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RENATA PARISSI BUAINAIN

IDENTIFICAÇÃO DE VARIANTES GÊNICAS DE BASE ÚNICA NOS GENES *SCN1A* E *MIR-146A* EM PACIENTES COM EPILEPSIA DO LOBO TEMPORAL

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RENATA PARISSI BUAINAIN - R.A.: 001 2020 10047

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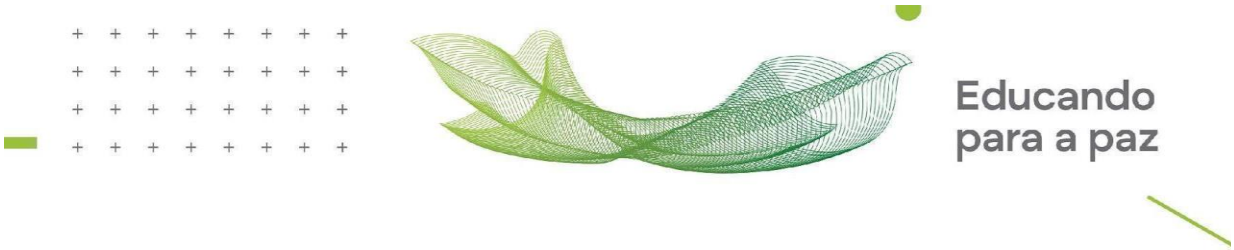

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Universidade São Francisco

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Dedico este trabalho aos meus familiares que souberam respeitar a minha ausência em diversos momentos e, sobretudo, aos meus pais (*in memoriam*) que me ensinaram o valor da educação.

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Pesquisadores Colaboradores:

Profa. Dra. Andréia de Melo Porcari

Programa de Pós-Graduação em Ciências da Saúde, USF

Laboratório de Espectroscopia de Massa

Prof. Dr. Carlos Tadeu Parisi de Oliveira

Departamento de Neurocirurgia

Hospital São Francisco na Providência de Deus

Bragança Paulista, SP

Prof. Me. Evaldo Pasquini Landi

Departamento de Hematologia e Oncologia, USF

Chefe do Hemonúcelo do Hospital São Francisco na Providência de Deus

Bragança Paulista, SP

Prof. Dr. Fernando Augusto Lima Marson

Programa de Pós-Graduação em Ciências da Saúde, USF

Laboratório de Biologia Celular e Molecular de Tumores e Compostos Bioativos

Laboratório de Genética Médica e Humana.

Profa. Dra. Juliana Mozer Sciani

Programa de Pós-Graduação em Ciências da Saúde, USF

Laboratório de Farmacologia Molecular e Compostos Bioativos

Profa. Dra. Karen Antônia Girotto Takazaki

Departamento de Neurologia

Universidade São Francisco

Bragança Paulista, SP

Profa. Dra. Manoela Marques Ortega

Programa de Pós-Graduação em Ciências das Saúde, USF

Laboratório de Biologia Celular e Molecular de Tumores e Compostos Bioativos

Laboratório de Genética Médica e Humana.

Profa. Dra. Raquel Girardello

Programa de Pós-Graduação em Ciências da Saúde, USF

Laboratório de Microbiologia e Imunologia

Alunos:

André Rodrigues Sodré (Iniciação Científica)

Gabriel Alves Bonafé (Doutorando)

Jéssica Silva dos Santos (Doutoranda)

Programa de Pós-Graduação em Ciências da Saúde, USF

Laboratório de Biologia Celular e Molecular de Tumores e Compostos Bioativos

Laboratório de Genética Médica e Humana.

Alunos:

Alexandre Varão Moura (Doutorando)

Pedro Henrique Godoy Sanches (Doutorando)

Programa de Pós-Graduação em Ciências da Saúde, USF

MS^4 *Life* Laboratório de Espectroscopia de Massa

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Epígrafe

"O poder nasce do querer. Sempre que o homem aplicar a veemência e a perseverante energia de sua alma a um fim, vencerá os obstáculos e, se não atingir o alvo fará, pelo menos, coisas admiráveis."

José de Alencar

RESUMO

A suscetibilidade à epilepsia e sua resistência a fármacos foram relacionadas a variantes de nucleotídeo único (SNVs) no gene *SCN1A* e no microRNA(miR)-146a. O *SCN1A* é responsável pela tradução da proteína NaV1.1 do canal de sódio e miR-146a regula o processo inflamatório pela via NF-kB. As SNVs rs2298771 e rs3812718 no gene *SCN1A* e rs2910164 no miR146a foram estudadas em diferentes populações com resultados conflitantes; além disso, não há estudos correlacionando tais SNVs e o risco para epilepsia de lobo temporal (ELT) resistente ou responsiva à fármacos no Brasil. O objetivo do estudo foi associar os genótipos das SNVs com a suscetibilidade à ELT, resistente e responsiva. Após análise de 1.272 prontuários, foram incluídos 70 pacientes com ELT [55 responsivos e 15 resistentes]. Também, foram adicionados ao estudo 50 amostras de tecido cerebral de pacientes com ELT resistentes obtidas por amidalohipocampectomia. Um total de 240 doadores de sangue sadios foram selecionados como população controle. A extração de ácidos desoxirribonucleicos foi realizada a partir de leucócitos dos pacientes com ELT e dos controles; e a partir dos tecidos parafinados das amostras de tecido cerebral. A identificação dos genótipos foi realizada pela reação em cadeia da polimerase em tempo real. A expressão relativa dos genes *SCN1A* e *MIR-146A* foi realizada a partir de ácidos ribonucleicos de leucócitos ou tecidos parafinados de pacientes com diferentes genótipos para as SNVs. Fisher ou X^2 avaliaram a diferença nas frequências genotípicas e o risco para a doença pela razão das chances (OR) com intervalo de confiança de 95% (IC95%). Foi observada maior frequência do genótipo AA versus AG+GG (42,50% vs. 26,67%, *p*-valor=0,059) em comparação com controles para a SNV rs2298771; além disso, uma maior frequência do alelo A (68,30% vs. 58,50%, *p*-valor=0,014) em pacientes em comparação com controles. Pacientes responsivos aos fármacos apresentam maior frequência do genótipo AA versus AG+GG (47,27% vs. 26,67%, *p*-valor=0,005) e maior risco para doença [2,47(IC95%=1,35-4,50)] em comparação aos controles. Maior frequência do alelo A foi observada em respondedores (77,00% vs. 58,50%, *p*-valor=0,035) em comparação aos controles. O nível de expressão relativa de *SCN1A* foi maior em CT (*p*-valor=0,004) e CC (*p*-valor=0,002) em comparação ao genótipo TT. Frequência aumentada do genótipo variante (CC) para a SNV rs2910164 foi observada em pacientes com ELT (15,00% vs. 5,42%, *p*-valor=0,009) e em pacientes com ELT responsivos (21,81% vs. 5,42%, *p*-valor=0,003) em comparação a controles. Pacientes responsivos a drogas com genótipos GC (*p*-valor=0,049) ou CC (*p*-valor=0,039) apresentaram expressão relativa de *MIR146A* menor do que pacientes resistentes aos fármacos. Pacientes com genótipos combinados rs2910164-CC e rs2298771-AA (7,5% vs. 1,7%) apresentaram OR=4,76 (IC95%=1,30-21,62) em comparação com indivíduos controles. Pacientes responsivos aos fármacos apresentaram maior risco para os genótipos combinados rs2910164-CC e rs2298771-AA (9,1% vs. 1,7%; OR=5,849 (IC95%=1,21-30,57) em comparação aos controles. O genótipo AA para a SNV rs2298771 (gene *SCN1A*) e o CC para rs2910164 (gene *MIR-146a*) demonstraram maior risco para o desenvolvimento da ELT responsiva devido, provavelmente, a ativação exacerbada de NF-kB e perda de função de *SCN1A* em pacientes com genótipos CC/TT para as SNVs.

***Palavras-chaves**: Epilepsia do Lobo Temporal. Biomarcadores Farmacológicos. Genética Médica. Canal de Sódio Disparado por Voltagem NAV1.1. MicroRNAs.*

ABSTRACT

Single nucleotide variants (SNVs) have been related to the susceptibility to epilepsy and drug resistance in the *SCN1A* gene and microRNA(miR)-146a. The *SCN1A* is responsible for translating the sodium channel protein NaV1.1 and miR-146a regulates the inflammatory process by modulating the NF-kB pathway. Thus, the SNVs rs2298771 and rs3812718 in the *SCN1A* gene and rs2910164 in the miR146a were studied in different populations with conflicting results; moreover, there are no studies correlating such SNVs and the risk for drug-resistant or drug-responsive temporal lobe epilepsy (TLE) in Brazil. We aimed to identify the different genotypes of the above SNVs and associate them with susceptibility to TLE with resistance and responsiveness to treatment. After analyzing 1,272 medical records, 70 patients with TLE were included (55 drug-responsive and 15 drug-resistant). Also, 50 brain tissue samples from patients with resistant TLE obtained by hippocampectomy were added to the study. A total of 240 healthy blood donors were selected as a control population. The extraction of deoxyribonucleic acids was from leukocytes of patients with TLE and control individuals; and also, from the paraffin-embedded tissues of brain tissue samples. Identification of genotypes was performed by real-time polymerase chain reaction. The relative expression of the *SCN1A* and *MIR-146A* genes was performed using ribonucleic acids extracted from leukocytes or paraffin-embedded tissues from patients with different genotypes for the SNVs. Fisher or X^2 evaluated the difference in genotypic frequencies and risk for disease by odds ratio (OR) with a 95% confidence interval (95%CI). Increased frequency of AA versus AG+GG genotypes (42.50% vs. 26.67%, p-value=0.059) when compared to controls was observed for SNV rs2298771; in addition, a higher frequency of the A allele (68.30% vs. 58.50%, p-value=0.014) in patients when compared to controls. Drug-responsive patients have a higher frequency of the AA versus AG+GG genotype (47.27% vs. 26.67%, p-value=0.005) and a higher risk for the disease [2.47(95%CI=1.35-4 .50)] when compared to controls. A higher frequency of A allele was also observed in drug-responsive patients (77.00% vs. 58.50%, p-value=0.035) compared to controls. The relative expression level of *SCN1A* was significantly higher in CT (p-value=0.004) and CC (p-value=0.002) when compared to the TT genotype. Increased frequency of variant genotype CC for SNV rs2910164 was observed in TLE patients (15.00% vs. 5.42%, p-value=0.009) and in drug-responsive TLE (21.81% vs. 5.42%, p-value=0.003) when compared to controls. Drug-responsive patients harboring GC (p-value=0.049) or CC (p-value=0.039) genotypes presented lower relative *MIR146A* expression when compared to drug-resistant patients. Patients carrying the combined rs2910164-CC and rs2298771-AA genotypes (7.5% vs. 1.7%) presented OR=4.76 (95%CI=1.30-21.62) when compared to controls. Drug-responsive patients also presented a higher risk for the combined rs2910164-CC and rs2298771-AA genotypes (9.1% vs. 1.7%; OR=5.849 (95%CI=1.21-30.57) when compared to controls. The AA genotype for SNV rs2298771 (*SCN1A* gene) and the CC genotype for rs2910164 (*MIR-146a*) showed a higher risk for the drug-responsive TLE probably due to the increased NF-kB activation and *SCN1A* loss-of-function in patients harboring CC/TT genotypes.

Keywords*: Temporal Lobe Epilepsy. Biomarkers, Pharmacological. Genetics, Medical. NAV1.1 Voltage-Gated Sodium Channel. MicroRNAs.*

Lista de Símbolos e Abreviações

ABCB1: Adnosine Triphosphate-Binding Cassette Sub-Family B Member 1

ABCC2: Adnosine Triphosphate Binding Cassette Sub-Family C Member 2

ARX: Aristaless Related Homeobox

ATP: *Adnosine Triphosphate*

CDKL5: X-linked cyclin-dependent kinase-like

CHRNA4: Cholinergic Receptor Nicotinic Alpha 4 Subunit

CHRNB2: Cholinergic Receptor Nicotinic Beta 2 Subunit

CLCN2: Chloride Voltage-Gated Channel 2

CNV: *Copy Number Variation*

CI: *Confidence Interval*

CYP1A1: Cytochrome P450 Family 1 Subfamily A Member 1

CYP2C9: Cytochrome P450 Family 2 Subfamily C Member 9

DNA: Ácido Desoxirribonucleico

EEG: Eletroencefalograma

ELT: Epilepsia de Lobo Temporal

FAE: Fármaco Antiepilético

GABA: Ácido Gama-Aminobutírico

GABRA1: Gamma-Aminobutyric Acid Type A Receptor Subunit Gamma1

GABRA2: Gamma-Aminobutyric Acid Type A Receptor Subunit Gamma2

GABRA3: Gamma-Aminobutyric Acid Type A Receptor Subunit Gamma3

GAT3: GABA transporter type 3

HLA-B1502: Human Leukocyte Aantigen B1502

IBGE: *Brazilian Institute of Geography and Statistics*

IC: Intervalo de Confiança

IL: *Interleukin*

ILAE: *International League Against Epilepsy*

IRAK:2 Interleukin 1 Receptor Associated Kinase 2

IRAK1: Interleukin 1 Receptor Associated Kinase 1

IRM: Imagem por Ressonância Magnética

Kb: kilobase

KCNQ2: Potassium Voltage-Gated Channel Subfamily Q Member 2

KCNQ3: Potassium Voltage-Gated Channel Subfamily Q Member 3

miR: MicroRNA

NF-kB: *Nuclear Factor Kappa B*

OR: Odds Ratio

Pb: pares de bases

PCDH19: Protocadherin 19

PCR: Reação em Cadeia da Polimerase

PET: Tomografia por Emissão de Pósitrons

RA: Registro Acadêmico

RNA: Ácido Ribonucleico

SCN1A: Sodium Voltage-Gated Channel Alpha Subunit 1

SCN1B: Sodium Voltage-Gated Channel Beta Subunit 1

SCN2A: Sodium Voltage-Gated Channel Alpha Subunit 2

SIRT1: Antiapoptotic Protein Silent InformationRregulator 1

SLC2A1: Solute Carrier Family 2 Member 1

SLC6A4: Solute Carrier Family 6 Member 4

SNV: *Single Nucleotide Variant*

SPECT: Tomografia por Emissão de Fóton Único

STK9: Serine Threonine-Protein Kinase 9

STXBP1: Syntaxin Binding Protein 1

TNF: *Tumor Necrosis Factor*

TRAF6: *Tumor Necrosis Factor Receptor Associated Factor 6*

USF: Universidade São Francisco

UA: Unidades arbitrárias

WHO: *World Health Organization*

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SUMÁRIO

1. INTRODUÇÃO

A epilepsia é uma doença do sistema nervoso central, crônica, podendo acometer indivíduos de todas as idades e prejudicar diretamente a qualidade de vida dos indivíduos afetados (STAFSTROM; CARMANT, 2015). É uma doença de acometimento mundial e definida por pelo menos duas crises epilépticas não provocadas ocorrendo em um intervalo superior a 24 horas. Também, pode ser definida por uma crise epiléptica não provocada com probabilidade de ocorrência de crises similar ao risco de recorrência, que em geral é de pelo menos 60%, após duas crises epilépticas não provocadas em 10 anos ou o indivíduo apresenta o diagnóstico de uma síndrome epiléptica. Ainda, uma crise epiléptica é a ocorrência transitória de sinais e sintomas decorrentes da atividade anormal excessiva ou síncrona no cérebro (FISHER et al., 2014).

1.1 Incidência e Prevalência da Epilepsia

De acordo com Fiest et al. (2017), a incidência média mundial da epilepsia ativa é de 5,4 por 1.000 pessoas e a incidência média mundial ao longo da vida, de aproximadamente 7,0 por 1.000 pessoas. Os autores também observaram uma incidência média de epilepsia de 56,8 por 100.000 pessoas por ano sendo a incidência mais alta na adolescência. A incidência mais alta foi em homens, sendo 7,3 por 1.000, comparado com 6,8 por 1.000 mulheres. A taxa de incidência de epilepsia também foi maior nos países subdesenvolvidos (6,7 por 1.000) em comparação aos países com alta renda (5,5 por 1.000). Nesse mesmo estudo, a prevalência geral de epilepsia ao longo da vida foi de 7,60 por 1.000 habitantes e a prevalência de epilepsia ativa foi de 6,38 por 1.000 habitantes (FIEST et al., 2017). Em 2016, havia 45,9 milhões de indivíduos com epilepsia ativa de natureza idiopática ou secundária, globalmente, sendo que destes, 24 milhões apresentaram somente epilepsia ativa idiopática (BEGHI, 2019). Assim, atualmente a Organização Mundial de Saúde (WHO, 2022) estima cerca de 50 milhões de pessoas diagnosticadas com epilepsia no mundo e uma estimativa de incidência anual de 2,4 milhões de novos casos diagnosticados (JALLON, 2002).

