Cannabis use and risk of schizophrenia: a Mendelian randomization study

Running title: Causal link between cannabis use and schizophrenia

Julien Vaucher[1*], MD, Brendan J. Keating[2], PhD, Aurélie M. Lasserre[3], MD, Wei Gan[4,5], PhD, Donald M. Lyall[6], PhD, Joey Ward[6], BSc, Daniel J. Smith[6], MD, Jill P. Pell[6], MD, Naveed Sattar[7], MD, PhD, Guillaume Paré[8,9,10,11], MD, Michael V. Holmes[12,13*], MD, PhD

1 Service of Internal Medicine, University Hospital of Lausanne, Lausanne, Switzerland
2 Department of Surgery, University of Pennsylvania, Philadelphia, PA, United States
3 Centre for Psychiatric Epidemiology and Psychopathology (CEPP), University Hospital of Lausanne, Prilly, Switzerland
4 Oxford Centre for Diabetes, Endocrinology, and Metabolism, Churchill Hospital Campus, University of Oxford, Oxford, UK
5 Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, UK
6 Institute of Health & Wellbeing, University of Glasgow, Scotland, UK
7 Institute of Cardiovascular and Medical Science, University of Glasgow, Scotland, UK
8 Population Health Research Institute, Hamilton Health Sciences, Department of Clinical Epidemiology and Biostatistics, McMaster University, Hamilton, ON, Canada
9 Population Genomics Program, Chanchlani Research Centre, McMaster University, Hamilton, ON, Canada
10 Department of Pathology and Molecular Medicine, McMaster University, Hamilton, ON, Canada
11 Thrombosis and Atherosclerosis Research Institute, McMaster University, Hamilton, ON, Canada
12 Clinical Trial Service Unit & Epidemiological Studies Unit (CTSU), Nuffield Department of Population Health, University of Oxford, UK
13 Medical Research Council Population Health Research Unit at the University of Oxford, UK

*Corresponding authors:

Dr Julien Vaucher
Service of Internal Medicine
Lausanne University Hospital (CHUV)
46, Rue du Bugnon
1011 Lausanne
Switzerland
T : 0044 77 1 777 25 24
E : julien.vaucher@chuv.ch

Dr Michael V. Holmes
Clinical Trial Service Unit & Epidemiological Studies Unit (CTSU)
Nuffield Department of Population Health
University of Oxford
United Kingdom
T : 0044 1865 74 3644
E : michael.holmes@ndph.ox.ac.uk

Abstract word count: 242
Total word count (excl. abstract and references): 3,493
Number of figures: 4
Number of references: 31

ABSTRACT

Cannabis use is observationally associated with an increased risk of schizophrenia, however whether the relationship is causal is not known. To determine the nature of the association between cannabis use on risk of schizophrenia using Mendelian randomization (MR) analysis, we used ten genetic variants previously identified to associate with cannabis use in 32,330 individuals. Genetic variants were used in a MR analyses of the association of genetically determined cannabis on risk of schizophrenia in 34,241 cases and 45,604 controls from predominantly European descent. Estimates from MR were compared to a meta-analysis of observational studies reporting effect estimates for ever use of cannabis and risk of schizophrenia or related disorders. Genetically determined use of cannabis was associated with increased risk of schizophrenia (OR of schizophrenia for users vs. non-users of cannabis: 1.37; 95%CI, 1.09 to 1.67; P-value=0.007). The corresponding estimate from observational analysis was 1.50 (95% CI, 1.10 to 2.00; P-value for heterogeneity = 0.88). The genetic instrument did not show evidence of pleiotropy on MR-Egger (Egger test, P-value=0.292) nor on multivariable MR accounting for tobacco exposure (OR of schizophrenia for users vs. non-users of cannabis, adjusted for ever vs. never smoker: 1.41; 95% CI, 1.09-1.83). Furthermore, the causal estimate remained robust to sensitivity analyses. These findings strongly support a causal association between genetically determined use of cannabis and risk of schizophrenia. Such robust evidence may inform public health message about the risks of cannabis use, especially regarding its potential mental health consequences.

INTRODUCTION

Cannabis is the most widely misused illicit drug with estimated 182 million consumers in 2013 globally.[1] Several high-profile observational studies have reported a

positive, dose-dependent association between cannabis use and risk of schizophrenia, especially in young people in whom cannabis use is particularly high.[2] The lifetime risk of schizophrenia is around 0.7%, and the natural history of disease carries a high risk of long-term symptoms and disability together with a reduced life expectancy.[3] In addition, schizophrenia represents a high economic burden with an estimated cost of $63 billion/year in the United States.[4] Clarifying the causal role between cannabis use and risk of schizophrenia is therefore important to understanding the health impacts of cannabis exposure and to inform on potential preventative strategies to alleviate the burden of disease from schizophrenia.[5]

A substantial body of observational evidence supports the hypothesis that cannabinoids play a role in the development of schizophrenia.[2] Prospective observational studies, with decades of follow-up and accounting for a large number of potential confounding factors (such as demographic, family history, personal history, socio-economic or other environmental markers) have consistently demonstrated that exposure to cannabis is associated with increased risk of schizophrenia or related disorders.[2] These findings have been reinforced by basic research experiments that point to cannabis altering various neurotransmission pathways linked to pathogenesis of psychotic disorders and by interfering with neurodevelopment in adolescents.[6] Despite this, any causal link between cannabis use and psychotic disorders remains controversial as observational findings can always be hampered by confounding (where another risk factor associated with cannabis actually causes disease) and/or reverse causality bias (where individuals affected by schizophrenia may be more prone to consume cannabis).[2, 7] Moreover, cannabis use is intimately associated with tobacco consumption and the latter has been observationally related to risk of schizophrenia, which may confound the link between cannabis and the disease.[8]

In the setting where a randomized trial – representing the optimal method to test a clinical hypothesis – of a harmful exposure (such as cannabis consumption) would be unethical, a genetic approach represents a valid alternative to assess causality free from confounding or reverse causality bias.[9] Using Mendelian randomization principles, causality between an exposure (such as cannabis use) and an outcome (e.g. schizophrenia) can be tested through use of genetic markers that associate with the exposure, employed as instrumental variables providing certain assumptions are met.[10] Recent developments of Mendelian randomization facilitate assessing the robustness of the genetic instrument by testing for presence of pleiotropy (where genetic markers associate with the outcome through more than one causal pathway, also known as horizontal, or directional, pleiotropy). Egger Mendelian randomization (MR-Egger) provides a statistical test for presence of pleiotropic effects of the genetic instrument, and a causal estimate that takes this into account , whereas multivariable MR provides a causal estimate for an exposure that statistically adjusts for a potential pleiotropic effect of the genetic marker(s) with a risk factor (e.g. tobacco consumption).[11, 12]

We used single nucleotide polymorphisms (SNPs) associated with ever use of cannabis reported in a recent genome-wide association study (GWAS),[13] as instrumental variables to clarify the causal role of cannabis consumption on risk of schizophrenia. We then assessed for presence of pleiotropy of the genetic instrument through MR-Egger and adjusted for potential shared pathways and/or confounding by tobacco consumption in multivariable MR. We additionally conducted sensitivity analyses by restricting to SNPs with putative functional roles and by sequentially excluding each SNP from the analysis. Finally, we compared the causal estimate to a meta-analysis of observational studies.

PARTICIPANTS AND METHODS

Observational analysis between ever use of cannabis and risk of schizophrenia

Observational studies reporting an association between cannabis use and risk of schizophrenia were selected from a recent and comprehensive review of the literature (published in 2016) and a meta-analysis from 2007 reporting prospective studies showing an association between cannabis use and schizophrenia.[2, 14] As only one study reported schizophrenia as an outcome,[15] we slightly broadened our inclusion criteria to also include studies reporting related disorders (schizophreniform disorder and psychotic symptoms). To identify additional studies that may be eligible for inclusion since the meta-analysis from 2007, we conducted a PubMed search (**Figure S1**).

To compare with the causal estimate (see below), we restricted to studies that reported ever use of cannabis (compared to never users of cannabis) as an exposure and a corresponding risk estimate for schizophrenia or related disorders and identified four studies that met these criteria.[15-18] We found one additional study in which the definition of the exposure was similar (any use of cannabis, provided that individuals have consumed cannabis ≥5 times) and also included it in the analysis.[19] The pooled effect estimate was derived using a random-effects meta-analysis of study summary estimates. **Tables S1 and S2** summarize the main characteristics of included and excluded studies, respectively.

Genetic markers associated with ever use of cannabis

We used the 10 leading SNPs from a recent GWAS (contributing studies outlined in **Table S3**), comprising data of participants from European ancestry predominantly, on cannabis use (phenotype defined as ever use of cannabis during participants' lifetime) to obtain the gene-exposure (SNP-cannabis) association estimates and their corresponding standard errors (SE) (**Table S4**).[13] Although none of the SNPs surpassed a conventional

genome-wide significance threshold (P-values comprised between 4.6×10^{-7} and 3.1×10^{-6} in the discovery analysis), estimates were directionally consistent across the vast majority of contributing studies (**Table S4**). These SNPs can individually, and cumulatively, be considered as valid instruments for Mendelian randomization analysis.[20]

Association between cannabis-associated genetic markers and risk of schizophrenia

The gene-outcome (SNP-risk of schizophrenia) association estimates were obtained using the publicly available GWAS repository on schizophrenia from the Psychiatric Genomics Consortium (http://www.med.unc.edu/pgc/downloads). **Table S5** describes the contributing studies. SNPs were directly matched with the 10 SNPs associated with ever use of cannabis. The number of individuals and the relationships between datasets are presented in **Figures S2**. We used the same reference allele for each SNP to orientate cannabis and schizophrenia estimates.

Statistical analysis

Mendelian randomization analysis was conducted by first generating an instrumental variable (IV) estimate for each SNP. The IV estimate for each SNP was generated by dividing the association of each SNP with risk of schizophrenia by the corresponding association with risk of ever use of cannabis and the standard error was estimated using the delta method.[21] We pooled instrumental variable estimates across SNPs using a fixed-effects meta-analysis. Estimates of the association of each SNP with ever use of cannabis were not transformed. In order to generate a Mendelian randomization estimate for 'users vs. non-users' of cannabis (as opposed to a per-1-log unit increase in ever use of cannabis), we transformed the summary estimate from meta-analysis using estimates of risk of schizophrenia in the population, and the prevalence of schizophrenia in never users of cannabis, as previously described.[22] A full description of the methodology is provided in the **Supplement**.

Characteristics of the genetic instrument

(i) Strength of instrument and power to detect a causal effect

In Mendelian randomization analyses, but especially in the context where multiple SNPs that did not achieve GWAS significance are used cumulatively, there are certain characteristics that need to be tested.

First, a concern might be weak instrument bias. Conventionally, when using datasets that overlap for the SNP-exposure and SNP-outcome, this can generate biased estimates and yield an inflated causal estimate (arising from correlation of the error terms of SNP-exposure and SNP-outcome).[23] However, in our case, there was only minimal overlap (4.7%) between the datasets used to derive the effect estimates for SNPs with ever use of cannabis and risk of schizophrenia (**Figure S2**), minimizing the possibility of weak instrument bias yielding a false positive association. I.e. in the context of non-overlapping datasets, weak instrument bias result in a false negative association.[23]

We estimated instrument strength by calculating the proportion of variance in use of cannabis explained by each SNP. We then derived the F-statistic of each SNP individually and cumulatively (full details provided in the **Supplement**).

