MicroRNAs as potential biomarkers in temporal lobe epilepsy and mesial temporal lobe epilepsy

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Abstract  
Temporal lobe epilepsy is the most common form of focal epilepsy in adults, accounting for one third of all diagnosed epileptic patients, with seizures originating from or involving mesial temporal structures such as the hippocampus, and many of these patients being refractory to treatment with anti-epileptic drugs. Temporal lobe epilepsy is the most common childhood neurological disorder and, compared with adults, the symptoms are greatly affected by age and brain development. Diagnosis of temporal lobe epilepsy relies on clinical examination, patient history, electroencephalographic recordings, and brain imaging. Misdiagnosis or delay in diagnosis is common. A molecular biomarker that could distinguish epilepsy from healthy subjects and other neurological conditions would allow for an earlier and more accurate diagnosis and appropriate treatment to be initiated. Among possible biomarkers of pathological changes as well as potential therapeutic targets in the epileptic brain are microRNAs. Most of the recent studies had performed microRNA profiling in body fluids such as blood plasma and blood serum and brain tissues such as temporal cortex tissue and hippocampal tissue. A large number of microRNAs were dysregulated when compared to healthy controls and with some overlap between individual studies that could serve as potential biomarkers. For example, in adults with temporal lobe epilepsy, possible biomarkers are miR-199a-3p in blood plasma and miR-142-5p in blood plasma and blood serum. In adults with mesial temporal lobe epilepsy, possible biomarkers are miR-153 in blood plasma and miR-145-3p in blood serum. However, in many of the studies involving patients who receive one or several anti-epileptic drugs, the influence of these on microRNA expression in body fluids and brain tissues is largely unknown. Further studies are warranted with children with temporal lobe epilepsy and consideration should be given to utilizing mouse or rat and non-human primate models of temporal lobe epilepsy. The animal models could be used to confirm microRNA findings in human patients and to test the effects of targeting specific microRNAs on disease progression and behavior. 

Key Words: adults; biomarkers; blood plasma; blood serum; children; hippocampal tissue; mesial temporal lobe epilepsy; microRNA; temporal cortical tissue; temporal lobe epilepsy

Introduction  
Epilepsy is a group of neurological disorders characterized by recurrent, unprovoked epileptic seizures (International League Against Epilepsy, 1993). Affecting around 50–60 million patients globally (Moshe et al., 2014; Wang et al., 2015), epilepsy is one of the most disabling medical disorders. Genetic and familial studies strongly suggest that a neurobiological basis may underlie the pathophysiology of epilepsy, but its etiology is not well understood (Pitkänen and Lukasiuk, 2011). While epilepsy can begin at any age, it most commonly begins in childhood or older adulthood. People over 65 years of age have the highest incidence of epilepsy of any age, accounting for nearly 25% of cases of new-onset epilepsy. Due to changing age demographics, there is an increasing number of older adults with epilepsy. There are many causes of epilepsy and seizures in and older people, these include brain insults such as stroke, traumatic brain injury, brain tumor; Alzheimer’s disease, and lifestyle risk factors such as alcohol, smoking, sleep deprivation, stress, and depression (No author listed, 2022). Worldwide it is estimated that 10.5 million children under 15 years have active epilepsy (Guerini, 2006). In the adult, the conditions leading to or arising from epilepsy may result in a loss or interference with previously acquired functions. By contrast, in a child, there may be interference with development rather than a pronounced loss of function. A child may fail to develop a skill, no longer progressing normally along the developmental continuum; the child may have a decreased rate of acquiring a behavior; or the child may actually regress or lose previously acquired developmental skills (Smith, 2010). 

The most frequent form of focal epilepsy in adults is temporal lobe epilepsy (TLE) (Coan et al., 2014; Bernhardt et al., 2015), accounting for one third of all diagnosed epileptic patients, with seizures usually originating from or involving mesial temporal structures such as the hippocampus, and with up to 70% of these patients being refractory to drug treatment with anti-epileptic drugs (AEDs) (Callaghan et al., 2007; de Boer et al., 2008; Yang et al., 2020). Seizures result from abnormal excessive neuronal discharges in the brain and can produce symptoms ranging in severity from a brief sensory experience to a major convulsive episode (Chang and Lowenstein, 2003). Status epilepticus (SE) occurs when seizures cannot be terminated by inhibitory mechanisms, (John Hopkins Medicine, 2022). It is one of the most frequent neurological emergencies with an incidence of about 20/100,000, with the potential to cause brain injury after 30–60 minutes, and a case fatality rate of around 15% (Kneke et al., 2001; Trinke et al., 2015). A high risk of memory deficits is associated with TLE (Oygbye et al., 2004) and up to 80% of patients with drug-resistant TLE demonstrates episodic memory impairments on neuropsychological testing (Helmstaedter, 2002) that often negatively affect daily functioning and quality of life (Giovagnoli et al., 2014). TLE is the most common childhood neurological disorder (Gataullina et al., 2015) occurring in 33–82 children per 100,000 children per year (Wu et al., 2019). Compared with adults, the symptoms of TLE in children are greatly affected by age and brain development. The changing symptoms cause great challenges for doctors trying to identify and effectively treat children with TLE (Nickels et al., 2012). 

The neuropsychological manifestations of early versus later onset of epilepsy are different. Neuropsychological and magnetic resonance imaging (MRI) measures in two groups of adults with TLE were compared. One group had a mean age of seizure onset at 7.8 years of age and the other group at 23 years of age. The analyses were controlled for the duration of epilepsy, presence of precipitating injury, secondary generalized epilepsy, depression, comorbid medical conditions, and the number of AEDs. The early-onset patients performed worse on all cognitive measures (intelligence, language, visual perception, memory, executive function) compared to late-onset patients and controls, with few differences between late-onset patients and controls. A difference in white matter volume was found between the two patient groups, with the decrease in brain tissue volume in the early-onset group not limited to the temporal lobe but observed both ipsilateral and contralateral to the side of seizure onset (Hermann et al., 2002). 

