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Research article

Diet-derived circulating antioxidants and risk of epilepsy: A study combining metabolomics and mendelian randomization

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ABSTRACT

Background: Previous studies offer inconclusive results on the association between diet-derived circulating antioxidants and epilepsy.
Objective: This study aims to assess oxidative stress presence in epilepsy patients' circulation and investigate the causal link between diet-derived circulating antioxidants and epilepsy.
Methods: Untargeted metabolomics analysis was conducted on plasma samples from 62 epileptic patients and 20 healthy individuals to evaluate oxidative stress based on metabolite alterations in epilepsy patients' circulation. Two-sample Mendelian Randomization (MR) analysis examined the causation between diet-derived circulating antioxidants (measured by absolute levels and relative metabolite concentrations) and epilepsy, utilizing the inverse-variance weighted (IVW) method as the primary outcome, with complementary MR analysis methods (MR Egger, weighted median, weighted mode, and simple mode).
Results: Untargeted metabolomics analysis revealed elevated circulating oxidizing metabolites (palmitic acid, oleic acid, linoleic acid, and myristic acid) and reduced reducing metabolites (glutamine) in epilepsy patients, providing robust evidence of oxidative stress. The IVW analysis indicated significantly reduced epilepsy risk (odds ratio: 0.552; 95% confidence interval: 0.335–0.905, $P = 0.018$) with genetically determined higher absolute circulating β-carotene. However, other diet-derived circulating antioxidants (lycopene, retinol, ascorbic acid, and selenium) and antioxidant metabolites (α-tocopherol, γ-tocopherol, ascorbic acid, and retinol) did not significantly associate with epilepsy risk. Additional MR analysis methods and heterogeneity assessments confirmed the results' robustness.
Conclusion: This study provides compelling evidence of oxidative stress in epilepsy patients' circulation. However, the majority of diet-derived circulating antioxidants (lycopene, retinol, ascorbic acid, vitamin E, and selenium) are unlikely to causally associate with reduced epilepsy risk, except for β-carotene.

1. Introduction

Epilepsy is a common neurological disorder affecting over 70 million people worldwide, with 80% of these patients residing in low- and middle-income countries, consequently imposing a considerable financial burden [1]. Although current antiepileptic drugs are

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effective in controlling seizures, long-term application could lead to drug tolerance, eventually developing into drug-refractory epilepsy [2]. Therefore, further exploration of mechanisms is urgently needed for the development of novel therapeutic approaches. Previous studies have highlighted that the pathological process of epilepsy is frequently associated with oxidative stress [3]. Neuronal damage is induced by oxidative stress, leading to the formation of a new network composed of surviving neurons, newly generated neurons, and proliferating glial cells. When this network facilitates the development and propagation of epilepsy, seizures ensue, resulting in the demise of newly formed neurons and establishing a vicious cycle [4–6]. Metabolomics, which investigates small molecule metabolites (<1000 Da) present in biological systems, serves as a powerful tool for the comprehensive assessment of dynamic metabolite alterations following endogenous or exogenous perturbations. Metabolomics analysis enables the understanding of the metabolic characteristics of organisms under physiological or pathological conditions [7]. In this study, untargeted metabolomics methods were employed to comprehensively and indiscriminately detect differential circulating metabolites in both epilepsy patients and healthy individuals. The findings revealed an elevation in the levels of oxidizing substances (palmitic acid, oleic acid, linoleic acid, and myristic acid) and a reduction in the levels of the reducing substance (glutamine) in the circulation of epileptic patients, indicating the presence of oxidative stress. Regrettably, currently, there is no optimal medication available to mitigate neuronal death by modulating oxidative stress and subsequently ameliorate epilepsy.

Animal model studies of epilepsy provide substantial evidence supporting the role of antioxidants in mitigating epilepsy by combating the detrimental effects of excessive reactive oxygen species (ROS) in mitochondria and inhibiting cell death [8,9]. As a result, antioxidants were regarded as a promising neuroprotective strategy for epileptic disorders [10]. Compared to pharmaceutical regulation of the endogenous antioxidant enzyme system, directly consuming antioxidants through the diet offers a simpler and less toxic approach. The primary dietary sources of antioxidants include β-carotene, lycopene, retinol, ascorbate acid, vitamin E, and selenium [11]. However, these diet-derived circulating antioxidants were controversial over the impact on epilepsy in previous studies. Studies conducted on pentylenetetrazol (PTZ) and high-fat diet-induced rat epilepsy models have indicated that vitamin E has the potential to attenuate mitochondrial oxidative stress in the brain [12–14]. Two clinical studies conducted on patients with epilepsy have demonstrated that long-term administration of vitamin E can reduce the frequency of seizures by approximately 60% in individuals with refractory epilepsy [15,16]. These findings suggest that vitamin E may be a potential treatment option for epilepsy. However, other randomized, double-blind clinical trials have reported that vitamin E supplementation for a duration of three months did not have an impact on the occurrence of seizures, regardless of the seizure type [17]. Additionally, the potential supplementary role of vitamin C in epileptic patients should be taken into consideration. Vitamin C, easily transported across the blood-brain barrier, has been shown to reduce hippocampal injury during seizures, working in collaboration with other antioxidants such as alpha-tocopherol. Furthermore, vitamin C also acts as a neuroprotective factor by enhancing cell membrane stability and reducing lipid peroxidation [18]. In various animal models of epilepsy, such as pilocarpine-induced, PTZ-induced, and penicillin-induced epilepsy，vitamin C has been confirmed to decrease oxidative stress and inhibit autophagy, thereby improving seizures and brain damage [19,20]. Moreover, long-term administration of β-carotene and vitamin A in mice was found to exhibit antiepileptic effects [21]. Studies in PTZ-induced epileptic models have also indicated that lycopene may play a protective role [22,23]. The function of selenium as an antioxidant trace element is thought to be realized by selenoproteins with antioxidant activity and the ability to promote neuronal cell survival. A case-control study found a significant reduction in mean serum selenium in patients with refractory epilepsy [24]. It is also shown that selenium deficiency increases the risk of seizures, and selenium supplementation may help alleviate seizures [25].

