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Evaluation of MicroRNAs in Pediatric Epilepsy

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What is already known on this topic?

- *The exact pathophysiology of epilepsy is unclear.*
- *More than 2000 microRNAs have been defined, and more than half of all known miRNAs are expressed in the brain.*
- *Recent research showed that microRNAs have a role in epilepsy pathogenesis.*

What this study adds to this topic?

- *The results showed that the expression of microRNA-155 and microRNA-223 was higher in children with epilepsy.*
- *The expression of the same microRNAs was higher in drug-resistant epilepsy patients than in healthy controls.*
- *The expressions of microRNA-146a, microRNA-155, and microRNA-223 were higher in drug-resistant patients than in drug-responsive children.*

ABSTRACT

Objective: The pathophysiology of epilepsy remains unknown. Recent research has shown that microRNA expression changes in epileptic adults. In the present work, we aimed to identify serum microRNA expression in drug-responsive and resistant children with idiopathic generalized epilepsy.

Materials and Methods: The study included 43 (20 male and 23 female) epilepsy patients and 66 (43 male and 23 female) control subjects. The mean ages of the groups were 113.41 ± 61.83 and 105.46 ± 62.31 months, respectively. Twenty-eight epileptic patients were classified as drug resistant. Thirteen of the controls were the siblings of patients with epilepsy. The study only included children with idiopathic generalized epilepsy who had normal brain magnetic resonance imaging. The serum microRNA expressions (microRNA-181a, microRNA-155, microRNA-146, and microRNA-223) were investigated. Expressions of serum microRNA-181a, microRNA-155, microRNA-146, and microRNA-223 were previously investigated in epilepsy patients and children with febrile seizures. Therefore, these microRNAs were chosen. The expressions of serum levels of microRNAs were determined using quantitative real-time polymerase chain reaction.

Results: The results indicated that the expressions of serum microRNA-155 and microRNA-223 were elevated in epileptic children ($P < .05$). The expression of the same microRNAs was also elevated in individuals with drug-resistant epilepsy compared to healthy controls ($P < .05$). microRNA-146a, microRNA-155, and microRNA-223 expressions were higher in drug-resistant patients than in drug-responsive children ($P < .05$). A logistic regression study determined that an increase of microRNA-155 was a risk for epilepsy, while a decrease of microRNA-146a risk for epilepsy.

Conclusion: Few researchers have investigated the function of microRNAs in the development of childhood epilepsy. Our findings revealed that epilepsy patients have abnormal microRNAexpression.

Keywords: Epilepsy, microRNA, drug resistant, children

INTRODUCTION

MicroRNAs (miRNA) are non-coding RNAs that primarily target messenger RNAs (mRNAs).[1]

They are valuable markers for epilepsy. The first factor is their enrichment in the brain. Although more than 2000 miRNAs have been identified, more than half of these are expressed in the human brain. Second, the presence of brain-enriched microRNAs in a biofluid such as blood would highly suggest a neurologic brain injury disease.[2]

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Epilepsy is a chronic neurological disorder with incidence rates in children ranging from 0.5 to 8 per 1000 person-years.[3-7] Epilepsy's specific pathophysiology is still a mystery. It might be linked to glial fibroblast expansion, inflammation, pathological circuit re-formation, and neuronal death. MicroRNAs may nuclear factor-kappa B (NF-kB) be involved in the occurrence and development of epilepsy by regulating these pathological processes.[8-11]

Studies revealed that miR-146a regulates the expression of NF-kB, interleukin-1 (IL-1), and interferon-α (INF-α) at the post-transcriptional level and affects the inflammatory reaction after an epileptic seizure. Increased miR-146a levels in the epileptic brain may alleviate inflammation.[12]

MicroRNAs-155 also has a role in the regulation of inflammatory pathways in epilepsy. In children with epilepsy, the expression levels of both miR-155 and tumor necrosis factor-α (TNF-α) are increased.

The increased TNF-α levels act as a feedback loop to regulate miR-155 expression. Thus, these 2 molecules interact to mediate the inflammatory process.[13] Glial cells have supportive and protective effects on neurons and are mainly involved in the material metabolism of neurons. Therefore, their dysfunction may be an important cause of epilepsy.

MicroRNA-155 is abundantly expressed in glial cells, and its expression is significantly increased in the brain tissue of epilepsy patients.[12]

Previous studies have found altered expressions of many different microRNAs in epilepsy, such as miR-34, miR-132, miR-146a, miR-199a, miR-375, miR-10b, miR-29a, and miR125a.[12,14-16] Recently, De Beneditis et al[17] compared microRNA expressions of epilepsy patients with controls and showed that miR-142, miR-146a, and miR-223 were significantly upregulated in patients.

