Experimed 2022; 12(1): 1-5

ORIGINAL ARTICLE

DOI: 10.26650/experimed.2021.1033564

Assessment of miR-1179 As a Potential Biomarker in Juvenile Myoclonic Epilepsy

Seda Süsgün[1,2,3], Ceyhun Toruntay[1,4], Alişan Bayrakoğlu[5], Ferda Uslu[5], Emrah Yücesan[1]

[1]Department of Medical Biology, Faculty of Medicine, Bezmialem Vakif University, Istanbul, Turkiye
[2]Graduate School of Health Sciences, Istanbul University, Istanbul, Turkiye
[3]Department of Genetics, Aziz Sancar Institute of Experimental Medicine, Istanbul University, Istanbul, Turkiye
[4]Department of Molecular Biology and Genetics, Graduate School of Natural and Applied Sciences, Yildiz Technical University, Istanbul, Turkiye
[5]Department of Neurology, Faculty of Medicine, Bezmialem Vakif University, Istanbul, Turkiye

ORCID ID: S.S. 0000-0001-9689-3111; C.T. 0000-0002-4743-0257; A.B. 0000-0001-9620-2237; F.U. 0000-0002-2124-5037; E.Y. 0000-0003-4512-8764

Cite this article as: Susgun S, Toruntay C, Bayrakoglu A, Uslu F, Yucesan E. Assessment of miR-1179 as a potential biomarker in juvenile myoclonic epilepsy. Experimed 2022; 12(1): 1-5.

ABSTRACT

Objective: Juvenile myoclonic epilepsy (JME) is one of the most common childhood types of epilepsy and comprises 5-10% of all epilepsies. Altered expression levels of microRNAs (miRNAs) have been reported in epilepsy as in many diseases. As is known, miRNAs regulate gene expression post-transcriptionally and have potential as diagnostic biomarkers due to their stability in clinical samples. Herein, this study aimed to evaluate miR-1179 levels of JME patients and assess the potential of miR-1179 as a diagnostic biomarker.

Materials and Methods: Twenty patients and 20 healthy controls were recruited in this study and total RNA was extracted from peripheral blood samples of participants. Quantitative real-time polymerase chain reaction (qRT-PCR) was performed to calculate the relative expression level of miR-1179. Additionally, receiver operating characteristic (ROC) curve was conducted to evaluate the diagnostic value of miR-1179 in JME.

Results: Expression levels of miR-1179 were statistically significantly increased in patients with JME compared to healthy controls ($p<0.0001$). ROC analysis revealed that miR-1179 is a well diagnostic biomarker with an area under the curve (AUC) of 0.89.

Conclusion: miR-1179 may be considered a remarkable biomarker in the diagnosis of JME. The interaction between miR-1179 and its target *Calmodulin 1 (CALM1)* should be reinforced through functional studies. Further research in larger cohorts will help to enlighten the etiopathogenesis of JME.

Keywords: Juvenile myoclonic epilepsy, miR-1179, ROC curve, biomarker

INTRODUCTION

Epilepsy, which is estimated to affect over 50 million people worldwide, is a serious and chronic neurological disease that impacts public health. Epilepsy is a term that refers to an enduring predisposition to epileptic seizures which are a transient occurrence of symptoms caused by synchronous or abnormal excessive electrical functioning in the brain (1, 2). Juvenile myoclonic epilepsy (JME) is an idiopathic generalized epilepsy syndrome that affects 5-10% of individuals with epilepsy. Onset of JME is related to age, generally manifests between 12-18 years, with gender bias towards female (3). The genetic inheritance of JME is not entirely clarified, a multifactorial mechanism is assumed, however, several genes have been associated with JME (4). The heterogeneous and multifactorial nature of epilepsy leads to challenges in determining biomarkers for diagnosis and prognosis. However, determining biomarkers is crucial and would help to develop targeted therapies. In this context, there are several notable studies evaluating the biomarker potential of microRNAs (miRNAs) in epileptogenesis (2).

Corresponding Author: Emrah Yücesan **E-mail:** emrahyucesan@yahoo.com / eyucesan@bezmialem.edu.tr
Submitted: 07.12.2021 **Revision Requested:** 27.12.2021 **Last Revision Received:** 30.12.2021 **Accepted:** 05.01.2022

miRNAs are small non-coding regulatory RNAs with their role in regulating post-transcriptional target gene expression through mRNA degradation or translational repression (5). miRNAs have a main role in various biological processes and are notable due to their potential in disease diagnostics and use as therapeutics. In particular, the stability of miRNAs in clinical samples has drawn attention to be used as disease biomarkers (6, 7). Altered expression levels of miRNAs have been associated with various conditions such as cardiovascular diseases, cancer, sepsis, and neurological diseases (8).

