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Metabolite Associations with Childhood and Juvenile Absence Epilepsy: A Bidirectional Mendelian Randomization Study

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ABSTRACT

Background: The precise involvement of metabolites in the pathogenesis of Childhood absence epilepsy (CAE) and juvenile absence epilepsy (JAE) remains elusive. Consequently, this investigation introduces bidirectional Mendelian randomization (MR) as a tool to explore causality and underlying mechanisms.
Methods: Bidirectional MR analysis was conducted employing a comprehensive set comprising 1091 human blood metabolites and 309 metabolite ratios, systematically probing potential causal associations with JAE and CAE. Genome-wide association study (GWAS) data pertaining to these epileptic conditions were meticulously obtained from the International League Against Epilepsy (ILAE) consortium. Sensitivity analyses were rigorously performed to evaluate for heterogeneity and pleiotropy. Reverse MR analysis was also conducted to verify the direction of causality, and no significant reverse causal relationships were identified.
Results: Following rigorous genetic variant selection, significant associations were identified based on $P_{IVW} < .05$, $P_{WM} < .05$, and $P_{MR\text{-}Egger} < .05$ criteria in MR analysis. Only 1 metabolite, (2 or 3)-decaonate levels, exhibited an association with JAE ($P = .005$, OR = 0.987, 95% CI = 0.978-0.996). Childhood absence epilepsy was associated with 5 metabolites: X-23648 ($P = .012$, OR = 0.982, 95% CI = 0.968-0.996), X-21845 levels ($P = .045$, OR = 1.018, 95% CI = 1.001-1.035), 2'-*o*-methylcytidine ($P = .008$, OR = 0.995, 95% CI = 0.991-1.001), 2'-*o*-methyluridine ($P = .007$, OR = 0.995, 95% CI = 0.99-0.999), and spermidine-to-pyruvate ratio ($P = .014$, OR = 0.973, 95% CI = 0.954-0.992). No evidence of reverse causality was found between JAE and CAE and the aforementioned metabolites.
Conclusion: The study establishes causal relationships between the aforementioned 6 metabolites and CAE and JAE. This integration of genomics with metabolism offers novel insights into epilepsy mechanisms and has important implications for screening and prevention.

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INTRODUCTION

Epilepsy affects approximately 60 million individuals worldwide, making it one of the most prevalent neurological disorders. In high-income countries, approximately 50 children per 100 000 are diagnosed with epilepsy annually, constituting 25% of newly diagnosed cases.[1,2] Epilepsy encompasses over 40 seizure types, as classified by the International League Against Epilepsy (ILAE), with clinical manifestations ranging from perceptual alterations to motor manifestations or loss of consciousness. Our focus in this context is on Childhood Absence Epilepsy (CAE) and Juvenile Absence Epilepsy (JAE), which represent distinct entities within the spectrum of idiopathic generalized epilepsies (IGEs).[3]

Childhood absence epilepsy is a prevalent subtype of pediatric epilepsy, affecting around 10-17% of cases within this population. This condition is characterized by recurrent absence seizures that typically commence between the ages of 4 and 10, often peaking around 6-7 years.[4,5] These seizures are marked by brief staring spells,

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frequently accompanied by rhythmic eye blinking or motor automatisms, followed by prompt restoration to baseline awareness and activity. On electroencephalography (EEG), CAE displays a recognizable pattern of generalized 3 Hz spike-and-wave discharges, which are bilaterally symmetric and synchronous in nature.[6]

Conversely, JAE stands as a recognized entity within the spectrum of IGEs, distinguished by absence seizures as the primary seizure type. Onset typically occurs after the age of 9 years and is characterized by specific EEG features, including generalized spike waves/polyspikes amidst a normal background.[7,8] Despite its prevalence, accounting for approximately 15% of all IGEs, JAE remains comparatively understudied and may be underdiagnosed.[9] Its clinical presentation often involves absence seizures with less frequency and reduced alteration of consciousness compared to CAE. Additionally, generalized tonic-clonic seizures may manifest early in the disease course, alongside potential occurrences of myoclonus. Juvenile absence epilepsy affects both genders equally, with an estimated prevalence ranging from 0.2% to 2.4% of all epilepsies.[10]

Concurrently, the metabolites, comprising a diverse array of small molecules reflecting the intermediates or end products of metabolic processes, emerge as a pivotal domain in understanding disease etiology and therapeutic interventions. Influenced by genetics, diet, lifestyle, and disease states, metabolites wield significant influence over disease susceptibility and therapeutic targets.[11-13] Utilizing human genetics, particularly Mendelian randomization (MR), offers a potent approach to unraveling the causal implications of metabolites in disease pathogenesis. Mendelian randomization leverages genetic variants as instrumental variables to examine metabolite-exposure-disease relationships, while mitigating confounding biases inherent in observational studies.[14] This methodological framework holds promise in elucidating the intricate interplay between the plasma metabolome and disease outcomes, offering insights into novel intervention strategies and therapeutic avenues.[15]

However, there has been limited recent research employing Mendelian randomization methods to investigate the causal association between the plasma metabolome and JAE and CAE. Prior investigations have explored the causal nexus between 486 metabolites and various subtypes of epilepsy (including generalized and focal epilepsy) within European cohorts, revealing evidence of causality for 4 specific metabolites.[16] Another independent study has reported an association between serum levels of 25-hydroxyvitamin D and adolescent absence epilepsy, albeit without observed associations with other forms of epilepsy. Notably, these epilepsy data were sourced from the ILAE Consortium.[17]

