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Alzheimer Disease and Epilepsy: A Mendelian Randomization Study

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## Abstract

### Background and Objectives

Observational studies suggested a bidirectional relationship between Alzheimer's disease (AD) and epilepsies. However, it remains debated whether and in which direction a causal association exists. The present study aims to explore the relationship between genetic predisposition to AD, CSF biomarkers of AD (Aβ42, pTau) and epilepsies with two-sample, bi-directional Mendelian randomization (MR) method.

### Methods

Genetic instruments were obtained from large-scale genome-wide meta-analysis of AD ($N_{case/proxy}$ = 111,326, $N_{control}$ = 677,663), CSF biomarkers of AD (Aβ42 and pTau, N = 13,116) and epilepsy ($N_{case}$ = 15,212, $N_{control}$ = 29,677) of European ancestry. Epilepsy phenotypes included all epilepsy, generalized epilepsy, focal epilepsy, childhood absence epilepsy, juvenile absence epilepsy, juvenile myoclonic epilepsy, generalized epilepsy with tonic-clonic seizures, focal epilepsy with hippocampal sclerosis, and lesion-negative focal epilepsy. Main analyses were performed using generalized summary data-based mendelian randomization (GSMR). Sensitivity analyses included IVW, MR-PRESSO, MR-Egger, weighted mode, and weighted median.

### Results

For forward analysis, genetic predisposition to AD was associated with an increased risk of generalized epilepsy (OR = 1.053, 95% CI: 1.002 ~ 1.105, $P$ = 0.038), and focal epilepsy with hippocampal sclerosis (OR = 1.013, 95% CI: 1.004~1.022, $P$ = 0.004). These associations were

consistent across sensitivity analyses and replicated using a separate set of instrumental SNPs from another AD GWAS. For reverse analysis, there was a suggestive effect of focal epilepsy with hippocampal sclerosis on AD (OR = 3.994, 95%CI: 1.172~13.613, $P$ = 0.027). In addition, genetically predicted lower CSF Aβ42 was associated with an increased risk of generalized epilepsy (β = 0.090, 95% CI: 0.022 ~ 0.158, $P$ = 0.010).

**Discussion**

The present MR study supports a causal link between AD, amyloid pathology, and generalized epilepsy. This study also indicates a close association between AD and focal epilepsy with hippocampal sclerosis. More effort should be made to screen seizure in AD, unravel its clinical implications, and explore its role as a putative modifiable risk factor.

**Key words**: Alzheimer's disease; epilepsy; Mendelian randomization; generalized epilepsy; focal epilepsy with hippocampal sclerosis

**Abbreviations**

| | |
|---|---|
| AD | Alzheimer's disease |
| CAE | Childhood Absence Epilepsy |
| Focal HS | focal epilepsy with hippocampal sclerosis |
| GSMR | generalized summary data-based mendelian randomization |
| GTCS | generalized epilepsy with tonic-clonic seizures |
| GWAS | Genome-Wide Association Study |
| HS | hippocampal sclerosis |
| IVW | Inverse Variance Weighted |

| JAE | Juvenile Absence Epilepsy |
| JME | Juvenile Myoclonic Epilepsy |
| MR | Mendelian Randomization |
| MR-PRESSO | MR pleiotropy residual sum and outlier |
| MTL | mesial temporal lobe |
| SNPs | single nucleotide polymorphisms |

## Introduction

Accumulating evidence indicates a close association between Alzheimer's disease (AD) and epilepsies. Preclinical studies indicated a vicious cycle between AD and seizures. On the one hand, amyloid-β demonstrated epileptogenic potential even in the early stages of the amyloid cascade [1]. Additional factors contribute to the increased seizure susceptibility in AD might include excitatory/inhibitory imbalance, locus coeruleus degeneration, neuroinflammation, vascular dysregulation, and metabolic alteration [2]. On the other hand, epileptiform activity and chronic hyperexcitability promotes amyloid plaque deposition and tau hyperphosphorylation [3].

In human, epidemiological studies indicated a bi-directional association of epilepsy with AD [4–8]. A recent meta-analysis identified a 1.8-fold increased risk of AD in people with epilepsy, while patients with AD are at a 3.1-fold higher risk of epilepsy [9]. However, these retrospective studies are prone to confounding effects, such as cerebrovascular insults, education level, and use of different antiepileptic drugs with varying effect on cognition.

Several studies have depicted seizures in AD. In an earlier retrospective study which included 446 individuals with pathologically diagnosed AD, 17% had a new-onset seizure after clinical

diagnosis of AD, with 89% presenting with generalized tonic-clonic seizures [10]. In recent years, with the aid of long-term EEG, focal seizures with impaired awareness was reported to be the most common type [11], even presenting at prodromal stage [12]. Notably, temporal lobe epilepsy (TLE) and AD shares several critical pathological and neuroimaging features, including pathological phosphorylation of amyloid and tau [13,14], and hippocampal sclerosis (HS) [15].

In addition, evidence indicated a putative link between childhood onset epilepsies and AD. In an amyloidogenic mouse model, network hyperexcitability and increased seizure susceptibility was found at juvenile stage, long before the onset of cognitive decline and amyloid deposition [16]. In human, task-related hippocampal hyperactivity was also evident in asymptomatic young adults carrying autosomal dominant AD mutations [17], asymptomatic young adults carrying *APOE* ε4 allele [18,19], and asymptomatic adults at genetic risk of late-onset AD [20]. Furthermore, five decades after childhood-onset epilepsy, there was increased amyloid burden [21] and altered brain metabolism resembling possible preclinical AD [22]. However, these studies are limited by sample sizes and the possibility of confounding and reverse causality.