Nos Estados Unidos a prevalência foi de 8,4 casos de epilepsia por 1.000 indivíduos, com taxas significativamente mais altas em pessoas com menos de 5 ou mais de 60 anos de idade e a incidência foi de 79,1 por 100.000 habitantes (HELMERS et al., 2015).

No Brasil existem poucos estudos epidemiológicos sobre a epilepsia, sendo inexistentes os estudos de incidência. Em 1986, um estudo observou a prevalência em São Paulo em torno de 11,9

para 1.000 indivíduos (MARINO; CUKIERT, 1986) e em Porto Alegre, em 1992, a prevalência foi de 16,5 por 1.000 (FERNANDES et al., 1992). Em 2002, no Rio de Janeiro, a prevalência de epilepsia ativa foi de 5,1 por 1.000 pessoas (GOMES et al., 2002). Entretanto, em um único estudo os autores calcularam, usando como base a população total brasileira e a média de prevalência mundial de pessoas, a quantidade de epilépticos no Brasil, chegando-se ao número aproximado de 9 milhões (NETO; MARCHETTI, 2005). Segundo o Instituto Brasileiro de Geografia e Estatística (IBGE, 2022) há no Brasil cerca de 203 milhões de pessoas e a WHO, 2022 estima que 2% da população brasileira tenha epilepsia.

Mais recentemente, nosso grupo realizou um estudo epidemiológico dos casos de epilepsia na cidade de Bragança Paulista, São Paulos, e verificou uma prevalência total de 1,30 pacientes/1.000 habitantes (BUAINAIN et al., 2022).

1.2 Classificação das epilepsias

A nova classificação da Liga Internacional Contra a Epilepsia (ILAE), utiliza o tipo de crise epiléptica, o tipo de epilepsia e as síndromes epilépticas (FISHER et al., 2017). As epilepsias são classificadas em focais, generalizadas, focais e generalizadas e desconhecidas. O termo epilepsia focal refere-se a crises epilépticas originadas e limitadas a um hemisfério cerebral. Elas podem ser discretamente localizadas ou mais amplamente distribuídas neste hemisfério. As crises generalizadas têm início em algum ponto do cérebro e rapidamente tornam-se bilaterais (FISHER et al., 2017). Crise convulsiva é um termo popular, não oficial, para designar atividade motora durante uma crise epiléptica. Por definição, entende-se como crise convulsiva qualquer crise epiléptica com abalos motores, à medida que as crises epilépticas não-convulsivas são interpretadas como ausência de abalos motores. Clinicamente, a classificação da crise epiléptica começa com a determinação de sua manifestação inicial, podendo esta ser focal (em um hemisfério, anteriormente denominada crise parcial) ou generalizada (em ambos os hemisférios), embora, alguns casos a presença de um início obscuro com ausência de adequação nos critérios diagnósticos levem a classificação como desconhecida. As crises de início focal têm origem em um determinado ponto de um hemisfério cerebral. Podem ser classificadas em perceptivas (quando o indivíduo tem percepção de si e do meio à sua volta) e não perceptivas (quando o indivíduo não tem percepção de si próprio e do meio à sua volta). As crises focais ainda são subclassificadas em início motor e não motor (FISHER et al. 2017). Os sintomas motores incluem automatismo (uma atividade motora

mais ou menos coordenada e repetitiva, normalmente ocorre quando a cognição está prejudicada e o indivíduo fica amnésico após a crise), atônico (diminuição repentina de tônus muscular com duração de 1 a 2 segundos), clônica (contração involuntária e repetitiva envolvendo os mesmos grupos musculares, com grande duração), espasmos epilépticos (uma flexão, extensão ou uma mistura de flexão e extensão de músculos predominantemente proximais e do tronco), hipercinética (envolve predominantemente membros proximais ou músculos axiais, produzindo movimentos balísticos sequenciais e irregulares, como pedalar), mioclônica (contrações súbitas únicas ou múltiplas e breves de músculos de variada topografia) e tônica (um aumento sustentado na contração muscular, durando alguns segundos a minutos). Os sintomas não motores manifestam-se por alteração autonômica (sensação consistente com o envolvimento do sistema nervoso autônomo, incluindo funções cardiovasculares, termorreguladoras, vasomotoras e dentre outros), alteração de comportamento (perda de discernimento social, apatia, episódio maníaco e dentre outros), alteração cognitiva (consiste em linguagem prejudicada ou outros domínios cognitivos, ou características positivas como *déjá-vus*, alucinações e ilusões), alteração emocional (envolvem, ansiedade, medo ou alegria) e alteração sensorial (uma experiência sensitiva não causada por estímulos apropriados do mundo externo). O termo de focal para bilateral substitui o antigo termo "parcial com generalização secundaria". Crise epiléptica de início generalizado é definida como originada em algum local de uma rede neuronal com rápido envolvimento de redes distribuídas bilateralmente (FISHER et al. 2017). As crises epilépticas de início generalizado, podem ser divididas em crises motoras, subdivididas em tônico-clônica (uma sequência que consiste numa fase tônica e posteriormente uma fase clônica), mioclônico-tônico-clônico (consiste numa fase mioclônica, uma tônica e uma clônica), mioclônico-atônico (uma fase mioclônica e uma fase atônica) e não motores, subdividido em atípicas (possui apresentação clínica atípica e ondas atípicas no eletroencefalograma) e típica (FISHER et al. 2017; SCHFFER et al., 2017) (Figura 1).

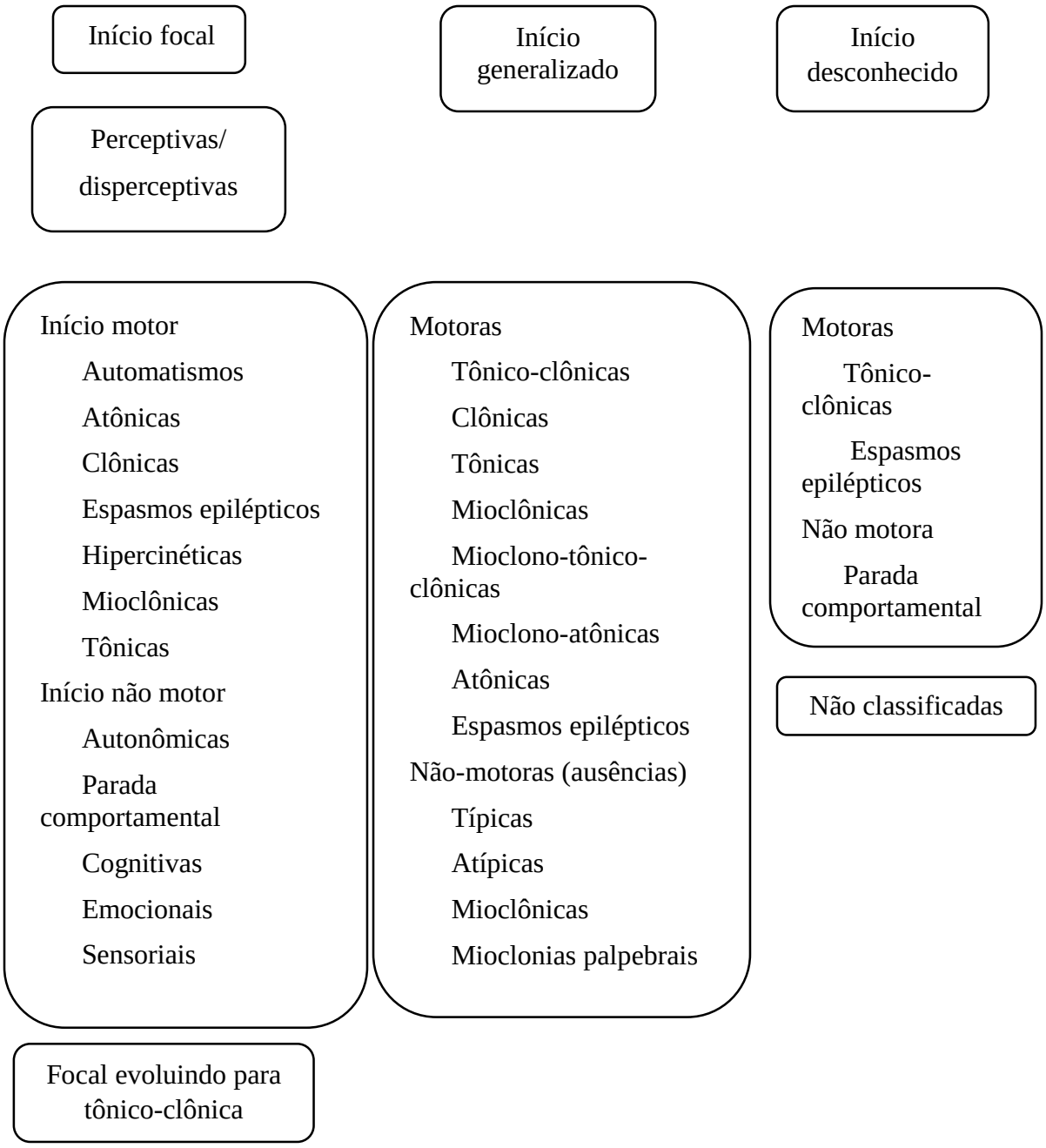

FIGURA 1. Classificação dos tipos de crises da ILAE 2017 (FISHER et al. 2017).

1.3 Epilepsia do lobo temporal

Existem poucos estudos epidemiológicos sobre ELT. Destaca-se o de Semah et al. (1998), que analisou a localização do foco epiléptico em 806 pacientes com epilepsia focal e destes casos, 66% tinham ELT, 25% epilepsia do lobo frontal, 2% epilepsia do lobo parietal, 3% epilepsia do lobo occipital e 3% epilepsia multilobar. Entretanto, a ELT mesial (ELTm) é a síndrome epiléptica mais comum em adultos e aproximadamente 40% destes são de difícil controle das crises

(ENGLOT; MORGAN; CHANG, 2020). É classificada como epilepsia focal e as crises originam-se do hipocampo e ou amígdala, denominados de zona epileptogênica (ENGLOT; MORGAN; CHANG, 2020). Pacientes com ELTm sofrem frequentemente com alterações cognitivas, psiquiátricas e comportamentais o que pode afetar suas atividades de vida diária, trabalho e relações interpessoais (CAPLAN, 2019).

1.4 Etiologia das epilepsias

De acordo com a ILAE, as etiologias de crise epiléptica são agrupadas em seis categorias, sendo estrutural, genética, infecciosa, metabólica, imunológica e um grupo de etiologia desconhecida (FISHER et al., 2017).

Resumidamente, a epilepsia estrutural, refere-se quando um achado na neuroimagem está em concordância com a alteração eletroencefalográfica e a manifestação clínica da crise epiléptica (LAPALME-REMIS; CASCINO, 2016). Na genética, a semiologia da crise, uma história familiar relevante e achados típicos no eletroencefalograma (EEG) são suficientes para inferir uma etiologia genética (FALCO-WALTER; SCHEFFER; FISHER, 2018). A epilepsia de etiologia infecciosa é causada por infecções bacterianas, virais, parasitárias ou fúngicas no sistema nervoso central (VEZZANI et al., 2016) e não atribuídas à crise epiléptica na infecção aguda, a qual seria provocada e não espontânea (FALCO-WALTER; SCHEFFER; FISHER, 2018). Doenças metabólicas podem causar crises por deficiência de substratos essenciais para o metabolismo e função da membrana, por acúmulo de substâncias tóxicas intracelular e alteração da osmolalidade intracelular (MESSING; SIMON, 1986). As principais deficiências metabólicas relatadas foram a deficiência da biotinidase e holocarboxilase sintase, a deficiência de folato cerebral, alterações da creatina, crises responsivas à ácido folínico, deficiência do transportador do tipo 1 da glicose, alterações mitocondriais, a epilepsia piridoxina-dependente, alterações do ciclo da uréia, a acidúria glutárica, a deficiência do cofator molibdênio, a hiperglicemia cetótica e não-cetótica e deficiência da dehidrogenase semialdeído succínica (LEE et al., 2018). A epilepsia autoimune, caracteriza-se por alterações inflamatórias ou história pessoal ou familiar de doença autoimune ou presença de anticorpos neuronais no soro ou líquido cefalorraquidiano (GRECO et al., 2016). O tipo de epilepsia desconhecida, engloba pacientes cuja etiologia permanece desconhecida após extensa investigação (FALCO-WALTER; SCHEFFER; FISHER, 2018).

Na epilepsia do lobo temporal (ELT), o tipo de lesão mais comumente observada é a esclerose mesial temporal. Os achados patológicos característicos são perda de neurônios excitatórios e inibitórios em subcampos específicos, brotamento axonal e reorganização sináptica e alterações na função e estrutura glial. Esta sequência de mudanças afeta o balanço entre excitação e inibição no circuito límbico até que se seguem crises epilépticas espontâneas (THIJS et al., 2019).

1.5 Diagnóstico

Os dados clínicos obtidos na anamnese são importantes para determinar em que circunstância ocorreu a crise epilética (STAFSTROM; CARMANT, 2015). Os sinais e sintomas das crises epilépticas são muito variados e incluem alterações sensoriais, motoras, autonômicas e da consciência. A semiologia das crises epilépticas determina a sua classificação (NOACHTAR; PETERS, 2009). O exame físico geral pode determinar uma doença sistêmica subjacente implicada na etiologia da epilepsia. O exame neurológico pode indicar sinais focais inferindo numa etiologia focal para as crises epilépticas. A avaliação metabólica, genética e do líquido cefalorraquidiano podem trazer informações relevantes (STAFSTROM; CARMANT, 2015).

O EEG de superfície ou intracraniano registra a atividade elétrica normal do cérebro, bem como suas alterações focais (epilepsia focal) e ou generalizadas (epilepsia generalizada). O EEG pode ser realizado de diversas formas, de acordo com a semiologia das crises epilépticas. Assim, o EEG deve ser realizado preferencialmente em vigília, em sono, com hiperpnéia e com fotoestimulação, os quais aumentam a sensibilidade do exame (BENBADIS et al., 2020). Em alguns pacientes o EEG de superfície pode ser repetidamente normal, principalmente nos pacientes com ELT. Nessas situações podem ser necessárias a monitorização eletroencefalográfica por vídeo (vídeo-EEG), por horas ou dias e, ou monitorização eletroencefalográfica intracraniana (BENBADIS et al., 2020).

A imagem por ressonância magnética (IRM) é o exame mais útil para o diagnóstico etiológico das epilepsias na busca de lesões estruturais e suas localizações. A IRM funcional é considerada um exame não invasivo que detecta alterações no nível de oxigênio sanguíneo durante a execução de uma tarefa pelo paciente, como a linguagem, a sensibilidade e a motricidade (LAPALME-REMIS; CASCINO, 2016). Nas epilepsias do lobo temporal a IRM é capaz de detectar a gliose na esclerose hipocampal. Se analisados quatro parâmetros simultaneamente

(espessura, área de superfície, volume e curvatura da substância cinzenta), a acurácia da IRM aumenta para mais de 96% nestes pacientes (SIDHU; DUNCAN; SANDER, 2018).

Os exames denominados tomografia por emissão de pósitrons (sigla em inglês PET) e tomografia por emissão de fóton único (sigla em Inglês SPECT) são modalidades de neuroimagem funcional realizadas pela injeção intravenosa de um radiofármaco emissor de pósitrons e a análise subsequente da sua distribuição em um determinado corte do corpo. A PET utiliza a fludeoxiglicose como ligante para analisar o consumo de glicose no metabolismo cerebral na área epileptogênica, a qual evidencia hipometabolismo no período interictal. Já a SPECT utiliza tecnécio-99m-hexametilpropileno amina oxima para mensurar o fluxo sanguíneo na área epileptogênica, o qual estará aumentado durante o período ictal. Estas técnicas são úteis em pacientes com epilepsia e IRM normal (LAPALME-REMIS; CASCINO, 2016).

