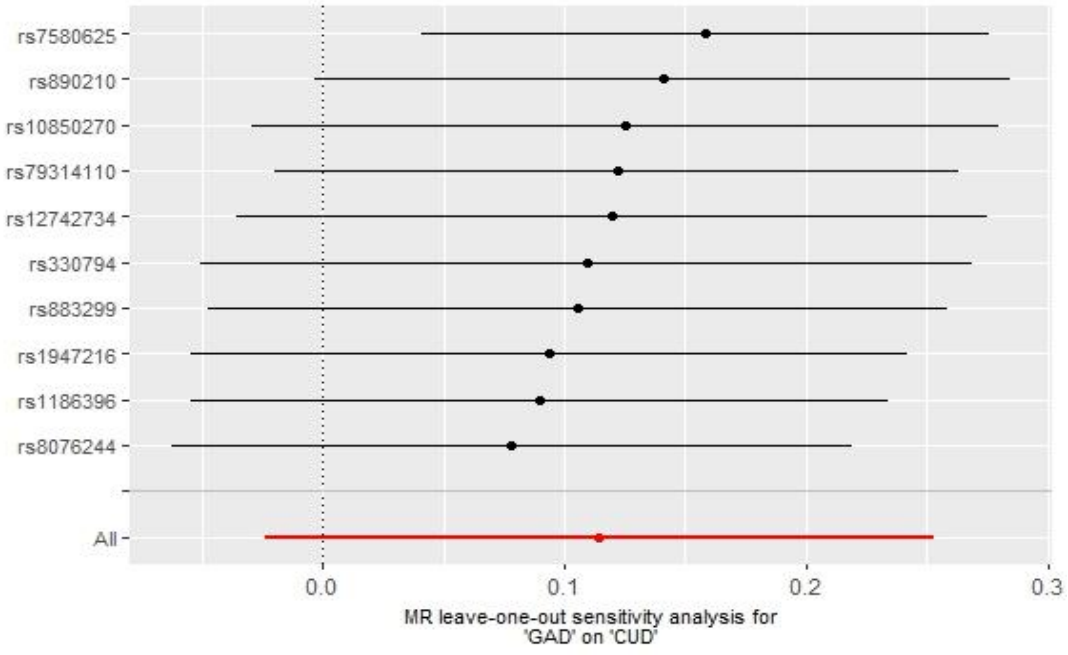

Figure S6-1. Scatter plot for CUD as the exposure and SP as the outcome

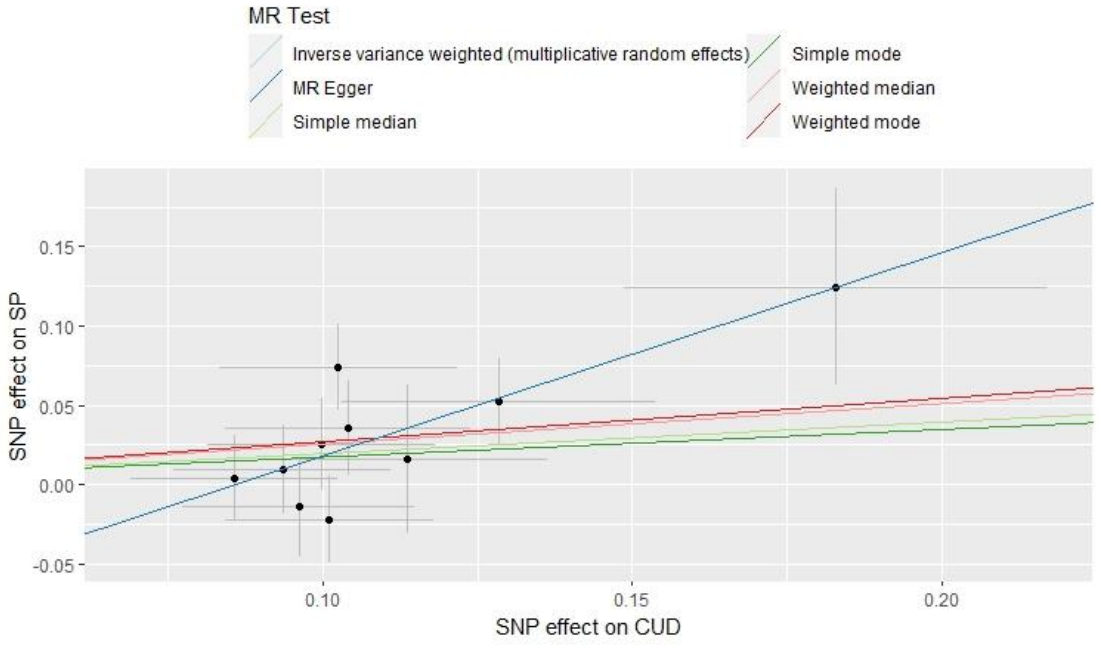

Figure S6-2. Forest plot for CUD as the exposure and SP as the outcome

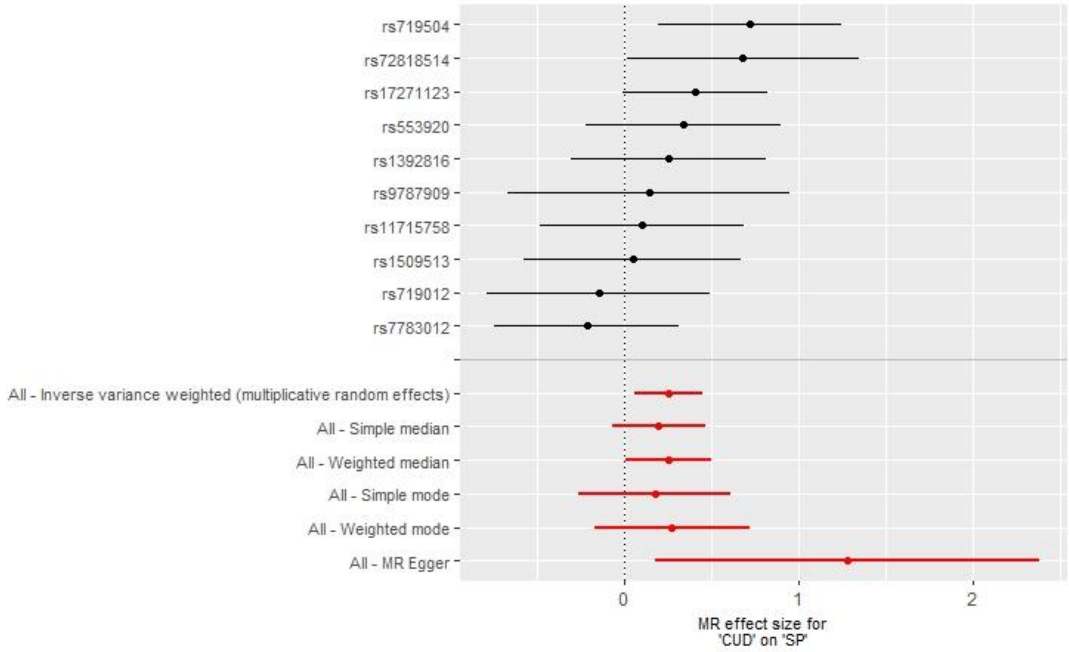

Figure S6-3. Funnel plot for CUD as the exposure and SP as the outcome