Treatment for epilepsy aims to control seizures, improve patients' quality of life, and prevent epilepsy-related morbidity and mortality.[18] Today, anti-epileptic drugs (AED) with various mechanisms of action are used to treat epilepsy.[19,20] Despite receiving the best medical care possible and the most potent AEDs, about one-third of children with epilepsy still experience seizures.[21,22] The results of studies showed that microRNA might have a role in developing drug-resistant epilepsy.[17,23]

On the other hand, microRNA-based treatment modalities are being developed. The leading approach to reducing miRNA expression is antisense oligonucleotides antimRs. Experimental animal research reported that antimRs-based treatment reduces seizure frequency.[24]

Most of the previous research about the role of miRNAs in epilepsy was conducted in adult patients. There are few studies on this topic done with children.[25,26] Recently Liu et al[27] reported higher relative expression levels of miR-155 in serum exosomes in children with epilepsy than in controls. It was also found that the expression of miR-155 in children with epilepsy was correlated with the course of the disease and the degree of abnormal electroencephalography (EEG).

In the present study, we aimed to evaluate miRNA expression in children with idiopathic generalized drug-resistant epilepsy and compare seizure-free children with 1 AED and healthy controls.

MATERIALS AND METHODS

Subjects

This prospective study was carried out in 3 different cities between October 1, 2018, and October 1, 2021. The ethics committee of Eskişehir Osmangazi University approved the study (IRB No. 2018/49, Date: 27.08.2018), and a university research grant provided the funding. Written informed consent was obtained from the parents of all the participating children. The children's medical background and demographic details were noted. Detailed physical examinations were performed, including neurological examinations.

EPILEPSY GROUP

Inclusion Criteria

(1) Pediatric neurologists at university hospitals diagnosed with epilepsy; (2) ages 1-18 years old; (3) normal brain magnetic resonance imaging (MRI); (4) patients with idiopathic generalized epilepsy; (5) negative findings from the genetic epilepsy panel, which includes tests for the most common genetic variants (SCNA, KCNQ, etc.); and (6) drug-resistant epilepsy defined as failure of adequate trials of 2 tolerated and appropriately chosen and used AED schedules (whether as monotherapies or in combination) to achieve sustained seizure freedom according to criteria's of International League Against Epilepsy (ILAE);[28]

Exclusion Criteria

(1) Patients younger than 12 months and older than 18 years old; (2) abnormal MRI findings; (3) children with focal epilepsy; and (4) positive genetic mutation in epilepsy panel.

Control Group

Sixty-six voluntary healthy age-matched children were included in the control group. Thirteen of them were siblings of patients.

Collection of Blood Samples

Three milliliters of blood was collected at least 1 week after the last seizure, and the serum was immediately separated by centrifugation and stored at −80°C in a freezer.

Quantitative Real-Time Polymerase Chain Reaction

Expressions of serum miR-181a, miR-155, mir-146, and miR-223 were previously investigated in epilepsy patients and children with febrile seizures.[29,30] Therefore, these miRNAs were chosen.

Pre-designed primers were used for real-time polymerase chain reaction (PCR) to determine the miRNA expression levels of all samples (Table 1).

Total RNA Extraction

Using a TRIzolTM Reagent kit (Thermo, St. Louis, USA), the manufacturer's instructions extracted total RNA from 200 µL of human serum samples. The samples were examined further following the measurement of the nucleic acid content in the total RNA samples using fluorescence spectrophotometry (Colibri Microvolume Spectrometer, Titertek-Berthold,

Table 1. miRNA Primer List	
miRNA	**Target Sequences (5'->3')**
hsa-miR-146a	UGAGAACUGAAUUCCAUGGGUU
hsa-miR-223	UGAUCAGUUUGUCAAAUACCCA
hsa-miR-155	UUAAUGCUAAUCGUGAUAGGGGUU
hsa-miR-181	AACAUUCAACGUGUCGGUGAGU

Germany). RNA concentrations ranged from 100 to 400 ng/μL, and the total RNA purity was verified using A260/A280 ratios (range 1.81-1.97).

Poly(A) Polymerization and Reverse Transcription

The experiment was carried out in a VeritiTM 384-Well Thermal Cycler using the Poly(A) Polymerase, Yeast (ABM, Canada) and OneScript® Plus cDNA Synthesis (ABM, Canada) kit methods (Life Technologies, United States).