Herein, the expression levels of miR-1179 have been investigated in patients with JME. According to the miRTarBase database, *Calmodulin 1* (*CALM1*) is one of the target genes of miR-1179. It is estimated that hsa-miR-1179 may bind to the 3'UTR sequence of the *CALM1* gene at positions 1469–1491, 2032–2051, or 2378–2398 for miRNA-target interaction predicted by miRanda (9). According to the STRING database, the CALM1 protein and the CACNA1A protein interact which is validated by experimental determination, text mining, and co-expression data (Figure 1) (10). Previously, genetic changes in *CACNA1A* have been associated with the pathogenesis of JME (11).

In the context of this study, peripheral blood samples were collected from individuals diagnosed with JME and healthy controls, and the relative expression levels of miR-1179 were compared using the quantitative real-time polymerase chain reaction (qRT-PCR) method. To the best of this researcher's knowledge, miR-1179 has not been analyzed in any neurological disease yet. The study findings suggest that miR-1179 should be further analyzed with large-scale research to enlighten its role in the etiopathogenesis of JME.

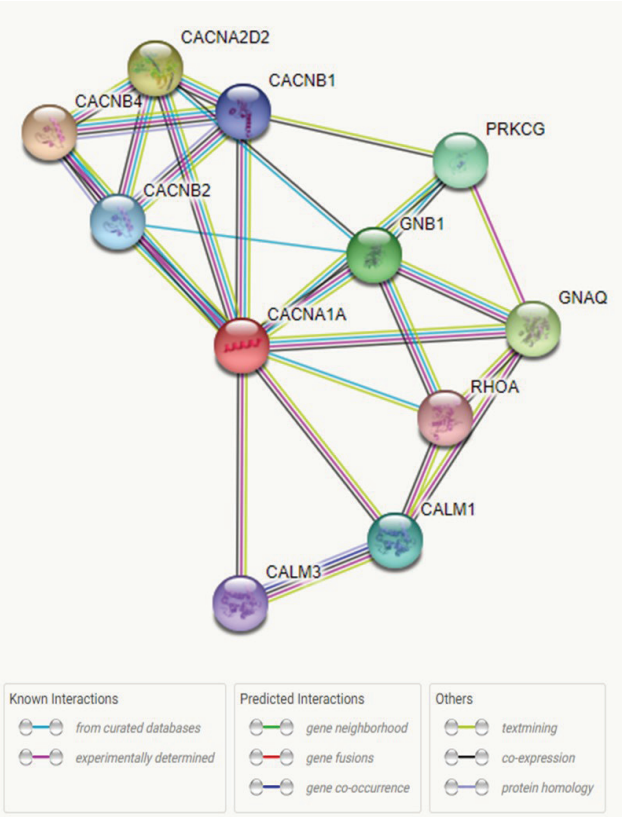

Figure 1. Interaction between CACNA1A and CALM1 proteins was visualized by the STRING database. Each color line indicates different interaction evidence.

MATERIALS AND METHODS

Patients Recruitment

This study protocol was approved by the ethics committee of Bezmialem Vakif University Faculty of Medicine (06.04.2021-E.11759), and written informed consent was obtained from all subjects. In the present study, 20 patients with JME (female:13, male:7) who were examined in Bezmialem Vakif University, Faculty of Medicine, Department of Neurology, and 20 healthy controls (female:12, male:8) without any neurological findings were recruited.

RNA Extraction and qRT-PCR

Five ml of peripheral blood samples was obtained in EDTA tubes from all participants (20 sample and 20 controls). Total RNAs were extracted using the QIAamp RNA Blood Mini Kit (Qiagen GmbH, Hilden, Germany) according to recommendations of the manufacturer. The quality and quantity of RNA were assessed via the Multiskan GO (Thermo Fisher Scientific, Boston, MA, USA). Two hundred fifty ng of the total RNA was converted to cDNA through a miRNA All-in-One cDNA Synthesis Kit (AbmGood, Vancouver, BC, Canada). The cDNAs were diluted 1:10 and used as templates for qRT-PCR. A qRT-PCR was performed on the Bio-Rad CFX96 Connect Real-Time PCR instrument (Bio-Rad Laboratories, Inc., California, USA) using BlasTaq 2X qPCR MasterMix (AbmGood), and data was obtained by Bio-Rad CFX Maestro software. The reaction conditions were as follows: 95˚C for 3 min, and 40 cycles of 95°C for 15 s, 60°C for 10 s, 72°C for 50 s. The miR-1179 forward primer (#MPH01026) and universal 3' miRNA reverse primer (#MPH00000) were purchased from AbmGood. A RNU6 was used as a reference gene. Relative miRNA expression levels were calculated by the $2^{-\Delta Ct}$ method (12).