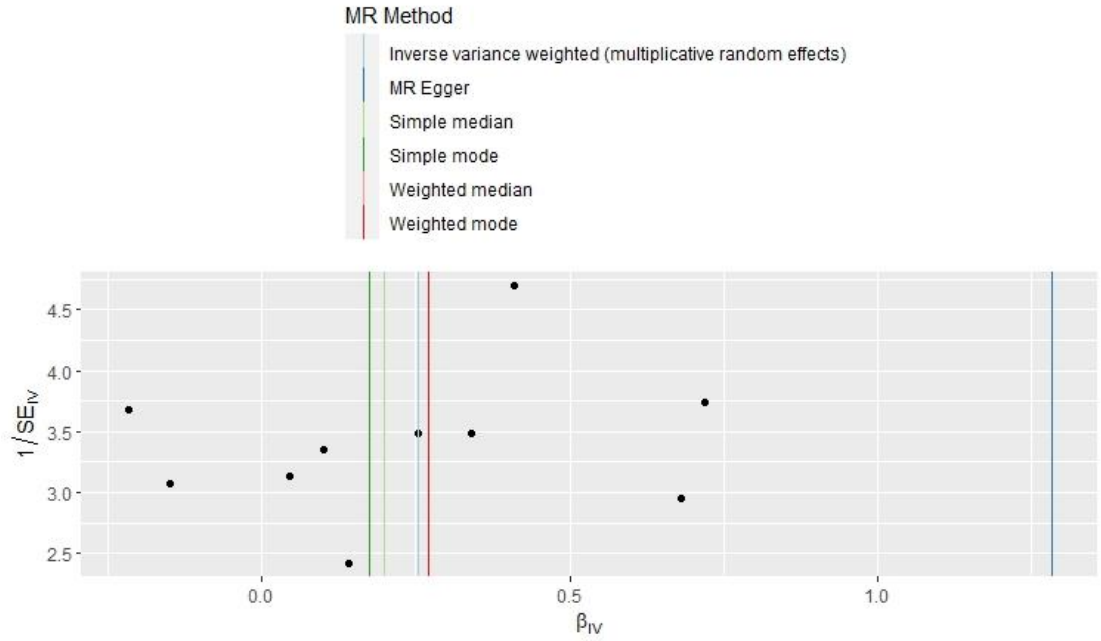

Figure S6-4. Leave-one-out plot for CUD as the exposure and SP as the outcome

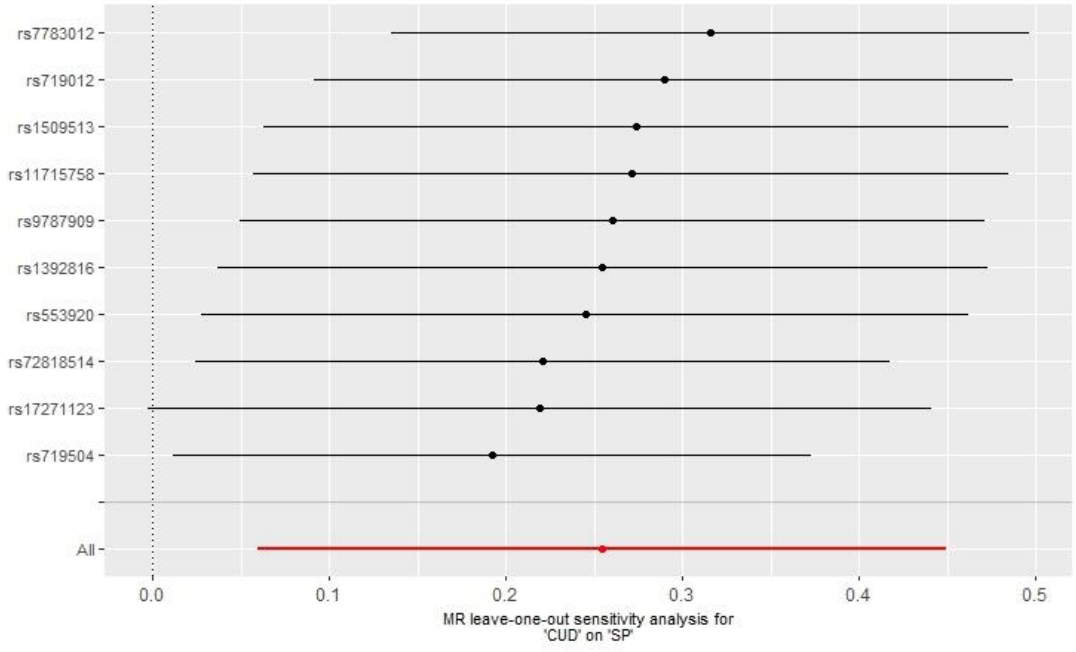

Figure S6-5. Scatter plot for SP as the exposure and CUD as the outcome

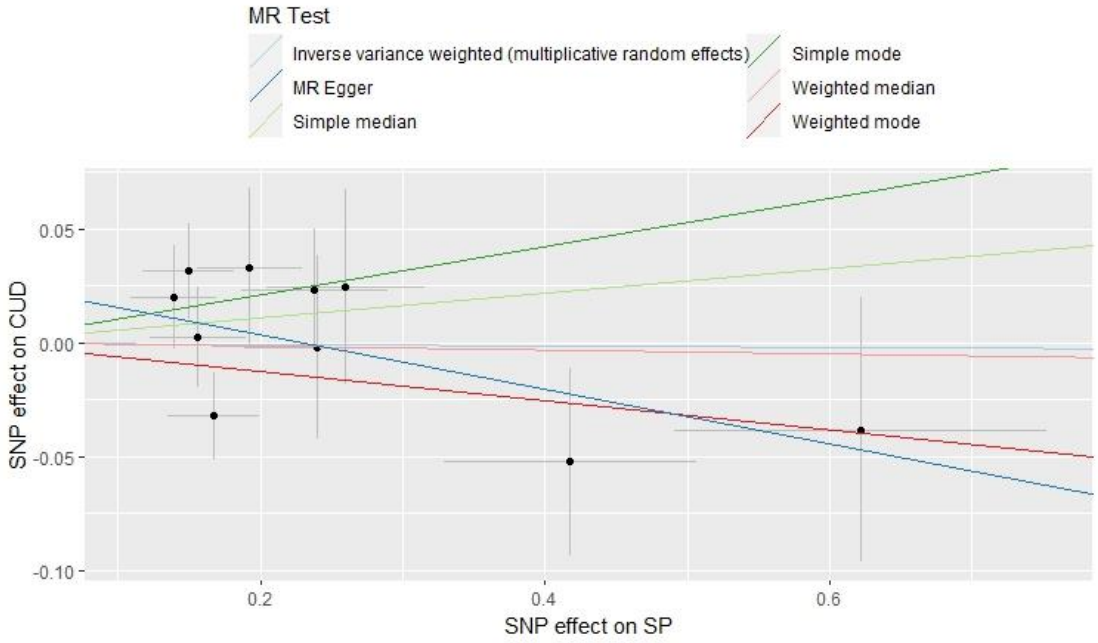

Figure S6-6. Forest plot for SP as the exposure and CUD as the outcome