Given the close relationship between epilepsy and AD, it is essential to verify a true causal association, as such an association would indicate a potentially modifiable cause, or previously underrecognized consequence of AD. However, it remains debated whether epilepsy drives AD or vice versa [23]. In contrast to a causal association, it is also likely that there is a shared mechanism driving both conditions (e.g., neuroinflammation, neurovascular unit dysfunction). In addition, retrospective studies are potentially limited by confounding effect. For example, certain anti-epileptic drugs, which might deteriorate cognition, are likely to contribute to the observed association between epilepsy and AD.

Mendelian Randomization (MR) uses genetic variants that are robustly associated with exposure as instrumental variables to estimate the causal effect of a suspected exposure on outcome. When the underlying assumptions are satisfied, the MR approach minimizes several inherent limitations of conventional observational studies, including unobserved confounding and reverse causality [24].

In this study, we investigated the bi-directional causal effect of AD and epilepsies by performing two-sample MR analyses. We tested the hypotheses that (1) AD drives epilepsy; (2) epilepsy drives AD. We also studied the association between genetically predicted CSF biomarkers of AD (Aβ42, pTau) and epilepsy.

# Methods

## Data Sources

GWAS summary statistics for epilepsy and subtypes were derived from the International League Against Epilepsy Consortium on Complex Epilepsies in 2018 [25]. Seizures and epilepsy syndromes were diagnosed according to ILAE classification. All cases were assessed and classified into subtypes according to EEG, imaging, and clinical histories by epilepsy specialist at each participating site. Phenotype categories included genetic generalized epilepsy, focal epilepsy, and unclassified epilepsy. 95.5% of the participants were of European ancestry. Z-scores were calculated for all epilepsy, focal epilepsy, and generalized epilepsy using fixed-effect trans-ethnic meta-analyses. We transformed z-scores into β and SE as previously described [26].

Genetic generalized epilepsy was further classified into (1) Childhood Absence Epilepsy (CAE), (2) Juvenile Absence Epilepsy (JAE), (3) Juvenile Myoclonic Epilepsy (JME), (4) generalized

epilepsy with tonic-clonic seizures (GTCS) alone, with spike and wave EEG, (5) not otherwise specified. Sub-phenotypes of focal epilepsy included (1) focal epilepsy with hippocampal sclerosis (focal HS), (2) lesion other than HS, (3) lesion negative, (4) not otherwise specified. For each sub-phenotypes, a BOLT-LMM analysis which only included White individuals was performed. When analyzing the effect of AD on epilepsies, we used all epilepsy, generalized epilepsy, focal epilepsy, CAE, JAE, JME, GTCS, focal epilepsy with HS, and lesion-negative focal epilepsy as outcome.

For AD, we used the latest AD GWAS meta-analysis dataset by Bellenguez *et al.*[27], which included both patients with AD and proxy cases i.e., people with a family history of AD. To replicate the positive findings, we used an earlier GWAS dataset by Lambert *et al.*[28], which only enrolled late-onset AD cases, and did not include any proxy cases.

Genetic predictors of CSF levels of Aβ42 and pTau were obtained from a recent study initiated by the European Alzheimer & Dementia Biobank including 13,116 European ancestry individuals from 31 study cohorts [29]. CSF levels were measured by each cohort separately. Participants spanned the full spectrum of AD, including subjective cognitive decline, mild cognitive impairment, and dementia. Details of lab procedures of CSF samples could be found from original studies. CSF levels were log10 transformed and normalized within cohorts and CSF assay type. Associations were adjusted for sex, age, assay type and 10 ancestry principal components. There was no sample overlap between epilepsy and AD, CSF biomarkers and AD datasets as we know of.

## Instrument selection and GSMR

For genetical instruments of AD and epilepsy, we used single nucleotide polymorphisms (SNPs) that were strongly associated with each exposure ($P < 5 \times 10^{-8}$) as instruments. Linkage disequilibrium-based clumping and standard quality control (imputation info score, call rate, Hardy-Weinberg equilibrium test, assessment of heterogeneity) has been performed by each original GWAS study. When an exposure-associated variant was not found in the outcome GWAS, we attempted to find a SNP proxy in strong linkage disequilibrium ($R^2 > 0.8$) using CEU reference samples from 1000 Genomes. We removed SNPs that were significantly associated with outcome ($P < 5 \times 10^{-5}$). Steiger filtering [30] was also performed to ensure variants demonstrated stronger association with the outcome than with the exposure. SNPs were harmonized to ensure the effect alleles were identical in both exposure and outcome data. Nonbiallelic and palindromic SNPs (A/T, G/C) were excluded. SNPs with a minor allele frequency < 0.01 were excluded due to potentially low confidence.

Generalized summary data-based MR (GSMR) was used as the primary analysis, which excludes potentially pleiotropic SNPs by the HEIDI outlier removal test [31]. The GSMR method also accounts for weak linkage disequilibrium between instrumental SNPs. 503 European samples from Phase 3 of 1000 Genomes project were used as a reference.

Three SNPs for all epilepsy, one for focal epilepsy, 11 for generalized epilepsy, one for JME, two for CAE, and two for focal HS were GWAS level significant and used as proxy for each epilepsy phenotypes. For JME, there was only one GWAS-level significant SNP (rs1046276), which was also strongly associated with AD ($P = 2.12 \times 10^{-4}$). Therefore, the effect of JME on AD was not analyzed.