We estimated power to detect the same magnitude of association reported in the observational studies, using a two-sided alpha of 0.05. Power was 100% and is presented in **Table S6**.

(ii) Assessment of directional pleiotropy

We tested for presence of unmeasured pleiotropy of the genetic instruments using MR-Egger as described by Bowden *et al*.[11] Essentially, this uses the same principles of testing for small study bias in meta-analysis. The methodology was similar as for conventional Mendelian randomization analysis (described above), with the exception that all alleles (and

corresponding estimates) were oriented in the direction of an increase in the exposure prior to the analyses. The standard error was obtained by bootstrap resampling 10,000 times.

Sensitivity analyses

As tobacco consumption has been related to risk of schizophrenia and shares a strong genetic correlation with cannabis in Stringer et al,[8, 13] we conducted a multivariable MR – to adjust for shared pathways with and/or potential confounding by tobacco - using summary statistics for the association of each of the 10 cannabis-related SNPs with tobacco (ever vs. never smokers) derived from 111,898 participants (51,984 ever smokers and 59,914 never smokers) from the UK Biobank (http://www.ukbiobank.ac.uk). Selection of participants and genotyping are described in the **Supplement**. Multivariable MR was conducted by regressing the SNP-cannabis estimates on SNP-schizophrenia estimates adjusting for SNP-tobacco estimates.[12] The standard error was obtained by bootstrap resampling 10,000 times.

We conducted two sensitivity analyses. First, we assessed the robustness of the summary causal estimate to inclusion of SNPs by sequentially removing each SNP from the Mendelian randomization analysis.

Second, we restricted the analyses to two SNPs (rs73067624 and rs4471463) located within two genes (*KCNT2* [1q31] and *NCAM1* [11q23], respectively) that were associated with ever use of cannabis in the gene-based tests of associations in Stringer *et al*.[13] These two genes are potentially functional: *KCNT2* encodes a potassium voltage-gated channel that may play a role in addiction.[13, 24] Previous studies have found that markers linked to *KCNT2* are related to cocaine dependence and opioid consumption.[24] *NCAM1* regulates pituitary growth hormone secretion and is implicated in dopaminergic neurotransmission,[13] and has been associated with dependence to nicotine, alcohol and heroin.[25]

All statistical analyses were conducted using Stata v.13.1 (Stata Corp, TX, USA).

RESULTS

Observational association between ever use of cannabis and risk of schizophrenia and related disorders

One prospective study met our primary research criteria and reported that ever use of cannabis (compared to no use) was associated with an odds ratio (OR) for schizophrenia of 1.50 (95% CI, 1.10-2.00). When meta-analysing this estimate with other prospective observational studies reporting related traits, including schizophreniform disorder and psychotic symptoms (encompassing a total of 1,326 cases and 58,263 controls), ever use of cannabis was associated with a 43% increase in the risk of schizophrenia or related disorders (OR, 1.43; 95% CI, 1.19-1.67; I^2=0%) using random-effects modelling (**Figure 1**).

Association between genetically determined ever use of cannabis and risk of schizophrenia

The 10 SNPs associated with ever use of cannabis explained 1.0% of its variance. There was a positive genetic association between ever use of cannabis and risk of schizophrenia (**Figure S3**). In Mendelian randomization analysis based on 34,241 cases of schizophrenia and 45,604 controls, ever use of cannabis was causally associated with risk of schizophrenia (Log OR per-1-log unit increase in ever use of cannabis, 0.07; 95% CI, 0.02-0.13; P-value=0.007) (**Figure 2**). Applying population-based estimates, this translated to a 37% increase in the risk of schizophrenia (OR for users vs. non-users of cannabis, 1.37; 95% CI, 1.09-1.67) (**Figure 3**). The Mendelian randomization estimate was consistent with estimates derived from observational analyses restricted to schizophrenia alone (test for heterogeneity, chi^2=0.23; P-value=0.634) or schizophrenia and related disorders combined (test for heterogeneity, chi^2=0.10; P-value=0.755) (**Figure 3**)

Assessment of pleiotropy of the genetic instrument

No evidence of unmeasured pleiotropy of the genetic instrument was identified using MR-Egger (P-value for pleiotropy=0.292). The estimates derived from MR-Egger and conventional Mendelian randomization are presented in **Figures S4** and **S5**.

Adjusting for the association of SNPs in the genetic instrument for smoking in multivariable MR did not show evidence of shared pathways and/or confounding with a causal estimate of schizophrenia from users of cannabis that remained stable (OR, 1.41; 95% CI, 1.09-1.83) (**Figure 3**).

Sensitivity analyses

To further test the stability of the Mendelian randomization estimate to inclusion of SNPs that could individually distort the genetic association between cannabis use and schizophrenia, we sequentially removed each SNP from the analysis. The direction and significance of the summary association between ever use of cannabis and risk of schizophrenia remained unchanged using this approach (**Figure 4**). Furthermore, restricting the analysis to two putative functional SNPs (rs73067624 and rs4471463) showed a persistent causal association (OR for users vs. non-users of cannabis, 1.88; 95% CI, 1.00-3.21) (**Figure S6**).

DISCUSSION

This study is the first to clarify that genetically determined use of cannabis is causally associated with increased risk of schizophrenia. This finding strongly corroborates many previous prospective observational studies that identified cannabis users to be at increased risk of schizophrenia, but that could not tease out correlation from causality. As cannabis is the leading drug of misuse, this finding is timely to draw attention to the potential mental

health consequences of cannabis use and to provide more robust scientific evidence to inform the public health debate on cannabis legalization.

During the last 30 years, epidemiological observations have consistently demonstrated a strong, positive and dose-dependent association between cannabis use and risk of psychotic disorders.[2, 14] The direction and the strength of the association persisted even after adjusting for measured confounders and with long periods (~25 years) of follow-up (to attempt to minimize confounding and reverse causality bias, respectively). Our meta-analysis of prospective observational studies confirmed these findings in a magnitude that tallies remarkably closely with previous reports.[14] Despite the consistency of observational data, clarifying whether or not cannabis use causally influences risk of schizophrenia has remained challenging. This is because observational studies, even accounting for confounding factors, can be affected by biases that can undermine the validity (such as residual confounding).[2] As such, the ability to answer the question on causality has been at an impasse, as a randomized controlled trial, (considered the gold standard to test a hypothesis), is not possible for ethical reasons, as it would involve exposing participants to a potentially harmful exposure (a similar scenario to examining whether alcohol protects against risk of cardiovascular disease).[26] In this setting, Mendelian randomization can provide pivotal information on causality, that can be of public health importance and inform public health guidelines.[27] Our findings strongly support the large body of evidence from observational studies that exposure to cannabis plays a causal role in the development of schizophrenia.

Our findings are supported by studies that show that expression of schizophrenia-associated cerebral cannabinoid receptors are modified by cannabis use[28] and that cortical maturation is altered by cannabis use in adolescents.[29] More compellingly, small randomized

trials involving human participants in laboratory conditions suggest that exposure to delta-9-tetrahydrocannabinol (THC) confers a risk to developing symptoms that mimic psychotic disorders.[6] Observationally and genetically tobacco use is strongly correlated with cannabis use and has been proposed to act synergistically with cannabis to establish addiction.[8] Moreover, the association between cannabis and psychotic experiences has been shown to be modulated by tobacco use, i.e. accounting for tobacco use reduces the cannabis-schizophrenia relationship.[30] Hence, the lack of confounding by tobacco consumption, as tested by multivariable MR analysis, strengthens the findings of a primary association between cannabis use and risk of schizophrenia. Finally, our sensitivity analysis restricting to two genes with presumptive functional roles in drug dependence may suggest that cannabis affects addiction mechanisms that in turn influence the risk of schizophrenia. However, against this theory is the observation that other drugs of addiction are less associated to risk of schizophrenia or related disorders.[31] Moreover any influence of addictive mechanisms would not undermine our findings, since cannabis exposure may be necessary to establish dependence, and addiction mechanisms could lie on the same causal pathway (**Figure S7**).

Limitations include that our study did not permit investigation of the risk of schizophrenia in relation to the quantity, type or route of administration of cannabis. Second, the precise mechanisms explaining of how some of the genetic markers under analysis alter cannabis use (or dependence) are remain unknown; however, this is not a necessary requirement to conduct a Mendelian randomization analysis using multiple loci. Third, the SNPs used in the analysis did not reach conventional genome-wide association significance thresholds. However, directions of effect were consistent in the vast majority of studies (**Table S4**) and combining individual SNPs for an analysis such as this remains valid provided the genetic instrument does not suffer from weak instrument bias. In that regard,

in the context of conducting summary-level Mendelian randomization analysis using non-overlapping data sources for the exposure and outcome (as we report here), weak instrument bias would bias the effect towards the null (i.e. opposite to weak instrument bias in overlapping datasets).[23] This greatly increases confidence in the Mendelian randomization estimate that we report. Furthermore, our sensitivity analyses were robust to various approaches to test for stability of the causal estimates. Fourth, MR-Egger may have been underpowered to detect directional pleiotropy of the genetic instrument (if it were present).[11] Against presence of major directional pleiotropy of any individual SNP is our analysis that excluded each SNP in turn, and to which our causal estimate remained robust. It is noteworthy that, despite these potential limitations, this study represents the closest approximation to a randomized trial on the effect of ever use of cannabis and risk of schizophrenia.

In summary, a genetic approach – representing an alternative approach to assess causality when a randomized trial would be unethical – strongly supports the notion that use of cannabis is causally related to risk of schizophrenia. This may help inform public health debate on cannabis use and preventive strategies to alleviate the burden of disease from schizophrenia.

ACKNOWLEDGEMENT

The publicly available GWAS results on schizophrenia were obtained from the Psychiatric Genomics Consortium, University of North Carolina at Chapel Hill (https://www.med.unc.edu/pgc/results-and-downloads). The authors express their gratitude to the participants and research teams that permitted to build the publicly available dataset from the Schizophrenia Working Group of the Psychiatric Genomics Consortium, the International Cannabis Consortium, and the UK Biobank.

Sources of support: JV is supported by the Swiss National Science Foundation (P2LAP3_155086). AML is supported by the Swiss National Science Foundation (323530_151479). This research has been conducted using the UK Biobank resource which has been funded by the Wellcome Trust medical charity, Medical Research Council, Department of Health of Scottish Government, the Northwest Regional Development Agency, the Welsh Assembly Government and the British Heart Foundation.

CONFLICT OF INTEREST

No disclosures were reported.

REFERENCES

1. United Nations Office on Drugs and Crime, World Drug Report 2015 (United Nations publication, Sales No. E.15.XI.6). http://www.unodc.org/documents/wdr2015/World_Drug_Report_2015.pdf. *Accessed 2016, 16 May*.

2. Gage SH, Hickman M, Zammit S. Association Between Cannabis and Psychosis: Epidemiologic Evidence. *Biol Psychiatry* 2016; **79:** 549-556.

3. Owen MJ, Sawa A, Mortensen PB. Schizophrenia. *Lancet* 2016; **14:** 1121-1126.

4. McEvoy JP. The costs of schizophrenia. *J Clin Psychiatry* 2007; **14:** 4-7.

5. Csete J, Kamarulzaman A, Kazatchkine M, Altice F, Balicki M, Buxton J *et al.* Public health and international drug policy. *Lancet* 2016; **387**(10026)**:** 1427-1480.

6. Sherif M, Radhakrishnan R, D'Souza DC, Ranganathan M. Human Laboratory Studies on Cannabinoids and Psychosis. *Biol Psychiatry* 2016; **79:** 526-538.