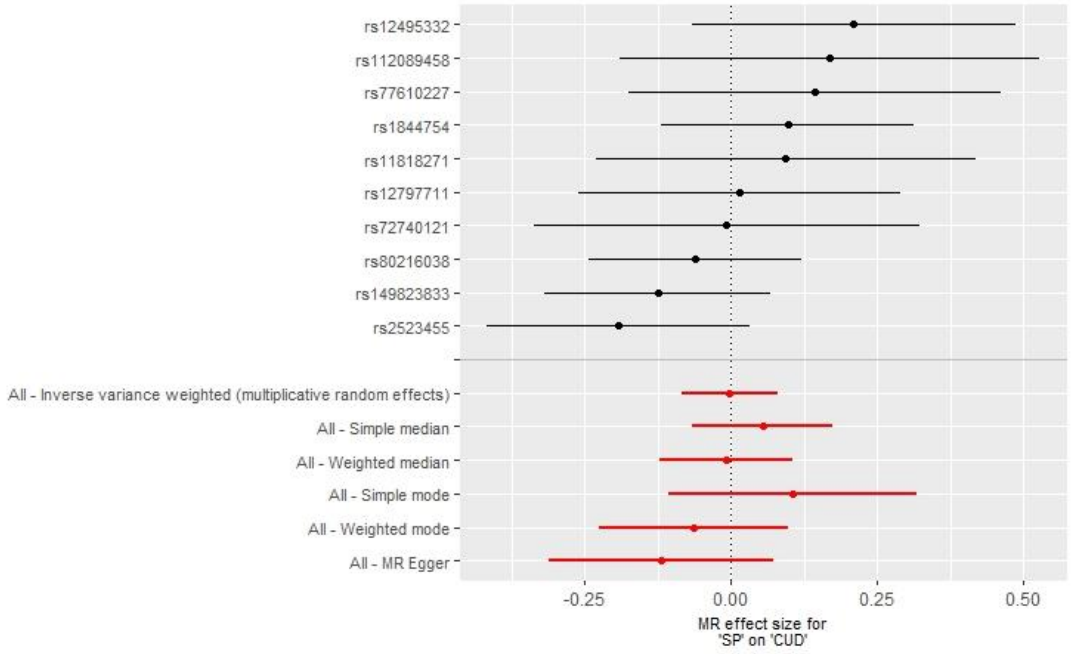

Figure S6-7. Funnel plot for SP as the exposure and CUD as the outcome

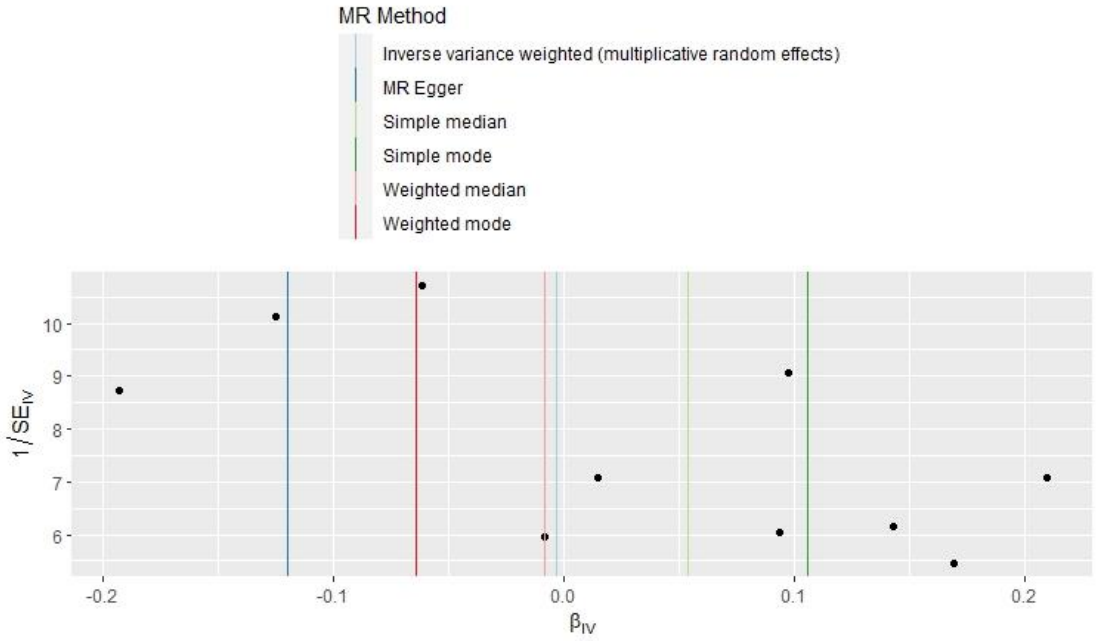

Figure S6-8. Leave-one-out plot for SP as the exposure and CUD as the outcome

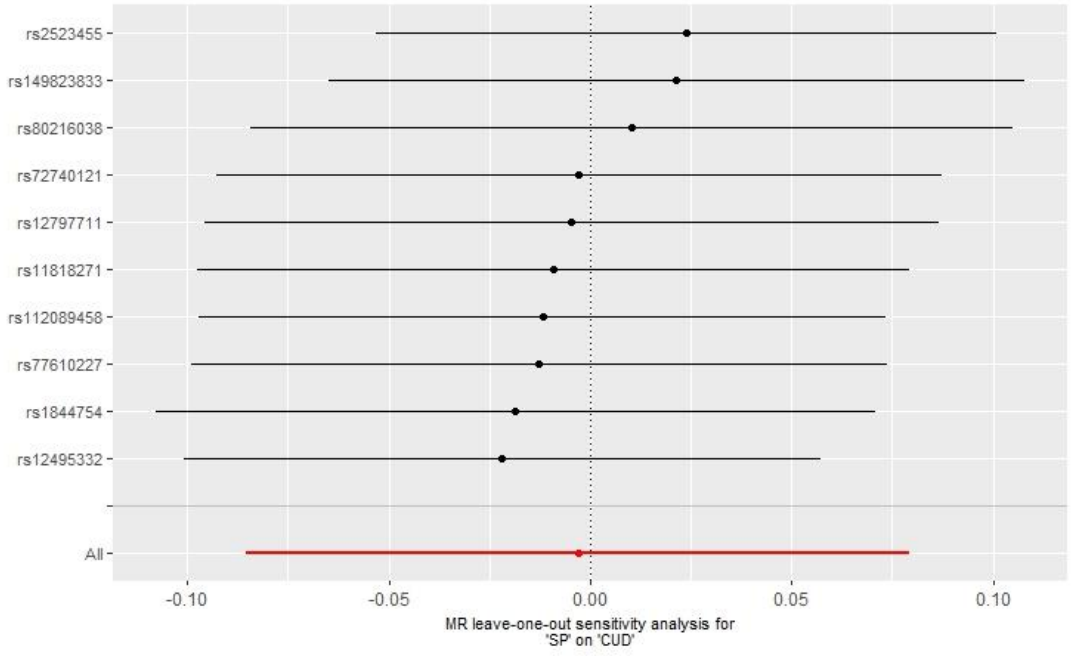

Figure S7-1. Scatter plot for CUD as the exposure and AG as the outcome