1.6 Tratamento

Setenta por cento dos pacientes com epilepsia recém diagnosticada podem ser tratados com êxito. Os fármacos antiepiléticos (FAEs) utilizados para o tratamento da epilepsia atuam diminuindo a atividade elétrica cerebral e prevenindo a despolarização através do bloqueio de canais de sódio ou cálcio, potencializando a função do canal de potássio, inibindo a excitação mediada pelo neurotransmissor glutamato ou promovendo a inibição mediada pelo ácido gama-aminobutírico (GABA) (STAFSTROM; CARMANT, 2015).

Em relação ao tratamento medicamentoso da epilepsia é preciso considerar o risco de recorrência de crises epilépticas, o tipo e a etiologia das crises, a escolha do FAE, a idade e o sexo do paciente, o uso de outras medicações e o custo e a duração do tratamento (GAVVALA e SCHUELE, 2016; JOHNSON, 2018).

Ainda, deve-se considerar que o primeiro FAE escolhido deve ser eficaz, bem tolerado e de fácil uso pelo paciente (GAVVALA e SCHUELE, 2016), sendo que a monoterapia é frequentemente a melhor opção, uma vez que, a politerapia pode promover interações entre os FAEs e toxicidade a longo prazo (THIJS et al., 2019).

Os FAEs evoluíram na última década com melhora no perfil de tolerabilidade, mas não da eficácia. Assim, podem ser classificados em primeira geração (fenobarbital, fenitoína, carbamazepina e ácido valpróico), segunda geração (gabapentina, lamotrigina, levetiracetam, oxcarbazepina, tiagabina, topiramato e zonisamida) e terceira geração (eslicarbazepina,

lacosamida, perampanel, pregabalina e rufinamida) (FRENCH et al., 2004). Apesar dos fármacos de terceira generação, 30% dos pacientes ainda são refratários à terapia medicamentosa (Vidaurre e Herbst, 2019).

De acordo com as recomendações da academia americana de neurologia, a carbamazepina e a lamotrigina são fármacos de escolha para o tratamento da epilepsia focal do adulto e o ácido valpróico para a epilepsia generalizada do adulto (KANNER et al., 2018). A epilepsia refratária a medicamentos é definida como a falha do uso adequado de dois FAEs bem tolerados e apropriadamente escolhidos, em monoterapia ou em combinação, em promover o controle definitivo das crises epilépticas (KWAN, 2010).

Se confirmado o diagnóstico da epilepsia refratária, um tratamento não farmacológico pode ser uma alternativa. Isto pode incluir intervenção cirúrgica, técnicas de neuromodulação e dieta cetogênica. O tratamento cirúrgico visa remover ou desconectar uma área circunscrita do cérebro tentando obter um melhor controle das crises epilépticas (THIJS et al., 2019). O resultado pós operatório é avaliado de acordo com a classificação de Engel. Assim, Engel I, pacientes livres de crises incapacitantes; Engel II, raras crises incapacitantes; Engel III, melhora significativa das crises em termos quantitativos (redução do número de crises >50% e/ou aumento do intervalo entre as crises maior que 50% que previamente a cirurgia); Engel IV, sem melhora significativa em termos quantitativos (redução das crises <50%, piora das crises após a cirurgia e/ou ausência de mudança no pós-operatório) (ENGEL, 1995). Recentemente, o uso do canabidiol tem sido cada vez mais difundido como opção terapêutica para os casos de epilepsia refratários, podendo reduzir em até 50% o número de crises epilépticas (ARZIMANOGLOU et al., 2020).

Mais de dois terços dos indivíduos têm epilepsia focal do lobo temporal (SIDHU; DUNCAN; SANDER, 2018; JOHNSON, 2018). A ELT é o tipo mais comum de epilepsia refratária à FAEs (ALLONE et al. 2017). As crises podem se originar da parte mesial ou lateral, da região temporal ou de ambas, eventualmente com extensão para áreas vizinhas. As crises ocorrem com ou sem evolução para tônico-clônica bilateral originando-se do lobo temporal, baseado em achados eletro-clínicos (MUHLHOFER et al., 2017).

1.7 Genética das epilepsias

A epilepsia pode ser resultado tanto de anormalidades genéticas primárias ou secundárias, quanto de desordens estruturais e metabólicas, as quais podem também possuir causas genéticas

(WANG, et al., 2017). Foi estimado que mais da metade das epilepsias apresentam base genética (PAL; PONG; CHUNG, 2010), tanto que a epilepsia generalizada idiopática foi denominada epilepsia generalizada genética pela ILAE (BERG, et al., 2010), além de definirem as epilepsias genéticas como crises que ocorrem por causa de um defeito genético presumido ou conhecido (BERG, et al., 2010).

A maioria das epilepsias familiares, como a epilepsia generalizada genética e a ELT familiar não possuem herança mendeliana (CROMPTON, et al., 2010; HELBIG, et al., 2008) mas, padrão de herança mais complexo, no qual o fenótipo é determinado por mutações/polimorfismos em um gene e talvez por interação ambiental (THOMAS; BERKOVIC, 2014).

Mutações foram descritas no primeiro gene envolvido com a epilepsia, o *CHRNA4* (*cholinergic receptor nicotinic alpha 4 subunit*) em 1994, um dos genes para epilepsia do lobo frontal noturna autossômica dominante (HELBIG; HEINZEN; MEFFORD, 2016). Entretanto, de acordo com pesquisas feitas na base de dados da Herança Mendeliana do Homem Online (https://www.omim.org), há cerca de 84 genes classificados como genes epilépticos (WANG, et al., 2017) devido a identificação frequente de diferentes mutações. Existem inúmeros espectros de expressão fenotípicas para a epilepsia, podendo variar de epilepsias monogênicas com pouco ou sem efeitos ambientais e as epilepsias poligênicas complexas e herdadas (THOMAS; BERKOVIC, 2014). Os principais genes identificados e relacionados com os distúrbios epilépticos monogênicos estão representados no quadro 1 (SCHULTE, et al., 2006).

Os canais iônicos são proteínas de membrana que formam uma espécie de poro, na qual há o transporte passivo de um íon específico para dentro da membrana; este tipo de transporte ocorre devido a diferença de concentração iônica extra e intracelular e também ao potencial de membrana (HATTA; SAKAMOTO; HORIO, 2002). Ainda, a crise epiléptica é um período de excitação anormal de uma população de neurônios de maneira interativa. O balanço entre sistemas que facilitam a excitação e mecanismos que perturbam o sistema de inibição podem levar ao surgimento das crises (SCHARFMAN, 2007). Canais iônicos comandam o processo de excitabilidade dos neurônios, além da comunicação entre eles pela liberação de neurotransmissores. Desta forma, mutações em genes que codificam esses canais e suas subunidades acessórias apresentam um papel importante em várias desordens que se associam à hiper ou hipoexcitabilidade neuronal (STEINLEIN, 2004).

Quadro 1. Principais genes identificados e relacionados com os distúrbios epilépticos monogênicos.

Tipos de genes	**Sigla**	**Nomenclatura dos genes**
Genes codificadores do canal de cloreto	*CLCN2*	*Chloride Voltage - Gated Channel 2*
Genes codificadores do canal de potássio dependente de voltagem	*KCNQ2* *KCNQ3*	*Potassium Voltage - Gated Channel Subfamily Q Member 2* *Potassium Voltage - Gated Channel Subfamily Q Member 3*
Genes codificadores do canal de sódio dependente de voltagem	*SCN1A* *SCN2A* *SCN1B*	*Sodium Voltage-Gated Channel Alpha Subunit 1 and 2* *Sodium Voltage-Gated Channel Beta Subunit 1*
Genes codificadores das subunidades alfa do receptor neuronal de acetilcolina	*CHRNA4* *CHRNA2*	*Cholinergic Receptor Nicotinic Alpha 4 Subunit* *Cholinergic Receptor Nicotinic Alpha 2 Subunit*
Genes codificadores da subunidade alfa-1 do receptor do ácido gama-aminobutírico	*GABRA1* *GABGR2*	*Gamma-Aminobutyric Acid Type A Receptor Subunit Alpha1* *Gamma-Aminobutyric Acid Type A Receptor Subunit Gamma2*

A variabilidade na penetrância e expressividade associadas às mutações nas epilepsias monogênicas sugerem combinação entre mutações gênicas e fatores ambientais (DIBBENS; HERON; MULLEY, 2007). A principal forma de agrupar os genes relacionados com a epilepsia é pela idade de início das manifestações epiléticas (BLUME-CHAIR, et al., 2001; BERG; CROSS, 2010). Assim, a epilepsia com manifestação no primeiro ano é conhecida como epilepsia neonatal familiar benigna e é causada por mutações nos genes *KCNQ2* e *KCNQ3*; a epilepsia com manifestação após o primeiro ano, é chamada de epilepsia neonatal familiar infantil benigna e relacionada com mutações no gene *SCN2A* (ZARA, et al., 2013). Ainda, mutações nos genes *STXBP1* (*Syntaxin Binding Protein 1*) e *ARX* (*Aristaless Related Homeobox***)** estão relacionadas com a síndrome de *Ohtahara*, *West* e *Dravet*, as quais as crises epilépticas se apresentam após o primeiro ano de vida, mas com padrão de herança mais complexo (VEERAMAH, et al., 2013; LEMKE; FEUX; DOUGHTY, 2012). Durante a infância, entre 1 e 10 anos, as epilepsias generalizadas como, epilepsia mioclônica juvenil e epilepsias com crises tônico-clônicas

generalizadas, são as mais comuns, sendo que as bases genéticas para essas epilepsias ainda não foram descritas (GUERRINI, 2006). Epilepsias focais foram associadas a danos estruturais focais (DHIMAN, 2017). Entretanto, a análise genética em inúmeras famílias com epilepsias focais demonstrou que há vários genes causadores, como por exemplo na epilepsia do lobo frontal noturna autossômica dominante, na qual os genes envolvidos podem ser *CHRNA4*, *CHRNA2* e *CHRNB2* (*Cholinergic Receptor Nicotinic Beta 2 Subunit***)** (BECCHETTI, et al., 2015). Alguns outros tipos de epilepsia, bem como os genes causadores e idade da primeira manifestação estão apresentados na Figura 2.

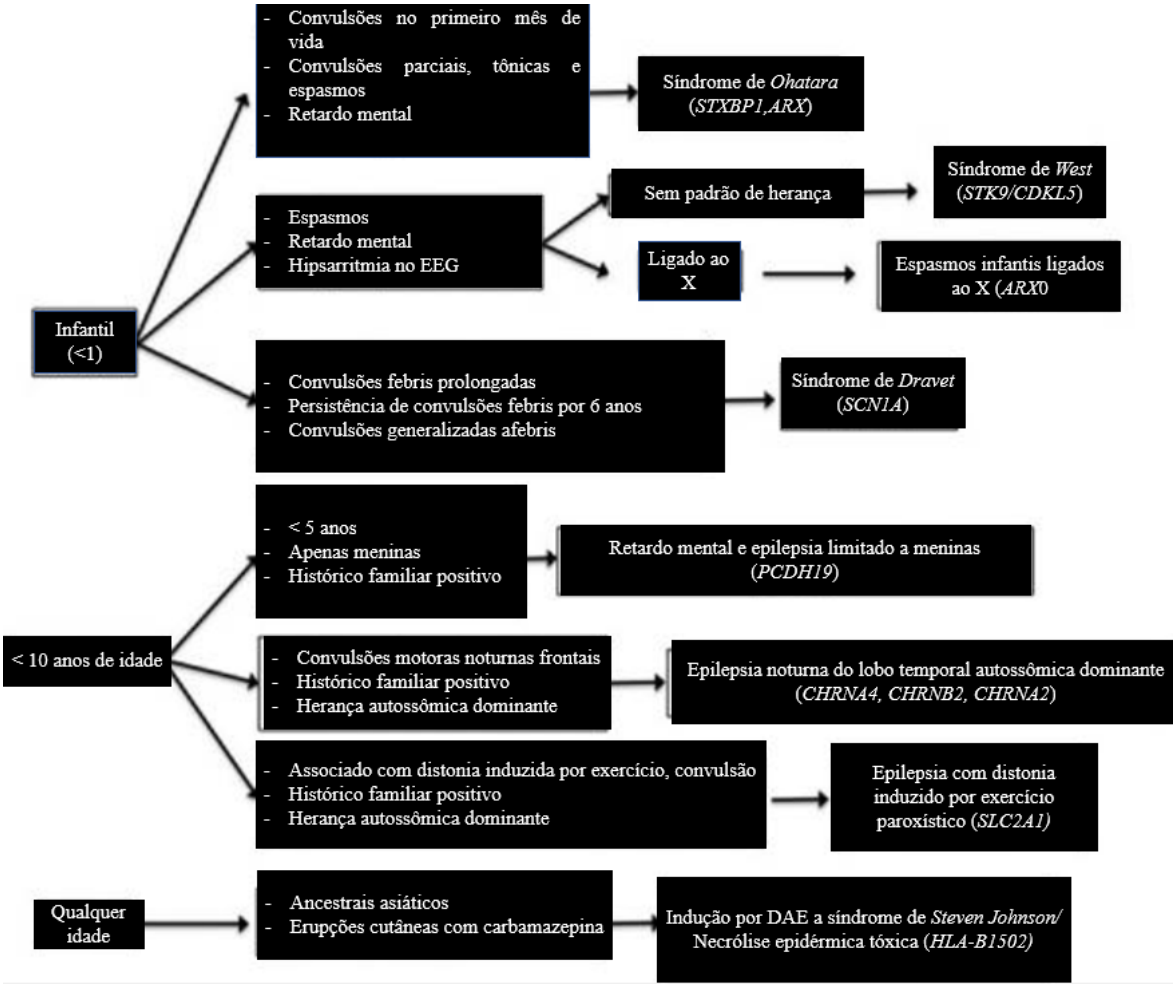

FIGURA 2. **Esquema com a correlação entre a faixa etária, o padrão clínico e o estudo genético das epilepsias.** A figura esquematiza com mais detalhes a idade das primeiras convulsões ou idade de aparição das convulsões (coluna da esquerda), as características de cada síndrome (segunda coluna da esquerda para direita), os nomes das síndromes epilépticas e genes relacionados (coluna da extrema direita) Fonte: DHIMAN, 2017.

Os avanços genéticos têm proporcionado transformações no conhecimento das causas e no tratamento das epilepsias. Mutações genéticas têm sido identificadas como principais fatores patogênicos em epilepsias familiares e idiopáticas. Parente de primeiro grau com quadro semelhante sugere alteração genética única (herança Mendeliana) enquanto história familiar de síndromes epilépticas diferentes sugerem que a mutação genética resulta em diferentes apresentações clínicas ou que membros daquela família não dividem a mesma causa e tipo de epilepsia. O modo de herança (transmissão autossômica dominante, autossômica recessiva, ligada ao X ou materna-mitocondrial) refina a lista de síndromes epilépticas e genes causadores (NOLAN; FINK, 2018). Aproximadamente 20 a 30% dos casos de epilepsia são de causa adquirida tais como acidente vascular cerebral, tumor ou trauma; entretanto, 70 a 80% dos casos acredita-se atribuir a um ou mais fatores genéticos (HILDEBRAND et al., 2013).

1.8 microRNAs e epilepsia

MicroRNAs (miRs) representam uma nova classe de RNAs (ácido ribonucleico) de fita simples, de 18-22 nucleotídeos, que desempenham um papel regulador fundamental na expressão gênica a nível pós-transcricional (GULYAEVA; KUSHLINSKIY, 2016). Os miRs podem ser isolados de células, tecidos e fluidos orgânicos como plasma, lágrima e urina (LU; ROTHENBERG, 2018). Acredita-se que a patogênese da epilepsia envolva expressões de genes que controlam a sinapse, a inflamação e a morte celular (MA, 2018). Assim, Ma (2018), realizou uma extensa revisão bibliográfica (2000 a 2017) relatando os miRs identificados na epilepsia e a expressão aumentada de miR-181a e miR-145a foi correlacionada com ativação da apoptose neuronal induzida por crise e gliose reativa, respectivamente. A expressão significativa de miR-124 reduziu a gravidade da crise e prolongou a latência de início das crises epiléticas em ratos, inferindo um efeito anti-epiléptico. O miR-199a-5p e miR-128 regularam as crises e seus danos pela ação moduladora do gene *SIRT1* (*antiapoptotic protein silent information regulator* 1). Finalmente, miR-194-5p, miR-30b-5p e miR-301a-3p foram relacionados com o diagnóstico de pacientes com epilepsia farmacorresistentes, em contraste com o miR-4521, um promissor biomarcador no soro e tecido cerebral para epilepsia refratária a FAEs e displasia cortical focal.