In this investigation, our objective is to explore the causal association between 1400 plasma metabolites and JAE and CAE using a bi-directional Mendelian randomization framework. The aim is to alleviate the common biases often observed in observational studies. Given the context of disease prevention and treatment, Mendelian randomization methods offer a promising alternative to randomized clinical trials, which may not always be feasible. Consequently, the findings of this study hold potential for informing and prioritizing candidate drug targets, particularly when substantiated by genetic evidence.[18,19] To evaluate causality directionality and mitigate reverse causation, a bi-directional strategy was adopted, treating the 1400 plasma metabolites as exposures and JAE/CAE as outcomes, and vice versa. This study adheres to the reporting guidelines outlined in the STROBE-MR checklist forMendelian randomization studies.[20]

MAIN POINTS

- Causal Relationships Between Metabolites and Epilepsy: The study establishes causal relationships between specific metabolites, including (2 or 3)-decanoate and spermidine, and the risk of CAE and JAE. This is achieved through a bidirectional MR approach, which provides evidence supporting the involvement of these metabolites in epilepsy pathogensis.
- Bidirectional Mendelian Randomization Approach: The research utilizes a robust bidirectional Mendelian randomization analysis to explore the causal associations between 1400 plasma metabolites and epilepsy. This approach mitigates confounding factors and reverses causality, ensuring that the identified associations are likely genuine causal relationships rather than coincidental correlations.
- Identification of Significant Metabolites: The study identifies six significant metabolites linked to CAE and JAE. For JAE, the study highlights (2 or 3) decanoate as a key metabolite, while for CAE, metabolites such as X-23648, X-21845, 2'-Oo-methylcytidine, 2'-Oo-methyluridine, and the spermidine to pyruvate spermidine to pyruvate spermidine ratio are identified as significant. These findings suggest that these metabolites could serve as biomarkers for the respective conditions.
- Implications for Screening and Prevention: The findings of the study have important implications for the screening and prevention of CAE and JAE. By identifying specific metabolites that are causally linked to these conditions, the research opens up potential pathways for early diagnosis and targeted preventive measures, which could significantly improve clinical outcomes for patients with these forms of epilepsy.

MATERIAL AND METHODS

Study Design

In this study, all voluntarily participated in the survey. The study was approved by the Ethics Committee of Jewish General Hospital (Approval Number EC202209101).

In this study, we conducted a thorough investigation into the relationship between 1400 metabolites and JAE and

CAE utilizing a robust Mendelian randomization approach. A rigorous Mendelian randomization analysis involves scrutinizing 3 pivotal hypotheses: (1) genetic instrumental variables demonstrate a substantial association with the exposure under examination; (2) genetic instrumental variables are free from any known or unknown confounding factors and bear no relevance to the outcome; and (3) the impact of instrumental variables on the findings is solely mediated by the exposure under examination.[21] To address these hypotheses, we employed a bidirectional analytical approach to identify genetically significant single nucleotide polymorphisms (SNPs) associated with 1091 metabolites and 309 metabolite ratios sourced from the Canadian Longitudinal Study on Aging (CLSA) cohort. To prevent sample overlap, metabolomic data and genetic information for JAE and CAE were extracted from distinct genome-wide association study (GWAS) datasets. Figure 1 provides a schematic depiction of this bidirectional MR investigation.[15,22]

Genome-Wide Association Study Data Sources for 1400 Plasma Metabolome

Genome-wide association study summary statistics for blood metabolites and metabolite ratios were obtained from the GWAS Catalog. Specifically, we utilized data from the European GWAS: GCST90199621–90201020. The analysis involved 8299 unrelated individuals of European descent from The CLSA. A total of approximately 15.4 million SNPs were tested for association with 1091 blood metabolites and 309 metabolite ratios.[15,22]

Genome-Wide Association Study Data for Juvenile Absence Epilepsy and Childhood Absence Epilepsy

The primary analysis relied on data from the ILAE consortium, comprising 793 cases of CAE and 415 cases of JAE, alongside 29 677 control subjects for each group. Nearly 86% of participants were of European descent, with approximately half being female. The datasets for CAE and JAE included genetic information from 4 979 765 and 4 986 340 SNPs, respectively.[23]

Selection of Instrumental Variables

Initially, we set a genome-wide significance threshold of $P < 5 \times 10^{-8}$ to detect highly correlated SNPs linked to the 1400 plasma metabolites, particularly those related to JAE and CAE. However, due to the limited number of SNPs identified for specific metabolites when considered as exposure variables, we revised the threshold to a slightly higher level of $P < 1 \times 10^{-5}$. To ensure the selection of independent SNPs and minimize the impact of linkage disequilibrium (LD), we established an LD parameter (r^2) threshold of 0.001 and a genetic distance of 10 000 kb. The strength of the association between instrumental variables and exposure factors was assessed using the F statistic, which was calculated as shown in formula (A). To address potential bias from weak instruments, we restricted our analysis to SNPs with an F statistic greater than 10.[21,24]