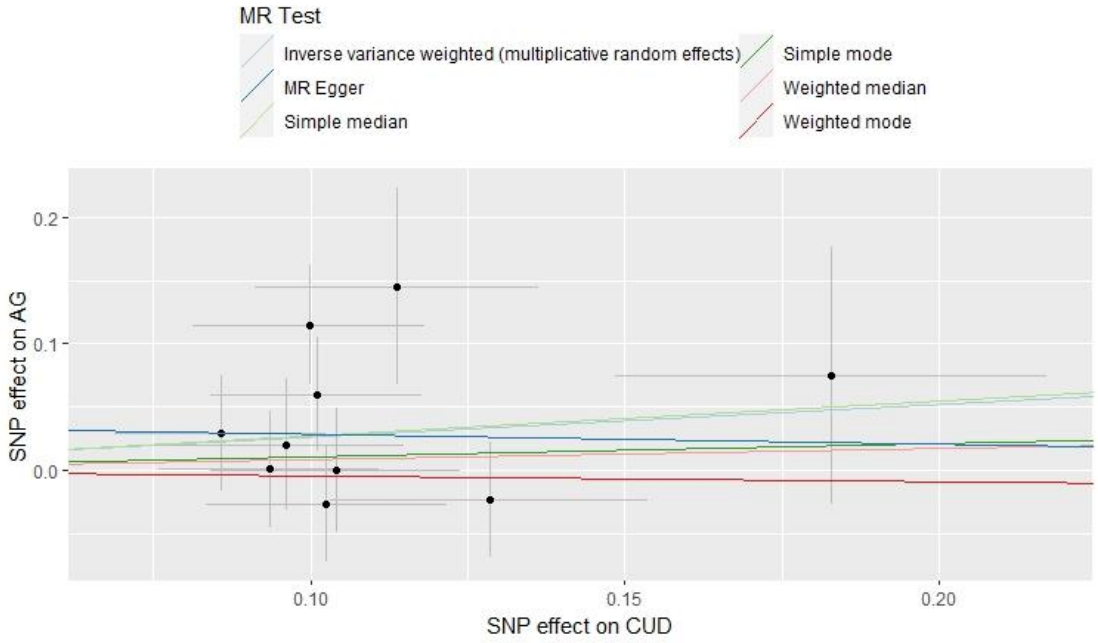

Figure S7-2. Forest plot for CUD as the exposure and AG as the outcome

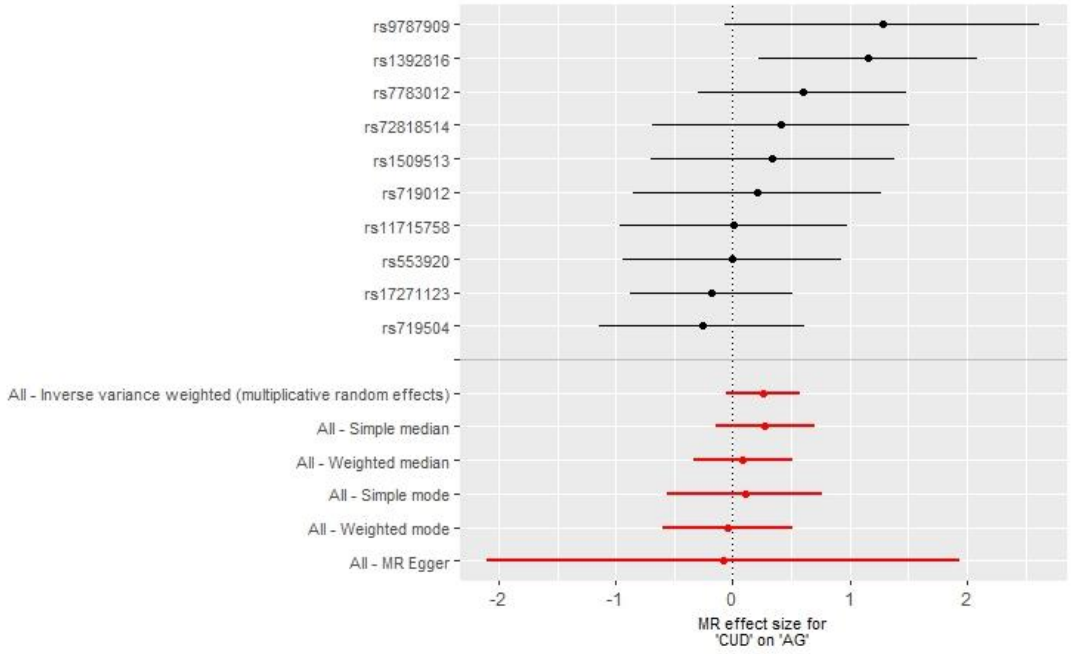

Figure S7-3. Funnel plot for CUD as the exposure and AG as the outcome

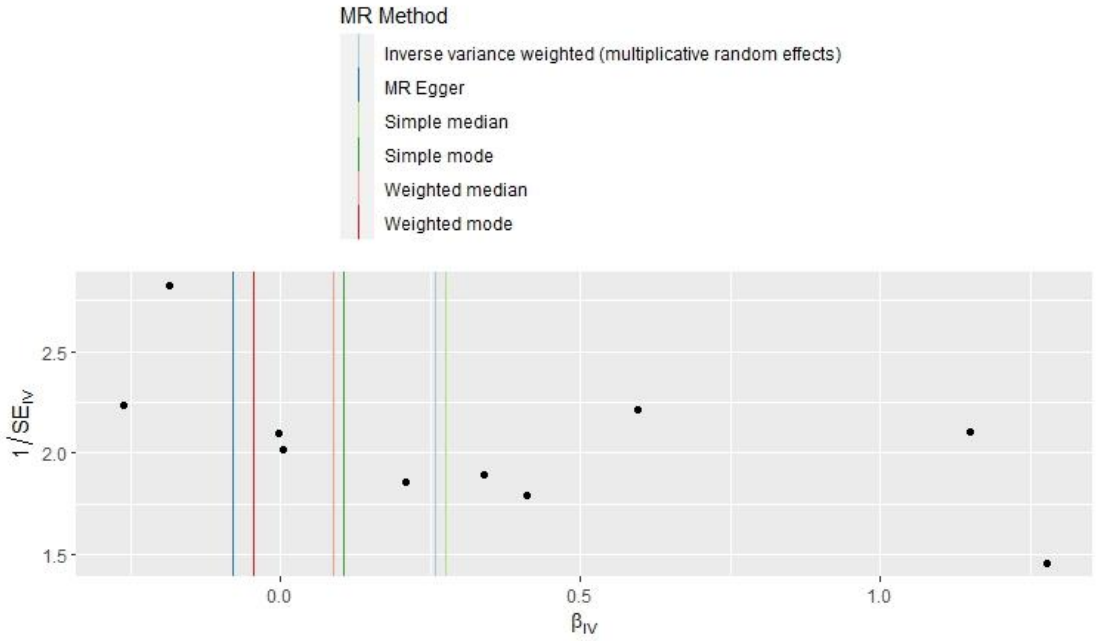

Figure S7-4. Leave-one-out plot for CUD as the exposure and AG as the outcome