Generally, the existing results are inconsistent, leading to uncertainty regarding the association between diet-derived circulating antioxidants and the risk of epilepsy. We should realize the current studies are primarily limited to early research stages, such as animal experiments, and have limited clinical evidence. However, clinical observational studies are difficult to avoid potential confounding bias between exposure and outcomes [26]. Randomized Controlled Trials (RCTs) are considered the most reliable method to investigate the causal relationship between exposure and outcome. Unfortunately, currently, there are no RCTs investigating the association between diet-derived circulating antioxidants and the risk of epilepsy.

The Mendelian randomization (MR) design employs genetic instruments as instrumental variables (IVs) to differentiate between correlation and causality in observed data. It achieves this by minimizing residual confounding and reducing the potential for reverse causality. Since genetic variation is randomly assigned at conception, individual traits are typically uncorrelated with one another. This process is analogous to the random assignment of participants to experimental and control groups in a randomized controlled trial. It ensures that individuals with genetic variation, which contributes to higher levels of risk factors, are evenly distributed between the groups, reducing the potential confounding effects of these risk factors. MR analysis also mitigates the issue of reverse causality since alleles are fixed and remain unaffected by the onset or progression of the disease [27]. Thus, we performed a two-sample MR to clarify whether genetically predicted diet-derived circulating antioxidants are causally associated with the risk of epilepsy.

2. Materials and methods

2.1. Untargeted metabolomics analysis

2.1.1. Inclusion criteria

1. Patients who had been diagnosed with various types of epilepsy based on the 2017 International League against Epilepsy Diagnostic Criteria;
2. Age of 16 years or older and a minimum follow-up time of 3 months;

3. Good mental state and the ability to cooperate well with examinations and treatments;
4. Written informed consent obtained from the patient or their legal guardian.

2.1.2. Exclusion criteria

1. Patients with febrile convulsions, syncope, functional neurologic disorder, and other non-epileptic diseases;
2. Age younger than 16 years, loss of follow-up, or follow-up time less than 3 months;
3. Presence of serious systemic diseases such as liver, kidney, and heart dysfunction, thyroid diseases, and blood system diseases;
4. History of alcoholism, family history of mental illness, or inability to cooperate with the examination.

2.1.3. Experimental group and plasma samples collection

62 epilepsy patients (EP) who met the inclusion and exclusion criteria were included in the study. Additionally, 20 non-epileptic adults who were recruited during the same period and matched for sex and age were selected as the normal control group (NC). To minimize the impact of diurnal variation, blood samples were collected during specific daytime hours, precisely between 9 a.m. and 10:00 a.m. A volume of 5 mL of venous blood was obtained from each subject after fasting, using a heparin anticoagulant tube. The collected blood samples were temporarily stored at 4 °C and within 1 h, transported to the specimen library of China-Japan Union Hospital of Jilin University for plasma preparation. Subsequently, the plasma samples were frozen at −80 °C for further analysis.

2.1.4. Plasma untargeted metabolomics analysis

2.1.4.1. Plasma samples preparation for metabolomics. In our approach to plasma sample preparation, we focused on ensuring optimal extraction of a wide range of metabolites. To this end, we mixed 100 μL of plasma with 400 μL of cold methanol-acetonitrile (v/v, 1:1), a solvent system chosen for its efficiency in extracting both hydrophilic and hydrophobic metabolites. The mixture was then sonicated for 1 h in ice baths to enhance cell lysis and metabolite release, followed by incubation at −20 °C for 1 h to precipitate proteins. Centrifugation at 4 °C (14,000 g, 20 min) facilitated the removal of proteins and other insoluble components. The supernatants, rich in metabolites, were collected and dried under vacuum to concentrate the metabolites for subsequent UHPLC-MS/MS analysis.

2.1.4.2. UHPLC-MS/MS analysis. For metabolomics profiling, a UPLC-ESI-Q-Orbitrap-MS system (Shimadzu Nexera X2 LC-30AD, Thermo Scientific Q-Exactive Plus) was employed to maximize the detection of diverse metabolites. The selection of the 2.1 mm × 100 mm ACQUIY UPLC BEH Amide 1.7 μm column (Waters) and the specific mobile phases (25 mM ammonium acetate/ammonium hydroxide in water and 100% acetonitrile) was based on their effectiveness in hydrophilic interaction liquid chromatography (HILIC), crucial for separating a broad range of metabolites, including those relevant to oxidative stress pathways. MS data acquisition was performed in both positive and negative ion modes using electrospray ionization (ESI) in a heated ESI source. The instrument settings included specific parameters for spray voltage, capillary temperature, sheath gas, aux gas, probe heater temp, and S-Lens RF Level. Instrument settings were finely tuned to optimize ionization efficiency and resolution, enhancing the detection sensitivity and accuracy. Full MS scans were conducted at a resolution of 70,000 (m/z 200), while MS/MS scans were performed at a resolution of 17,500 (m/z 200). Quality control (QC) samples were utilized for data normalization, and blank samples and QC samples were regularly injected for QC purposes.