O *miR-146a* foi observado com expressão aumentada em astrócitos do cérebro humano, podendo inibir genes relacionados com as vias inflamatórias durante a epileptogênese (ARONICA et al., 2010). O envolvimento do *miR-146a* em vias inflamatórias, especialmente a via de

sinalização do fator nuclear kappa B (NF-kB), foi relacionado com a neuroinflamação (IORI; ARONICA; VEZZANI, 2017). Ainda, a modulação da expressão do *miR-146a* após exposição a interleucina (IL)-1β indicou que este *miR* regulou negativamente a expressão de genes pertencentes a via NF-kB, como o *IRAK1* (*Interleukin 1 Receptor Associated Kinase 1*), *IRAK2* (*Interleukin 1 Receptor Associated Kinase 2*) e *TRAF6* (*Tumor Necrosis Factor Receptor Associated Factor 6*), promovendo a expressão de *TNF-α* (*Tumor Necrosis Factor α)* e *IL* (*Interleukin*)*-6* (ARONICA et al., 2010). Além disso, ambos, *miR-146a* e *IL-1β* foram observados com expressão elevada em tecidos cerebrais de pacientes com ELT (OMRAN, et al., 2012).

1.9 Variantes gênicas de base única

Mutações genéticas consistem em variações na sequência de nucleotídeos de uma molécula de DNA (ácido desoxirribonucleico) que não estão presentes na maioria da população (frequência <1%) enquanto polimorfismos genéticos são variações na sequência do DNA presentes em >1% da população. As mutações podem ser alterações na sequência de DNA, como substituição, deleção ou inserção de nucleotídeos, variando de um nucleotídeo único até um limite definido de modo arbitrário de aproximadamente 100 kilobases (kb). Os polimorfismos de DNA podem ser classificados de acordo com a forma como a sequência de DNA varia entre os diferentes alelos. Eles podem ser polimorfismos de inserção ou deleção quando resulta de variações causadas por inserção ou deleção em qualquer parte ou variando de um único par de bases (pb), até aproximadamente 1.000 pb. Outro tipo de polimorfismo inclui a variação no número de cópias (CNVs). As CNVs consistem em variações no número de cópias de segmentos grandes do genoma, entre 1.000 pb a muitas centenas de pares de kilobases. Os mais simples e comuns de todos os polimorfismos são as variantes de nucleotídeo único (SNVs). Assim, um lócus caracterizado por uma SNV geralmente tem apenas dois alelos, que correspondem a duas bases diferentes que ocupam uma localização específica no genoma (NUSSBAUM; MCINNES; WILLARD, 2016).

Ainda, SNVs que alteram aminoácidos de genes codificadores de proteínas podem influenciar drasticamente a função das proteínas e desempenhar um papel vital na fisiopatologia de doenças (HUANG, 2015). Embora as SNVs sejam observadas em média uma vez a cada 1.000 pb no genoma, a distribuição é desigual e a grande maioria das SNVs são encontradas em íntrons e em sequências que estão a alguma distância de genes conhecidos (WILLARD; KOOCHEKPOUR, 2013). No entanto, há ainda um número significativo de SNVs em éxons e em

outros elementos funcionais, totalizando cerca de 100.000 SNVs exônicos documentados (WILLARD; KOOCHEKPOUR, 2013).

Conforme o exposto, as SNVs podem influenciar na expressão gênica e estarem relacionadas a suscetibilidade a doenças como o câncer e epilepsia (WILLARD, KOOCHEKPOUR, 2013; JIMENEZ-MATEOS, HENSHALL; 2013). Os genes envolvidos com a epilepsia estão representados no quadro 2 (CHOUCHI et al., 2017).

Quadro 2. Principais genes envolvidos com a epilepsia.

Tipos de genes	**Sigla**	**Nomenclatura dos genes**
Genes transportadores de FAEs como aqueles codificadores de proteínas transportadoras de membrana dependente de ATP	*ABCB1* *ABCC2*	*ATP-Binding Cassette Sub-Family B Member 1* *ATP-Binding Cassette Sub-Family C Member 2*
Genes-alvo de FAEs como as subunidades alfas do receptor do GABA	*GABRA1* *GABRA2* *GABRA3*	*Gamma-Aminobutyric Acid Type A Receptor Subunit Alpha1, 2 and 3*
Genes responsáveis por canais de sódio	*SCN1A* *SCN2A*	*Sodium Voltage-Gated Channel Alpha Subunit 1 and 2*
Genes responsáveis pela tradução de enzimas do metabolismo carcinogênico conhecidos como citocromo p450	*CYP1A1* *CYP2C9* *GSTM1*	*Cytochrome P450 Family 1 Subfamily A Member 1* *Cytochrome P450 Family 2 Subfamily C Member 9* glutationa S-transferase mu 1
Genes codificadores de proteínas envolvidas com transporte como o transportador de GABA tipo 3	*GAT3*	*GABA transporter type 3*
Gene transportador de soluto família 6 membro 4	*SLC6A4*	*Solute Carrier Family 6 Member 4*

Legenda: FAE: fármaco-anti-epiléptico; ATP: trifosfato de adenosina; GABA: ácido gama-aminobutírico.

1.10 SNVs no *SCN1A*

Zhi, Wu e Yang (2018), realizaram uma metanálise para avaliar a associação entre a SNV rs3812718 no gene *SCN1A* e epilepsia e observaram risco significativo para a doença em pacientes portadores dos genótipos homozigoto dominante (AA) e homozigoto variante (GG). Em contraste,

Ma et al. (2018) avaliaram a SNV rs3812718 no gene *SCN1A* e observaram um risco aumentado para epilepsia generalizada com crise febril em pacientes portadores do alelo G.

A farmacorresistência na epilepsia tem sido recentemente relacionada a SNVs, incluindo a SNV IVS5-91G>A (rs3812718) no gene *SCN1A*, com resultados contrastantes. Em uma metanálise, não foi observada a associação entre os genótipos da SNV rs3812718 e farmacorresistência à fármacos bloqueadores de canais de sódio (BAO; LIU; XIAO, 2018). Markovic et al. (2019) demonstraram ausência de correlação entre os genótipos para a SNV rs3812718 e eficácia da lamotrigina (fármaco bloqueador de canal de sódio) em pacientes com epilepsia focal. Angelopoulo et al., 2017 observaram associação entre o genótipo GG para a SNV rs3812718 e responsividade à monoterapia sem evidência de efeito na epilepsia farmacorresistente em pacientes com epilepsia. Em respeito à responsividade ao FAE carbamazepina, Zhang, Liu e Ye (2021) não encontraram associação entre a SNV rs3812718 por metanálise. Wang et al., 2018 encontraram em crianças portadores do genótipo TT para a SNV rs3812718 um alto risco para desenvolver resistência ao FAE valproato de sódio, sendo que o genótipo recessivo pode diminuir o risco desta resistência ao medicamento.

Bao, Liu e Xiao (2018) demonstraram que o genótipo AA para a SNV rs2298771 no gene *SCN1A*, está associado significativamente com a farmacorresistência a fármacos bloqueadoras de canais de sódio. Da mesma maneira, Zhang, Liu e Ye (2021) evidenciou uma forte associação do genótipo GG no mesmo polimorfismo, com resistência à carbamazepina em pacientes asiáticos. Entretanto, nenhuma associação de risco com os diferentes genótipos da referida SNVcom o metabolismo ou resistência à carbamazepina foi detectada por Zhao et al. (2021). Shi et al. (2019) não evidenciaram associação entre os genótipos da SNV rs2298771 e resposta ao ácido valpróico, bem como Mousavi et al. (2022) que não observaram relação com epilepsia famacorresistente em crianças. Liu et al. (2020), encontraram uma menor frequência de resistência ao valproato de sódio no subgrupo de epilepsia focal em crianças com o genótipo AG para a SNV rs2298771 em comparação com aquelas crianças portadoras do genótipo AA. No estudo de Ashfaq et al. (2022), o polimorfismo rs2298771 não esteve relacionado a farmacorresistência à fármacos bloqueadoras de canais de sódio nas crianças paquistanesas com epilepsia.

Recentemente, Abduljabbar et al. (2023), avaliaram 72 pacientes com epilepsia e subdividiram em grupos farmacorresistentes e farmacorresponsivos. Concluíram que os

polimorfismos rs2298771 (alelo G), rs3812719 (alelo C) e rs2195144 (alelo T) no gene *SCN1A* estão associados à resistência aos fármacos anti-epilépticos em pacientes epilépticos da Jordânia e os resultados mostram que os polimorfismos identificados numa população podem não ser os mesmos em outra.

1.11 SNVs no *miR-146a*

As principais SNVs estudadas no miR-146a foram a n.60G>C (rs2910164), n.-411A>G (rs57095329) e g.159879978C>T (rs2431697) (CUI et al., 2015; WANG et al., 2017; ZHOU et al., 2014; MANNA et al., 2013, BOSCHIERO et al., 2020). Particularmente, a SNV rs2910164 foi relacionado com doenças inflamatórias como o lúpus eritematoso sistêmico, a síndrome de Behcet´s, a colite ulcerativa, a sepse e a asma (JIMENEZ-MORALES et al., 2012; ZHOU et al., 2014).

Foi observado que os diferentes genótipos da SNV rs2910164 podem modular o nível de expressão do *miR146a* maduro (ZHOU et al., 2014). Apenas dois estudos avaliaram a SNV rs2910164 na epilepsia, os quais não evidenciaram correlação com a ELT em pacientes caucasianos italianos (MANNA et al., 2013) e a epilepsia resistente a fármacos em pacientes chineses (CUI et al., 2015). Abdel-Rasol et al., 2023 recentemente publicou um estudo com a SNV rs57095329 no *miR-146a* em pacientes com epilepsia e controles e uma sub-análise entre grupos resistentes e responsivos. Constatou que os genótipos AG e GG estavam em maior número no grupo resistente enquanto o genótipo AA estava em maior número no grupo responsivo.

Nosso grupo investigou a correlação entre as SNVs rs2910164 e rs57095329 no *miR-146a* e a suscetibilidade a epilepsia farmacologicamente resistente, em amostras de tecido epileptogênico. Os resultados demonstraram que o genótipo GC do SNV rs2910164 parece estar associado com a suscetibilidade à epilepsia farmacologicamente resistente devido a expressão significativamente diminuída de *miR-146a* nos pacientes portadores deste genótipo, favorecendo a via de sinalização NF-kB (BOSCHIERO et al., 2020). A SNV rs57095329 não foi observado como envolvida com o risco da doença nas amostras de tecido epileptogênico avaliados.

A ELT é a epilepsia mais frequente do adulto e também o tipo de crise que apresenta maior farmacorresistência, tornando-a uma doença limitante para os pacientes no aspecto social, pessoal, laboral e familiar e um grande impacto econômico no seu tratamento. Com o avanço da genética nas últimas duas décadas, vários estudos têm procurado explicar a farmacorresistência nestas

epilepsias, incluindo análises de SNVs em genes ou em miRs, relacionados com a fisiopatologia da doença e resposta terapêutica aos FAEs. Os genes *SCN1A* e *miR146a* individualmente foram anteriormente correlacionados com epilepsias farmacorresistentes com resultados conflitantes. Entretanto, não há na literatura estudos evidenciando a associação de ambos os genes na influência das epilepsias farmacorresistentes e farmacorresponsivas. Assim, o presente projeto investigou a correlação entre SNVs nos genes *SCN1A* e *MIR146a* e a suscetibilidade a epilepsia farmacorresistente e farmacorresponsiva, devido à ausência de estudos similares na população Brasileira.

2. OBJETIVOS

2.1 Objetivos gerais

- Realizar revisão da literatura de estudos caso-controles que avaliaram variantes de nucleotídeo único (SNVs) localizadas em miRs ou em sequência 3´UTR de genes-alvos de miRs e que poderiam influenciar a suscetibilidade à epilepsia;
- Realizar levantamento epidemiológico dos casos de epilepsia no serviço de neurologia do Hospital Universitário São Francisco na Providência de Deus (HUSF), de 01 de janeiro de 2010 a 31 de março de 2021.

2.2 Objetivos específicos

- Identificar os diferentes genótipos herdados para as SNVs c.3184A>G (rs2298771) e IVS5-91G>A (rs3812718) no gene *SCN1A* e n.60G>C (rs2910164) no *miR-146a* como fatores preditores de suscetibilidade para o desenvolvimento de epilepsia, bem como de sua resistência ou não ao tratamento e, como possíveis fatores de risco e prognósticos.
- Identificar a frequência das variantes gênicas das SNVs rs2298771 e rs3812718 no gene *SCN1A*, em amostras de DNA a partir de sangue periférico de pacientes ELT farmacorresistentes ou farmacorresponsivos ou de tecido cerebral de pacientes farmacorresistentes pela reação em cadeia da polimerase (PCR) em tempo real;
- Identificar a frequência da variante gênica SNV rs2910164 no *miR-146a*, em amostras de DNA a partir de sangue periférico de pacientes ELT farmacorresistentes ou farmacorresponsivos ou de tecido cerebral de pacientes farmacorresistentes pela PCR em tempo real;
- Correlacionar as frequências dos genótipos das SNVs acima com o risco para a epilepsia farmacorresistentes e farmacorresponsivas;
- Analisar a expressão dos genes *MIR146a* e *SCN1A* em amostras de RNAs a partir de sangue periférico de pacientes ELT farmacorresistentes e farmacorresponsivos ou de tecido cerebral de pacientes farmacorresistentes que apresentarem diferentes genótipos para as SNVs estudadas, pela PCR quantitativa.

3 CAPÍTULO 1: publicado

Título do artigo: *Single-Nucleotide Variants in microRNAs Sequences or in their Target Genes Might Influence the Risk of Epilepsy: A Review.*

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O objetivo do artigo foi revisar estudos de caso-controle que investigaram a relação entre SNVs nas sequências de microRNAs (miRs) ou em seus genes-alvo e a suscetibilidade à epilepsia a partir de 1º de janeiro de 2010 a 31 de outubro de 2020. As principais SNVs observadas associadas ao risco de epilepsia resistente a medicamentos foram as SNVs n.60G>C (rs2910164) e n.-411A> G (rs57095329), ambas localizadas no *miR-146a*. As SNVs rs2910164 e rs57095329 podem modificar o nível de expressão do *miR-146a* maduro e o risco de epilepsia. A SNV rs57095329 pode estar correlacionada com a resistência à fármacos quando um número maior de pacientes é avaliado. Assim, nós concluímos que a principal desvantagem da maioria dos estudos é o pequeno número de indivíduos inscritos, o que carece de poder amostral.

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REVIEW PAPER

Single-Nucleotide Variants in microRNAs Sequences or in their Target Genes Might Influence the Risk of Epilepsy: A Review

Renata Parissi Buainain[1,2] · Matheus Negri Boschiero[1,2] · Bruno Camporeze[1,2] · Paulo Henrique Pires de Aguiar[1,2,3] · Fernando Augusto Lima Marson[1,2] · Manoela Marques Ortega[1,2]

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Abstract

Single-nucleotide variant (SNV) is a single base mutation at a specific location in the genome and may play an import role in epilepsy pathophysiology. The aim of this study was to review case–control studies that have investigated the relationship between SNVs within microRNAs (miRs) sequences or in their target genes and epilepsy susceptibility from January 1, 2010 to October 31, 2020. Nine case–control studies were included in the present review. The mainly observed SNVs associated with drug-resistant epilepsy (DRE) risk were SNVs n.60G > C (rs2910164) and n.-411A > G (rs57095329), both located at miR-146a mature sequence and promoter region, respectively. In addition, the CC haplotype (rs987195-rs969885) and the AA genotype at rs4817027 in the MIR155HG/miR-155 tagSNV were also genetic susceptibility markers for early-onset epilepsy. MiR-146a has been observed as upregulated in human astrocytes in epileptogenesis and it regulates inflammatory process through NF-κB signaling by targeting tumor necrosis factor-associated factor 6 (*TRAF6*) gene. The SNVs rs2910164 and rs57095329 may modify the expression level of mature miR-146a and the risk for epilepsy and SNVs located at rs987195-rs969885 haplotype and at rs4817027 in the MIR155HG/miR-155 tagSNV could interfere in the miR-155 expression modulating inflammatory pathway genes involved in the development of early-onset epilepsy. In addition, SNVs rs662702, rs3208684, and rs35163679 at 3′untranslated region impairs the ability of miR-328, let-7b, and miR-200c binding affinity with paired box protein PAX-6 (*PAX6*), BCL2 like 1 (*BCL2L1*), and DNA methyltransferase 3 alpha (*DNMT3A*) target genes. The SNV rs57095329 might be correlated with DRE when a larger number of patients are evaluated. Thus, we concluded that the main drawback of most of studies is the small number of individuals enrolled, which lacks sample power.

