Check for updates

Received: 15 May 2024 | Revised: 17 September 2024 | Accepted: 17 September 2024

DOI: 10.1111/epi.18134

Epilepsia®

RESEARCH ARTICLE

Circulating microRNAs and isomiRs as biomarkers for the initial insult and epileptogenesis in four experimental epilepsy models: The EPITARGET study

Erwin A. van Vliet[1,2] | **Mirte Scheper**[1] | **James D. Mills**[1,3,4] | **Noora Puhakka**[5] | **Kinga Szydlowska**[6] | **Manuela Ferracin**[7,8] | **Francesca Lovisari**[9,10] | **Marie Soukupova**[9] | **Silvia Zucchini**[9] | **Prashant K. Srivastava**[11] | **Michael R. Johnson**[12] | **Katarzyna Lukasiuk**[6] | **Jan A. Gorter**[2] | **Eleonora Aronica**[1] | **Asla Pitkänen**[5] | **Michele Simonato**[9,10]

[1]Department of (Neuro)pathology, Amsterdam Neuroscience, Amsterdam UMC, University of Amsterdam, Amsterdam, The Netherlands

[2]Swammerdam Institute for Life Sciences, Center for Neuroscience, University of Amsterdam, Amsterdam, The Netherlands

[3]Department of Clinical and Experimental Epilepsy, UCL Queen Square Institute of Neurology, London, UK

[4]Chalfont Centre for Epilepsy, Buckinghamshire, UK

[5]A. I. Virtanen Institute for Molecular Sciences, University of Eastern Finland, Kuopio, Finland

[6]Laboratory of Epileptogenesis, Nencki Institute of Experimental Biology, Warsaw, Poland

[7]Department of Medical and Surgical Sciences, University of Bologna, Bologna, Italy

[8]IRCCS Azienda Ospedaliero–Universitaria di Bologna, Bologna, Italy

[9]Department of Neuroscience and Rehabilitation, University of Ferrara, Ferrara, Italy

[10]Division of Neuroscience, IRCCS San Raffaele Scientific Institute, Milan, Italy

[11]National Heart and Lung Institute, Imperial College London, London, UK

[12]Division of Brain Sciences, Imperial College London, London, UK

Correspondence
Erwin A. van Vliet, Center for Neuroscience, Swammerdam Institute for Life Sciences, University of Amsterdam, Science Park 904, Amsterdam 1098 XH, the Netherlands.
Email: e.a.vanvliet@uva.nl

Funding information
Seventh Framework Programme, Grant/Award Number: FP7- HEALTH project 602102 [EPITARGET]

Abstract

Objective: Structural epilepsies can manifest months or years after the occurrence of an initial epileptogenic insult, making them amenable for secondary prevention. However, development of preventive treatments has been challenged by a lack of biomarkers for identifying the subset of individuals with the highest risk of epilepsy after the epileptogenic insult.

Methods: Four different rat models of epileptogenesis were investigated to identify differentially expressed circulating microRNA (miRNA) and isomiR profiles as biomarkers for epileptogenesis. Plasma samples were collected on day 2 and day 9 during the latency period from animals that did or did not develop epilepsy during long-term video-electroencephalographic monitoring. miRNAs

Erwin A. van Vliet, Mirte Scheper, and James D. Mills share first authorship.

Eleonora Aronica, Asla Pitkänen, and Michele Simonato share last authorship.

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.
© 2024 The Author(s). *Epilepsia* published by Wiley Periodicals LLC on behalf of International League Against Epilepsy.

and isomiRs were identified and measured in an unsupervised manner, using a genome-wide small RNA sequencing platform. Receiver operating characteristic analysis was performed to determine the performance of putative biomarkers.

Results: Two days after an epileptogenic insult, alterations in the levels of several plasma miRNAs and isomiRs predicted epileptogenesis in a model-specific manner. One miRNA, miR-3085, showed good sensitivity (but low specificity) as a prognostic biomarker for epileptogenesis in all four models (area under the curve = .729, sensitivity = 83%, specificity = 64%, $p < .05$).

Significance: Identified plasma miRNAs and isomiRs are mostly etiology-specific rather than common prognostic biomarkers of epileptogenesis. These data imply that in preclinical and clinical studies, it may be necessary to identify specific biomarkers for different epilepsy etiologies. Importantly, circulating miRNAs like miR-3085 with high negative predictive value for epileptogenesis in different etiologies could be useful candidates for initial screening purposes of epileptogenesis risk.

KEYWORDS

amygdala stimulation, perforant pathway stimulation, pilocarpine, posttraumatic epilepsy, status epilepticus, traumatic brain injury

1 | INTRODUCTION

Structural epilepsies can manifest themselves months or even years after an epileptogenic insult (e.g., stroke, tumor, trauma, or status epilepticus [SE]). The latency time between the epileptogenic insult and the first spontaneous seizure presents a window of opportunity for preventive treatments. However, even the most promising well-characterized antiepileptogenic therapies have not yet been tested clinically. This partly relates to lack of biomarkers that would indicate a subcohort of subjects being at the highest risk of epileptogenesis, thus reducing the size of the study cohort and, consequently, the cost. Moreover, optimizing the treatment cohort would reduce the number of subjects exposed to possible adverse effects of treatments, some of which can be invasive.[1]

Biomarkers have been defined by a joint US Food and Drug Administration and National Institutes of Health working group as "a defined characteristic that is measured as an indicator of normal biological processes, pathological processes, or responses to an exposure or intervention, including therapeutic interventions" (https://www.ncbi.nlm.nih.gov/books/NBK326791/). Biomarkers can be classified according to their use (e.g., susceptibility/risk and prognostic biomarkers would be useful to predict the development of epilepsy following an epileptogenic insult) and come in different forms (e.g., molecular, imaging, physiologic). Molecular biomarkers (genetic, proteins, small molecules) are particularly attractive, because they can be measured in easily accessible compartments, such as blood, plasma, serum, saliva, or urine.[2]

Key points

- Circulating miRNA and isomiR profiles were screened as biomarkers for epileptogenesis in four different rat models of epileptogenesis.
- Two days after an epileptogenic insult, alterations of several miRNAs and isomiRs predicted epileptogenesis in a model-specific manner.
- miR-3085 showed good sensitivity (but low specificity) as a prognostic biomarker for epileptogenesis in all four models.
- Identified plasma miRNAs and isomiRs are mostly etiology-specific rather than common prognostic biomarkers of epileptogenesis.

Among small molecules, circulating microRNAs (miRNAs) have recently attracted much attention as potential biomarkers of epileptogenesis.[3] miRNAs are small, non-coding RNAs that regulate gene expression by binding to target mRNA molecules and inducing their degradation or blocking their translation into proteins.[4] Not surprisingly, given their involvement in the control of protein levels, miRNAs have been shown to play important roles in various biological processes, including development,

15281167, 2024, 11, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/epi.18134 by Test, Wiley Online Library on [29/05/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

differentiation, cell proliferation, and apoptosis. It has been shown that miRNAs are involved in the regulation of synaptic plasticity and that alterations in miRNA expression levels are associated with epilepsy.[5–7] For example, some miRNAs have been shown to regulate the expression of genes involved in synaptic transmission, excitability, and neuroinflammation, all of which are important in the development and maintenance of epilepsy.[7–11] In addition, miRNAs have been implicated in the regulation of neurogenesis and neuroplasticity, which are also believed to be important in the pathogenesis of epilepsy.[8–11] Therefore, miRNAs represent a promising target for the development of new therapeutic strategies.[8,12–15]

Because miRNAs are found in stable forms in biological fluids, such as blood and cerebrospinal fluid, and their levels in these fluids may change in disease states, they are attractive candidates as noninvasive or minimally invasive biomarkers for various pathological conditions, including epilepsy.[3] For example, it has been shown that changes in the serum levels of certain miRNAs may be used to distinguish patients with epilepsy from healthy controls as well as patients with drug-resistant versus drug-responsive epilepsy.[1,11,16,17] Furthermore, the relevant role of miRNAs was demonstrated in the prediction of the early development of autism spectrum disorder or intellectual disabilities in patients with tuberous sclerosis complex.[18] Finally, preclinical studies demonstrated that circulating miRNAs are dysregulated during epileptogenesis[8–11] and that circulating brain-enriched miRNAs reflect the extent of cortical injury in the brain after traumatic brain injury (TBI).[19]

Alternative forms of miRNAs can arise due to differences in the processing of miRNA precursors; these modified miRNAs are known as isomiRs.[20] Compared to their cognate miRNAs, isomiRs can differ in length and/or sequence, including editing of the miRNA sequence, and can have distinct functional capabilities.[20,21] isomiRs can also be differentially regulated in disease states, potentially serving as biomarkers for diagnosis, prognosis, and monitoring.[22,23]