2.1.4.3. Data preprocessing and filtering. The raw MS data from the NC and EP groups underwent processing using MS-DIAL for peak alignment, retention time correction, and peak area extraction. Metabolites were identified based on accurate mass and MS/MS data matching against the human metabolome database (HMDB), MassBank, and other public databases, as well as a self-built metabolite standard library. Variables with more than 50% non-zero measurement values in at least one group were retained for further analysis.

2.1.4.4. Multivariate statistical analysis. R version 3.4.1 was used for multivariate data analyses and modeling. The data were mean-centered using Pareto scaling. Orthogonal Partial Least Squares Discriminant Analysis (OPLS-DA) was employed to construct models and generate a score plot for visualizing group separation. Variable importance in projection (VIP) scores from OPLS-DA were used to identify metabolites with high discriminatory power. Metabolites with a VIP score >1 were considered significant. Fold change (FC) analysis and T-test were conducted to compare metabolite abundance between groups, with a significance level of P-value <0.05. Differentially abundant metabolites were used to generate HMDB classification ring maps. Receiver Operating Characteristic (ROC) curve analysis was performed to assess the discriminatory ability and accuracy of the metabolites in distinguishing between groups.

3. MR analysis

3.1. Study design and data sources

The two-sample MR study design enables estimation of the causal effect of the exposure on the outcome by utilizing genome-wide association study (GWAS) summary statistics from two independent studies. This design is based on three fundamental assumptions: (1) A strong correlation should exist between the genetic instruments and the exposure. (2) The genetic instruments solely influence the outcome through the exposure. (3) The genetic instruments are free from any recognized confounders related to the exposure-

outcome association [28,29]. In this study, we conducted a two-sample MR analysis to evaluate the causal effect of diet-derived circulating antioxidants (including β-carotene, lycopene, retinol, vitamin E, ascorbic acid, and selenium) on epilepsy (Fig. 1).

3.2. IVs and outcome GWAS

The genetic dataset for epilepsy outcome includes 212,453 individuals, with a total of 2143 epilepsy patients, and involves 8,885,805 single nucleotide polymorphisms (SNPs). Further information regarding the study design, phenotype definition, genotyping, and quality control can be accessed on the website: https://gwas.mrcieu.ac.uk/datasets/bbj-a-115/. The present study examined six key dietary-derived antioxidants as exposures, which included β-carotene, lycopene, retinol, ascorbate, vitamin E (α- and γ-tocopherol), and selenium. We considered both authentic absolute levels of antioxidants in blood and their corresponding circulating metabolites, which were quantified as relative concentrations in plasma or serum. β-carotene, lycopene, retinol, and ascorbate were identified as absolute antioxidant levels, while α-tocopherol, γ-tocopherol, ascorbate, and retinol were utilized as antioxidant metabolites. GWAS was searched to extract prominent SNPs as genetic IVs. Subsequently, all included IVs were harmonized, and genetic variants that were absent from the outcome GWAS dataset were excluded rather than substituted with proxies. To validate the first assumption in a MR study, the strength of the IVs was assessed using the R^2 value, which represents the proportion of variance in the phenotype explained by genetic variants, along with the mean F-statistic [30,31]. A threshold of F-statistic >10 was used to determine a strong association between IVs and exposures. Importantly, the studies that contributed data to these GWAS meta-analyses had obtained ethical approval from the respective institutional review boards. For this study, we only extracted summarized data from these studies, eliminating the need for additional ethics approval.

3.3. Absolute circulating antioxidants

In a GWAS of 2344 participants from the Nurses' Health Study, three SNPs (linkage disequilibrium [LD] <0.2 as specified in the study; $P < 5 \times 10^{-8}$) were found to be associated with plasma β-carotene levels [32]. A GWAS conducted on 441 older Amish adults revealed the identification of five SNPs (LD < 0.001; $P < 5 \times 10^{-6}$) associated with circulating lycopene levels [33]. A GWAS involving 5006 Caucasian individuals from two cohorts identified fifteen SNPs (LD < 0.001; $P < 5 \times 10^{-8}$) associated with circulating retinol levels [34]. In a study involving more than 52,018 European participants, a SNP ($P = 2.0 \times 10^{-7}$) was identified in relation to ascorbate levels [35]. Furthermore, in a GWAS conducted on 8340 European individuals, fifteen SNPs ($P < 5 \times 10^{-6}$) were identified [36].

3.4. Circulating antioxidant metabolites

SNPs for each metabolite were extracted from published GWAS with suggestive genome-wide significance level ($P < 1 \times 10^{-5}$) [37,38]. Importantly, eleven SNPs for α-tocopherol (n = 7276), thirteen for γ-tocopherol (n = 5822), and fourteen for ascorbate (n = 2063) were obtained from a cohort of 7824 adult individuals participating in two European population studies. Additionally, twenty-four SNPs for retinol (n = 1957) were derived from a subset of 1960 individuals of European descent. LD between all SNPs associated with the same exposure was evaluated. When LD was observed (LD > 0.001), the variant with the smallest *P* value was chosen.