A combination of clinical examination, patient history, electroencephalographic (EEG) recordings, and brain imaging is used in the diagnosis of TLE, which requires a range of technical expertise and is resource-intensive (Sidhu et al., 2018; Amin and Benbadis, 2019; Galovic et al., 2019). A correct diagnosis is critical and informs the choice of therapy among other factors (Moshé et al., 2015). EEG has only limited diagnostic gain unless performed within specialist epilepsy monitoring units where continuous
Mesial temporal lobe epilepsy with hippocampal sclerosis (mTLE-HS) is the most common and well-defined form of symptomatic focal epilepsy. The precise diagnosis of mTLE-HS should be based on clinical history, electrophysiological findings, neuroimaging, and video-EEG. However, electrophysiological findings are often nonspecific or absent (Engel et al., 2017). Therefore, the standard of care includes an epilepsy workup, which can take months to years to determine if surgical drug treatment is a viable option. This delay can put patients at risk of developing TLE, including a potential epilepsy-inciting event such as traumatic brain injury, stroke, or SE (Garner et al., 2019; Engel and Pitkänen, 2020; Klein and Tylkowski, 2020; Löscher, 2020). Although effective surgical treatment is available, only 20–40% of patients are candidates for drug treatment and the offer of surgical treatment is still limited. The diagnosis should always be made based on a combination of factors and the semiological pattern of seizures is the only mandatory criterion (Wieser, 2009). Expression of miR-32-3p was also significantly downregulated in TLE patients (Engel et al., 2012). A molecular biomarker that could distinguish epilepsy and SE from other neurological conditions would allow for an earlier and more accurate diagnosis and appropriate treatment (Heide and Lowenstein, 2014; Pitkänen et al., 2016; Walker et al., 2016).

The underlying mechanisms of epileptogenesis are still unclear; hence, a better understanding of involved biological processes is fundamental for novel treatment strategies and therapeutics. Among potential therapeutic targets as well as possible biomarkers of pathological changes in the epileptic brain are microRNAs (miRNAs). MiRNAs have been implicated in various pathophysiological processes, including neuroinflammation and neurodegeneration (e.g., Alzheimer’s and Parkinson’s disease) (Hutvágner and Zamore, 2002; Pillai, 2005). The monitoring of miRNA expression might disclose the onset or progression of the disease, making miRNAs a useful biomarker. Numerous studies of miRNA expression in TLE have provided evidence for their importance in epilepsy pathogenesis (Brennan and Henshall, 2018). Abnormal expression of miRNAs is related to pathways of inflammation, cell death, synaptic reorganization, and neuronal excitability, thus influencing the pathogenesis of epilepsy (Choo et al., 2016). Mesial temporal lobe epilepsy with hippocampal sclerosis is associated with alterations in miRNA expression, particularly in the hippocampus. More than 20 miRNAs were differentially expressed in hippocampi and peripheral blood from both animal models and tissue resected specimens from patients with drug-resistant epilepsy (Korotkov et al., 2017). Several factors can influence the levels of miRNA expression in the blood such as motor phenomena, use of AEDs, and specific epileptic syndromes, among others, which makes identifying a specific biomarker for epilepsy challenging (Raoof et al., 2018). Thus, published data are often conflicting, and few studies specifically examine correlations between miRNA and mTLE-HS (Korotkov et al., 2017; Raoof et al., 2018). Here we published studies on 451 patients with epilepsy in comparison to healthy subjects to identify potential biomarkers for this disease (Wang et al., 2015a, b; Avanisi et al., 2017; Yan et al., 2017). We chose to analyze recent literature on the levels of miRNA expression in cerebrospinal fluid, blood, blood plasma exosomes, and brain tissue samples from patients that could serve as diagnostic biomarkers to distinguish patients from controls, monitor disease severity, and potential therapeutic targets.

MicroRNAs in Epilepsy

We performed a PubMed search for original research articles published from January 1 to August 30, 2021 on miRNA biomarkers compared to healthy controls in cerebrospinal fluid, whole blood, blood plasma, blood plasma exosomes, blood serum, and brain tissue samples. The studies involved in the review and its contents are shown (Figure 1). A total of 22 articles were found for this review, which we discuss in detail below.

Figure 1 | Flow diagram indicating how the review was performed and its contents.

**Temporal lobe epilepsy in adults**

Cerebrospinal fluid (CSF): Raoof et al. (2017) collected CSF samples from three different centers and performed both a discovery and validation phase analysis. The discovery phase involved 15 TLE, 15 SE, and 15 controls, and with the OpenArray platform the most abundant miRNAs were miR-19b-3p, miR-23b-3p, miR-30b-5p, miR-30c-5p, miR-150-5p, miR-204-5p, miR-223-3p, miR-320a and miR-483-5p. Overall, the most abundant and most consistently detected miRNAs in each of the three sets of samples were generally very similar. A number of significantly regulated miRNAs were detected which included 5 differentially expressed miRNAs in TLE compared to controls (1 downregulated and 4 upregulated) and 15 miRNAs in SE samples compared to controls (7 downregulated and 8 upregulated). The validation phase was performed with CSF samples from 14 TLE, 17 SE, and 25 controls. A fourth group of samples from 25 patients with other neurological diseases was included (the majority were patients with Alzheimer’s disease and multiple sclerosis). By RT-PCR assay, levels of miR-19b-3p were significantly lower in TLE samples compared to controls, SE, and patients with other neurological diseases, indicating that decreased CSF levels of miR-19b-3p may be a biomarker of TLE. Levels of miR-451a were higher in SE compared to controls, TLE, and other neurological disease samples, indicating increased CSF levels of miR-451a as a biomarker of SE. Expression of miR-21-5p was also significantly higher in SE samples compared to TLE and control samples, suggesting this may be a non-specific biomarker of brain metabolism due to seizure activity. Although miR-21-5p was at higher levels in SE compared to other neurological diseases, this was not significant. Levels of miR-886-3p were significantly higher during validation in TLE samples compared to samples from patients with other neurological diseases. The levels of miR-204-5p and miR-223-3p were not significantly different between the groups. While there was no influence of age on CSF levels of miR-19b-3p, miR-21-5p, or miR-451a, there was a stronger correlation for miR-886-3p indicating the dysregulation of miR-886-3p could be biased by age. Receiver operating characteristics curve analysis (ROC) showed that miR-451a was the best performing single miRNA with the area under the curve (AUC) 0.91 for distinguishing between TLE and SE samples. MiR-21-5p also showed good distinction between SE and control samples with AUC 0.83. Levels of miR-19b-3p showed good separation between TLE and each of the other two types of samples, in addition all three miRNAs gave AUC 0.83 for TLE compared to all other groups. When the comparison was made between SE and other groups, the combination miR-21-5p and miR-451a (AUC 0.85) was as accurate as all three miRNAs combined.