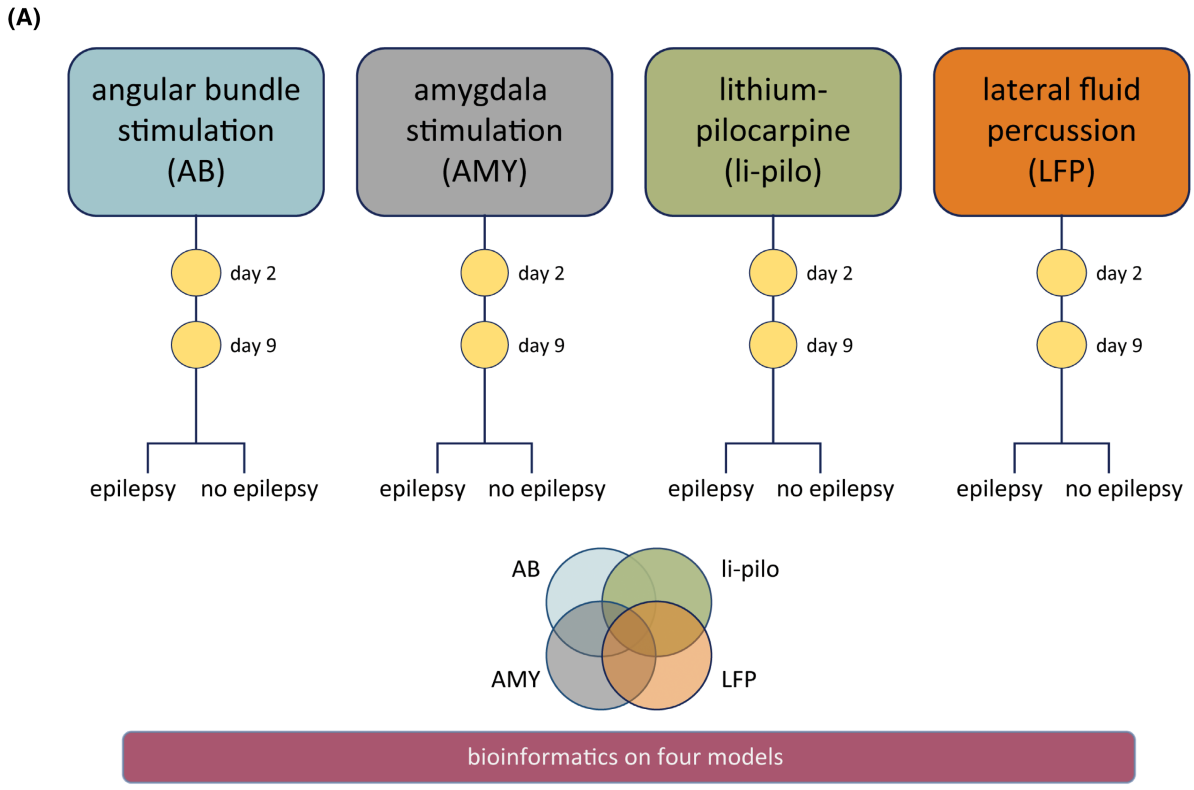
Although circulating biomarkers have been a hot topic for several large European Union-funded epilepsy-related research projects,[24] the use of miRNAs/isomiRs as biomarkers of epileptogenesis is still in the early stages of development. Specifically, it is still unclear whether circulating biomarkers can be prognostic for the development of epilepsy, because most preclinical studies performed so far have investigated blood samples of animals after SE. These animals, although still in the latency period during sample collection, were all expected to develop epilepsy; that is, no comparison of samples from animals that will or will not develop epilepsy was performed. Furthermore, all but one[8] animal study were limited to a single etiology,[9,10,25,26] including our recent previous studies.[19,27] Importantly, it is still unknown whether the putative prognostic value of identified miRNAs is etiology-common or etiology-specific, as none of the studies have employed multiple different models of epileptogenesis. Finally, isomiRs have not been analyzed thus far.

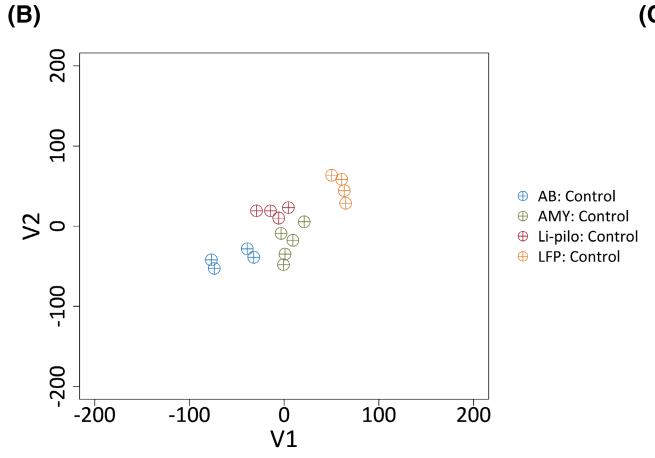
Therefore, the aim of this study was twofold: (1) to identify differentially expressed plasma miRNA and isomiR profiles in four different models of structural epilepsy, by comparing samples collected during the latency period from animals that subsequently did or did not develop epilepsy; and (2) to compare the data from the four models to identify commonalities and etiology-specific alterations. We hypothesized that changes in the levels of plasma miRNAs/isomiRs at early post-SE and/or TBI time points represent prognostic biomarkers of the development of epilepsy, and that some of them are common to all four models.

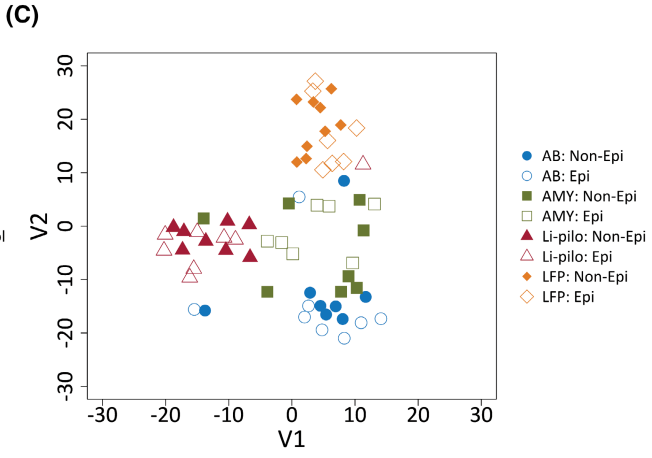
This work was conducted using a multicenter design developed within a European Union FP7-funded research consortium (EPITARGET).

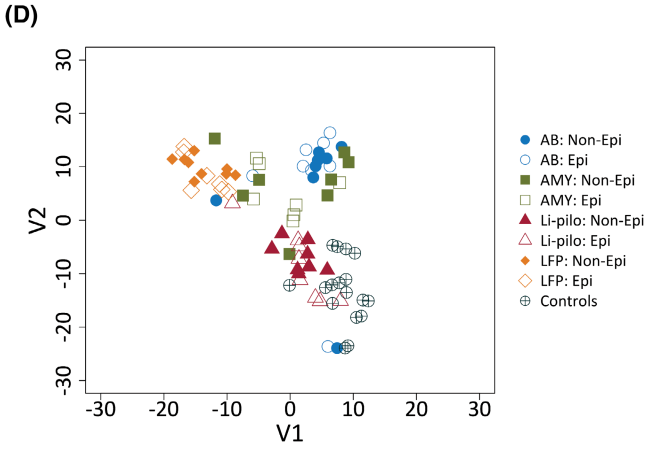
FIGURE 1 (A) Study design. The EPITARGET animal cohort included in total 80 adult male Sprague Dawley rats that were divided in four cohorts: (1) the electrical post-status epilepticus (SE) model in which the angular bundle is stimulated (AB), (2) the electrical post-SE model, in which the amygdala is stimulated (AMY); (3) the chemical post-SE model, based on lithium and pilocarpine peripheral administration (Li-pilo); and (4) the lateral fluid percussion injury model (LFP), in which a transient fluid pressure pulse against the exposed dura is used to induce traumatic brain injury (TBI). Analysis of each SE model included sham-operated experimental controls ($n = 4$), rats with SE that developed epilepsy ($n = 8$), and rats with SE that did not develop epilepsy ($n = 8$). Similarly, analysis of the TBI model included sham-operated experimental controls ($n = 4$), rats with TBI that developed epilepsy ($n = 7$), and rats with TBI that did not develop epilepsy ($n = 9$). For each model, blood samples were withdrawn from the same individuals at 2 and 9 days post-SE or post-TBI. Plasma was prepared for small RNA sequencing, followed by a bioinformatics comparison of the four models. Video-electroencephalographic recordings were made to discriminate which animals developed epilepsy and which did not. (B–D) Principal component analysis (PCA) of four epilepsy models according to circulating miRNA expression 2 days after the initial insult. PCA of normalized read counts (counts/million) shows that the plasma miRNA expression profile 2 days after the initial insult separates the samples from the four experimental models as well as the control samples into different clusters. In contrast, the PCA did not separate rats with epilepsy (Epi) and without epilepsy (Non-Epi) for any of the models. (A) Control samples only. (B) All samples, excluding control samples. (C) all samples, including control samples.