Keywords Epilepsy · microRNAs (miRs) · Single-nucleotide variants (SNVs) · Susceptibility

Abbreviations

3′-UTR	3′untranslated region
AARS	Alanyl-tRNA synthetase
ALDH7A1	Aldehyde dehydrogenase 7 family member A1
ALG13	Asparagine-linked glycosylation 13
BCL2L1	BCL2 like 1
CI	Confidence interval
DNA	Deoxyribonucleic acid
DNMT3A	DNA methyltransferase 3 alpha
DRE	Drug-resistant epilepsy
NF-kB	Factor nuclear kappa B
GABA	Gamma-aminobutyric acid
IFN-Ɣ	Interferon-gamma
IL-1	Interleukin 1
IL-1β	Interleukin 1 beta
IRAK1	Interleukin 1 receptor associated kinase 1
ILAE	International league against epilepsy
MTLE	Mesial temporal lobe epilepsy
miRs	MicroRNAs
NMDA	N-Methyl-D-aspartate
OR	Odds ratio
OMIM	Online mendelian inheritance in man

✉ Manoela Marques Ortega
manoela.ortega@usf.edu.br

[1] Laboratory of Cell and Molecular Tumor Biology and Bioactive Compounds, Post Graduate Program in Health Science, São Francisco University (USF), Avenida São Francisco de Assis, 218, Jardim São José, Bragança Paulista, São Paulo 12916-900, Brazil

[2] Laboratory of Human and Medical Genetics, Post Graduate Program in Health Science, USF, Avenida São Francisco de Assis, 218, Jardim São José, Bragança Paulista, São Paulo 12916-900, Brazil

[3] Departament of Neurosurgery, Hospital Santa Paula, São Paulo, São Paulo, Brazil

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PAX-6	Paired box protein PAX-6
PCR	Polymerase chain reaction
RISC	RNA-induced silencing complex
RNA	Ribonucleic acid
SNVs	Single-nucleotide variants
SCN1A	Sodium voltage-gated channel alpha subunit 1
*SCN2*A	Sodium voltage-gated channel alpha subunit 2
SCN1B	Sodium voltage-gated channel beta Subunit 1
TlE	Temporal lobe epilepsy
TNF-α	Tumor necrosis factor alpha
*TRAF*6	Tumor necrosis factor receptor (TNFR)-associated actor 6
WHO	World Health Organization

Introduction

Epilepsy is a chronic brain disorder defined by at least two unprovoked seizures that occur within 24 h (Fisher et al. 2014). The disease affects about 50 million people worldwide at all ages (WHO 2019). The seizures are divided into focal, generalized, and unknown onset, according to the International league against epilepsy (ILAE) classification (Scheffer et al. 2017). The focal seizure is more common than generalized in children and adults (Beghi 2020), and the temporal lobe epilepsy (TLE) is the most common focal epilepsy subtype (Johnson 2019). In addition, TLE is the most common type of drug-resistant epilepsy (DRE) (Asadi-Pooya et al. 2017).

ILAE (Scheffer et al. 2017) has defined six etiologic categories for epilepsy as (a) structural etiology, a finding on neuroimaging reasonably inferred to cause the patient's seizures (Lapalme-Remis and Cascino 2016); (b) variant in a gene or copy number variant, which is pathogenic for epilepsy. The family history and typical features as electroencephalography and seizure semiology might be sufficient for genetic etiology (Hildebrand et al. 2013); (c) infectious etiology for patients with epilepsy due to the neurocysticercosis, human immunodeficiency virus, cytomegalovirus or cerebral toxoplasmosis (Vezzani et al. 2016); (d) metabolic epilepsies for patients with epilepsy due to a metabolic derangement such as pyridoxine-dependent seizures and cerebral folate deficiency (Parikh et al. 2015); (e) auto-immune diseases as encephalitis, which has been linked to both neuronal intracellular and neuronal cell surface antibodies (Toledrano and Pittock 2015); (f) unknown etiology for patients whose etiology remains unclear (Falco-Walter et al. 2018).

The genomic technology advances have greatly increased the knowledge on the epilepsy basis and genetic changes. Wang et al. (2017a) have evaluated the Online Mendelian Inheritance in Man (OMIM) database and the authors have found 84 epilepsy-related genes, being the sodium voltage-gated channel alpha subunit 1A (*SCN1A*) gene, the mainly observed one (Perucca and Perucca 2019). The most common epilepsy genes were ion-channel genes (*SCN1A, SCN1B, SCN2A*, others), totalizing 28 of the 84 epilepsy-related genes. Mutations in enzyme/enzyme-modulator genes as alanyl-tRNA synthetase (*AARS*), aldehyde dehydrogenase 7 family member A1 (*ALDH7A1*), and asparagine-linked glycosylation 13 (*ALG13*) ranked as the second cause (25/84 epilepsy-related genes). The remaining genes were involved in transport, receptor binding, cell adhesion, signal transduction/molecule, membrane trafficking, cytoskeleton, nucleic acid binding, and other unknown functions (Wang et al. 2017a).

Recently, the role of microRNAS (miRs) in the epilepsy pathophysiology have been also described as biomarkers and novel therapy approaches for epilepsy (Ma 2018). Interestingly, single-nucleotide variants (SNVs) in miRs sequences or in their 3′untranslated region (3′-UTR) target genes might influence the risk for epilepsy and expression on their target genes, increasing diseases susceptibility, including epilepsy (Tao et al. 2015; Li et al. 2016b; Panjwani et al. 2016; Xiao et al. 2019; Boschiero et al. 2020). Thus, the aim of this study was to review case–control studies, which investigated the relationship between SNVs in miRs and in their target genes and risk for epilepsy.

The Biogenesis of miRs

The biogenesis of miR begins in the cell nucleus, from the transcription of DNA to pri-miR, by the action of the enzymes PASHA and DROSHA. The pri-miR undergoes action of the enzyme exportin-5 and it is exported to the cell cytoplasm where it gives rise to the pre-miR. This is catalyzed by another enzyme, Dicer, finally forming the mature miR. Mature miR is associated with a complex or set of enzymes called RNA-induced silencing complex (RISC) and suppresses or inhibits protein synthesis by cleavage of messenger RNAs (mRNAs) or by preventing translation of mRNAs, inhibiting protein production (Hata and Kashima 2016).

SNVs in miRs and Epilepsy

MiRs, discovery in 1980 (Horvitz and Sulston 1980) and subsequently existence confirmed in 2001 (Lee and Ambros 2001), ushered a new era in molecular biology. MiRs are short non-coding regulatory RNAs with 19 to 25 nucleotides (nt) in size, responsible for post-transcriptional silencing regulating of their target genes expression (Lu and Rothenberg 2018). Base-pairing occurs between the miR and

target gene, often within the 3′-UTR of the mRNA, resulting in recruitment of additional factors that lead to either degradation of the mRNA or inhibition of translation (Krol et al. 2010; Meister 2013). In mammals, 60% of the mRNAs have a known seed sequence for miR-binding; thus, in the brain, miRs are particularly abundant and control neurogenesis (Kosik 2006). In Dicer knockout mouse model, the biogenesis of miR is blocked, leading to neuronal loss and premature animal death (Schaefer et al. 2007).

Noteworthy, the majority of the known miRs are expressed in the brain and many such as miR-124 has elevated expression in the brain cells, but less detectable in other tissues (Lagos-Quintana et al. 2002; Miska et al. 2004; Shao et al. 2010; Ludwig et al. 2016). Furthermore, excitatory and inhibitory neurons, astrocytes, microglia, and oligodendrocytes express specific miRs (He et al. 2012; Jovicic et al. 2013). In contrast, individual miRs loss can also be sufficient to produce central nervous system phenotypes as the loss of miR-9 that results in brain development defects (Shibata et al. 2011), the loss of miR-124, which results in hippocampus neurodegeneration (Sanuki et al. 2011), and the postnatal deletion of miR-128 from dopaminergic neurons results in epilepsy (Tan et al. 2013).

Recently, the role of miRs in the epilepsy pathophysiology have been described on synaptic structure and function (miR-134, miR-128, miR-203 and miR-139), neurogenesis and neuronal migration (miR-134, miR-128, miR-124 and miR-137), inflammation (miR-146 and miR-22), transcription (miR-132, miR-124 and miR-199), and cell death (miR-34a and miR-184) (Brennan and Henshall 2018).

The SNVs in miRs are examples of point mutations that could affect miR function in three possible ways: altering transcription of the primary miR transcript, processing primary miR (pri-miR) and precursor miR (pre-miR), and by their effects on the modulation of miR-mRNA interplay (Saunders et al. 2007; Duan et al. 2007). Subsequently, SNVs in miRs have been associated with several brain pathogenesis like Parkinson's disease, Alzheimer's disease, or other neurodegenerative diseases (Quinlan et al. 2017; Wang et al. 2017b; Dehghani et al. 2018) and might also increase the risk for epilepsy (Manna et al. 2013). SNV is a substitution of a single nucleotide that occurs at a specific position in the genome and the most common source of genetic polymorphism in the human genome accounts about 90% of all polymorphisms (Dabhi and Mistry 2014).

In the present review, only six case–control studies have evaluated SNVs in miRs sequence and risk for epilepsy (Table 1). The most evaluated SNVs associated with epilepsy susceptibility were SNVs n.60G > C (rs2910164) and n.-411A > G (rs57095329), both located at miR-146a mature sequence and promoter region, respectively (Manna et al. 2013; Cui et al. 2015; Issac et al. 2015; Li et al. 2016b; Boschiero et al. 2020). In addition, the CC haplotype (rs987195-rs969885) and the AA genotype at rs4817027 in the MIR155HG/miR-155 tagSNV were also genetic susceptibility markers for early-onset epilepsy (Tao et al. 2015).

Neuroinflammatory signaling is partially controlled by miR-146a and overexpression of miR-146a following status epilepticus potently suppresses recurrent seizures in mice models (Iori et al. 2017). In addition, miR-146a has been observed to be upregulated in human epileptic astrocytes (Lukiw et al. 2008) and it regulates inflammatory process through the nuclear factor kappa B (NF-κB) signaling by targeting tumor necrosis factor-associated factor 6 (*TRAF6*) gene (Taganov et al. 2006; Hou et al. 2009). The SNVs rs2910164 and rs57095329 in the miR-146a may alter the expression level of the mature miR-146a (Zhou et al. 2014; Boschiero et al. 2020) and the risk of epilepsy.

Only four studies have evaluated the association of epilepsy risk and the SNV rs2910164 in the pre-miR-146a (Manna et al. 2013; Cui et al. 2015; Issac et al. 2015; Boschiero et al. 2020). (Manna et al. 2013) tested the rs2910164 and susceptibility to TLE in an Italian population cohort and analysis comparing genotypes and alleles' frequencies in patients and controls showed no significant differences, including clinical characteristics. (Cui et al. 2015) evaluated the SNV rs2910164 in Chinese TLE and non-TLE patients and the authors found that the SNV rs2910164 was not associated with epilepsy in both groups. (Issac et al. 2015) has examine whether SNV rs2910164 effected the proinflammatory cytokine, serum high-mobility group box 1 levels, in Egyptian children presenting febrile seizures. The authors discovered that rs2910164 polymorphism was not associated with elevated risk of febrile seizures. However, higher high-mobility group box 1 levels in rs2910164 CC compared to GG genotype was observed. Finally, (Boschiero et al. 2020) have observed an increased frequency of rs2910164 GC in brain tissues from DRE patients with two times risk for epilepsy. The Brazilian population is extremely mixed (dos Santos et al. 2013), which may explain the contrasting results. Thus, the discrepancy among the studies might be due to ethnic variation and differences in number of recruited patients.

Only three groups (Cui et al. 2015; Li et al. 2016b; Boschiero et al. 2020) have studied the SNV rs57095329 in patients with epilepsy. The study of (Cui et al. 2015) described that the rs57095329 A allele was associated with a reduced risk of seizures frequency in Chinese DRE patients. In contrast, (Li et al. 2016b) observed in Chinese childhood epilepsy patients that the G allele of rs57095329 could increase drug-resistance risk and seizure severity, but no genotype risk association was observed by authors. (Boschiero et al. 2020) have included only DRE patients and, most of the patients and controls were equally heterozygous for

Table 1 Association between single-nucleotide variants (SNVs) within microRNAs (miRs) sequences and epilepsy

References	Population	Associated disease	SNV	miRs	miRs-target genes	Genotypes Risk	Putative risk alleles	OR (95%CI)
Boschiero et al. (2020)	Brazil	Control vs. TLE/DRE	rs2910164 G > C	*miR-146a*	*NF-kB*	**GG/GC (p = 0.04)**		1.98 (1.19–3.57)
						GG/CC ($p = 0.06$)		1.18 (0.27–3.97)
						GC/GG + CC (p = 0.023)		**1.90 (1.10–3.9)**
						GG/GC + CC (p = 0.047)		0.54 (0.30–0.96)
						CC/GG + GC ($p = 1.00$)		0.81 (0.20–2.52)
							C/G ($p = 0.283$)	1.98 (1.19–3.57)
			rs57095329 A > G			AA/GA + GG ($p = 0.597$)		–
						GA + GG/AA ($p = 0.587$)		–
						AA + AG/GG ($p = 0.703$)		1.46 (0.24–6.33)
						AA + GG/GA ($p = 0.703$)		1.73 (0.38–16.24)
							A/G ($p = 0.721$)	1.08 (0.72–1.60)
Manna et al. (2016)	Italy	Control vs. MTLE	rs531564 C > G	*miR-124*	*Neuronal differentiation*	CC/CG/GG ($p = 0.579$)	C/G ($p = 0.293$)	1.21 (0.85–1.71)
Li et al. (2016b)	China	Control vs. epilepsy	rs57095329 A > G	*miR-146a*	*NF-kB*	AA/GA ($p = 0.945$)	–	1.01 (0.69–1.49)
						AA/GG ($p = 0.089$)	–	1.27 (0.97–1.66)
						AA/GA + GG ($p = 0.405$)	–	1.16 (0.8–1.64)
						AA + GA/GG ($p = 0.087$)	–	1.58 (0.94–2.69)
			rs2292832 T > C	*miR-149*	*TNF-α NF-kB*	TT/TC ($p = 0.914$)		0.98 (0.68–1.41)
						TT/CC ($p = 0.433$)		1.12 (0.85–1.48)
						TT/TC + CC ($p = 0.837$)		1.04 (0.74–1.46)
						TT + TC/CC ($p = 0.356$)		1.28 (0.76–2.16)

Table 1 (continued)

References	Population	Associated disease	SNV	miRs	miRs-target genes	Genotypes Risk	Putative risk alleles	OR (95%CI)
			rs11614913 T > C	*miR-196a2*	*TNF-α NF-kB*	TT/TC ($p = 0.986$)		1.00 (0.67–1.52)
						TT/CC ($p = 0.696$)		1.05 (0.82–1.34)
						TT/TC + CC ($p = 0.895$)		1.03 (0.70–1.51)
						TT + TC/CC ($p = 0.566$)		1.13 (0.75–1.69)
			rs3746444 A > G	*miR-499*	*TNF-α NF-kB*	AA/GA ($p = 0.917$)		1.02 (0.69–1.51)
						AA/GG ($p = 0.438$)		0.88 (0.63–1.22)
						AA/GA + GG ($p = 0.817$)		0.96 (0.67–1.37)
						AA + GA/GG ($p = 0.422$)		0.77 (0.40–1.46)
		DRE vs. drug responsive	rs57095329 A > G	*miR-146a*	*NF-kB*	**AA/GA (p = 0.005)**		**2.34 (1.30–4.21)**
						AA/GG (p = 0.002)		**1.79 (1.24–2.59)**
						AA/GA + GG (p < 0.001)		**2.63 (1.56–4.43)**
						AA + GA/GG (p = 0.017)		**2.34 (1.17–4.67)**
							G (p < 0.001)	**2.36 (1.61–3.47)**
			rs2292832 T > C	*miR-149*	*TNF-α NF-kB*	TT/TC ($p = 0.849$)	–	0.95 (0.55–0.64)
						TT/CC ($p = 0.962$)		0.99 (0.67–1.47)
						TT/TC + CC ($p = 0.849$)		1.05 (0.63–1.75)
						TT + TC/CC ($p = 0.969$)		1.02 (0.49–2.11)
			rs11614913 T > C	*miR-196a2*	*TNF-α NF-kB*	TT/CT ($p = 0.992$)	–	0.99 (0.54–1.86)
						TT/CC ($p = 0.894$)		0.99 (0.67–1.47)
						TT/TC + CC ($p = 0.902$)		1.05 (0.63–1.75)