Detection of miRNAs by Quantitative Polymerase Chain Reaction

Expression levels of miRNAs for each sample were determined by using ABI 7500 FAST qPCR System (Life Technologies, United States). Real-time PCR was performed in duplicate for each miRNA and included non-template control. Human miRNAs (miR181, miR155, mir146a, and miR223) designs (Diagen, Ankara, Turkey) were used for the study, and endogenous control small ncRNA SNORD47 was chosen as the internal control for normalization. Analysis of real-time PCR data was made possible by calculating the standard curve and CT values. The fold change data's normal distribution was shown by the logarithmic adjustment, which helped normalize the expression levels in cases of over- and under-expression of genes and lessen the effect of extreme values.[31,32]

Statistical Analysis

Fold change medians of miRNA expression levels were shown. Due to the small sample size and Shapiro-test Wilk's results that were not normally distributed, non-parametric tests were conducted. The 2 groups' levels of cytokines and miRNA were compared using the Mann–Whitney *U* test. For further evaluation to determine the effects of independent variables on the dependent variable, a model was constructed with a categorical dependent variable (2 groups), and binary logistic regression analysis was performed. Statistical significance was defined as a *P*-value less than .05. Analysis was done using the Statistical Package for Social Sciences (SPSS), version 15.0 software (SPSS Inc.; Chicago, IL, USA).

RESULTS

Sociodemographic and Clinical Features of the Study Group

The study was conducted with 109 children (43 patients and 66 controls). Sixty-three males and 46 females were included. Sixty-five percent of (28/43) idiopathic generalized epilepsy patients were drug resistant. Among healthy controls, 13 children were healthy siblings of epilepsy patients. The study group's mean age was 108.60 ± 61.96 months. The mean ages of epilepsy patients and controls were 113.41 ± 61.83 (4-218) months and 105.46 ± 62.31 (5-216), respectively. Statistical analysis revealed no significant difference between the mean ages of epilepsy patients and controls ($P > .05$). In addition, no difference was found between the mean age of resistant and drug-responsive patients (Table 2).

microRNA Expressions

The serum miRNA expression analysis revealed an alteration of miR-155 and miR-223 in epilepsy patients compared to the controls (Table 3).

Considering children with drug-resistant epilepsy, the same miRNAs were higher than controls ($P < .05$).

The results showed that the expression of serum miR-146a, miR-155, and miR-223 in drug-resistant epilepsy patients was higher than in drug-responsive ones (Table 4).

There was no statistically significant difference in the expression of serum miRNAs between drug-resistant epilepsy patients and their healthy siblings ($P > .05$).

The logistic regression analysis revealed that an alteration of miR-155 was a positive risk factor for epilepsy, whereas an alteration of miR-146a was a negative risk factor for epilepsy (Table 5).

The results of logistic regression showed that also age does not affect miRNA expression ($P > .05$).

DISCUSSION

Epilepsy is a chronic neurological disease. The exact etiology of epilepsy is still unclear. There is growing evidence of microRNAs have a role in the pathogenesis of epilepsy.

miRNAs can shape neuronal excitability through modulating the expression of specific ion channels, changing the biophysical and firing properties of individual neurons. MicroRNAs can also target ion channels associated with neuroplasticity.

Table 2. Sociodemographic Features of the Study Group				
	Epilepsy Patients		**Controls**	***P***
Age (months) (mean ± SD; minimum–maximum)	113.41 ± 61.83 (4-218)		105.46 ± 62.31 (5-216)	.52
Gender (boys/girls)	20/23		43/23	.08
	Drug Responsive	Drug Resistant		
Disease duration median (range) (years)	1.2 (0.8-2.9)	3.4 (1.1-13)	N/A	N/A
AED treatment				
Monotherapy	13	3	N/A	N/A
Polytherapy	2	25		
AED, anti-epileptic drug; N/A, not available.				

Table 3. Serum miRNA 146a, 155, 181, and 223 Expressions

miRNAs	Epilepsy patients Fold Change Median $2^{-\Delta\Delta Ct}$ (minimum–maximum)	Controls Fold Change Median $2^{-\Delta\Delta Ct}$ (minimum–maximum)	*P*
miR-146a	−0.99 (−4.88 to 2.18)	−0.43 (−5.88 to 10.21)	>.05
miR-155	13.37 (10.33 to 16.08)	−4.18 (−14.25 to 17.28)	**<.05**
miR-181	−0.60 (−3.55 to 15.31)	0.36 (−5.92 to 7.29)	>.05
miR-223	3.29 (−0.40 to 6.99)	0.01 (−7.92 to 7.75)	**<.05**