15281167, 2024, 11, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/epi.18134 by Test, Wiley Online Library on [29/05/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License









15281167, 2024, 11, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/epi.18134 by Test, Wiley Online Library on [29/05/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

2 | MATERIALS AND METHODS

2.1 | Study design

The study design is summarized in Figure 1A. Eighty adult male Sprague Dawley rats were included in the study, divided in four cohorts: (1) the electrical post-SE model, in which the angular bundle was stimulated (henceforth AB); (2) the electrical post-SE model in which the amygdala was stimulated (AMY); (3) the chemical post-SE model, in which lithium–pilocarpine was injected peripherally (Li-pilo); and (4) the lateral fluid percussion injury (LFP) model, in which a transient fluid pressure pulse against the exposed dura was used to induce severe TBI. For each SE model, samples were obtained from experimental controls ($n=4$), rats with SE that subsequently developed epilepsy ($n=8$), and rats with SE that did not subsequently develop epilepsy ($n=8$). Similarly, for the TBI model, sham-operated experimental controls ($n=4$), rats with TBI that developed epilepsy ($n=7$), and rats with TBI that did not develop it ($n=9$) were included. For each model, blood samples were withdrawn from the same individuals 2 and 9 days post-SE/TBI, which were used to obtain plasma for small RNA sequencing. Continuous video-electroencephalographic (vEEG) recordings were performed to discriminate animals that did or did not develop epilepsy and to allocate samples ex post in the proper group.

The four epilepsy models were established in four laboratories participating in this study (AB in Amsterdam, the Netherlands; AMY in Warsaw, Poland; Li-pilo in Ferrara, Italy; LFP in Kuopio, Finland), each laboratory following internally approved procedures. Sample collection and processing were instead conducted using common, identical procedures in all laboratories.[28] All experiments were approved by ethical committees and performed in accordance with the guidelines of the European Community Council Directives 2010/63/EU as well as the ARRIVE and the NC3Rs (National Centre for the Replacement, Refinement, and Reduction of Animal Research) guidelines.[29] For details about the four epilepsy models, blood sampling, and small RNA sequencing, see Supplementary Methods.

All RNA sequencing data used in this paper are publicly available at the European Nucleotide Archive (https://www.ebi.ac.uk/ena/browser/home) under the accession number PRJEB78561.

3 | RESULTS

3.1 | Initial insult and epileptogenesis

See Supplementary Results.

3.2 | miRNA and isomiR plasma analysis 2 days after an epileptogenic insult

3.2.1 | Principal component analysis

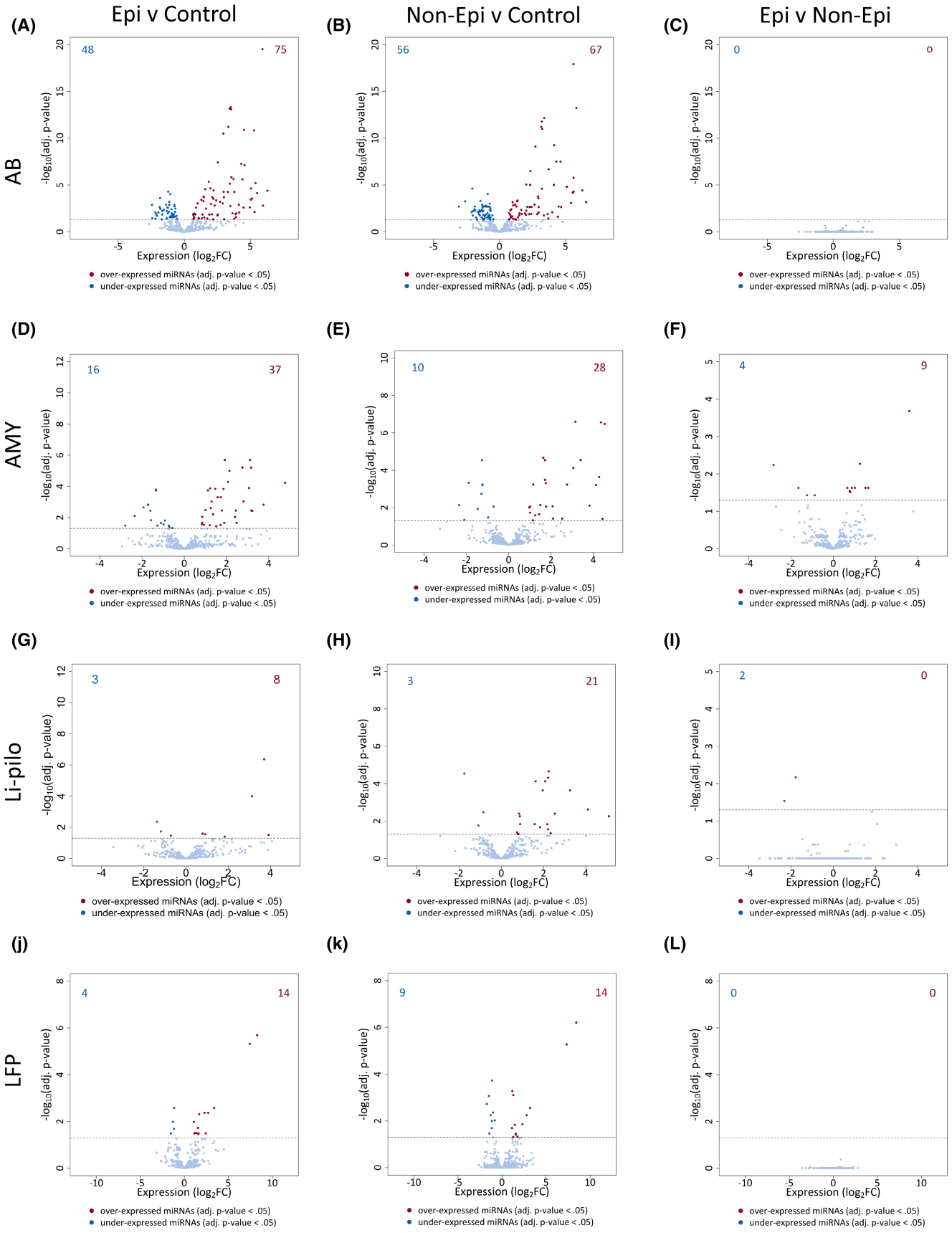
Unsupervised principal component analysis (PCA) on normalized read counts (counts/million) was conducted to assess the plasma miRNA expression profile 2 days following the initial insult in the four experimental models as well as in control samples. As shown in Figure 1B–D, the PCA clearly demonstrated distinct clustering of samples from the four experimental models, highlighting a unique miRNA expression pattern associated with each model. In contrast, when comparing rats set to develop epilepsy (Epi) and those without epilepsy (Non-Epi) within each experimental model, PCA analysis did not reveal significant differentiation, suggesting that the presence or absence of epilepsy did not exert a substantial influence on miRNA expression profiles for the tested time point. This pattern was consistently observed in the isomiR analysis as well, as indicated in Figure S1.

3.2.2 | Differential expression analysis

Differential expression analysis showed a total of 205 differentially expressed (DE) miRNAs (71 downregulated, 134 upregulated) across all four models when Epi samples were compared to control samples (Figure 2A,D,G,J), as well as 208 DE miRNAs (78 downregulated, 130 upregulated) when Non-Epi samples were compared to control samples (Figure 2B,E,H,K). The AB and AMY models displayed the highest number of DE miRNAs, whereas these numbers were lower for the Li-pilo and LFP models. A comparison between the Epi and Non-Epi samples showed an overall

FIGURE 2 Differential expression analysis (DEA) of miRNAs 2 days after the initial insult. DEA showed that many miRNAs were upregulated (red dots) or downregulated (blue dots) 2 days after the initial insult in each model when samples from rats with epilepsy (Epi) were compared to controls (A, D, G, and J) or when samples from rats without epilepsy (Non-Epi) were compared to controls (B, E, H, and K). This was most evident for the AB and the AMY models. Fewer differentially expressed miRNAs were found in the Li-pilo and LFP models. When Epi and Non-Epi animals were compared (C, F, I, and L), only four miRNAs were downregulated and nine upregulated for the AMY model, and two miRNAs were downregulated and zero upregulated for the Li-pilo model, whereas no miRNA was upregulated or downregulated for the AB and LFP models. AB, angular bundle; AMY, amygdala; Li-pilo, lithium–pilocarpine; LFP, lateral fluid percussion.