Statistical Analyses

The GraphPad Prism 8.0 (GraphPad Software, Inc., CA, USA) was used to perform statistical analyses with a 95% confidence level and 0.05 significance level. Whether the data were distributed normally was tested with the Shapiro-Wilk test. When comparing the data with non-normal distribution between two independent groups the Mann-Whitney test was used. Additionally, to assess the diagnostic value of miRNAs, receiver operating characteristic (ROC) curves were generated, and area under the curve (AUC) values were calculated.

RESULTS

In the present study, the patients with JME comprise seven males and 13 females, and age-matched controls comprise eight males and 12 females. The averages of the cycle threshold (Ct) values obtained by the qRT-PCR study are

presented in Table 1. Accordingly, expression levels of miR-1179 in the samples obtained from peripheral blood were statistically significantly increased in patients compared to healthy controls ($p<0.0001$, Figure 2). The AUC value was determined as 0.89 at the 95% confidence level by a ROC curve analysis ($p<0.0001$, Figure 3).

Table 1. The average expression levels of miR-1179.

	miR-1179 Ct_{mean}	RNU6 Ct_{mean}	ΔCt	$2^{-\Delta Ct}$
Patients	34.34	28.34	6.00	0.02
Controls	33.56	26.31	7.25	0.01

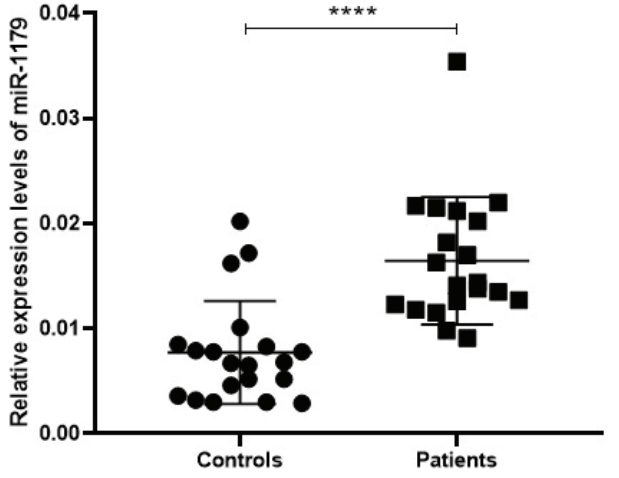

Figure 2. Relative expression levels of miR-1179 in patients and controls. **** indicates $p<0.0001$.

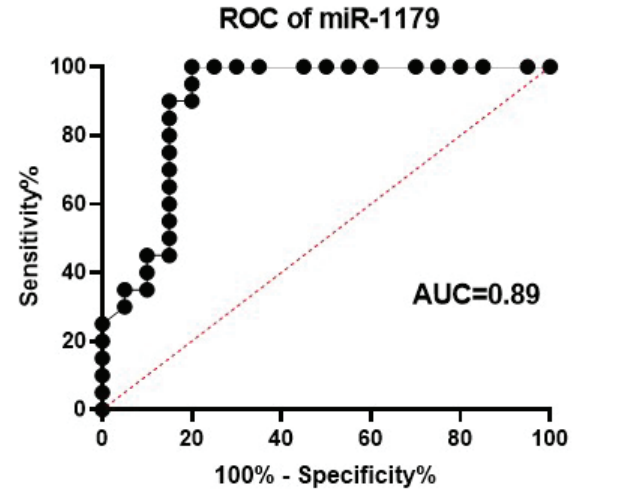

Figure 3. ROC curve analysis results reveal the diagnostic performance of miR-1179.

DISCUSSION

Epileptogenesis which includes many molecular and cellular processes is a complex process that has not been enlightened. Different epilepsy syndromes may have different etiology. Therefore, the determination of biomarkers associated with epilepsy is quite important to be used for diagnosis, prognosis, and treatment (13).

Applications of miRNA-based biomarkers have recently been an extensive and remarkable field of investigation. miRNAs are the most investigated subtypes of regulatory small non-coding RNAs. miRNAs are promising biomarkers thanks to their stability in extracellular areas, high stability in pre-clinical samples, and tissue specificity (14). Lots of research suggests that miRNAs have shown altered expression levels and/or dysfunction in various conditions with different types of cancer, cardiovascular disease, Parkinson's disease, epilepsy, multiple sclerosis, Alzheimer's disease, glioblastoma, and myasthenia gravis (8).

