Detection of Deregulated miRNAs in Childhood Epileptic Encephalopathies

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Abstract

Aims

The term "epileptic encephalopathy" is used to describe a possible relationship between epilepsy and developmental delay. The pathogenesis of developmental encephalopathies, independent of epilepsy, can be defined by genetic control mechanisms. The aim of this study was to investigate the use of miRNAs as serum biomarkers for the determination and discrimination of epileptic encephalopathies.

Methods

Whole blood samples obtained from 54 individuals in 2 groups designated as epileptic encephalopathy patients group (n=24) and healthy controls (n=30) were included in this study. The expression levels of 10 miRNAs were determined using qRT-PCR. After the determination of expression levels the correlation of upregulated miRNA levels and Ki67 index was calculated using Pearson correlation test.

Results

The comparison of epileptic encephalopathy patients group with healthy controls revealed the upregulation of one miRNAs (hsa-miR-324-5p) and downregulation of three miRNAs (hsa-miR-146a-5p, hsa-miR-138-5p, hsa-miR-187-3p).

Conclusion

It has been determined that miRNAs with altered expression are an important factor in the formation of epileptic seizures and seizure-induced neuronal death. The fact that processes that play a key role in epiloptogenesis are under the control of miRNAs causes miRNAs to become meta-controllers of gene expression in the brain. We thouhgt that further studies are needed to prove that especially, hsa-miR-146a-5p, hsa-miR-138-5p and hsa-miR-187-3p can be used as epileptic encephalopathy biomarkers. Detection of disease-specific miRNAs could contribute to the development of presicion treatments.

Introduction

Epilepsy is a common neurological disorder in childhood defined as seizures that occur as a result of neuronal discharge in a specific region of the brain (Li 2020). The incidence of the disease in children varies between 33-82 per 100,000 per year (Wu 2019). Drug-resistant epilepsy (DRE) is defined as the persistence of seizures despite adequate anti-epileptic treatment with two or more antiepileptic drugs at efficacious daily doses, alone or in combination, and affects approximately 30% of children with epilepsy. Nowadays it has been defined as not pharmacoresponsive epilepsies. The most severe forms of treatment-resistant epilepsy usually begin in childhood (Patel 2016). In patients with epilepsy related to a genetic etiology, drug resistance is particularly frequent, especially in those with severe developmental and epileptic encephalopathies (Guery 2021).

Epileptic encephalopathy is defined by the presence of frequent epileptiform activity that causes slowing or regression of developmental skills. It is known that many of the epileptic encephalopathies have a molecular genetic basis. The fact that genetic etiology alone can cause developmental impairment means that individualized treatments should be developed to prevent the devastating consequences of this disease group. (Scheffer and Liao 2020). According to the 2010 ILAE (International League Against Epilepsy) classification and terminology commission, epileptic encephalopathy is defined as conditions in which epileptic activity causes serious cognitive and behavioral disorders and may worsen over time, beyond what is expected from the existing pathology (Berg 2010). Childhood EE are classified as infantile spasms, Dravet syndrome, Lennox-Gastaut syndrome, epileptic encephalopathy with Electrical Status during Slow Sleep (ESES), and Landau-Kleffner syndrome, etc. The common feature of these syndromes is that they are generally resistant to antiseizure drugs (ASD) (Engel 2001). The response to (ASD) is generally poor despite high dose polytherapy The most common application used in the treatment of EE patients is adrenocorticotropic hormone (ACTH). However, ACTH causes side effects with high morbidity and mortality, such as hyperglycemia, hypertension, immunodeficiency, and iatrogenic Cushing's syndrome (Khan 2012). However, because the quality of life is driven by several factors in patients with DRE, including the tolerability of the treatment, ASD management should try to optimize efficacy while anticipating the risks of drug-related adverse events. All patients with DRE should be evaluated at least once in a tertiary epilepsy center, especially to discuss eligibility for non-pharmacological therapies (Guery 2021). However, a second handicap from DRE is the risk of sudden unexpected death (SUDEP) and adverse effects on quality of life (QOL) at the individual patient level and family level, which should be considered in therapeutic management.