7. Large M, Di Forti M, Murray R. *Cannabis: debated schizophrenia link*. *Nature* 2015;**527**:305.

8. Rabin RA, George TP. A review of co-morbid tobacco and cannabis use disorders: possible mechanisms to explain high rates of co-use. *The American journal on addictions / American Academy of Psychiatrists in Alcoholism and Addictions* 2015; **24:** 105-116.

9. Burgess S, Timpson NJ, Ebrahim S, Davey Smith G. *Mendelian randomization: where are we now and where are we going? Int J Epidemiol 2015;**44**:379-88.*, vol. .

10. Burgess S, Butterworth A, Thompson SG. Mendelian randomization analysis with multiple genetic variants using summarized data. *Genet Epidemiol* 2013; **37:** 658-665.

11. Bowden J, Davey Smith G, Burgess S. Mendelian randomization with invalid instruments: effect estimation and bias detection through Egger regression. *International journal of epidemiology* 2015; **44:** 512-525.

12. Burgess S, Dudbridge F, Thompson SG. *Re: "Multivariable Mendelian randomization: the use of pleiotropic genetic variants to estimate causal effects"*. *Am J Epidemiol* 2015 Feb 15;**181**:290-1.

13. Stringer S, Minica CC, Verweij KJ, Mbarek H, Bernard M, Derringer J *et al.* Genome-wide association study of lifetime cannabis use based on a large meta-analytic sample of 32 330 subjects from the International Cannabis Consortium. *Transl Psychiatry* 2016; **29:** 36. doi: 10.1038/tp.2016.

14. Moore TH, Zammit S, Lingford-Hughes A, Barnes TR, Jones PB, Burke M *et al.* Cannabis use and risk of psychotic or affective mental health outcomes: a systematic review. *Lancet* 2007; **370:** 319-328.

15. Zammit S, Allebeck P, Andreasson S, Lundberg I, Lewis G. Self reported cannabis use as a risk factor for schizophrenia in Swedish conscripts of 1969: historical cohort study. *BMJ* 2002; **325**(7374).

16. Tien AY, Anthony JC. Epidemiological analysis of alcohol and drug use as risk factors for psychotic experiences. *J Nerv Ment Dis* 1990; **178:** 473-480.

17. van Os J, Bak M, Hanssen M, Bijl RV, de Graaf R, Verdoux H. Cannabis use and psychosis: a longitudinal population-based study. *Am J Epidemiol* 2002; **156:** 319-327.

18. Arseneault L, Cannon M, Poulton R, Murray R, Caspi A, Moffitt TE. Cannabis use in adolescence and risk for adult psychosis: longitudinal prospective study. *BMJ* 2002; **325**(7374)**:** 1212-1213.

19. Henquet C, Krabbendam L, Spauwen J, Kaplan C, Lieb R, Wittchen HU *et al.* Prospective cohort study of cannabis use, predisposition for psychosis, and psychotic symptoms in young people. *BMJ* 2005; **330. doi 10.1136/bmj.38267.664086.63.**

20. Burgess S, Small DS, Thompson SG. A review of instrumental variable estimators for Mendelian randomization. *Stat Methods Med Res* 2015;17. doi: 10.1177/0962280215597579.

21. Thomas DC, Lawlor DA, Thompson JR. *Re: Estimation of bias in nongenetic observational studies using "Mendelian triangulation" by Bautista et al. Ann Epidemiol 2007;17:511-3.*

22. Ross S, Gerstein HC, Eikelboom J, Anand SS, Yusuf S, Pare G. Mendelian randomization analysis supports the causal role of dysglycaemia and diabetes in the risk of coronary artery disease. *Eur Heart J* 2015; **36:** 1454-1462.

23. Burgess S, Davies NM, Thompson SG. Bias due to participant overlap in two-sample Mendelian randomization. *Gen Epidemiol* 2016. In press.

24. Gelernter J, Kranzler HR, Sherva R, Koesterer R, Almasy L, Zhao H *et al.* Genome-wide association study of opioid dependence: multiple associations mapped to calcium and potassium pathways. *Biol Psychiatry* 2014; **76:** 66-74.

25. Yang BZ, Kranzler HR, Zhao H, Gruen JR, Luo X, Gelernter J. Haplotypic variants in DRD2, ANKK1, TTC12, and NCAM1 are associated with comorbid alcohol and drug dependence. *Alcohol Clin Exp Res* 2008; **32:** 2117-2127.

26. Holmes MV, Dale CE, Zuccolo L, Silverwood RJ, Guo Y, Ye Z *et al.* Association between alcohol and cardiovascular disease: Mendelian randomisation analysis based on individual participant data. *BMJ* 2014;**10**. doi: 10.1136/bmj.g4164.

27. Piepoli MF, Hoes AW, Agewall S, Albus C, Brotons C, Catapano AL *et al.* 2016 European Guidelines on cardiovascular disease prevention in clinical practice: The Sixth Joint Task Force of the European Society of Cardiology and Other Societies on Cardiovascular Disease Prevention in Clinical Practice (constituted by representatives of 10 societies and by invited experts): Developed with the special contribution of the European Association for Cardiovascular Prevention & Rehabilitation (EACPR). *Eur Heart J* 2016; **23**.

28. Dean B, Sundram S, Bradbury R, Scarr E, Copolov D. Studies on [3H]CP-55940 binding in the human central nervous system: regional specific changes in density of cannabinoid-1 receptors associated with schizophrenia and cannabis use. *Neuroscience* 2001; **103:** 9-15.

29. French L, Gray C, Leonard G, Perron M, Pike GB, Richer L *et al.* Early Cannabis Use, Polygenic Risk Score for Schizophrenia and Brain Maturation in Adolescence. *JAMA Psychiatry* 2015; **72:** 1002-1011.

30. Gage SH, Hickman M, Heron J, Munafo MR, Lewis G, Macleod J *et al.* Associations of cannabis and cigarette use with psychotic experiences at age 18: findings from the Avon Longitudinal Study of Parents and Children. *Psychol Med* 2014; **44:** 3435-3444.

31. Dekker N, Meijer J, Koeter M, van den Brink W, van Beveren N, Kahn RS *et al.* Age at onset of non-affective psychosis in relation to cannabis use, other drug use and gender. *Psychol Med* 2012; **42:** 1903-1911.

FIGURES TITLES AND LEGENDS

Figure 1 Meta-analysis of prospective observational studies reporting an association between use of cannabis and risk of schizophrenia or related disorders

Random-effects meta-analysis of observational studies. Studies are sorted by type of outcome. Odds ratios (OR) and 95% confidence intervals (CI) express the risk of schizophrenia or psychotic symptoms for ever use of cannabis (compared to never use). For additional information on each study, see **Table S1**. Dunedin, Dunedin Multidisciplinary Health & Development Study; NEMESIS, Netherlands Mental Health Survey and Incidence Study; SC, Swedish Cohort; EDSP, Early Developmental Stages of Psychopathology Study; ECA, Epidemiologic Catchment Area.

Figure 2 Meta-analysis of the association of genetically-determined use of cannabis and risk of schizophrenia for the 10 SNPs in the genetic instrument

Fixed-effect meta-analysis of the instrumental variable estimates for each of the 10 SNPs associated with ever use of cannabis. Log odds ratios (Log OR) and 95% confidence intervals (CI) express the risk of schizophrenia per-1-log unit increase in ever use of cannabis. The exponentiated summary estimate corresponds to an OR for schizophrenia of 1.08 (95%CI: 1.02, 1.14) per 1-log unit increase in ever use of cannabis. The method to derive the population-based OR of schizophrenia among users of cannabis compared to non-users (OR 1.55; 95%CI, 1.14, 2.00), as presented in the main text and **Figure 3**, is described in the **Supplement**.

Figure 3 Comparison of observational (blue) and causal (red) estimates for use of cannabis and risk of schizophrenia

Two observational estimates are provided according to a stringent definition of schizophrenia (as reported in the Swedish cohort[15]) or to an outcome comprising studies reporting risk of schizophrenia or psychotic symptoms (derived from the meta-analysis reported in **Figure 1**) for ever use of cannabis. Causal estimates represent population-based associations derived by conventional (**Figure 2**) and multivariable MR. The total number of cases and controls in each analysis are presented.

Figure 4 Sensitivity analysis of the association of use of cannabis and risk of schizophrenia by sequentially removing each SNP from the analysis

Plot of the Mendelian randomization summary estimates derived after sequential removal of each SNP from the analysis. The red vertical line represents the causal effect estimate (derived from Mendelian randomization) when including the 10 SNPs in the analysis (presented in **Figure 3**). Odds ratios (OR) and 95% confidence intervals (CI) represent the population-based risk of schizophrenia in users of cannabis (compared to non-users).

Figure 1 Meta-analysis of prospective observational studies reporting an association between use of cannabis and risk of schizophrenia or related disorders

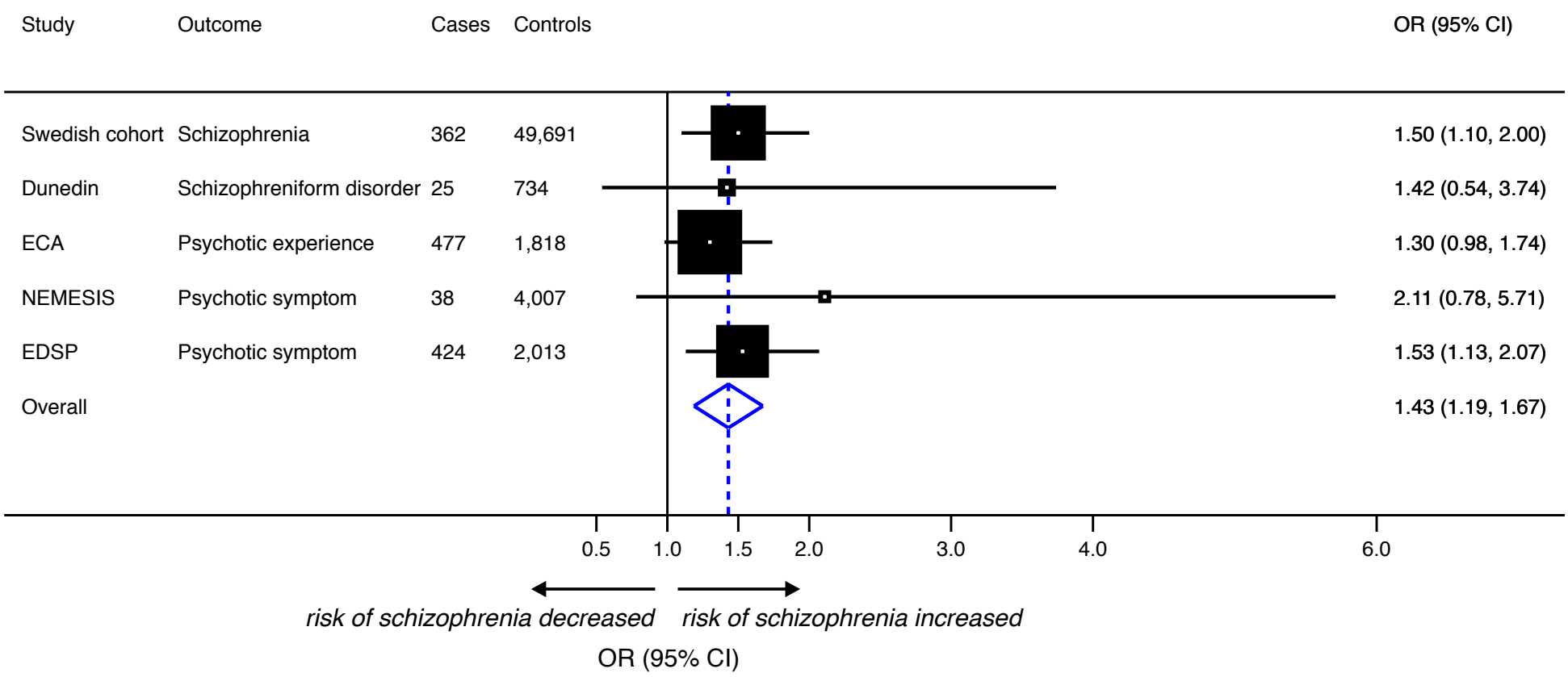


Figure 2 Meta-analysis of the association of genetically-determined use of cannabis and risk of schizophrenia for the 10 SNPs in the genetic instrument

