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# Differential expression of *miR-34a, 451, 1260, 1275* and *1298* in the neocortex of patients with mesial temporal lobe epilepsy

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A B S T R A C T

Mesial temporal lobe epilepsy (mTLE) is the most common epilepsy syndrome which will eventually become pharmacologically intractable partial-onset seizures. Regulation of gene expression is an important process in the development of this pathology where microRNAs (miRs) are involved. The role of miRs has been widely studied in the hippocampus of rodents and patients. However, little is known about its differential expression in other brain regions such as the neocortex. The temporal neocortex plays a major role in the generation and propagation of seizures and in synaptic disruption, impairing the excitatory and inhibitory balance. Therefore, we assessed the expression of *miR-146a, 34a, 1260, 1275, 1298, 451, 132* and *142-3p* in the neocortex of 12 patients with mTLE and compared them with miRs expression found in 10 control samples. We noted a significant decrease in the expression of *miR-34a* and *1298* in patients with mTLE and a -1.49 to -7.0 fold change respectively compared with controls. Conversely, we observed a significant increase in the expression of *miR-451, 1260* and *1275* in patients with a 25.67, 4.09 and a 7.07 fold change respectively compared to controls. Using Pearson correlation, we explored the association between the clinical features of mTLE patients and controls with miRs expression. In the control group we found a significant correlation only with age and *miR-146a* expression ($r = 0.733$). The analysis of mTLE patients showed a negative correlation between expression of *miR-1260* ($r = -0.666$) and *miR-1298* ($r = -0.651$) and age. Furthermore, we found a positive correlation between *miR-146a* expression with seizure frequency ($r = 0.803$) and a positive correlation between *miR-146a* and *451* expression with number of antiepileptic drugs used for presurgical treatment ($r = 0.715$ and $0.611$ respectively), thus suggesting a positive correlation with disease severity. These miRs are associated with biological processes such as apoptosis, drug resistance, inflammation, inhibitory and excitatory synaptic transmission, axonal guidance and signaling of neurotrophins. Therefore, deepening our understanding of the targets involved in these miRs will help to elucidate the role of the neocortex in epilepsy.

## 1. Introduction

Epilepsy is a brain disorder that affects 1- 2% of the population worldwide and is characterized by spontaneous and recurrent epileptic seizures (Escalaya et al., 2015). Mesial temporal lobe epilepsy (mTLE) is the most frequent type of epilepsy, it has a typical ictal clinical semiology and the focus may be located not only in the hippocampus but also in extrahippocampal structures (Haneef et al., 2014; Téllez-Zenteno and Hernández-Ronquillo, 2011). mTLE is the most common type of drug-resistant epilepsy which frequently displays favorable

responses to surgery, and when accompanied by hippocampal sclerosis, 70-80% of patients remain seizure-free after surgery (Asadi-Pooya et al., 2016; Pohlen et al., 2017). Cerebral cortex malformations are also responsible for approximately 25 - 40% of drug-resistant epilepsies (Alonso-Nanclares et al., 2005; Dogini et al., 2013). Most temporal lobe seizures originate in mesial structures, mainly in the hippocampus, while the remaining originate in temporal neocortical regions (Bercovici et al., 2012). The temporal neocortex plays a significant role in the generation and propagation of seizures (Chagnac-Amitai and Connors, 1989; Chervin et al., 1988). The temporal neocortex of

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**Table 1**
Description of the patient's clinical features (n = 12).

| Patient | Gender | Age (years) | Seizure onset age (years) | Epilepsy duration (years) | Seizures/month | AEDs | Diagnostic | RIN |
|---|---|---|---|---|---|---|---|---|
| 500 | F | 21 | 3 | 18 | 4 | (3) CLB, LVT, LCM | mTLE L | 7.1 |
| 501 | M | 53 | 6 | 47 | 130 | (4) AVP, CBZ, CNZ [a] | mTLE L | 8.3 |
| 510 | F | 29 | 12 | 17 | 15 | (2) CBZ, LVT | mTLE R | 7.6 |
| 520 | M | 37 | 2 | 35 | 30 | (3) CBZ, LMG, TPM | mTLE + CD R | 8.2 |
| 522 | M | 39 | 31 | 8 | 4 | (3) LMG, CNZ, LVT | mTLE R | 7.0 |
| 523 | F | 57 | 12 | 45 | 5 | (3) CBZ, CNZ, LVT | mTLE R | 7.3 |
| 534 | F | 29 | 6 | 23 | 8 | (3) CNZ, LVT [a] | mTLE L | 8.0 |
| 537 | F | 29 | 4 | 25 | 5 | (2) AVP, LMG | mTLE + CD L | 7.9 |
| 539 | M | 38 | 34 | 4 | 3 | (3) AVP, CBZ, LCM | mTLE L | 7.0 |
| 541 | F | 13 | 9 | 4 | 76 | (4) OXCBZ, LMG, CLB, LCM | mTLE + CD R | 7.4 |
| 547 | M | 41 | 6 | 35 | 15 | (3) TPM [a] | mTLE L | 7.0 |
| 551 | F | 25 | 18 | 7 | 99 | (3) [a] | mTLE L | 8.0 |

Abbreviations: FFemale, MMale, mTLEMesial Temporal Lobe Epilepsy, CDCortical dysplasia, RRight, LLeft, RINRNA Integrity Number.