Whole blood: 30 TLE patients were recruited by Xiao et al. (2018) of which 14 were normal, and 9 had HS. MiR-10 had disease course > 10 years and 20 had disease course < 10 years, and 10 were drug-resistant and 20 were drug-responsive. A control group consisted of 30 subjects. Peripheral venous blood was collected into an EDTA tube and stored at −80°C prior to analysis. Total RNA extraction profiles were constructed primarily focusing on lncRNAs and miRNAs. Differentially methylated miRNAs in TLE included novel miRNAs (45%) and known miRNAs (55%). Several miRNA families and clusters were differentially methylated between TLE and controls including 3 miRNA families and 3 chromosomal clusters hypermethylated in TLE: one chromosomal cluster, miR-23b and miR-27b, from chromosome 9; let 7 family...
comprising of miRNAs from one chromosomal cluster, let-7a, let-7d, and let-7f, from chromosome 9; mir-515 family comprising of miRNAs from one chromosomal cluster, mir-517b, mir-518a, and mir-516a, from chromosome 19; and miR-653 from chromosome 10. TLE patients can be divided into different subgroups: drug resistant or drug responsive, MNI negative or hippocampal sclerosis, and disease course > 10 years or < 10 years. A set of miRNAs and IncRNAs was found to be differentially expressed in TLE patient groups compared to controls. The expression of miR-142 and miR-223 was significantly upregulated in drug resistant patients (p = 0.003) compared to controls. The difference was not significant. The relative expressions of miR-142 and miR-223 were significantly upregulated in serum samples collected 3 months prior to surgery that included an assessment of verbal episodic memory performance. The lack of significance was probably due to the reduced numerosity of the population. In the drug responsive group, there was a significant positive association between miR-142 and miR-132, age and age of onset, disease duration and left hippocampal volume, and right hippocampal volume and left hippocampal volume. The association was observed between left hippocampal volume and age of onset and disease duration. ROC analysis showed an AUC of 0.80 for miR-142, an AUC of 0.75 for miR-223, and an AUC of 0.80 for combined miR-142 and miR-223 to discriminative drug responsive vs. nonresponsive TLE patients.

Temporal lobe tissue: Busch et al. (2020) examined mRNA expression in left (language dominant) temporal lobe tissue resected from 23 drug resistant TLE patients for treatment of their seizures. Given the known association between APOE polymorphisms and memory performance (i.e., APOEe4 detrimental, APOEe2 protective [Small et al, 2004]), only patients who were homozygous for APOEe3 allele (wildtype) were included in the study. All patients underwent a comprehensive neuropsychological assessment 2 months prior to surgery that included an assessment of verbal episodic memory. Patients were then separated into two memory groups based on a
mean composite delayed memory score (combined delayed story recall and word-list learning tasks) (Banks et al., 2019; Jonaitis et al., 2019). Patients with mean scores < 85 were classified as having “impaired” memory (n = 10) and those with scores ≥ 85 were classified as having “intact” memory (n = 13). The two memory groups were well matched on demographic and disease-related variables. The impaired memory group demonstrated significantly delayed verbal memory scores than the intact memory group. Using ROC analysis followed by receiver operating characteristic analysis, 4 differentially expressed miRNAs were identified in brain tissues of patients with impaired memory compared to those from patients with intact memory. Both miR-1237-5p and miR-6805-5p were downregulated, whereas miR-3939 and miR-138-5p were upregulated in patients with impaired memory compared to those from patients with intact memory.

Sun et al. (2016) used microarray to analyze miRNAs in the temporal cortex collected from nine refractory TLE patients who underwent surgical removal of the lesion in the first phase of the study. Among the differentially expressed miRNAs, miR-148a-3p and miR-935 were significantly downregulated and miR-153 was significantly upregulated in temporal cortex tissue of TLE patients compared to temporal lobe tissue obtained from eight patients with hypertension who required emergency surgery to clear intracranial hematoma. In the second phase, qRT-PCR was used to validate the differentially expressed miRNAs in tissue samples from 13 refractory TLE patients and 16 controls (both groups included patients from the first phase). Consistent with the microarray results, miR-129-2-3p was significantly increased in tissue samples from the refractory TLE patients compared to controls. The expression of miR-935 in tissue samples of the refractory TLE patients was not significantly increased. By ROC analysis, the expression of miR-129-2-3p in temporal lobe tissue was a useful biomarker for differentiating TLE patients from controls with AUC 0.929.

Hippocampal granule cells: 14 drug resistant TLE patients, who were candidates to epilepsy surgery, were recruited by Zucchini et al. (2014). All patients underwent tailored temporal lobe resection to remove the epileptogenic area according to the findings during presurgical investigation. Surgery consisted of removing the temporal pole, the anterior neocortical lateral convolutions, the uncus-entorhinal area, and the hippocampus and parahippocampal gyrus. The main surgical specimens (hippocampus and/or temporal pole) were removed "en bloc". Specimens were spatially oriented, formalin fixed and paraffin embedded. They were dewaxed and stained with hematoxylin and eosin. Neuropathological evaluation was performed using the most recent classifications of HS, granule cell pathology (GCP), and focal cortical dysplasias (Blümcke et al., 2009, 2011, 2013), applying the recommended histochemical and immunohistochemical stains. Specimens were excluded if no GCP or no HS were found. In the brains from the 10 patients, four different neuroepithelial features could define GCP type 2. (1) Dispersion: rows of granule cells spread into the molecular layer and the distance between granule cells is increased; (2) ectopic granule cells: single or clusters of granule cells are dispersed into the molecular layer; (3) clusters: ectopic granule cell forms clusters within the molecular layer; (4) bilaminar: two granule cell layers, separated by a cell-free gap (Blümcke et al., 2009). While patterns of granule cell loss (thinning and/or cell free gaps, GCP 3) occur isolated, patterns of architectural abnormalities (GCP 2) can appear with cell loss. Therefore, only sections in which no cell loss was detected (based on neuronal nuclei staining) were included in the analysis. 10 µm thick sections were cut using a microtome and paraffin-embedded granule cell layer of the dentate gyrus was laser dissected. Microdissected cells were captured in microtub transfer film. Granule cells were collected from at least 3–4 slices per patient to obtain an adequate quantity of RNA. Materials from all sections of the same patient were used to perform RT-qPCR. The sections analyzed for which no cell loss was detected were on poly-drug therapy, and neuropathological examination showed that these patients had HS type 1 (Blümcke et al., 2013), which was associated with no granule cell pathology (no GCP) in five patients and with granule cell pathology (GCP type 2) in the other five. GCP consisted of granule cell dispersion in four cases and bilaminar granule cell layer in one case. By miRNA microarray, 12 miRNAs were differentially expressed in patients without GCP compared to patients with GCP type 2. Of these, six had relatively higher expression in tissue from patients without GCP and six were higher in those with GCP type 2. Differential expression of a subset of three miRNAs (miR-338-5p, miR-219-5p, and miR-487a) was validated in an extended cohort of 47 patients (n = 24 GCP type 2 and 23 controls). Using qRT-PCR, expression levels of all these miRNAs were different in the two groups, confirming microarray findings. The levels of miR-338-3p and miR-219-5p were decreased in GCP type 2 but did not reach significance. In contrast, miR-487a expression was significantly lower in GCP type 2. Using RT-qPCR, expression levels of ANTXR1 miRNAs were increased in the GCP type 2 group. An increase of ANTXR1 immuno-reactivity was observed in the granule cell layer of patients with GCP type 2, as compared with those with no GCP.

