Epilepsy Research 218 (2025) 107656

Contents lists available at ScienceDirect

Epilepsy Research

journal homepage: www.elsevier.com/locate/epilepsyres

Exploring metabolic biomarkers and pathways in pharmacoresistant epilepsy: A systematic review

Zheng-Dong Lim [a,1], Nur Asyiqin Syafiqa Abdullah [a], Kheng-Seang Lim [b,2], Paul Chi-Lui Ho [c,3], Alina Arulsamy [d,4], Si-Lei Fong [b,5], Hui-Yin Yow [a,e,*,6]

[a] Department of Pharmaceutical Life Sciences, Faculty of Pharmacy, Universiti Malaya, Kuala Lumpur 50603, Malaysia
[b] Division of Neurology, Department of Medicine, Faculty of Medicine, Universiti Malaya, Kuala Lumpur 50603, Malaysia
[c] School of Pharmacy, Monash University Malaysia, Subang Jaya, Selangor 47500, Malaysia
[d] Jeffrey Cheah School of Medicine and Health Sciences, Monash University Malaysia, Subang Jaya, Selangor 47500, Malaysia
[e] Precision Medicine and Omics Centre (PrOmiC), Faculty of Pharmacy, Universiti Malaya, Kuala Lumpur 50603, Malaysia

ARTICLE INFO

Keywords:
Epilepsy
Pharmacoresistant epilepsy
Drug-resistant epilepsy
Metabolic pathway

ABSTRACT

Drug-resistant epilepsy (DRE) is characterized by the failure to attain sustained seizure freedom despite adequate trials of two antiseizure medication (ASM) regimens that are well tolerated and appropriately chosen and administered, either as monotherapies or in combination. Despite being a cornerstone of epilepsy treatment, ASMs are ineffective in achieving seizure remission in nearly one-third of patients, who are consequently classified as having DRE. This systematic review aims to determine potential metabolic biomarkers and pathways linked to DRE, which could inform personalized treatment and optimize therapeutic outcomes. A comprehensive search of databases, namely Medline, Web of Science and the Cochrane Central Register of Controlled Trials (CENTRAL) based on predefined inclusion and exclusion criteria yielded 29 eligible studies after full-text screening. The risk of bias from these studies was reviewed using the Office of Health Assessment and Translation (OHAT) risk of bias rating tool. Key information, including study groups, sample size, model types, and main findings were tabulated. Several metabolites were identified, including amino acids (glycine, glutamate, isoleucine), organic acids (lactate), and glucose, which may serve as potential biomarkers for DRE. MetaboAnalyst 6.0 pathway analysis identified the alanine, aspartate and glutamate metabolism, as well as phenylalanine, tyrosine and tryptophan biosynthesis pathways, emerged with significant impact score ($\geq$0.5, $p < 0.05$). The findings highlight the promising role of these metabolites and pathways as predictive biomarkers for DRE and potential therapeutic targets for novel drug development.

1. Introduction

Epilepsy is a complex neurological disorder characterized by recurring seizure episodes. Drug-resistant epilepsy (DRE) is a particularly challenging form of the condition, defined by the International League Against Epilepsy (ILAE) as the failure to attain sustained seizure freedom despite adequate trials of two antiseizure medication (ASM) regimens that are well tolerated and appropriately chosen and administered, either as monotherapies or in combination (Kwan et al., 2010). DRE is also known as pharmacoresistant, medically refractory or intractable epilepsy when patients do not respond to standard medical treatments (Kwan et al., 2010).

Currently, numerous novel agents with varied seizure-modulating mechanisms are being explored in preclinical or clinical stages

* Corresponding author at: Department of Pharmaceutical Life Sciences, Faculty of Pharmacy, Universiti Malaya, Kuala Lumpur 50603, Malaysia.
E-mail addresses: limzhengdong@gmail.com (Z.-D. Lim), asyiqinsyafiqa@gmail.com (N.A.S. Abdullah), kslimum@gmail.com (K.-S. Lim), paul.ho@monash.edu (P.C.-L. Ho), alina.arulsamy@monash.edu (A. Arulsamy), fongsilei@gmail.com (S.-L. Fong), huiyin.yow@um.edu.my (H.-Y. Yow).
[1] https://orcid.org/0009-0006-4735-5847
[2] https://orcid.org/0000-0002-2787-2365
[3] https://orcid.org/0000-0001-9213-7116
[4] https://orcid.org/0000-0003-0993-5622
[5] https://orcid.org/0000-0003-1217-6380
[6] https://orcid.org/0000-0002-9455-0817

https://doi.org/10.1016/j.eplepsyres.2025.107656
Received 22 May 2025; Received in revised form 13 August 2025; Accepted 1 September 2025
Available online 4 September 2025

alongside emerging therapies that show potential disease-modifying or antiepileptogenic effects (Löscher and Klein, 2021). However, despite advancements in drug development, nearly one-third of patients still have uncontrolled seizures, highlighting the limitations of existing treatments (Guery and Rheims, 2021; Löscher and Schmidt, 2011). The impact of DRE extends far beyond uncontrolled seizures, significantly affecting patients' quality of life, socioeconomic status and mental health (Allers et al., 2015; Chatterjee et al., 2020; Cianchetti et al., 2018). Moreover, DRE is linked to a higher risk of mortality, particularly sudden unexpected death in epilepsy (Callaghan et al., 2014; Harden et al., 2017).

The underlying mechanisms of drug resistance in epilepsy remain elusive, presenting a critical research gap (Janmohamed et al., 2020; Anyanwu and Motamedi, 2018). Metabolomics has become a promising tool for unraveling the complex pathogenesis of DRE. This cutting-edge scientific technique involves the comprehensive analysis of metabolites – small molecules including amino acids, organic acids, sugars, nucleotides, steroids, and lipids (Eid, 2022; Chen et al., 2022). Preliminary research has identified several key metabolites of interest. A narrative review by Lai et al. (2022) highlighted metabolomics allows researchers to identify and quantify metabolic changes in biological samples, such as blood or cerebrospinal fluid (Lai et al., 2022). Glutamate, lactate and citrate as metabolites consistently altered in patients and animal models of epilepsy, suggesting their potential as biomarkers for the condition (Lai et al., 2022). In the context of DRE, aspartic acid, glutamine, glutamate, pyruvic acid, palmitic acid and gamma-aminobutyric acid (GABA) were reported to be associated with drug resistance (Kong et al., 2025).

There is an urgent clinical necessity to discover reliable metabolic biomarkers and pathways that could provide insights into DRE's pathogenesis (French et al., 2021). This systematic review aims to bridge the critical knowledge gap by comprehensively examining existing evidence on metabolomic investigations in DRE and characterizing the metabolomic landscape of DRE. Ultimately, this review could enhance comprehension of DRE and the pathogenesis of different epilepsy subtypes, paving the way for personalized treatment strategies.

2. Methods

2.1. Eligibility criteria

The research question was formulated using the four components of the Population, Intervention, Comparison and Outcome (PICO) framework, alongside the inclusion and exclusion criteria of the studies (Table 1). To ensure the selection of relevant and accessible studies, additional exclusion criteria were applied, such as excluding studies not written in English and studies where the full text is not available (even upon request from the authors).

Table 1
The eligibility criteria based on the PICO framework.

Domain	Inclusion criteria	Exclusion criteria
Population	• Studies on animal DRE models. • Studies on DRE patients.	• Studies not related to epilepsy patients or animal epilepsy models.
Intervention	• Epilepsy treated with antiseizure medications, keto diet or surgery. • Original article, short communication.	• Non-intervention-related publications (i.e. reviews, editorial letters, thesis papers, conference papers, case studies).
Comparison	• Non-DRE cases or models in which there is a response towards antiseizure medications. • Patients who are seizure-free even without antiseizure medications therapy.	• Studies that do not separate the DRE group from the non-DRE group when making a comparison with the control group.
Outcome	• Identification of metabolic biomarkers and pathways in DRE by using analytical platforms, e.g., nuclear magnetic resonance spectroscopy and mass spectrometry.	• Outcomes which are not related to identification of metabolic biomarkers and pathways in DRE (e.g. docking studies, identification of antibodies).

2.2. Search strategy

The relevant databases were used to comprehensively search existing studies exploring potential metabolic biomarkers and pathways in DRE, namely Medline, Web of Science and Cochrane Central Register of Controlled Trials (CENTRAL). Search terms relevant to the research question were used for search and retrieval purposes. The search terms included both Medical Subject Headings (MeSH) terms and keywords for Medline and solely keywords for the other two databases stated above (**Online Resource 1**). The keywords and MeSH terms were generated from an initial general search of the databases to find out possible synonyms used for the important terms. Previous systematic reviews done on DRE or epilepsy and/or metabolomics were examined to obtain the keywords appropriate to search the databases. No restrictions on the publication date were applied during the search. All relevant articles published up to March 1, 2024, were identified.

2.3. Selection of articles

A comprehensive search identified 2646 articles from Medline, 646 papers from Web of Science and 353 papers from CENTRAL. All records were imported into the Rayyan software (https://www.rayyan.ai/) (Ouzzani et al., 2016). After the initial retrieval, duplicates (n = 225) were identified and removed using Rayyan. The remaining articles (n = 3420) were screened by the titles and abstracts by two reviewers (NASA and YHY) independently. Each article was labeled as 'included', 'excluded' or 'maybe'. Articles labeled as 'maybe', where eligibility could not be determined from the title and abstract, were obtained in full text and evaluated further, with final inclusion determined through reviewer consensus. The articles included (n = 33) underwent thorough screening of their full texts, following the established inclusion and exclusion criteria, as outlined in the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow method (Fig. 1) (Page et al., 2021). Any conflicts between the two reviewers were resolved through discussion until a consensus was reached. The study protocol was registered with the International Prospective Register of Systematic Reviews (PROSPERO) (https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42024560628).

2.4. Data extraction

Information regarding the authors, year of study, study design, study group, sample type sample size, metabolic analysis type, statistical analysis and key findings related to the metabolites analyzed were extracted and organized into tables. In cases of discrepancies in the data extraction process, consensus was achieved through deliberation among reviewers. A meta-analysis was deemed unsuitable owing to considerable heterogeneity among the included studies concerning the study populations, animal DRE models, and outcomes. The Preferred Reporting Items for Systematic Reviews and Meta-Analyses extension for Systematic Reviews (PRISMA-SR) flow method was employed to document the rationale, methodology and results of the systematic review (**Online Resource 2**) (Page et al., 2021).

2.5. Bias assessment

The included articles were evaluated for risk of bias using The Office of Health Assessment and Translation (OHAT) risk of bias rating tool based on six types of bias (i.e. selection bias, confounding bias,

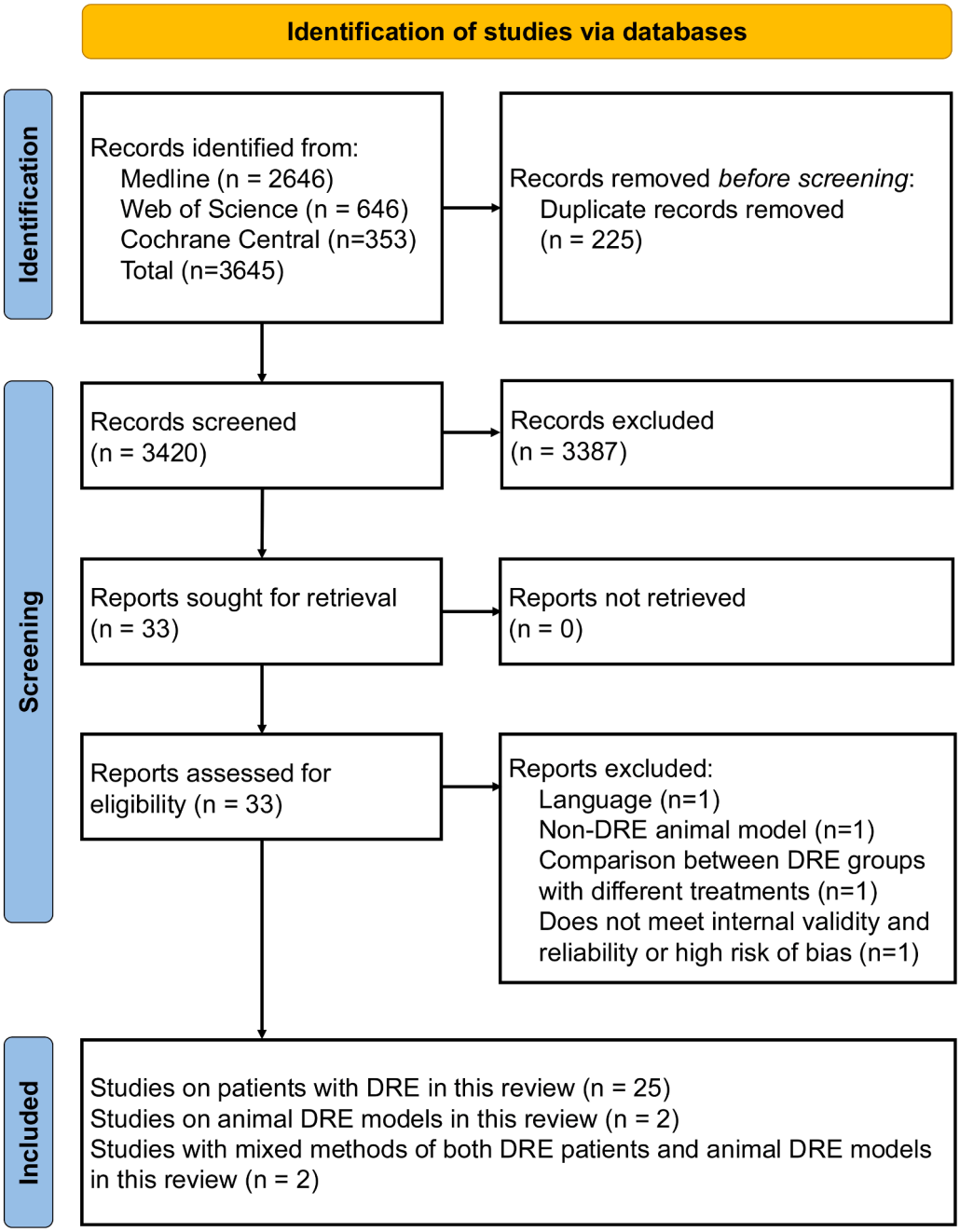

Fig. 1. PRISMA flow diagram of included studies.

performance bias, attrition or exclusion bias, detection bias and selective reporting bias) (National Institutes of Environmental Health Sciences, 2019, 2024). Each study was meticulously assessed through a set of questions, with potential biases categorized as 'definitely low risk of bias', 'probably low risk of bias', 'probably high risk of bias' and 'definitely high risk of bias'. The risk of bias assessment was reviewed and evaluated independently by NASA and YHY to enhance reliability and any discrepancies found were discussed to form a consensus.