Table 4. Serum Expression of miRNAs in Drug-Resistant and Drug-Responsive Patients

miRNAs	Drug-Resistant Patients Fold Change Median $2^{-\Delta\Delta Ct}$ (minimum–maximum)	Drug-Responsive Patients Fold Change Median $2^{-\Delta\Delta Ct}$ (minimum–maximum)	*P*
miRNA-146a	−0.09 (−4.88 to 2.18)	−1.81 (−2.76 to −0.02)	**<.05**
miRNA-155	13.72 (11.08 to 16.08)	12.15 (10.33-15.19)	**<.05**
miRNA-181	−0.78 (−3.55 to 15.31)	2.16 (2.50-10.48)	>.05
miRNA-223	3.61 (−0.40 to 6.99)	2.07 (−0.16 to 5.32)	**<.05**

Table 5. Logistic Regression Analysis of Expressions of miRNAs

miRNAs	β	Standard Error	*P*	OR	95% CI (Minimum–Maximum)
miR-181	0.030	0.086	0.727	1.031	0.87-1.221
miR-155	0.303	0.125	**0.015**	**1.354**	1.061-1.729
miR-146a	−0.635	0.295	**0.031**	**0.530**	0.297-0.945
miR-223	0.279	0.263	0.289	1.322	0.789-2.215

miRNA, microRNA; OR, odds ratio.

Another mechanism by which miRNAs can modulate circuit function is synaptic scaling via interactions with genes associated with synaptic transmission. Apoptosis, inflammation, and pathological circuit re-formation are other mechanisms contribute to the pathophysiology of epilepsy, in which miRNAs might be involved.[10,11,33] Brain injury in patients with epilepsy can promote the release of inflammatory factors and induce inflammatory reactions. These inflammatory factors (e.g., IL-1, INF-α, and TNF-α) can destroy the blood–brain barrier and aggravate damage to the nervous system and also excite neurons and promote repeated seizures.[11]

In the present multicenter study, expression levels of miR181a, miR155, miR146a, and miR223 were searched. The expressions of these miRNAs were previously investigated in epilepsy patients and children with febrile seizures.[29,30] Therefore, these miRNAs were chosen. The present research showed an alteration of miR-155 and miR-223 in epilepsy patients compared to the controls.

It was reported that miR-155 is connected to the control of inflammatory pathways in epilepsy. In individuals with temporal lobe epilepsy (TLE), it was discovered that miR-155 TNF-α expression levels were both increased. The elevated TNF-α levels control miR-155 expression through a feedback loop. These 2 molecules, therefore, work together to mediate the inflammatory process.[13] On the other hand, miR-155 is expressed in glial cells, and its expression is significantly increased in the brain tissue of epilepsy patients.[12]

Research has demonstrated that miR-155 suppresses several genes necessary for microglia activation, phagocytosis, and inflammatory signaling, enabling the microglia to perform beneficial activities in models of motor neuron disease.[34]

Cai et al[35] have shown that injection of antagomirs of miR-155 improves postictal behavior in animal models of pilocarpine-induced status epilepticus.

MicroRNA-223 was another miR that was found to be altered in epilepsy cases in our study. De Benedittis et al[17] discovered that the expression of 3 microRNAs (miR-142, miR-146a, and miR-223) was increased in patients. According to the results of this investigation, patients' miR-223 expression was also changed. Also, in a recent study, it was discovered that miR-223 expression was increased in people with TLE and animal models of epilepsy.[36]

We found no difference in the expression of miR-146a and miR-223 levels between patients and controls. A review done by Ghafouri-Fard et al[37] reported increased expressions of miR-132, miR-146a, miR-181a, and miR-155 in epilepsy.

Increased miR-146a levels in the epileptic brain may suppress inflammation higher levels of pro-inflammatory cytokines, such as IL1, IL6, and TNF- α, as well as higher expression of miR146a, were seen in an animal model of epilepsy. They proposed that miR146a acts as a negative feedback modulator to regulate the synthesis of these inflammatory cytokines.[38]

Silencing miR-181a produces neuroprotection against hippocampus neuron cell apoptosis post-status epilepticus in children with temporal lobe epilepsy.[39]

Our results showed the difference in expressions of miR-146a, miR-155, and miR-223 between drug-responsive and drug-resistant cases. In general, approximately 30% of patients with epilepsy suffer from drug-resistant epileptic seizures despite the use of appropriate drugs.[40] In our study, 65% of patients were drug resistant. This higher ratio might be caused by the centers where research was conducted being tertiary hospitals.