3.5. Statistical analysis

The primary methods employed in this study to evaluate the relationship between diet-derived circulating antioxidants and epilepsy were the inverse-variance weighted (IVW) method, the weighted median (WM) method, and MR-Egger. The IVW method,

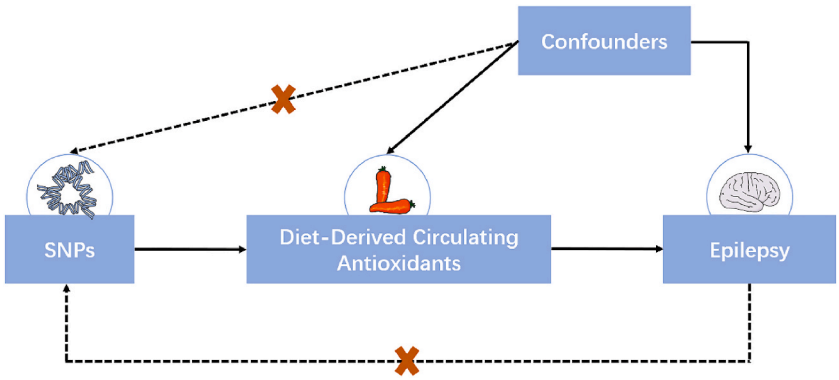

Fig. 1. Mendelian randomization model of diet-derived circulating antioxidants, (β-carotene, lycopene, retinol, ascorbate acid, vitamin E, and selenium), assessed through absolute levels and relative metabolite concentrations, and epilepsy. The design is under the assumption that the genetic variants are associated with addictive behaviors, but not with confounders, and the genetic variants influence epilepsy only through addictive behaviors. SNP: single nucleotide polymorphism.

assuming the effectiveness and independence of all SNPs, constrained the regression intercept to zero. It utilized the inverse of outcome variance as weights for fitting [39]. When all genetic variations meet the assumptions of IVs, the IVW method provides highly precise results and is widely regarded as the gold standard for evaluating causal effects [40]. Similar to the IVW method, MR-Egger employs weighted linear regression of outcome coefficients on exposure coefficients, taking into account the presence of an intercept term [41]. The WM method calculates the median of the distribution function derived from sorting SNP effect values based on their weights. The robustness of WM results persists even when 50% of the SNPs represent ineffective genetic variations [42]. In this study, the *P*-value of IVW results was the primary indicator for assessing the causal effect between exposure and outcome, while other methods were employed to support the evaluation of MR effect values. The consistency of directional effects with the IVW method across different methods indicated robust findings. Subsequently, IVW was employed to assess heterogeneity and investigate discrepancies among individual IVs by quantifying heterogeneity using Cochran's Q test statistic [43]. All statistical analyses were performed utilizing the "Two-Sample MR" package in R software (version 4.2.1).

4. Result

4.1. Sample characteristic

The study consisted of a total of 82 subjects: 62 epilepsy patients for EP group and 20 healthy volunteers served as NC group. There were no significant differences in age and sex among the two groups. (Table 1).

4.2. Untargeted metabolomic differential metabolite analysis

Firstly, we constructed an OPLS-DA model based on the untargeted metabolomics results of EP and NC groups (Fig. 2A). The results revealed significant inter-group differences and good intra-group reproducibility in the differential metabolites. The differentially expressed metabolites, identified based on the criteria of VIP score >1.0 and $P < 0.05$, have the potential to serve as biomarkers for diagnosing epilepsy. We identified a total of 264 circulating metabolites exhibiting significant differences between the NC and EP groups, with 125 up-regulated and 139 down-regulated (Supplementary Table 1, Fig. 2B). The HMDB classification ring maps indicated that the differential metabolites of epilepsy patients are mostly concentrated in lipids and lipid-like molecules (30.34%) and organic acids and derivatives (13.79%) (Fig. 2C). Additionally, we conducted a comprehensive analysis of the metabolites in these two categories. Through this analysis, we identified five representative metabolites that exhibited significant changes. These findings provide evidence for the occurrence of cyclic oxidative stress in epilepsy patients. In the context of fatty acid metabolism, the EP group exhibited higher circulating levels of palmitic acid, oleic acid, linoleic acid, and myristic acid compared to the NC group. With respect to amino acid metabolism, the circulating levels of glutamine were lower in epileptic patients compared to the NC group (Fig. 2D). The subsequent analysis of ROC curves revealed that these five differential metabolites demonstrated relatively high accuracy in distinguishing between the EP and NC groups [Fig. 3(A–E)].

4.3. Selection of SNPs

The summary information of SNPs identified for dietary-derived circulating antioxidants and their metabolites can be found in Supplemental Table 2. Retinol and ascorbate are available in both absolute circulating antioxidant forms and as metabolites. The F-statistics for all SNPs utilized in the current study exceeded 10.

4.4. Dietary-derived circulating antioxidants and epilepsy

The IVW analysis method revealed that genetically determined higher levels of absolute circulating β-carotene were associated with a reduced risk of epilepsy (odds ratio [OR], 0.552; 95% confidence interval [CI]: 0.335–0.905; $P = 0.018$). A subsequent heterogeneity test using the IVW method indicated no evidence of heterogeneity among the SNPs, and the results remained stable (Cochran's $Q = 1$, $P = 0.736$). No causal effect on epilepsy was observed for the other dietary-derived circulating antioxidants, as

Table 1
Demographic and clinical characteristics of enrolled subjects.