2.6. Pathway analysis

By using significantly impacted metabolites reported in the included studies, metabolic pathway analyses were conducted for human, mouse and rat studies using MetaboAnalyst 6.0 software (https://www.metaboanalyst.ca) with reference to the Kyoto Encyclopedia of Genes and Genomes (KEGG) database. Metabolic pathways were considered relevant and important based on an impact value threshold of ≥ 0.1 and statistical significance set at $p < 0.05$. A subanalysis of human subjects was performed based on predefined criteria, including age groups, epilepsy types and interventions. A comparison of the impacted pathways among DRE patient subgroups was conducted. Additionally, a comparison of metabolic pathways between DRE patients and animal DRE models was also conducted. The altered metabolic pathways in DRE patients, their subgroups and animal DRE models were identified by integrating the results of pathway analyses.

3. Results

3.1. Overall description of the included studies

This review encompassed 29 studies in total, comprising 25 studies on DRE patients, two on animal DRE models, and two employing mixed methods with DRE patients and animal DRE models. The studies were published in the years ranging from 1992 to 2023, with 17 (54 %) studies published within the last five years.

3.2. Metabolomic studies of DRE patients

The characteristics of the 27 included articles are shown in Table 2 and Table 3. The studies employ diverse research methodologies, utilizing different sample types, analytical techniques, and subject populations. Table 2 shows studies where the sample type is brain imaging (11/27, 40.7 %). Table 3 shows studies where the sample type is anything other than brain imaging, including blood (3/27, 11.1 %), plasma (7/27, 25.9 %), cerebrospinal fluid (CSF) (4/27, 14.8 %), brain tissue (3/27, 11.1 %), urine (3/27, 11.1 %), fecal matter (1/27, 3.7 %), microdialysis collections (1/27, 3.7 %), and dried blood spots (1/27, 3.7 %). Some studies used multiple types of samples. Sample sizes of patients with DRE in these studies range from 5 to 48.

The included studies varied in their comparison groups, subject populations and experimental designs. Most studies compared differences between DRE patients and healthy controls (11/27, 40.7 %), while others compared with non-DRE patients (2/27, 7.4 %) or both (2/27, 7.4 %). The subject populations also differed, with some studies investigating only adult patients (12/27, 44.4 %), others focusing on children's cohorts (12/27, 44.4 %), and a few including a broader age group encompassing both adults and children (3/27, 11.1 %). Experimental designs further varied, with most studies investigating certain epilepsy subtype, namely temporal lobe epilepsy (TLE) (10/27, 37.0 %), while others examined the effect of interventions, including ASMs such as vigabatrin (1/27, 3.7 %), carbamazepine and clobazam (1/27, 3.7 %), valproic acid (1/27, 3.7 %), as well as non-pharmacological approaches like the ketogenic diet (KD) (6/27, 22.2 %), vagus nerve stimulation (VNS) (1/27, 3.7 %), and transcranial direct current stimulation (tDCS) (1/27, 3.7 %). These methodological variations contribute to the heterogeneity of findings while providing a comprehensive perspective of the metabolic landscape of epilepsy.

3.2.1. Metabolic pathways in DRE patients

Pathway analyses were performed on 27 studies involving DRE patients along with the subgroup analysis (Fig. 2). The subgroups included adults with TLE, children on KD, adults with unclassified epilepsy (either study did not specify the type of epilepsy or examined multiple types of epilepsy in adults), children not receiving KD, patients undergoing treatment with vigabatrin, carbamazepine and clobazam, valproic acid, VNS and tDCS. Pathway analysis was not conducted for the subgroups of patients undergoing treatment with vigabatrin, VNS and tDCS because of the limited number of significantly impacted metabolites found in the respective studies, which was insufficient for a meaningful analysis. The altered metabolic pathways were tabulated in **Online Resource 3**, with important pathways identified based on impact value (≥ 0.1) and p-value (< 0.05).

A comparison of the altered metabolic pathways across subgroups and DRE patients showed no common pathway shared by all groups (Fig. 3). Notably, alanine, aspartate and glutamate metabolism and glycine, serine and threonine metabolism were altered in at least three subgroups. None of the subgroups fully recapitulated each other, but the metabolic pathways altered in adults with TLE and adults with unclassified epilepsy were all affected in the human DRE group. Cysteine and methionine metabolism was unique to children on KD, while the citrate cycle (TCA cycle) was specific to children not receiving KD and patients treated with valproic acid. Starch and sucrose metabolism was uniquely altered in children on KD and patients receiving carbamazepine and clobazam. An overview of all altered pathways and related metabolites in DRE patients and subgroups was presented in Fig. 4.

3.3. Metabolomic studies of animal DRE models

The 4 included metabolomic studies utilizing animal DRE models are detailed in Table 4. The animal models used were rat (1/4, 25 %) and mice (3/4, 75 %). In study with rat, pilocarpine model was employed. In studies using mice, the models used were the knockout-mouse model of the mammalian INDY [SLC13A5] homolog, Aldh7a1-knockout mouse model, and in another study, the mice were colonized with microbes from the post-KD-treated pediatric epilepsy patients' gut microbiota. The types of samples analyzed included plasma, CSF, brain tissue, and fecal matter.

In the study by Wen et al. (2021) using Wistar rats of the pilocarpine-induced model of DRE, it was found that similar to humans, there is an increase in L-tyrosine and citric acid, as well as a decrease in L-alanine and L-arginine (Wen et al., 2021). Like in humans, the mouse models in Engelke et al. (2021) also show an increase of 2-OPP and 6-oxoPIP in plasma and brain tissues. Unlike in humans, there were also higher levels of piperideine-6-carboxylate, α-aminoadipic semialdehyde, and pipecolic acid in the mice's brain tissues (Engelke et al., 2021). Another study using a mouse model by Henke et al. in 2020 showed a significant increase in citrate, aspartate and glutamate levels in the CSF but with reduced citrate levels in the parahippocampal cortex, suggesting reduced neuronal uptake and altered citric acid cycle (TCA) metabolism (Henke et al., 2020). In another study, Lum et al. (2023) colonized mice with microbes from the fecal matter of children with epilepsy after their KD diet and showed that metabolites associated with fatty acids β-oxidation, such as palmitoleoylcarnitine and oleoylcarnitine increased whereas kynurenine decreased similarly to the changes seen in human patients when KD was initiated (Lum et al., 2023).

3.3.1. Metabolic pathways in animal DRE models

Pathway analyses were performed on three studies using mouse models and two studies using rat models of DRE (Fig. 5). The altered metabolic pathways were tabulated with important pathways identified based on impact value (≥ 0.1) and p-value (< 0.05) (**Online Resource 4**). Alanine, aspartate and glutamate metabolism, arginine biosynthesis and TCA cycle were perturbed in mouse models, while only phenylalanine, tyrosine and tryptophan biosynthesis was perturbed in rat models.

3.4. Comparison between DRE patients and animal DRE models

In total, 12 important altered pathways were identified in DRE patients, three in mouse models and one in rat models (**Online Resource 5**). Notably, no single pathway was commonly involved across all three species. However, overlaps were observed between species: phenylalanine, tyrosine and tryptophan biosynthesis was shared between DRE patients and rat models, while alanine, aspartate and glutamate metabolism and arginine biosynthesis were shared between DRE patients and mouse models. The TCA cycle was unique in the mouse model.

3.5. Bias assessment

The risk of bias assessment was detailed in **Online Resource 6**. Only five out of the 30 articles (if including the one article excluded due to high risk of bias) (16.7 %) had 'Definitely low risk of bias' and 'Probably low risk of bias' only in all the domains. Most of the studies had ratings of 'Probably high risk of bias' due to their relatively small sample size (19/30, 63.3 %). Confounding bias is another domain where articles were rated 'Probably high risk of bias' as several studies (11/30, 36.7 %) fail to adjust for or control the confounding variables that may affect the results and interpretation of their study. One study (Fountas et al., 2012) was rated 'Definitely high risk of bias' in one of the domains (detection)

Table 2
The characteristics of articles with human participants using brain imaging.

Study	Study Design	Study Group	Sample Size (n and diagnosis)	Sample Type	Sampling Time	Sample Analytical Platform	Statistical Analysis	Main Findings
Abuhaiba et al. (2022)	Prospective single-blinded repeated measure design study	Adult DRE given sham tDCS then real tDCS	n = 7 (DRE of temporal lobe onset)	Brain imaging	Post-tDCS	MRS	Wilcoxon signed-rank test	↓: GABA in the EZ after real c-tDCS stimulation vs. sham tDCS ($p = 0.02$) & was correlated to decreased IED frequency ↑: Glutathione in the EZ after real c-tDCS stimulation vs. sham tDCS
Campos et al. (2010)	Cohort study	Adult PWE (drug-responder & non-drug-responder) vs. control	n = 46 (PWE, responder, n = 25, non-responder, n = 21) n = 27 (control)	Brain imaging	1–2 years post-treatment	^{1}H-MRS	ANOVA	↓: NAA/Cr in both hippocampi of the non-drug-responder group vs. control ($p < 0.001$)
Fadaie et al. (2016)	Cohort study	Adult patients with refractory TLE vs. healthy control	n = 5 (refractory TLE) n = 4 (control)	Brain imaging	60 min postictal phase	^{1}H-MRS	MWU	↓: NAA/(Cho + Cr), NAA/Cr and NAA/Cho after ictus in ipsilateral hippocampus (vs. contralateral hippocampus and control data)
Guye et al. (2002)	Cohort study	Patients with drug-resistant unilateral TLE vs. healthy control	n = 10 (drug-resistant unilateral TLE) n = 15 (control)	Brain imaging	Interictal period	^{1}H-MRS	MWU	↓: NAA/(Cho+Cr) in all regions involved in SEEG electrophysiological epileptic abnormalities vs. control ($p < 0.05$)
Hetherington et al. (1995)	Cohort study	Adult patients with intractable TLE vs. healthy control	n = 10 (intractable TLE) n = 10 (control)	Brain imaging	Prior surgical implantation	MRSI	*t*-test	↑: Cho/NAA (in 8 patients vs. control). Cr/NAA in all patients' affected hippocampus vs. control ($p < 0.05$, *t*-test). Cr from the Cr/NAA-defined lesion in 9 patients ($p < 0.05$ in 3 patients) ↓: NAA signal of the Cr/NAA-defined lesion in 5 patients ($p < 0.05$)
Hugg et al. (1996)	Cohort study	Adult patients with medically refractory TLE vs. healthy control	n = 10 (medically refractory TLE) n = 10 (healthy control)	Brain imaging	1 year post-temporal lobectomy	^{1}H-MRS	*t*-test	↑: Cr/NAA (unilaterally in 8 patients, bilaterally in 2 patients) ($p < 0.05$)
Kuzniecky et al. (1992)	Cohort study	Adult patients with intractable TLE vs. healthy control	n = 7 (intractable TLE) n = 5 (control)	Brain imaging	≥ 48 h after partial seizure; if secondary generalization, ≥ 7 days after seizure	^{31}P NMR	One-way ANOVA	↓: PCr/P_i in epileptogenic temporal lobe compared with control and unaffected contralateral temporal lobe
Nakamura et al. (2022)	Cohort study	Adult patients (aged 19–87) with glioblastoma	n = 23 (4 with seizures remission, 7 with refractory seizures, 12 with no seizures)	Brain imaging	1 week preoperative	MRS	Two-tailed Student's *t*-test	↑: Lactate (patients with refractory seizures after treatment vs. patients with no seizures after treatment) ↑: Glutamate and NAA (patients with epilepsy vs. patients with no epilepsy)
Pan et al. (1999)	Prospective study	Children with intractable epilepsy before and after KD	n = 7 (4 Lennox-Gastaut syndrome, 1 absence, 1 primary generalized tonic-clonic, & 1 partial complex)	Brain imaging	Pre- and 1–4 months post-KD	^{31}P NMR	Paired Student's two-tailed *t*-test	↑: PC/γ-ATP, PCr/P_i after KD
Petroff et al. (1999)	Cohort study	Adult patients with refractory complex partial seizures given VGB	n = 6 (refractory complex partial seizures) n = 8 (control)	Brain imaging	Pre- and post-treatment	^{1}H NMR	Student's *t*-test two-tailed	↑: GABA (acute), homocarnosine (within 1 week)
Sokól and Flakus (2004)	Cohort study	Patients (aged 10 – 33 years) with DRE vs. healthy control	n = 16 (DRE) n = 18 (control)	Brain imaging	N/A	^{1}H NMR	N/A	↑: Glucose, alanine, taurine ↓: NAA