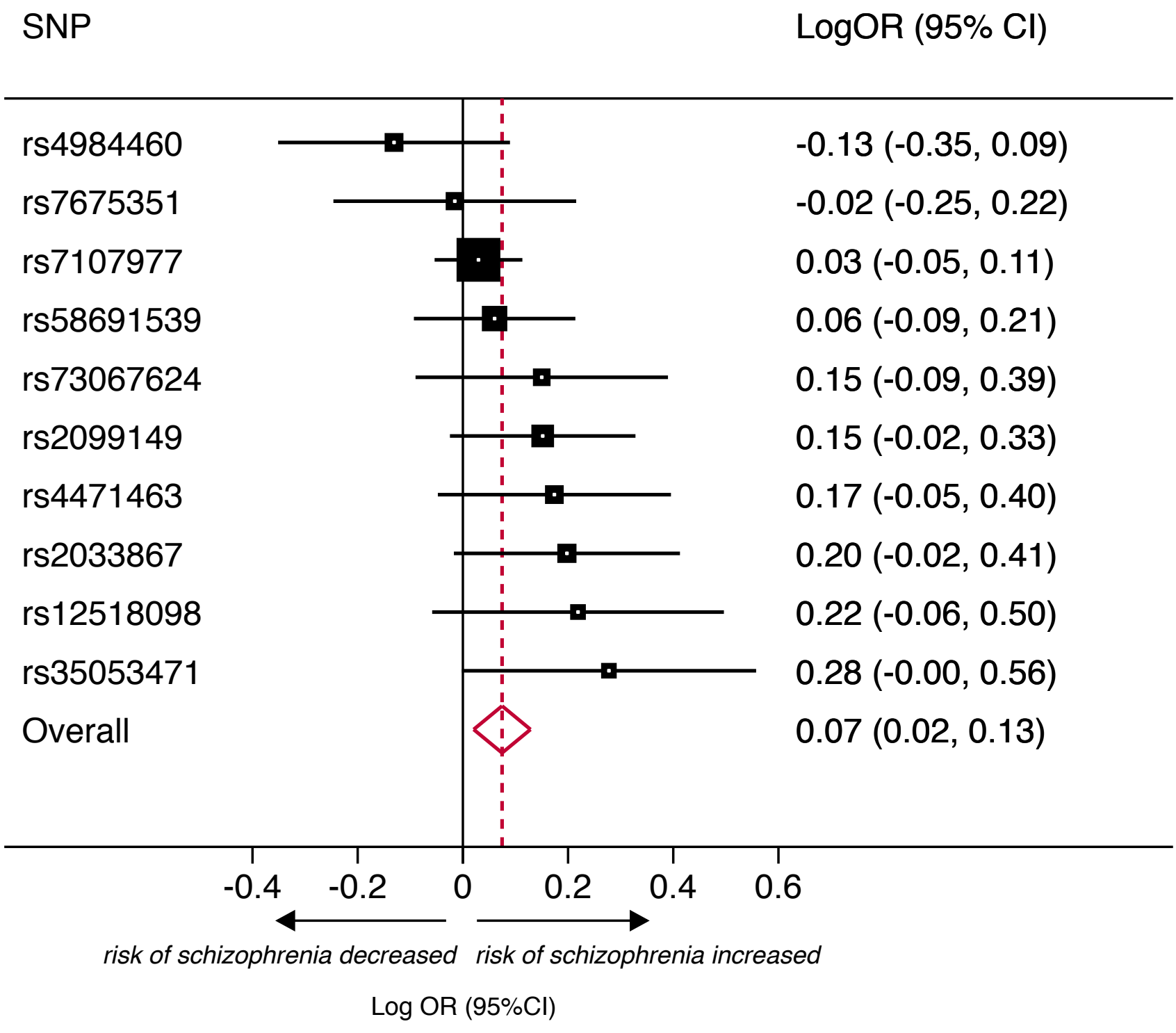


Figure 3 Comparison of observational (blue) and causal (red) estimates for use of cannabis and risk of schizophrenia

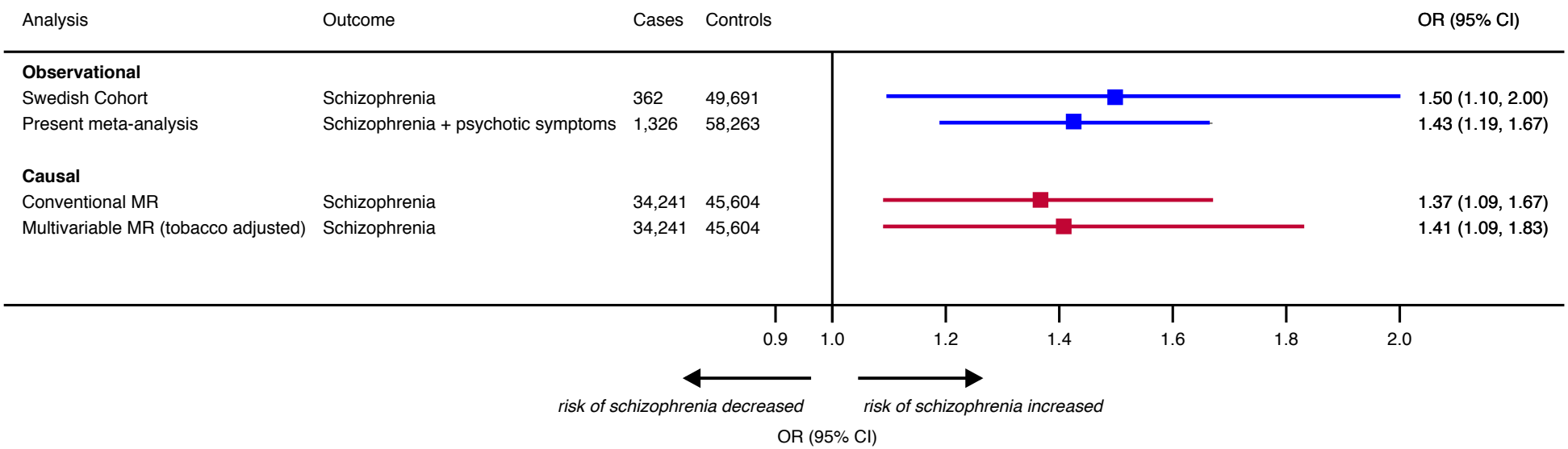
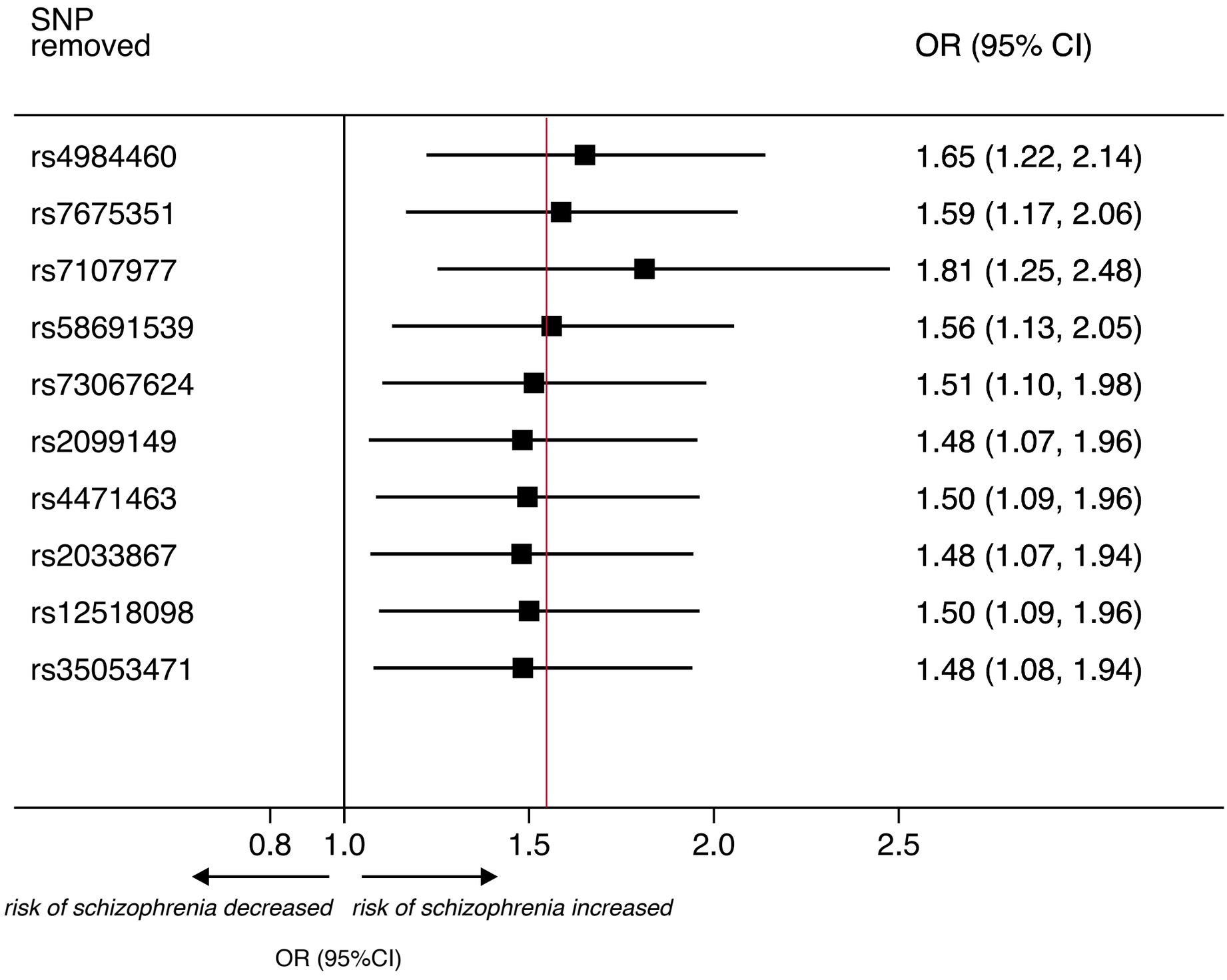


Figure 4 Sensitivity analysis of the association of use of cannabis and risk of schizophrenia by sequentially removing each SNP from the analysis



Supplement

Cannabis use and risk of schizophrenia: a Mendelian randomization analysis

by Julien Vaucher, MD, Brendan J. Keating, PhD, Aurélie M. Lasserre, MD, Wei Gan, PhD, Donald M. Lyall[6], PhD, Joey Ward[6], BSc, Daniel J. Smith[6], MD, Jill P. Pell[6], MD, Naveed Sattar[7], MD, PhD, Guillaume Paré, MD, Michael V. Holmes, MD, PhD