15281167, 2024, 11, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/epi.18134 by Test, Wiley Online Library on [29/05/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License



15281167, 2024, 11, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/epi.18134 by Test, Wiley Online Library on [29/05/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

lower number of DE miRNAs (Figure 2C,F,I,L). In this comparison, only the AMY model (four downregulated, nine upregulated) and the Li-pilo model (two downregulated, zero upregulated) displayed differential expression, whereas this was not the case for either the AB or the LFP model.

For isomiRs, a similar pattern was observed (Figure S2) for Epi samples and Non-Epi samples as compared to control samples, although many more upregulated and downregulated isomiRs were found as compared to the number of DE miRNAs. When Epi and Non-Epi rats were compared, the majority of DE isomiRs were found for the AMY model (nine downregulated, 20 upregulated), followed by the AB model (one upregulated isomiR).

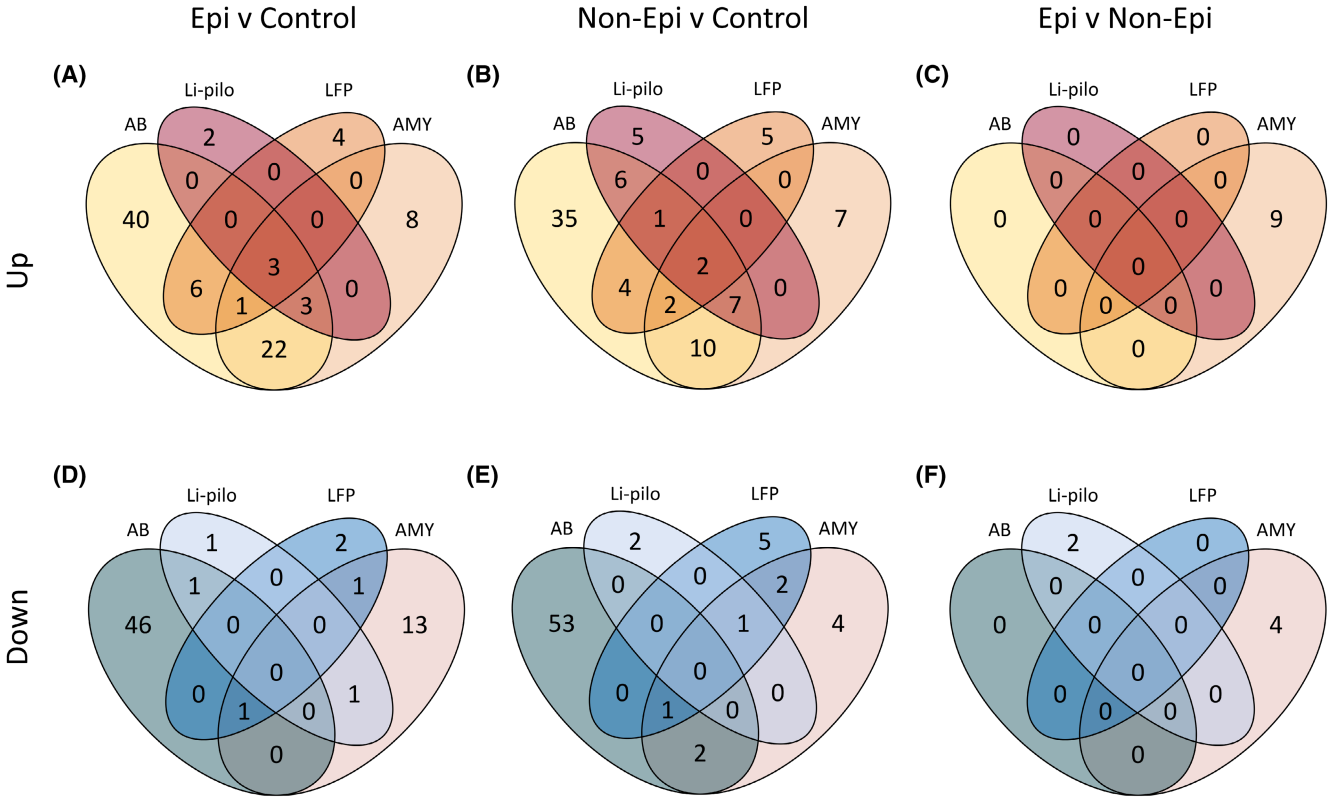
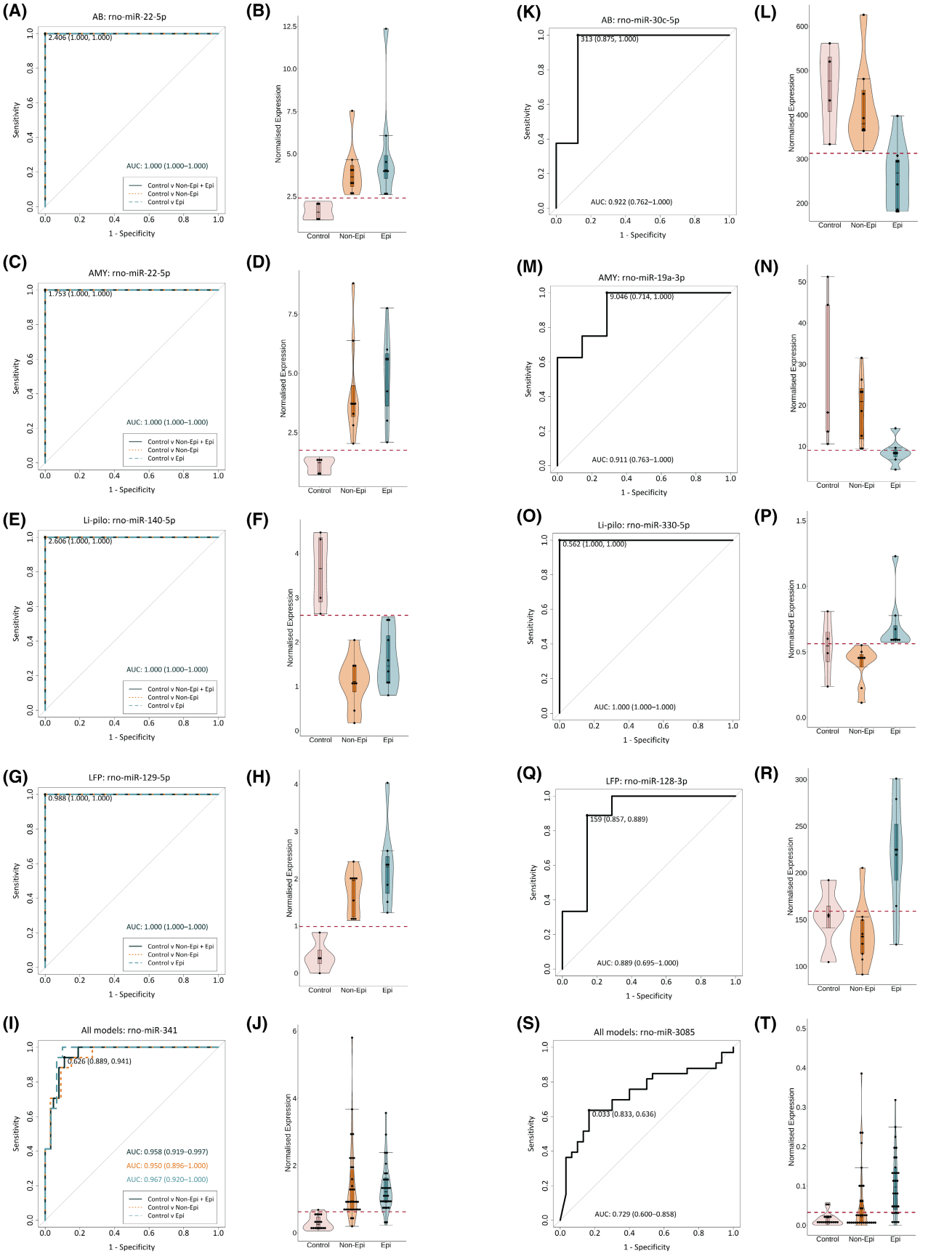


FIGURE 3 Venn diagram analysis of miRNA expression 2 days after the initial insult. Venn diagram analysis shows that most upregulated (Up) and downregulated (Down) miRNAs are model-specific. Samples taken from rats that subsequently developed epilepsy (Epi) are compared to control samples (A and D), samples from rats that did not develop epilepsy (Non-Epi) are compared to control samples (B and E), and samples from Epi rats are compared with samples from Non-Epi rats (C and F). Only three upregulated miRNAs were found to be in common for all four models when Epi samples were compared with control samples, and two upregulated miRNAs when Non-Epi samples were compared with control samples. The AB and AMY models had the most miRNAs in common. AB = angular bundle, AMY, amygdala; Li-pilo, lithium–pilocarpine; LFP, lateral fluid percussion.