[a] The clinical history was incomplete for these patients.

patients with mTLE presents synaptic disruption affecting it's excitatory and inhibitory balance (DeFelipe et al., 1998; Marco and DeFelipe, 1997). This disturbance is associated with changes in GABAergic neurotransmission in the neocortex of patients (Avoli et al., 1995; Teichgräber et al., 2009) and may be explained by the impaired inhibitory control of the GABAergic system over pyramidal cells (DeFelipe, 1999). Additionally the neocortex surrounding the epileptic focus of patients with mTLE shows a decrease in 5-HT1A receptor binding in layers I-II associated with high excitability and seizure activity (Rocha et al., 2007). Moreover cortical thinning is a feature that has been observed in patients with mTLE spanning diverse cortical structures such as the mesiotemporal, limbic, and central sensorimotor cortices (Alhusaini et al., 2019).

It is thought that neuronal death, inflammation, changes in the function of ionic channels, neurogenesis and gliosis are involved in the epileptogenic process (McNamara et al., 2006). Experimental and human epilepsy are associated with aberrant production protein involving a prominent transcriptional suppression process (Gorter et al., 2006). MiRs are considered important regulators of protein synthesis during and after epileptic seizures (Alsharafi et al., 2015). They are a specific type of small RNAs that negatively regulate genic expression by binding the 3´UTR region of specific mRNA sequences and prevent their translation (Brennan and Henshall, 2018). MiRs are involved in several biological processes such as proliferation, differentiation, metabolism, apoptosis and development (Behm-Ansmant et al., 2006). MiR expression in human samples has been assessed using several tools such as TaqMan and low-density array influencing the sensitivity and specificity to detect them (McKiernan 2012) resulting in a diversity of expression profiles. For example, the differential expression of over 1000 miRs has been reported in the hippocampus and other brain regions in humans and experimental epilepsy (Mooney et al., 2016). Also it has been demonstrated that miR expression patterns can vary depending on their location within different brain regions (Pichardo-Casas et al., 2012). In this work we focus on the expression analysis in the neocortex of four miRs widely investigated in epilepsy (*miR-132, 146a. 34a* and *142-3p*) and another four lesser studied ones, whose participation in this pathology was demonstrated recently (*miR-451, 1260, 1275* and *1298*). *MiR-132* was the first to be linked with seizure activity when it was observed that its expression increased in the hippocampus of mice after status epilepticus induction by pilocarpine (Jimenez-Mateos and Henshall, 2013). The expression of *miR-146a* also increases due to an inflammatory response in animal models of mTLE and patients (Aronica et al., 2010). The expression of *miR-34a* increases in the hippocampus of rodents after the induction of status epilepticus preceding epilepsy, and induces apoptosis since this miR negatively regulates the expression of BCL-2 (Hu et al., 2012). Furthermore, *miR-142-3p* downregulation was identified in rat brain after status epilepticus induction (Wang et al., 2016). In a more recent study, the differential expression

of some miRs, including *miR-1260, 1275, 1298* and *451* was identified in the hippocampus of patients with intractable mTLE and hippocampal sclerosis. The same work suggested that these miRs are involved in processes such as neurotrophin signaling, axonal guidance, regulation of potassium channel expression and GABA action (Bencurova et al., 2017). The analysis of these miRs focused on the hippocampus, an area frequently affected in pharmacoresistant mTLE. However due to the temporal neocortex participation in the generation and propagation of seizures and synaptic disruption we hypothesize that the expression pattern of these miRs in the neocortex can be different to that found in the hippocampus. In accordance with the aforementioned, the aim of this work was to assess the differential expression of *miR-146a, 34a, 1260, 1275, 1298, 451, 132* and *142-3p* in the temporal neocortex of mTLE patients compared to that of control subjects. We also explored its correlation with patients´ clinical features including age, gender, epilepsy duration, age at onset of epileptic seizures, seizure frequency and number of antiepileptic drugs used in the presurgery treatment.

## 2. Methods

### 2.1. Patients´ and controls´ characteristics and sample tissues

The mTLE group included 12 tissue specimens of 7 female and 5 male patients with an age range of 13-57 years, age of seizure onset was 2-34 years, duration of epilepsy 4-47 years, seizure frequency 3-130 monthly seizures and the presurgery pharmacological treatment included 2-4 antiepileptic drugs. All the experimental group had a confirmed diagnosis of mTLE, additionally 25 % presented cortical dysplasia. All mTLE patients underwent a comprehensive presurgical evaluation including video electroencephalogram (EEG), magnetic resonance imaging (MRI) and single photon emission computed tomography (SPECT) at the epilepsy surgery program, Instituto Nacional de Neurología y Neurocirugía "Manuel Velasco Suárez" (INNN). Scalp EEG showed lateralizing and focalizing interictal epileptiform activity. Video-EEG displayed at least two complex partial seizures and interictal SPECT offered information regarding the hypoperfusion area. Through MRI using a 1.5- or 3-T machine we identified mesial sclerosis (see Table1). This study was approved by the scientific committees of the institutions involved in the present research and informed consent was obtained from each patient in the experimental group. Temporal neocortex tissue was collected immediately after its resection, quickly frozen in pulverized dry ice, transported in RNA later (76104, QIAGEN) and stored at −70 ℃. The results were compared with those of 10 control tissues obtained from subjects without brain disorders and a maximum 20 hours of postmortem interval according to the guidelines of Mexico City's Medical Forensic Unit (Servicio Medico Forense, SE-MEFO, for its acronym in Spanish). The age range for control subjects was 12-73 years and their clinical characteristics are described in