Table of Contents

Supplementary analysis

The population-based risk of schizophrenia among users compared to non-users of cannabis

The method to derive the odds ratio of genetically determined use of cannabis on risk of schizophrenia in the population is based on Ross *et al.*[1]

Given:

(A) Prevalence of cannabis use in the European Union population = 0.133.[2] This figure tallies with prevalence of ever use of cannabis in the Swedish cohort (=0.108),[3] which thus allows comparison between the observational and causal estimates

(B) Prevalence of schizophrenia in non-users of cannabis = 0.006 (as retrieved from the Swedish cohort)[3]

(C) Odds ratio for schizophrenia associated with genetically determined cannabis use (users vs. non users) at the population level

(D) Genetic association with schizophrenia as function of genetic association with use of cannabis (where causal genetic effects are expressed as Log OR per allele for both schizophrenia and use of cannabis)

The following can be calculated:

(E) Calculated population prevalence of schizophrenia: **(A × B × C) + (1 - A) × B**

(F) Estimated prevalence of schizophrenia in individuals with a theoretical increase in risk of use of cannabis of *e* = 2.72 fold: **(A × exp(1) × B × C) + (1 - A × exp(1)) × B**

(G) Estimated odds ratio for schizophrenia per *e* = 2.72 fold increase in risk of use of cannabis: **F/E**

It results that:

(H) $$\exp(\mathrm{D}) = \mathrm{G} = \frac{F}{E} = \frac{(\mathbf{A} \times \mathbf{exp}(\mathbf{1}) \times \mathbf{B} \times \mathbf{C}) + (\mathbf{1} - \mathbf{A} \times \mathbf{exp}(\mathbf{1})) \times \mathbf{B}}{(\mathbf{A} \times \mathbf{B} \times \mathbf{C}) + (\mathbf{1} - \mathbf{A}) \times \mathbf{B}}$$

As C is the only unknown variable, the association between genetically determined use of cannabis (cannabis users vs. non-users) and risk of schizophrenia (expressed as an odds ratio) at the population level can be calculated using algebraic transformations and (H) can be simplified into:

(I) $$\mathbf{C} = \mathbf{1} + \frac{(\mathbf{1} - \mathbf{exp}(\mathbf{D}))}{(\mathbf{ex\,p}(\mathbf{D}) - \mathbf{exp}(\mathbf{1})) \times \mathbf{A}}$$

Proportion of the variance in use of cannabis

The proportion of variance (conceptually similar to the R^2) in use of cannabis was computed for each SNP based on the formula provided by Shim *et al*.:[4]

$$R^2 = \frac{2\beta^2 \times MAF \times (1 - MAF)}{2\beta^2 \times MAF \times (1 - MAF) + \left(se(\beta)\right)^2 \times 2N \times MAF \times (1 - MAF)}$$

with β, effect size (beta coefficient) for a given SNP, MAF, minor allele frequency, se(β), standard error of effect size, and *N*, sample size.

UK Biobank: general description, genotyping, selection of participants and statistical analysis

General description

The UK Biobank cohort is a large prospective cohort of 502,628 participants with phenotypic information, of whom around 152,249 have genetic information available as of July 2017 with the remainder due to be released in Q3 2016 .[5] In the analysis, we reported on cross-sectional data at baseline. All participants attended one of 22 assessment centres from 2006 to 2010 where they completed a series of physical, sociodemographic, and medical assessments. Participants self-reported their smoking status as never, past or current. (A minority of n=299 refused to answer and these were removed).

Genotyping

UK Biobank genotyping was conducted by Affymetrix using a bespoke BiLEVE Axiom array for ~50,000 participants, and the remaining ~450,000 (for the purposes of this study 100,000) on a further updated bespoke Affymetric Axiom array (based on the 1st array). The two are extremely similar, sharing over 95% marker content. We controlled for array type as a covariate. Further information on the genotyping process is available on the UK Biobank website (http://www.ukbiobank.ac.uk/scientists-3/genetic-data), which includes detailed technical documentation (http://www.ukbiobank.ac.uk/wp-content/uploads/2014/04/UKBiobank_genotyping_QC_documentation-web.pdf). UK Biobank provide recommendations, which we followed, for which participants to exclude from analysis based on whether: the sample failed quality control; had significant missing data or heterozygosity. We used ten (UK Biobank provided) genetic principal components to account for population stratification. All SNPs present in the current analysis were in Hardy Weinberg equilibrium.

Selection of participants and statistical analysis

Of the 152,249 baseline participants, there were 112,197 participants who satisfied the inclusion criteria of: passed quality control; were Caucasian; had no first cousins or closer in the cohort, and had no mismatch between reported/genetically estimated sex and ethnicity. Of these 111,898 had smoking-related data. The mean age was 56.90 (SD = 7.93) years, and 53,122 (47.4%) were male. There were 59,914 never smokers and 51,984 ever smokers (46.46%).

Risk of ever smoking per SNP (0;1;2 dose model) was adjusted for age, sex, ten genetic principal components, batch, assessment centre and array.

All analyses were conducted with PLINK and STATA v.13.

Table S1 Summary of the observational studies included in the analysis of cannabis use and risk of schizophrenia, presented by year of recruitment

Study	Full name (country)	Year(s) of recruitment	Years of follow-up	Cases	Controls	Exposure (as reported in the text)	Outcome (source)	Adjustments	Reference
Swedish cohort	Cohort of Swedish conscripts (Sweden)	1969-70	26	362	49,691	Cannabis ever (use)	Schizophrenia (Swedish national hospital discharge)	Psychiatric diagnosis at conscription, IQ score, poor social integration, disturbed behaviour, cigarette smoking	3
Dunedin	Dunedin Multidisciplinary Health and Development Study (New Zealand)	1972-73	26	25	735	Cannabis users by age 18	Schizophreniform disorder (DSM-IV)	-	6
ECA	Epidemiologic Catchment Area Program (US)	1980-84	1	477	1,818	Use of marijuana (vs. No use)	Self-reported psychotic experiences (Diagnostic Interview Schedule)	Sex, being in school, level of education, marital status, employment, presence at baseline of depressive episodes, manic episodes, agoraphobia and obsessive-compulsive disorder	7
EDSP	Early Developmental Stages of Psychopathology Study (Germany)	1995	4	424	2,013	Any use (≥5 times)	Psychotic symptoms (Münich version of the composite international diagnostic interview [M-CIDI])	Age, sex, socioeconomic status, living in city, childhood trauma, predisposition to psychosis at baseline, other drug use, tobacco, alcohol, predisposition for psychosis at follow-up and depression at baseline and follow-up	8
NEMESIS	Netherlands Mental Health Survey and incidence Study (Netherlands)	1996	3	38	4,007	Baseline any use (of cannabis)	Any psychosis (based on the Brief Psychiatric Rating Scale)	Age, sex, ethnic group, level of education, unemployment and marital status, use of other drugs	9

Table S2 Summary of the observational studies excluded from the analysis of cannabis use and risk of schizophrenia with reasons for exclusion presented by year of recruitment

Study	Full name/description (country)	Year(s) of recruitment	Years of follow-up	Exposure (definition)	Outcome (source)	Reason(s) for exclusion	Reference (PMID)
CHDS	Christchurch Health and Development Study (New Zealand)	1977	21	Cannabis dependence (dependent vs. not dependent) based on *DSM-IV* diagnostic for cannabis dependence and frequency of cannabis use (from never use to daily use)	Psychotic symptoms (Symptom Checklist 90 [SCL-90] – 10 items)	Dependence severity (based on a count of *DSM-IV* cannabis dependence criteria) not available	Fergusson et al, Psychol Med, 2003 (12537032) and Fergusson et al. Addiction, 2005 (15733249)
Zürich Study	Zürich Study (Switzerland)	1978	30	Frequency of cannabis use in adolescence (3 levels : none ; casual ; regular)	Schizophrenia nuclear symptoms subscale (SCL-90-R)	Lifetime use (ever vs. never users) not available	Rössler et al, Addiction, 2012 (22151745)
California	California inpatient hospital admissions	1990-2000	10	Any cannabis-related ICD-9 diagnostic code within a medical recode	Readmission with any schizophrenia diagnoses (ICD-9)	Use of inpatient data - hospitalization with any record of cannabis use and subsequent hospitalization for schizophrenia.	Callaghan et al., Am J Psychiatry, 2012 (22193527)
ALSPAC	Avon Longitudinal Study of Parents and Children	1991-92	16	Cumulative cannabis use at age 16 years (4 levels: never; 1-20 times; 21-60 times; >60 times)	Psychotic experiences (semi-structured interview based on PLIKSi)	Lifetime use (ever vs. never users) not available	Gage et al., Psychol Med, 2014 (25066001)
NPMS	British National Psychiatric Morbidity Survey	2000	1.5	Cannabis use (3 levels: not used in past year, used in past year but no report of dependence; dependence) corresponding to ever use of cannabis <u>but only over the past one year</u> *and* cannabis dependence (dependent vs. not dependent)	Psychotic symptoms (Psychosis Screening Questionnaire)	Lifetime use (ever vs. never users) and dependence severity (based on a count of *DSM-IV* cannabis dependence criteria) not available	Wiles et al., Br J Psychiatr, 2006 (16738341)

Table S3 Studies included in the cannabis-GWAS (Stringer *et al.*[10])

Study	**Country**	***N total***	**Reference/PMID**
Discovery			
ALSPAC	UK	2976	22507743
BLTS	Australia	721	23187020
CADD	USA	853	Not published
EGCUT1	Estonia	2765	15133739
EGCUT2	Estonia	970	15133739
FinnTwin	Finland	1029	23298696
HUVH	Spain	981	25284319
MCTFR	USA	6241	23363460
NTR	Netherlands	4653	20477721
QIMR	Australia	6778	17988414
TRAILS	Netherlands	1226	18763693
Utrecht	Netherlands	1173	20925969
Yale Penn European American	USA	1964	24166409
Replication			
Radar	Dutch	338	25466800
SYS	Canada	551	25454417
TwinsUK	UK	2078	twinsuk.ac.uk
Yale Penn African American	US	2660	24166409

ALSPAC, Avon Longitudinal Study of Parents and Children; BLTS, Brisbane Longitudinal Twin Study; CADD, Center on Antisocial Drug Dependence; EGCUT, Estonian Genome Center University of Tartu; FinnTwin, Finnish Twin Cohort (FinnTwin12 & FinnTwin16); HUVH, Hospital Universitari Vall d'Hebron; MCTFR, Minnesota Center for Twin and Family Research; NTR, Netherlands Twin Register; QIMR, Queensland Institute of Medical Research Berghofer adults; TRAILS, TRacking Adolescents'Individual Lives Survey; Utrecht, Utrecht Cannabis Cohort (CannabisQuest); Radar, Research on Adolescent Development and Relationships ; SYS, Saguenay Youth Study.