## MR assumptions and sensitivity Analyses

The MR method was based on three key assumptions, including that the instruments were strongly associated with exposure, were independent of confounders, and affect outcome through the exposure and not through alternative pathways. All the instrumental variables in the current analyses demonstrated an F-statistic > 10, indicating a low risk of weak instrument bias. For sensitivity analyses, we used SNPs retained after HEIDI outlier removal test, and performed Inverse Variance Weighted (IVW), MR-Egger, weighted-median, weighted-mode, MR-PRESSO (MR pleiotropy residual sum and outlier). These methods each rely on distinct assumptions. Horizontal pleiotropy was examined using MR-Egger regression. When the Egger intercept significantly deviated from zero, there was evidence of horizontal pleiotropy. Median- and mode- based method is more robust to outliers and provides an estimate when up to 50% weights are from invalid instruments. Pleiotropy was also examined using the MR-PRESSO [32] Global test and *P* values were calculated according to 1000 simulations. If significant global heterogeneity was found, then a local outlier test was performed to detect outlying SNPs. When there was only one SNP available as the instrument, the Wald ratio was reported.

Leave-one-out analyses was performed to evaluate if the MR result is relied on a particular variant. Cochran's Q was also calculated to assess evidence of heterogeneity. We depicted forest plots and funnel plots to visually inspect heterogeneity and horizontal pleiotropy.

When levels of CSF Aβ42 and pTau were exposure, we clumped SNPs at $r^2 < 0.1$ and used 5e-8 as significance level. As sensitivity analysis, different clumping thresholds (0.001, 0.01, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8) and different GWAS significance levels (5e-8, 5e-6) was used. As GSMR

accounts for weak correlation due to linkage disequilibrium, loosening clumping threshold might increase the number of genetic instruments and allow for an increased statistical power.

MR analyses were performed using R (version 4.1.0). Packages used included gsmr [31] (version 1.0.9), TwoSample MR [33] (version 0.5.6), MRPRESSO [32] (version 1.0), and LdlinkR [34] (version 1.2.2). Results were reported following the STROBE-MR guideline [35]. All hypothesis testings were two-sided. The threshold for significance was $P < 0.05$. Given the exploratory nature of our study, we did not perform correction of multiple comparisons. For statistically significant associations, we performed a series of sensitivity analyses as well as replication study using separate datasets.

## Standard Protocol Approvals, Registrations, and Patient Consents

This study is based on publicly available data. The summary statistics for AD, CSF biomarkers, and epilepsy did not contain any personal information, and the GWAS have obtained ethical approval from relevant ethics review boards. Written consent to be enrolled in the databases was obtained from study participants, or for those with substantial cognitive decline, from a caregiver, legal guardian, or other proxy by each original GWAS study. Patient consents for the current analysis were waived because of the retrospective nature of the study.

## Data Availability

Summary statistics of AD by Bellenguez *et al.* and CSF biomarkers were downloaded from the European Bioinformatics Institute GWAS Catalog (https://www.ebi.ac.uk/gwas/) under accession no. GCST90027158 (AD), GCST90129599 (CSF Aβ42), and GCST90129600 (CSF pTau). Summary statistics of AD by Lambert *et al.* were downloaded from the NIAGADS Data Storage Site (https://www.niagads.org/igap-summary-statistics-lambert-et-al-2013). Summary

statistics of epilepsy and subtypes by the International League Against Epilepsy Consortium on Complex Epilepsies were downloaded from https://www.epigad.org/gwas_ilae2018_16loci.html.

Data used to generate the main result was presented on eTable 1. The bi-directional MR analysis results, heterogeneity test (Q statistics) results, Egger intercept results, MR-PRESSO global test results were presented on eTables 2–5, respectively. Codes are available upon reasonable request.

# Results

Basic information of the contributing GWAS was summarized in Table 1.

## Effect of AD on epilepsies

The effects of AD on epilepsies were shown on Figure 1. Notably, genetic predisposition to AD was associated with elevated risk of focal HS (OR = 1.013, 95% CI: 1.004~1.022, $P_{\text{GSMR}} = 0.004$) (Figure 1). Extensive sensitivity analyses yielded similar estimates in magnitude and direction ($P_{\text{IVW}} = 0.001$, $P_{\text{MR-PRESSO}} = 0.003$, $P_{\text{Weighted median}} = 0.011$, $P_{\text{Weighted mode}} = 0.056$) (Figure 2). Although estimates using the Egger method was not significant ($P_{\text{Egger}} = 0.433$), this method estimates horizontal pleiotropy at the cost of reduced power and precision [36]. No evidence of pleiotropy ($P_{\text{Egger intercept}} = 0.969$; $P_{\text{MR-PRESSO global test}} = 0.777$) or heterogeneity ($P_{\text{IVW\_Q}} = 0.786$, $P_{\text{Egger\_Q}} = 0.743$) was found. In addition, the leave-one-out analysis also suggested that the causal effect was not driven by a particular instrumental SNP (Figure 2C). Funnel plot demonstrated no evidence of asymmetry, indicating a low risk of directional pleiotropy (Figure 2D).

To further verify the association between AD and focal HS, we performed a replication analysis using instrumental SNPs from an earlier AD GWAS dataset by Lambert *et al.*, which did not include proxy cases [28]. The results indicated similar causal effect in magnitude and direction

($P_{\text{GSMR}} = 0.009$, $P_{\text{IVW}} = 0.020$, $P_{\text{MR-PRESSO}} = 0.033$, $P_{\text{Weighted median}} = 0.005$, $P_{\text{Weighted median}} = 0.029$),

further validating the causal association.

In addition, AD was associated with an increased risk of generalized epilepsy (OR = 1.053, 95%

CI: 1.003~1.105, $P_{\text{GSMR}} = 0.038$). Sensitivity analyses yielded similar estimates in magnitude

and direction ($P_{\text{IVW}} = 0.025$, $P_{\text{MR-PRESSO}} = 0.035$, $P_{\text{Weighted median}} = 0.061$, $P_{\text{Weighted mode}} = 0.079$)

(Figure 3). No evidence of pleiotropy ($P_{\text{Egger intercept}} = 0.480$; $P_{\text{MR-PRESSO global test}} = 0.661$) or

heterogeneity ($P_{\text{IVW\_Q}} = 0.659$, $P_{\text{Egger\_Q}} = 0.634$) was found. The leave-one-out analysis and funnel

plot was demonstrated on Figure 3, C and D. Using instrumental SNPs from Lambert *et al.* [28],

the estimated effect of AD on generalized epilepsy was in similar direction and marginally

significant (OR = 1.039, 95% CI: 0.991~1.089, $P_{\text{GSMR}} = 0.114$).