Table 1 (continued)

References	Population	Associated disease	SNV	miRs	miRs-target genes	Genotypes Risk	Putative risk alleles	OR (95%CI)
						TT + TC/CC ($p = 0.775$)		1.02(0.49–2.11)
			rs3746444 A > G	*miR-499*	*TNF-α NF-kB*	AA/GA ($p = 0.837$)	–	1.06 (0.60–1.88)
						AA/GG ($p = 0.595$)	–	0.86 (0.499–1.49)
						AA/GA + GG ($p = 0.968$)	–	1.01 (0.592–1.73)
						AA + GA/GG ($p = 0.529$)	–	1.41 (0.48–4.13)
Cui et al. (2015)	China	Control vs. total cases	rs2910164 G > C	*miR-146a*	*NF-kB*	CC/CG/GG ($p = 0.150$)	C/G ($p = 0.328$)	1.15 (0.89–1.48)
		Control vs. TLE				CC/CG/GG ($p = 0.265$)	C/G ($p = 0.567$)	1.09 (0.825–1.45)
		Control vs. no-TLE				CC/CG/GG ($p = 0.282$)	C/G ($p = 0.214$)	1.28 (0.87–1.88)
		Control vs. total cases	rs57095329 A > G	*miR-146a*	*NF-kB*	AA/GA/GG ($p = 0.754$)	C/G ($p = 0.523$)	1.12 (0.82–1.54)
		Control vs. TLE				AA/GA/GG ($p = 0.968$)	C/G ($p = 0.862$)	1.04 (0.74–1.47)
		Control vs. no-TLE				AA/GA/GG ($p = 0.410$)	C/G ($p = 0.241$)	1.35 (0.83–2.20)
		Control vs. DRE	rs2910164 G > C	*miR-146a*	*NF-kB*	CC/CG/GG($p = 0.650$)	C/G ($p = 0.506$)	1.14 (0.78–1.66)
		Control vs. DRE	rs57095329 A > G	*miR-146a*	*NF-kB*	**AA/GA/GG ($p = 0.026$)**	G ($p = 0.011$)	–
Tao et al. (2015)	China	Control vs. DRE	rs969885 C > T	*miR-155*	*Inflammatory pathways*	CC/CT/TT ($p = 0.536$)	C/T ($p = 0.548$)	0.79 (0.36–1.72)
						CC/CT + TT ($p = 0.717$)		0.85 (0.36–2.04)
						CC + CT/TT ($p = 0.233$)		**5.07 (0.35–73.13)**
			rs12483428 T > C	*miR-155*	*Inflammatory pathways*	TT/TC/CC ($p = 0.516$)	T/C ($p = 0.511$)	1.27 (0.63–2.57)
						TT/TC + CC ($p = 0.542$)		1.29 (0.57–2.90)
						TT + TC/CC ($p = 0.705$)		1.55 (0.16–14.83)

Table 1 (continued)

References	Population	Associated disease	SNV	miRs	miRs-target genes	Genotypes Risk	Putative risk alleles	OR (95%CI)
			rs987195 C > G	*miR-155*	*Inflammatory pathways*	CC/CG/GG ($p = 0.118$)	C/G ($p = 0.097$)	1.59 (0.92–2.75)
						CC/CG + GG ($p = 0.081$)		1.98 (1.92–4.28)
						CC + CG/GG ($p = 0.448$)		1.48 (0.54–4.03)
			rs4817027 G > A	*miR-155*	*NF-kB*	GG/GA/AA ($p = 0.074$)	G/A ($p = 0.094$)	1.72 (0.91–3.24)
						GG/GA + AA ($p = 0.213$)		1.63 (0.76–3.52)
						GG + GA/AA ($p = 0.024$)		**13.13 (1.40–123.83)**
Manna et al. (2013)	Italy	Control vs. TLE	rs2910164 G > C	*miR-146a*	*NF-kB*	GG/GC/CC ($p = 0.536$)	G/C ($p = 0.361$)	1.10 (0.89–1.36)
						GG/CG		1.17 (0.88–1.55)
						GG/CC		1.10 (0.65–1.8)

SNVs single-nucleotide variants, *miRs* microRNAS, *vs.* versus, *OR* odds ratio with 95% confidence intervals, *TLE* temporal lobe epilepsy, *DRE* drug-resistant epilepsy, *MTLE* mesial temporal lobe epilepsy, *TNF-α* tumor necrosis factor alpha, *NF-KB* factor nuclear Kappa B

the SNV rs57095329 with no genotype risk association. Epilepsy is a multifactorial disorder in which genetic susceptibility and environmental factors may be implicated; larger patients cohort are needed to confirm the possible clinical association of rs57095329.

Recently, it was investigated the association of SNVs rs2292832, rs11614913, and rs3746444 in the precursor sequences of miR-149, miR-196a2, and miR-499, respectively in neurodegenerative disorder as Parkinson (Haixia et al. 2012). Interestingly, the three miRs also modulate genes related to inflammation pathways including tumor necrosis factor-α (TNF-α), toll-like receptor signaling, and cytokine response (Haixia et al. 2012). Li et al. (2016b) have hypothesized that the SNVs rs2292832, rs11614913, and rs3746444 located at miRs precursor sequences may also contribute to childhood epilepsy risk. Thus, the authors have genotyped the three SNVs in a hospital-based case–control studies in a Chinese population and no interrelation with epilepsy risk was observed.

Furthermore, the effect of the SNV g.9903189C/G (rs531564) located at primary miR-124 on susceptibility to mesial temporal lobe epilepsy (MTLE), most common refractory epilepsy form, was investigated using a case control study in Italian population (Manna et al. 2016). The neuron-specific miR-124 have been showed to be essential for neuronal differentiation (Makeyev et al. 2007). Recently, miR-124 has been found to be upregulated in the acute and chronic seizure stages of MTLE (Peng et al. 2013). Therefore, (Manna et al. 2016) have determined whether SNV rs531564 could influence risk to MTLE patients. No statistically significant differences were found in the allele or genotype distributions of the miR-124 rs531564 polymorphism in patients and control groups evaluated.

Above studies were the first and unique to evaluate SNVs rs2292832, rs11614913, and rs3746444 in Chinese with epilepsy and the SNV rs531564 in Italian MTLE susceptibility, respectively. The findings need to be reproduced in a larger patients' cohort and other populations.

Both miR-146a and miR-155 are the most involved in the inflammatory process of epilepsy. Recently, a positive association between SNV rs2910464 in the miR-146a and Brazilian patients with DRE was evaluated by our team (Boschiero et al. 2020). The first report that MIR155HG/miR-155 tag SNVs are related to DRE was provided by Tao and collaborators (Tao et al. 2015). MiR-155 is a transcription product of its host gene, *MIR155HG*, and its expression could be affected by polymorphisms located at both *MIR155HG* and miR-155 genes in multiple sclerosis (Paraboschi et al., 2011). Thus, (Tao et al. 2015) have evaluated Chinese Han DRE patients and healthy individuals for the 4 tag SNVs rs969885, rs12483428, rs987195, and rs4817027, located at *MIR155HG/miR-155*. Their study has showed that the CC haplotype (rs987195-rs969885) is a genetic susceptibility

marker for early-onset epilepsy. In addition, the authors have found that the AA genotype (rs4817027) and the CC haplotype (rs987195-rs969885) were genetic susceptibility markers for DRE. On the contrary, the CG haplotype (rs987195-rs969885) was a genetic protective factor against DRE. The results are compatible with the inflammatory mechanism of DRE.

In conclusion, most of the studies presented here were unique and the findings need to be reproduced in a larger patients' cohort in different populations. In addition, the GC and CC genotypes for SNV rs2910164 in miR-146a, the CC haplotype (rs987195-rs969885) and the AA genotype at rs4817027 for MIR155HG/miR-155 tag SNV, were genetic susceptibility markers for DRE or early-onset epilepsy, confirming the role of both miR-146a and miR-155 with inflammation response in the pathogenesis of epilepsy. MiR-146a is a NF-κB trans-activational target and negatively regulates interleukin 1 receptor associated kinase 1 (*IRAK1)* and *TRAF6*, being identified as a powerful innate immune and pro-inflammation regulator (Jazdzewski et al. 2008). The expression of *miR-155*, an inflammatory modulator, is significantly increased in the brain in an immature rat model of status epilepticus and in children with MTLE (Ashhab et al. 2013), suggesting that the inflammatory role of miR-155 is involved in the development of early-onset epilepsy.

In fact, an increasing amount of evidence has supported the hypothesis that inflammatory processes within the epileptic brain might constitute a common and crucial mechanism in the pathology of seizures (Vezzani 2014). Brain injury leads to the activation of the microglial cells, which increases the release of proinflammatory cytokines as interleukin (IL)-1, interferon-gamma (IFN-γ), and TNF-α, which further activate the NF- kB mediated pathway. At the same time, there is also a damage to the gamma-aminobutyric acid (GABA) GABAergic neurons in the brain, which leads to a relative increase in the excitatory transmitter like glutamate. Increased activation of the glutamate receptor lead to increase in the oxidative stress that ultimately activates the NF- kB through proinflammatory pathway (Singh et al. 2018). As a consequence of this action, N-methyl-D-aspartate (NMDA) receptor-mediated Ca^{2+} influx into neurons is enhanced by IL-1, and this effect plays a role in promoting excitotoxicity and seizure generation (Viviani et al. 2003; Balosso et al. 2008). Lubin and collaborators (Lubin et al. 2007) have found that inhibition of NF-kB significantly decreased seizure threshold in treated rats suggesting that NF-kB activation is neuroprotective following a variety of brain insults and neurodegenerative conditions, supporting the proposal that proinflammatory cytokines and the NF-κB pathway have a role in the pathogenesis of status epilepticus development (Zhang et al. 2018).

As previously commented, SNVs in miRs related to epilepsy might affect the levels of proteins associated with the disorder. However, most of the studies did not involve additional experiments to assess the miRs and its predicted targets expression, once obtaining tissue samples of epileptogenic foci is difficult. Thus, only (Boschiero et al. 2020) have evaluated the miR-146a expression level in the epileptogenic tissues, considering the different genotypes for the SNV rs2910164. The authors have observed lower miR-146a expression in the GC and CC genotypes compared to GG genotype. Also, *TRAF6* gene expression level was higher in GC and CC than in GG genotype.

SNVs in miRs Target Genes

The miR: mRNA pairing consequence is a protein expression loss, resulting from either decreased transcript levels or translational repression (Winter et al. 2009). Many mRNAs contain conserved miR target sites in their 3′-UTR. The average size of human highly expressed neuronal genes is 1300 nt, whereas for genes specific to non-neuronal tissue it is 700 nt (Lewis et al. 2005; Sood et al. 2006), while the efficient miR-binding site consists of 6–8 nt. The composition of specific miRs associated with the 3′-UTR of a mRNA along with the efficiency of miR pairing to their target sequences impacts the mRNA's half-life and influences protein levels (Filipowicz et al. 2008; Bartel 2009) Considering the complexity of miRNA: mRNA pairing, the introduction of a SNV into a 3′-UTR can introducing or removing miR target sequences or changing the binding efficiency. In addition, the introduction or removal of miR target sites may affect binding to other miR target sequences in the SNV's close proximity, which could have unpredicted effects on the mRNA half-life.

There are only 3 studies that have observed SNVs in the 3′-UTR of miRs target genes in epilepsy (Table 2). One study has observed that the SNV rs662702 of miRNA-328 binding site in the 3′-UTR of paired box protein PAX-6 (*PAX6*), which is known to result in increased *PAX6* expression, conferred the increased risk of centrotemporal spikes of Rolandic epilepsy (Panjwani et al. 2016).

Also, Li et al. (2016a) have investigated if genetic variants in 3′-UTR of *SCN1A*, affecting the miR-mRNA 3′-UTR interaction and *SCN1A* gene repression, potentially associated with epilepsy. The authors identified twelve variants, NM_001202435.1:n.6277A > G, n.6568_6571del, n.6761C > T, n.6874A > T, n.6907 T > C, n.6978A > G, n.7065_7066insG, n.7282 T > C, n.7338_7344del, n.7385 T > A, n.7996 C > T, and n.8212C > T in 3′-UTR of *SCN1A* gene. The authors have observed that the genotype distribution of n.7282 T > C was significantly different in the male group, being the homozygous variant (CC) and

heterozygous (CT) much less frequent in male patients than in male controls (Table 2). Other two variants, n.7996C > T and n.8212C > T did not significantly distribute genotypes differently between cases and controls. In female subset, three variants were distributed relatively even in the patient and control group, n.7282 T > C, n.7996 C > T, and n.8212 C > T (Table 2). The genetic variant n.6978 A > G was fully deviated (variant GG, 100%) from that of the homozygous genotype (AA). The homozygous variants genotypes frequencies of n.6277 A > G, n.6568_6571del, n.6761 C > T, n.6874 A > T, n.6907 T > C, n.7065_7066insG, n.7338_7344del, and n.7385 T > A were quite low, one or two cases in some gender group (male group or female group).

More recently, (Xiao et al. 2019) have experimentally confirmed that SNV rs3208684 A > C in 3′-UTR of BCL2 like 1 (*BCL2L1*) impairs the ability of let-7b binding affinity with *BCL2L1*. Previous study have demonstrated that *BCL2L1*, an anti-apoptotic member of the Bcl-2 family, it was found to be overexpressed in human TLE, conferring a survival property to neural cells (Henshall et al. 2000). In addition, it was reported that let-7b could act as a key regulator in the intrinsic apoptotic pathway by targeting *BCL2L1* (Yan et al. 2017), since it was also verified previously that Let-7b is downregulated in TLE (McKiernan et al. 2012).

Using Luciferase report assays, Xiao and colleagues (Xiao et al. 2019) have demonstrated that miR-200c targeted 3′-UTR of the DNA methyltransferase 3 alpha (*DNMT3A*) gene expression and the SNV rs35163679, within the miR-200c binding site, influenced the ability of miR-200c binding affinity with *DNMT3A*. Previously, it was reported increased *DNMT3A* expression in patients with intractable TLE (Zhu et al. 2012). *DNMT3A* is a member of the DNA methyltransferase enzyme family, which promotes de novo methylation during development and regulate synaptic function in mature central nervous system neurons (Feng et al. 2010).

In conclusion, SNVs in the 3′-UTR of miRs target genes may be potential molecular pathological mechanisms of TLE and therapeutic targets; however, case–control studies including different ethnic populations need to be performed to confirm the results.

The SNV n.-411A > G (rs57095329) in *miR-146a* as a Risk Factor for DRE

As pointed out before, most of the studies were unique and the findings need to be reproduced in a larger patients' cohort in different populations. However, after a literature review, three similar studies for SNV rs57095329 at miR-146a was identified in DRE patients (Cui et al. 2015; Li et al. 2016b; Boschiero et al. 2020). In this context, we input all data for the SNV rs57095329 in a dataset, aiming first to compare the results and then, to have a better design to identify an association between SNV rs57095329 and DRE. Thus, we performed one subgroup data including all Chinese and Brazilian DRE patients versus healthy Chinese and Brazilian individuals.

The comparative association of the SNV rs57095329 in patients with DRE and controls groups are showed in Table 3. The percentage of different genotypes individually for the evaluated SNV was similar in the two Chinese studies; however, it was different for Brazilian patients (Boschiero et al. 2020).