FIGURE 4 (A–J) Receiver operating characteristic (ROC) analysis of insult-specific miRNA expression 2 days after the initial insult. ROC analysis shows that specific miRNAs can be detected for each model that separate control samples from samples taken from rats that subsequently developed epilepsy (Epi), from rats that did not develop epilepsy (Non-Epi), and from all injured rats (Epi + Non-Epi combined; injury-specific miRNAs; [A–H] area under the curve [AUC] = 1, $p < .05$). Here represented are the ROC analyses of miR-22-5p for the AB and AMY models, miR-140-5p for the Li-pilo model, and miR-129-5p for the LFP model. miR-341 separates with high sensitivity (>90%) and specificity (100%) Epi (AUC = .967, $p < .05$) as well as Non-Epi (AUC = .950, $p < .05$) and Epi + Non-Epi (AUC = .958, $p < .05$) from control samples for all models (I and J). (K–T) ROC analysis of epilepsy-specific miRNA expression 2 days after the initial insult. ROC analysis reveals specific miRNAs for each model that separate samples taken from rats that subsequently developed epilepsy (Epi) from those without epilepsy (Non-Epi) with high sensitivity and specificity (epilepsy-specific miRNAs; K-R). Here represented are the ROC analyses of miR-30c-5p (AUC = .922, $p < .05$) for the AB model, miR-19a-3p (AUC = .911, $p < .05$) for the AMY model, miR-330-5p (AUC = 1, $p < .05$) for the Li-pilo model, and miR-128-3p (AUC = .889, $p < .05$) for the LFP model. miR-3085 separates Epi from Non-Epi (AUC = .729, $p < .05$) for all models (S and T), although with a lower sensitivity (83%) and specificity (64%) as compared to the sensitivity and specificity of miRNAs specific for each separate model. AB, angular bundle; AMY, amygdala; Li-pilo, lithium–pilocarpine; LFP, lateral fluid percussion.

15281167, 2024, 11, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/epi.18134 by Test, Wiley Online Library on [29/05/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License



15281167, 2024, 11, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/epi.18134 by Test, Wiley Online Library on [29/05/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

3.2.3 | Venn diagram analysis

Most upregulated and downregulated miRNAs were model-specific when Epi samples were compared to control samples (Figure 3A,D), as well as for Non-Epi samples versus control samples (Figure 3B,E) and for Epi versus Non-Epi samples (Figure 3C,F). Only three upregulated miRNAs (rno-miR-129-5p, rno-miR-129-1-3p, rno-miR-129-2-3p) were found to be in common for all four models when Epi samples were compared to control samples, and two upregulated miRNAs (rno-miR-129-5p, rno-miR-410-3p) when Non-Epi samples were compared to control samples. The AB and AMY models had the most miRNAs in common.

A similar pattern was observed for isomiRs (Figure S3). Only two isomiRs were found to be upregulated (miR-128-3p_trim2, miR-127-3p_trim4) in all four models for Epi samples versus control samples and one isomiR (miR-127-3p_trim4) for Non-Epi samples versus control samples. Also, for isomiRs, the AB and AMY models had the most differentially expressed isomiRs in common.

3.2.4 | Receiver operating characteristic analysis

Receiver operating characteristic (ROC) analysis showed that, for each model, specific miRNAs could be detected that separated Epi as well as Non-Epi and Epi + Non-Epi samples from control samples with 100% sensitivity and 100% specificity (Figure 4A–H; area under the curve [AUC] = 1, $p < .05$): for example, miR-22-5p for the AB as well as for the AMY model, miR-140-5p for the Li-pilo model, and miR-129-5p for the LFP model. These miRNAs may be viewed as insult-related biomarkers, because the changes were observed in all animals that received the epileptogenic insult, irrespective of whether they would eventually develop epilepsy. Furthermore, miR-341 could separate with high sensitivity (>90%) and specificity (100%) Epi samples (AUC = .967, $p < .05$) as well as Non-Epi (AUC = .950, $p < .05$) and Epi + Non-Epi samples (AUC = .958, $p < .05$) from control samples for all models (Figure 4I,J).

ROC analysis also revealed specific miRNAs for each model that separated Epi from Non-Epi samples with high sensitivity and specificity (Figure 4K–R): for example, miR-30c-5p (AUC = .922, $p < .05$) for the AB model, miR-19a-3p (AUC = .911, $p < .05$) for the AMY model, miR-330-5p (AUC = 1, $p < .05$) for the Li-pilo model, and miR-128-3p (AUC = .889, $p < .05$) for the LFP model. These miRNAs may be viewed as epileptogenesis-specific biomarkers (i.e., prognostic biomarkers that predict the development of epilepsy), because changes in their levels were observed in plasma from Epi but not from Non-Epi or control rats. miR-3085 could separate Epi from Non-Epi samples (AUC = .729, $p < .05$) in all models (Figure 4S,T), with a lower sensitivity (83%) and especially specificity (64%) as compared to the sensitivity and specificity of miRNAs specific for each separate model. A top 10 list of miRNAs is provided for each model individually and all models together in Table S1.

To determine whether a set of different miRNAs may better serve as a paramount biomarker signature of epileptogenesis across all models, two different combinations were tested (see Table S1). First, we combined the miRNAs with the highest AUCs for each model, namely, miR-30c-5p for AB, miR-19a-3p for AMY, miR-330-5p for Li-pilo, and miR-128-3p for LFP (Figure 5A). Second, we combined the five miRNAs with the highest AUCs for all models, namely, miR-3085, miR-487b-3p, miR-98-5p, miR-872-3p, and miR-138-5p (Figure 5B). However, the sensitivity and/or specificity, as well as the AUC value, remained low with both approaches (respectively 53% and 76%, AUC = .625; and 83% and 64%, AUC = .762).

Insult-related, model-specific isomiRs could be detected that separated Epi as well as Non-Epi samples and Epi + Non-Epi samples from controls with 100% sensitivity and 100% specificity (Figure S4A–H; AUC = 1, $p < .05$): for example, miR-133c_A_5prime for the AB model, miR-10a-5p_trim4 for the AMY model, miR-486_trim1 for the Li-pilo model, and miR-182_trim3 for the LFP model. Furthermore, miR-127-3p_trim4 could separate with 97% sensitivity and 100% specificity Epi samples (AUC = .998, $p < .05$) as well as Non-Epi samples (AUC = .998, $p < .05$) and Epi + Non-Epi samples (AUC = .998, $p < .05$) from controls for all models (Figure S4I,J).

ROC analysis also revealed epileptogenesis-specific isomiRs for each model that separated Epi from Non-Epi samples with high sensitivity and specificity (Figure S5A–H): for example, miR-186-5p_T_3prime (AUC = 1, $p < .05$) for the AB model, miR-423-3p_A_3prime (AUC = .982, $p < .05$) for the AMY model, miR-3596d_AA_5prime (AUC = .922, $p < .05$) for the Li-pilo model, and miR-19b-3p_trim3 (AUC = .905, $p < .05$) for the LFP model. miR-98-5p_trim2 could separate Epi from Non-Epi samples (AUC = .759, $p < .05$) for all models (Figure S5I,J), although with a much lower sensitivity (57%) as compared to the sensitivity of model-specific isomiRs. A top 10 list of isomiRs is provided for each model individually and all models together in Table S2.

A combination of the best isomiRs of each model was plotted as a single ROC curve (Figure S6A), as well as a combination of the best five isomiRs based on AUCs across all models (Figure S6B). However, the sensitivity and/or specificity, and the AUC value, were low with both

15281167, 2024, 11, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/epi.18134 by Test, Wiley Online Library on [29/05/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

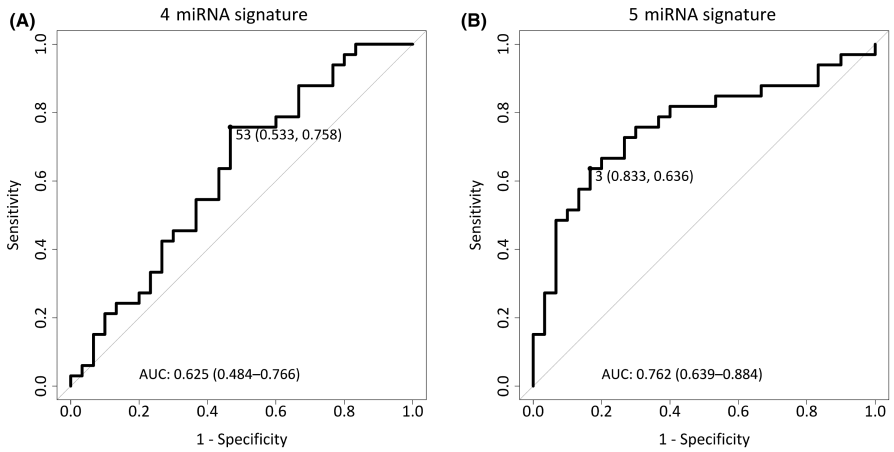


FIGURE 5 Combination of multiple miRNA signatures. (A) Combination of the best receiver operating characteristic (ROC) curves of independent models as one ROC curve and (B) combination of five highest miRNAs based on areas under the curve (AUCs) across all models.

approaches (respectively 87% and 39%, AUC = .604; and 70% and 70%, AUC = .700).