^{1}H-MRSI proton magnetic spectroscopic imaging, *ASM* antiseizure medication, *ANOVA* analysis of variance, *Cho* choline, *Cho/NAA* choline/N-acetylaspartate ratio, *Cr* creatine, *Cr/NAA* creatine/N-acetylaspartate ratio, *c-tDCS* cathodal-transcranial direct current stimulation, *DRE* drug-resistant epilepsy, *EZ* epileptogenic zone, *GABA* gamma-aminobutyric acid, *IED* epileptiform interictal discharge, *KD* ketogenic diet, *MRS* magnetic resonance spectroscopy, *MRSI* magnetic resonance spectroscopic imaging, *MWU* Mann–Whitney *U* test, *N/A* not available, *NAA* N-acetylaspartate, *NAA/(Cho + Cr)* N-acetylaspartate/(choline + creatine) ratio, *NAA/Cho* N-

acetylaspartate/choline ratio, *NAA/Cr* N-acetylaspartate/creatine ratio, *NMR* nuclear magnetic resonance, *PC/γ-ATP* phosphocreatine/γ-adenosine triphosphate ratio, *PCr/P_i* phosphocreatine/inorganic phosphate ratio, *PWE* patients with epilepsy, *SEEG* stereoelectroencephalography, *tDCS* transcranial direct current stimulation, *TLE* temporal lobe epilepsy, *VGB* vigabatrin, ↑ increased level, ↓ decreased level

as no statistical analysis was conducted for their results (Fountas et al., 2012). Thus, this particular study was excluded from this review. Generally, the majority of the retained studies satisfied most internal validity criteria for bias control.

4. Discussion

Metabolomics provides insights into disease pathophysiology and biomarker discovery. To date, multiple metabolomic studies have investigated alterations in metabolic pathways associated with DRE. However, findings remain heterogeneous, with variations in study design, analytical platforms and sample sources. Hence, this review consolidated findings from 29 metabolomic studies, critically evaluating the potential metabolites altered in DRE as a predictive model for patients to have DRE and for possible targets for future novel ASMs for this type of epilepsy patients.

The most reported metabolites in the DRE studies included amino acids (glycine, glutamate, isoleucine), organic acids (lactate), and glucose. These metabolites were significantly associated with the altered metabolic pathways identified through pathway analysis conducted in this review. Notably, alanine, aspartate, and glutamate metabolism pathways were altered not only in DRE patients (including the subgroups of adults with TLE, adults with unclassified epilepsy and children not receiving KD) but also in the DRE mouse model. Glutamate and aspartate emerged as the key metabolites disrupted across both DRE patients and mouse models. Elevated glutamate levels were reported in four human studies. It is crucial to highlight that these studies compared DRE patients to healthy controls or assessed differences between epileptic and non-epileptic brain regions rather than comparing DRE patients to those responsive to ASMs. As such, the findings may reflect general epilepsy-related metabolic changes rather than drug resistance-specific alterations. Consistently, one mouse model-based study reported an increase in glutamate levels. These findings were congruent with the report by Lai et al. (2022), which highlighted elevated glutamate concentrations in the clinical epilepsy population and animal models (Lai et al., 2022). As the predominant excitatory neurotransmitter in the mammalian central nervous system (CNS), glutamate can cause neuronal hyperexcitability if excessively accumulated (McKenna, 2013). Its interaction with ionotropic glutamate receptors may contribute to epilepsy development (Alcoreza et al., 2021).

Alterations in glycine, serine and threonine metabolism were observed across multiple DRE patient subgroups, including adults with TLE, children on KD and children not receiving KD, with notable changes in glycine levels. Glycine, the most basic nonessential amino acid, is integral to several biological processes, including the formation of DNA, collagen and phospholipids. Additionally, it also plays a key role in synthesizing glutathione, a potent scavenger of free radicals in the nervous system, while contributing to energy release. Acting as both an inhibitory and excitatory neurotransmitter, elevated glycine levels can result in excito-neurotoxicity, triggering seizures, and causing brain damage (Murki et al., 2006).

Phenylalanine, tyrosine and tryptophan biosynthesis, phenylalanine metabolism and tyrosine metabolism were significantly impacted in DRE patients and these pathways are closely interconnected. Phenylalanine is converted to tyrosine, forming the basis for tyrosine metabolism, which produces catecholamine neurotransmitters, including dopamine and norepinephrine. These neurotransmitters are crucial for maintaining excitatory and inhibitory balance in the brain, and disruptions in their levels can lower the seizure threshold (Akyuz et al., 2021; Ozdemir, 2024; Rezaei et al., 2017). Furthermore, tyrosine metabolism contributes to energy production through the TCA cycle which is often impaired in epilepsy (McDonald et al., 2018; Parkhitko et al., 2020; Rho and Boison, 2022). Hence, the interplay between these pathways may affect neuronal excitability due to the influences of catecholamine neurotransmitters and energy depletion.

Glucose, a key metabolite involved in starch and sucrose metabolism, was found to be altered in a few studies. Guo et al. (2023), in their investigation of the metabolome of pediatric patients, revealed that higher glucose levels were correlated with treatment resistance in epilepsy among those who did not respond to valproic acid polytherapy, compared to those who responded to valproic acid monotherapy (Guo et al., 2023). Interestingly, starch and sucrose metabolism remained unaffected in this subgroup. Their findings provide credence to the theory that abnormalities in the brain's TCA cycle and glucose metabolism pathways contribute significantly to both seizure initiation and progression. In contrast, starch and sucrose metabolism was altered in the subgroups of children on KD and patients treated with carbamazepine and clobazam. Masino et al. (2021) reported decreased glucose levels in children responsive to KD compared to those who were not responsive to KD (Masino et al., 2021). Similarly, Godoi et al. (2022) showed that patients with intractable mesial temporal lobe epilepsy (mTLE) were found to have lower glucose levels than patients with mTLE responsive to carbamazepine in combination with clobazam (Godoi et al., 2022). This may be attributed to the nervous tissue's preferential and intensive use of glucose. Additionally, neurotransmitters such as glutamate, acetylcholine, and GABA rely on energy metabolism (Deutch and Roth, 2014). It stands to reason that an alteration in energy metabolism may contribute to the development of seizures and epilepsy.

Isoleucine levels were reported to be decreased in blood and plasma (Godoi et al., 2022; Hung et al., 2021). Conversely, Ong et al. (2021) observed elevated isoleucine levels in the microdialysis collection samples (Ong et al., 2021). However, this review did not identify any altered pathway directly involving isoleucine. There is a suggestion that an abnormal alteration in isoleucine could result in elevated glutamate levels in nervous tissue. This elevation may contribute to heightened hyperexcitability and excitotoxicity, ultimately leading to neuroinflammation, a phenomenon frequently associated with epilepsy (Evangeliou et al., 2009; Dufour et al., 2001).

Furthermore, the organic acid lactate levels were reportedly different in patients with DRE. Boguszewicz et al. (2019) postulated that alterations in the levels of lactate could potentially be linked, at least partially, to heightened oxidative stress (Boguszewicz Ł et al., 2019). In situations of tissue hypoxia, mitochondrial oxidation is compromised, which is a primary factor contributing to elevated levels of branched-chain amino acids. This leads to the excess production and underutilization of lactate both globally and in localized areas. Lai et al. (2022) reported an increased lactate level in patients with epilepsy and epileptic models in their narrative review, which indicates that lactate is not specific to DRE. They also explained that elevated lactate levels could manifest in individuals diagnosed with various other CNS conditions, including ischemic stroke, mitochondrial encephalomyopathy, and migraine (Lai et al., 2022).

Cysteine and methionine metabolism pathway was uniquely altered in children on KD. It plays a crucial role in maintaining redox balance and neurotransmitter function, both of which are relevant to epilepsy. Cysteine is essential for transsulfuration and redox metabolism, particularly in the production of glutathione. However, glutathione synthesis also affects other important processes, as it requires not only cysteine but also glutamate and glycine. Since glutamate and glycine are neurotransmitters, changes in cysteine and glutathione levels can influence their availability, potentially disrupting the balance between excitatory and inhibitory signals. This imbalance, often driven by excessive glutamate, may contribute to epilepsy (Brister et al., 2022). Deceased

Table 3
The characteristics of the articles with human participants using sample types other than brain imaging.

Study	Study Design	Study Group	Sample Size (n and diagnosis)	Sample Type	Sampling Time	Sample Analytical Platform	Statistical Analysis	Main Findings
Akiyama et al. (2023)	Observational study	DRE children aged between 8 months to 17 years 3 months at baseline & 2 – 4 weeks after KD	n = 11 (West syndrome, Dravet syndrome, generalized epilepsy, Lennox–Gastaut syndrome, and focal epilepsy)	Plasma & urine	Pre- and 2–4 weeks post-KD	GC-MS/MS & LC-MS/MS	Wilcoxon Signed-rank test, MWU, PLS-DA	GC-MS/MS plasma ↑: 3-Hydroxybutyric acid, acetoacetic acid, 3-hydroxyisobutyric acid, acetylglycine, 2-hydroxybutyric acid, citramalic acid ↓: Glyoxylic acid, psicose, cystine, 2′-deoxyuridine, hippuric acid LC-MS/MS plasma ↑: Glutamic acid, 2-aminobutyric acid, triglycerides and diglycerides, and acylcarnitines (C2, C4:OH, and C18). ↓: Cysteine, cystine, homoarginine, ceramide, lysophosphatidylcholine acyl, hexosylceramide GC-MS/MS urine ↑: 3-Hydroxybutyric acid, 3-hydroxyisobutyric acid, 2-hydroxybutyric acid, 3-aminoisobutyric acid, 3-hydroxypropionic acid, glycerol 3-phosphate
Boguszewicz Ł et al. (2019)	Cohort study	Children with epilepsy (aged 7 – 48 months) vs. control	n = 28 (children with epilepsy; with seizure, n = 12, without seizure, n = 16) n = 20 (control)	Blood	Between 6 and 9 a.m.	^{1}H NMR	OPLS-DA, cv-ANOVA, Student's *t*-test, MWU, LDA, LOO	↑: N-acetyl-glycoproteins, creatine, lipids, glycine & lactate ↓: Citrate
Detour et al. (2018)	Observational study	Patients with medically intractable mTLE	n = 48 (medically intractable mTLE) Specifically: noHS, n = 10 HS1, n = 20 HS2, n = 6 GG, n = 7 DNT, n = 5 noHS vs. HS1 & HS2, GG & DNT	Hippocampal biopsies	Intraoperative	HRMAS NMR	Kruskal-Wallis, Pearson's chi-square	↑: glutamine, glutamate, and glutathione (HS1 & HS2 vs. noHS) ↓: acetate, alanine, arginine, ascorbate, glycine, NAA, phosphocholine, taurine, total choline, and valine (HS1 & HS2 vs. noHS) In the HS1 & HS2, increased seizure occurrence was linked to a low level of NAA and aspartate. ↑: alanine, arginine, ascorbate, lactate (GG & DNT vs. noHS) ↓: acetate, glutamine, and glutathione (GG & DNT vs. noHS)
Engelke et al. (2021)	Cohort study	Pyridoxine-dependent epilepsy patients (age ranged from 3 – 28 years) vs. control (non-inborn error of metabolism)	n = 7 (NGMS & IRIS-MS) n = 15 (LC-MS/MS)	Plasma, urine, CSF and/or dried blood spots Brain tissue samples from cerebral cortex (postmortem)	Previously collected for routine screening or treatment follow (Not specified)	NGMS, IRIS-MS & LC-MS/MS	2-sided *t*-test, MWU	↑: 2-OPP and 6-oxoPIP in plasma, urine, CSF, and brain tissue
Godoi et al. (2022)	Cohort study	Adult patients (range from 26 – 70 years) with mTLE treated with carbamazepine in combination with clobazam vs. control	n = 28 (mTLE, 20 of which are refractory to treatment) n = 28 (control)	Plasma	N/A	^{1}H NMR	*t*-test, PCA, PLS-DA, LOO, ROC, VIP	Patients vs. control: ↑: glucose ↓: saturated lipids, isoleucine, beta-hydroxybutyrate, and proline Refractory vs responsive mTLE: ↑: lipoproteins, lactate ↓: glucose, unsaturated lipids, isoleucine, and proline