The etiology of epileptic encephalopathy is complex. Early diagnosis of IE in infants and children depends on a high level of suspicion and continuous monitoring. Risk factors include etiologies, clinical seizures, semiology, comorbidities (change in movements, social interaction), systemic symptoms (eg. markers), and research findings (eg EEG, metabolic scans, genetic studies) (Scheffer 2017). It is now accepted that most patients with DEE have a genetic etiology (Happ 2020; Hu 2012). Many of these genetic variants have been identified in patients previously thought

to be constitutive or 'idiopathic'. Genetic analysis of a DEE cohort (n = 197) revealed that almost a third had pathogenic variants in known or novel genes (Scheffer 2016). An increasing number of genetic variants are implicated in the development of DEE (Wu 2019).

Other etiologies associated with IE include structural (eg, neurocutaneous diseases, cortical developmental disorders, brain tumors), metabolic (eg, vitamin-dependent epilepsies, amino acid disorders, non-ketotic hyperglycemia), and immune disorders (eg Rasmussen syndrome). (Sisodiya 2020). We included children with EE whose cause could not be found in our study. Known causes were excluded by performing a metabolic and epilepsy genetic panel on these patients beforehand.

As a result of the analysis of brain tissue obtained from patients and animal models, it was determined that gene expressions were greatly changed in the affected brain regions. Understanding what controls gene expression may open new avenues for the treatment or prevention of epileptic encephalopathy (Fisher 2014). Therefore, the development of new treatment targets and strategies in epileptic encephalopathies will reduce the risk of mortality and morbidity due to seizures and drugs used by individuals with this disease. For this purpose, identifying the relevant pathways and molecular mechanisms that coordinate gene expression is crucial for a better understanding of the pathogenic process and the development of new therapeutic approaches.

microRNA (miRNA) is a class of small (19-25 nucleotide), single-stranded, endogenous, non-protein-coding short RNA molecules that regulate gene expression either by promoting mRNA degradation or attenuating protein translation at the posttranscriptional level (Reschke 2015). miRNAs are initially transcribed by polymerase II (Pol II) as primary transcripts (pri-miRNA), processed into pre-miRNA, and finally converted to mature miRNA by RNases called Drosha and Dicer, respectively. The functional strand of mature miRNA is loaded into the RNA-induced silencing complex (RISC) containing the Argonaute 2 (Ago-2) protein. The RISC-loaded miRNA is then directed to silencing the target mRNA via mRNA degradation or translation inhibition. A given miRNA can have several binding sites to the same mRNA, and a single mRNA can be targeted by more than one miRNA, thus producing stronger effects. In line with this view, a single miRNA can regulate the expression of hundreds of genes, thus present important effects on cellular functions (Esquela and Kerscher 2006). They mainly function to lower protein levels in cells through sequence-specific binding to target mRNAs, leading to transcript degradation or translational repression.

More than 50% of the miRNAs identified are expressed in the brain. The brain has a variety of miRNAs that are crucial for the establishment and maintenance of normal development and cell phenotype. Acute and chronic nervous system diseases, including epilepsy, are associated with dysregulation of key components of the miRNA biogenesis pathway and altered expression of miRNA. miRNAs are implicated in many brain functions important for epileptogenesis, including cell death, neurogenesis, and synaptic plasticity (Karnati 2015). Studies on associating the development of epilepsy with miRNA have gained momentum in the last 10 years. Large-scale miRNA profiling studies that characterize changes in the expression of different miRNAs are carried out, especially in animal models and subsequent experimental studies with human blood and tissue samples (Mooney 2016). Recently, functional studies in rodents have shown that miRNAs can have potent effects on brain excitability, seizures, and epilepsy. It has been reported that targeting miRNAs, which play a key role in epilepsy, suppress or exacerbates seizures and alters brain excitability, which has a potential for miRNA-based therapeutics in epilepsy (Reschke and Henshall 2015).

In this study, it was aimed to determine the expression levels of 10 different miRNAs (hsa-miR-23a-3p, hsa-miR-34a-5p, hsa-miR-132-3p, hsa-miR-146a-5p, hsa-miR-134-5p, hsa-miR-30a-5p, hsa-miR-138-5p, hsa-miR-324-5p, hsa-miR-330-3p, hsa-miR-187-3p) thought to be deregulated in childhood epileptic encephalopathy patients and to reveal the disease formation process.