3.3 | miRNA and isomiR plasma analysis 9 days after an epileptogenic insult

See Supplementary Results and Figure 6.

4 | DISCUSSION

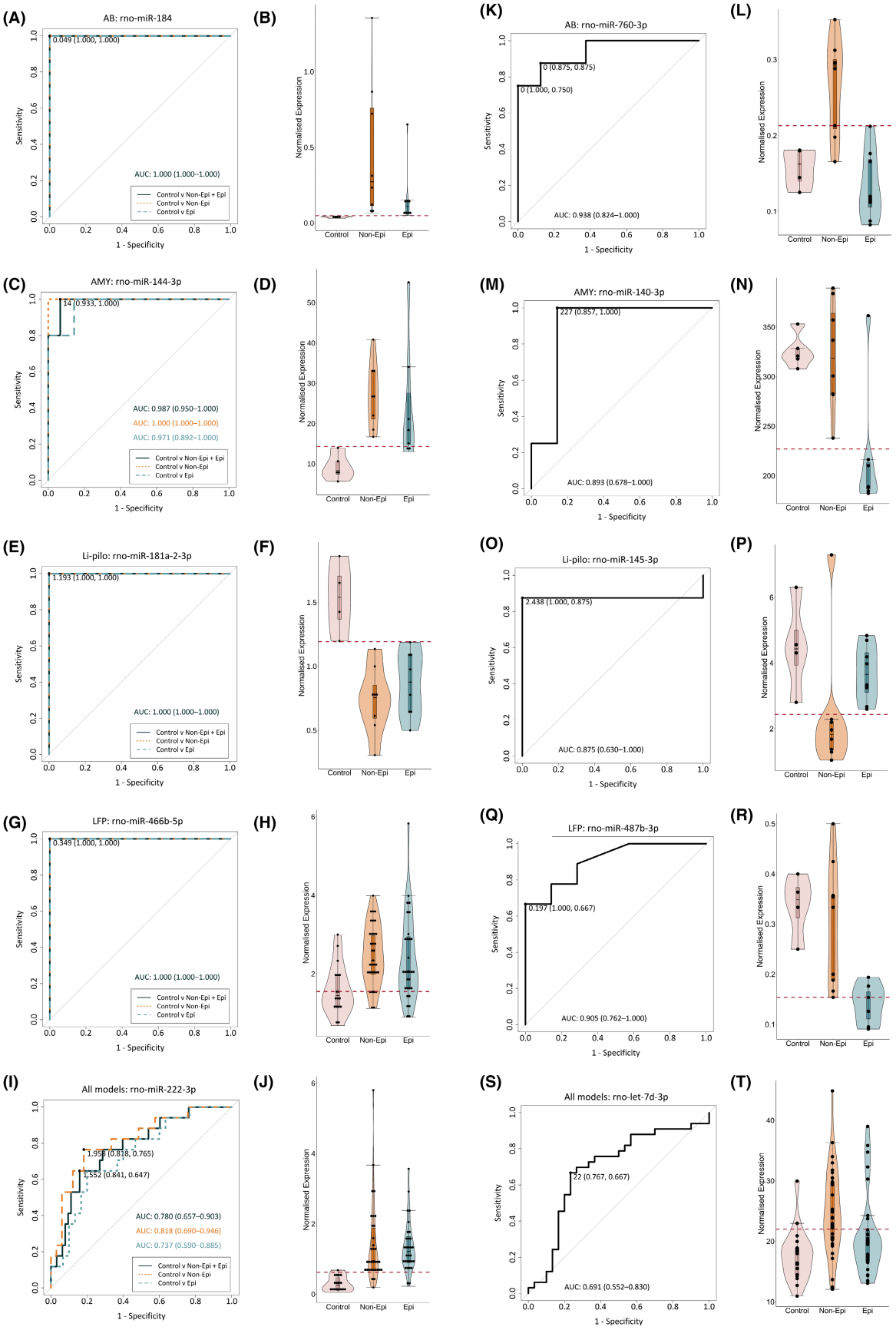
There is a great need for identification of diagnostic and prognostic biomarkers of epileptogenesis to pinpoint subjects at high risk of developing epilepsy after an epileptogenic insult and in need of more intensive follow-up. Epileptogenesis biomarkers would also be critical for stratification of the study cohorts for clinical antiepileptogenesis trials, making them more affordable. From the ethical point of view, biomarkers would also reduce unnecessary treatment and exposure to adverse events.

Among the biomarker modalities (electrophysiologic, imaging, molecular, and cellular), molecules in biological fluids are particularly attractive, due to the ease of sample collection and to their low costs.[1] Plasma miRNAs offer distinct advantages over other circulating molecules.[3] First, they are relatively stable in biofluids. In plasma, they are found mainly bound to argonaute2, the key effector protein of miRNA-mediated silencing, or within macrovesicles (exosomes).[30] Second, they are easy to measure, and loss of signal is minimal even after multiple freeze–thaw cycles.[31] Third, they are enriched in the brain, where specific cell types express specific miRNAs[32,33] and from where they can be distributed into the circulation within exosomes.[34] Fourth, their levels in the brain are altered in various neurological disorders, including epilepsy.[6] Consequently, presence of brain-enriched miRNAs in the peripheral blood can be hypothesized to depend upon type and/or location of brain injury or injury/disease progression.

Multiple studies have been conducted to identify alterations in plasma or serum levels of specific miRNAs or sets of miRNAs in association with epilepsy. Most of these studies have aimed to discover diagnostic biomarkers by analyzing blood samples of patients or animals in which epilepsy was already established.[3] Thus far, only four studies (three in animals, one in humans) were designed to measure miRNA levels in plasma during the latency period, to identify diagnostic or prognostic biomarkers of epileptogenesis.[1] Animal studies identified plasma miRNAs that were altered specifically in the latency period, but not during established chronic epilepsy. For example, miR-21-5p was upregulated during the latency period in the AB model,[9] whereas miR-9a-3p was upregulated and miR-598-5p downregulated in the pilocarpine model.[10] Other miRNAs underwent changes in the latency period that were observed also in chronic epilepsy. For example, miR-142-5p was downregulated in the AB model,[9] miR-142-3p was downregulated in the pilocarpine model,[10] miR-93-5p was upregulated and miR-574-3p was downregulated in the mouse intra-amygdala kainate model and pilocarpine model as well as in the rat perforant path stimulation model.[8] In the human study, performed in a small cohort of patients that suffered intracerebral hemorrhage (ICH), downregulation of two miRNAs (miR-4317 and miR-4325) differentiated patients with post-ICH seizures from those without seizures at 1 year.[35] Even though these studies have identified promising miRNA biomarker candidates, they were not assessed in ROC analysis or validated in separate cohorts. Moreover, the miRNAs identified differed between studies, and their ability to distinguish between the animals that did or did not develop epilepsy was not investigated.

The present study was designed to overcome many of these limitations. First, this is a multicenter study (only one of the previous studies was multicenter).[8] Animals were carefully characterized for the development of

15281167, 2024, 11, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/epi.18134 by Test, Wiley Online Library on [29/05/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License



epilepsy after diverse epileptogenic etiologies; long-term vEEG monitoring allowed identification of animals that did or did not develop epilepsy (herein termed Epi and Non-Epi). All blood sampling procedures were strictly standardized,[28] and all samples were analyzed together. Data were carefully analyzed, including an ROC analysis.

15281167, 2024, 11, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/epi.18134 by Test, Wiley Online Library on [29/05/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

FIGURE 6 (A–J). Receiver operating characteristic (ROC) analysis of insult-specific miRNA expression 9 days after the initial insult. ROC analysis shows that specific miRNAs can be detected for each model that separate control samples from samples taken from rats that subsequently developed epilepsy (Epi), from those that did not develop epilepsy (Non-Epi), and from all injured animals combined (Epi + Non-Epi; injury-specific miRNAs). (K–T) ROC analysis of epilepsy-specific miRNA expression 9 days after the initial insult. ROC analysis reveals specific miRNAs for each model that separate samples taken from rats that subsequently developed epilepsy (Epi) from those that did not develop epilepsy (Non-Epi) with high sensitivity and specificity (epilepsy-specific miRNAs). AB, angular bundle; AMY, amygdala; AUC, area under the curve; Li-pilo, lithium–pilocarpine; LFP, lateral fluid percussion.

Finally, for the first time in epilepsy studies, we included the isomiRs in the analysis. The duration of vEEG monitoring in our study was long, albeit with breaks, meaning that the risk of a false negative misclassification of a rat as nonepileptic is low. However, that monitoring was non-continuous remains a caveat.