(*continued on next page*)

Table 3 (*continued*)

Study	Study Design	Study Group	Sample Size (n and diagnosis)	Sample Type	Sampling Time	Sample Analytical Platform	Statistical Analysis	Main Findings
Guo et al. (2023)	Single-centre, retrospective cohort study	Children responders to VPA monotherapy (RE group) vs. children that do not respond to VPA polytherapy (NR group)	n = 53 (RE group) n = 37 (NR group)	Plasma	30 min before the next scheduled dose	Metabolomic analysis: (HILIC)-LC-MS/MS Lipidomic analysis: (RP)-LC–MS	PLS-DA, VIP	↓: FAs, glycerophospholipids (phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol) and citric acid, L-thyroxine (NR vs. RE group) ↑: TG, glucose and 2-oxoglutarate levels (NR vs. RE group)
Hung et al. 2021	Prospective study	Children with DRE on KD at baseline, 3rd, 6th, 9th, and 12th months of KD therapy	n = 13 (responds to KD) n = 9 (no response to KD)	Blood	Pre- and 3, 6, 9 and 12 months post-KD	Tandem mass spectrometry	GEE, ROC	After KD therapy compared to baseline ↑: C2, C4:OH, glycine ↓: C3, C4-DC, C5, C5:OH, ketogenic amino acids (phenylalanine, tyrosine, leucine/isoleucine), methionine, valine *free carnitine reduced at month 9 but increased toward baseline after KD therapy KD responders vs. those with no response to KD ↓: free carnitine, C2 (at baseline, at 3 months), C3, C4:OH (at baseline), C5, C5-DC, C16, leucine-isoleucine ↑: β-hydroxybutyrate
Klinkenberg et al. (2014)	Randomized, active-controlled, double-blind, add-on study	Children (aged between 4 – 18 years) with DRE underwent VNS	n = 21 (high-output therapeutic stimulation) n = 20 (low-output active control)	Plasma & CSF	Pre-implantation, 20 weeks and 39 weeks post-stimulation	LC-MS/MS	Kendall's tau, MWU, Wilcoxon signed-rank test, *t*-test	↑: Anthranilic acid
Lum et al. (2023)	Prospective study	Children aged 1 – 10 with refractory epilepsy within 1 day before KD and 1 month after KD	n = 10	Fecal matter	Pre- and 1 month post-KD	GC/MS, LC/MS and LC-MS/MS	Student's *t*-test, Wilcoxon signed-rank test, ANOVA	↑: C16:1 & C18:1 ↓: Kynurenine
Marafi et al. (2021)	Cohort study	Children with DEEs vs. control	n = 10 (5 of which is DRE)	Plasma, urine & CSF	N/A	LC-MS	N/A	Plasma ↓: Plasma glutamine, N-acetylglutamine, urea ↑: N-acetylglutamine, N-acetylasparagine, 1-palmitoylglycerol (16:0) CSF ↓: aspartate and cysteine ↑: N-acetylglutamine, N-acetylasparagine, 1-palmitoylglycerol (16:0)
Masino et al. (2021)	Retrospective study	Children (range 1 – 18 years) with DRE on KD (responders to KD vs. non responders)	n = 5 (responds to KD) n = 5 (does not respond to KD)	CSF	Pre- and 3 months post-KD; around 8 a.m., soon after breakfast	UPLC-MS/MS, GC-MS	ANOVA, paired and unpaired t-tests	Responders vs. non responders: ↑: Ketone bodies (β-hydroxybutyrate and acetoacetate) monohydroxy FAs (γ-hydroxybutyrate, 3-hydroxyoctanoate), hexanoic acid ↓: Glucose, fructose, sorbose
Murgia et al. (2017)	Cohort study	Adults PWE (drug-responder), PWE (non-drug-responder), vs. control	n = 18 (drug-responder) n = 17 (non-drug-responder) n = 35 (control)	Blood	After overnight fasting	^{1}H NMR	PCA, OPLS-DA, MWU, cv-ANOVA	Control vs. drug-responders ↑: acetoacetate, acetone, acetate Control vs. non-drug-responders ↑: acetate, acetoacetate, acetone, alanine, choline, glutamate, scyllo-inositol, 2-OH-butyrate, 2-OH-valerate, 3-OH-butyrate ↓: glucose, citrate, lactate

(*continued on next page*)

Table 3 (*continued*)

Study	Study Design	Study Group	Sample Size (n and diagnosis)	Sample Type	Sampling Time	Sample Analytical Platform	Statistical Analysis	Main Findings
Ong et al. (2021)	Prospective study	Adult patients with drug-resistant focal epilepsy (baseline & hourly samples during the 12-h period surrounding a seizure event)	n = 30	Micro-dialysis collection	Continuously for up to 8 days post-operative	LC-MS/MS	Change point analysis	↑: Glutamate, isoleucine, valine, leucine in epileptic regions vs. nonepileptic regions, glutamine in epileptic regions and nonepileptic regions
Peng et al. (2023)	Cross sectional study	Children with NECP vs. children with CPE	n = 8 (NECP) n = 13 (CPE, of which 5 are DRE)	Fecal matter	N/A	Q-Exactive mass spectrometer	Wilcoxon rank-sum test, Fisher's exact test, *t*-test, fold change analysis, and PLS-DA	↑: kynurenic acid, 2-oxindole, dopamine, 2-hydroxyphenylalanine, 3,4-dihydroxyphenylglycol, L-tartaric acid, D-saccharic acid (CPE vs. NECP) ↑: indole, homovanillic acid (DRE vs. non-DRE)
Wu et al. (2017)	Cross sectional study	High versus low/non-spiking regions in patients with intractable epilepsy (range 3 – 16 years)	n = 9	Brain tissue	Intraoperative	^{1}H HR-MAS MRS	GEE, ROC, PCA, Pearson correlations	↑: Creatine + phosphocreatine, choline in epileptic brain regions ↓: Lactate
Żarnowska et al. (2019)	Prospective study	Children with intractable epilepsy before and after KD (at 3, 6 and 12 months)	n = 16	Plasma	Pre- and 3, 6 and 12 months post-KD	HPLC	Repeated measures ANOVA, MWO, Student's *t*-test	↑: Kynurenic acid ↓: Kynurenine

(HILIC)-LC–MS/MS hydrophilic interaction liquid chromatography, *(RP)-LC–MS* reverse-phase liquid chromatography, *^{1}H HR-MAS MRS* proton high-resolution magic angle spinning magnetic resonance spectroscopy, *2-OPP* 2S,6S-/2S,6R-oxopropylpiperidine-2-carboxylic acid, *6-oxoPIP* 6-oxopiperidine-2-carboxylic acid, *ANOVA* analysis of variance, *C16:1* palmitoleoyl carnitine, *C16* palmitoyl carnitine, *C18:1* oleoylcarnitine, *C18* stearoylcarnitine, *C2* acetylcarnitine, *C3* propionate acylcarnitine, *C4:OH* 3-hydroxybutyrylcarnitine, *C4-DC* methylmalonyl carnitine, *C5* isovaleryl carnitine, *C5:OH* 3-hydroxyisovalerylcarnitine, *C5-DC* glutarylcarnitine, *CPE* cerebral palsy with epilepsy, *CSF* Cerebrospinal fluid, *cv-ANOVA* analysis of variance of the cross-validated residuals, *DEEs* developmental and epileptic encephalopathies, *DNT* dysembryoplastic neuroepithelial tumors, *DRE* drug-resistant epilepsy, *FAs* fatty acids, *GC-MS* gas chromatography-mass spectrometry, *GC-MS/MS* gas chromatography-tandem mass spectrometry, *GEE* generalized estimating equation, *GG* gangliogliomas, *HRMAS NMR* ^{1}H high-resolution magic angle spinning nuclear magnetic resonance, *HS* hippocampal sclerosis, *HS1* hippocampal sclerosis type 1, *HS2* hippocampal sclerosis type 2, *IRIS-MS* mass spectrometer, infrared ion spectroscopy, *KD* ketogenic diet, *LC-MS* liquid chromatography-mass spectrometry, *LC-MS/MS* liquid chromatography-tandem mass spectrometry, *LDA* linear discriminant analysis, *LOO* leave-one-out validation, *mTLE* mesial temporal lobe epilepsy, *MWU* Mann–Whitney *U* test, *N/A* not available, *NAA* N-acetylaspartate, *NECP* non-epileptic cerebral palsy, *NGMS* next-generation metabolic screening, *NMR* nuclear magnetic resonance, *noHS* normal epileptiform tissue group without hippocampal sclerosis, *OPLS-DA* orthogonal partial least-squares discriminant analysis, *PCA* principal component analysis, *PLS-DA* partial least-squares discriminant analysis, *PWE* patients with epilepsy, *ROC* receiver operator characteristics, *TG* triglycerides, *UPLC-MS/MS* ultra-high performance liquid chromatography-tandem mass spectrometry, *VIP* variable importance in projection, *VNS* vagus nerve stimulation, *VPA* valproic acid, ↑ increased level, ↓ decreased level

cysteine levels were reported to be attributed to KD in enhancing mitochondrial activity and fatty acid oxidation, leading to increased oxidative stress. To counteract this, glutathione production may be upregulated, resulting in higher cysteine utilization (Napolitano et al., 2020).

Disruptions in glycerophospholipid metabolism were found in DRE patients, the subgroups of children not receiving KD and patients receiving valproic acid. Glycerophospholipids such as phosphatidylcholine and phosphatidylethanolamine are essential components of cell membranes, and their imbalance can impact neuronal processes, including membrane fusion, neurotransmitter uptake and release, endocytosis and exocytosis (Lai et al., 2022). In addition, choline levels, a product of glycerophospholipid metabolism, were found to be altered. From the human brain imaging studies, it is clear that there is commonly a decrease in N-acetylaspartate/ (creatine + choline), N-acetylaspartate/choline and N-acetylaspartate/creatine ratio. Studies suggest that reductions in metabolite levels in such instances as brain imaging predominantly signify functional neuronal changes (i.e., neuronal and glial dysfunction) and are not necessarily indicative of degeneration or damage of neurons in seizure-affected regions (Cendes et al., 1997; Knowlton et al., 2002). However, it is worth noting that the decrease in these metabolite ratios was typically reported in studies comparing DRE patients to healthy controls. Thus, these alterations are likely non-specific and could potentially be altered in patients with epilepsy, regardless of their resistance to ASMs.

As highlighted in 3.4, some overlaps in metabolic alterations were observed between animal models and human studies. While these diverse animal models provide metabolic insights into specific aspects of DRE pathophysiology, their translational relevance remains largely model-dependent, with each capturing distinct mechanisms of the DRE. For instance, the ALDH7A1 knockout model mirrors the key biochemical and behavioral phenotype of human pyridoxine-dependent epilepsy (Engelke et al., 2021; Al-Shekaili et al., 2020), a condition typically unresponsive to standard ASMs. Whereas, the knockout model for the *SLC13A5* reflects the pathogenic mutations in human *SLC13A5* gene (mammalian INDY homolog), which is associated with severe neonatal encephalopathy and DRE (Henke et al., 2020). Each model perturbs different metabolic pathways and notable differences across species and experimental models further underscore the limitations of direct extrapolation. This is primarily attributed to multifactorial nature of drug resistance mechanisms in epilepsy, which are not mutually exclusive and may be mechanistically interconnected (Perucca et al., 2023).