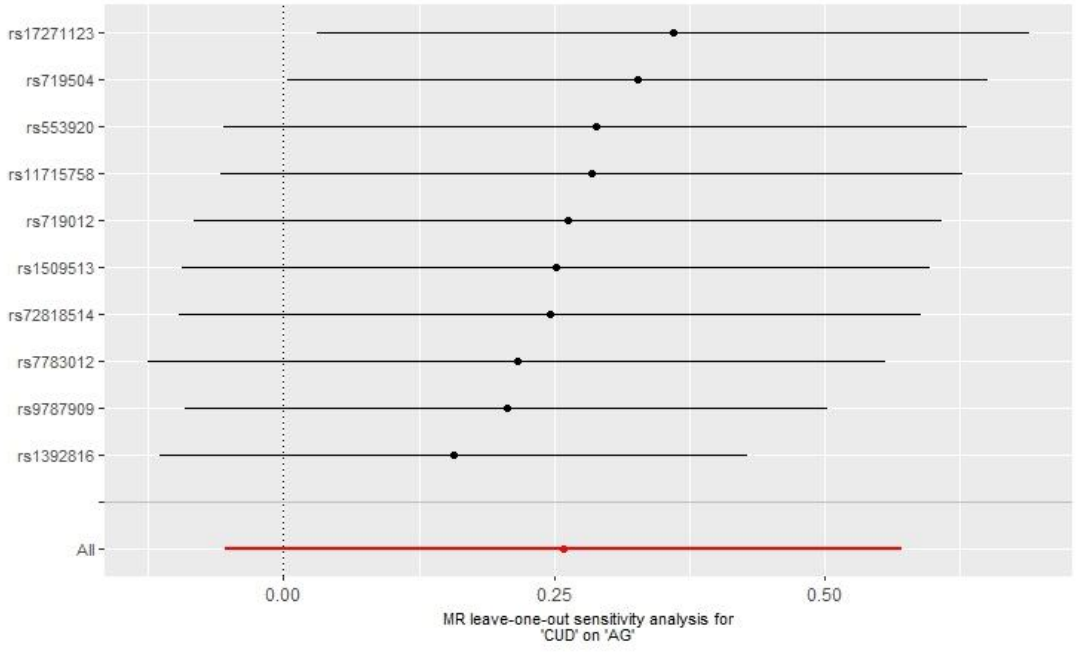

Figure S7-5. Scatter plot for AG as the exposure and CUD as the outcome

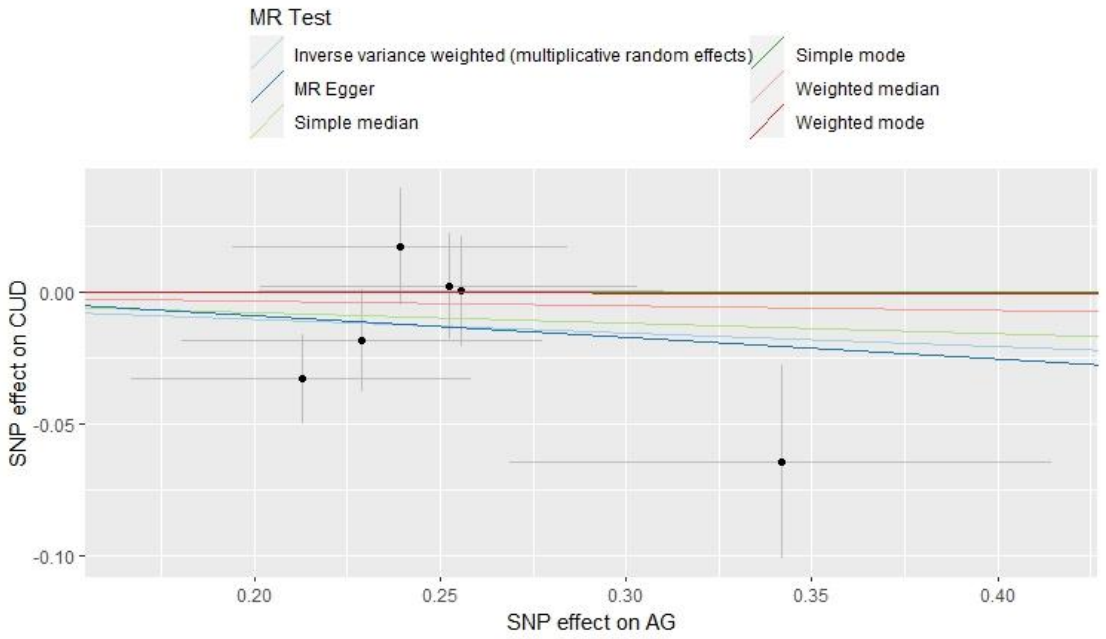

Figure S7-6. Forest plot for AG as the exposure and CUD as the outcome

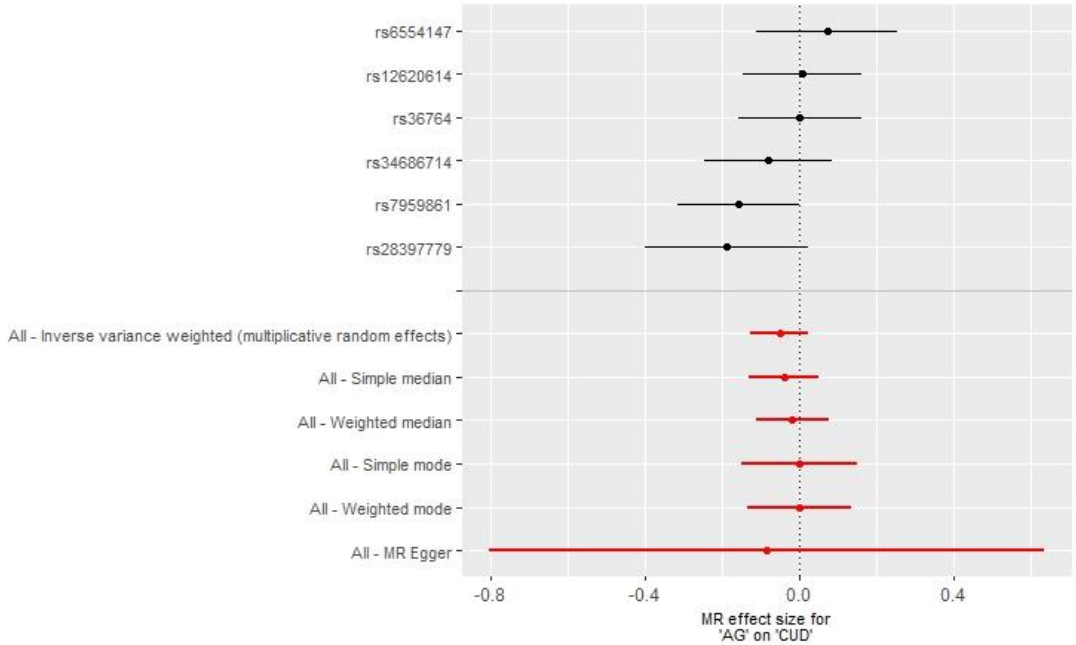

Figure S7-7. Funnel plot for AG as the exposure and CUD as the outcome