In children

Blood plasma: miRNA expression in blood plasma samples from 59 children diagnosed with mesial temporal lobe epilepsy (mTLE) (Li et al., 2020) using qRT-PCR. The expression of miR-194-1a-3p in blood plasma was significantly lower in children with TLE compared to the control group. By ROC analysis, using the expression level of miR-194-1a-3p in plasma gave AUC 0.671 with sensitivity 0.862 and specificity 0.65 at the cut-off value of 0.859, indicating the diagnostic value of miR-194-1a-3p for the clinical diagnosis of children with TLE.

Blood serum: Yu et al. (2021) examined miRNA expression in blood serum samples from 98 TLE children and 72 healthy, age and gender matched children. By qRT-PCR, the level of miR-148a-3p expression in serum was significantly increased in TLE children compared with healthy controls. TLE children were divided into two groups according to the median expression value of miR-148a-3p, giving a low miR-148a-3p expression group (n = 45) and a high miR-148a-3p expression group (n = 53). MiR-148a-3p expression was significantly correlated with febrile seizure (FS) history and seizure frequency (per month) in TLE children. No significant associations were shown between miRNAs expression and other clinical features, including age, gender, course of the disease, interictal EEG, unilateral temporal, bilateral temporal, and seizure duration(s). TLE children were divided into the FS-positive group (n = 38) and the FS-negative group (n = 60). qRT-PCR showed that the level of miR-148a-3p expression was significantly increased in the FS-positive group compared to non-FS children. By ROC analysis, using the level of miR-148-3p expression for distinguishing TLE from controls gave AUC 0.928, sensitivity 0.837, and specificity 0.917 at the cut-off value of 1.015, which indicated high diagnostic accuracy of miR-148a-3p. Furthermore, ROC analysis showed that the level of miR-148a-3p expression could distinguish TLE children with FS history from non-FS children giving AUC of 0.891, sensitivity of 0.842, and specificity of 0.883 at the cut-off value of 1.485.

Using qRT-PCR assay, Wu et al. (2021) found the level of miR-29a in blood serum to be significantly lower in 65 TLE children compared with 70 healthy, age and gender matched patients. According to the test results, 11 cases had developed mesial sclerosis. TLE children with significantly decreased serum miR-29a expression compared to the control group. Furthermore, the level of miR-15a-5p expression was compared in the clinical groups of TLE patients according to the results of EEG and MRI. There was no significance for miR-15a-5p expression in bilateral temporal lobe epilepsy patients (n = 108) compared with unilateral temporal lobe epilepsy patients (n = 47). Also, there was no significance for miR-15a-5p expression in mesial sclerosis patients (n = 11) compared to no abnormality (n = 52). These data indicated that miR-15a-5p expression did not vary in different subgroups of TLE patients. ROC analysis to determine the diagnostic value of serum miR-15a-5p for TLE gave AUC 0.908, sensitivity 0.825, and specificity 0.881 at the cut-off of 1.650, indicating that serum miR-15a-5p might be a sensitive biomarker to distinguish TLE patients from healthy controls.

Mesial temporal lobe epilepsy

In adults

Blood plasma: Using RT-qPCR, Gong et al. (2018) showed that blood plasma miR-153 expression in 22 patients with refractory mesial temporal lobe epilepsy (mTLE) was significantly lower compared to 20 controls, who had never been diagnosed with epilepsy or seizures and had undergone surgical treatment for head trauma or cerebral hemorrhage.

Avanzini et al. (2017) performed both a discovery and validation phase analysis to identify blood biomarkers other than miR-153. In the discovery phase, 31 out of 15,140 mTLE, 13 focal cortical dysplasias type II patients, and 16 healthy control subjects were included. Using qRT-PCR, only miR-134 was significantly downregulated in plasma of patients with mTLE compared to controls. No difference was found in plasma levels of miR-134 in patients with focal cortical dysplasias compared to controls. The expression levels of miR-23a and miR-31 were not different among the groups analyzed. In the validation phase, miR-134 was significantly downregulated in the plasma of 65 mTLE patients when compared to 83 healthy controls without epilepsy. miR-134 was downregulated both in drug responsive mTLE patients (n = 27) and drug resistant mTLE patients (n = 38) when compared to controls. No difference was observed in plasma miR-134 levels between drug responsive and drug resistant mTLE patients, miR-134 was not significantly different between mTLE patients with (n = 48) or without (n = 17) the presence of signs indicating HS on MRI. Seizure frequency was also found to have no statistically significant effect on plasma miR-134 levels using a score of up to 1 seizure per month. After adjusting for clinical phase, ROC analysis, in the discovery phase, plasma levels of miR-134 could discriminate patients with mTLE from controls with AUC of 0.75, sensitivity of 0.65 and specificity of 0.75. In the validation phase, the accuracy for identifying patients with mTLE was indicated by AUC 0.671 with a sensitivity of 0.75 and specificity of 0.58.

Blood plasma miRNA expression was examined in mTLE patients in both a discovery and validation phase by Li et al. (2016). In the discovery phase, miR-153 expression was significantly decreased in the plasma of 32 surgical patients with mTLE compared with 18 age-matched surgical controls. No significant differences in miR expression were observed between these surgical patients and controls. The mTLE patients had undergone anterior temporal lobectomy, while the controls had undergone surgical treatment for
The expression levels of miR-143-3p, miR-145-5p, miR-365a-3p, and miR-532-3p showed a significant increase of expression 30 minutes postictally compared to the baseline levels. Linear regression analysis of these miRNAs revealed a significant correlation between total seizure duration and relative changes in miRNA expression levels in two of the four miRNAs (miR-143-3p, 145-5p). For these two miRNAs, seizure duration was inversely correlated with the relative increase of the miRNA in blood. Examination of miRNA expression in a subgroup of mTLE patients with seizures occurring during sleep demonstrated that in four mTLE patients miRNA expression levels were clearly higher as compared to the remaining mTLE patients 30 minutes after the BCS. Further analysis of miRNA expression in these subgroup of patients revealed that miR-6781 was significantly deregulated in mTLE patients not only at the 30 minutes time-point post-seizure but also at the 3–6 hours and 20–28 hours time-points. At 3–6 hours following the BSC no significantly deregulated miRNAs were detected. However, the results demonstrated the presence of miR-4322 and miR-6781 expression levels at 30 minutes, 3–6 hours, 20–28 hours, and 3–6 days. Thirteen miRNAs were selected for qPCR validation in these four patients based on pre-seizure and post-seizure expression profiling. Ten of them were differentially expressed at 30 minutes, 3–6 hours, and 20–28 hours, another three at 30 minutes and 3–6 hours. One candidate, miRNA-663b, was significantly increased 3–6 hours after the BSC.