Demographic and clinical characteristics	NC (n = 20)	EP (n = 62)	*P*-value
Age (mean ± SD)	28.05 ± 4.17	33.24 ± 16.04	0.158[a]
Male/Female Ratio	10/10	36/26	0.527 [b]
Seizure type (n, %)			
Focal	–	18 (29.0)	
Generalized	–	32 (51.6)	
Mixed	–	12 (19.4)	

NC: Normal control; EP: Epilepsy.
n: number of samples; SD: Standard deviation.
[a] P value calculated using T-test.
[b] P value calculated using chi-square test.

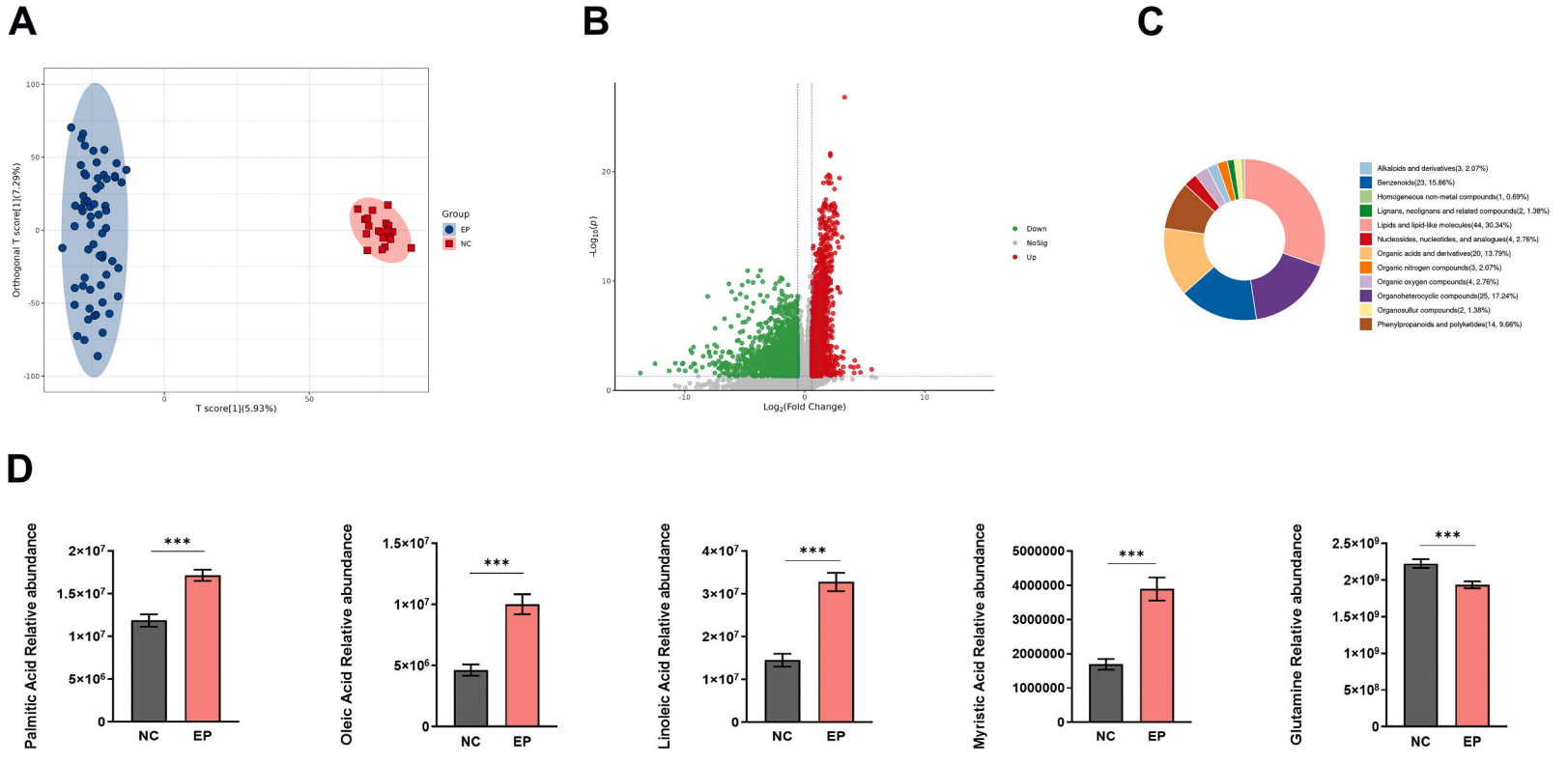

Fig. 2. Untargeted metabolomic differential metabolites analysis between NC and EP groups. (A) OPLS-DA score. (B) Volcano plot. (C) HMDB classification ring maps. (D) Relative abundance of oxidized metabolites (palmitic acid, oleic acid, linoleic acid, and myristic acid) and reducing metabolites (glutamine).