Material And Methods

Study design

A total of 54 samples from 30 healthy controls and 24 epileptic encephalopathy patients were included in the study. Blood samples were taken from volunteers who were prediagnosed in Health Sciences University, Dr. Behçet Uz Children's Training and Education Hospital, Department of Pediatry, Division of Pediatric Neurology outpatient clinics, aged between 1 month and 18 years, who underwent electroencephalogram (EEG), brain magnetic resonance imaging (MRI) and metabolic tests and were diagnosed with EE. Control group samples were taken from healthy individuals matched for age and sex. The study was approved by the Clinical Research Ethics Committee of Izmir Dr. Behçet Uz Childrens Training and Education Hospital (Project No: 2019/084, Ethical Approval No: 2019/07-07). After all, individuals included in the study or their parents read and signed the informed consent form, 2 ml peripheral blood samples were taken into EDTA tubes for miRNA isolation and centrifuged at 3000 rpm for 10 minutes for serum separation from blood. Serum samples were stored at -80°C until the study was carried out and transferred to Health Sciences University Medical Biology Department by the cold chain for miRNA isolation.

miRNA extraction and cDNA synthesis

miRNA isolation was performed from serum samples using the miRNeasy Serum/Plasma Advanced Kit (Qiagen, Hilden, Germany, Cat. No: 217204) according to the manufacturer's instructions. Next, cDNA synthesis from miRNAs was performed using the miRCURY LNA RT Kit (Qiagen, Hilden, Germany, Cat. No: 339340) according to the manufacturer's instructions. To determine the purity and concentration of cDNA samples, cDNA control PCR and melting curve analysis were performed.

qRT-PCR

Pre-designed primers were used for Real-time PCR to determine miRNA expression levels of all samples (Table 1.). In total, a gene panel was constructed with specific primers and one housekeeping primer for 10 different miRNAs (miRCURY LNA miRNA Custom PCR Panels, Qiagen, Hilden, Germany). Real-time PCR reactions were performed in triplicate on a Light-Cycler 480 II (Roche) instrument following the manufacturer's protocol using the miRCURY LNA SYBR Green PCR Kit (Qiagen, Hilden, Germany, Cat. No: 339346). Thermal cycling conditions were performed at 95◦C for 10 minutes, then at 95◦C for 15 seconds, at 60◦C for 1 minute, and at 72◦C for 10 seconds for 45 cycles. PCR specificity was confirmed by melting curve analysis.

Table 1
miRNA primer list

miRNA	Target sequence (5'-> 3')
hsa-miR-23a-3p	AUCACAUUGCCAGGGAUUUCC
hsa-miR-34a-5p	UGGCAGUGUCUUAGCUGGUUGU
hsa-miR-132-3p	UAACAGUCUACAGCCAUGGUCG
hsa-miR-146a-5p	UGAGAACUGAAUUCCAUGGGUU
hsa-miR-134-5p	UGUGACUGGUUGACCAGAGGGG
hsa-miR-30a-5p	UGUAAACAUCCUCGACUGGAAG
hsa-miR-138-5p	AGCUGGUGUUGUGAAUCAGGCCG
hsa-miR-324-5p	CGCAUCCCCUAGGGCAUUGGUGU
hsa-miR-330-3p	GCAAAGCACACGGCCUGCAGAGA
hsa-miR-187-3p	UCGUGUCUUGUGUUGCAGCCGG

Statistical analysis

SPSS 25.0 was used for statistical analysis. The significance of miRNA expression levels was calculated by performing a t-test on 2-Δct values for each different miRNA. For the analysis of continuous variables, the Shapiro-Wilk test and Levene's test was facilitated to investigate the distribution and variance homogeneity. Parametric continuous values were compared with t-test and ANOVA while non-parametric variables were compared with Mann-Whitney U and Kruskal-Wallis tests. Categorical variables were analyzed by chi-square tests. Significant correlations between the expression data and clinical parameters were assessed by calculating the Pearson correlation coefficient. The obtained data were analyzed with an online software program (miRCURY LNA miRNA PCR Panels & Assay) developed by Qiagen GeneGlobe Data Analysis Center. Expression levels of each miRNA were evaluated using the Ct (Threshold Cycle) value. These results were normalized for each sample using the SNORA66 housekeeping gene (ΔCt). The relative amount of each miRNA to SNORA66 was described by the equation ΔCt = (Ct miRNA - Ct SNORA66). Fold changes between control and patient were calculated as ΔΔCt. The N-fold differential expression of the miRNA gene of the patient sample compared with the control was expressed as $2^{-\Delta\Delta Ct}$. Mean values of fold difference for epileptic encephalopathy patients were compared with mean values obtained from control samples. In this study, miRNAs with fold change ≥ 2 were considered deregulated.