$$(A)\ F = \left(\frac{\beta}{SE}\right)^2$$

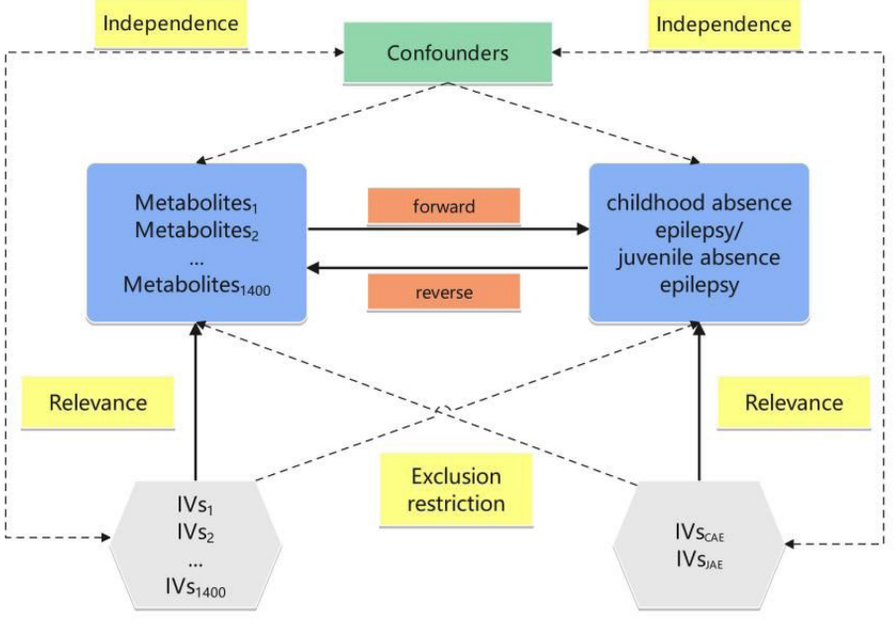

Figure 1. Bidirectional Mendelian randomization (MR) analysis schematic diagram. Selecting 1400 plasma metabolites with differential abundance as significant instrumental variables for exploring their bidirectional causal relationships with JAE and CAE. The 3 fundamental assumptions of Mendelian randomization analysis are represented by acyclic graphs.

(A) Formula for calculating the *F* statistic, where β represents the effect size and *SE* denotes the standard error.

Statistical Analysis

Using the "fastMR" package within R software (version 4.3.2), we conducted a bidirectional 2-sample MR study to explore the relationship between 1400 metabolites and both JAE and CAE. Our MR analysis employed a range of methodologies, encompassing the random-effects variance-weighted model (IVW), MR-Egger,[25] weighted median,[26] and weighted mode. While the random-effects IVW model served as the primary approach, it was supplemented by MR-Egger, weighted median, and weighted mode analyses to ensure robustness in our findings. To assess heterogeneity in SNP effects concerning the 1400 plasma metabolome, JAE, and CAE, we utilized both the I^2 index and Cochran's Q statistic in the IVW analyses. The proportion of heterogeneity within the total variance was determined by equation (B),[27] A *P*-value exceeding .05 indicated the absence of significant heterogeneity. Furthermore, both the MR-Egger method and a "leave-one-out" analysis were employed to investigate horizontal pleiotropy. The MR-Egger method is utilized to assess pleiotropy by examining the intercept term, while the "leave-one-out" analysis examines whether the exclusion of individual SNPs affects the causal relationship between exposure and outcome. A *P*-value exceeding .05 suggested no evidence of horizontal pleiotropy. Our initial analysis fulfilled the criteria of $P_{IVW} < .05$ and $P_{WM} < .05$, thus rendering it significant. Subsequently, we conducted a more rigorous examination, requiring associations to meet the criteria of $P_{IVW} < .05$, $P_{WM} < .05$, and $P_{MR\text{-}Egger} < .05$ for classification as significant.

$$(B)\ I^2 = \frac{Q - df}{Q} \times 100\%$$

(B) Formula for calculating the I^2 statistic, where Q represents Cochran's Q statistic and *df* is the degrees of freedom.

Reverse MR Analysis

To verify the directionality of the causal relationships, we conducted a reverse Mendelian randomization analysis. This analysis considered CAE and JAE as exposures and metabolites as outcomes. The results indicated that no significant reverse causal relationships exist between CAE and JAE and the metabolites. These findings confirm the primary direction of causality established in our study.

RESULTS

Out of the 1400 analyzed metabolites, only 2 valid SNPs were identified with a genome-wide significance cutoff of 5×10^{-8}. To ensure an adequate number of SNPs for subsequent MR analysis, the threshold for the 1400 metabolites was increased to $P < 1 \times 10^{-5}$. In the selection process of instrumental SNPs for MR analysis, a total of 1400 metabolites or metabolite ratios were chosen, with each compound or ratio associated with varying numbers of SNPs, ranging from 5 to 20. None of the SNPs were excluded based on F statistics, indicating the absence of weak instruments. The distribution and significance of these associations are visually summarized in Figures 2 and 3. Figure 2 presents the circular heat map of IVW,