The main finding of the present study is that at 2 days after the initial insult, several miRNAs and isomiRs can be identified in plasma that predict with a high sensitivity and specificity those animals that will and those that will not develop epilepsy. As our experimental setting also included sham-operated control animals, we were able to identify changes in miRNAs and isomiRs that were attributable to the epileptogenic insult per se rather than epileptogenesis. Interestingly, most of the injury or epileptogenesis changes in miRNAs and isomiRs were model-specific. We could identify minor commonalities only between models generated by electrical stimulation of brain regions in the mesial temporal lobe (the AB and AMY models). No commonality was found in miRNAs and isomiRs changes when compared to the chemical model (Li-pilo) or the TBI model (LFP). In translational terms, this suggests that circulating small RNAs may, at best, be developed as etiology-specific biomarkers of epileptogenesis, although we cannot exclude strain-, husbandry-, or site-specific effects.

It may be noted that these emerging insult-specific miRNAs biomarkers of epileptogenesis are novel and not identified in previous studies. Except for miR-93-5p (as mentioned earlier), none of the identified miRNA biomarkers had been previously reported as dysregulated during the latency period. Also, previously identified miRNA biomarker candidates were not on the top 10 list of the best miRNA predictors of epilepsy development identified in the present study. The lack of alignment with previous studies is not unexpected, because of differences in the models and species analyzed. For example, we used rats for the AB model, whereas Brennan and colleagues used mice.[8] Here, we investigated the lithium–pilocarpine model, whereas Roncon and coworkers investigated the pilocarpine model.[10] However, the most important differences with previous studies may relate to study designs. In previous studies, sampling was not performed longitudinally. Moreover, SE (that was used as the epileptogenic insult) induced spontaneous seizures in the large majority of the animals. Here, instead, we longitudinally sampled the animals at two early time points and monitored them for a prolonged period of time to detect unprovoked seizures. Consequently, samples could be allocated to the Epi or Non-Epi group.

Contrary to expectations, we were unable to identify a single miRNA or isomiR, or any combination thereof, that predicted epilepsy development with high sensitivity and specificity independent of the initial epileptogenic insult. The only hit that displayed a borderline interest was miR-3085. It separated Epi from Non-Epi animals in all models with an AUC of .729 and with 64% specificity and 83% sensitivity. This indicates a good negative predictive value, that is, a probability of identifying animals that do not have ongoing epileptogenesis and will not develop epilepsy. The expression of miR-3085 in human and rodents is brain-enriched but very low. Interestingly, the proinflammatory factor interleukin-1 induces the expression of miR-3085-3p in brain, at least in part via nuclear factor κB (NFκB). In a feed-forward mechanism, miR-3085-3p then potentiates NFκB signaling.[36] Furthermore, it was also differentially regulated in brain tissue of a mouse model of Gaucher disease brains, linked to mitochondrial dysfunction.[37] These observations, even though requiring validation in an independent study, provide an interesting tool for a staged use of epileptogenesis biomarkers.[1] That is, noninvasive, low-cost, blood-based biomarkers could serve as a screening tool to determine indication for higher cost, more invasive diagnostic procedures such as imaging or EEG. A screening tool to exclude individuals who will likely not develop epilepsy from further clinical investigation would need to have a high negative predictive value but not necessarily a high positive predictive value. Recently, an ad hoc working group for Alzheimer disease (AD) suggested that a candidate biomarker for primary care screening in AD should have a negative predictive value of >90% but a positive predictive value of ~50%.[38] Based on these considerations, miR-3085 may be worthy of further investigations.

5 | CONCLUSIONS

Our study has identified plasma miRNAs and isomiRs that can serve as diagnostic biomarkers for specific epileptogenic insults, distinct from those miRNAs and

15281167, 2024, 11, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/epi.18134 by Test, Wiley Online Library on [29/05/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

isomiRs that can serve as prognostic biomarkers for epilepsy development. This implies that miRNA and isomiR regulation can be epileptogenesis-specific and not merely insult-related. The other major finding relates to etiology specificity of epileptogenesis biomarkers. The only exception was miR-3085, which showed a moderate AUC in differentiation of Epi animals from non-Epi animals in all four models. Etiology specificity is not entirely surprising, as not only the pathology of the epileptogenic insult, but also the progression of the epileptogenic process, differs dramatically in different preclinical and clinical structural epilepsies. This suggests that a given biomarker may be detectable at a given time point in the process induced by a given epileptogenic event. Interestingly, a greater overlap between models and human structural epilepsies has been observed in the chronic phase of epilepsy, suggesting a progressive convergence of initially heterogeneous processes.[3,8]

The present study offers several testable hypotheses for future investigations. First, we analyzed the total levels of miRNAs and isomiRs. However, it is known that, in plasma, these molecules are mainly found in association with proteins or in exosomes (see preceding text). Analysis of the argonaute2-bound and the exosome fractions could provide a better diagnostic yield for biomarker analysis. Second, validation of specific miRNAs and isomiRs in an independent cohort is warranted. Ideally, this should include human samples, even if the follow-up of at-risk individuals after an epileptogenic event can be challenging. In this respect, miR-3085 and the most predictive model-specific miRNAs and isomiRs can be tested. Third, the brain origin of the candidate miRNA and isomiR biomarkers needs to be verified. At a more advanced stage of research, specificity for epileptogenesis versus other neurological conditions and association with clinical variables (e.g., age, sex, comorbidities, treatments) should be evaluated, following the steps of a recently published roadmap for the identification of biomarkers of epileptogenesis.[1]

AUTHOR CONTRIBUTIONS

Conception and design: Michele Simonato, Asla Pitkänen, Eleonora Aronica, and Katarzyna Lukasiuk. *Acquisition of data*: Erwin A. van Vliet, Noora Puhakka, Kinga Szydlowska, Francesca Lovisari, and Marie Soukupova. *Analysis of data*: Mirte Scheper, James D. Mills, Manuela Ferracin, Prashant K. Srivastava, and Michael R. Johnson. *Supervision*: Silvia Zucchini, Jan A. Gorter, and Katarzyna Lukasiuk. *Writing–original draft*, *writing–review and editing*: all authors.

ACKNOWLEDGMENTS

We thank Jasper Anink, Mario Barbieri, Jarmo Hartikainen, Merja Lukkari, Karolina Nizinska, and Maciej Olszewski for their excellent technical assistance, Niina Lapinlampi for her help in setting up the RedCap database for data collection, and Danijela Koppers-Lalic for advice on isomiR analysis.

FUNDING INFORMATION

This research was funded by a grant from the European Community (FP7- HEALTH project 602102 [EPITARGET]).

CONFLICT OF INTEREST STATEMENT

None of the authors has any conflict of interest to disclose. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in European Nucleotide Archive at https://www.ebi.ac.uk/ena/browser/home, reference number PRJEB78561.

ORCID

Erwin A. van Vliet https://orcid.org/0000-0001-5747-3202
Mirte Scheper https://orcid.org/0000-0002-0676-4701
James D. Mills https://orcid.org/0000-0002-9910-2933
Noora Puhakka https://orcid.org/0000-0001-6151-3510
Kinga Szydlowska https://orcid.org/0000-0001-9019-7676
Manuela Ferracin https://orcid.org/0000-0002-1595-6887
Francesca Lovisari https://orcid.org/0000-0002-6455-7110
Marie Soukupova https://orcid.org/0000-0002-0489-1977
Silvia Zucchini https://orcid.org/0000-0001-9010-6447
Prashant K. Srivastava https://orcid.org/0000-0003-1454-5493
Michael R. Johnson https://orcid.org/0000-0002-5848-9342
Katarzyna Lukasiuk https://orcid.org/0000-0002-1582-0920
Jan A. Gorter https://orcid.org/0000-0003-3842-1700
Eleonora Aronica https://orcid.org/0000-0002-3542-3770
Asla Pitkänen https://orcid.org/0000-0002-8163-5416
Michele Simonato https://orcid.org/0000-0002-3586-2693

15281167, 2024, 11, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/epi.18134 by Test, Wiley Online Library on [29/05/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