Table S4 Summary of the 10 SNPs associated with use of cannabis (by increasing beta)

SNP	Chr	Position	EAF	Effect allele	Other allele	Sample size	Beta	SE	p-value	Number of studies directionally consistent**
rs35053471	3	47124761	0.62	T	A	31301	0.090	0.022	2.7×10^{-6}	11/12
rs12518098	5	60864467	0.68	C	G	32330	0.090	0.023	3.0×10^{-6}	12/13
rs4471463*	11	112983595	0.45	C	T	32330	0.100	0.021	1.5×10^{-6}	10/13
rs4984460	15	96424399	0.25	G	T	32330	0.110	0.023	4.6×10^{-7}	9/13
rs7675351	4	141218757	0.14	C	A	24912	0.130	0.033	1.4×10^{-6}	10/11
rs73067624*	1	196333461	0.10	C	T	24191	0.160	0.041	3.1×10^{-6}	10/10
rs2099149	12	30479358	0.19	G	T	22902	0.170	0.034	9.8×10^{-7}	8/9
rs2033867	2	175188281	0.06	A	G	22612	0.230	0.050	2.6×10^{-6}	5/5
rs58691539	2	52753909	0.09	G	T	12210	0.290	0.062	2.1×10^{-6}	4/4
rs7107977	11	915764	0.60	A	G	8759	0.290	0.064	1.9×10^{-6}	6/6

* represent the two SNPs used in the sensitivity analysis and corresponding to two genes (*KCNT2* for rs73067624 and *NCAM1* for rs4471463), with a putative functional role, that were associated with ever use of cannabis in the gene-based tests of associations in ever use of cannabis-GWAS.[10]

** represents directional consistency of the beta coefficients across studies under analysis.

None of the 10 SNPs were in linkage disequilibrium (R^2<0.1) based on SNP Annotation and Proxy Search (SNAP, Broad Institute, MA, US).[11]

Chr, chromosome; EAF, effect allele frequency; SE, standard error. Beta coefficient corresponds to log odds of ever use of cannabis.

Table S5 Studies included in the Schizophrenia-GWAS (reproduced from the supplementary material of Ripke *et al.*[12])

Study	Country	Cases	Controls	Reference/PMID
Umeå	Sweden	341	577	Not published
Umeå	Sweden	193	704	Not published
TOP	Norway	377	403	19571808
Uni. of Edinburgh	UK	367	284	19571811
Denmark	-	876	871	19571808
PEIC, WTCCC2	Seven countries	574	1812	23871474
PEIC, WTCCC2	Spain	150	236	23871474
New York, US & Israel	US & Israel	325	139	20489179
Ireland	Ireland	264	839	19571811
WTCCC2	Ireland	1291	1006	22883433
GRAS	GRAS	1067	1169	20819981
EGCUT	Estonia	234	1152	15133739
EGCUT controls	Estonia	347	310	15133739, 4166486
EGCUT controls	Estonia	636	636	15133739,24166486
EGCUT controls	Estonia	256	130	15133739,24166486
EGCUT controls	Estonia	1154	2310	15133739,24166486
MGS	US, Australia	2638	2482	19571809
London	UK	509	485	19571811
Hubin	Sweden	265	319	19571808
Bulgaria	-	195	608	Not published
Toronto/Lilly (MIGen)	Canada & US	526	1644	Not published
Israel	-	894	1594	24253340
WTCCC controls	Six countries	157	245	22885689
New York	US	190	190	17522711
ASRB	Australia	456	287	21034186
Cardiff	UK	396	284	19571811
CLOZUK	UK	3426	4085	22614287
CLOZUK	UK	2105	1975	22614287
Netherlands	-	700	607	19571808
Finland	-	186	929	19571808
Finnish	-	360	1082	Not published
Portugal	-	346	215	19571811
CIDAR	US	67	65	24424392
Pfizer	-	662	1172	Not published
Bonn/Mannheim	Germany	1773	2161	19571808
Munich	Germany	421	312	19571808
Aberdeen	UK	719	697	19571811
CATIE	US	397	203	18347602
sw1	Sweden	215	210	23974872
sw234	Sweden	1980	2274	23974872
sw5	Sweden	1764	2581	23974872
sw6	Sweden	975	1145	23974872
CogUK	UK	530	678	21850710
NIMH CBDB	US	133	269	11381111
NIMH CBDB	US	497	389	11381111
Denmark	-	471	456	19571808
Bulgaria	-	649	649	22083728
Six countries	-	516	516	22885689
Bulgaria	-	70	70	Not published
Japan	-	492	427	20832056
STCRP	Singapore	868	938	Not published
China	-	476	2018	24043878

For additional information on each study (incl. complete study name), see Ripke *et al.*[12] Cases included individuals with schizophrenia or schizoaffective disorder. In addition, for some studies, cases were defined based on hospital discharge records, patients having treatment-resistant schizophrenia, or registered to use clozapine.

Table S6 Power (two-sided α=0.05) for conventional Mendelian randomization analysis

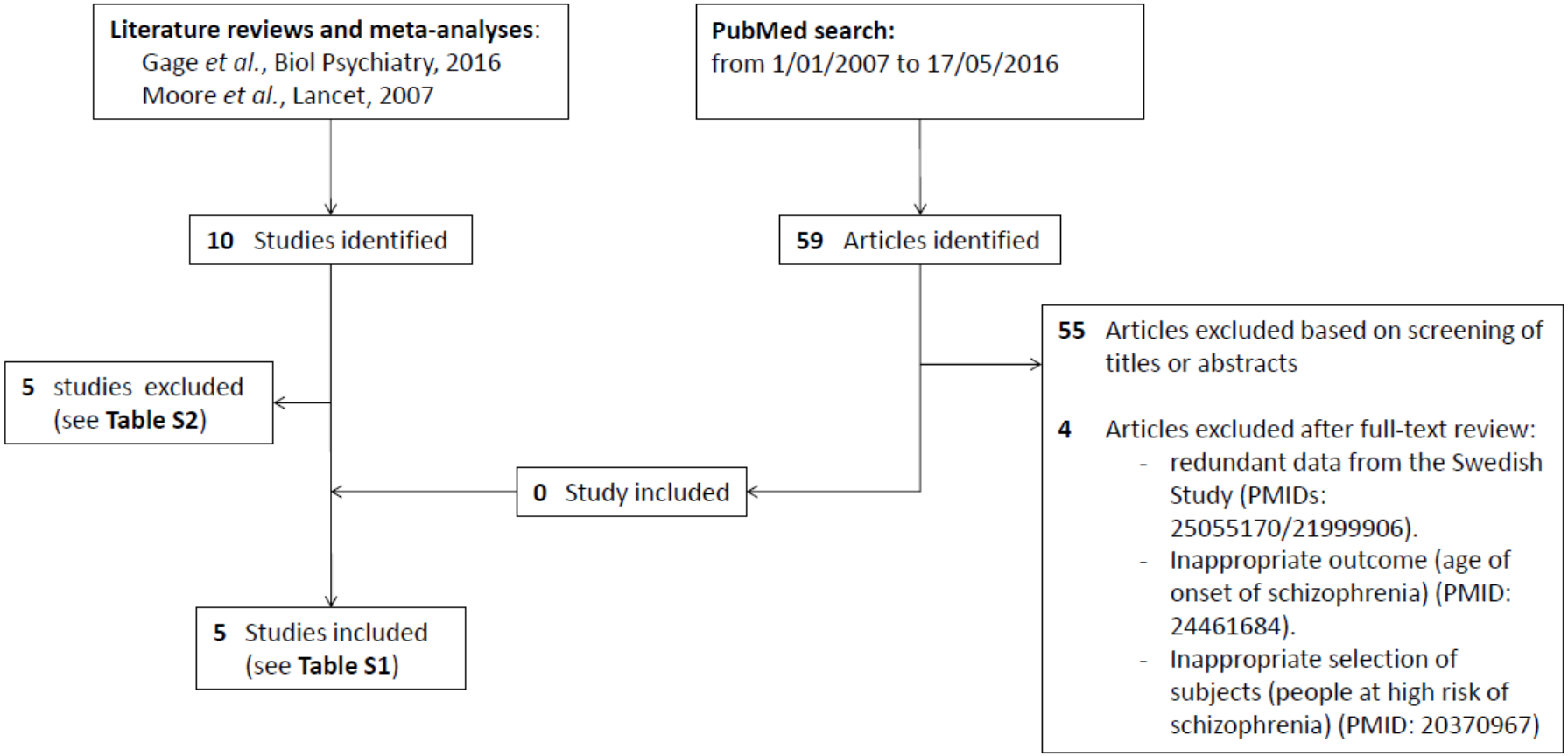
Exposure	Actual N (Schizophrenia-GWAS)	Proportion of cases (Schizophrenia-GWAS)	Observational OR	R2 of instrument	N required for 80% power	Power at actual N
Cannabis use	79,845	0.429	1.500*	0.010	18,120	1.0

Power calculation was based on the method developed by Brion et al.[13]

* from Swedish cohort.[3]

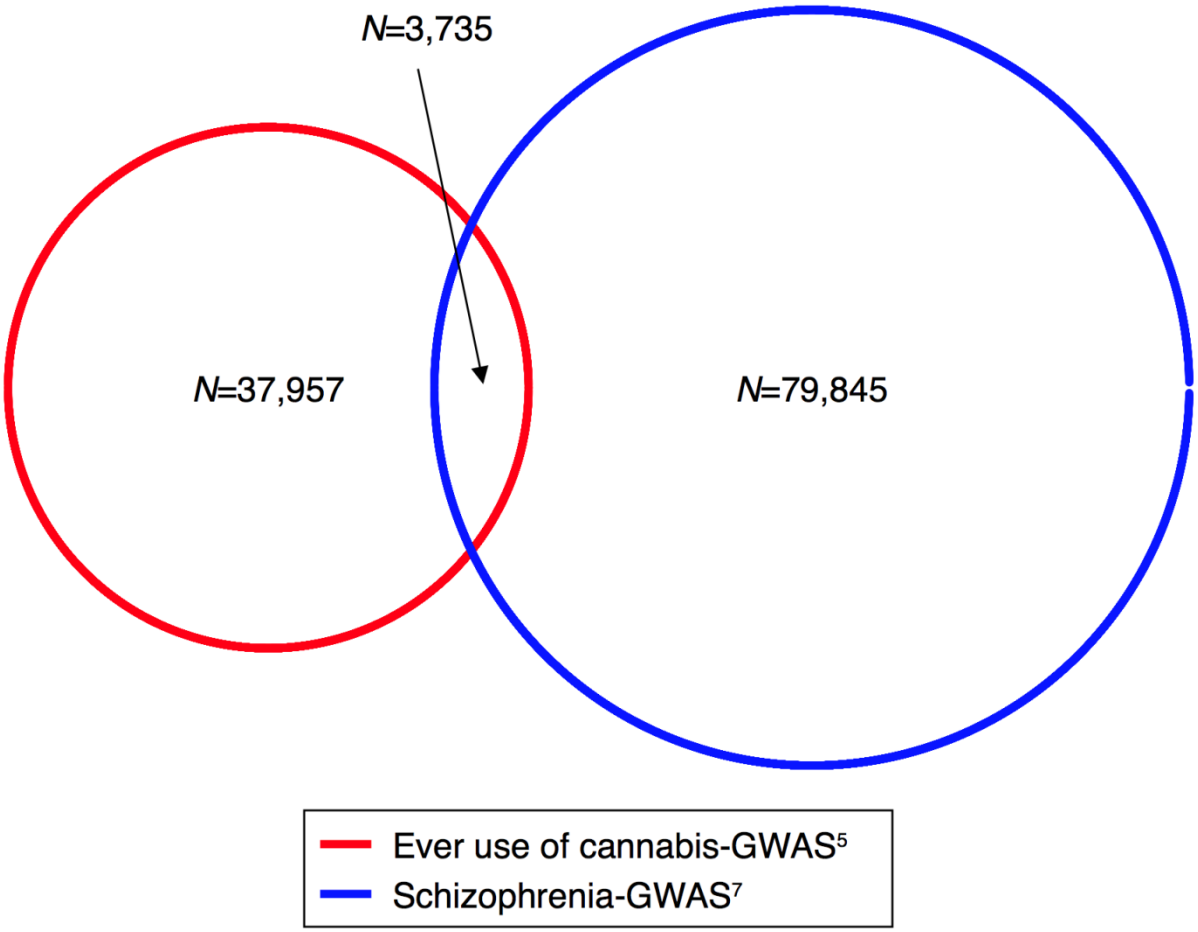
Power at OR=1.37 (=causal estimate) is 0.99.