Results

Demographic and clinical characteristics of the study population

The demographic and clinical characteristics of both case and control groups were summarized in Table 2. The mean ages for EE patients and healthy control groups were 0.1 ± 8.77 and 0.1 ± 11.02 respectively. When the EEG findings of the patients were examined, 7 patients showed Lennox-Gastaut syndrome, 2 patients showed generalized epileptic activity, 2 patients showed Focal ESES, 6 patients showed focal epileptic activity and 5 patients showed modified hypsarrhythmia. When the patients were evaluated in terms of developmental and behavioral disorders, mental retardation was found in 18 patients, growth retardation in 4 patients (cognitive retardation in 1 patient, global growth retardation in 1 patient, growth and development retardation in 2 patients), autism in 1 patient, and normal psychomotor development in 1 patient.

Table 2
The demographic and clinical characteristics of both case and control groups were demonstrated.

Patient No	Age(month) Sex	Age at onset of seizures	Types of seizures	IED in EEG	MRI of brain	Applied treatment modalities	Muscle tone	Other manifestations
1	90/M	4 years	T, M	Generalized	Normal	ASDs, KD	N	Ataxia, hypogammaglobulinemia
2	150/M	2.5 years	A, M, Abs	Focal + Generalized	Periventricular leukomalacia	ASDs, HT, VNS	N	
3	218/F	10 years	M	Focal + Generalized	Normal	ASDs, HT, VNS, KD	N	Gallstone
4	139/M	2 days	ES, T, M	Focal	Parieto-occipital leukomalacia	ASDs, HT	N	Microcephaly, nystagmus
5	84/F	12 months	T, C, GTC	Focal + Generalized	Parietal subcortical leukomalacia	ASDs, KD	N	
6	216/F	9 months	T, C, A, Abs	Focal + Generalized	Agyria, pachygyria, lissencephaly	ASDs, HT, VNS		
7	183/F	2 years	A, M, Abs	Focal + Generalized	Arachnoid cyst	ASDs, HT	N	
8	144/M	8 months	T, C, gelastic	Multifocal + Generalized	Parieto-occipital leukomalacia	ASDs	Hypotonic	
9	168/M	7 months	Focal to bilateral TC	Focal + Generalized	Lissencephaly, pachygyria, brain stem & cerebellar atrophy	ASDs	N	
10	144/M	12 months	BA, Automatism	Multifocal + Generalized	Diffuse cerebral atrophy, mild ventriculomegaly	ASDs, KD	Hypotonic	Tracheostomy, gastrostomy
11	84/F	12 months	M, GTC, BA, Automatism	Focal + Generalized	Diffuse cerebral atrophy	ASDs	Hypotonic	Gastrostomy
12	132/F	6 months	T, M	Focal + Generalized	Diffuse cerebral & cerebellar atrophy, ischaemic changes	ASDs, KD	Hypotonic	Gastrostomy
13	39/F	7 months	ES, T, M, BA	Multifocal + Generalized	Multicystic encephalomalacia, hypoplasia of CC	ASDs, HT, KD	Hypotonic	Microcephaly
14	122/F	3 months	ES, T, M, BA	Focal	Diffuse cerebral atrophy, mild ventriculomegaly	ASDs, KD	Hypotonic	Microcephaly
15	39/M	8 months	ES, T, BA	Focal + Generalized	Periventricular leukomalacia	ASDs	Hypotonic	Microcephaly
16	306/M	2.5 years	M, BA	Focal + Generalized	Normal	ASDs	N	Ataxia
17	84/M	2 years	ES, Focal to bil. TC, M	Focal + Generalized	Diffuse cerebral atrophy, hypomyelination	ASDs	Hypotonic	Microcephaly, gastrostomy
18	84/F	3 months	T, M	Focal + Generalized	Hypomyelination	ASDs	Hypotonic	
19	66/F	2 years	M, Abs	Generalized	Normal	ASDs	N	
20	147/M	6 months	T, M	Multifocal	Normal	ASDs	Hypotonic	Microcephaly, nystagmus
21	153/F	2 months	ES, C	Focal	Normal	ASDs, HT, KD	Hypotonic	