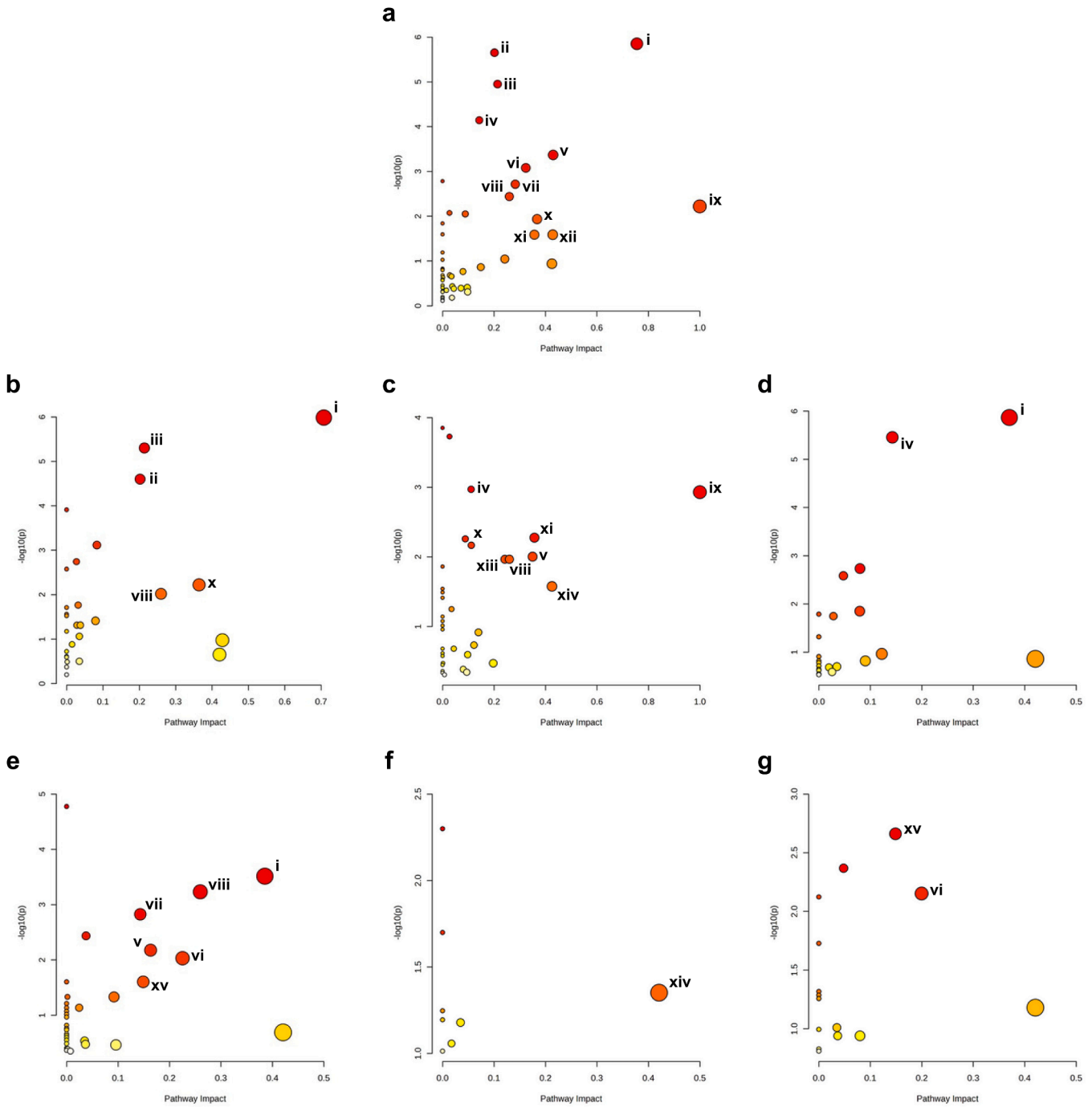

Fig. 2. Summary of pathway analysis of DRE patients and various subgroups performed by MetaboAnalyst 6.0 using significantly impacted metabolites reported in studies on (a) human, (b) TLE in adults, (c) KD in children, (d) adults (unclassified), (e) children without KD, (f) carbamazepine & clobazam and (g) valproic acid. Important metabolic pathways were identified based on impact values ($\geq$0.1) and p ($<$0.05): (i) Alanine, aspartate and glutamate metabolism; (ii) Arginine biosynthesis; (iii) Arginine and proline metabolism; (iv) Butanoate metabolism; (v) Glyoxylate and dicarboxylate metabolism; (vi) Glycerophospholipid metabolism; (vii) Tyrosine metabolism; (viii) Glycine, serine and threonine metabolism; (ix) Phenylalanine, tyrosine and tryptophan biosynthesis; (v) Glutathione metabolism; (xi) Phenylalanine metabolism; (xii) Taurine and hypotaurine metabolism; (xiii) Cysteine and methionine metabolism; (xiv) Starch and sucrose metabolism; (xv) Citrate cycle (TCA cycle).

Therefore, careful selection and evaluation of the specific model employed and its inherent limitations are essential when interpreting and generalizing preclinical findings to the human condition.

To our best knowledge, only one narrative review on metabolomics in epilepsy has been published. However, it was not specific to DRE and aimed to unearth potential diagnostic biomarkers for epilepsy (Lai et al., 2022). Thus, this review was conducted in the context of DRE according to the established protocols of a systematic review. It is important to note that distinguishing whether metabolites changes are due to drug resistance or treatment effects remains challenging. Many of the included studies involved patients receiving different ASM regimens, while others did not specify the ASM regimen in their inclusion criteria. Given that different types of ASMs can independently alter the metabolites, this variability could confound the observed metabolomic signatures (Murgia et al., 2017).

The studies included exhibit substantial heterogeneity across

Pathways \ Subgroups	Human (n=27)	Adults with TLE (n=10)	Children on KD (n=6)	Adults with unclassified epilepsy (n=3)	Children without KD (n=6)	Vigabatrin* (n=1)	Carbamazepine & clobazam (n=1)	Valproic acid (n=1)	VNS* (n=1)	tDCS* (n=1)
Alanine, aspartate and glutamate metabolism	■	■		■	■					
Arginine and proline metabolism	■	■								
Arginine biosynthesis	■	■								
Butanoate metabolism	■		■	■						
Citrate cycle (TCA cycle)					■			■		
Cysteine and methionine metabolism			■							
Glutathione metabolism	■	■	■							
Glycerophospholipid metabolism	■				■			■		
Glycine, serine and threonine metabolism	■	■	■		■					
Glyoxylate and dicarboxylate metabolism	■		■		■					
Phenylalanine metabolism	■		■							
Phenylalanine, tyrosine and tryptophan biosynthesis	■		■							
Starch and sucrose metabolism			■				■			
Taurine and hypotaurine metabolism	■									
Tyrosine metabolism	■				■					

* Too few metabolites to perform pathway analysis
n is the number of studies included for each subgroup

Fig. 3. Important altered metabolic pathways (impact value ≥0.1 and $p < 0.05$) involved in different subgroups of human DRE. The green cells indicate pathways associated with each specific subgroup. The pathways were summarized by pathway analysis using significantly impacted metabolites by MetaboAnalyst 6.0.

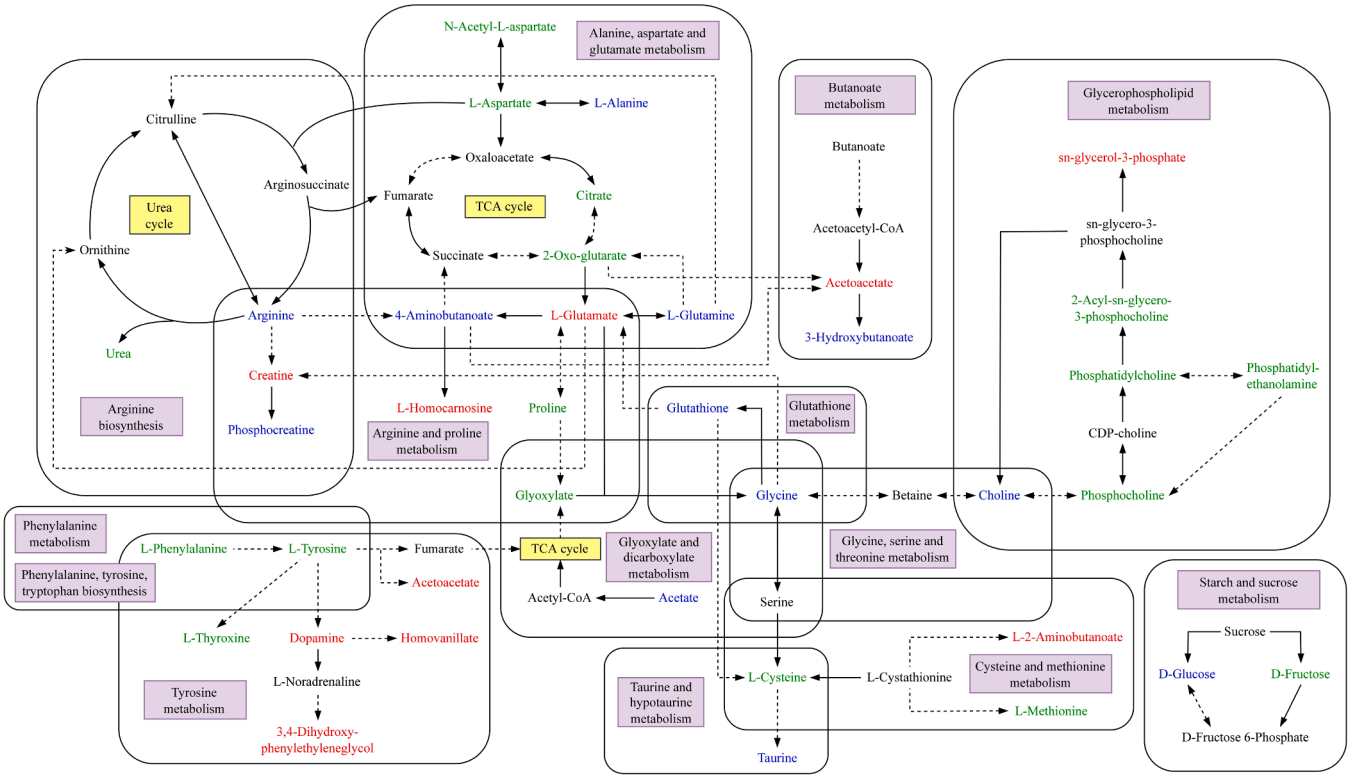

Fig. 4. The altered metabolic pathways and associated metabolites in DRE patients and various subgroups. Dotted arrows represent multiple steps between metabolites, whereas solid arrows indicate a single-step conversion. Double-sided arrows denote bidirectional conversion, while single-sided arrows indicate unidirectional conversion. Metabolites highlighted in color indicate those reported to be significantly altered in DRE patients, with red, blue, and green representing increased levels, both increased and decreased levels, and decreased levels, respectively (adapted from Kanehisa et al., 2024 (Kanehisa et al., 2024); Lai et al., 2022 (Lai et al., 2022)).

multiple domains, including study design, study population, sample types and analytical platforms. The high degree of variability makes it difficult to synthesize a coherent and definitive metabolomic signature for DRE. Patient demographics, such as age and sex contribute to metabolic variations (Adav and Wang, 2021; Brennan and Gibbons, 2020). Distinct baseline metabolic profiles between pediatric and adult populations, as well as between males and females, can influence metabolomic outcomes and complicate data interpretation. In addition,

Table 4
The characteristics of animal DRE studies.

Study	Model and animal	Sample Size	Sample Type	Sampling Time	Sample Analytical Platform	Statistical Analysis	Main Findings
Engelke et al. (2021)	Aldh7a1-knockout mouse model, aged 14 months	Aldh7a1-knockout, n = 4 Wild type, n = 4	Plasma and brain tissues	N/A	NGMS, MS	MWU	↑: 2-OPP, 6-oxoPIP in plasma and brain tissues ↑: P6C, α-AASA, and pipecolic acid in brain tissues
Henke et al. (2020)	Knockout-mouse model of the mammalian INDY [SLC13A5] homolog, SLC13A5	Knockout (n = 10), wildtype (n = 10)	CSF, plasma & brain tissue	After 4 h of fasting in the morning	GC-MS	ANOVA, MWU, Kolmogorov-Smirnov test	No significant change in organic acid levels in plasma in knockout vs. wildtype ↑: Citrate levels in CSF of SLC13A5 −/− knockout mice (significant) compared to SLC13A5 + /+ wildtype mice. Glutamate and aspartate in the CSF of SLC13A5 −/− mice ↓: Citrate levels in parahippocampal cortex in SLC13A5 −/− mice. Lactate and succinate levels in CSF of SLC13A5 −/− mice compared to SLC13A5 + /+ mice
Lum et al. (2023)	Germ-free mice colonized with microbes from the post-KD-treated pASMiatric epilepsy patients' microbiota	Colonized with microbes (each human donor sample, n = 10, was inoculated into 13 – 15 GF mice), control (n = unknown)	Mouse fecal matter	4 days post-inoculation	GC/MS, LC/MS and LC-MS/MS	Student's *t*-test, Wilcoxon signed-rank test, ANOVA	↑: Palmitoleoyl carnitine, oleoylcarnitine (C18:1) ↓: Kynurenine
Wen et al. (2021)	Wistar rats/Pilocarpine model	Epilepsy group, n = 12 Control, n = 12	Rat plasma	Postictal	UPLC-MS/MS	N/A	↑: Cytosine, adenosine, L-tyrosine, citric acid, fructose ↓: L-alanine, L-arginine

2-OPP 2S,6S-/2S,6R-oxopropylpiperidine-2-carboxylic acid, *6-oxoPIP* 6-oxopiperidine-2-carboxylic acid, *ANOVA* analysis of variance, *CSF* cerebrospinal fluid, *GC-MS* gas chromatography-mass spectroscopy, *KD* ketogenic diet, *LC-MS/MS* liquid chromatography tandem mass spectrometry, *LC-MS* liquid chromatography-mass spectroscopy, *MS* mass spectrometer, *MWU* Mann–Whitney *U* test, *N/A* not available, *NGMS* next-generation metabolic screening, *P6C* piperideine-6-carboxylate, *PCA* principal component analysis, *UPLC-MS/MS* ultra-high performance liquid chromatography-tandem mass spectrometry, *α-AASA* α-aminoadipic semialdehyde, ↑ increased level, ↓ decreased level