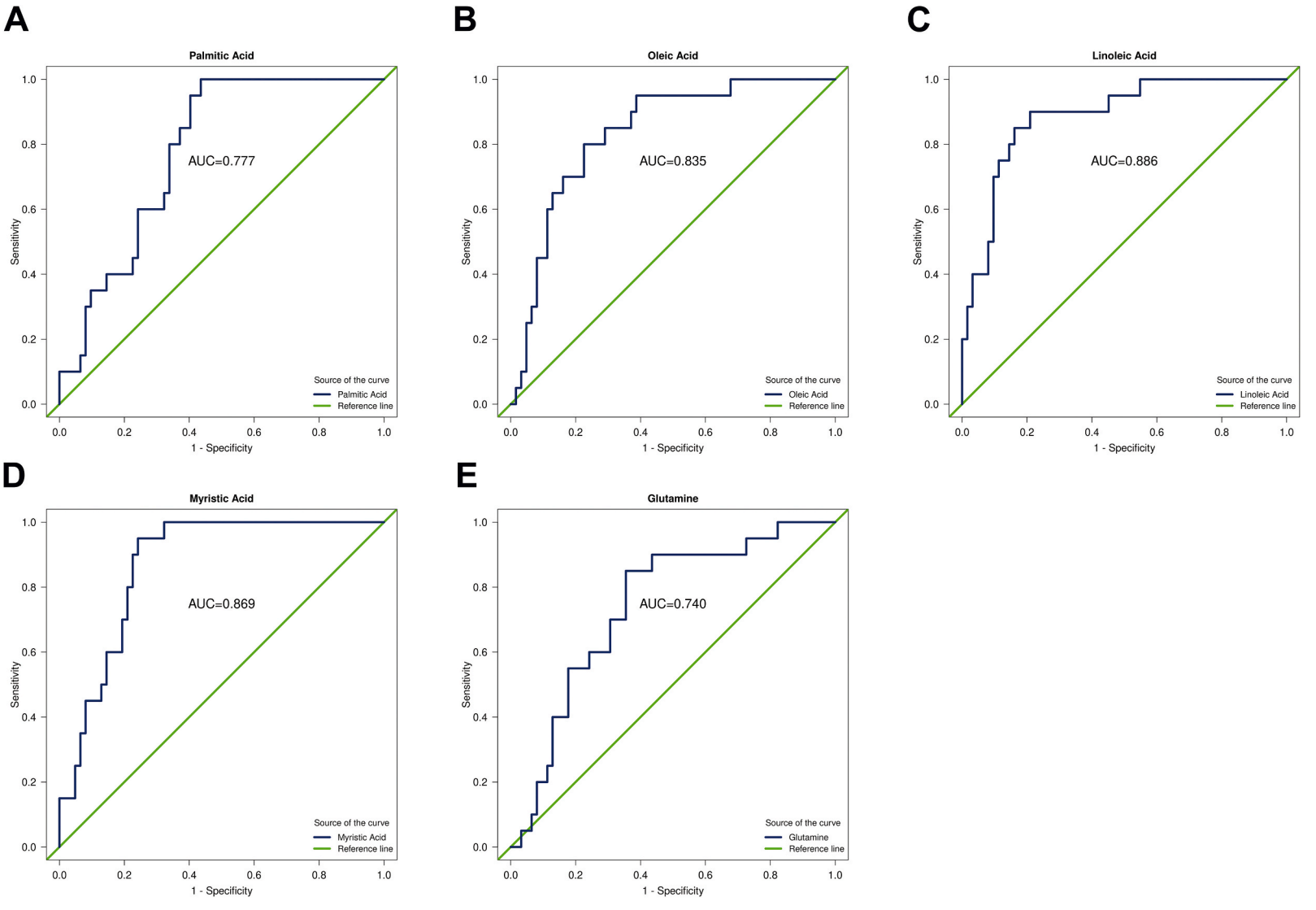

Fig. 3. Receiver Operating Characteristic curve analysis. (A) Palmitic acid. (B) Oleic acid. (C) Linoleic acid. (D) Myristic acid). (E) Glutamine.

indicated by the IVW approach (Absolute circulating antioxidants: lycopene: OR, 0.994; 95% CI, 0.895–1.103, $P = 0.904$; retinol: OR, 0.537; 95% CI, 0.089–3.257, $P = 0.499$; ascorbic acid: OR, 0.974; 95%, CI 0.899–1.054, $P = 0.510$; selenium: OR, 1.016; 95% CI, 0.848–1.217, $P = 0.862$; Circulating antioxidant metabolites: α-tocopherol: OR, 1.437; 95% CI, 0.640–3.228, $P = 0.380$; γ-tocopherol: OR, 0.687; 95% CI, 0.431–1.095, $P = 0.114$; ascorbic acid: OR, 0.932; 95%, CI 0.655–1.326, $P = 0.694$; retinol: OR, 1.062; 95% CI, 0.971–1.162, $P = 0.188$). The same results were observed when employing alternative MR approaches (Fig. 4).

5. Discussion

In this study, we performed a comprehensive untargeted metabolomic analysis on peripheral blood samples collected from both epilepsy patients (n = 62) and healthy individuals (n = 20), aiming to assess the occurrence of oxidative stress in the circulation of patients with epilepsy based on metabolite alterations. Blood samples have extensive utility in clinical research pertaining to diverse diseases, encompassing epilepsy. Due to their accessibility, they are frequently utilized as a surrogate for brain tissue. Moreover, blood samples can effectively capture the comprehensive physiological alterations that transpire pre- and post-disease onset [44,45]. Our research findings revealed increased expression of palmitic acid, oleic acid, linoleic acid, and myristic acid as well as decreased expression of glutamine in the epilepsy group, as compared to the NC group. Subsequent analysis using ROC curves revealed that these five differential metabolites exhibited high accuracy in discriminating between the two groups of subjects. Extensive research has been conducted on the association between oleic acid and oxidative stress. The use of oleic acid to induce ROS generation in HepG2 liver cancer cells is a widely recognized model for studying oxidative stress [46]. Moreover, studies have shown that oleic acid can stimulate the generation of ROS in mouse dermal fibroblasts, which subsequently triggers lipid peroxidation [47]. Palmitic acid has been implicated in inflammation. A study conducted by Wen et al. [48] demonstrated that palmitic acid can activate NOD-like receptor thermal protein domain associated protein 3 (NLRP3), leading to increased ROS production in macrophages and subsequent attenuation of the Adenosine 5'-monophosphate-activated protein kinase (AMPK) signal. The AMPK signal functions as a negative regulator