A study comprising 77 drug-resistant and 81 drug-responsive epilepsy patients found that miR-194-5p, -301a-3p, -30b-5p, -342-5p, and -4446-3p were significantly deregulated in the drug-resistant group compared to the drug-responsive group and the control group. Among these 5 microRNAs, miR-301a-3p showed the highest diagnostic value for drug-resistant epilepsy and was inversely correlated with seizure severity.[30] An adult investigation found that miR-142, miR-146a, and miR-223 were significantly upregulated in patients, and miR-142 and miR-223 expression levels showed well in distinguishing drug-sensitive vs. drug-resistant TLE cases.[17] Our results showed that miR-146a expression is significantly altered in drug-resistant patients. Leontariti et al[41] also showed elevated serum levels of miR-146a and miR-134 in drug-resistant patients and found that they were a risk factor for developing refractory epilepsy. Drug resistance is significantly influenced by multidrug transporters,

such as the multidrug resistance gene 1 (MDR1), which in astrocytes encodes a P-glycoprotein. Hypoxia-inducible factor-1-α (HIF-1-α). targets and controls MDR1 expression. According to studies, there is a lower concentration of AEDs in the brain as a result of the coordinated overexpression of HIF-1 and MDR1. Through HIF-1, miRNAs have an impact on the pharmacoresistance pathways.[23]

Most of the previous investigations about the role of miRNAs in epilepsy were conducted in adult patients.[14-16] There are few studies on this topic done with children that are limited.[8,17,25,26,42] Elnady et al[26] looked at the expression of miR-106b and miR-146a in 20 healthy controls and 30 children with epilepsy. Upregulation of miRNAs was observed in patients more than in controls. A systematic review identified only 10 manuscripts performed with 225 children, and 21 different miRNAs were studied previously[24]. Recently, Liu et al[27] searched serum exosome levels of miR-155, and they reported a higher relative expression level in the pediatric epilepsy group as compared to the healthy control group. They also revealed that the expression of miR-155 in children with epilepsy was correlated with the course of the disease and degree of abnormal EEG. In the present research, the relationship between EEG findings and miRNA expressions was not analyzed.

Expressions of miRNAs might have a role in the pathogenesis of not only epilepsy but also epileptic encephalopathy. A study from Turkey conducted with 54 children revealed that the comparison of the epileptic encephalopathy patients' group with healthy controls revealed the upregulation of miR-324-5p and downregulation of miR-146a-5p, miR-138-5p, and miR-187-3p).[43]

Although patients with epileptic encephalopathy were excluded in the present study, the results of both researches showed an alteration of expression of miR-146a.

According to our best, all control groups in previous research, including adults and children, were composed of healthy nonrelatives. In the current study, 13 of the 66 healthy controls were siblings of epilepsy patients. This fact supports our study even though there was no statistically significant difference between the expression of miRNAs in drug-resistant epilepsy patients and their unaffected siblings. Frye et al[44] searched miRNA expression levels in children with autism spectrum disorder (ASD), their typically developed healthy siblings and unrelated controls. They documented 25 miRNAs were upregulated and 43 miRNAs were down-regulated in ASD cases than their typically developed healthy siblings. It must be remembered that it is nearly impossible to interpret a single miRNA's molecular and biophysical functions fully. One miRNA can target many transcripts, and many different miRNAs can target 1 gene. Similarly, miRNAs can target and be targeted by other aspects of epigenetic mechanisms, such as DNA methylation.

MicroRNA expression varies with age.[45] An association between miR-223 overexpression and age of onset in drug-resistant patients has been reported previously.[17] This point might explain the differences in results of pediatric and adult research about miRNA expression in epilepsy. Genetic polymorphisms might also affect the role of miRNAs in the pathogenesis of epilepsy.[46] According to a recent study by Boschiero et al[47] revealed that rs2910164 polymorphism in the miR-146a gene might be associated with susceptibility to drug-resistant epilepsy due to the decreased MIR146a expression.

Most of the previous research was conducted in patients with abnormal findings such as cortical dysplasia and mesial tempol sclerosis.[12,25,48]

A recent study done by Nomair et al[49] included patients with idiopathic generalized and focal epilepsy and documented that both serum miR-146a and miR132 expressions were significantly higher in patients than healthy controls in both groups. However, there was no significant difference in serum miR-146a and miR-132 relative expressions between generalized and focal groups. We found that the expression of serum miR-146a, miR-155, and miR-223 in drug-resistant epilepsy patients was higher than in drug-responsive ones.

They also reported that miR-146 expression was significantly different between drug-responsive and drug-resistant cases among patients with focal epilepsy.

Only children with normal neuroimaging and negative epilepsy panel were included in the study. We thought that this might be a crucial feature of the present study because these inclusion criteria may allow the research group to be more homogenized. We did not compare the results expressions of miRNA according to epileptic syndromes. This might be limitation of present study.