**Table 2**
Description of control subject's clinical characteristics (n = 10).

| Autopsies | Gender | Age (years) | Postmortem delay (h) | Cause of death | RIN |
|-----------|--------|-------------|----------------------|----------------|-----|
| A5 | F | 73 | 13 | Pneumonia | 7.0 |
| A7 | M | 45 | 18 | Injury | 8.5 |
| A11 | F | 12 | 14 | Injury | 7.3 |
| A12 | F | 40 | 20 | Injury | 7.0 |
| A13 | F | 33 | 17 | Injury | 7.0 |
| A17 | M | 25 | 18 | Injury | 7.0 |
| A18 | M | 55 | 15 | Thoracic trauma | 8.3 |
| A21 | M | 16 | 18 | Injury | 7.4 |
| A29 | M | 41 | 9 | Generalized visceral congestion | 7.0 |
| A30 | F | 40 | 7 | Bilateral pneumonia | 7.0 |

Abbreviations: C: Control, F: Female, M: Male, RIN: RNA Integrity Number.

Table 2. All control tissues were identically treated as that of patients. All procedures were performed following the Declaration of Helsinki, Ethical Principles for Medical Research Involving Human Subjects.

## 2.2. RNA isolation

Neocortex tissue (100 mg) was homogenized in 1 mL of Trizol®RT (15596-018, Sigma-Aldrich) and total RNA was extracted according to the manufacturer's instructions. RNA quality was determined by the RNA integrity number (RIN) measured on an Agilent 2100 (Agilent Technologies, Waldbronn Germany) using an Agilent RNA 6000 Nano Kit (5067-1512, Agilent Technologies). All RNA samples used RIN ≥ 7.0.

## 2.3. Quantitative reverse-transcriptase polymerase-chain-reaction (qRT-PCR)

RNA samples were used for reverse transcription (RT) reaction, 20 ng of total RNA were added to 15 μl of RT master mix using the TaqMan® MicroRNA Reverse Transcription Kit (4366597, Applied Biosystems). The RT reaction was performed under the following conditions: (1) 16 °C during 30 min; (2) 42 °C during 30 min; (3) 85 °C during 5 min. For each RT reaction, a blank control was prepared using all reagents, and RNA was replaced with an equivalent water volume. The qPCR reaction was carried out using the TaqMan™ Universal Master Mix II with UNG (4440038, Applied Biosystems). Reactions were incubated at (1) 50 °C during 2 min and 95 °C during 10 min, followed by (2) 40 cycles of 95 °C during 15 s and 60 °C during 60 s, and (3) melting curve analysis from 55 to 99 °C. The universal U48 primers used a housekeeping gene for relative expression analysis of *miR-146a, 34a, 1260, 1275, 1298, 451, 132* and *142-3p*. Relative expression analysis was performed using the Pfaffl method since the calculated miR efficiencies were different (Pfaffl, 2001).

## 2.4. Statistical analysis

The relative expression of miR was shown as mean ± SEM (Standard Error of Mean) and a Mann Whitney test was performed to determine significant differences between groups. Unpaired t test was performed for Ct comparisons. The selection of Mann Whitney or Unpaired t test was determined depending on the data normality which was evaluated with the Kolmogorov-Smirnov normality test. Pearson correlation analysis was performed between the relative expression of miR of patients and the control group according to their clinical characteristics (age at onset of epileptic seizures, epilepsy duration, seizure frequency, number of antiepileptic drugs for the presurgery treatment,
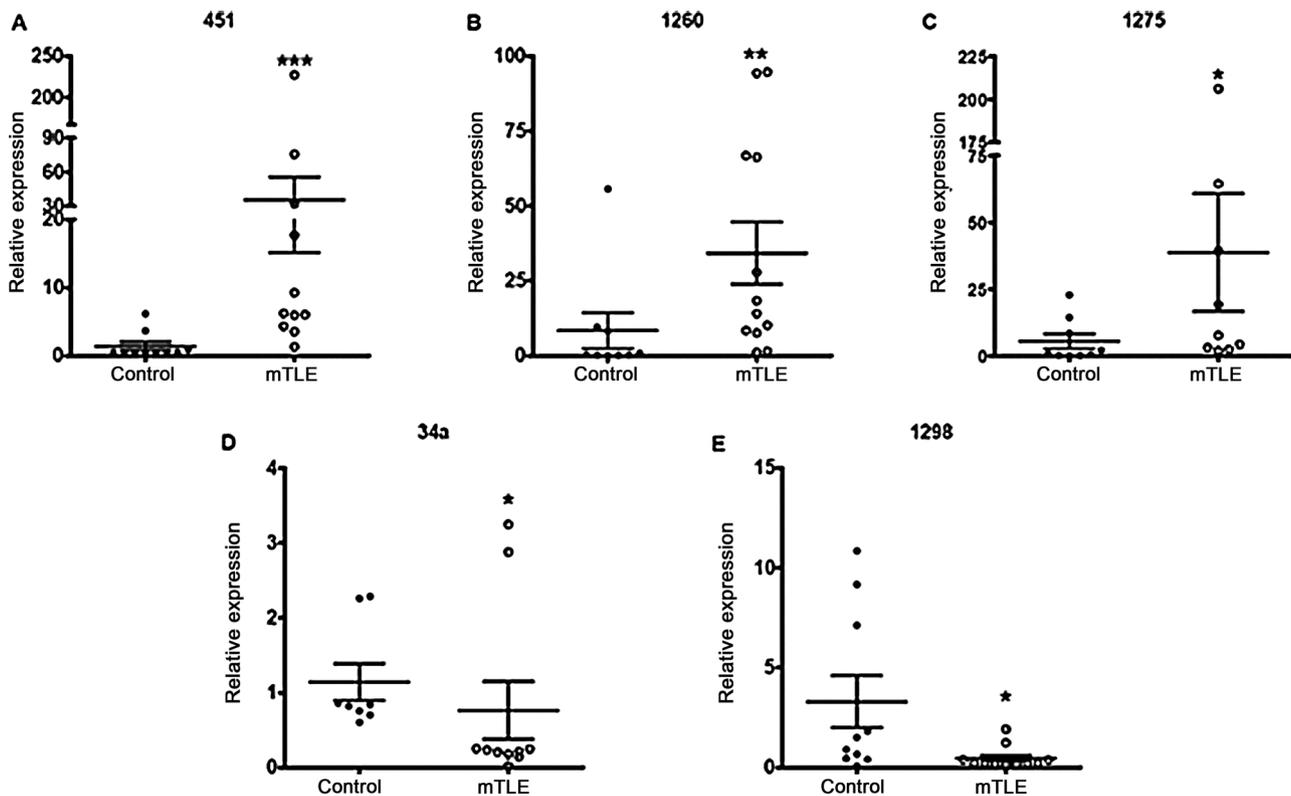