REFERENCES

1. Simonato M, Agoston DV, Brooks-Kayal A, Dulla C, Fureman B, Henshall DC, et al. Identification of clinically relevant biomarkers of epileptogenesis—a strategic roadmap. Nat Rev Neurol. 2021;17:231–42.
2. Pitkanen A, Ekolle Ndode-Ekane X, Lapinlampi N, Puhakka N. Epilepsy biomarkers—toward etiology and pathology specificity. Neurobiol Dis. 2019;123:42–58.
3. Enright N, Simonato M, Henshall DC. Discovery and validation of blood microRNAs as molecular biomarkers of epilepsy: ways to close current knowledge gaps. Epilepsia Open. 2018;3:427–36.
4. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell. 2004;23(116):281–97.
5. Dogini DB, Avansini SH, Vieira AS, Lopes-Cendes I. MicroRNA regulation and dysregulation in epilepsy. Front Cell Neurosci. 2013;4(7):172.
6. Henshall DC, Hamer HM, Pasterkamp RJ, Goldstein DB, Kjems J, Prehn JHM, et al. MicroRNAs in epilepsy: pathophysiology and clinical utility. Lancet Neurol. 2016;15:1368–76.
7. Srivastava PK, Roncon P, Lukasiuk K, Gorter JA, Aronica E, Pitkanen A, et al. Meta-analysis of microRNAs dysregulated in the hippocampel dentate gyrus of animal models of epilepsy. eNeuro. 2017;4(6):e0152-17.2017 1-22.
8. Brennan GP, Bauer S, Engel T, Jimenez-Mateos EM, Del Gallo F, Hill TDM, et al. Genome-wide microRNA profiling of plasma from three different animal models identifies biomarkers of temporal lobe epilepsy. Neurobiol Dis. 2020;144:105048.
9. Gorter JA, Iyer A, White I, Colzi A, van Vliet EA, Sisodiya S, et al. Hippocampal subregion-specific microRNA expression during epileptogenesis in experimental temporal lobe epilepsy. Neurobiol Dis. 2014;62:508–20.
10. Roncon P, Soukupova M, Binaschi A, Falcicchia C, Zucchini S, Ferracin M, et al. MicroRNA profiles in hippocampal granule cells and plasma of rats with pilocarpine-induced epilepsy—comparison with human epileptic samples. Sci Rep. 2015;18(5):14143.
11. Korotkov A, Mills JD, Gorter JA, van Vliet EA, Aronica E. Systematic review and meta-analysis of differentially expressed miRNAs in experimental and human temporal lobe epilepsy. Sci Rep. 2017;14(7):11592.
12. Iori V, Iyer AM, Ravizza T, Beltrame L, Paracchini L, Marchini S, et al. Blockade of the IL-1R1/TLR4 pathway mediates disease-modification therapeutic effects in a model of acquired epilepsy. Neurobiol Dis. 2017;99:12–23.
13. Morris G, Reschke CR, Henshall DC. Targeting microRNA-134 for seizure control and disease modification in epilepsy. EBioMedicine. 2019;45:646–54.
14. Vangoor VR, Reschke CR, Senthilkumar K, van de Haar LL, de Wit M, Giuliani G, et al. Antagonizing increased miR-135a levels at the chronic stage of experimental TLE reduces spontaneous recurrent seizures. Neuroscience. 2019;39:5064–79.
15. Veno MT, Reschke CR, Morris G, Connolly NMC, Su J, Yan Y, et al. A systems approach delivers a functional microRNA catalog and expanded targets for seizure suppression in temporal lobe epilepsy. Proc Natl Acad Sci USA. 2020;7(117):15977–88.
16. Pitkanen A, Paananen T, Kyyriainen J, Das Gupta S, Heiskanen M, Vuokila N, et al. Biomarkers for posttraumatic epilepsy. Epilepsy Behav. 2021;121:107080.
17. Martinez B, Peplow PV. MicroRNAs as potential biomarkers in temporal lobe epilepsy and mesial temporal lobe epilepsy. Neural Regen Res. 2023;18:716–26.
18. Scheper M, Romagnolo A, Besharat ZM, Iyer AM, Moavero R, Hertzberg C, et al. miRNAs and isomiRs: serum-based biomarkers for the development of intellectual disability and autism spectrum disorder in tuberous sclerosis complex. Biomedicine. 2022;10(8):1838.
19. Heiskanen M, Das Gupta S, Mills JD, van Vliet EA, Manninen E, Ciszek R, et al. Discovery and validation of circulating microRNAs as biomarkers for Epileptogenesis after experimental traumatic brain injury-the EPITARGET cohort. Int J Mol Sci. 2023;1:24.
20. Gebert LFR, MacRae IJ. Regulation of microRNA function in animals. Nat Rev Mol Cell Biol. 2019;20:21–37.
21. Morin RD, O'Connor MD, Griffith M, Kuchenbauer F, Delaney A, Prabhu AL, et al. Application of massively parallel sequencing to microRNA profiling and discovery in human embryonic stem cells. Genome Res. 2008;18:610–21.
22. Koppers-Lalic D, Hackenberg M, de Menezes R, Misovic B, Wachalska M, Geldof A, et al. Non-invasive prostate cancer detection by measuring miRNA variants (isomiRs) in urine extracellular vesicles. Oncotarget. 2016;19(7):22566–78.
23. Glogovitis I, Yahubyan G, Wurdinger T, Koppers-Lalic D, Baev V. isomiRs-hidden soldiers in the miRNA regulatory army, and how to find them? Biomol Ther. 2020;30:11.
24. Pitkanen A, Henshall DC, Cross JH, Guerrini R, Jozwiak S, Kokaia M, et al. Advancing research toward faster diagnosis, better treatment, and end of stigma in epilepsy. Epilepsia. 2019;60:1281–92.
25. Chen M, Zhao QY, Edson J, Zhang ZH, Li X, Wei W, et al. Genome-wide microRNA profiling in brain and blood samples in a mouse model of epileptogenesis. Epilepsy Res. 2020;166:106400.
26. Brindley E, Heiland M, Mooney C, Diviney M, Mamad O, Hill TDM, et al. Brain cell-specific origin of circulating microRNA biomarkers in experimental temporal lobe epilepsy. Front Mol Neurosci. 2023;16:1230942.
27. Szydlowska K, Bot A, Nizinska K, Olszewski M, Lukasiuk K. Circulating microRNAs from plasma as preclinical biomarkers of epileptogenesis and epilepsy. Sci Rep. 2024;6(14):708.
28. van Vliet EA, Puhakka N, Mills JD, Srivastava PK, Johnson MR, Roncon P, et al. Standardization procedure for plasma biomarker analysis in rat models of epileptogenesis: focus on circulating microRNAs. Epilepsia. 2017;58:2013–24.
29. Lidster K, Jefferys JG, Blumcke I, Crunelli V, Flecknell P, Frenguelli BG, et al. Opportunities for improving animal welfare in rodent models of epilepsy and seizures. J Neurosci Methods. 2016;15(260):2–25.
30. Arroyo JD, Chevillet JR, Kroh EM, Ruf IK, Pritchard CC, Gibson DF, et al. Argonaute2 complexes carry a population of circulating microRNAs independent of vesicles in human plasma. Proc Natl Acad Sci USA. 2011;22(108):5003–8.
31. McDonald JS, Milosevic D, Reddi HV, Grebe SK, Algeciras-Schimnich A. Analysis of circulating microRNA: preanalytical and analytical challenges. Clin Chem. 2011;57:833–40.
32. He M, Liu Y, Wang X, Zhang MQ, Hannon GJ, Huang ZJ. Cell-type-based analysis of microRNA profiles in the mouse brain. Neuron. 2012;12(73):35–48.
33. Jovicic A, Roshan R, Moisoi N, Pradervand S, Moser R, Pillai B, et al. Comprehensive expression analyses of neural

15281167, 2024, 11, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/epi.18134 by Test, Wiley Online Library on [29/05/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

cell-type-specific miRNAs identify new determinants of the specification and maintenance of neuronal phenotypes. J Neurosci. 2013;20(33):5127–37.
34. Levy E. Exosomes in the diseased brain: first insights from in vivo studies. Front Neurosci. 2017;11:142.
35. Iwuchukwu I, Nguyen D, Beavers M, Tran V, Sulaiman W, Fannin E, et al. MicroRNA regulatory network as biomarkers of late seizure in patients with spontaneous intracerebral hemorrhage. Mol Neurobiol. 2020;57:2346–57.
36. Le L, Niu L, Barter MJ, Young DA, Dalmay T, Clark IM, et al. The role of microRNA-3085 in chondrocyte function. Sci Rep. 2020;14(10):21923.
37. Dasgupta N, Xu YH, Li R, Peng Y, Pandey MK, Tinch SL, et al. Neuronopathic Gaucher disease: dysregulated mRNAs and miRNAs in brain pathogenesis and effects of pharmacologic chaperone treatment in a mouse model. Hum Mol Genet. 2015;15(24):7031–48.
38. Hampel H, O'Bryant SE, Molinuevo JL, Zetterberg H, Masters CL, Lista S, et al. Blood-based biomarkers for Alzheimer disease: mapping the road to the clinic. Nat Rev Neurol. 2018;14:639–52.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: van Vliet EA, Scheper M, Mills JD, Puhakka N, Szydlowska K, Ferracin M, et al. Circulating microRNAs and isomiRs as biomarkers for the initial insult and epileptogenesis in four experimental epilepsy models: The EPITARGET study. Epilepsia. 2024;65:3406–3420. https://doi.org/10.1111/epi.18134

15281167, 2024, 11, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/epi.18134 by Test, Wiley Online Library on [29/05/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License