**Effect of epilepsies on AD**

For reward analysis, there was a significant effect of focal HS on AD (OR = 3.994, 95%CI:

1.172~13.613, $P_{\text{IVW}} = 0.027$) (Figure 1). We attempted to replicate this finding using the

Lambert *et al*. 2013 AD GWAS dataset, the estimated effect was not significant, and in opposite

direction ($P_{\text{IVW}} = 0.252$). Since there were only two instrumental SNPs for focal HS, no

sensitivity analysis was performed.

**Effect of CSF Aβ42 and pTau on epilepsies**

The effect of CSF Aβ42 and pTau on epilepsies were displayed on Figure 4. Lower CSF Aβ42

was linked to increased risk of generalized epilepsy (β = 0.090, 95%CI: 0.022 ~ 0.158, $P_{\text{GSMR}} =$

0.010). Sensitivity analyses indicated similar estimates in magnitude and direction ($P_{\text{IVW}} = 0.014$,

$P_{\text{MR-PRESSO}} = 3E\text{-}4$, $P_{\text{Weighted median}} = 0.021$, $P_{\text{Weighted mode}} = 0.139$). No evidence of pleiotropy

($P_{\text{Egger intercept}} = 0.298$; $P_{\text{MR-PRESSO global test}} = 0.996$) or heterogeneity ($P_{\text{IVW\_Q}} = 0.975$,

$P_{\text{Egger\_Q}} = 0.993$) was found. Furthermore, using different levels of GWAS significance threshold

(5e-8, 5e-6) and clumping threshold, from rigorous to liberal, the estimated associations by

GSMR were consistent in direction and magnitude (Figure 5, eTable 6).

For reverse MR, no significant effect of epilepsy on CSF biomarkers was observed (eTable 2).

## Discussion

In this study, we found a causal association between AD and generalized epilepsy, this

association was robust to sensitivity analyses and further validated by a significant association

between lower CSF Aβ42 and increased risk of generalized epilepsy. We also found that AD was

causally linked to focal epilepsy with HS, which was robust to sensitivity analyses and replicated

using a separate set of instrumental variables. There was also evidence of potential causal effect

of focal epilepsy with HS on AD, although association in this direction was less compelling.

The causal association between AD and generalized epilepsy was consistent with previous

observations. Generalized epilepsy have been observed in 15-40% patients with AD at various

disease stages [23]. The epilpetogenic property of Aβ pathology has been demonstrated in various

amyloidogenic mouse models and also wild type models with exogenous Aβ [1]. In human patients

with late-onset epilepsy of unknown origin, there were lower levels of CSF Aβ42 [39,40]. In

patients with AD, Aβ-related alterations to large-scale network structure suggested propensity to

generalized seizures according to a study using computational modelling with

electrophysiological data [41]. The current study provides novel evidence that amyloid pathology is

causally implicated in epileptogenesis in human.

Interestingly, there was a significant effect of AD on generalized epilepsy, but not on the subgroups CAE, JAE, JME, and GTCS. 34% generalized epilepsy cases in the dataset belongs to the "Generalized epilepsy, not otherwise specified, with spike and wave EEG" subcategory, and this nonspecific subcategory might contribute most to the observed association. In addition, there was also limited sample sizes of each subgroup, which might limit the statistical power. Nevertheless, the potential association between early-life epilepsy and late-life neurodegeneration remains to be elucidated.

Our finding that AD was causally linked to focal epilepsy with HS adds to the previous body of literature that hippocampus and mesial temporal lobe (MTL) might account for network hyperexcitability and clinical seizures in AD. In an animal model of AD, spontaneous nonconvulsive seizure activity was detected in hippocampal network [42]. In AD patients without a history of clinically overt seizures, a majority of subclinical epileptiform discharges were detected in the temporal lobes [43]. In patients with early-stage AD and no clinical seizure, left temporal hyperexcitability was detected, while AD patients with comorbid clinically overt seizures demonstrated temporal hyperexcitability in both hemispheres [44].

The casual effect of AD on focal epilepsy with HS has several important implications. First, focal epilepsy with HS commonly presents as focal onset seizures with or without impaired awareness presenting with déjà vu, amnestic spells, fluctuation in cognition. Clinically, it is challenging to distinguish these nonconvulsive seizures from other transient events, including delirium, hallucinations, sundowning, syncope, transient ischemic attack, metabolic disturbances, which are also commonly observed in elderly or demented patients. Therefore, a thorough and goal-directed history taking, and long-term overnight EEG monitoring might facilitate the recognition of nonconvulsive seizures in patients with AD. Second, a large percent of MTL

spikes and seizures are subclinical, and are poorly transmitted to the scalp EEG electrodes [45,46]. The causal association between AD and focal epilepsy with HS indicates an urgent need to develop electrophysiological biomarkers of MTL hyperexcitability [44]. Third, most of the studies in AD excluded patients with personal history of seizure at enrollment. If seizure is an integral part of AD, future studies and trials may consider enrolling subjects with co-morbid seizure.

Causal association is not necessarily exclusively unidirectional, and a potential bidirectional causal association has previously been reported [47]. In the current study, we found that focal epilepsy with HS might be causally linked to AD. This finding is in accordance with previous observations that TLE gives rise to AD-like pathological changes [13,14]. There was also an anecdotal report of a patient with drug-resistant HS diagnosed with AD nine years after temporal lobe resection [13]. In the current study, the suggestive causal effect of focal HS on AD implies that well controlled focal HS would probably mitigate the risk of AD. Furthermore, targeting hyperexcitability is a feasible strategy to prevent or slow the progression of AD [48], more bench and bedside studies are needed.