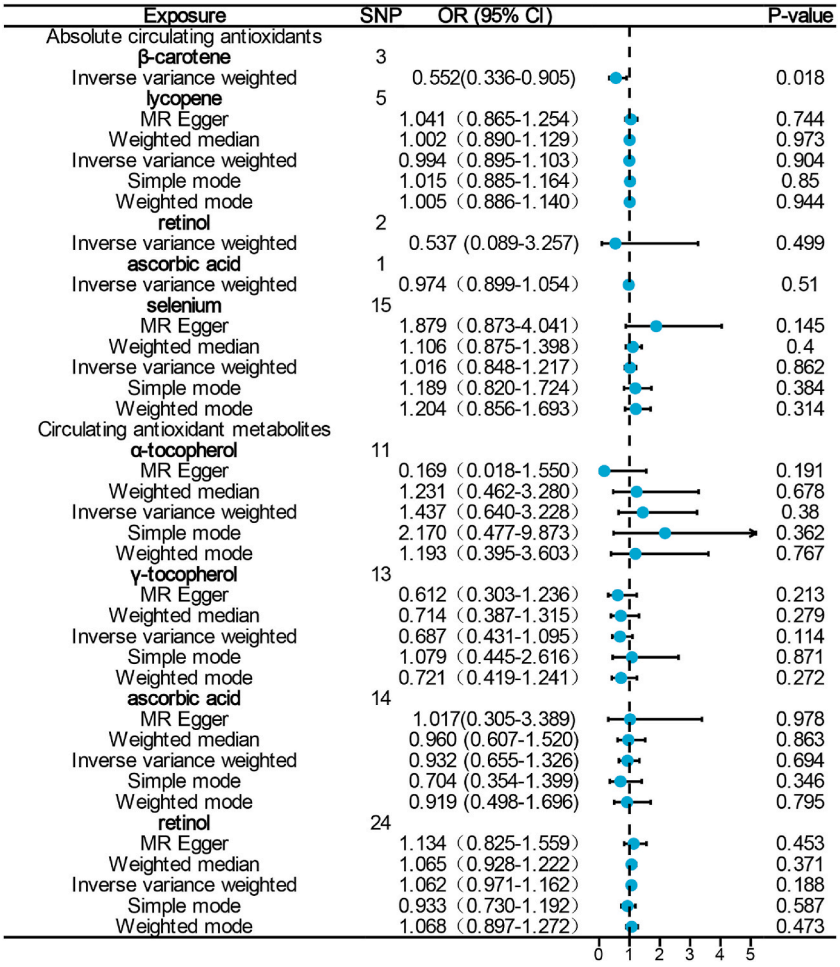

Fig. 4. Forest plot. The summary results of diet-derived circulating antioxidants, (β-carotene, lycopene, retinol, ascorbate acid, vitamin E, and selenium), assessed through absolute levels and relative metabolite concentrations, and epilepsy using various mendelian randomization methods.

of both ROS generation and inflammation. However, in the presence of elevated ROS levels, this regulatory mechanism becomes disrupted, leading to further ROS production and perpetuating a vicious cycle. Additionally, palmitic acid can also induce ferroptosis in osteoblasts through the activation of the Methyltransferase like 3/Apoptosis Signal-regulating Kinase 1-protein 38 signaling pathway [49]. High concentrations (500 μg/L) of linoleic acid have been found to exert substantial inhibitory effects on algal growth. Moreover, research has indicated that the exposure of algae to high concentrations of linoleic acid leads to a marked elevation in reactive oxygen species (ROS), which could potentially facilitate the occurrence of ferroptosis [50]. In addition, research has demonstrated a positive correlation between the content of myristic acid and ROS levels in tumor cells [51]. Glutamine plays a vital role in the biosynthesis of glutathione, thereby enhancing the body's antioxidant capacity. It functions as a crucial reducing agent and antioxidant. In normal physiological conditions, glutamine accounts for over 60% of the free amino acids present in the human body, and the body can synthesize surplus glutamine to fulfill its requirements. However, numerous disease states are associated with a substantial reduction in glutamine levels, resulting in a diminished capacity for reduction in the body [52]. In general, our plasma untargeted metabolomics results suggest that oxidative stress is indeed present in the circulation of epilepsy patients.