Abbreviations: A: atonic, Abs: absence, BA: behavior arrest, C: clonic, ES: epileptic spasm, GTC: generalized tonic-clonic, M: myoclonic, T: tonic, TC: tonic-clonic, ASDs: anti-seizure drugs, HT: hormonal theraphy (glucocorticoids or corticotropin), KD: ketogenic diet, VNS: vagal nerve stimulation

Patient No	Age(month) Sex	Age at onset of seizures	Types of seizures	IED in EEG	MRI of brain	Applied treatment modalities	Muscle tone	Other manifestations
22	151/M	12 months	C	Focal + Generalized	Normal	ASDs	Hypotonic	
23	62/F	10 months	ES, T, M, C	Focal + Generalized	Normal	ASDs, HT	Hypotonic	Microcephaly
24	36/F	8 months	Focal to bil. TC, T, Autonomic	Focal + Generalized	Hypomyelination	ASDs	N	

Abbreviations: A: atonic, Abs: absence, BA: behavior arrest, C: clonic, ES: epileptic spasm, GTC: generalized tonic-clonic, M: myoclonic, T: tonic, TC: tonic-clonic, ASDs: anti-seizure drugs, HT: hormonal theraphy (glucocorticoids or corticotropin), KD: ketogenic diet, VNS: vagal nerve stimulation

miRNA deregulation in epileptic encephalopathy patients

To determine the expression levels of 10 different miRNAs (Table 3.) that are thought to be deregulated in epileptic encephalopathy patients compared to healthy controls, serum miRNA expression levels were determined by the qRT-PCR method in 30 healthy controls and 24 epileptic encephalopathy patients.

Table 3
Fold regulations and p values

miRNA	Fold regulation	p değeri
hsa-miR-23a-3p	-5.29	0.94
hsa-miR-34a-5p	-2.31	0.26
hsa-miR-132-3p	1.12	0.33
hsa-miR-146a-5p	-31.44	***0.0001**
hsa-miR-134-5p	-1.16	0.06
hsa-miR-30a-5p	-4.32	0.07
hsa-miR-138-5p	-8.22	***0.003**
hsa-miR-324-5p	2.07	***0.03**
hsa-miR-330-3p	-2.09	0.051
hsa-miR-187-3p	-37.22	***0.00007**

Differential expression in epileptic encephalopathy patient group in comparison to healthy controls

In comparison to control group, 8 miRNAs have shown significant deregulation in epileptic encephalopathy patient group (Figure 1). Out of these 8 miRNAs 1 was upregulated while 7 miRNAs were downregulated (Figure 2.). No significant difference was found in 2 miRNAs. The most substantially upregulated miRNA was hsa-miR-324-5p (FC324-5p=2.07, p324-5p=0.0032158), while, as downregulated hsa-miR-187-3p, hsa-miR-146a-5p, hsa-miR-138-5p, hsa-miR-23a-3p, hsa-miR-30a-5p, hsa-miR-330-3p, and hsa-miR-34a-5p have shown the most fold change (FC187-3p=-37.22, p187-3p=0.000079; FC146a-5p= -31.44, p146a-5p= 0.000102; FC138-5p= -8.22, p138-5p= 0.003782, FC23a-3p= -5.29, p23a-3p= 0.943002, FC30a-5p= -4.32, p30a-5p= 0.074398, FC330-3p= -2.09, p330-3p= 0.051443, FC34a-5p= 2.31, p34a-5p= 0.265964).

Discussion

Children with EEs are in the risk group in terms of developmental problems, mental status disorders, comorbidities such as physical problems. The cognitive outcome is not only related to the cause but also appropriate treatments, such as hormonal therapy or early surgical treatment in cases with focal cortical dysplasia (Sisodiya 2020). In our study, only one patient had normal neuromotor development, and most of the remaining patients had mental motor retardation, as well as any autism, growth and developmental retardation, global developmental retardation, cognitive retardation.