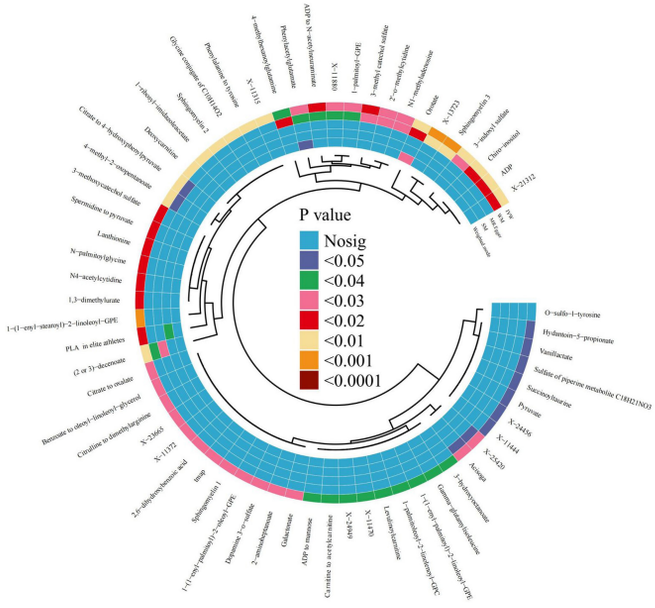

Figure 2. The circular heat map of IVW, WM, Mendelian randomization-Egger, SM, and weighted mode for juvenile absence epilepsy exposure (IVW < 0.05)

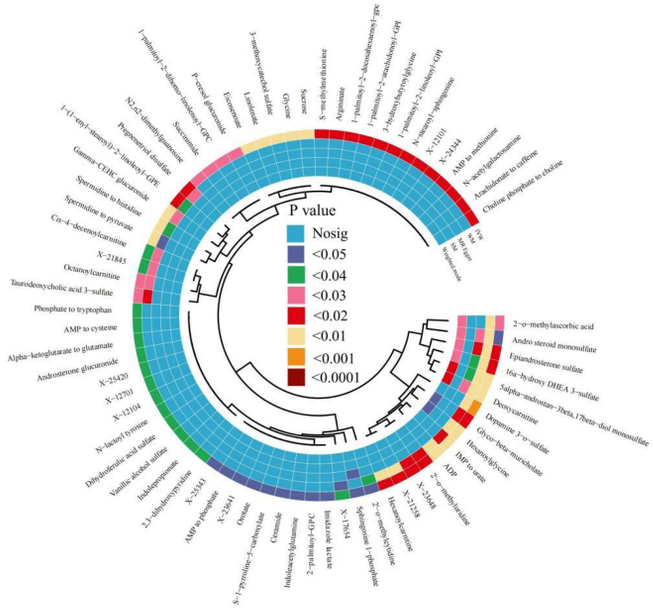

Figure 3. The circular heat map of IVW, WM, Mendelian randomization-Egger, SM, and weighted mode for childhood absence epilepsy exposure (IVW < 0.05).

WM, MR-Egger, SM, and weighted mode analyses for JAE exposure, highlighting significant associations (IVW < 0.05). Similarly, Figure 3 provides a corresponding circular heat map for CAE exposure, detailing the significant metabolites under the same criteria.

Utilizing IVW and WM methodologies, we identified significant associations between JAE and CAE with various metabolites across distinct metabolic pathways, all with *P*-values below .05. In JAE, key associations were observed in lipid metabolism (e.g., sphingomyelin), carbohydrate metabolism (e.g., chiro-inositol), neurotransmitter metabolism (e.g., 3-methyl catechol sulfate), fatty acid metabolism, RNA modification, and energy metabolism. For CAE, metabolites significantly associated with the condition spanned lipid metabolism (e.g., epiandrosterone sulfate), carbohydrate metabolism, neurotransmitter metabolism, hormone metabolism, RNA modification, and cellular homeostasis. Comprehensive details for both conditions are available in Supplementary Figure 1. Notably, Cochran Q tests indicated no significant heterogeneity and leave-one-out analyses affirmed the stability of these associations. Furthermore, reverseMendelian randomization analysis revealed no significant causal effects in the opposite direction for either JAE or CAE.

Further analysis adhered to stringent significance criteria (IVW < 0.05, WM < 0.05, MR-Egger < 0.05). Specifically, (2 or 3)-decenoate levels were exclusively associated with JAE, while X-23648, X-21845, 2'-*o*-methylcytidine, 2'-*o*-methyluridine, and spermidine to pyruvate ratio were significantly linked to CAE. Cochran Q-derived *P* values and I^2 indicated homogeneity in causal associations. The robustness of these associations was further confirmed through "leave-one-out" analyses, as shown in Supplementary Figure 2 for CAE as the outcome and Supplementary Figure 3 for JAE as the outcome (IVW < 0.05, WM < 0.05, MR-Egger < 0.05). As illustrated in Figure 4, the MR analysis forest plots depict these associations. Notably, reverse MR analysis demonstrated no significant associations between CAE, JAE, and previously significant metabolite ratios.

DISCUSSION

By integrating metabolomics and genomics data, we have offered valuable insights for identifying potential biomarkers for JAE through Mendelian randomizationstudies. 2-decanoate Decenoate and 3-decanoate are also known as metabolites of fatty acids.[28,29] Fatty acids play a crucial role in the nervous system, profoundly influencing the formation and functionality of myelin sheaths. Alongside cholesterol and phospholipids, fatty acids constitute a significant portion of myelin lipids, forming its fundamental structure.[30] Their presence enhances myelin viscosity, stabilizing its lipid and protein composition, thus protecting neuronal axons and facilitating nerve impulse propagation. Additionally, fatty acids are involved in the initiation and compaction of myelin, increasing its thickness and stability to further shield neurons from external injury.[31] Serving as primary constituents of neuronal cell membranes, fatty acids modulate membrane fluidity, stability, and permeability, thereby influencing neuronal electrical signal conduction and intercellular communication.[32] Moreover, fatty acids are integral to neuronal energy metabolism, producing ATP through oxidative metabolism to support normal neuronal function. Furthermore, fatty acids can influence neuronal signaling pathways and the synthesis and release of neurotransmitters, crucially regulating neuronal excitability and inhibition, thus modulating the stability and functionality of neuronal networks.[33]