**Fig. 1.** Expression profiling of miR in the neocortex of patients with mTLE. A-C) Relative expression shows an increase of *miR-451* (nC = 10, nP = 11), *miR-1260* (nC = 9, nP = 12) and *miR-1275* (nC = 9, nP = 9) in the neocortex of patients compared to control tissues. D, E) Relative expression shows a decrease of *miR-34a* (nC = 8, nP = 10) and *miR-1298* (nC = 10, nP = 12) in the neocortex of patients compared to control tissues. Mann Whitney test *p = 0.0343 (34a), *p = 0.0161 (1298), **p = 0.0008 (451), **p = 0.0062 (1260), *p = 0.04 (1275) vs control. nC: n control, nP: n patients.

age and gender). Alpha levels were set at p < 0.05. All statistical analyses were performed using GraphPad Prism software.

## 3. Results

### 3.1. Control group clinical characteristics and miR expression

The control group included 10 subjects, 5 female and 5 males, with an age range of 12 to 73 years. The postmortem interval was between 7 h and 20 h and the cause of death include: 70% injuries, 20% pneumonia and 10% generalized congestion. In this group, miR expression levels were 1.14 ± 0.25, 3.29 ± 1.295, 1.38 ± 0.6175, 8.38 ± 6.042, 2.03 ± 1.021, 1.677 ± 0.6101, 2.765 ± 0.8831 and 5.470 ± 2.720 for *miR-34a*, 1298, 451, 1260, 146a, 132, 142-3p, 1275 respectively (see Fig. 1, supplementary Fig. 1 and Supplementary Table 1). Using Pearson correlation, we evaluated the influence of postmortem delay in miR expression and no correlation was found in any miR expression profile, thus indicating that the time of collection of tissues did not affect RNA integrity, being comparable with mTLE tissues since RIN in both groups were similar (Table 1–3). Age showed a positive correlation (r = 0.733, p = 0.025) with *miR-146a* expression (see Fig. 2C, Table 3).

### 3.2. MiR expression in the neocortex of mTLE patients

In mTLE patients, five of the eight miRs assessed, namely *miR-34a, 1298, 451, 1260* and *1275*, showed a significant differential expression compared with autopsies. *MiR-34a* expression was 0.7635 ± 0.3848 (p = 0.0343) showing a decrease of -1.49 fold change compared with that of controls. *MiR-1298* expression was 0.470 ± 0.1582 (p = 0.0161) also demonstrating a decrease of -7.0 fold change. On the other hand, *miR-451* expression in patients was 35.36 ± 20.24 (p = 0.0008) showing an increase of 25.62 fold change compared with controls. *MiR-1260* and *1275* expression exhibited a similar pattern with a mean of 34.25 ± 10.36 (p = 0.0062) and 38.69 ± 22.11 (p = 0.04), that represented an increase of 4.09 and 7.07 fold change respectively (Fig. 1). The expression values of *miR-132, 142-3p* and *146a* did not show significant differences when compared with the control group suggesting that these miRs are not affected by mTLE in the neocortex (see supplementary Fig. 1 and supplementary Table 1).