Confusion might arise when using the term "HS" in the context of AD and dementia. HS is a pathological endpoint that is associated with various underlying brain diseases, including TLE, HS-Aging, tauopathy, non-tauopathy FTD, and cerebrovascular disease [49]. As overlap and discrepancies between HS-Aging and AD are gaining increasing attention, we would like to emphasize that the observed association between "focal HS" and AD does not represents such an association. The "focal HS" subgroup in the current study was derived from the ILAE 2018 GWAS and represented a group of patients with focal epilepsy and evidence of HS on imaging.

Limitations of the current study included as follows. First, clinical diagnosis of AD with different criteria at different centers was prone to inconsistency. Non-Alzheimer's dementia, when incorrectly included, could drive a spurious association. Second, the MR method was unable to explore the time-specific effect. It remains unclear if the epileptogenic process presents before, in parallel with, or after the onset of AD. As emerging evidence indicates that seizures could precede the onset of cognitive decline in AD [40,50], more electrophysiological studies in AD patients at various disease stages are needed. Third, although we used the largest available GWAS data, there is a relatively limited sample size for epilepsy subtypes and CSF biomarkers. Studies enrolling larger sample size are needed to replicate the current findings. In addition, this study constitutes of participants of European ancestry. Future studies using GWAS data from other ethnicities (e.g., Biobank Japan, China Kadoorie Biobank) are needed to test the generalizability across different populations.

In conclusion, using two-sample MR, we provide novel evidence that AD was causally linked to generalized epilepsy and focal epilepsy with hippocampal sclerosis. Our finding highlights that AD and amyloid pathology gives rise to epilepsies. More effort should be made to screen seizure in AD and understand its clinical implications.

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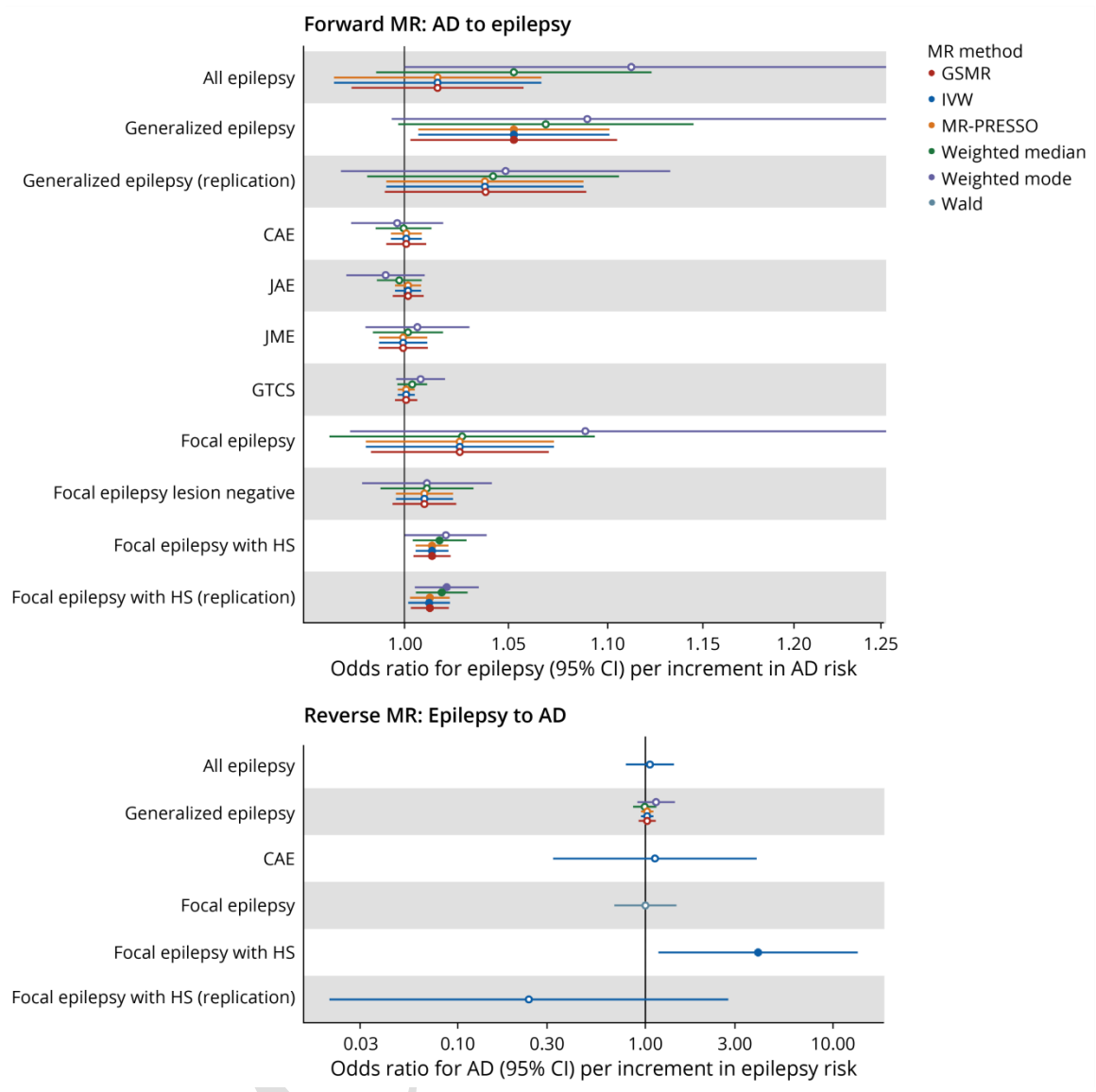
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**Figure 1. Bidirectional associations between AD and epilepsies estimated by mendelian randomization**