Figure S1 Flowchart of observational meta-analysis



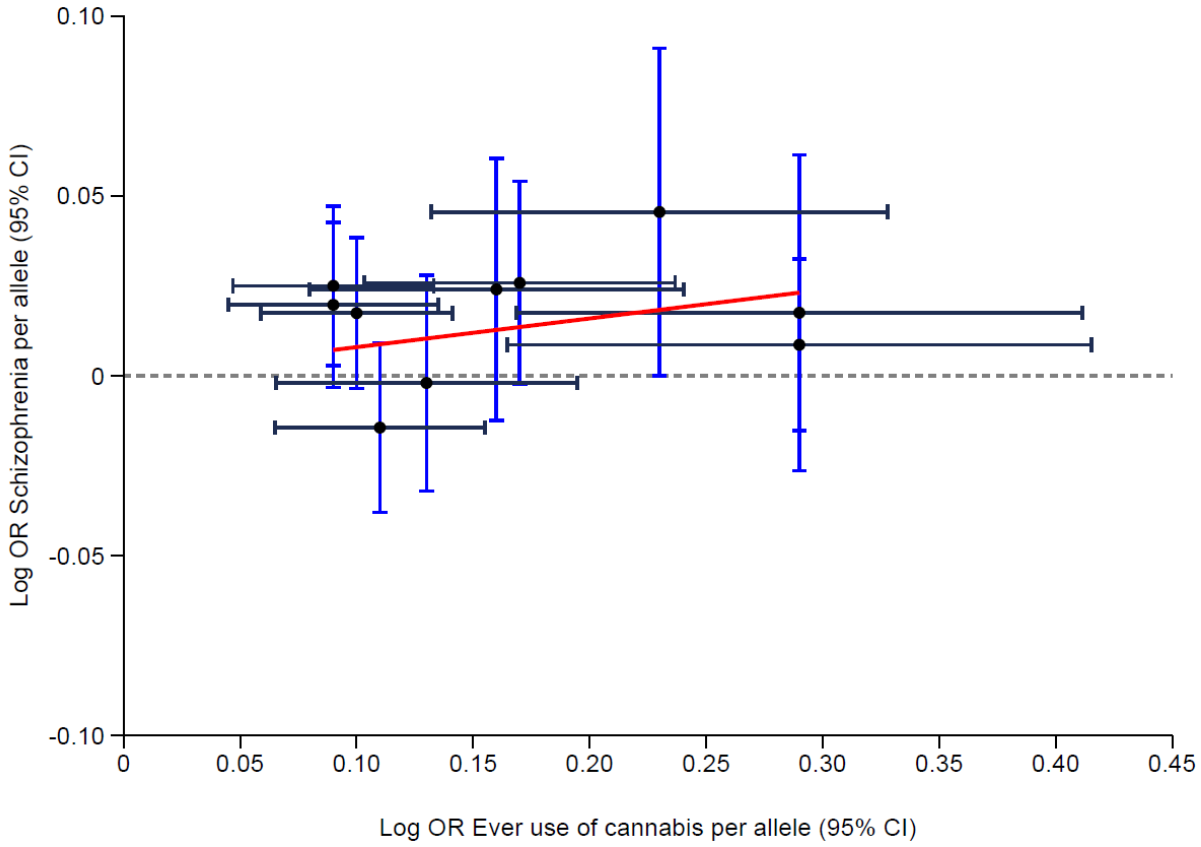
PubMed Search was conducted on 17 May 2016 to retrieve additional prospective observational studies reporting an association between cannabis use and risk of schizophrenia since the last meta-analysis on the subject published in 2007. PubMed terms applied for the search: ("cannabis"[MeSH Terms] OR "marijuana"[MeSH Terms]) AND "schizophrenia"[MeSH Terms] AND (("2007/01/01"[PDAT] : "2016/12/31"[PDAT]) AND "humans"[MeSH Terms]).

Figure S2 Venn diagram showing the number of individuals and the overlap between the cannabis-GWAS and the schizophrenia-GWAS



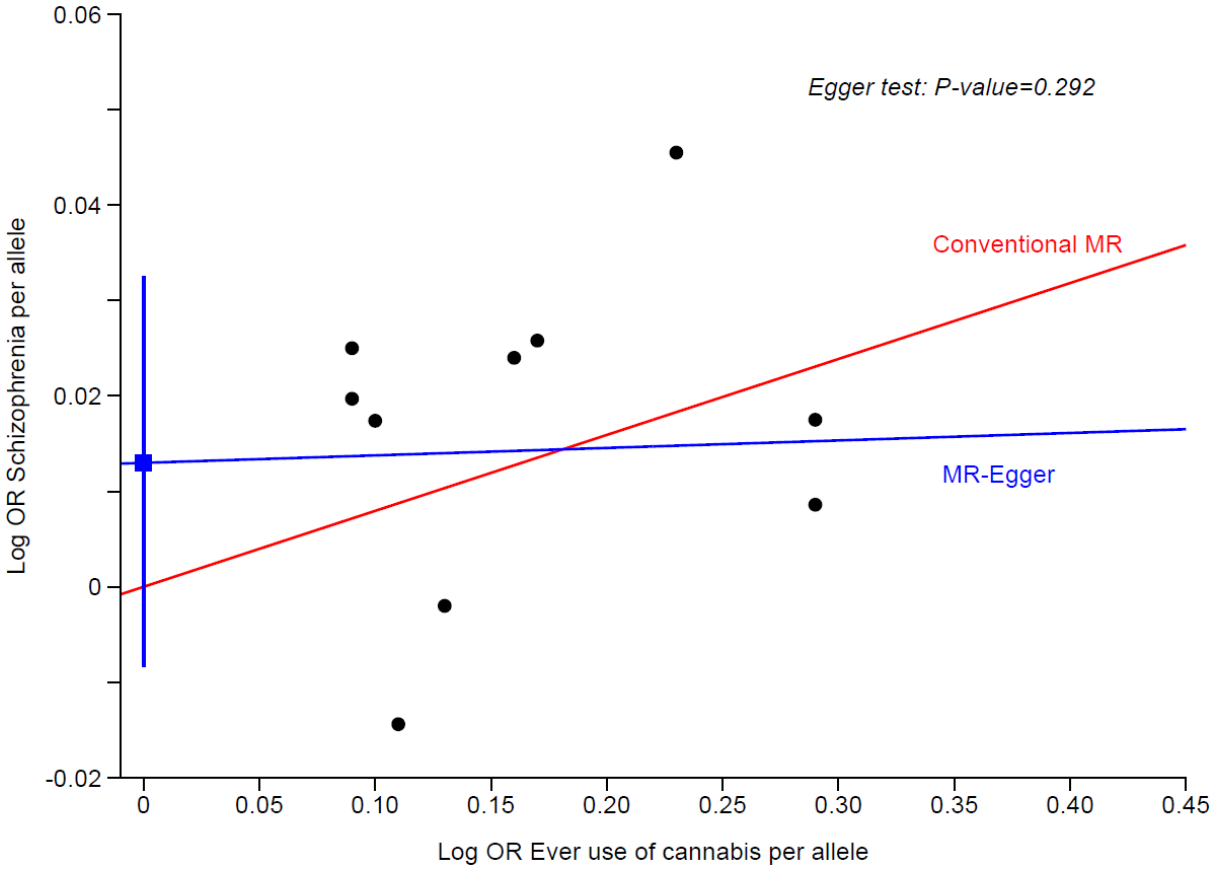
Based on the data provided in Stringer *et al.*[10] and Ripke *et al.*[12], only the EGCUT study (Estonian Genome Center University of Tartu (*N*=3,735) – see **Tables S3 & S5**) contributed to both GWAS.

Figure S3 Pair-wise association plot of the 10 SNPs associated with cannabis use and risk of schizophrenia



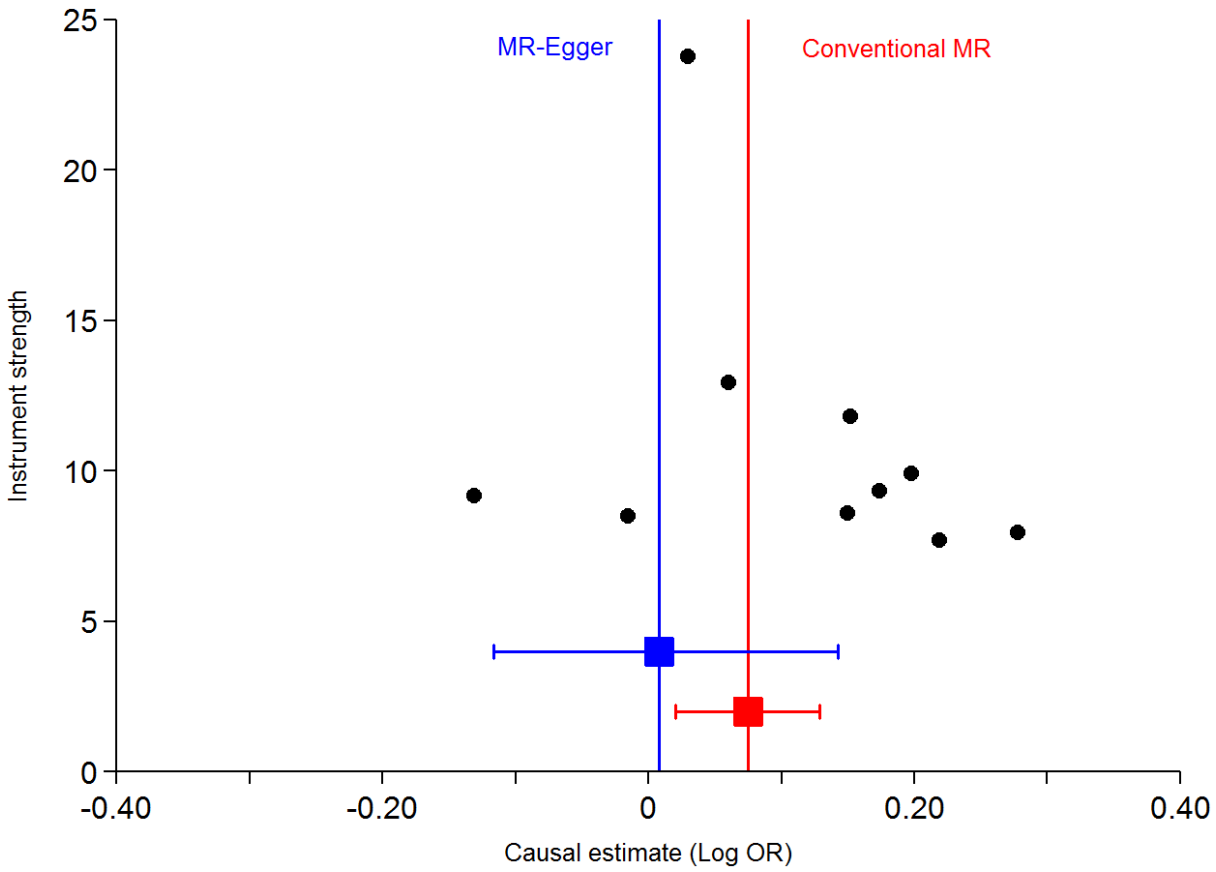
The red line represents the regression slope of the causal effects estimates (derived by the inverse-variance weighted approach as proposed by Bowden *et al.*).[14]