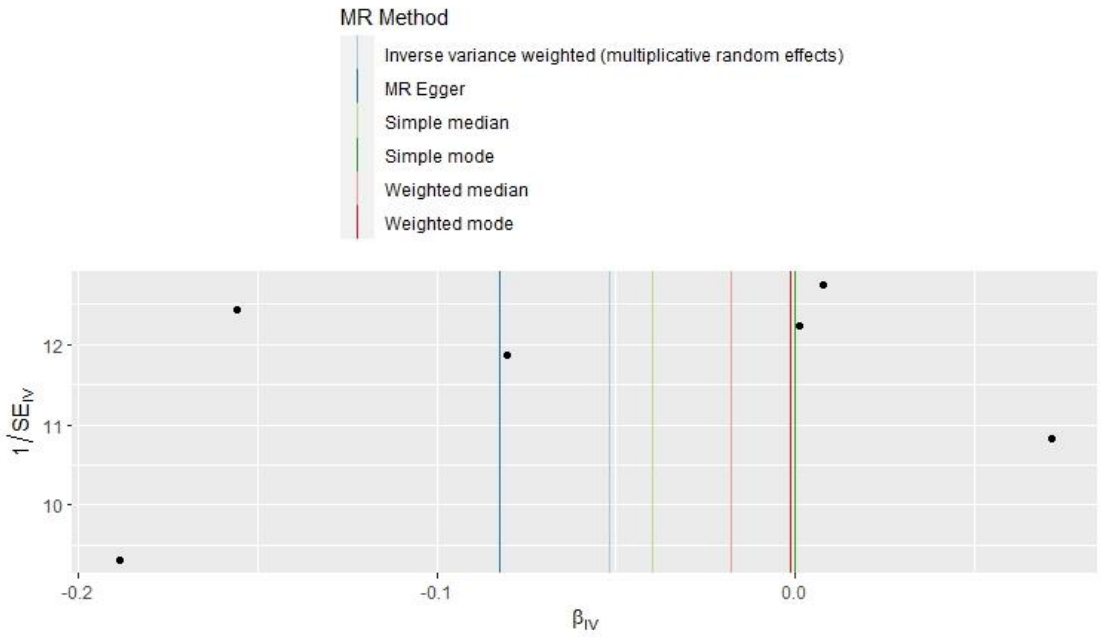

Figure S7-8. Leave-one-out plot for AG as the exposure and CUD as the outcome

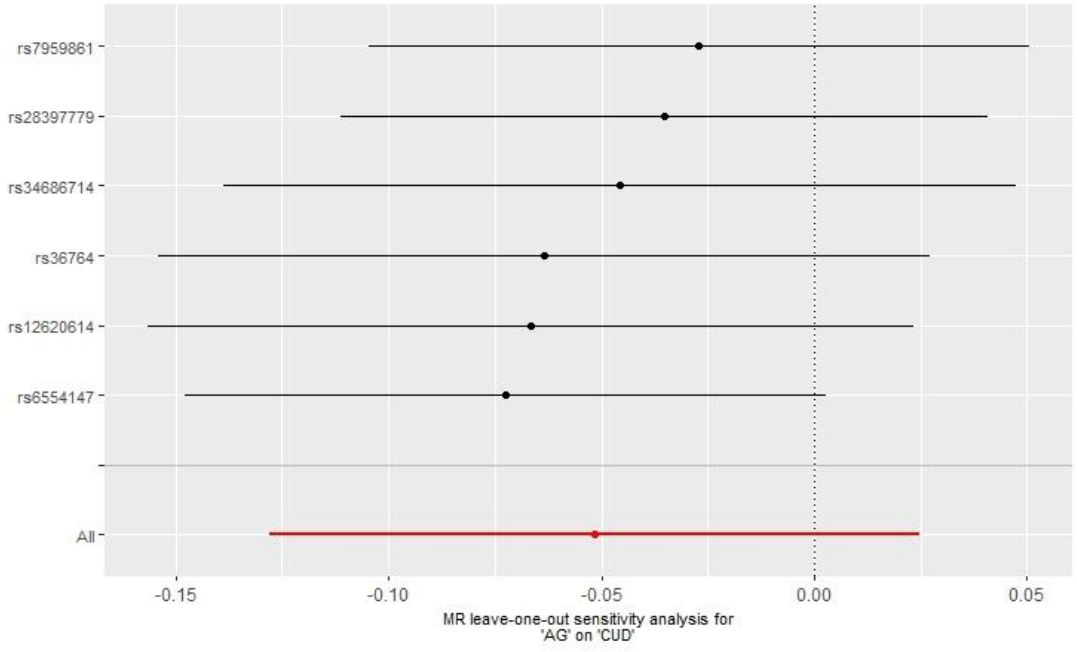

Figure S8-1. Scatter plot for CUD as the exposure and PD as the outcome

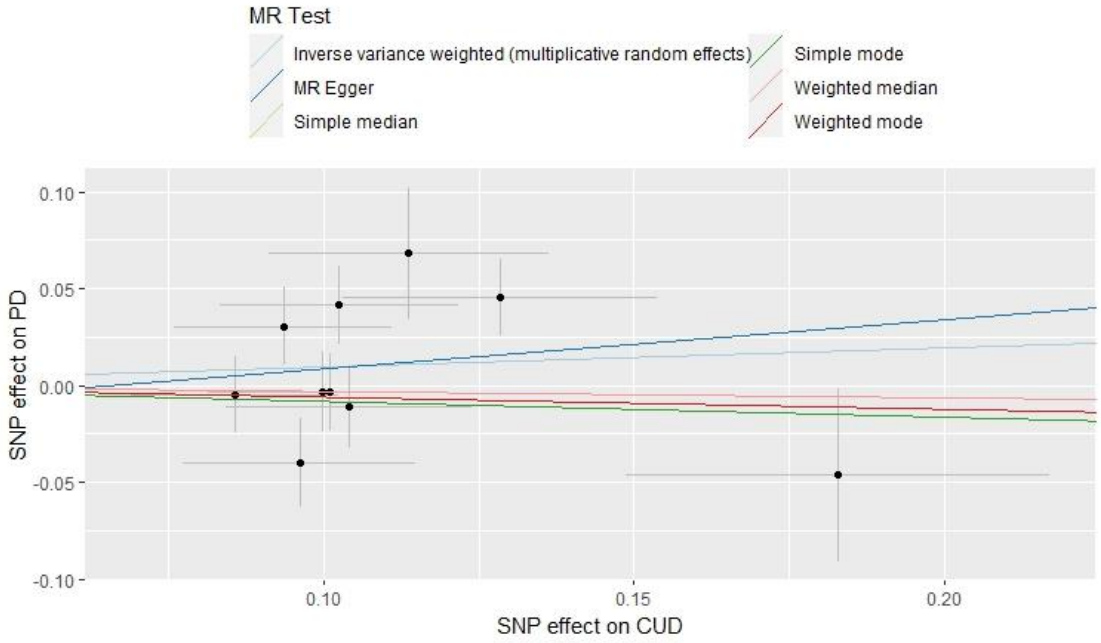

Figure S8-2. Forest plot for CUD as the exposure and PD as the outcome