Temporal cortex tissue: Gong et al. (2018) investigated miRNA expression in temporal cortex tissue resected from 22 patients with refractory mTLE who had undergone anterior temporal lobectomy. Temporal neocortical tissues without abnormal pathological changes were obtained from 20 controls, who were validated by RT-qPCR in 56 mTLE patients with/without surgery compared to the remaining mTLE patients 30 minutes after the BCS. Further analysis of miRNA expression in these subgroup of patients revealed that miR-6781 was significantly deregulated in mTLE patients not only at the 30 minutes time-point post-seizure but also at the 3–6 hours and 20–28 hours time-points. At 3–6 hours following the BSC no significantly deregulated miRNAs were detected. However, the results demonstrated the presence of miR-4322 and miR-6781 expression levels at 30 minutes, 3–6 hours, 20–28 hours, and 3–6 days. Thirteen miRNAs were selected for qPCR validation in these four patients based on pre-seizure and post-seizure expression profiling. Ten of them were differentially expressed at 30 minutes, 3–6 hours, and 20–28 hours, another three at 30 minutes and 3–6 hours. One candidate, miRNA-663b, was significantly increased 3–6 hours after the BSC.

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potentially introduce a defect in brain development (Henschall, 2014; Rao and Pak, 2016). For this reason, patient data were categorized and analyzed in 4 categories based on their epilepsy onset age: (a) all patients together; (b) the first unprovoked seizure before the age of 10 (childhood); (c) between 10 and 19 years of age (adolescent); (d) at the age of 20 years or later (adult onset). Massive parallel sequencing data processing and differential expression analysis were performed. A search of the massive parallel sequencing datasets for differentially expressed miRNAs in patients with the TLE onset within the ages of 2 and 9 years discovered 123 miRNAs significantly dysregulated in patients. In the adolescent-onset category, 130 miRNAs were significantly dysregulated in TLE. The category with onset age above 20 contained 80 dysregulated miRNAs. 49 miRNAs were significantly dysregulated in all age categories. Since all patients underwent surgery in adulthood, the period between epilepsy onset and sample collection differed among childhood-onset (mean 33.2 years), adolescent-onset (mean 23 years), and adult-onset (mean 72 years) patients. The duration of epilepsy might have affected the expression of detected miRNA, and correlations were found between the expression of miR-142-3p, miR-135a-5p, and miR-484 (all upregulated) with the duration of epilepsy but these associations were low. qPCR assay validated the upregulation of miR-29a-5p and miR-142-3p across all categories in patients and let-7b-3p, miR-135a-5p, and miR-140-5p increased in patients with TLE onset in adolescence. In addition, qPCR confirmed increased expressions of miR-142-5p, miR-193a-5p, and miR-203a-3p in one or more expression sets in patients along with the group without onset categorization.

Hippocampal tissue samples from 20 patients who underwent amygdalo-hippocampectomy due to resistant mTLE-HS were analyzed for miRNA expression by António et al. (2019). Patients were evaluated and classified into two groups in terms of surgical prognosis: one group consisted of 10 patients with favorable outcome and the other consisted of 10 patients with unfavorable outcome (Engel III-IV). Hippocampal tissue samples from nine autopsied individuals were used as controls. The autopsied individuals had no neurological or psychiatric disorders, typically died within 15 hours after the episode. By microarray analysis, a comparison of the samples from the two groups of patients (satisfactory outcome vs. unsatisfactory outcome) revealed that some miRNAs were differentially expressed in the hippocampal samples, and these miRNAs were selected for further investigation: miR-145, miR-181c, miR-199a, and miR-1183. By qPCR analysis, only miR-145 expression was significantly different between the mTLE-HS and control groups and was decreasing in hippocampal tissue samples from autopsied patients. Comparing the groups (I vs. III-IV) revealed no significant differences in the expression levels of the four miRNAs.

Bencuova et al. (2017) performed miRNA analysis on hippocampal tissue samples from 19 patients with left-sided mTLE and 14 patients with right-sided mTLE. All patients were referred for their medical intractability and fulfilled the diagnostic criteria for mTLE-HS. The diagnosis was made according to the ILAE criteria. Visual inspections of the MRI scans revealed unilateral HS concurrent with the EEG lateralization of the epileptogenic zone in all patients. None of the patients revealed other brain structural lesions on MRI scans or had undergone previous intrasural brain surgery. Paraffin-embedded control hippocampal tissue samples were obtained from 10 postmortem cases without hippocampal aberrations. Surgically resected and autopsy tissues were macroscopically inspected, and they were fixed in neutral buffered formalin, grossly inspected, and carefully oriented, and their proportions measured. Hippocampal specimens were dissected into 2–3-mm-thick tissue slices along the anterior-posterior axis. Representative tissue samples were routinely processed and paraffin-embedded. Formalin-fixed paraffin-embedded tissue sections were stained with hematoxylin and eosin and evaluated using light microscopy. The presence of neuronal depletion and gliosis in HS tissue samples was confirmed using neuronal nuclei and glial fibrillary acidic protein immunohistochemistry. Total RNA was isolated from formalin-fixed paraffin-embedded hippocampal specimens from mTLE-HS patients. Comparisons of the Engel outcomes with the age at seizure onset were carried out in 16 mTLE-HS patients and 8 post-mortem controls. Samples were identically treated: they were fixed in 10% neutral buffered formalin, processed and paraffin embedded. Formalin-fixed paraffin-embedded hippocampal samples from mTLE-HS patients. Comparisons of the Engel outcomes with the age at seizure onset were carried out in 16 mTLE-HS patients and 8 post-mortem controls. Samples were identically treated: they were fixed in 10% neutral buffered formalin, processed and paraffin embedded. Formalin-fixed paraffin-embedded hippocampal samples from mTLE-HS patients. Comparisons of the Engel outcomes with the age at seizure onset were carried out in 16 mTLE-HS patients and 8 post-mortem controls. Samples were identically treated: they were fixed in 10% neutral buffered formalin, processed and paraffin embedded. Formalin-fixed paraffin-embedded hippocampal samples from mTLE-HS patients. Comparisons of the Engel outcomes with the age at seizure onset were carried out in 16 mTLE-HS patients and 8 post-mortem controls. Samples were identically treated: they were fixed in 10% neutral buffered formalin, processed and paraffin embedded. Formalin-fixed paraffin-embedded hippocampal samples from mTLE-HS patients. Comparisons of the Engel outcomes with the age at seizure onset were carried out in 16 mTLE-HS patients and 8 post-mortem controls. Samples were identically treated: they were fixed in 10% neutral buffered formalin, processed and paraffin embedded. Formalin-fixed paraffin-embedded hippocampal samples from mTLE-HS patients. Comparisons of the Engel outcomes with the age at seizure onset were carried out in 16 mTLE-HS patients and 8 post-mortem controls. Samples were identically treated: they were fixed in 10% neutral buffered formalin, processed and paraffin embedded. Formalin-fixed paraffin-embedded hippocampal samples from mTLE-HS patients. Comparisons of the Engel outcomes with the age at seizure onset were carried out in 16 mTLE-HS patients and 8 post-mortem controls. Samples were identically treated: they were fixed in 10% neutral buffered formalin, processed and paraffin embedded.
those for adult mTLE patients, with certain individual miRNAs having altered expression both in studies with adult TLE patients and those with adult mTLE patients. MiR-654-3p was upregulated in the blood plasma of TLE pre-seizure vs. control (Raoof et al., 2018) and upregulated in blood serum of TLE vs. control and TLE drug resistant vs. TLE drug responsive (De Benedittis et al., 2021), as well as being upregulated in hippocampal tissue of mTLE vs. control (Bencurova et al., 2017; Baloun et al., 2020). Similarly, miR-339-5p was upregulated in blood plasma in TLE pre-seizure vs. control (Raoof et al., 2018) and upregulated in hippocampal tissue of mTLE-HS vs. control (Bencurova et al., 2017). There was a possible inconsistency in the reported findings for blood plasma miR-134 which was upregulated in TLE severe vs. control and no difference for TLE moderate vs. TLE mild vs. control (Wang et al., 2017) but was downregulated in mTLE vs. control (Avansini et al., 2017). This would need to be further investigated.