The advent of powerful genomic technologies allows us to add genomic sequence data to all other data for diagnosis; where appropriate, other "omics" data (eg epigenomic information) can be added to them. The topic of precision medicine is currently largely driven by genetic discoveries into the causes of some rare, severe, typically early-onset epilepsies, including developmental and epileptic encephalopathies. The list of genes carrying pathogenic rare variants is growing. These discoveries have in some cases led to a better understanding of disease biology, and rational

treatment strategies have sometimes been developed, including avoiding existing ASDs or reusing drugs that were not previously licensed for use in epilepsy (Demarest 2018). However, most such reports were anecdotal and short-term, and a precision medicine approach using a theoretically ideal treatment for most of the newly disclosed genetic epilepsies did not exist. (Sisodiya, 2020). However, the approach of identifying the cause of particular epilepsy and establishing a rational treatment option remains attractive, and a new strategy may be found for treatment-resistant epileptic encephalopathies.

In recent epilepsy genetics studies, miRNAs are important regulators of gene expression (Mooney 2016). The failure of pre-clinical trials targeting genes encoding proteins that are effective in the development of epilepsy to prevent epileptogenesis has contributed to the prominence of miRNAs (Pitkanen 2011). The fact that processes that play a key role in epileptogenesis, were such as neuronal death, gliosis, inflammation, and reorganization of neuronal networks, are under the control of miRNAs, causing miRNAs to become meta-controllers of gene expression in the (Dogini 2013). Therefore, researchers are trying to determine their potential as biomarkers by manipulating miRNAs in vivo in order to understand the function of miRNAs that play a role in epilepsy. Although miRNA studies have been performed in adulthood, especially in mesial temporal lobe epilepsy, this rate is very limited in childhood epilepsy. It has been determined that miRNAs with altered expression are an important factor in the formation of epileptic seizures and seizure-induced neuronal death (Jimenez-Mateos 2012). Approximately 100 miRNAs with varying expression levels were detected in miRNA profile screening studies performed with tissues isolated from the hippocampus resected from patients with temporal lobe epilepsy (Kan 2012; McKiernan 2012).

According to the results of these studies, miR-146a and miR-132 are up-regulated, while miR-30a/b, miR-138, miR-324, miR-330 (Bot 2013), and miR-187 (Gorter 2014) are down-regulated. Jimenez-Mateos et al. detect that the expression level of miR-134 was increased in epilepsy and silencing of miR-134 caused a significant reduction in induced and spontaneous seizures. Limk1, the target gene of miR-134, is an important protein that plays a role in the control of the dendritic structure, which is a critical contact point for excitatory transmission in the central nervous system. miR-146a expressed in atrocities is thought to negatively regulate inflammation by targeting IRAK and TRAF protein family members (Iyer 2012). An article made with cell lines published by Qin et al in July 2021 reported that the miR-138-5p gene is a miRNA targeting caspase-3 to control apoptosis. In this study, it was reported that miR-138-5p is directly associated with the proliferation and survival of human multiple myeloma cells. We think that miR-138-5p, which was found to be downregulated in our study, triggers apoptosis, negatively affects cell survival, and causes damage to many brain functions that are important for epileptogenesis (Qin 2021; Zeng 2021). Evidence obtained from both in-vitro and in-vivo experiments indicated that miR-187-3p is involved in CFC memory (Zhao 2020). In an article published in 2017 by Suya Zhang et al, the potential binding sites of miRNAs and their target genes were analyzed. rno-miR-187-3p was downregulated in stimulated groups compared to controls. Seven distinct genes were identified in the genome as predicted target genes of rno-miR-187-3p. The results obtained in our study overlap with these results (Zhang 2017). In an article by F. Dakterzada et al. in Alzheimer's patients and published in 2020, miR-324-5p expression was found to be upregulated (Dakterzada 2020). The upregulation of the miR-324-5p gene, which is known to cause cortical dysplasia, which is reported to be upregulated in many cancer types such as chronic pancreatitis, colon cancer, and breast cancer, was also found to be upregulated in our study, in line with the literature (Kalser 2018; Wang 2017).

miR-23a, which is generally determined to be up-regulated in epilepsy profile screening studies, provides control of transcription factors that play a role in apoptosis, inflammation, and differentiation. miR-34a plays a role in promoting apoptosis, and silencing of miR-34a during epilepsy protects the hippocampus by generating an anti-apoptotic signal (Na 2020).