AD: Alzheimer's disease; CAE: Childhood Absence Epilepsy; focal HS: focal epilepsy with hippocampal sclerosis; GTCS: generalized epilepsy with tonic-clonic seizures; JAE: Juvenile Absence Epilepsy; JME: Juvenile Myoclonic Epilepsy; MR: mendelian randomization

Forward MR: AD to epilepsy

Odds ratio for epilepsy (95% CI) per increment in AD risk

Reverse MR: Epilepsy to AD

Odds ratio for AD (95% CI) per increment in epilepsy risk

**Figure 2. Association between Alzheimer's disease and focal epilepsy with hippocampal sclerosis estimated by the mendelian randomization**

A: Forest plot of focal epilepsy with hippocampal sclerosis for each 1 SD increase of AD risk.

B: Scatter plot showing the effect of genetic instruments on AD risk against their effect on focal epilepsy with hippocampal sclerosis.

C: There was no substantial change of IVW causal estimate after removing any of the instrumental SNPs.

D: The funnel plot showed no asymmetry.
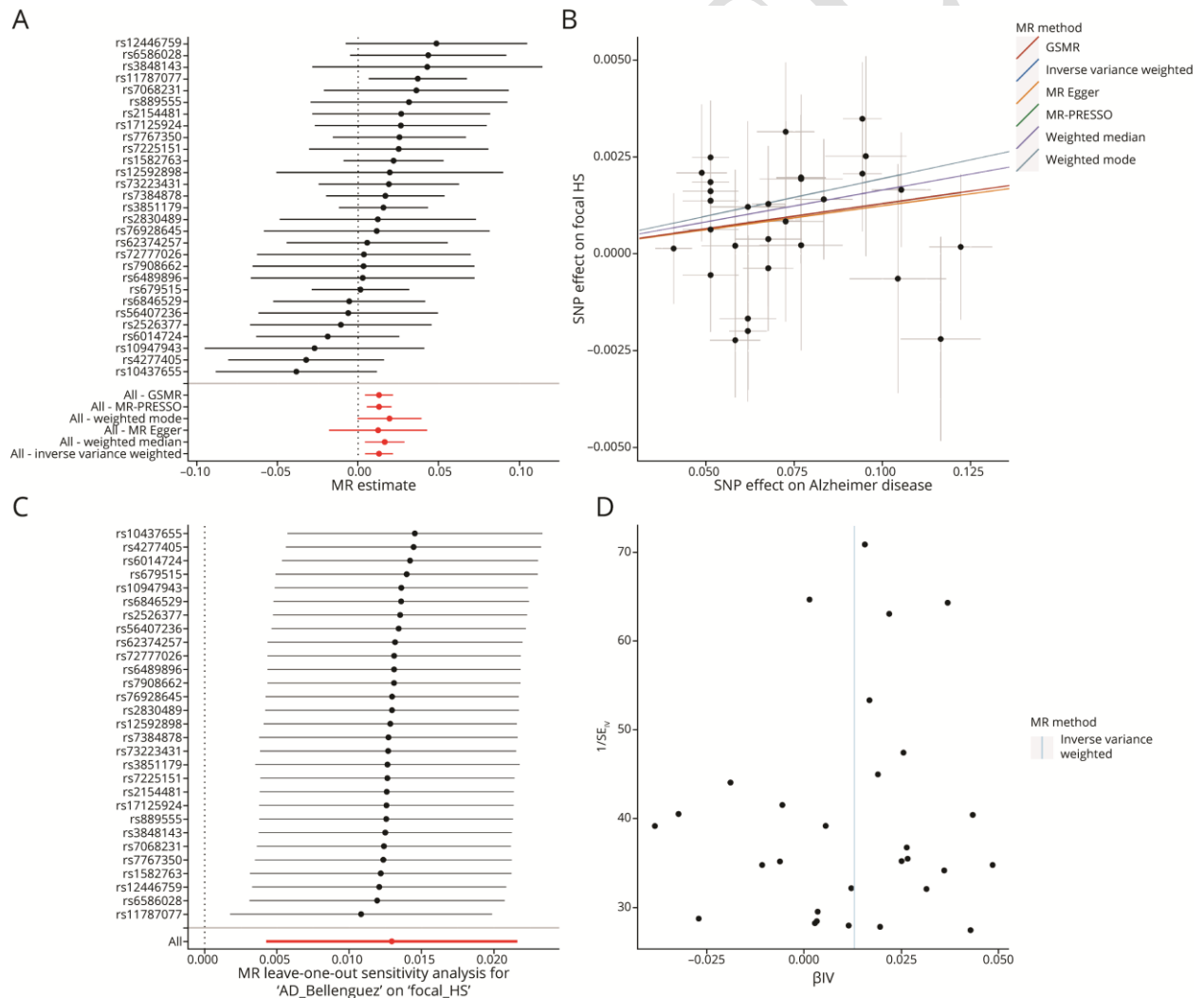
**Figure 3. Association between Alzheimer's disease and generalized epilepsy estimated by the mendelian randomization**

A: Forest plot of generalized epilepsy for each 1 SD increase of AD risk.

B: Scatter plot showing the effect of genetic instruments on AD risk against their effect on generalized epilepsy.

C: There was no substantial change of IVW causal estimate after removing any of the instrumental SNPs.