### 3.3. Correlation between miRs expression and patient´s clinical features

Finally, we analyzed whether age at onset of epileptic seizures, epilepsy duration, seizure frequency, number of antiepileptic drugs for the presurgery treatment or age could be associated with the miRs expression by Pearson correlation. Of the clinical features analyzed, we found a negative correlation between patient's age and the expression levels of *miR-1298* and *1260*, with a Pearson r of -0.651 (p = 0.022) for *miR-1298* and -0.666 (p = 0.018) for *miR-1260* (Fig. 2A and B, Table 3). The decrease in the expression of *miR-1298* and *1260* with age was only observed among patients with mTLE. Seizure frequency showed a positive correlation with *miR-146a* expression levels with a Pearson r of 0.803 (p = 0.009) (see Fig. 3A, Table 3). We also found a positive correlation between *miR-451* and *146a* expression and the number of antiepileptic drugs used in the presurgical treatment with a Pearson r of 0.611 (p = 0.046) and 0.715 (p = 0.03) respectively (see Fig. 3B and C, Table 3).

## 4. Discussion

We assessed the differential expression of *miR-1298, 451, 142-3p, 34a, 1260, 1275, 132* and *146a* in the temporal neocortex of patients with mTLE and found a significant decrease in the expression of *miR-34a* and *1298* and a significant overexpression of *miR-451*, 1260 and *1275* in the neocortex of patients compared to that of control tissue. In the case of *miR-34a*, our results in the neocortex were not correlated with the ones reported in the hippocampus in which an overexpression in the acute, latent and chronic stages of epilepsy was observed (Bot et al., 2013; Hu et al., 2012; Risbud and Porter, 2013; Sano et al., 2012). It has been documented that *miR-34a* expression is regulated by p53 and promotes apoptosis by regulation of BCL-2 (Jimenez-Mateos and Henshall, 2013; Sano et al., 2012). Nevertheless, this function is controversial as one of these studies reported a decrease in neuronal apoptosis when *miR-34a* was suppressed; whereas another study did not show an effect (Hu et al., 2012; Sano et al., 2012). Since we found a
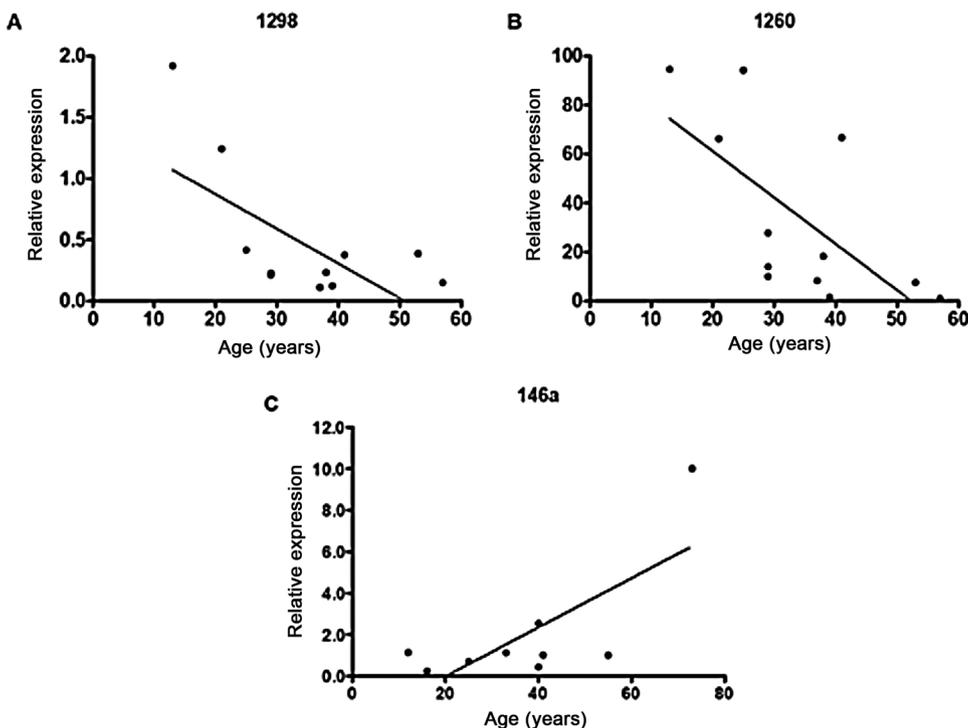


**Fig. 2.** Association between the expressions of miR with age. A, B) The relative expression of *miR-1298* and *1260* showed a negative correlation with the patient's age (n = 12). C) *miR-146a* expression correlates in a positive way with age in the control group (n = 9). Pearson correlation, p = 0.022, r = -0.651 (1298); p = 0.018, r = -0.666 (1260); p = 0.025, r = 0.733 (146a).

**Table 3**

P value and Pearson r from correlation analysis between the miR expressions and clinical features.