The present study has some limitations. In the present study, the AEDs of patients were not considered. The opinions on the results of the impact of anti-epileptic therapy on a reliable miRNA expression level are not consistent. Rats were given phenobarbital for 2 weeks by Haenisch et al.[14] but the measured levels of microRNAs did not significantly alter. However, some studies show that AEDs can impact the concentrations of certain miRNAs. Several miRNAs decrease in response to phenobarbital or valproate.[50,51] An animal model analyzed the effect of carbamazepine and diazepam before and after therapy and reported none of the 5 miRNAs showed a statistically significant change.[52]

In our research, blood samples were collected during the interictal period. A sampling at several time points in patients with epilepsy is still not very common. There are few studies in which miRNA expression measurements were carried out at least twice.[25] Brennan et al[52] analyzed plasma samples in patients with TLE which were taken during the seizure-free period (on admission and 24 hours after the seizure). Their results showed that the levels of most miRNAs were different between the 2 samples, which indicated their relationship to epilepsy rather than seizure. An investigation searched changes in miRNA expression before and within 30 minutes, 3-6 hours, 20-8 hours, and 3-6 days after seizure in patients with TLE. Analysis showed that the change was transient and associated with seizures.[53]

In conclusion, epilepsy is a chronic neurological disease, and its pathophysiology is unclear. Despite the development of new drugs, some patients could not be seizure-free. The results of the present study revealed that miRNAs-146a, -181a, -155, and

223, especially miR-155 and miR-223, might be involved in epilepsy pathogenesis, and expression levels of miRNAs might be useful in predicting drug resistance.

Ethics Committee Approval: The study was approved by the Ethical Committee of Eskişehir Osmangazi University (IRB Number 2018/13, Date:13.08.2018).

Informed Consent: Written informed consent was obtained from the parents of all the participating children.