Interestingly, after the association between Chinese and Brazilian samples, it was observed significantly genotype differences between patient and control groups. Thus, increased frequency of AA genotype was observed in patients compared to controls [55.98% versus (vs.) 41.60%, $p\text{-}value \leq 0.01$] with 1.78 [95% confidential interval (CI) = 1.43–2.22] risk for DRE (Table 3). The A allele presented significantly risk for the disease compared to G allele (68.37% vs. 61.34%, $p\text{-}value \leq 0.01$) with an Odds ratio (OD) of 1.36 (95%CI = 1.13–1.65).

Our results highlighted that the SNV rs57095329 might be correlated with DRE when a larger number of patients are evaluated. Thus, we concluded that the main drawback of most of studies is the small number of individuals enrolled, which lacks sample power. Epilepsy is a multifactorial disorder in which genetic susceptibility and environmental factors may be implicated; larger cohort from different countries including patients with DRE and patients' drug-responsiveness are needed to confirm the possible association of SNV rs57095329.

Conclusions

- The most evaluated SNVs associated with DRE risk were SNVs n.60G > C (rs2910164) and n.-411A > G (rs57095329), both located at miR-146a mature sequence and promoter region, respectively.
- MiR-146a has been identified to be involved in the upregulation of inflammatory responses in human astrocytes in epileptogenesis through NF-κB signaling by targeting *TRAF6* gene and miR-155 has been reported as inflammatory pathway genes modulator in early-onset epilepsy development.
- The CC haplotype (rs987195-rs969885) and the AA genotype at rs4817027 in the MIR155HG/miR-155 tag SNV were associated with early-onset epilepsy.
- SNVs rs662702, rs3208684, and rs35163679 at 3′-UTR impairs the ability of miR-328, let-7b, and miR-200c binding affinity with *PAX6*, *BCL2L1*, and *DNMT3A* target genes, indicating that SNVs in 3′-UTR of target genes may be potential molecular pathological mechanisms of

Table 2 Association between single-nucleotide variants (SNVs) in the 3′untranslated region (UTR) of microRNAs (miRs) target genes and epilepsy

References	Population	Methods	SNVs	3′-UTR genes	miRs	Putative risk alleles	**OR (95%IC)**
Panjwani et al. (2016)	US, Canada, Argentina, France and the UK	Control vs. Rolandic epilepsy	rs662702 C > T	*PAX6*	*miR-328*	**CC/CT/TT ($p = 2.6 \times 10^{-4}$)**	**12.29 (3.20–7.22)**
Li et al. (2016a)	China	Control vs. epileptic patients	n.6277A > G	*SCN1A*	–	–	–
			n.6568_6571del	*SCN1A*	–	–	–
			n.6761C > T	*SCN1A*	–	–	–
			n.6874A > T	*SCN1A*	–	–	–
			n.6907 T > C	*SCN1A*	–	–	–
			n.6978A > G	*SCN1A*	–	–	–
			n.7065_7066insG	*SCN1A*	–	–	–
			n.7282 T > C	*SCN1A*	–	**TT/CC + CT ($p < 0.05$) (Male patient)** TT/CT/TT ($p > 0.05$) (*Female patient*)	0.42 (1.61–0.11) 1.50 (0.36–1.17)
			n.7338_7344del	*SCN1A*	–	–	–
			n.7385 T > A	*SCN1A*	–	–	–
			n.7996 C > T	*SCN1A*	–	CC + CT/TT ($p > 0.05$) CC/CT/TT ($p > 0.05$) (*Female patient*)	0.875 (0.89–0.62) 0.91 (0.86–0.68)
			n.8212C > T	*SCN1A*	–	CC/CT + TT ($p > 0.05$) CC/CT/TT ($p > 0.05$) (Female *patient*)	0.77 (1.12–0.60) 1.03 (0.94–1.01)
Xiao et al. (2019)	–	Luciferase report assay	rs3208684 A > C	*BCL2L1*	*let-7b*	–	–
		Luciferase report assay	rs35163679	*DNMT3A*	*miR-200c*	–	–

SNVs single-nucleotide variants, *3´-UTR* 3′untranslated region, *vs.* versus, *OR* odds ratio with 95% confidence intervals, *US* United States of America, *UK* United Kingdom, *miRs* microRNAS, *DNMT3A* DNA methyltransferase 3 alpha, *PAX6* paired box protein PAX-6, *BCL2L1* BCL2 like 1, *SCN1A* sodium voltage-gated channel alpha subunit 1

TLE; however, case–control studies including different ethnic populations need to be performed.

- SNV rs57095329 might be correlated with DRE when a larger number of patients are evaluated. Thus, we concluded that the main drawback of most of studies is the small number of individuals enrolled, which lacks sample power.

Table 3 Comparative association of the single-nucleotide variant n.-411A > G (rs57095329) in *miR-146A* in patients with drug-resistant epilepsy and health control groups

Genotypes	Patients n (%) A	Controls n (%) A	Odds ratio (95%CI)		
			Additive (AA vs. GA vs. GG)	Dominant (GA + GG vs. AA)	Recessive (AA + GA vs. GG)
AA	0 (0.00)	5 (2.14)	NA	NA	Reference
GA	58 (95.08)	221 (94.44)	NA	NA	Reference
GG	3 (4.92)	8 (3.42)	NA	NA	1.46 (0.242–6.33)
p-value by model			0.597*	0.587*	0.703*
Genotypes	Patients n (%) B	Controls n (%) B	Additive	Dominant	Recessive
AA	160 (59.93)	152 (56.93)	NA	1.13 (0.80–1.60)	Reference
GA	89 (33.33)	76 (28.46)	NA	Reference	Reference
GG	18 (6.74)[a]	39 (14.61)	NA	Reference	0.42 (0.24–0.76)
p-value by model			≤0.01**	0.482** (0.405#)	0.003** (0.087#)
Genotypes	Patients n (%) C	Controls n (%) C	Additive	Dominant	Recessive
AA	163 (65.46)	155 (62.25)	NA	1.15 (0.80–1.66)	Reference
GA	79 (31.73)	86 (34.54)	NA	Reference	Reference
GG	7 (2.81)	8 (3.21)	NA	Reference	0.87 (0.31–2.44)
p-value by model			0.754**	0.456**	0.793**
Genotypes	Patients n (%)—Total	Controls n (%)—Total	Additive	Dominant	Recessive
AA	323 (55.98)[b]	312 (41.60)	NA	1.79 (1.43–2.22)	Reference
GA	226 (39.17)	383 (51.07)	NA	Reference	Reference
GG	28 (4.85)	55 (7.33)	NA	Reference	0.65 (0.40–1.03)
p-value by model			≤0.01**	≤0.01**	0.068**
Allele	Patients n (%)—Total		Controls n (%)—Total		Allelic analysis
A	323 (68.37)		695 (61.34)		1.36 (1.13–1.65)
G	254 (31.63)		438 (38.66)		Reference
p-value					≤0.01**

*Fisher's test

**Chi-square

#Adjusted odds ratio based on age and sex. *OR* odds ratio, *95%CI* 95% confidence interval, *NA* not applicable

[A]Boschiero et al. 2020

[B]Li et al. 2016a, b

[C]Cui et al. 2015

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Data Availability The data and material will be available on request.

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethics Approval This study was approved by the Ethic Committee of Universidade São Francisco (USF) (CAAE: 90786718.1.0000.5514). 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.

Consent for Publication All the authors gave the consent for publication.

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4. CAPÍTULO 2: publicado

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O objetivo do artigo foi apresentar o primeiro estudo baseado em evidências sobre o perfil epidemiológico de indivíduos com epilepsia no serviço de neurologia do Hospital Universitário São Francisco na Providência de Deus (HUSF) mapeando as características desta doença em um centro de referência na região do Sudeste do Brasil. Nossa taxa de prevalência da epilepsia foi menor do que outros estudos na região sudeste do Brasil. A etiologia estrutural predominou em nossa região em comparação com a causa desconhecida, que é mais frequente em outras regiões do Brasil e no mundo. As diferenças podem ser atribuídas à alta prevalência de neuroinfecção, principalmente neurocisticercose, em nossa região e por sermos um centro de referência para traumatismo cranioencefálico.

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Epidemiologic Profile of Patients With Epilepsy in a Region of Southeast Brazil: Data From a Referral Center

Renata Parissi Buainain[1,2*†], *Carlos Tadeu Parisi Oliveira*[3], *Fernando Augusto Lima Marson*[1,2†] *and Manoela Marques Ortega*[1,2†]

[1] *Laboratory of Cell and Molecular Tumor Biology and Bioactive Compounds, São Francisco University, São Paulo, Brazil,* [2] *Laboratory of Human and Medical Genetics, São Francisco University, São Paulo, Brazil,* [3] *São Francisco University Hospital, São Paulo, Brazil*

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Mireya Fernandez-Fournier, University Hospital La Paz, Spain

***Correspondence:**
Renata Parissi Buainain
renata.buainaim@usf.edu.br

†ORCID:
Fernando Augusto Lima Marson
orcid.org/0000-0003-4955-4234
Manoela Marques Ortega
orcid.org/0000-0003-4609-7074
Renata Parissi Buainain
orcid.org/0000-0003-0244-4011

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Introduction: Epilepsy affects about 50 million people worldwide, 80% of whom live in low- and middle-income countries. In Brazil, epidemiological studies are outdated and restricted to specific regions, mostly due to the continental size of country.

Objective: We aimed to present the first evidence-based study on the epidemiological aspects of individuals with epilepsy, mapping the characteristics of this disease in a referral center in a region of Southeast Brazil.

Methods: A retrospective study was carried out from January 2010 to March 2021. Patients were selected according to the International League Against Epilepsy Criteria.

Results: From a total of 618 selected patients, 317 (51.3%) were men and 301 (48.7%) were women with an average age of 34.03 ± 20.66 years. The average age at the first seizure was 15.16 ± 17.61 years. The prevalence ratio was 1.30 cases/1,000 habitants. Childhood febrile seizure was present in 44 patients (7.9%) and family history of epilepsy in 231 (37.4%) patients. The predominant type of seizure was focal in 401 (64.9%) patients. The most frequent etiologies were structural in 254 (41.1%) patients and unknown in 238 (38.5%) patients. Most of the patients' treatments were based on anti-seizure drugs in monotherapy [389 (62.9%)] with 398 (64.4%) drug-responsive patients.

Conclusions: Our epilepsy prevalence rate was lower than other studies in the Southeast Region of Brazil. In addition, the structural epilepsy type was predominant in our study compared with unknown causes, which is more frequent in other Brazilian regions and worldwide studies. The differences may be attributed to our region, which presents a high prevalence of neuroinfection, specially neurocysticercosis, and a referral center for traumatic brain injury. Moreover, the contrasting results reinforce the need for an adequate epidemiological assessment of epilepsy incidence in a region of Southeast Brazil.

Keywords: epilepsy, epidemiology, prevalence, neurocysticercosis, neuroinfection, traumatic brain injury

KEY POINTS

- In line with previous Brazilian studies, in our study we found similar race (white prevalence), the average age of 34.06 ± 20.66 years, average age at seizures' onset of 15.16 ± 17.61 years, the presence of childhood febrile seizures (7.9%), focal type seizures (64.9%), monotherapy treatment (62.9%), and drug-responsiveness (64.4%).
- Our epilepsy prevalence rate was lower than other studies in the Southeast Region of Brazil, which reinforces the need for an adequate epidemiological assessment of epilepsy incidence in Southeast Brazil, the most populated region.
- The most frequent etiology was structural (41.1%) compared with previous Brazilian and worldwide studies, where unknown (up to 50.0%) was more prevalent. The difference between etiologies can be attributed to the high prevalence of neuroinfection as neurocysticercosis and due to our hospital being a referral center for traumatic brain injury.
- Our study population presented a prevalence ratio of 1.3 cases/1,000 habitants.

INTRODUCTION

Epilepsy is a chronic condition characterized by unprovoked recurrent epileptic seizures (1) and may affect the individuals of all ages, sexes, races, income groups, and geographical areas (2). Epilepsy affects about 50 million people worldwide, 80% of whom live in low- and middle-income countries (2). In 2017, the prevalence rate of epilepsy in developing countries was 8.75 per 1,000 individuals and in developed countries was 5.18 per 1,000 individuals (3).

The prevalence rate of epilepsy in developing countries is usually higher than in developed countries, mostly due to higher neurocysticercosis and head trauma rates and the lack of available treatments (4). In addition, despite the very-low cost of effective anti-seizure drugs, more than 75% of people with epilepsy in low-income countries do not receive any treatment (2). The Global Burden of Disease Study of Epilepsy (GBDE) estimated over 125,000 deaths associated with epilepsy in 2016, of which 81% were in low- and middle-sociodemographic index countries (5).

In fact, between 1990 and 2016, there was a non-significant change in the age-standardized prevalence of idiopathic epilepsy. In contrast, a significant decrease in age-standardized mortality rates and age-standardized disability-adjusted life-years rates were observed (5). Thus, to reduce the rate of epileptic seizures in low-income countries, it would be necessary to facilitate access to the anti-seizure treatments and effective new drugs development (5).

Garcia-Martin and Serrano-Castro (6) conducted an epilepsy epidemiological review study in Spain and Latin America due to the similarity of the population. Despite changes in the economic development and health conditions, the authors concluded that there was no evidence of any changes in the epidemiology of epilepsy in Spain and Latin America in the last 15 years. In addition, Bolivia, Colombia, Ecuador, and Peru, known as the endemic areas of cysticercosis, showed the highest prevalence and incidence rates of epilepsy (6).

To the best of our knowledge, only 11 epidemiological epilepsy population studies have been conducted in Brazil between 1986 and 2018 (7–17) (**Table 1**). In Brazil, epidemiological studies are rare besides being outdated and restricted to specific regions due to the continental size of country, difficulty in collaboration between the referral centers, and diagnostic errors. This study aimed to present the prevalence of epilepsy in a region of Southeast Brazil, describing the patients' characteristics of this disease in the referral center as the first evidence-based epidemiological aspect in this Brazilian region.

Abbreviations: IBGE, brazilian institute of geography and statistics; CT, computed tomography; CI, confidence interval; ICD 10, international classification of diseases 10; ILAE, international league against epilepsy; LTE, lifetime epilepsy; MRI, magnetic resonance imaging; USF, são francisco university; HUSF, são francisco university medical school hospital; GBDE, the global burden of disease study of epilepsy; USA, united states of america; WHO, world health organization.

METHODS

Ethical Committee

The study is a retrospective study and based on the observational database analysis. The study was approved by the Ethics Committee of São Francisco University, Bragança Paulista, São Paulo, Brazil (approval #28258920.7.0000.5514).

Patient's Selection

A retrospective study of patients with epilepsy was carried out from January 2010 to March 2021. Patients of all ages selected according to the inclusion criteria in the "Inclusion and exclusion criteria" section below were enrolled in the study. Data were collected from São Francisco University Hospital (HUSF), Bragança Paulista, located in Southeast Brazil, São Paulo. The data obtained were used for an epidemiological study of the region (rural and urban areas), which is composed of 11 municipalities served by the city of Bragança Paulista that contributes about 64.5% of the total population, according to the Brazilian Institute of Geography and Statistics (18) (**Supplementary Table 1**).

The selection of patients with epilepsy was performed from an electronic medical record system at the hospital, which contains all numbers of patients' medical records. We used the current International Classification of Diseases 10 (ICD 10), according to the World Health Organization (WHO), namely, G40 (G40.0, G40.1, G40.2, G40.3, G40.4, G40.5, G40.6, G40.7, G40.8, and G40.9) to define epilepsy. The study included medical records from patients from 1 January 2010 to 31 March 2021. Further, all medical records (1,272) were evaluated by two authors (Buainain, RP and Oliveira, CTP), considering anamnesis, personal and family history of epilepsy, clinical and neurological physical examination, electroencephalogram, and brain images, such as magnetic resonance imaging (MRI) and computed tomography (CT) to confirm the epilepsy diagnosis and to perform the seizures classification.

TABLE 1 | Prevalence of epilepsy in epidemiological studies in Brazil between 1986 and 2021.