| Study | Analysis method | Comparison, and number of subjects | Altered miRNA expression |
|-------|-----------------|------------------------------------|--------------------------|
| In adults | | | |
| CSF | Real-time PCR | TLE vs. control | Downregulated: miR-19b-3p |
| Raoof et al., 2017 | Real-time PCR | TLE vs. SE | Downregulated: miR-19b-3p |
| Raoof et al., 2017 | Real-time PCR | TLE vs. CND | Upregulated: miR-886-3p |
| Raoof et al., 2017 | Real-time PCR | SE vs. control | Downregulated: miR-19b-3p |
| Raoof et al., 2017 | Real-time PCR | SE vs. TLE | Upregulated: miR-451a, miR-21-5p |
| Raoof et al., 2017 | Real-time PCR | SE vs. CN | Upregulated: miR-451a |

| Blood plasma | | | |
| Brennan et al., 2020 | Real-time PCR | TLE pre-seizure vs. control | Upregulated: miR-93a-5p, miR-199a-3p |
| Brennan et al., 2020 | Real-time PCR | TLE post-seizure vs. control | Upregulated: miR-93a-5p, miR-199a-3p |
| Brennan et al., 2020 | Real-time PCR | TLE post-seizure vs. TLE pre-seizure | Upregulated: miR-142-5p |
| Raoof et al., 2018 | Real-time PCR | TLE post-seizure vs. control | Downregulated: miR-27a-3p, miR-328-3p |
| Raoof et al., 2018 | Real-time PCR | TLE pre-seizure vs. control | Upregulated: miR-199a-3p, miR-126-5p, miR-339-5p, miR-654-3p, miR-4433b-3p |
| Raoof et al., 2018 | Real-time PCR | TLE pre-seizure vs. TLE post-seizure | Upregulated: miR-199a-3p, miR-126-5p, miR-339-5p, miR-654-3p, miR-4433b-3p |
| Wang et al., 2017 | Real-time PCR | TLE severe vs. control | Upregulated: miR-93a-5p, miR-199a-3p |
| Wang et al., 2017 | Real-time PCR | TLE moderate vs. control | No difference in miR-134 |
| Wang et al., 2017 | Real-time PCR | TLE mild vs. control | No difference in miR-134 |
| Sun et al., 2016 | Real-time PCR | TLE vs. control | Upregulated: miR-129-2-3p |

| Blood serum | | | |
| De Benedittis et al., 2021 | Real-time PCR | TLE vs. control | Upregulated: miR-142, miR-146a, miR-223 |
| De Benedittis et al., 2021 | Real-time PCR | TLE drug resistant vs. TLE drug responsive | Upregulated: miR-142, miR-223 |

| Temporal lobe tissue | | | |
| Busch et al., 2020 | Small RNA seq + differential transcript analysis | TLE impaired memory vs. TLE intact memory | Upregulated: miR-3939, miR-6750-5p |
| Sun et al., 2016 | Real-time PCR | TLE vs. control | Downregulated: miR-1237-5p, miR-6805-5p |

| Hippocampal granule cells | | | |
| Zucchini et al., 2014 | Real-time PCR | GCP type 2 vs. without GCP | Downregulated: miR-487a |

| In children | | | |
| Blood plasma | | | |
| Niu et al., 2021 | Real-time PCR | TLE vs. control | Downregulated: miR-194-5p |

| Blood serum | | | |
| Yu et al., 2021 | Real-time PCR | TLE vs. control | Upregulated: miR-148a-3p |
| Yu et al., 2021 | Real-time PCR | TLE FS-positive vs. TLE FS-negative | Upregulated: miR-148a-3p |
| Wu et al., 2021 | Real-time PCR | TLE vs. control | Downregulated: miR-29a |
| Li et al., 2020 | Real-time PCR | TLE vs. control | Downregulated: miR-15a-5p |