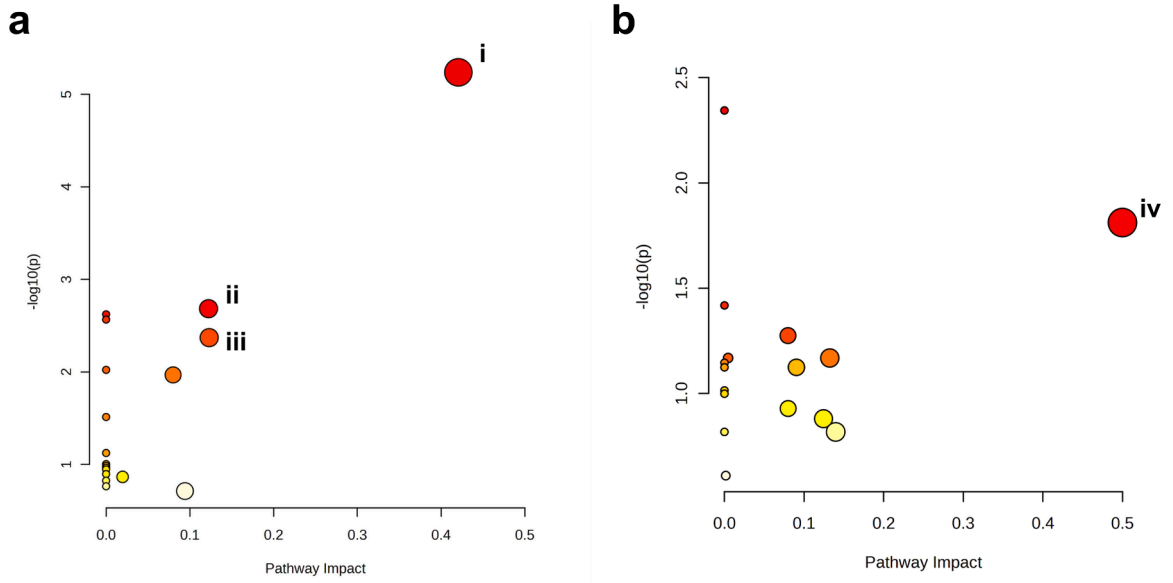

Fig. 5. Summary of pathway analysis of animal DRE models performed by MetaboAnalyst 6.0 using significantly impacted metabolites reported in studies on (a) mice and (b) rats. Important metabolic pathways were identified based on impact values (≥ 0.1) and p (< 0.05): (i) Alanine, aspartate and glutamate metabolism; (ii) Arginine biosynthesis; (iii) Citrate cycle (TCA cycle); (iv) Phenylalanine, tyrosine and tryptophan biosynthesis.

metabolite profiles vary depending on the biological matrix analyzed. For instance, cerebrospinal fluid may better reflect central nervous system activity, whereas plasma is more susceptible to peripheral and dietary influences (Lehmann, 2021). Analytical platforms, such nuclear magnetic resonance and liquid chromatography-mass spectrometry further differ their sensitivity and metabolite coverage, often detecting non-overlapping subsets of metabolites (Emwas et al., 2019). These methodological differences reduce the comparability of findings across studies and limit the validity of identified metabolic signatures.

The timing of biological sample collection relative to treatment

critically affects the interpretation of metabolomic data, as therapeutic interventions, such as ASMs, dietary modifications, and surgical procedures, can significantly influence metabolic profiles (Eid, 2022; Saigusa et al., 2021; Shibutami and Takebayashi, 2021). In this review, substantial variability was observed in sampling time across studies, complicating comparisons across studies and may confound the identification of disease-related biomarkers. Pre-treatment sampling is better suited to distinguish baseline disease biomarkers from those associated with treatment response (Beger et al., 2020). Furthermore, a notable limitation of this review is that the majority of included studies compared patients with epilepsy to non-epileptic controls, rather than directly comparing DRE to drug-sensitive epilepsy cohorts. As a result, the findings of this review primarily describe the metabolites associated with epilepsy, rather than pharmacoresistance.

The inconsistency in the definition of DRE among the included studies is another limitation of this review. While several studies applied the ILAE definition, others did not specify their criteria, and some used alternative definitions to describe their study populations. Such variations can affect the interpretation of findings, as differences in classification criteria may lead to the inclusion of patients with differing degrees of treatment resistance, seizure control history, or underlying pathology. This heterogeneity in patient characteristics could contribute to variability in metabolomic profiles, potentially confounding comparisons across studies. In addition, most of the studies included in this review employed cross-sectional designs, which limit the ability to infer causality between metabolic alterations and DRE. Longitudinal studies are warranted to address these limitations as they involve repeated measurements of the same individuals over time and are more appropriate for examining changes, trajectories, and temporal relationships (Kim, 2021).

The small sample sizes prevalent across most included studies also introduce a significant risk of bias and limit the statistical power to detect robust metabolic changes. Due to the complexity of metabolomics research, there is a lack of standard methods to estimate the sample size required (Nyamundanda et al., 2013). Hence, determining the correct sample sizes to be used in metabolomics is crucial to truly capture the metabolites. Another limitation of this review is the exclusive consideration of English-language publications. Excluding papers in other languages might introduce some degree of publication bias. In light of the limitations mentioned, we emphasize that the identified metabolites and altered pathways in DRE should be considered preliminary hypotheses rather than definitive findings.

This systematic review is crucial for bridging the research gap regarding the underlying mechanisms of DRE by synthesizing findings from existing metabolomic research and identifying the possible key metabolic biomarkers. By providing a comprehensive summary of current evidence, this review aimed to enhance the understanding of essential metabolites and pathways in DRE patients as predictive biomarkers and to inform the development of novel therapeutic strategies, ultimately improving clinical outcomes for patients. Future research should prioritize the development of standardized protocols for metabolomic studies in DRE to enhance comparability and reproducibility across studies. Direct comparative studies between drug-resistant and drug-sensitive epilepsy cohorts are needed to better elucidate metabolic signatures specific to pharmacoresistance. Additionally, conducting multicenter studies with adequate sample sizes, particularly with well-defined and homogeneous subgroups in terms of timing and comparison groups, would provide more robust data and help validate the existing findings. Standardized protocols and larger sample sizes could minimize bias and confounding factors and increase the reliability of results. Further studies to elucidate the mechanisms underlying these metabolic changes will be vital for advancing targeted therapies and creating predictive models for DRE.

The review highlights several promising metabolic pathways and biomarkers, offering potential avenues for clinical translation. One of the application lies in the development of diagnostic biomarker panels. Metabolites consistently altered in DRE such as glutamate and glycine levels, perturbations in phenylalanine and tyrosine metabolism, and disruptions in glucose metabolism could form the basis for such panels. While brain-based studies provide valuable insights, their clinical utility is limited, attributed to key drawbacks, as brain tissues are difficult to obtain (Eid, 2022), and spectroscopic imaging, though non-invasive, is time-consuming and technically complex, requiring technologist intervention (Weinberg et al., 2021). In contrast, biomarkers identified in more accessible biofluids like plasma, serum, or urine offer a more feasible approach (Whitlock et al., 2022). This would allow clinicians to make more informed treatment decisions, potentially reducing the duration of ineffective ASMs trials and improving patient outcomes.

Beyond diagnosis, metabolic profiling also holds promise for treatment stratification. By identifying biochemical pathways predominantly disrupted in individual patients, clinicians may tailor therapies that directly target these abnormalities, such as perampanel may benefit for those with disturbances in glutamatergic signaling (Singh Rana et al., 2023), zonisamide for those with mitochondrial-related metabolic dysfunction (Condello et al., 2013; Grover et al., 2013) and ketogenic diets for those with impaired energy metabolism (D'Andrea Meira et al., 2019). In addition, the metabolic alterations identified also offer novel therapeutic targets for drug development. This could yield new treatments that address the underlying mechanisms of resistance, potentially overcoming the limitations of current ASMs.

5. Conclusion

This study reviewed the potential metabolites and pathways associated with DRE. The metabolites that may potentially be used for targeted therapies and predictive models are amino acids (glycine, glutamate, isoleucine), organic acids (lactate), and glucose. However, it is important to note that some metabolites such as glutamate and lactate may not be specific to DRE and could potentially represent general biomarkers of epilepsy rather than pharmacoresistance. The altered pathways with higher impact scores ($\geq$0.5, $p < 0.05$) include alanine, aspartate and glutamate metabolism; and phenylalanine, tyrosine and tryptophan biosynthesis. Further studies exploring the underlying mechanisms of these metabolic changes will be crucial in developing targeted therapies and predictive models for DRE.

CRediT authorship contribution statement

Alina Arulsamy: Writing – review & editing, Supervision. **Fong Si Lei:** Writing – review & editing, Supervision. **Yow Hui Yin:** Writing – review & editing, Supervision, Funding acquisition, Data curation, Conceptualization. **Lim Zheng Dong:** Writing – original draft, Software, Methodology, Formal analysis. **Nur Asyiqin Syafiqa Abdullah:** Writing – original draft, Methodology, Formal analysis, Data curation. **Lim Kheng Seang:** Writing – review & editing, Supervision, Conceptualization. **Ho Paul Chi Lui:** Writing – review & editing, Supervision, Conceptualization.

Funding

This research was funded by the Ministry of Higher Education (MOHE) Malaysia for the Fundamental Research Grant Scheme (FRGS/1/2024/SKK15/UM/02/4). MOHE provided financial support but did not influence the content of the work.

Declaration of Generative AI and AI-assisted technologies in the writing process

The authors declare that no generative artificial intelligence (AI) tool or service were utilized in the preparation or editing of this work. The authors take full responsibility for the content of this publication.

Declaration of Competing Interest

The authors have no relevant financial or non-financial interests to disclose.

Acknowledgments

The authors acknowledge Universiti Malaya for providing the support and resources for this study.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.eplepsyres.2025.107656.

Data availability

All data generated or analyzed during this study are included in this article (and online resource files).

References

Abuhaiba, S.I., Duarte, I.C., Castelhano, J., Dionísio, A., Sales, F., Edden, R., Castelo-Branco, M., 2022. The impact of cathodal tDCS on the GABAergic system in the epileptogenic zone: a multimodal imaging study. Front. Neurol. 13, 935029. https://doi.org/10.3389/fneur.2022.935029.

Adav, S.S., Wang, Y., 2021. Metabolomics signatures of aging: recent advances. Aging Dis. 12 (2), 646–661. https://doi.org/10.14336/ad.2020.0909.

Akiyama, M., Akiyama, T., Saigusa, D., Hishinuma, E., Matsukawa, N., Shibata, T., Tsuchiya, H., Mori, A., Fujii, Y., Mogami, Y., Tokorodani, C., Kuwahara, K., Numata-Uematsu, Y., Inoue, K., Kobayashi, K., 2023. Comprehensive study of metabolic changes induced by a ketogenic diet therapy using GC/MS- and LC/MS-based metabolomics. Seizure. Eur. J. Epilepsy 107, 52–59. https://doi.org/10.1016/j.seizure.2023.03.014.

Akyuz, E., Polat, A.K., Eroglu, E., Kullu, I., Angelopoulou, E., Paudel, Y.N., 2021. Revisiting the role of neurotransmitters in epilepsy: an updated review. Life Sci. 265, 118826. https://doi.org/10.1016/j.lfs.2020.118826.

Alcoreza, O.B., Patel, D.C., Tewari, B.P., Sontheimer, H., 2021. Dysregulation of ambient glutamate and glutamate receptors in epilepsy: an astrocytic perspective. Front. Neurol. 12, 652159. https://doi.org/10.3389/fneur.2021.652159.

Allers, K., Essue, B.M., Hackett, M.L., Muhunthan, J., Anderson, C.S., Pickles, K., Scheibe, F., Jan, S., 2015. The economic impact of epilepsy: a systematic review. BMC Neurol. 15 (1), 245. https://doi.org/10.1186/s12883-015-0494-y.

Al-Shekaili, H.H., Petkau, T.L., Pena, I., Lengyell, T.C., Verhoeven-Duif, N.M., Ciapaite, J., Bosma, M., van Faassen, M., Kema, I.P., Horvath, G., Ross, C., Simpson, E.M., Friedman, J.M., van Karnebeek, C., Leavitt, B.R., 2020. A novel mouse model for pyridoxine-dependent epilepsy due to antiquitin deficiency. Hum. Mol. Genet. 29 (19), 3266–3284. https://doi.org/10.1093/hmg/ddaa202.

Anyanwu, C., Motamedi, G.K., 2018. Diagnosis and surgical treatment of drug-resistant epilepsy. Brain Sci. 8 (4), 49. https://doi.org/10.3390/brainsci8040049.

Beger, R.D., Schmidt, M.A., Kaddurah-Daouk, R., 2020. Current concepts in pharmacometabolomics, biomarker discovery, and precision Medicine. Metabolites 10 (4), 129. https://doi.org/10.3390/metabo10040129.

Boguszewicz Ł, Jamroz, E., Ciszek, M., Emich-Widera, E., Kijonka, M., Banasik, T., Skorupa, A., Sokół, M., 2019. NMR-based metabolomics in pediatric drug resistant epilepsy – preliminary results. Sci. Rep. 9 (1), 15035. https://doi.org/10.1038/s41598-019-51337-z.