The neurotoxic effects of fatty acids and their underlying mechanisms have been clarified. Excessive fatty acid accumulation can induce lipid peroxidation in neuronal cells, resulting in the production of detrimental oxidative byproducts. Moreover, an excessive influx of fatty acids can activate non-oxidative metabolic pathways in cells, leading to ceramide overproduction and subsequent cellular

Outcome	Exposure	nSNP	MR test	*P* value	Pleiotropy test	P for pleiotropy	Cochran's *Q* test	I^2	P for heterogeneity
JAE	(2 or 3)−decenoate levels	11	IVW	0.005			IVW	9.30%	0.597
		11	MR Egger	0.022	MR Egger	0.089	MR Egger	0.00%	0.356
		11	Weighted median	0.032					
CAE	X−23648 levels	15	IVW	0.012			IVW	0.00%	0.461
		15	MR Egger	0.031	MR Egger	0.433	MR Egger	1.29%	0.431
		15	Weighted median	0.008					
CAE	X−21845 levels	15	IVW	0.045			IVW	0.00%	0.897
		15	MR Egger	0.026	MR Egger	0.138	MR Egger	0.00%	0.792
		15	Weighted median	0.005					
CAE	2'−o−methylcytidine levels	8	IVW	0.008			IVW	0.00%	0.687
		8	MR Egger	0.038	MR Egger	0.453	MR Eggerr	0.00%	0.713
		8	Weighted median	0.005					
CAE	2'−o−methyluridine levels	12	IVW	0.007			IVW	0.00%	0.835
		12	MR Egger	0.029	MR Eggerr	0.301	MR Egger	0.00%	0.803
		12	Weighted median	0.004					
CAE	Spermidine to pyruvate ratio	11	IVW	0.014			IVW	5.50%	0.641
		11	MR Egger	0.012	MR Egger	0.089	MR Egger	0.00%	0.391
		11	Weighted median	0.006					

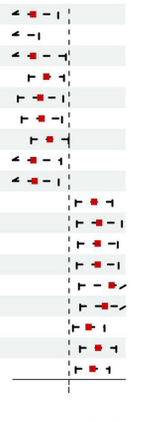

Figure 4. The figure represents a forest plot of Mendelian randomization analysis with plasma metabolites as exposure and juvenile absence epilepsy and childhood absence epilepsy as outcomes, along with tests for heterogeneity and horizontal pleiotropy.

toxicity. Additionally, studies have revealed that in cells lacking low-density lipoprotein, fatty acids can undergo conversion to acylcarnitines, inducing mitochondrial fragmentation and dysfunction, thus promoting the generation of reactive oxygen species. Lipid membrane peroxidation can also lead to lipid release, exacerbating the detrimental effects. Neurons and astrocytes collaborate in transporting fatty acids using lipoprotein particles to alleviate their neurotoxic effects. Under conditions of oxidative stress, neurons upregulate the expression of ApoE, a lipoprotein, to facilitate the disposal of fatty acids.[34] Moreover, astrocytes metabolize lipid particles via mitochondrial oxidative processes, lowering reactive oxygen species levels and thus safeguarding neurons against the neurotoxic effects of fatty acids.[35]

X−23648 and X−21845 represent 2 unidentified metabolites. The levels of 2'-*o*-methylcytidine and 2'-*o*-methyluridine, which are nucleoside modifications, participate in diverse cellular processes, including the regulation of RNA stability and gene expression.[36] Spermidine denotes a polyamine compound intricately involved in various cellular phenomena, including cell proliferation, differentiation, and programmed cell death.[37] Conversely, pyruvate plays a crucial role as a pivotal metabolite in the glycolytic pathway, serving as a precursor for the citric acid cycle and thus assuming a paramount role in cellular energy production. miRNAs, small non-coding RNA molecules, have the capacity to modulate cellular functionality by regulating gene expression. After maturation, these miRNAs are enclosed within the binding pocket of the Argonaute (AGO) protein, with AGO2 playing a pivotal role as an effector, thus forming the RNA-induced silencing complex (RISC). The miRNA-loaded RISC diligently searches target mRNAs for seed regions containing nucleotide complementarity, ultimately leading to the inhibition or degradation of the translation of the target. Within extracellular vesicles, including exosomes, AGO-bound miRNAs are abundant in bodily fluids such as plasma, potentially originating from various tissues, and their compositional variations suggest diagnostic potential.[14,38-42]

Spermidine (SPD) serves as a gliotransmitter released from astrocytes, exerting influence on network excitation and contributing to epileptiform activity. Inhibition of SPD synthesis in epilepsy models prevents seizure generation, highlighting its potential as an antiepileptic target. By enhancing the conversion of putrescine (PUT) to gamma-aminobutyric acid (GABA) in astrocytes, SPD facilitates GABA release through GAT-2/3 transporters, potentially elucidating its antiepileptic effect. Moreover, the antiepileptic drug levetiracetam may operate by augmenting surface expression of GAT-2/3, further implicating astrocytic GABA release in epilepsy modulation.[37] Additionally, spermidine exerts multiple protective actions on neurons. Firstly, it mitigates oxidative stress damage by enhancing superoxide dismutase (SOD) activity and reducing malondialdehyde (MDA) levels. Secondly, spermidine promotes autophagy by activating AMP-activated protein kinase (AMPK) and modulating autophagy-related proteins, facilitating the removal of cellular waste and damaged molecules. Furthermore, spermidine regulates mitochondrial function by controlling mitochondrial dynamics and proteins such as cytochrome c oxidase IV (COX IV), thereby ensuring an ample energy supply for neurons. Lastly, it suppresses the expression of apoptotic proteins, diminishes inflammatory factors, and reduces neuronal cell death and inflammation.[43]