Studies published in PubMed from 2000 to 2017 to determine miRNA biomarkers in epilepsy patients were compiled by Yihong Ma et al., and according to this review, decreasing expression levels of miR-134 could be a diagnostic biomarker for epilepsy. miR-181 level downregulates in the acute stage of epilepsy, and this data supports that miRNA could be used to specify the disease phase. In addition, miR-15a-5p, miR-128, miR-199a, miR-124, and miR-194-5p could be candidate diagnostic biomarkers in patients with epilepsy (15). In a recent study by Ferreira et al., serum levels of miR-155 and miR-146a were found up-regulated in patients with genetic generalized epilepsy compared to controls, therefore, these miRNAs were suggested as diagnostic biomarkers (13). Another study indicated that serum levels of miR-106b and miR-146a were statistically significantly increased in patients with focal and generalized epilepsy (16).

Taken together, miRNAs should be considered as remarkable biomarkers in the diagnosis and prognosis of epilepsy. Therefore, this study aimed to assess the diagnostic biomarker performance of miR-1179 in JME. To the best of this researcher's knowledge, the expression level of miR-1179 has not been evaluated in any neurological disorders to date. According to study results, miR-1179 was significantly upregulated in JME patients compared to healthy controls.

As known, ROC curve analysis is used as an efficient graphical tool to evaluate the diagnostic significance of biomarkers by calculating the sensitivity and the specificity of interested miRNA (17). In the present study, the AUC value was calculated through ROC curve analysis to evaluate miR-1179 as a diagnostic biomarker. Accordingly, this study suggests miR-1179 as a useful diagnostic biomarker in JME.

According to miRTarBase, hsa-miR-1179 targets *CALM1* mRNA from the 3'UTR (9). *CALM1* (ENSG00000198668) encodes Calmodulin-1 protein (Uniprot ID: P0DP23). Calcium (Ca^{2+}) flux

across cell membranes play a key role in the cellular responses while calmodulin (CaM) perceives Ca^{2+} concentration changes. CaM detects such alteration, and it relays this information to interaction partners. The accurate Ca^{2+} signaling is driven by three independent calmodulin-encoding genes (*CALM1-3*) in the genome and these three genes encode entirely the same functional CaM protein (18). CaM regulation of Ca^{2+} channels is the main point to Ca^{2+} signaling (19). The *CACNA1A* encodes a subunit of the voltage gated Ca^{2+} channel. Further, voltage dependent Ca^{2+} channels are involved in various biological processes such as releasing a neurotransmitter or hormone, muscle contraction, gene expression, and pathogenic changes in *CACNA1A* that have been associated with numerous diseases (MIM: 601011).

In 2001, Chioza et al. showed that *CACNA1A* was directly related to the etiopathogenesis of the idiopathic generalized epilepsy group which comprises JME (20). According to the STRING, which is a database that reliably presents protein-protein interactions based on both experimental and literature research data, the CACNA1A and CALM1 proteins are directly related (10). Consequently, it may be speculated that *CALM1* contributes to the etiology of JME. Based on this information, this study suggests that miR-1179 is involved in the pathogenesis of JME by targeting *CALM1* mRNA.

CONCLUSION

Considering calcium channelopathies in epilepsy, the low expression of *CALM1* due to increased expression of miR-1179 could be a role in the etiopathogenesis of JME. Additionally, this study emphasizes that miR-1179 could be a useful diagnostic biomarker in JME. To this researcher's knowledge, the present study may be the first in terms of evaluating miR-1179 in any neurological disease. Further analysis and functional studies in larger cohorts may enlighten the effect of miR-1179 on the pathogenesis of JME and could reveal its diagnostic value accurately.

Ethics Committee Approval: This study was approved by the ethics committee of Bezmialem Vakif University Faculty of Medicine (06.04.2021-E.11759).

Peer-review: Externally peer-reviewed.

Author Contributions: Conception/Design of Study - S.S., C.T., A.B., F.U., E.Y.; Data Acquisition - S.S., C.T., A.B., F.U., E.Y.; Data Analysis/Interpretation - S.S., E.Y.; Drafting Manuscript - S.S.; Critical Revision of Manuscript - E.Y.; Final Approval and Accountability - S.S., C.T., A.B., F.U., E.Y.

Financial Disclosure: This work was supported by the grants of Scientific Research Projects Coordination Unit of Bezmialem Vakif University (Grant reference number: 20211202).

Conflict of Interest: The authors have no conflict of interest to declare.