Subsequently, we employed five methods of MR analysis to evaluate the relationship between diet-derived circulating antioxidants and the risk of epilepsy in a large sample size. Our findings indicated that, except for β-carotene, diet-derived circulating antioxidants (lycopene, retinol, ascorbate, vitamin E (α-and γ-tocopherol) and selenium) did not demonstrate a causal effect on epilepsy. Due to the importance of diet-derived circulating antioxidants and the high incidence of epilepsy worldwide, it is important to clarify the influencing mechanisms and causal effects of diet-derived circulating antioxidants on epilepsy. β-Carotene, celebrated for its antioxidant prowess, is theorized to play a pivotal role in epilepsy management, leveraging its neuroprotective and immunomodulatory characteristics. This potent antioxidant is crucial in neutralizing oxidative stress in the brain, a frequent phenomenon during epileptic seizures [53–55]. It functions by eliminating free radicals, potentially reducing neuronal damage and alleviating neurotoxicity linked with seizures [56]. Furthermore, as a Vitamin A precursor, β-carotene bolsters key neural activities, such as synaptic plasticity and neuronal signaling, typically compromised in epilepsy [57]. The immunomodulatory attributes of β-carotene also command attention, especially considering the growing awareness of neuroinflammation's role in epilepsy's development [53,58,59]. By influencing immune responses, it could alter the progression or intensity of the disorder. Nonetheless, it is vital to acknowledge that these hypothesized mechanisms stem from theoretical and preclinical research. Substantial clinical evidence validating the effectiveness and safety of β-Carotene in epilepsy treatment remains to be gathered. Future investigations are essential to fully understand its specific function and interplay with established antiepileptic medications across the varied landscape of epilepsy. And there is not enough evidence to prove a definite causal relationship between antioxidants and epilepsy. There could be several potential explanations for this phenomenon. Firstly, previous observational studies were unable to exclude the possibilities of reverse causality and residual confounding. Factors like patients simultaneously taking multiple antioxidants or adhering to antiepileptic drugs could have influenced the results [13,18]. Secondly, the beneficial effects of antioxidants were primarily observed in animal models of epilepsy, and only a limited number of antioxidants have been evaluated as adjuvant therapies in epilepsy patients, with limited success thus far [15–17]. Lastly, the lack of a significant effect may be attributed to factors such as the timing, dosage, and duration of antioxidant supplementation, as well as the uncertain timing of epilepsy onset and its long-term progression [12]. Hence, the causal relationship between diet-derived circulating antioxidants and epilepsy remains unclear, highlighting the need for meticulously designed randomized controlled trials (RCTs) to assess the effectiveness of diet-derived circulating antioxidants in epilepsy patients.

5.1. Strengths and limitations

Our study's strength lies in its integrated approach, combining metabolomics and MR analysis, which allowed for a comprehensive exploration of the relationship between diet-derived circulating antioxidants and epilepsy risk. More importantly, MR analysis minimizes confounding bias and utilizes a wide range of epilepsy genetic data. By employing MR analysis, our study greatly mitigated concerns of reverse causality and residual confounding. We utilized diverse methods to ensure the reliability of our MR estimates, validate the MR hypothesis, and corroborate the robustness of our findings across multiple MR models with consistent directions and magnitudes. Additionally, through the use of complementary statistical methods, we found no evidence of heterogeneity in our analysis. Nevertheless, it is noteworthy that some limitations remain in this study. Firstly, the current GWAS database does not provide summary-level statistics for specific subtypes of epilepsy, such as drug-resistant or refractory epilepsy. As a result, further exploration of the underlying relationships among different epilepsy subtypes is hindered. Additionally, the study lacks specific clinical cohort data, which limits our ability to analyze potential variations in the relationship between antioxidants and epilepsy within specific populations or subgroups. While our analysis demonstrates a significant effect of β-carotene in decreasing the risk of epilepsy, we were unable to perform a detailed and convincing sensitivity analysis for β-carotene due to limited genetic variants. Consequently, the generalizability of our findings still needs further exploration by RCTs. Thirdly, we observed an insufficient number or absence of SNPs when employing a strict threshold to select exposed variants. Therefore, we opted for a more relaxed threshold. As a result, this had implications for the persuasiveness of our research findings, despite the F-statistic of each ultimately selected SNP exceeding 10. However, it is gratifying to note that this phenomenon has gained widespread acceptance in numerous studies [60,61].

6. Conclusion

This study provides valuable insights into the relationship between diet-derived circulating antioxidants and epilepsy. Our findings indicate the presence of oxidative stress in the circulation of individuals with epilepsy. However, the results suggest that the majority of these antioxidants (lycopene, retinol, ascorbate, vitamin E and selenium) are unlikely to have a causal association with a reduced risk

of epilepsy, except for β-carotene. This finding indicated potential clinical implications for clinicians that dietary supplementation with beta-carotene may be effective in patients with epilepsy.

Ethics approval and consent to participate

This untargeted metabolomics study was reviewed and approved by the Ethics Committee of China-Japan Union Hospital of Jilin University, with the approval number: [2023022701]. All participants/patients (or their proxies/legal guardians) provided informed consent to participate in the study.

Review and/or approval by an ethics committee was not needed for this MR study because only publicly available GWAS data were used in the MR analysis and the Ethics approval and consent to participate could be available in the original GWAS study.

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Consent for publication

Not applicable.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding authors.

CRediT authorship contribution statement

Zhen Liang: Writing – original draft, Data curation. **Yingyue Lou:** Writing – original draft. **Zhaoshi Zheng:** Methodology. **Qi Guo:** Software. **Songyan Liu:** Writing – review & editing, Funding acquisition, Conceptualization.

Declaration of competing interest

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List of abbreviations

ACN	Acetonitrile
AMPK	Adenosine 5‘-monophosphate-activated protein kinase
CI	Confidence interval
EP	Epilepsy patients
ESI	electrospray ionization
FC	Fold change
GWAS	Genome-wide association study
HMDB	Human metabolome database
IV	instrumental variable
IVW	inverse-variance weighted
MR	Mendelian Randomization
NC	Normal control
OPLS-DA	Orthogonal Partial Least Squares Discriminant Analysis
OR	Odds ratio
PTZ	Pentylenetetrazol
QC	Quality control
RCT	Randomized Controlled Trial
ROC	Receiver Operating Characteristic
ROS	Reactive oxygen species
SNP	Single nucleotide polymorphism
VIP	Variable importance in projection

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e26813.

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