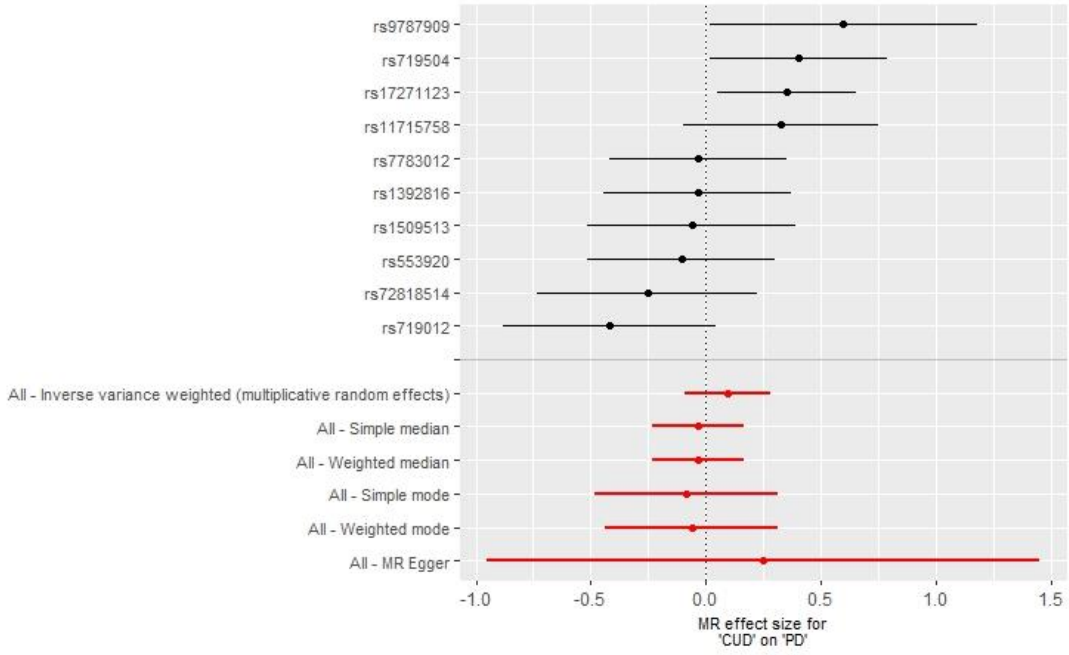

Figure S8-3. Funnel plot for CUD as the exposure and PD as the outcome

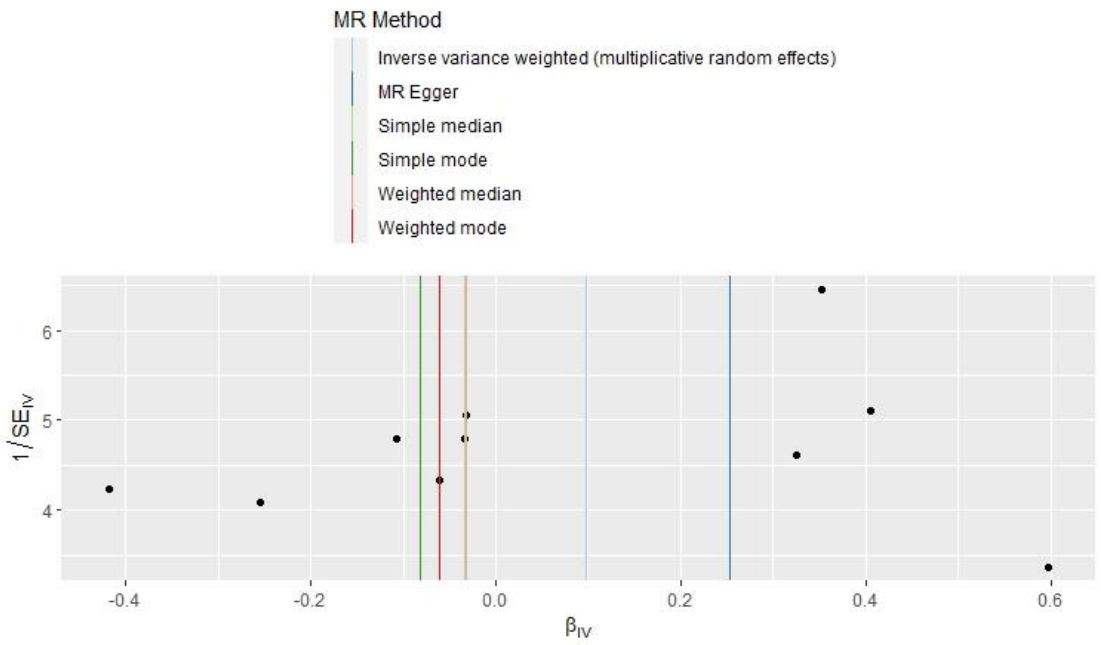

Figure S8-4. Leave-one-out plot for CUD as the exposure and PD as the outcome

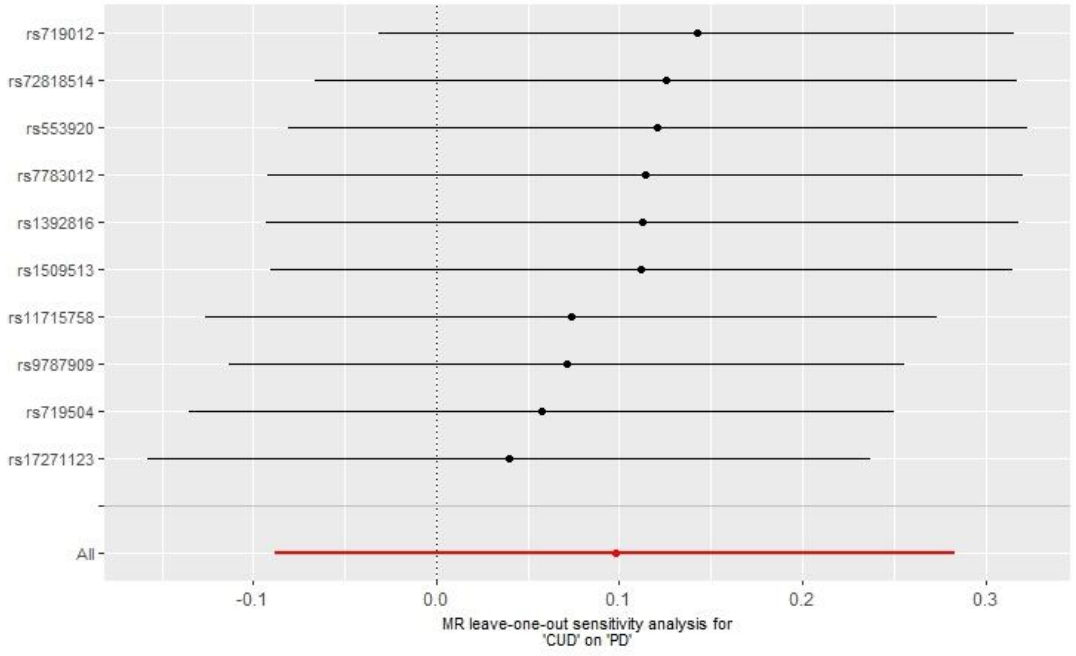

Figure S8-5. Scatter plot for PD as the exposure and CUD as the outcome