D: The funnel plot showed no asymmetry.
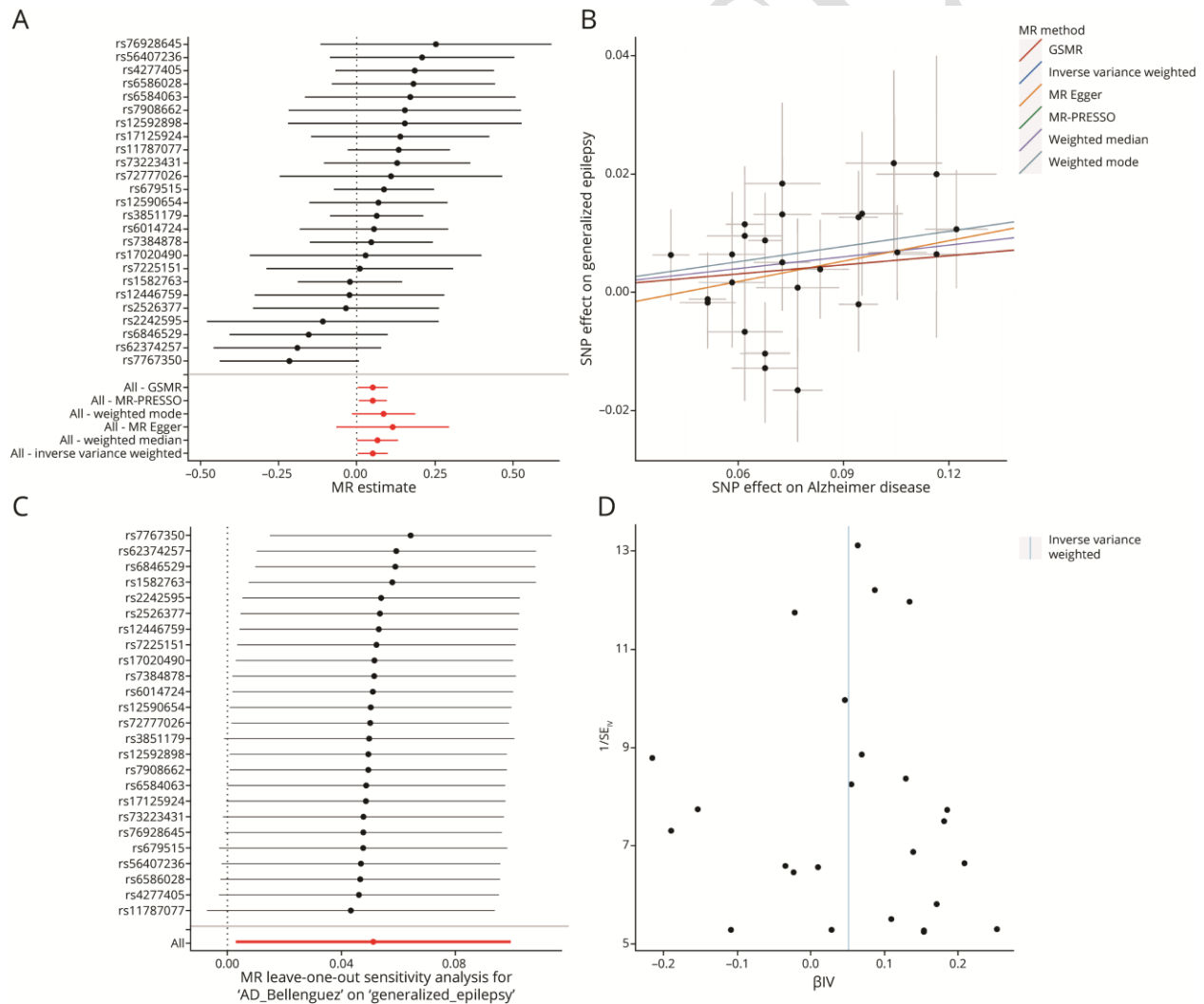
**Figure 4. Association between CSF Aβ42 estimated by mendelian randomization**

Note: for the association between Aβ42 and all epilepsy, between pTau and generalized epilepsy, the MR-PRESSO method found evidence of pleiotropy and the results presented here are outlier-corrected.
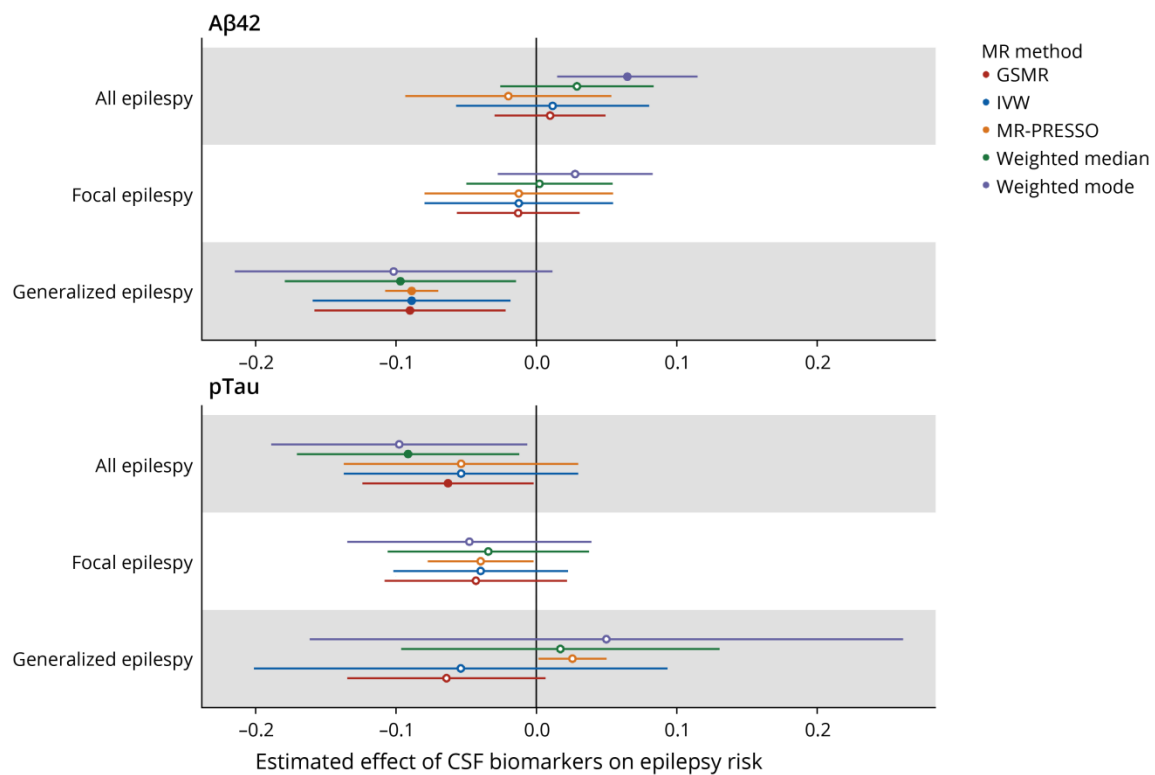