Developmental delay in children with epilepsy may be the result of intense epileptiform activity (seizures and EEG abnormalities) or a combination of related factors. However, these seizures do have an impact on the quality of life, as well as the patient’s safety, and as such need intervention. Therefore, the current “International League Against Epilepsy” classification has defined three electroclinical conditions: “developmental encephalopathy”, “epileptic encephalopathy” and “developmental and epileptic encephalopathy” (DEE). In particular, many biological pathways may play a role in the pathogenesis of DEEs. DNA repair, transcriptional regulation, axon myelination, metabolite and ion transport, and paroxysmal function may all be involved in DEE (Hamdan 2017; Raga 2021).

Although miRNA studies have been performed in adulthood, especially in mesial temporal lobe epilepsy, this rate is very limited in childhood epilepsy. In recent years, the fields of RNA-based therapy have advanced considerably and miRNAs offer powerful new features in this field. A study on miRNA expression only in cases with EE was not found in the literature review. Many biological pathways may play a role in the pathogenesis of DEEs. Among these, channelopathies and synaptic dysfunctions are the most common (He 2019). Causes of DEEs include disruption of other cellular functions, DNA repair, transcriptional regulation, axon myelination, metabolite and ion transport, and peroxisomal function (McTague 2016). Understanding the genetic causes of EE is essential for targeted therapies. By uncovering the pathogenetic mechanism and neurobiological processes, it may be possible to improve not only seizure control but also developmental outcome and disease-related comorbidities. However, there is a need to develop new and safe therapeutic drugs, particularly through preclinical research, to evaluate how these therapeutic interventions can be administered safely without causing unwanted side effects such as the currently widely prescribed ASDs (Tecuapetla 2020).

Conclusion

Determining the molecular mechanisms of these miRNAs, which play a role in the development of EE, is very important for the development of presicion treatments. In this study, we detected deregulated expression levels of four of the miRNAs. We hope that our study will shed light on further studies and that these disorders can be treated better with supportive researches.

Declarations

ETHICAL STATEMENT:

Ethics approval and consent to participate: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The study was approved by the Clinical Research Ethics Committee of Izmir Dr. Behçet Uz Pediatrics and Surgery Training and Research Hospital (Project No: 2019/084, Ethical Approval No: 2019/07-07). All procedures were followed in accordance with Helsinki Declaration of 1975, as revized in 2000. Informed consent was taken from all patients included to the study.

Consent for publication: Not applicable.

Availability of data and materials: The data used to support the findings of this study are available from the corresponding author upon request.

Competing interests: The authors declare they have no confict of interest.

Funding: Nor applicapable.

Authors' contributions: Aycan Unalp and Ender Coskunpinar designed the study; Aycan Unalp, Serdar Pekuz, Bahar Toklu Baysal, Selvinaz Edizer, Ceyda Hayretdag and Elif Gudeloglu obtained the samples; Aycan Unalp, Ender Coskunpinar, Kubra Gunduz, Serdar Pekuz, Bahar Toklu Baysal, Selvinaz Edizer, Ceyda Hayretdag and Elif Gudeloglu drafted and revised the manuscript; Ender Coskunpinar and Kubra Gunduz acquired, analyzed, and interpreted the data. All authors read and approved the manuscript.

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Authors' information (optional):

DATA AVAILABLITY STATEMENT

All data generated or analysed during this study are included in this published article (and its supplementary information files).

Ethics Approval

The study was approved by the Clinical Research Ethics Committee of Izmir Dr. Behçet Uz Pediatrics and Surgery Training and Research Hospital (Project No: 2019/084, Ethical Approval No: 2019/07-07).

Informed Consent

All procedures were followed following the Helsinki Declaration of 1975, as revised in 2000. Informed consent was taken from all patients included in neuron the study.