| miR | Gender | Age | Seizure onset | Epilepsy duration | Seizure frequency | No. Antiepileptic drugs | Postmortem delay |
|---|---|---|---|---|---|---|---|
| **34a** | | | | | | | |
| P: | 0.810 | 0.747 | 0.501 | 0.457 | 0.445 | 0.260 | – |
| | | r = -0.117 | r = 0.242 | r = -0.267 | r = -0.274 | r = -0.394 | – |
| A: | 0.985 | 0.112 | – | – | – | – | 0.550 |
| | | r = -0.605 | – | – | – | – | r = 0.250 |
| **451** | | | | | | | |
| P: | 0.927 | 0.112 | 0.697 | 0.094 | 0.182 | 0.046 * | – |
| | | r = -0.506 | r = 0.133 | r = -0.529 | r = 0.435 | r = 0.611 | – |
| A: | 0.841 | 0.305 | – | – | – | – | 0.285 |
| | | r = -0.361 | – | – | – | – | r = 0.376 |
| **1260** | | | | | | | |
| P: | 0.283 | 0.018 * | 0.624 | 0.156 | 0.265 | 0.426 | – |
| | | r = -0.666 | r = -0.158 | r = -0.437 | r = 0.350 | r = 0.254 | – |
| A: | 0.905 | 0.374 | – | – | – | – | 0.400 |
| | | r = -0.338 | – | – | – | – | r = 0.321 |
| **1298** | | | | | | | |
| P: | 0.432 | 0.022* | 0.454 | 0.238 | 0.372 | 0.088 | – |
| | | r = -0.651 | r = -0.239 | r = -0.369 | r = 0.283 | r = 0.514 | – |
| A: | 0.222 | 0.361 | – | – | – | – | 0.076 |
| | | r = -0.324 | – | – | – | – | r = 0.584 |
| **132** | | | | | | | |
| P: | 0.876 | 0.077 | 0.957 | 0.147 | 0.392 | 0.098 | – |
| | | r = -0.529 | r = 0.017 | r = -0.446 | r = 0.272 | r = 0.500 | – |
| A: | 0.884 | 0.690 | – | – | – | – | 0.534 |
| | | r = 0.210 | – | – | – | – | r = -0.322 |
| **146a** | | | | | | | |
| P: | 0.765 | 0.093 | 0.852 | 0.093 | 0.009 * | 0.030 * | – |
| | | r = -0.592 | r = 0.073 | r = -0.591 | r = 0.803 | r = 0.715 | – |
| A: | 0.111 | 0.025 * | – | – | – | – | 0.426 |
| | | r = 0.733 | – | – | – | – | r = -0.304 |
| **142-3p** | | | | | | | |
| P: | 1.000 | 0.069 | 0.787 | 0.219 | 0.330 | 0.083 | – |
| | | r = -0.542 | r = -0.088 | r = -0.384 | r = 0.308 | r = 0.520 | – |
| A: | 0.841 | 0.862 | – | – | – | – | 0.099 |
| | | r = 0.063 | – | – | – | – | r = -0.551 |
| **1275** | | | | | | | |
| P: | 0.905 | 0.124 | 0.650 | 0.127 | 0.759 | 0.271 | – |
| | | r = -0.552 | r = 0.176 | r = -0.548 | r = 0.120 | r = 0.412 | – |
| A: | 0.556 | 0.408 | – | – | – | – | 0.215 |
| | | r = -0.316 | – | – | – | – | r = 0.458 |

Abbreviations: P: Patients, C: Controls, *: Significant difference, –: Not applicable.

different expression profile of *miR-34a* in the neocortex compared with that reported in the hippocampus, we suggest that the regulation mechanism of this miR may change in different brain areas after neuronal injury due to epileptic seizures.

In our study, *miR-1298*, *1260* and *1275* showed significant changes in their expression in the neocortex of patients with mTLE. Changes in the expression of *miR-1298* have been reported in other neurodegenerative diseases such as Parkinson and Huntington. An increase in *miR-1298* expression in the hippocampus of APP / PS1 transgenic mice and a decrease in prefrontal cortex samples from patients with Huntington has also been shown (Hoss et al., 2016; Liu et al., 2014). In epilepsy research, there is a dearth of information regarding *miR-1298, 1260* and *1275*. Furthermore a study carried out in the hippocampus of patients with mTLE and hippocampal sclerosis (TLE-HS) reported a marked decrease in *miR-1298* expression and an increase in *miR-1260* and *1275* (Bencurova et al., 2017). This finding is similar to the results observed in the neocortex by our work group and suggests that *miR-1260, 1275* and *1298* may participate in the same signaling pathways associated with epilepsy both in the hippocampus and in the neocortex. By *in silico* prediction, semaphorin 3A and VAMP4 signaling pathways were associated with these miRs (Bencurova et al., 2017). The role of semaphoring 3A has been widely studied in epilepsy, noting that its expression is induced in the hippocampus of rats after status epilepticus by kainic acid (Barnes et al., 2003; Holtmaat et al., 2003). Semaphorin neuronal signaling and axonal guidance have been identified in a proteomic analysis of human epileptic neocortex. These two processes have

been involved in the changes associated with synaptic plasticity in epilepsy (Keren-Aviram et al., 2018). This suggests that *miR-1298, 1260* and *1275* in the neocortex may play a key role in synaptic plasticity and axonal guidance during epilepsy.