Brennan, L., Gibbons, H., 2020. Sex matters: a focus on the impact of biological sex on metabolomic profiles and dietary interventions. Proc. Nutr. Soc. 79 (2), 205–209. https://doi.org/10.1017/S002966511900106X.

Brister, D., Werner, B.A., Gideon, G., McCarty, P.J., Lane, A., Burrows, B.T., McLees, S., Adelson, P.D., Arango, J.I., Marsh, W., Flores, A., Pankratz, M.T., Ly, N.H., Flood, M., Brown, D., Carpentieri, D., Jin, Y., Gu, H., Frye, R.E., 2022. Central nervous system metabolism in autism, epilepsy and developmental delays: a cerebrospinal fluid analysis. Metabolites 12 (5), 371. https://doi.org/10.3390/metabo12050371.

Callaghan, B., Choi, H., Schlesinger, M., Rodemer, W., Pollard, J., Hesdorffer, D.C., Hauser, W.A., French, J., 2014. Increased mortality persists in an adult drug-resistant epilepsy prevalence cohort. J. Neurol. Neurosurg. Psychiatry 85 (10), 1084–1090. https://doi.org/10.1136/jnnp-2013-307074.

Campos, B.A.G., Yasuda, C.L., Castellano, G., Bilevicius, E., Li, L.M., Cendes, F., 2010. Proton MRS May predict AED response in patients with TLE. Epilepsia 51 (5), 783–788. https://doi.org/10.1111/j.1528-1167.2009.02379.x.

Cendes, F., Andermann, F., Dubeau, F., Matthews, P.M., Arnold, D.L., 1997. Normalization of neuronal metabolic dysfunction after surgery for temporal lobe epilepsy. Neurology 49 (6), 1525–1533. https://doi.org/10.1212/WNL.49.6.1525.

Chatterjee, A., Nair, R., Gandeti, R., Puppala, G.K., Chandran, V., Gorthi, S.P., Radhakrishnan, K., 2020. Socioeconomic consequences of drug-resistant epilepsy in an adult cohort from Southern India. Epilepsy Behav. 110, 107173. https://doi.org/10.1016/j.yebeh.2020.107173.

Chen, Y., Li, E.-M., Xu, L.-Y., 2022. Guide to metabolomics analysis: a bioinformatics workflow. Metabolites 12 (4), 357. https://doi.org/10.3390/metabo12040357.

Cianchetti, C., Bianchi, E., Guerrini, R., Baglietto, M.G., Briguglio, M., Cappelletti, S., Casellato, S., Crichiutti, G., Lualdi, R., Margari, L., Romeo, A., Beghi, E., 2018. Symptoms of anxiety and depression and family's quality of life in children and adolescents with epilepsy. Epilepsy Behav. 79, 146–153. https://doi.org/10.1016/j.yebeh.2017.11.030.

Condello, S., Currò, M., Ferlazzo, N., Costa, G., Visalli, G., Caccamo, D., Pisani, L.R., Costa, C., Calabresi, P., Ientile, R., Pisani, F., 2013. Protective effects of zonisamide against rotenone-induced neurotoxicity. Neurochem. Res. 38 (12), 2631–2639. https://doi.org/10.1007/s11064-013-1181-2.

D'Andrea Meira, I., Romão, T.T., Pires do Prado, H.J., Krüger, L.T., Pires, M.E.P., da Conceição, P.O., 2019. Ketogenic diet and epilepsy: what we know so far. Front. Neurosci. 13, 5. https://doi.org/10.3389/fnins.2019.00005.

Detour, J., Bund, C., Behr, C., Cebula, H., Cicek, E.A., Valenti-Hirsch, M.-P., Lannes, B., Lhermitte, B., Nehlig, A., Kehrli, P., Proust, F., Hirsch, E., Namer, I.-J., 2018. Metabolomic characterization of human hippocampus from drug-resistant epilepsy with mesial temporal seizure. Epilepsia 59 (3), 607–616. https://doi.org/10.1111/epi.14000.

Deutch, A.Y., Roth, R.H., 2014. Pharmacology and biochemistry of synaptic transmission: classical transmitters. In: Byrne, J.H., Heidelberger, R., Waxham, M.N. (Eds.), From Molecules to Networks, 3rd edn. Academic Press, Boston, pp. 207–237.

Dufour, F., Nalecz, K.A., Nalecz, M.J., Nehlig, A., 2001. Modulation of absence seizures by branched-chain amino acids: correlation with brain amino acid concentrations. Neurosci. Res. 40 (3), 255–263. https://doi.org/10.1016/S0168-0102(01)00232-2.

Eid, T., 2022. Harnessing metabolomics to advance epilepsy research. Epilepsy Curr. 22 (2), 123–129. https://doi.org/10.1177/15357597221074518.

Emwas, A.-H., Roy, R., McKay, R.T., Tenori, L., Saccenti, E., Gowda, G.A.N., Raftery, D., Alahmari, F., Jaremko, L., Jaremko, M., Wishart, D.S., 2019. NMR spectroscopy for metabolomics research. Metabolites 9 (7), 123. https://doi.org/10.3390/metabo9070123.

Engelke, U.F.H., van Outersterp, R.E., Merx, J., van Geenen, F.A.M.G., van Rooij, A., Berden, G., Huigen, M.C.D.G., Kluijtmans, L.A.J., Peters, T.M.A., Al-Shekaili, H.H., Leavitt, B.R., de Vrieze, E., Broekman, S., van Wijk, E., Tseng, L.A., Kulkarni, P., Rutjes, F.P.J.T., Mecinović, J., Struys, E.A., Jansen, L.A., Gospe Jr., S.M., Mercimek-Andrews, S., Hyland, K., Willemsen, M.A.A.P., Bok, L.A., van Karnebeek, C.D.M., Wevers, R.A., Boltje, T.J., Oomens, J., Martens, J., Coene, K.L.M., 2021. Untargeted metabolomics and infrared ion spectroscopy identify biomarkers for pyridoxine-dependent epilepsy. J. Clin. Investig. 131 (15), e148272. https://doi.org/10.1172/JCI148272.

Evangeliou, A., Spilioti, M., Doulioglou, V., Kalaidopoulou, P., Ilias, A., Skarpalezou, A., Katsanika, I., Kalamitsou, S., Vasilaki, K., Chatziioanidis, I., Garganis, K., Pavlou, E., Varlamis, S., Nikolaidis, N., 2009. Branched chain amino acids as adjunctive therapy to ketogenic diet in epilepsy: pilot study and hypothesis. J. Child Neurol. 24 (10), 1268–1272. https://doi.org/10.1177/0883073809336295.

Fadaie, F., Mobarakeh, N.M., Fesharaki, S.S.H., Harirchian, M.H., Kharazi, H.H., Rad, H. S., Habibabadi, J.M., 2016. 1H-MRS metabolite's ratios show temporal alternation in temporal lobe seizure: comparison between interictal and postictal phases. Epilepsy Res. 128, 158–162. https://doi.org/10.1016/j.eplepsyres.2016.08.015.

Fountas, K.N., Tsougos, I., Gotsis, E.D., Giannakodimos, S., Smith, J.R., Kapsalaki, E.Z., 2012. Temporal pole proton preoperative magnetic resonance spectroscopy in patients undergoing surgery for mesial temporal sclerosis. Neurosurg. Focus FOC 32 (3), E3. https://doi.org/10.3171/2012.1.FOCUS11327.

French, J.A., Bebin, M., Dichter, M.A., Engel Jr, J., Hartman, A.L., Jóźwiak, S., Klein, P., McNamara Sr, J., Twyman, R., Vespa, P., 2021. Antiepileptogenesis and disease modification: clinical and regulatory issues. Epilepsia Open 6 (3), 483–492. https://doi.org/10.1002/epi4.12526.

Godoi, A.B., do Canto, A.M., Donatti, A., Rosa, D.C., Bruno, D.C.F., Alvim, M.K., Yasuda, C.L., Martins, L.G., Quintero, M., Tasic, L., Cendes, F., Lopes-Cendes, I., 2022. Circulating metabolites as biomarkers of disease in patients with mesial temporal lobe epilepsy. Metabolites 12 (5), 446. https://doi.org/10.3390/metabo12050446.

Grover, N.D., Limaye, R.P., Gokhale, D.V., Patil, T.R., 2013. Zonisamide: a review of the clinical and experimental evidence for its use in parkinson's disease. Indian J. Pharmacol. 45 (6), 547–555. https://doi.org/10.4103/0253-7613.121266.

Guery, D., Rheims, S., 2021. Clinical management of drug resistant epilepsy: a review on current strategies. Neuropsychiatr. Dis. Treat. 17, 2229–2242. https://doi.org/10.2147/NDT.S256699.

Guo, H.-L., Wang, W.-J., Dong, N., Zhao, Y.-T., Dai, H.-R., Hu, Y.-H., Zhang, Y.-Y., Wang, J., Qiu, J.-C., Lu, X.-P., Chen, F., 2023. Integrating metabolomics and lipidomics revealed a decrease in plasma fatty acids but an increase in triglycerides in children with drug-refractory epilepsy. Epilepsia Open 8 (2), 466–478. https://doi.org/10.1002/epi4.12712.

Guye, M., LeFur, Y., Confort-Gouny, S., Ranjeva, J.-P., Bartolomei, F., Régis, J., Raybaud, C.A., Chauvel, P., Cozzone, P.J., 2002. Metabolic and electrophysiological alterations in subtypes of temporal lobe epilepsy: a combined proton magnetic resonance spectroscopic imaging and depth electrodes study. Epilepsia 43 (10), 1197–1209. https://doi.org/10.1046/j.1528-1157.2002.05102.x.

Harden, C., Tomson, T., Gloss, D., Buchhalter, J., Cross, J.H., Donner, E., French, J.A., Gil-Nagel, A., Hesdorffer, D.C., Smithson, W.H., Spitz, M.C., Walczak, T.S., Sander, J. W., Ryvlin, P., 2017. Practice guideline summary: sudden unexpected death in epilepsy incidence rates and risk factors. Neurology 88 (17), 1674–1680. https://doi.org/10.1212/WNL.0000000000003685.

Henke, C., Töllner, K., van Dijk, R.M., Miljanovic, N., Cordes, T., Twele, F., Bröer, S., Ziesak, V., Rohde, M., Hauck, S.M., Vogel, C., Welzel, L., Schumann, T., Willmes, D. M., Kurzbach, A., El-Agroudy, N.N., Bornstein, S.R., Schneider, S.A., Jordan, J.,

Potschka, H., Metallo, C.M., Köhling, R., Birkenfeld, A.L., Löscher, W., 2020. Disruption of the sodium-dependent citrate transporter SLC13A5 in mice causes alterations in brain citrate levels and neuronal network excitability in the hippocampus. Neurobiol. Dis. 143, 105018. https://doi.org/10.1016/j.nbd.2020.105018.

Hetherington, H., Kuzniecky, R., Pan, J., Mason, G., Morawetz, R., Harris, C., Faught, E., Vaughan, T., Pohost, G., 1995. Proton nuclear magnetic resonance spectroscopic imaging of human temporal lobe epilepsy at 4.1 t. Ann. Neurol. 38 (3), 396–404. https://doi.org/10.1002/ana.410380309.

Hugg, J.W., Kuzniecky, R.I., Gilliam, F.G., Faught, R.E., Morawetz, R.B., Hetherington, H.P., 1996. Normalization of contralateral metabolic function following temporal lobectomy demonstrated by 1H magnetic resonance spectroscopic imaging. Ann. Neurol. 40 (2), 236–239. https://doi.org/10.1002/ana.410400215.

Hung, P.-L., Lin, J.-L., Chen, C., Hung, K.-Y., Hsieh, T.-Y., Hsu, M.-H., Kuo, H.-C., Lin, Y.-J., 2021. An examination of serum acylcarnitine and amino acid profiles at different time point of ketogenic diet therapy and their association of ketogenic diet effectiveness. Nutrients 13 (1), 21. https://doi.org/10.3390/nu13010021.

Janmohamed, M., Brodie, M.J., Kwan, P., 2020. Pharmacoresistance – epidemiology, mechanisms, and impact on epilepsy treatment. Neuropharmacology 168, 107790. https://doi.org/10.1016/j.neuropharm.2019.107790.

Kanehisa, M., Furumichi, M., Sato, Y., Matsuura, Y., Ishiguro-Watanabe, M., 2024. KEGG: biological systems database as a model of the real world. Nucleic Acids Res. 53 (D1), D672–D677. https://doi.org/10.1093/nar/gkae909.

Kim, S., 2021. Cross-Sectional and longitudinal studies. In: Gu, D., Dupre, M.E. (Eds.), Encyclopedia of Gerontology and Population Aging, edn. Springer International Publishing, Cham, pp. 1251–1255.

Klinkenberg, S., van den Borne, C.J.H., Aalbers, M.W., Verschuure, P., Kessels, A.G., Leenen, L., Rijkers, K., Aldenkamp, A.P., Vles, J.S.H., Majoie, H.J.M., 2014. The effects of vagus nerve stimulation on tryptophan metabolites in children with intractable epilepsy. Epilepsy Behav. 37, 133–138. https://doi.org/10.1016/j.yebeh.2014.06.001.