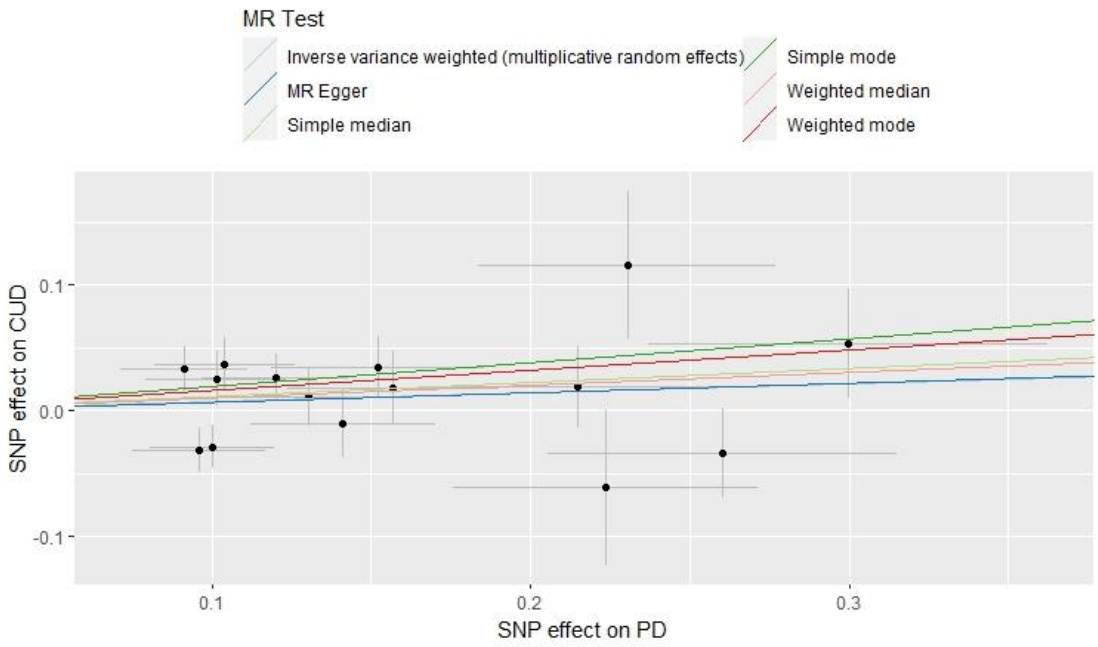

Figure S8-6. Forest plot for PD as the exposure and CUD as the outcome

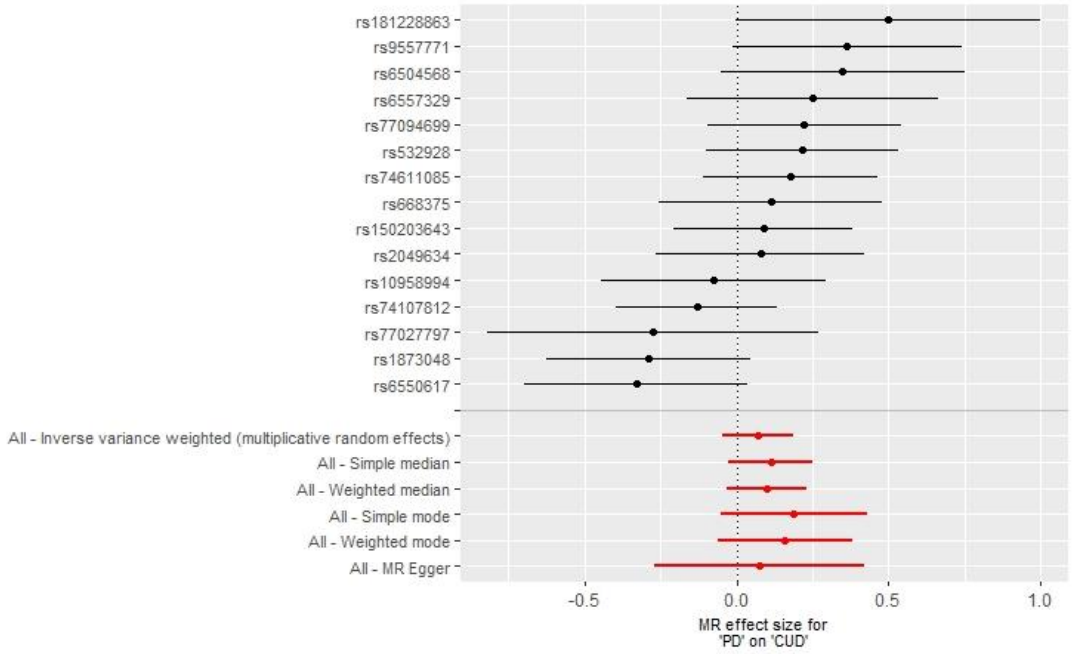

Figure S8-7. Funnel plot for PD as the exposure and CUD as the outcome

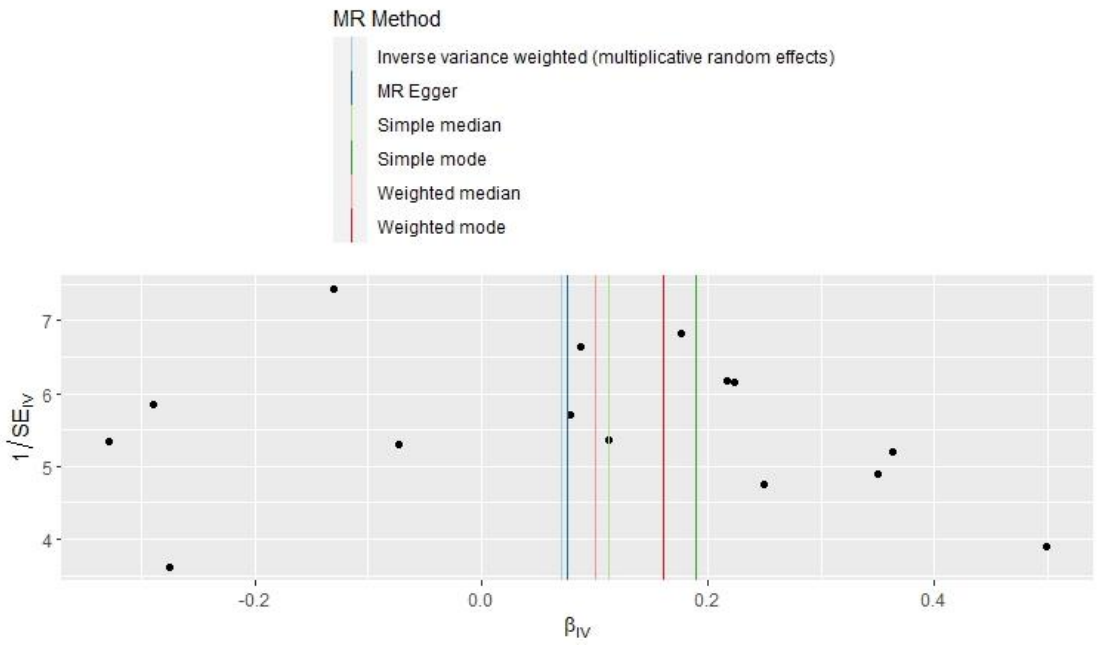

Figure S8-8. Leave-one-out plot for PD as the exposure and CUD as the outcome