*MiR-1298* is associated with ischemic processes as its expression increases together with *miR-1264* and *448* in rats after ischemia/reperfusion. Moreover, through KEGG analysis, the signaling pathways related to this cluster (1298/1264/448) were determined involving signaling of neurotrophins, adherent unions and axonic guidance (Uhlmann et al., 2017). In epilepsy, the brain-derived-neurotrophic-factor (BDNF) expression increases after epileptic seizures in brain areas that include the hippocampus, amygdala, the piriform and the entorhinal cortex. The role of BDNF in epilepsy involves potentiation of glutamatergic transmission, acute neuronal depolarization by Nav1.9 channels and a decrease in GABAergic transmission (Scharfman, 2005). Nevertheless, BDNF also favors and/or promotes the regeneration of neurons in the hippocampus after seizures. Neurotrophins may be also regulated by *miR-1275* since by *in silico* assay it was recognized as a regulator of neurptrophin-3 (NT-3) (Bencurova et al., 2017). The administration of neurotrophins such as FGF, NT-3 and NGF causes seizures and its neutralization delays neuronal kindling in animal models; however, other authors report opposing effects involving these factors in neuronal regeneration (Simonato et al., 2006). Finally, epileptic seizures lead to an increase in the expression of neurotrophin precursors (proneurotrophins) and their p75 receptor produce an imbalance that may provoke apoptosis (Friedman, 2010). These data highlight the
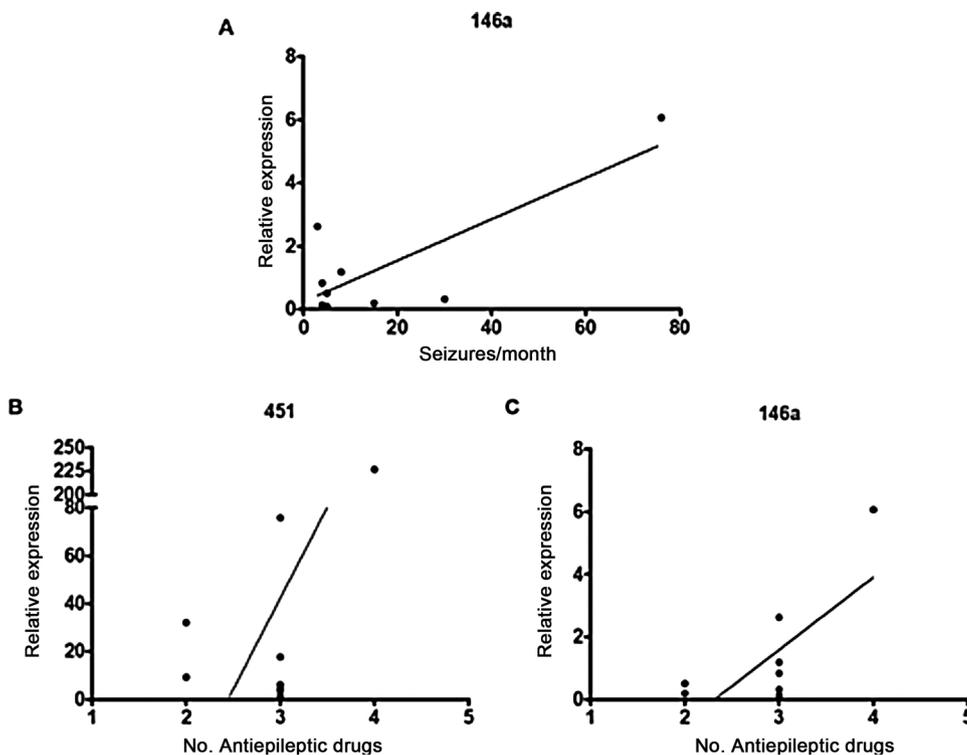
**Fig. 3.** Association between the expressions of miR with seizure frequency and antiepileptic drugs. A) The relative expression of *miR-146a* shows a positive correlation with seizure frequency (n = 9), Pearson correlation, p = 0.009, r = 0.803. B, C) The relative expression of *miR-451* and *146a* shows a positive correlation with number of antiepileptic drugs used for treatment (n = 11 and 9 respectively). Pearson correlation, p = 0.046, r = 0.611 (451); p = 0.030, r = 0.715 (146a).

need to further study the influence of *miR-1298* and *1275* in the BDNF regulation and other neurotrophins in the neocortex in epilepsy.

*MiR-1260* expression has been assessed mainly in cancer, in a study carried out with prostate cancer patients, different *miR-1260* targets were identified including proteins involved in the development and progression of epilepsy such as Bcl-2, FGF22, FGFR1, FOXO4, Cox2 and TRAF6 (Said et al., 2018). Specifically, it has been reported that FGF22 knockout mice are resistant to the induction of pentylenetetrazol generalized seizures (Lee and Umemori, 2013). Another target is FGFR1, its expression increases with the administration of kainic acid in different brain areas that include the hippocampus and cortex (Borght et al., 2011). FOXO4 is a transcription factor of the Forkhead box family identified as a tumor suppressor and its expression is associated with a decrease in the risk of seizure appearance among patients with low grade gliomas (Wang et al., 2017). Ciclooxigenase 2 (Cox2) plays a significant role in neurodegeneration, its expression increases in mTLE models and its inhibition prevents hippocampal damage after kainic acid administration (Sharma et al., 2009). TRAF6 is an element of the TGFβ signaling pathway involved in epilepsy and its expression increases in the hippocampus and cortex after pilocarpine administration in rats. The inhibition of this signaling process reduces the expression of IL-1β, apoptosis and improves the neuronal survival index (Tian et al., 2016). Il-1β signaling is a *miR-146a* target (Iyer et al., 2012), nevertheless in our study we did not find changes in the expression of this miR. It has been previously suggested, the overregulation of *miR-146a* represents an attempt to regulate the inflammatory response (Cava et al., 2018). We thereby suggest that the tissue analyzed in our study displayed low levels of inflammation, or that this process occurred before the sample was collected, or that the anti-inflammatory mechanism developed in patients could be *miR-1260* mediated.