**Figure 5. Estimates of associations between CSF Aβ42 and generalized epilepsy estimated by GSMR using different clumping thresholds and GWAS significance levels**

The estimated effect of CSF Aβ42 on generalized epilepsy remained in similar direction, magnitude and was consistently significant, except for clumping at 0.001. Vertical error bars represent standard error. Note: when GWAS significance level was set at 5e-8 and clumping threshold was set at 0.001, only one SNP was left, and a GSMR analysis was not performed.
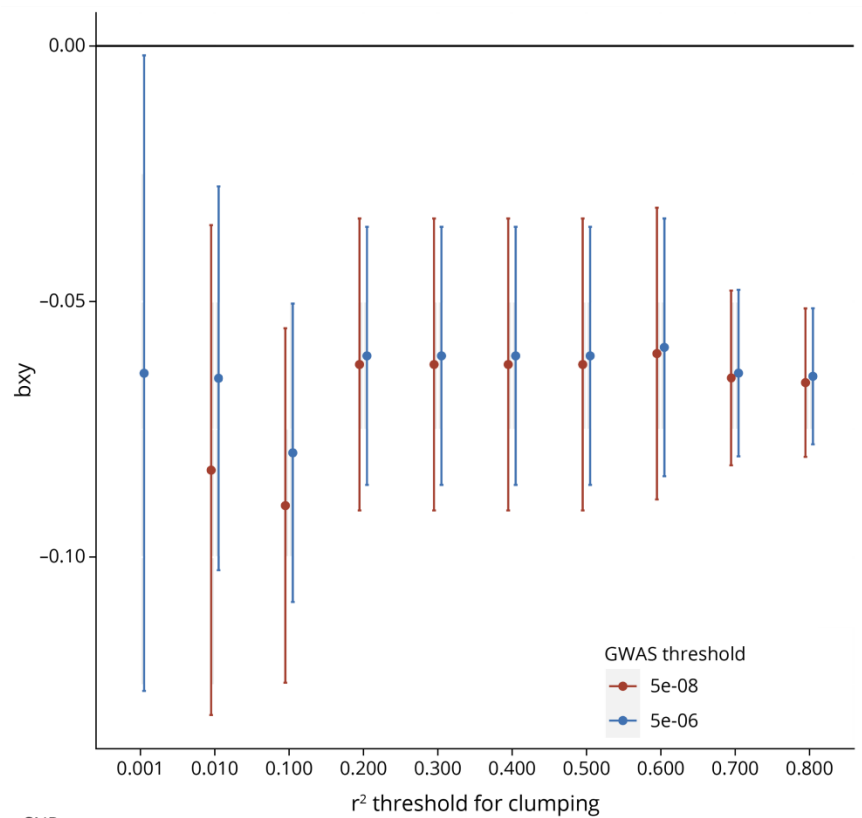
**Table 1 GWAS dataset used in the Mendelian randomization**

| Phenotype | Sample size | | Population (%European) | Publication |
|---|---|---|---|---|
| | *n* cases | *n* controls | | |
| All Epilepsy | 15,212 | 29,677 | 95.5% | Abou-Khalil *et al*. 2018 |
| Generalized Epilepsy | 3,769 | | 98.3% | |
| Focal Epilepsy | 9,671 | | 94.0% | |
| Childhood Absence Epilepsy | 778 | 24,218 | 100%[a] | |
| Juvenile Absence Epilepsy | 415 | | | |
| Juvenile Myoclonic Epilepsy | 1,177 | | | |
| GTCS alone, with spike and wave EEG | 225 | | | |
| Focal epilepsy with HS | 709 | | | |
| Focal epilepsy, lesion other than HS | 2,751 | | | |
| Focal epilepsy, lesion negative | 2,660 | | | |
| Alzheimer's disease | 39,106 diagnosed, 46,828 proxy in stage I [b], 25,392 cases in stage 2 | 401,577 in stage 1[b], 276,086 in stage 2 | 100% | Bellenguez *et al*. 2022 |
| | 17,008 in stage 1[b], 8,572in stage 2 | 37,154 in stage 1[b], 11,312 in stage 2 | 100% | Lambert *et al*. 2013 |
| CSF Aβ42, pTau | 13,116 individuals with subjective cognitive decline, mild cognitive impairment, and dementia | | 100% | Jansen *et al*. 2022 |

[a] GWAS of specific syndromes of genetic generalized epilepsy and phenotypes of focal epilepsy were limited to European ancestry.
[b] Stage 1 was used as outcome as these studies released publicly available stage 1 GWAS summary statistics. GWAS-level significant SNPs from meta-analysis of stage 1 and 2 were used as instrumental SNPs for exposure.
GTCS: generalized epilepsy with tonic-clonic seizures
HS: hippocampal sclerosis

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