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

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REFERENCES

1. Bushati N, Cohen SM. microRNA functions. *Annu Rev Cell Dev Biol*. 2007;23:175-205. [CrossRef]
2. Enright N, Simonato M, Henshall DC. Discovery and validation of blood microRNAs as molecular biomarkers of epilepsy: ways to close current knowledge gaps. *Epilepsia Open*. 2018;3(4):427-436. [CrossRef]
3. Ngugi AK, Kariuki SM, Bottomley C, Kleinschmidt I, Sander JW, Newton CR. Incidence of epilepsy: a systematic review and meta-analysis. *Neurology*. 2011;77(10):1005-1012. [CrossRef]
4. Russ SA, Larson K, Halfon N. A national profile of childhood epilepsy and seizure disorder. *Pediatrics*. 2012;129(2):256-264. [CrossRef]
5. Oka E, Ohtsuka Y, Yoshinaga H, Murakami N, Kobayashi K, Ogino T. Prevalence of childhood epilepsy and distribution of epileptic syndromes: a population-based survey in Okayama, Japan. *Epilepsia*. 2006;47(3):626-630. [CrossRef]
6. Aaberg KM, Gunnes N, Bakken IJ, et al. Incidence and prevalence of childhood epilepsy: a nationwide cohort study. *Pediatrics*. 2017;139(5). [CrossRef]
7. Beghi E. The epidemiology of epilepsy. *Neuroepidemiology*. 2020;54(2):185-191. [CrossRef]
8. Bencurova P, Baloun J, Hynst J, et al. Dynamic miRNA changes during the process of epileptogenesis in an infantile and adult-onset model. *Sci Rep*. 2021;11(1):9649. [CrossRef]
9. Yakovleva KD, Dmitrenko DV, Panina IS, et al. Expression profile of miRs in mesial temporal lobe epilepsy: systematic review. *Int J Mol Sci*. 2022;23(2):951. [CrossRef]
10. Chang BS, Lowenstein DH. Epilepsy. *N Engl J Med*. 2003;349(13):1257-1266. [CrossRef]
11. Vezzani A, Aronica E, Mazarati A, Pittman QJ. Epilepsy and brain inflammation. *Exp Neurol*. 2013;244:11-21. [CrossRef]
12. Wang J, Zhao J. MicroRNA dysregulation in epilepsy: from Pathogenetic involvement to diagnostic biomarker and therapeutic agent development. *Front Mol Neurosci*. 2021;14:650372. [CrossRef]
13. Ashhab MU, Omran A, Kong H, et al. Expressions of tumor necrosis factor-alpha and microRNA-155 in an immature rat model of status epilepticus and children with mesial temporal lobe epilepsy. *J Mol Neurosci*. 2013;51(3):950-958. [CrossRef]
14. Haenisch S, von Rüden EL, Wahmkow H, et al. miRNA-187-3p-mediated regulation of the KCNK10/TREK-2 potassium channel in a rat epilepsy model. *ACS Chem Neurosci*. 2016;7(11):1585-1594. [CrossRef]
15. Ma Y. The Challenge of microRNA as a biomarker of epilepsy. *Curr Neuropharmacol*. 2018;16(1):37-42. [CrossRef]
16. Brennan GP, Henshall DC. microRNAs in the pathophysiology of epilepsy. *Neurosci Lett*. 2018;667:47-52. [CrossRef]
17. De Benedittis S, Fortunato F, Cava C, et al. Circulating microRNA: the potential novel diagnostic biomarkers to predict drug resistance in temporal lobe epilepsy, a pilot study. *Int J Mol Sci*. 2021;22(2):702. [CrossRef]
18. Tenney JR. Epilepsy-Work-Up and Management in children. *Semin Neurol*. 2020;40(6):638-646. [CrossRef]
19. Dang LT, Silverstein FS. Drug treatment of seizures and epilepsy in newborns and children. *Pediatr Clin North Am*. 2017;64(6):1291-1308. [CrossRef]
20. Moavero R, Pisani LR, Pisani F, Curatolo P. Safety and tolerability profile of new anti-epileptic drug treatment in children with epilepsy. *Expert Opin Drug Saf*. 2018;17(10):1015-1028. [CrossRef]
21. Gonzalez-Giraldo E, Sullivan JE, eds. Advances in the treatment of drug-resistant pediatric epilepsy. *Semin Neurol*. 2020;40(2):257-262. [CrossRef]
22. Kumar G. Evaluation and management of drug resistant epilepsy in children. *Curr Probl Pediatr Adolesc Health Care*. 2021;51(7):101035. [CrossRef]
23. Bohosova J, Vajcner J, Jabandziev P, Oslejskova H, Slaby O, Aulicka S. MicroRNAs in the development of resistance to antiseizure drugs and their potential as biomarkers in pharmacoresistant epilepsy. *Epilepsia*. 2021;62(11):2573-2588. [CrossRef]
24. Morris G, O'Brien D, Henshall DC. Opportunities and challenges for microRNA-targeting therapeutics for epilepsy. *Trends Pharmacol Sci*. 2021;42(7):605-616. [CrossRef]
25. Rzepka-Migut B, Paprocka J. Prospects and limitations related to the use of microRNA as a biomarker of epilepsy in children: a systematic review. *Life (Basel)*. 2021;11(1). [CrossRef]
26. Elnady HG, Abdelmoneam N, Eissa E, et al. MicroRNAs as potential biomarkers for childhood epilepsy. *Open Access Maced J Med Sci*. 2019;7(23):3965-3969. [CrossRef]
27. Liu Y, Yu G, Ding YY, Zhang YX. Expression of miR-155 in serum exosomes in children with epilepsy and its diagnostic value. *Dis Markers*. 2022;2022:7979500. [CrossRef]
28. Kwan P, Arzimanoglou A, Berg AT, et al. Definition of drug resistant epilepsy: consensus proposal by the ad hoc task force of the ILAE commission on therapeutic strategies. *Epilepsia*. 2010;51(6):1069-1077. [CrossRef]
29. Çarman KB, Karal Y, Gül Mert G, et al. Expression of microRNA 146a, 155, 181 and 223 in febrile seizure. *Turk J Pediatr*. 2021;63(4):594-601. [CrossRef]
30. Wang J, Tan L, Tan L, et al. Circulating microRNAs are promising novel biomarkers for drug-resistant epilepsy. *Sci Rep*. 2015;5:10201. [CrossRef]
31. Willems E, Leyns L, Vandesompele J. Standardization of real-time PCR gene expression data from independent biological replicates. *Anal Biochem*. 2008;379(1):127-129. [CrossRef]
32. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-delta delta C(T)) Method. *Methods*. 2001;25(4):402-408. [CrossRef]
33. Manna I, Fortunato F, De Benedittis S, et al. Non-coding RNAs: new biomarkers and therapeutic targets for temporal lobe epilepsy. *Int J Mol Sci*. 2022;23(6):3063. [CrossRef]
34. Butovsky O, Jedrychowski MP, Cialic R, et al. Targeting miR-155 restores abnormal microglia and attenuates disease in SOD1 mice. *Ann Neurol*. 2015;77(1):75-99. [CrossRef]
35. Cai Z, Li S, Li S, et al. Antagonist targeting microRNA-155 protects against lithium-pilocarpine-induced status epilepticus in C57BL/6 mice by activating brain-derived neurotrophic factor. *Front Pharmacol*. 2016;7:129. [CrossRef]