Impairment of GABAergic synapses is a crucial factor in epilepsy. In this regard, *miR-1275* has been suggested as a regulator of Gamma-aminobutyric acid type A receptor (GABRA2) (Bencurova et al., 2017) playing a key role in the excitatory and inhibitory balance. Finally we found a *miR-451* overregulation, this result is partly consistent with that of a study carried out in cerebrospinal fluid (CSF) of patients with status epilepticus and mTLE in which the expression of *miR-451* was significantly increased in the CSF of patients with status epilepticus but not in patients with mTLE. Moreover this miR has been associated with antiepileptic drug resistance targeting the MDR1 transporter (Raoof et al., 2017). In this regard, our results emphasize the importance of *miR-451* in pharmacoresistance among patients with mTLE.

We tested the correlation of miRs expression and clinical features such as age, gender, age of seizure onset, duration of epilepsy, seizure frequency and number of antiepileptic drugs used. We observed a negative correlation between the age of the patients and *miR-1260* and *1298* relative expression, suggesting that the expression of these miRs is age-associated only in patients with epilepsy as we did not observe this correlation in the control group. The role of *miR-1260* and *1298* in aging has not been described yet. It has been shown that *miR-34a* expression increases with age thereby promoting apoptosis (Smith-Vikos and Slack, 2012), conversely our results suggest regulation changes during the epileptic process. On the other hand, *miR-146a* exhibited a positive correlation with age in the control group, suggesting the presence of an inflammatory process that may be age-related. The cause of death may also be implicated since two of the older subjects died due to pneumonia and the inflammatory process that accompanies this disease. In addition, *miR-146a* expression correlated with seizure frequency and number of antiepileptic drugs used for treatment. This miR is expressed in astrocytes and regulates neuroinflammation thereby proposing that its expression increases as a mechanism to compensate the inflammatory process induced after repeated seizures (Tiwari et al., 2018). We found a correlation between *miR-451* expression levels with the number of antiepileptic drugs used, in this regard *miR-451* is associated with drug resistance having MDR1 protein as a target (Raoof et al., 2017). We investigated if gender could influence miRs expression hence we performed a correlation analysis between female and male miRs expression both in controls and mTLE samples and did not find a correlation (see Table 3). We then compared the miRs relative expression by gender. We observed a significant decrease in *miR-1298* expression in female patients compared to female controls and noted a similar finding among our male participants, without statistical significance in the latter group (experimental and control groups). *MiR-451* and *1260* showed a significant increase among female patients

compared to female controls; a similar trend was noted among male participants without reaching statistical significance (see Supplementary Fig. 2).

Our study focused on the temporal neocortex miRs expression however this cerebral region is formed by different neuronal types and glial cells. The miR expression profile obtained from neocortical and cerebellar glutamatergic and GABAergic neurons proves that the major determinant of miRs expression is the neurotransmitter phenotype rather than its localization. One of the miRs identified in the expression profile was *miR-34a* detected in the parvalbumin positive GABAergic neurons in the neocortex (He et al., 2012). On the other hand, glial cells expressed several miRs including *miR-146a* which is produced by astrocytes during inflammation (Tiwari et al., 2018). Here we assessed miRs expression in the entire tissue, however future studies will need to target these miRs at specific neuronal types to have a precise understanding of their function in epilepsy.

Circulating miRs as diagnostic biomarkers and miRs expression have been analyzed in biofluids of epileptic subjects. *MiR-146a* and *34a* were overexpressed in animal and human plasma samples (Cava et al., 2018) and a study highlighted that *miR-142-3p* expression correlates in plasma and brain tissue in experimental epilepsy (Roncon et al., 2015). Nevertheless, the miR expression profile in the brain does not always correlate with findings in plasma, as was the case of *miR-134* and *miR-451* which were found upregulated in the hippocampus and CSF respectively but no change was observed in plasma (Raoof et al., 2017; Tiwari et al., 2018).

Study limitations included a limited sample size, the patient's clinical features that could have influenced the expression profile of the miRs and the study design as we were only able to measure the expression of the miRs using qPCR. The significant differences in the results could be amplified if we were able to increase our sample size and screen all miRs expressed in the neocortex of patients to avoid the exclusion of potential targets. Another limitation, and potential bias, was the collection time of the samples in the control group which ranged from 7 to 20 h post mortem compared to the tissue of patients that was obtained immediately after surgery and hence could be considered recently collected. This difference was not reflected in the values of the RIN in both groups, however the pattern of expression of the miRs could be modified and its exploration would need to be carried out at shorter postmortem intervals. Lastly, our results could be enriched with plasma confirmation and future assessment of these miRs could be studied in different biofluids of patients.

## 5. Conclusions

Our results show the differential expression of *miR-34a, 451, 1260, 1275* and *1298* in the neocortex of patients with mTLE and the negative association between *miR-1260* and *1298* with the age of the patients. *MiR-146a* and *451* also played a role in epilepsy severity since they correlated with seizure frequency and pharmacologic resistance. Our findings support the strategic role these miRs play in the neocortex during the development and progression of epilepsy.

## Declaration of Competing Interest

None of the authors have any conflict of interest to disclose.

## Acknowledgments

## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.eplepsyres.2019.106188.

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