Pyruvate plays a pivotal role in supporting neuronal function by facilitating tricarboxylic acid cycle (TCA) activity, ensuring sufficient ATP production. It achieves this by compensating for the depletion of alpha-ketoglutarate resulting from the release of neurotransmitters glutamate and GABA, both of which are derivatives of alpha-ketoglutarate. While neuronal pyruvate carboxylation holds significant quantitative importance, it must be counterbalanced by the decarboxylation of malate or oxaloacetate since there is no net CO_2 fixation in the brain. This decarboxylation process occurs in both neurons and astrocytes. Pyruvate supplementation has demonstrated a neuroprotective effect, particularly during energy deficiency, potentially through pyruvate carboxylation via malic enzyme, leading to an increase in dicarboxylates that can be metabolized for ATP production through the TCA cycle.[44]

Furthermore, pyruvate's involvement in cellular metabolism extends to its capacity to induce intracellular pH reduction. This acidification of the cytosol triggers autophagy and mitophagy, essential processes for maintaining cellular homeostasis and eliminating misfolded proteins and damaged mitochondria. Dysregulation of autophagy and mitophagy is implicated in various neurodegenerative disorders. Notably, organic compounds such as lactate and pyruvate, inherent to cellular metabolism, can effectively reduce intracellular pH at non-toxic concentrations. Incubation with lactate or pyruvate has been demonstrated to induce both short-term and long-term mitophagy and autophagy, offering protection against apoptotic and necrotic cell death in neurons and astrocytes, thereby preserving mitochondrial function. Therefore, pyruvate-induced cytosolic acidification serves as a mechanism to activate cell-protective autophagy and mitophagy, suggesting potential therapeutic strategies for neuroprotection.[45]

Several limitations must be acknowledged in this investigation. GWAS data originate predominantly from European Caucasians, limiting the generalizability of our findings to other ethnicities. Epidemiological research highlights varying incidence rates of JAE and CAE across regions. Uncertainty persists regarding significant disparities in our predictive factors due to insufficient genomic data from diverse regions and ethnicities. Our

Menedlian randomization study rigorously screened 1091 metabolites and 309 metabolite ratios, wherein 1180 serum metabolites had explicit structures and names, while 220 were unknown or partially characterized. Our study distinguishes itself by incorporating the largest sample size in serum metabolite and JAE and CAE Menedlian randomization analyses. It is crucial to recognize that the selected metabolite samples represent only a small portion of the vast and diverse blood metabolome.

CONCLUSION

The Menedlian randomization study emphasized the causal link between metabolites and JAE and CAE risk, along with the potential effect of cyclic metabolism disruptions on these conditions. In particular, (2 or 3)-decenoate, X-23648, X-21845, 2'-*o*-methylcytidine, 2'-*o*-methyluridine, and the spermidine-to-pyruvate ratio were identified as cyclic metabolism biomarkers suitable for screening and preventive clinical practice in JAE and CAE.

Data Availability Statement: The dataset used in this study is publicly available. Metabolomic and genetic information for the 1091 metabolites and 309 metabolite ratios were sourced from the Canadian Longitudinal Study on Aging (CLSA) cohort, as detailed in the article with PMID: 36635386. Data for childhood absence epilepsy (CAE) and juvenile absence epilepsy (JAE) were obtained from the GWAS study accessible via PMID: 30531953.

Ethics Committee Approval: This study utilized publicly available data. Metabolomic data were approved by the Jewish General Hospital Ethics Board (Protocol Number: 2021-2762). Genetic data (CAE and JAE) were approved by local ethics boards at respective sites.

Informed Consent: N/A.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – L.D., J.L., R.L; Design – L.D., J.L., R.L; Supervision – L.D.; Data Collection and/or Processing – H.Y., X.Z., H.Y., Q.W., J.Z., D.L., Y.W., D.W.; Analysis and/or Interpretation – R.L., H.Y., X.Z., H.Y., Q.W., J.Z., D.L., Y.W., D.W.; Literature Search – R.L.; Writing – J.L., R.L.; Critical Review – L.D., J.L.

Declaration of Interests: The authors have no conflicts of interest to declare.