Knowlton, R.C., Abou-Khalil, B., Sawrie, S.M., Martin, R.C., Faught, R.E., Kuzniecky, R.I., 2002. In vivo hippocampal metabolic dysfunction in human temporal lobe epilepsy. Arch. Neurol. 59 (12), 1882–1886. https://doi.org/10.1001/archneur.59.12.1882.

Kong, S.T., Lim, S.-H., Ching, J., Ho, P.C.-L., 2025. GC-MS uncovers unique metabolic markers of drug-resistant epilepsy in capillary but not venous dried blood spots. J. Pharm. Biomed. Anal. 253, 116561. https://doi.org/10.1016/j.jpba.2024.116561.

Kuzniecky, R., Elgavish, G.A., Hetherington, H.P., Evanochko, W.T., Pohost, G.M., 1992. In vivo 31P nuclear magnetic resonance spectroscopy of human temporal lobe epilepsy. –1586 Neurology 42 (8), 1586. https://doi.org/10.1212/WNL.42.8.1586.

Kwan, P., Arzimanoglou, A., Berg, A.T., Brodie, M.J., Allen Hauser, W., Mathern, G., Moshé, S.L., Perucca, E., Wiebe, S., French, J., 2010. Definition of drug resistant epilepsy: consensus proposal by the ad hoc task force of the ILAE commission on therapeutic strategies. Epilepsia 51 (6), 1069–1077. https://doi.org/10.1111/j.1528-1167.2009.02397.x.

Lai, W., Du, D., Chen, L., 2022. Metabolomics provides novel insights into epilepsy diagnosis and treatment: a review. Neurochem. Res. 47 (4), 844–859. https://doi.org/10.1007/s11064-021-03510-y.

Lehmann, R., 2021. From bedside to bench—practical considerations to avoid pre-analytical pitfalls and assess sample quality for high-resolution metabolomics and lipidomics analyses of body fluids. Anal. Bioanal. Chem. 413 (22), 5567–5585. https://doi.org/10.1007/s00216-021-03450-0.

Löscher, W., Klein, P., 2021. The pharmacology and clinical efficacy of antiseizure medications: from bromide salts to cenobamate and beyond. CNS Drugs 35 (9), 935–963. https://doi.org/10.1007/s40263-021-00827-8.

Löscher, W., Schmidt, D., 2011. Modern antiepileptic drug development has failed to deliver: ways out of the current dilemma. Epilepsia 52 (4), 657–678. https://doi.org/10.1111/j.1528-1167.2011.03024.x.

Lum, G.R., Ha, S.M., Olson, C.A., Blencowe, M., Paramo, J., Reyes, B., Matsumoto, J.H., Yang, X., Hsiao, E.Y., 2023. Ketogenic diet therapy for pediatric epilepsy is associated with alterations in the human gut microbiome that confer seizure resistance in mice. Cell Rep. 42 (12), 113521. https://doi.org/10.1016/j.celrep.2023.113521.

Marafi, D., Fatih, J.M., Kaiyrzhanov, R., Ferla, M.P., Gijavanekar, C., Al-Maraghi, A., Liu, N., Sites, E., Alsaif, H.S., Al-Owain, M., Zakkariah, M., El-Anany, E., Guliyeva, U., Guliyeva, S., Gaba, C., Haseeb, A., Alhashem, A.M., Danish, E., Karageorgou, V., Beetz, C., Subhi, A.A., Mullegama, S.V., Torti, E., Sebastin, M., Breilyn, M.S., Duberstein, S., Abdel-Hamid, M.S., Mitani, T., Du, H., Rosenfeld, J.A., Jhangiani, S.N., Coban Akdemir, Z., Gibbs, R.A., Taylor, J.C., Fakhro, K.A., Hunter, J.V., Pehlivan, D., Zaki, M.S., Gleeson, J.G., Maroofian, R., Houlden, H., Posey, J.E., Sutton, V.R., Alkuraya, F.S., Elsea, S.H., Lupski, J.R., 2021. Biallelic variants in SLC38A3 encoding a glutamine transporter cause epileptic encephalopathy. Brain 145 (3), 909–924. https://doi.org/10.1093/brain/awab369.

Masino, S.A., Ruskin, D.N., Freedgood, N.R., Lindefeldt, M., Dahlin, M., 2021. Differential ketogenic diet-induced shift in CSF lipid/carbohydrate metabolome of pediatric epilepsy patients with optimal vs. No anticonvulsant response: a pilot study. Nutr. Metab. 18 (1), 23. https://doi.org/10.1186/s12986-020-00524-1.

McDonald, T., Puchowicz, M., Borges, K., 2018. Impairments in oxidative glucose metabolism in epilepsy and metabolic treatments thereof. Front. Cell. Neurosci. 12, 274. https://doi.org/10.3389/fncel.2018.00274.

McKenna, M.C., 2013. Glutamate pays its own way in astrocytes. Front. Endocrinol. 4, 191. https://doi.org/10.3389/fendo.2013.00191.

Murgia, F., Muroni, A., Puligheddu, M., Polizzi, L., Barberini, L., Orofino, G., Solla, P., Poddighe, S., Del Carratore, F., Griffin, J.L., Atzori, L., Marrosu, F., 2017. Metabolomics as a tool for the characterization of Drug-Resistant epilepsy. Front. Neurol. 8, 459. https://doi.org/10.3389/fneur.2017.00459.

Murki, S., Roushan, F., Rao, A., 2006. Opsoclonus in a neonate with nonketotic hyperglycinemia. Internet J. Pediatr. Neonatol. 7 (2), 1–3.

Nakamura, Y., Inoue, A., Nishikawa, M., Ohnishi, T., Yano, H., Kanemura, Y., Ohtsuka, Y., Ozaki, S., Kusakabe, K., Suehiro, S., Yamashita, D., Shigekawa, S., Watanabe, H., Kitazawa, R., Tanaka, J., Kunieda, T., 2022. Quantitative measurement of peritumoral concentrations of glutamate, N-acetyl aspartate, and lactate on magnetic resonance spectroscopy predicts glioblastoma-related refractory epilepsy. Acta Neurochir. 164 (12), 3253–3266. https://doi.org/10.1007/s00701-022-05363-y.

Napolitano, A., Longo, D., Lucignani, M., Pasquini, L., Rossi-Espagnet, M.C., Lucignani, G., Maiorana, A., Elia, D., De Liso, P., Dionisi-Vici, C., Cusmai, R., 2020. The ketogenic diet increases in vivo glutathione levels in patients with epilepsy. Metabolites 10 (12), 504. https://doi.org/10.3390/metabo10120504.

National Institutes of Environmental Health Sciences (2019) Handbook for conducting a literature-based health assessment using OHAT approach for systematic review and evidence integration. ⟨https://ntp.niehs.nih.gov/whatwestudy/assessments/noncancer/handbook⟩. Accessed 30 April 2024.

Nyamundanda, G., Gormley, I.C., Fan, Y., Gallagher, W.M., Brennan, L., 2013. MetSizeR: selecting the optimal sample size for metabolomic studies using an analysis based approach. BMC Bioinforma. 14 (1), 338. https://doi.org/10.1186/1471-2105-14-338.

Ong, C., Damisah, E.C., Gruenbaum, S.E., Dhaher, R., Deng, Y., Sandhu, M.R.S., Zaveri, H.P., Spencer, D.D., Eid, T., 2021. Increased branched-chain amino acids at baseline and hours before a spontaneous seizure in the human epileptic brain. Epilepsia 62 (6), e88–e97. https://doi.org/10.1111/epi.16920.

Ouzzani, M., Hammady, H., Fedorowicz, Z., Elmagarmid, A., 2016. Rayyan—a web and mobile app for systematic reviews. Syst. Rev. 5 (1), 210. https://doi.org/10.1186/s13643-016-0384-4.

Ozdemir, E., 2024. Adrenergic receptor system as a pharmacological target in the treatment of epilepsy (Review). Med. Int. 4 (2), 20. https://doi.org/10.3892/mi.2024.144.

Page, M.J., McKenzie, J.E., Bossuyt, P.M., Boutron, I., Hoffmann, T.C., Mulrow, C.D., Shamseer, L., Tetzlaff, J.M., Akl, E.A., Brennan, S.E., Chou, R., Glanville, J., Grimshaw, J.M., Hróbjartsson, A., Lalu, M.M., Li, T., Loder, E.W., Mayo-Wilson, E., McDonald, S., McGuinness, L.A., Stewart, L.A., Thomas, J., Tricco, A.C., Welch, V.A., Whiting, P., Moher, D., 2021. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. Int. J. Surg. 88, 105906. https://doi.org/10.1016/j.ijsu.2021.105906.

Pan, J.W., Bebin, E.M., Chu, W.J., Hetherington, H.P., 1999. Ketosis and epilepsy: 31P spectroscopic imaging at 4.1 t. Epilepsia 40 (6), 703–707. https://doi.org/10.1111/j.1528-1157.1999.tb00766.x.

Parkhitko, A.A., Filine, E., Mohr, S.E., Moskalev, A., Perrimon, N., 2020. Targeting metabolic pathways for extension of lifespan and healthspan across multiple species. Ageing Res. Rev. 64, 101188. https://doi.org/10.1016/j.arr.2020.101188.

Peng, Y., Chiu, A.T.G., Li, V.W.Y., Zhang, X., Yeung, W.L., Chan, S.H.S., Tun, H.M., 2023. The role of the gut-microbiome-brain axis in metabolic remodeling amongst children with cerebral palsy and epilepsy. Front. Neurol. 14, 1109469. https://doi.org/10.3389/fneur.2023.1109469.

Perucca, E., Perucca, P., White, H.S., Wirrell, E.C., 2023. Drug resistance in epilepsy. Lancet Neurol. 22 (8), 723–734. https://doi.org/10.1016/s1474-4422(23)00151-5.

Petroff, O.A.C., Hyder, F., Collins, T., Mattson, R.H., Rothman, D.L., 1999. Acute effects of vigabatrin on brain GABA and homocarnosine in patients with complex partial seizures. Epilepsia 40 (7), 958–964. https://doi.org/10.1111/j.1528-1157.1999.tb00803.x.

Rezaei, M., Sadeghian, A., Roohi, N., Shojaei, A., Mirnajafi-Zadeh, J., 2017. Epilepsy and dopaminergic system. Physiol. Pharmacol. 21 (1), 1–14.

Rho, J.M., Boison, D., 2022. The metabolic basis of epilepsy. Nat. Rev. Neurol. 18 (6), 333–347. https://doi.org/10.1038/s41582-022-00651-8.

Saigusa, D., Matsukawa, N., Hishinuma, E., Koshiba, S., 2021. Identification of biomarkers to diagnose diseases and find adverse drug reactions by metabolomics. Drug Metab. Pharmacokinet. 37, 100373. https://doi.org/10.1016/j.dmpk.2020.11.008.

Shibutami, E., Takebayashi, T., 2021. A scoping review of the application of metabolomics in nutrition research: the literature survey 2000–2019. Nutrients 13 (11), 3760. https://doi.org/10.3390/nu13113760.

Singh Rana, Z., Suman, R., Veleri, S., Punnakkal, P., 2023. Mechanism of Anti-seizure medications and emerging trends in epilepsy treatment. Int. J. Drug Discov. Pharmacol. 2 (2), 1–16. https://doi.org/10.53941/ijddp.2023.100001.

Sokól, M., Flakus, L., 2004. Metabolic changes in epileptic patients-^{1}H NMR in vivo study. Phys. Med. 20, 21–23.

Weinberg, B.D., Kuruva, M., Shim, H., Mullins, M.E., 2021. Clinical applications of magnetic resonance spectroscopy in brain tumors: from diagnosis to treatment. Radiol. Clin. North Am. 59 (3), 349–362. https://doi.org/10.1016/j.rcl.2021.01.004.

Wen, C., Zhou, C., Jin, Y., Hu, Y., Wang, H., Wang, X., Yang, X., 2021. Metabolic changes in rat plasma after epilepsy by UPLC-MS/MS. Curr. Pharm. Anal. 17 (5), 573–583. https://doi.org/10.2174/1573412916666200206145207.

Whitlock, J.H., Soelter, T.M., Williams, A.S., Hardigan, A.A., Lasseigne, B.N., 2022. Liquid biopsies in epilepsy: biomarkers for etiology, diagnosis, prognosis, and

therapeutics. Hum. Cell 35 (1), 15–22. https://doi.org/10.1007/s13577-021-00624-x.

Wu, H.C., Dachet, F., Ghoddoussi, F., Bagla, S., Fuerst, D., Stanley, J.A., Galloway, M.P., Loeb, J.A., 2017. Altered metabolomic–genomic signature: a potential noninvasive biomarker of epilepsy. Epilepsia 58 (9), 1626–1636. https://doi.org/10.1111/epi.13848.

Żarnowska, I., Wróbel-Dudzińska, D., Tulidowicz-Bielak, M., Kocki, T., Mitosek-Szewczyk, K., Gasior, M., Turski, W.A., 2019. Changes in tryptophan and kynurenine pathway metabolites in the blood of children treated with ketogenic diet for refractory epilepsy. Seizure 69, 265–272. https://doi.org/10.1016/j.seizure.2019.05.006.