36. He Z, Chen H, Zhong Y, et al. MicroRNA 223 targeting ATG16L1 affects microglial autophagy in the kainic acid model of temporal lobe epilepsy. *Front Neurol*. 2021;12:704550. [CrossRef]
37. Ghafouri-Fard S, Hussen BM, Abak A, Taheri M, Jalili Khoshnoud R. Aberrant expression of miRNAs in epilepsy. *Mol Biol Rep*. 2022;49(6):5057-5074. [CrossRef]
38. Ebrahimi R, Golestani A. The emerging role of non-coding RNAs in neuroinflammation: implications in pathogenesis and therapeutic approaches. *J Cell Physiol*. 2022;37:1206-1224.
39. Ren L, Zhu R, Li X. Silencing miR-181a produces neuroprotection against hippocampus neuron cell apoptosis post-status epilepticus in a rat model and in children with temporal lobe epilepsy. *Genet Mol Res*. 2016;15(1). [CrossRef]
40. Asadi-Pooya AA, Tajbakhsh A, Savardashtaki A. MicroRNAs in temporal lobe epilepsy: a systematic review. *Neurol Sci*. 2021;42(2):571-578. [CrossRef]
41. Leontariti M, Avgeris M, Katsarou MS, et al. Circulating miR-146a and miR-134 in predicting drug-resistant epilepsy in patients with focal impaired awareness seizures. *Epilepsia*. 2020;61(5):959-970. [CrossRef]
42. Paul S, Ruiz-Manriquez LM, Ledesma-Pacheco SJ, et al. Roles of microRNAs in chronic pediatric diseases and their use as potential biomarkers: a review. *Arch Biochem Biophys*. 2021;699:108763. [CrossRef]
43. Ünalp A, Coskunpinar E, Gunduz K, et al. Detection of deregulated miRNAs in childhood epileptic encephalopathies. *J Mol Neurosci*. 2022;72(6):1234-1242. [CrossRef]
44. Frye RE, Rose S, McCullough S, et al. MicroRNA expression profiles in autism spectrum disorder: role for miR-181 in immunomodulation. *J Pers Med*. 2021;11(9):922. [CrossRef]
45. Baloun J, Bencurova P, Totkova T, et al. Epilepsy miRNA profile depends on the age of onset in humans and rats. *Front Neurosci*. 2020;14:924. [CrossRef]
46. Buainain RP, Boschiero MN, Camporeze B, de Aguiar PHP, Marson FAL, Ortega MM. Single-nucleotide variants in microRNAs sequences or in their target genes might influence the risk of epilepsy: a review. *Cell Mol Neurobiol*. 2022;42(6):1645-1658. [CrossRef]
47. Boschiero MN, Camporeze B, Santos JSD, et al. The single nucleotide variant n.60G>C in the microRNA-146a associated with susceptibility to drug-resistant epilepsy. *Epilepsy Res*. 2020;162:106305. [CrossRef]
48. Peng J, Omran A, Ashhab MU, et al. Expression patterns of miR-124, miR-134, miR-132, and miR-21 in an immature rat model and children with mesial temporal lobe epilepsy. *J Mol Neurosci*. 2013;50(2):291-297. [CrossRef]
49. Nomair AM, Mekky JF, El-Hamshary SA, Nomeir HM. Circulating miR-146a-5p and miR-132-3p as potential diagnostic biomarkers in epilepsy. *Epilepsy Res*. 2023;191:107089. [CrossRef]
50. Zhou R, Yuan P, Wang Y, et al. Evidence for selective microRNAs and their effectors as common long-term targets for the actions of mood stabilizers. *Neuropsychopharmacology*. 2009;34(6):1395-1405. [CrossRef]
51. Shizu R, Shindo S, Yoshida T, Numazawa S. MicroRNA-122 down-regulation is involved in phenobarbital-mediated activation of the constitutive androstane receptor. *PLoS One*. 2012;7(7):e41291. [CrossRef]
52. Brennan GP, Bauer S, Engel T, et al. Genome-wide microRNA profiling of plasma from three different animal models identifies biomarkers of temporal lobe epilepsy. *Neurobiol Dis*. 2020;144:105048. [CrossRef]
53. Surges R, Kretschmann A, Abnaof K, et al. Changes in serum miRNAs following generalized convulsive seizures in human mesial temporal lobe epilepsy. *Biochem Biophys Res Commun*. 2016;481(1-2):13-18. [CrossRef]