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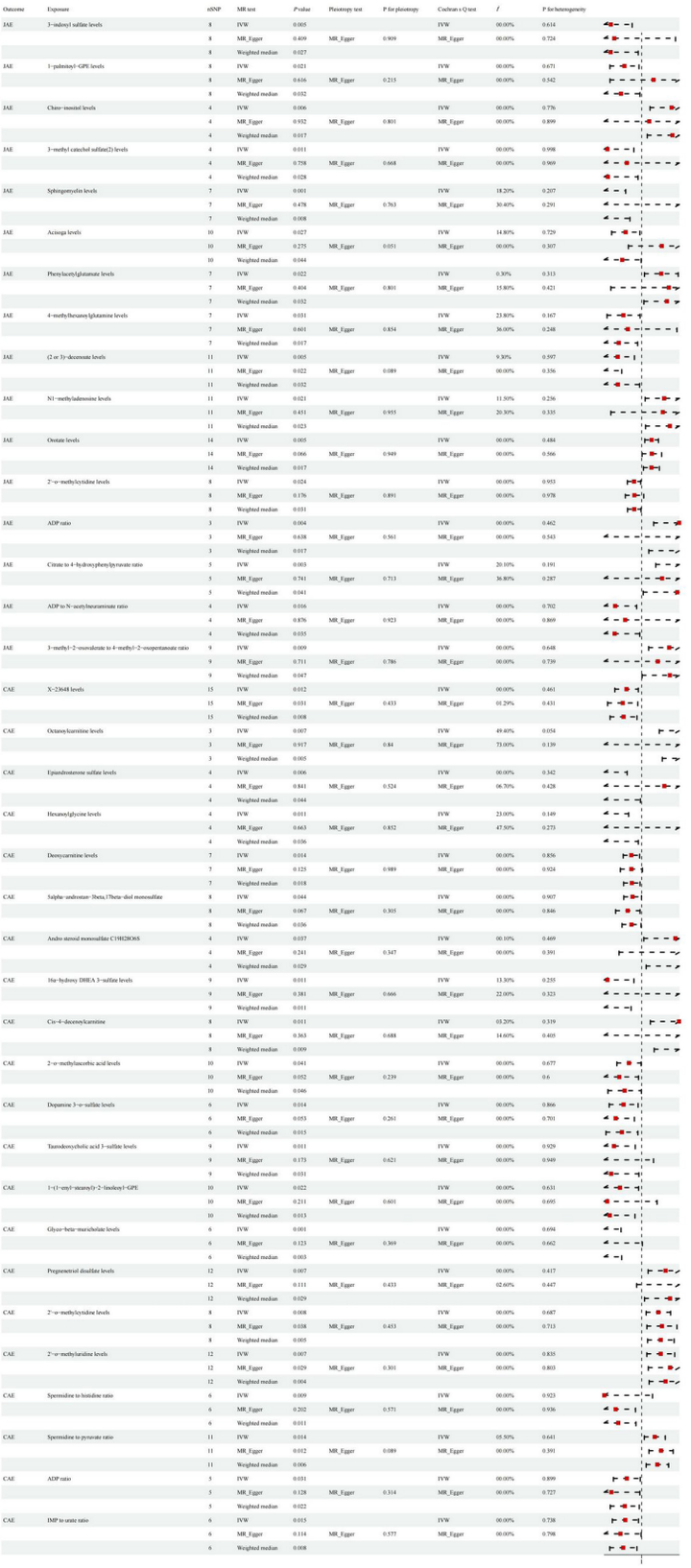
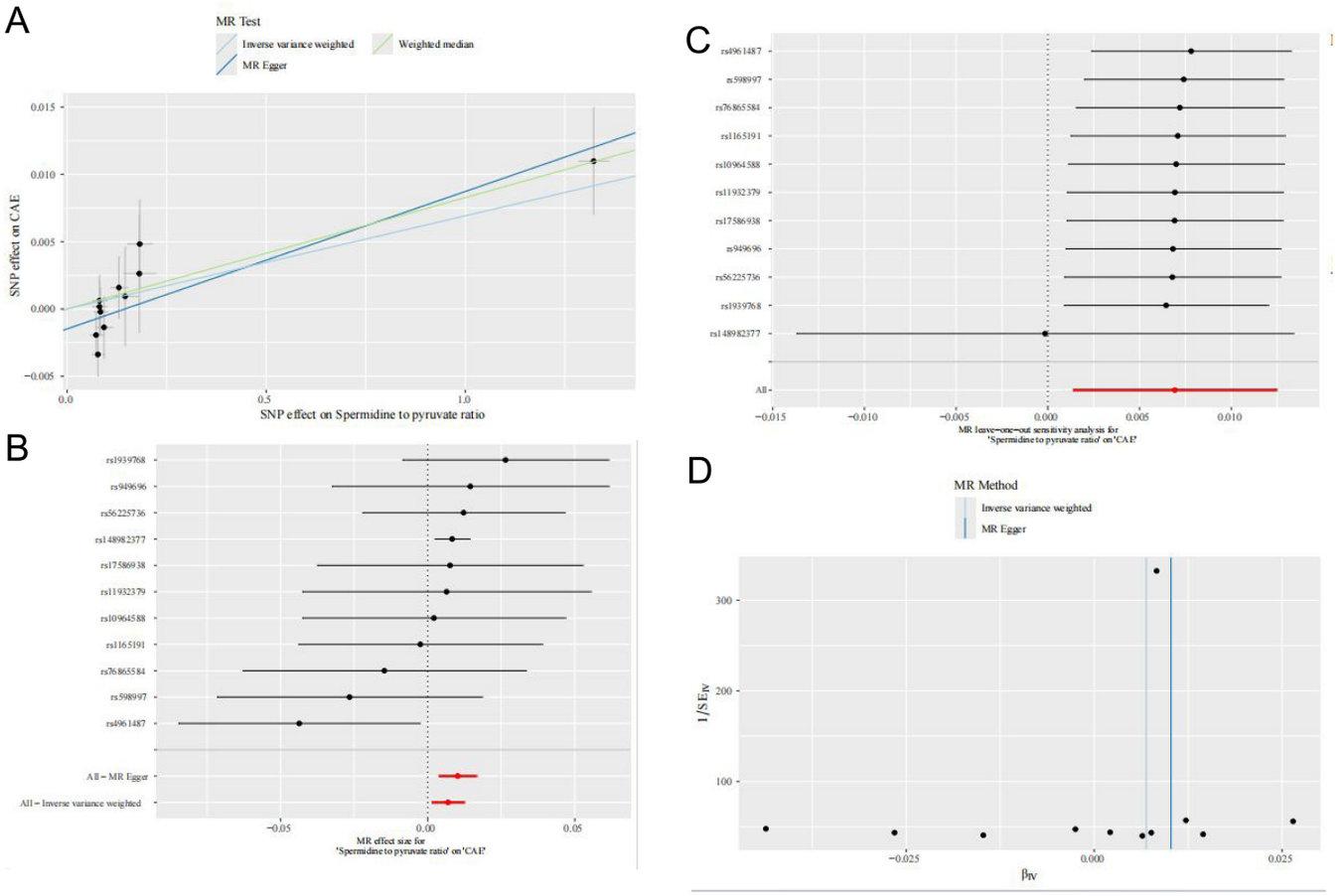
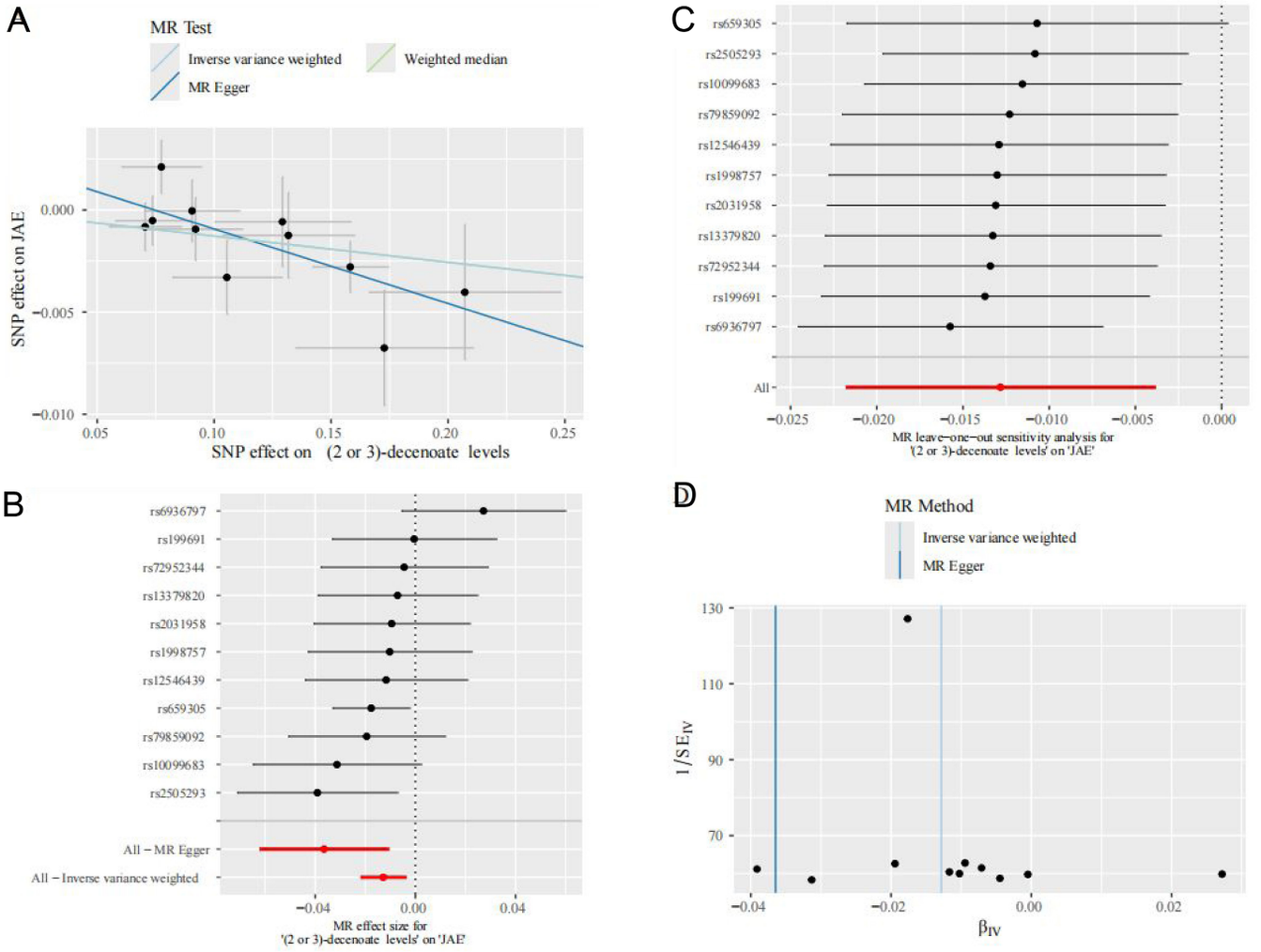


Supplementary Figure 1. Forest Plot of MR Analysis with Metabolites as Exposure and JAE and CAE as Outcomes (IVW < 0.05, WM < 0.05)



Supplementary Figure 2. Leave-One-Out Analysis with Metabolites as Exposure and CAE as Outcome (IVW < 0.05, WM < 0.05).



Supplementary Figure 3. Leave-One-Out Analysis with Metabolites as Exposure and JAE as Outcome (IVW < 0.05, WM < 0.05).