REFERENCES

1. Moshe SL, Perucca E, Ryvlin P, Tomson T. Epilepsy: new advances. Lancet 2015; 385(9971): 884-98. [CrossRef]
2. Pitkanen A, Loscher W, Vezzani A, Becker AJ, Simonato M, Lukasiuk K, et al. Advances in the development of biomarkers for epilepsy. Lancet Neurol 2016; 15(8): 843-56. [CrossRef]
3. Pietrafusa N, La Neve A, de Palma L, Boero G, Luisi C, Vigevano F, et al. Juvenile myoclonic epilepsy: Long-term prognosis and risk factors. Brain Dev 2021; 43(6): 688-97. [CrossRef]
4. Amrutkar C, Riel-Romero RM. Juvenile Myoclonic Epilepsy. 2021 Aug 11. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022.
5. Vishnoi A, Rani S. MiRNA Biogenesis and Regulation of Diseases: An Overview. Methods Mol Biol 2017; 1509: 1-10. [CrossRef]
6. Hydbring P, Badalian-Very G. Clinical applications of microRNAs. F1000Res 2013; 2: 136. [CrossRef]
7. Süsgün S, Karacan İ, Yücesan E. Optimization of One Step Reverse Transcription Quantitative PCR Method for miRNA Expression Analyses. Experimed 2021; 11(2): 113-9. [CrossRef]
8. Condrat CE, Thompson DC, Barbu MG, Bugnar OL, Boboc A, Cretoiu D, et al. miRNAs as Biomarkers in Disease: Latest Findings Regarding Their Role in Diagnosis and Prognosis. Cells 2020; 9(2). [CrossRef]
9. Huang HY, Lin YC, Li J, Huang KY, Shrestha S, Hong HC, et al. miRTarBase 2020: updates to the experimentally validated microRNA-target interaction database. Nucleic Acids Res 2020; 48(D1): D148-D54. [CrossRef]
10. Szklarczyk D, Gable AL, Nastou KC, Lyon D, Kirsch R, Pyysalo S, et al. The STRING database in 2021: customizable protein-protein networks, and functional characterization of user-uploaded gene/measurement sets. Nucleic Acids Res 2021; 49(D1): D605-D12. [CrossRef]
11. Dziadkowiak E, Chojdak-Lukasiewicz J, Olejniczak P, Paradowski B. Regulation of microRNA Expression in Sleep Disorders in Patients with Epilepsy. Int J Mol Sci 2021; 22(14). [CrossRef]
12. Riedel G, Rudrich U, Fekete-Drimusz N, Manns MP, Vondran FW, Bock M. An extended DeltaCT-method facilitating normalisation with multiple reference genes suited for quantitative RT-PCR analyses of human hepatocyte-like cells. PLoS One 2014; 9(3): e93031. [CrossRef]
13. Martins-Ferreira R, Chaves J, Carvalho C, Bettencourt A, Chorao R, Freitas J, et al. Circulating microRNAs as potential biomarkers for genetic generalized epilepsies: a three microRNA panel. Eur J Neurol 2020; 27(4): 660-6. [CrossRef]
14. Krauskopf J, Verheijen M, Kleinjans JC, de Kok TM, Caiment F. Development and regulatory application of microRNA biomarkers. Biomark Med 2015; 9(11): 1137-51. [CrossRef]
15. Ma Y. The Challenge of microRNA as a Biomarker of Epilepsy. Curr Neuropharmacol 2018; 16(1): 37-42. [CrossRef]
16. An N, Zhao W, Liu Y, Yang X, Chen P. Elevated serum miR-106b and miR-146a in patients with focal and generalized epilepsy. Epilepsy Res 2016; 127: 311-6. [CrossRef]
17. Hsu MJ, Chang YC, Hsueh HM. Biomarker selection for medical diagnosis using the partial area under the ROC curve. BMC Res Notes 2014; 7: 25. [CrossRef]
18. Jensen HH, Brohus M, Nyegaard M, Overgaard MT. Human Calmodulin Mutations. Front Mol Neurosci 2018; 11: 396. [CrossRef]

19. Mori MX, Vander Kooi CW, Leahy DJ, Yue DT. Crystal structure of the CaV2 IQ domain in complex with Ca2+/calmodulin: high-resolution mechanistic implications for channel regulation by Ca2+. Structure 2008; 16(4): 607-20. [CrossRef]

20. Chioza B, Wilkie H, Nashef L, Blower J, McCormick D, Sham P, et al. Association between the alpha(1a) calcium channel gene CACNA1A and idiopathic generalized epilepsy. Neurology 2001; 56(9): 1245-6. [CrossRef]