Figure S4 Scatter plot of the genetic association with cannabis use against genetic association with schizophrenia



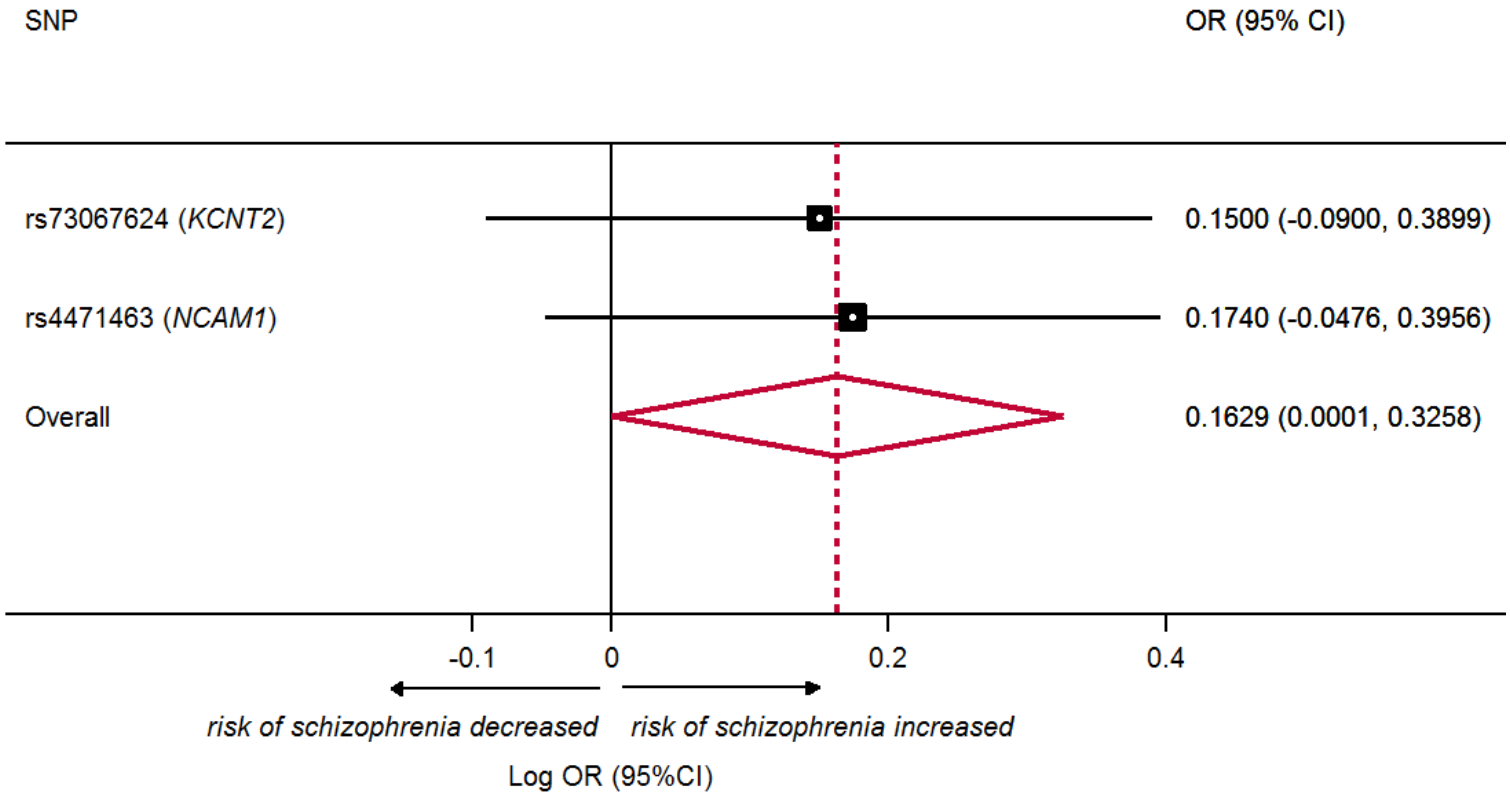
The conventional Mendelian randomization (Conventional MR in red) and Egger Mendelian randomization (MR-Egger in blue) causal effects estimates are presented as regression slopes. The constant and its 95% CI (obtained by bootstrap resampling 10,000 times) derived from Egger regression are shown as the blue square and vertical bar, respectively.

Figure S5 Funnel plot of the instrument strength (minor allele frequency corrected genetic association with cannabis use) against causal estimates of cannabis use on schizophrenia



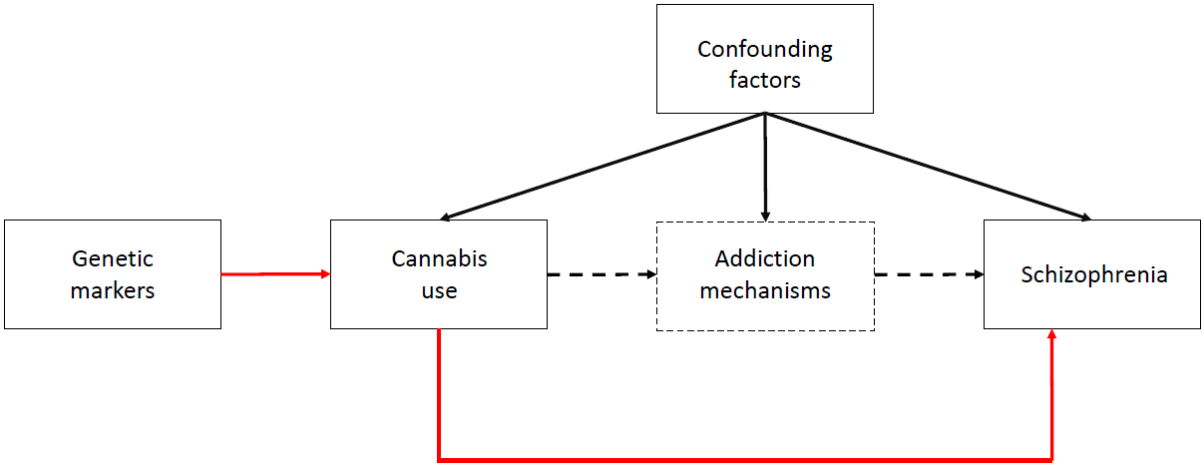
The instrument strength, representing the minor allele frequency corrected genetic association with ever use of cannabis is calculated by dividing the SNP-exposure association by the standard error of the SNP-outcome association for each SNP.[14] The conventional Mendelian randomization (Conventional MR in red) and Egger Mendelian randomization (MR-Egger in blue) causal effect estimates are presented.

Figure S6 Sensitivity analyses of the association of cannabis use and risk of schizophrenia restricting to two SNPs with putative functional roles



Fixed-effect meta-analysis of the causal estimates of use of cannabis-SNPs. Genes with presumptive function roles are indicated in brackets. Log odds ratios (Log OR) and 95% confidence intervals (CI) express the risk of schizophrenia per-1-log unit increase in ever use of cannabis and the corresponding combined OR (95% CI) is 1.18 (1.00-1.39). The method to derive the population-based OR of schizophrenia (OR 1.88; 95%CI, 1.00-3.21) among users of cannabis compared to non-users, as presented in the main text, is described on **pages 3-4** of the **Supplement**.

Figure S7 Conceptual framework representing the association between genetically determined cannabis use and risk of schizophrenia



The findings of our Mendelian randomization analysis – in line with previous randomized trials in laboratory conditions on cannabis and psychotic symptoms - support the pathway indicated with the solid red lines, where cannabis use causally influences the risk of schizophrenia. The use of genetic markers diminishes the possibility that confounding factors (e.g. demographic, parental history, personal history, socioeconomic or other environmental confounders) are explaining the association that we report (solid black lines). It is nonetheless possible that genetic markers associate with schizophrenia through different pathway(s), but this is not supported by the results of Egger Mendelian randomization that showed absence of unmeasured pleiotropy of the genetic markers employed. Finally, it is possible that genetically determined cannabis use influences addiction mechanisms which in turn determine the risk of schizophrenia (dashed black lines). Supported by our sensitivity analysis restricting on two genetic markers potentially associated with addiction, this hypothesis still represents a valid interpretation of the results, as cannabis use – which triggers addiction - would then lie on the causal pathway to schizophrenia.

Supplementary references

1. Ross S, Gerstein HC, Eikelboom J, Anand SS, Yusuf S, Pare G. Mendelian randomization analysis supports the causal role of dysglycaemia and diabetes in the risk of coronary artery disease. Eur Heart J 2015;36:1454-62.
2. European Monitoring Centre for Drugs and Drug Addiction (2016), European Drug Report 2016: Trends and Developments, Publications O ce of the European Union, Luxembourg (http://www.emcdda.europa.eu/system/files/publications/2637/TDAT16001ENN.pdf). Accessed 2016, Aug 06.
3. Zammit S, Allebeck P, Andreasson S, Lundberg I, Lewis G. Self reported cannabis use as a risk factor for schizophrenia in Swedish conscripts of 1969: historical cohort study. BMJ 2002;325.
4. Shim H, Chasman DI, Smith JD, et al. A multivariate genome-wide association analysis of 10 LDL subfractions, and their response to statin treatment, in 1868 Caucasians. PloS one 2015;10.
5. Sudlow C, Gallacher J, Allen N, et al. UK biobank: an open access resource for identifying the causes of a wide range of complex diseases of middle and old age. PLoS Med 2015;12.
6. Arseneault L, Cannon M, Poulton R, Murray R, Caspi A, Moffitt TE. Cannabis use in adolescence and risk for adult psychosis: longitudinal prospective study. BMJ 2002;325:1212-3.
7. Tien AY, Anthony JC. Epidemiological analysis of alcohol and drug use as risk factors for psychotic experiences. J Nerv Ment Dis 1990;178:473-80.
8. Henquet C, Krabbendam L, Spauwen J, et al. Prospective cohort study of cannabis use, predisposition for psychosis, and psychotic symptoms in young people. BMJ 2005;330. doi 10.1136/bmj.38267.664086.63.
9. van Os J, Bak M, Hanssen M, Bijl RV, de Graaf R, Verdoux H. Cannabis use and psychosis: a longitudinal population-based study. Am J Epidemiol 2002;156:319-27.
10. Stringer S, Minica CC, Verweij KJ, et al. Genome-wide association study of lifetime cannabis use based on a large meta-analytic sample of 32 330 subjects from the International Cannabis Consortium. Transl Psychiatry 2016;29:36. doi: 10.1038/tp.2016.
11. Johnson AD, Handsaker RE, Pulit SL, Nizzari MM, O'Donnell CJ, de Bakker PI. SNAP: a web-based tool for identification and annotation of proxy SNPs using HapMap. Bioinformatics 2008;24:2938-9. (http://www.broadinstitute.org/mpg/snap/ldsearch.php).
12. Schizophrenia Working Group of the Psychiatric Genomics Consortium. Biological insights from 108 schizophrenia-associated genetic loci. Nature 2014;511:421-7.
13. Brion MJ, Shakhbazov K, Visscher PM. Calculating statistical power in Mendelian randomization studies. International journal of epidemiology 2013;42:1497-501.
14. Bowden J, Davey Smith G, Burgess S. Mendelian randomization with invalid instruments: effect estimation and bias detection through Egger regression. International journal of epidemiology 2015;44:512-25.