CND: Control/other neurological diseases; CSF: cerebrospinal fluid; FS: febrile seizure; GCP: granule cell pathology; PCR: polymerase chain reaction; SE: status epilepticus; TLE: temporal lobe epilepsy. All the miRNAs listed are human miRNAs (hsa-miRs).
Table 1: Alterations of miRNA expression in blood plasma, blood plasma exosomes, blood serum, and brain tissues in adults with mTLE

| Study | Analysis method | Comparison, and number of subjects | Altered miRNA expression |
|-------|-----------------|------------------------------------|-------------------------|
| **Blood plasma** | | | |
| Gong et al., 2018 | Real-time PCR | mTLE vs. control | Downregulated: miR-153 |
| | | 22 vs. 20 | Downregulated: miR-153 |
| Avansini et al., 2017 | Real-time PCR | mTLE vs. control | Downregulated: miR-153 |
| | | 65 vs. 83 | Downregulated: miR-153 |
| Avansini et al., 2017 | Real-time PCR | mTLE vs. control | Downregulated: miR-153 |
| | | 27 vs. 83 | Downregulated: miR-153 |
| Avansini et al., 2017 | Real-time PCR | mTLE vs. control | Downregulated: miR-153 |
| | | 38 vs. 83 | Downregulated: miR-153 |
| Li et al., 2016 | Real-time PCR | mTLE vs. control | Downregulated: miR-153 |
| | | 56 vs. 101 | Downregulated: miR-153 |
| **Blood plasma exosomes** | | | |
| Yan et al., 2017 | Real-time PCR | mTLE-HS vs. control | Upregulated: miR-3613-5p |
| | | 40 vs. 40 | Downregulated: miR-4668-5p, miR-4322, miR-8071, miR-6781-5p, miR-197-5p |
| **Blood serum** | | | |
| Ioriatti et al., 2020 | Real-time PCR | mTLE-HS Engel I vs. control | Upregulated: miR-328-3p, miR-654-3p |
| | | 14 vs. 11 | Upregulated: miR-328-3p |
| Ioriatti et al., 2020 | Real-time PCR | mTLE-HS Engel III–IV vs. control | Upregulated: miR-328-3p |
| | | 14 vs. 11 | Upregulated: miR-328-3p |
| Ioriatti et al., 2020 | Real-time PCR | mTLE-HS Engel I + Engel III–IV vs. control | Upregulated: miR-328-3p |
| | | 28 vs. 11 | Upregulated: miR-328-3p |
| Surges et al., 2016 | PCR | mTLE-HS post-seizure 30 min vs. mTLE-HS pre-seizure | Upregulated: miR-143-3p, miR-145-3p, miR-365a-3p, miR-532-5p |
| | | 14 vs. 14 | Downregulated: miR-153 |
| **Temporal cortex tissue** | | | |
| Gong et al., 2018 | Real-time PCR | mTLE vs. control | Downregulated: miR-153 |
| | | 22 vs. 20 | Downregulated: miR-153 |
| Li et al., 2016 | Real-time PCR | mTLE vs. control | Downregulated: miR-153 |
| | | 32 vs. 18 | Downregulated: miR-153 |
| **Hippocampal tissue** | | | |
| Baloun et al., 2020 | PCR | mTLE vs. control | Upregulated: miR-129-2-3p, miR-142-3p, miR-142-5p, miR-193a-3p, miR-203a-3p, miR-484, miR-539-5p |
| | | 16 vs. 8 | Downregulated: miR-145 |
| Antônio et al., 2019 | Real-time PCR | mTLE vs. control | Upregulated: miR-1260a, miR-1260b, miR-1275, miR-129-1-3p, miR-129-2-3p, miR-142-3p, miR-142-5p, miR-144-5p, miR-150-5p, miR-191-5p, miR-193b-3p, miR-339-5p, miR-374b-5p, miR-443, miR-4454, miR-451a, miR-4792, miR-6087, miR-874-3p |
| | | 20 vs. 9 | Downregulated: miR-129-5p |
| Bencurova et al., 2017 | PCR | mTLE-HS vs. control | Upregulated: miR-129-2-3p, miR-142-3p, miR-142-5p, miR-193a-3p, miR-203a-3p, miR-484, miR-539-5p |
| | | 50 vs. 10 | Downregulated: miR-129-5p |

mTLE: Mesial temporal lobe epilepsy; mTLE-HS: mesial temporal lobe epilepsy with hippocampal sclerosis; PCR: polymerase chain reaction. Engel I and Engel III–IV refer to favorable and unfavorable outcome after surgery for epilepsy, respectively. All the miRNAs listed are human miRNAs (hsa-miRs).

With regard to possible non-invasive biomarkers of TLE/mTLE in adults, potential candidates are miR-129-2-3p, miR-142-5p, miR-145-3p, miR-153, miR-199a-3p, miR-339-5p, and miR-654-3p. Changes were also found for some of these miRNAs in the temporal lobe and hippocampal tissue, increasing the importance that they might have in epilepsy. Upregulation of miR-129-2-3p occurred in temporal lobe tissue in adults with TLE vs. control and in hippocampal tissue of adults with mTLE vs. control. Upregulation of miR-142-5p and miR-339-5p and downregulation of miR-145-3p were found in hippocampal tissue of adults with mTLE vs. control. Moreover, downregulation of miR-153 occurred in temporal cortex tissue of mTLE vs. control.

Many of the TLE patients in the articles reviewed had been receiving mono or poly drug therapy. In one study, patients did not receive AEDs for 2 weeks prior to miRNA profiling (Wang et al., 2017). In the patients with new-onset severe epilepsy, the blood plasma expression level of miR-134 was significantly higher than in healthy controls, and administration of valproate acid was found to decrease the plasma level of miR-134 in these patients so that it was no longer significantly greater than controls (Wang et al., 2017).

The limited overlap in findings of the reviewed studies could be due to several factors. The mean age of the adult TLE patients varied from 28.5 to 55.8 years and for adult mTLE patients from 24.9 to 43.1 years. The mean duration of epilepsy for adult TLE varied from new onset to 36.8 years and for adult mTLE patients from 11.5 to 27.4 years. Also as mentioned above, the use AEDs by adult TLE and mTLE patients varied. Where reported, the proportions of drug resistant and drug responsive TLE patients varied between different studies, e.g. in the study by Avansini et al. (2017), there were 27 drug sensitive and 38 drug resistant mTLE patients, while that of De Benedittis et al. (2021) comprised 17 drug sensitive and 10 drug resistant TLE patients. The levels of miRNA expression need to be measured separately in drug resistant and drug responsive patients as was done by Avansini et al. (2017) and De Benedittis et al. (2021). These are all important factors and together with other variables such as comorbid disease and the nature of the precipitating injury could influence the levels of miRNA expression (Tables 3 and 4). Furthermore, in some studies, there was a significant difference in mean age between mTLE patients and control subjects (e.g. Bencurova et al., 2017).