Declaration of interests

 The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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Figures

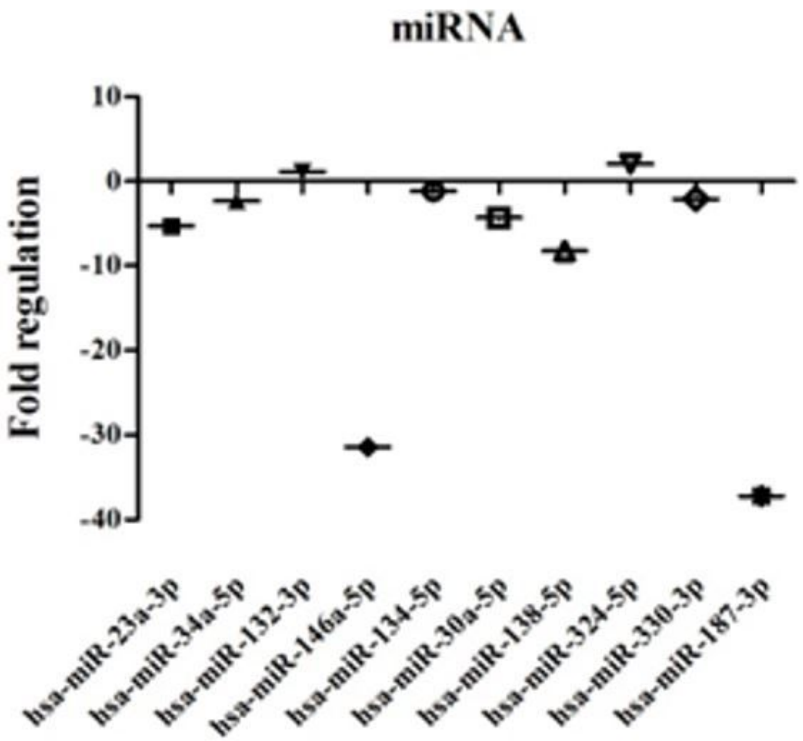

Figure 1

miRNA fold regulation graphic

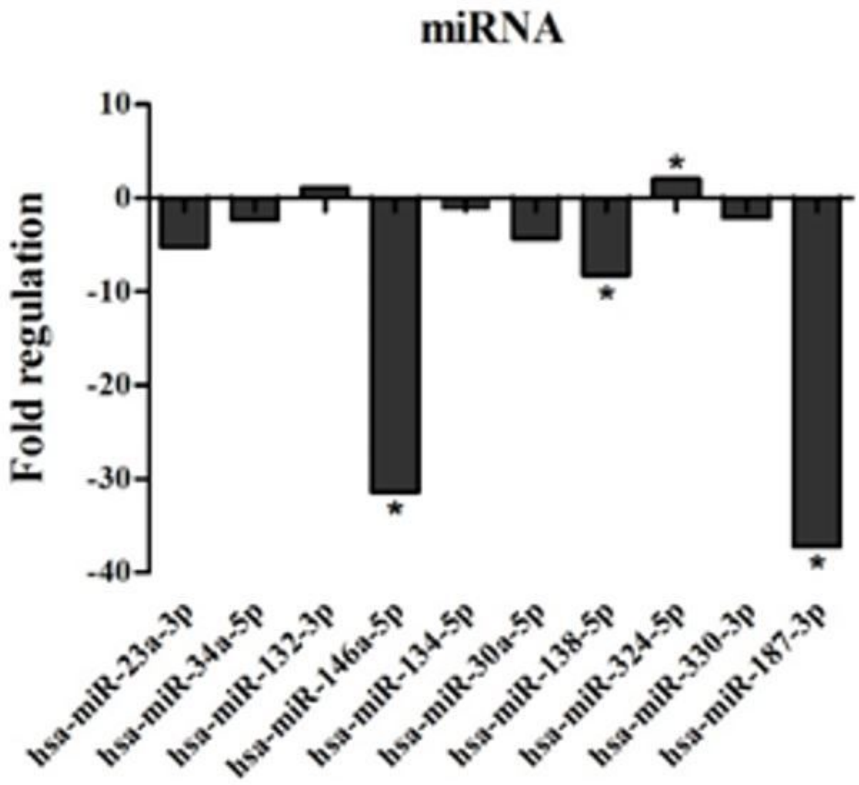

Figure 2

Fold regulation graphic