In a recent study involving a median 5-year follow-up period (range 2–13 years), it was reported that 26/112 (23.2%) TLE patients achieved seizure freedom after treatment with AEDs alone, while 86/112 (76.8%) were diagnosed as drug resistant (Yang et al., 2020). Possible treatment of TLE patients, especially those that are drug resistant, could be by the use of miRNA agonist (mimics) or antagonists (Inhibitors). Hippocampal cells treated in magnesium-free medium were used to examine the effects of overexpression of specific miRNAs on cell viability and the influence on cell apoptosis induced by TLE (Li et al., 2020). In addition, the miRNA profile in rats with adult-onset TLE closely resembled the profile in mTLE-HS patients (Baloun et al., 2020). The rat model of TLE involved inducing SE in male Wistar albino rats aged P12 (postnatal day 12), P25, or P60 by intraperitoneal injection with 127 mg/kg LiCl 24 hours prior to pilocarpine/saline injection (Kubová et al., 2004). Video-EEG monitoring 3 months after SE demonstrated that 25% of rats in the P12 group and 50% in the P25 group developed spontaneous seizures (Kubová et al., 2004). The animals developing SE could subsequently be treated with specific miRNA agonists and antagonists and the effects on epileptic behavior examined. SE has also been induced in male C57BL6/J mice (4–6 weeks of age) by intraperitoneal injection of 30 mg/kg kainic acid (Zhu et al., 2019). SE was defined as continuous tonic-clonic seizures following several discontinuous convulsive seizures. The seizure intensity was assessed on the Racine scale: Stage 1, mouth and facial movements; Stage 2, head nodding; Stage 3, forelimb clonus; Stage 4, seizures characterized by rearing and falling (Racine, 1972). The onset of SE was defined when stage 5 seizures continued for at least 5 minutes (Chen and Wasterlain, 2006; Trinka and Kälviäinen, 2017; Jain et al., 2019). Intracerebral delivery of miR-23a agomir or antagonir was performed in kainic acid-induced TLE mice and effects on spatial learning and memory were assessed (Zhu et al., 2019). Interestingly, a non-human primate model of TLE was described...
Table 3 | Ages, duration of epilepsy, and use of AEDs in TLE patients in studies of miRNAs in cerebrospinal fluid, whole blood, blood plasma, blood serum, and brain tissue samples

| Study                      | Ages of TLE patients (yr) | Duration of epilepsy (yr) | Number of AEDs used by patients |
|----------------------------|---------------------------|---------------------------|---------------------------------|
| **In adults**              |                           |                           |                                 |
| Cerebrospinal fluid        |                           |                           |                                 |
| Raiff et al., 2017         | 40.0±14.3                 | NR                        | NR                              |
| Whole blood                |                           |                           |                                 |
| Xiao et al., 2018          | 28.5±12.8                 | > 10 yr, 10               | NR                              |
| Blood plasma               |                           |                           |                                 |
| Brennan et al., 2020       | 39.4±11.3                 | 16.2±15.5                 | All patients on polydrug therapy|
| Raiff et al., 2018         | 44.2±11.9                 | NR                        | No medication at 2 wk before blood sampling |
| Wang et al., 2017          | 29.8±8.9                  | New-onset patients        | Some patients used more than 2 AEDs |
| Raiof et al., 2017         | NR                       | NR                        |                                 |
| Sun et al., 2016           | 55.7±9.1                  | 35.0±13.2                 | Some patients used more than 2 AEDs |
| **Blood serum**            |                           |                           |                                 |
| De Benedetis et al., 2021  | 43.7±17.1                 | 18.2±17.9                 | NR                              |
| **Temporal lobe tissue**   |                           |                           |                                 |
| Busch et al., 2020         | 44.3                      | 14.7                      | Almost all were taking at least 2 AEDs |
| Sun et al., 2016           | 55.8±9.1                  | 36.8±11.1                 | Some patients used more than 2 AEDs |
| **Hippocampal granule cells** |                         |                           |                                 |
| Zucchi et al., 2014        | 39.1±9.2                  | 25.9±12.2                 | Almost all were taking at least 2 AEDs |
| **In children**            |                           |                           |                                 |
| Blood plasma               |                           |                           |                                 |
| Niu et al., 2021           | 9.3±3.3                   | NR                        | NR                              |
| Blood serum                |                           |                           |                                 |
| Yu et al., 2021            | 9.6±3.2                   | 6.7±2.7                   | NR                              |
| Wu et al., 2021            | 10.0±2.6                  | NR                        |                                 |
| Li et al., 2020            | 9.8±2.8                   | NR                        |                                 |

Mean ± SD (or SE in some cases). AED: Anti-epileptic drug; NR: not reported; TLE: temporal lobe epilepsy.

Table 4 | Ages, duration of epilepsy, and use of AEDs in adults with mTLE in studies of miRNAs in blood plasma, blood plasma exosomes, blood serum, and brain tissue samples

| Study                      | Ages of mTLE patients (yr) | Duration of epilepsy (yr) | Number of AEDs used by patients |
|----------------------------|---------------------------|---------------------------|---------------------------------|
| Blood plasma               |                           |                           |                                 |
| Gong et al., 2018          | NR                       | NR                        | NR                              |
| Avansini et al., 2017      | NR                       | NR                        | At least 2 AEDs                 |
| Li et al., 2016            | 24.9±11.7                 | 11.5±5.2                  | All were taking 2 or more AEDs  |
| Blood plasma exosomes      |                           |                           |                                 |
| Yan et al., 2017           | 27.3                      | 13.7                      | At least 2 AEDs                 |
| Blood serum                |                           |                           |                                 |
| Iorio et al., 2020         | 33±6.8, 14                | NR                        | NR                              |
| Surges et al., 2016        | 37±7.0, 14                | NR                        | At least 2 AEDs                 |
| **Temporal cortex tissue**  |                           |                           |                                 |
| Gong et al., 2018          | NR                       | NR                        | NR                              |
| Li et al., 2016            | 24.9±11.7                 | 11.5±5.2                  | All were taking 2 or more AEDs  |
| **Hippocampal tissue**     |                           |                           |                                 |
| Baloum et al., 2020        | 40.2                      | 23.3                      | All were taking 2 or more AEDs  |
| António et al., 2019       | 35±29.0, 10               | NR                        | NR                              |
| Bencucov et al., 2017      | 38±28.9, 10               | NR                        |                                 |

Mean ± SD (or SE in some cases). AED: Anti-epileptic drug; mTLE: mesial temporal lobe epilepsy; NR: not reported.

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In conclusion, a large number of miRNAs were found to be dysregulated in CSF, blood plasma, blood serum, and brain tissue samples collected from patients diagnosed with TLE and mTLE, and with some overlap in findings for TLE and mTLE patients. However, the main difficulty with interpreting the study findings is determining to what extent the taking of AEDs by the TLE and mTLE patients had influenced the levels of miRNA expression in the body fluids and brain tissues examined. Further studies using the rat and mouse models of TLE could provide useful information, especially when performed with immature (childhood) and adult animals. Most of the articles reviewed were performed with samples collected from adult patients, and with relatively few from children. This needs to be addressed in future studies.

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