City—state	*N*	Prevalence of epilepsy	Prevalence of active epilepsy	Age group	References
São Paulo/São Paulo (urban area)	7,603 interviews	11.9/1,000	—	All ages	(7)
Florianópolis/Santa Catarina (epilepsy clinic)	120 medical records	294.8/1,000	—	>18 years	(8)
Paranatinga-Nobres/Mato Grosso (Indigenous Bakairi)	483 interviews	186/1,000	124/1,000	All ages	(9)
Rio de Janeiro/Rio de Janeiro (urban community)	982 interviews	16.3/1,000	5.1/1,000	All ages	(10)
São José do Rio Preto/São Paulo	17,293 interviews	18.6/1,000	8.2/1,000	All ages	(11)
São José do Norte/Rio Grande do Sul (rural and urban areas)	531 interviews	45.2/1,000	—	<5 years	(12)
Districts of Barão Geraldo-Jaguaré- Santo Antônio/São Paulo	54,102 interviews	9.2/1,000	5.4/1,000	All ages	(13)
District of Paraisópolis/São Paulo	22,013 interviews	9.7/1,000	8.7/1,000	0-16 years	(14)
Passo Fundo/Rio Grande do Sul	2,285 born between 2003 and 2007	6.52/1,000	5.33/1,000	0-4 years	(15)
Barra do Bugres/Mato Grosso (Semiurban region)	30,132 interviews	7.8/1,000	5.6/1,000	All ages	(16)
Pelotas/Rio Grande do Sul (Medicine Faculty at the Federal University of Pelotas)	101 interviews	—	673/1,000	12-75 years	(17)

Inclusion and Exclusion Criteria

The inclusion criteria for this study were based on the International League Against Epilepsy (ILAE) criteria: "at least two unprovoked (or reflex) seizures occurring >24 h apart" (1). Pure febrile seizures in childhood, single seizures, provoked seizures, neonatal seizures, and non-epileptic events were excluded. The febrile seizure was defined as "an event in infancy or childhood, usually occurring between 6 months and 5 years of age, associated with fever but without evidence of intracranial infection or defined cause" (19).

Statistical Analyses

The numerical data are shown as mean ± standard deviation (SD); median (percentile 25-75). The categorical data are shown as absolute frequency and percentage. The statistical analysis was done using Mann–Whitney and Fisher's exact tests, and an alpha of 0.05 was adopted in all analyses. The statistical analysis was performed with the Statistical Package for the Social Sciences software (IBM SPSS Statistics for Macintosh, Version 27.0).

RESULTS

Demographic Characteristics of the Patients With Epilepsy

The medical records of 1,272 patients were evaluated and only 618 (48.5%) patients were included in our study, according to the ILAE criteria. Bragança Paulista city presented 170,533 inhabitants and adding the comprehensive regions, it had a total population of 480,623 inhabitants, according to the IBGE, in 2020 (**Supplementary Table 1**). The 618 patients with epilepsy included in the present study correspond to a total prevalence ratio of 1.30 patients/1,000 habitants or an average prevalence of 11.69 patients/100,000 habitants/year.

The original cohort accounted for 618 individuals who met the criteria for defined epilepsy were 317 (51.3%) men and 301 (48.7%) women. No absolute predominance was observed (1.05:1.00) for sexes, and the percentages were nearly the same (51.3%).

Clinical Characteristics of the Patients With Epilepsy

The clinical information is shown in **Table 2**. Briefly, the average age of the patients was 34.03 ± 20.66 years, and the average age at the first seizure was 15.16 ± 17.61 years. The predominant race was White in 248 (40.1%) patients; childhood febrile seizure occurred in 44 (7.9%) patients, and the family history of epilepsy in 231 (37.4%) patients. We observed focal seizures in 401 (64.9%) patients considering the seizure type. The etiology of epilepsy was structural in 254 (41.1%) patients and unknown in 238 (38.5%) patients. Most of the patients' treatments were based on anti-seizure drugs in monotherapy [389 (62.9%)], and drug-responsive occurred in 398 (64.4%) patients.

Association Between the Demographic and Clinical Data Among Patients With Epilepsy

Significantly statistical data were observed when age (years) was compared with sex (male 32.25 ± 20.86; female 35.91 ± 20.31; $p = 0.011$) (**Table 3**; **Figure 1A**). The febrile seizure was present at the time of the first seizure at ages between 0 and 2 years (2.59 ± 5.67 years; $p < 0.001$) (**Table 3**; **Figure 1B**). Curiously, the patients without febrile seizures had an older age at the first seizure (16.63 ± 18.05 years). In addition, patients with any genetic syndrome were younger (22.31 ± 17.63 vs. 34.55 ± 20.64 years; $p = 0.002$) (**Table 3**; **Figure 1C**) and had first seizure early (5.95 ± 12.81 vs. 15.51 ± 17.68 years; $p < 0.001$) (**Table 3**; **Figure 1D**).

The monotherapy was commonly used among patients with generalized seizures (87.0%) followed by patients with focal seizures (56.8%) and focal and generalized seizures (50.0%) ($p = 0.009$) (**Table 4**).

TABLE 2 | Epidemiological and clinical data of 618 patients with epilepsy from a Brazilian University Hospital in Southeast São Paulo between January 2010 and March 2021.

Marker	Distribution (*n*, %) or numerical data*
Sex	
Male	317 (51.3%)
Female	301 (48.7%)
Race	
Asian	3 (0.5%)
White	248 (40.1%)
Black	12 (1.9%)
Mixed Black and White (Multiracial background)	15 (2.4%)
Not described/missing data	340 (55.0%)
Febrile seizure	
Absent	528 (85.4%)
Present	44 (7.9%)
Not described/missing data	41 (6.6%)
Family history	
Absent	226 (36.6%)
Present	231 (37.4%)
Not described/missing data	161 (26.1%)
Seizure type	
Focal	401 (64.9%)
Generalized	23 (3.7%)
Focal and generalized	8 (1.3%)
Not described/missing data	186 (30.1%)
Etiology of epilepsy	
Structural	254 (41.1%)
Genetic	3 (0.5%)
Infectious	8 (1.3%)
Metabolic	2 (0.3%)
Immune	1 (0.2%)
Unknown	238 (38.5%)
Not described/missing data	112 (18.1%)
Epilepsy therapy	
Monotherapy	389 (62.9%)
Polytherapy	23 (36.1%)
Not described/missing data	6 (1.0%)
Therapy response	
Drug responsive	398 (64.4%)
Drug-resistant	85 (13.8%)
Not described/missing data	135 (21.8%)
Age (years)	34.03 ± 20.66; 29.00 (16.00-49.00)
Age at first seizure (years)	15.16 ± 17.61; 9.00 (2.00-22.00)

** The numerical data are shown as mean ± standard deviation (SD); median (percentile 25–75).*

TABLE 3 | Clinical data in association with age and age at the first seizure in patients with epilepsy from a Brazilian University Hospital in Southeast São Paulo between January 2010 and March 2021.

Sex	Age (years)*	Age at first seizure (years)*
Male	32.25 ± 20.86; 27 (15.00-47.50)	14.61 ± 17.54; 8 (1.00-22.25)
Female	35.91 ± 20.31; 32 (20.00-50.00)	15.74 ± 17.71; 10 (2.00-21.75)
P-value	0.011	0.139
Febrile seizure		
Absent	35.39 ± 20.73; 31 (18.00-51.00)	16.63 ± 18.05; 11 (3.00-25.75)
Present	17.71 ± 11.06; 15 (8.50-24.00)	2.59 ± 5.67; 1 (0.00-2.00)
P-value	<0.001	<0.001
Syndrome		
Absent	34.55 ± 20.64; 30 (17.00-49.00)	15.51 ± 17.68; 9 (2.00-23.00)
Present	22.31 ± 17.63; 17.5 (11.50-30.00)	5.95 ± 12.81; 0 (0.00-7.00)
P-value	0.002	<0.001

** The numerical data are shown as mean ± standard deviation; median (percentile 25–75). The statistical analyses were done using the Mann–Whitney test. An alpha of 0.05 was adopted in all analyses.*

Table 5 presents the epilepsy etiology of all evaluated patients, being structural [male 131 (51.0%); female 12 (49.4%)] the leading cause of epilepsy. Monotherapy was statistically significant in the structural, genetic, metabolic, and unknown etiologies ($p < 0.001$); polytherapy prevailed in the infectious and immune etiologies ($p < 0.001$) (**Table 5**). In addition, the association between the epilepsy etiology and the resistance to treatment was significant ($p = 0.002$) (**Table 5**).

DISCUSSION

The present study aimed to describe the epidemiological profile of patients with epilepsy in a referral center in Southeast Brazil. In addition, we pointed out that since 1986, 11 epidemiological population survey studies have been conducted in Brazil (7–17) with wide variability in the epilepsy prevalence rate.

Among the studies that have been conducted in the Southeast Region of Brazil, the prevalence rates were between 9.2/1,000 and 18.6/1,000 individuals (7, 10, 11, 13, 14); in contrast, we had a prevalence rate of 1.30 cases/1,000 individuals. The contrasting results reinforce the need for an adequate epidemiological assessment of epilepsy incidence in Brazil, mainly in the Southeast region, which is the most populated region. In addition, the low prevalence of patients with epilepsy in our study can be explained by the fact that the data are from a single-center, and it cannot represent all patients with epilepsy in this part of the Southwest region of Brazil.

The epidemiological studies on epilepsy in Latin America in the last 10 years are rare. Recently, Alva-Díaz et al. (20) have published a meta-analysis in Latin America and the Caribbean, and the authors have found that the active epilepsy prevalence

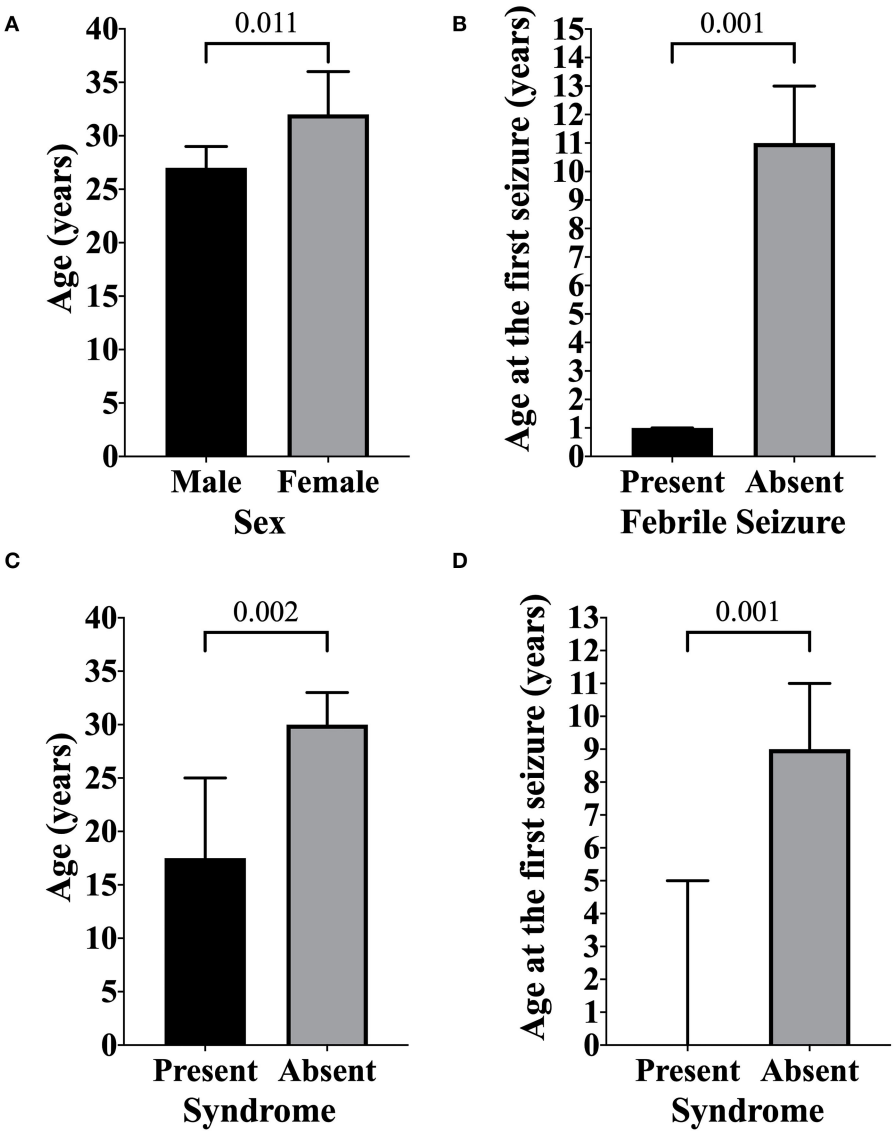

FIGURE 1 | Overview of the significant association between age and sex, genetic syndrome, and first seizure. **(A)** Significant statistical data were observed when age was compared with sex (male 27 years; female 32 years; $p = 0.011$), indicating that men present seizures earlier than women. **(B)** Childhood febrile seizure was present at the age of the first seizure at ages between 0 and 2 (1 year; $p < 0.001$). In addition, the age at first seizure in patients without febrile seizures was 11 years. **(C)** Patients with any genetic syndrome presented seizures earlier (17.5 vs. 30 years; $p = 0.002$). **(D)** Age at the first seizure in patients with genetic syndromes ranged between 0 and 7 years (present <1 year; absent 9 years; $p < 0.001$). The data are shown as median and 95% confidence interval (CI). The statistical analyses were done using the Mann–Whitney test. An alpha of 0.05 was adopted in all analyses.

was 9.06 per 1,000 individuals. Melcon et al. (21) have observed a prevalence rate of 3.8 per 1,000 individuals with active epilepsy in a town in the Province of Buenos Aires.

Fiest et al. (3) demonstrated, in a meta-analysis including 222 studies between 1985 and 2017, the prevalence of active epilepsy of 6.38 per 1,000 persons. In addition, the incidence rate of epilepsy was higher in low- to middle-income countries.

Our study found a quite predominance of epilepsy in men and a mean age of patients with epilepsy of 34 years, while two previous Brazilian studies, also from the Southeast region (11, 13), have observed the predominance rate of women patients (51.2 and 51.3%), with a mean age of 36.0 and 38.4 years, respectively. In addition, a third study in the Southern region of Brazil has observed a predominance rate of mens (55.0%) with a mean age of 16.6 years (22). In the Argentine study, the prevalence rate of epilepsy was 5.3/1,000 for male patients and 7.1/1,000 for female patients (21). González et al. (23) have analyzed patients with epilepsy in Paraguay found 51.9% of male patients. Moreover, a review study considering North, Central, and South America, Europe, and Asia, between 1985 and 2007 (24) has found a higher prevalence rate of epilepsy in men than in women.

TABLE 4 | Clinical data and the type of seizure in individuals with epilepsy from a Brazilian University hospital from January 2010 to March 2021.

Marker	Data	Focal	Generalized	Focal and generalized	*P*-value
Sex	Male	204 (94.9%)	8 (3.7%)	3 (1.4%)	0.250
	Female	197 (90.8%)	15 (6.9%)	5 (2.3%)	
Febrile seizure	Absent	354 (93.4%)	19 (5.0%)	6 (1.6%)	0.563
	Present	22 (91.7%)	2 (8.3%)	0 (0.0%)	
Therapy	Monotherapy	226 (56.8%)	20 (87.0%)	4 (50.0%)	0.009
	Polytherapy	172 (43.2%)	3 (13.0%)	4 (50.0%)	
Therapy response	Drug responsive	251 (77.5%)	19 (95.0%)	5 (71.4%)	0.123
	Drug resistant	73 (22.5%)	1 (5.0%)	2 (28.6%)	

The statistical analyses were done using the Fisher's exact test. An alpha of 0.05 was adopted in all analyses.

TABLE 5 | The clinical data and etiology of epilepsy in individuals with epilepsy from a Brazilian University hospital from January 2010 to March 2021.

Marker	Data	Structural	Genetic syndrome	Infectious	Metabolic	Immune	Unknown	*P*-value
Sex	Male	131 (51%)	2 (0.8%)	6 (2.3%)	1 (0.4%)	0 (0.0%)	117 (45.5%)	0.653
	Female	12 (49.4%)	1 (0.4%)	2 (0.8%)	1 (0.4%)	1 (0.4%)	121(48.6%)	
Therapy	Monotherapy	127 (50.4%)	2 (66.7%)	3 (37.5%)	2 (100%)	0 (0.0%)	176 (75.2%)	<0.001
	Polytherapy	125 (49.6%)	1 (33.3%)	5 (62.5%)	0 (0.0%)	1 (100%)	58 (24.8%)	
Therapy response	Drug responsive	152 (76.8%)	1 (100%)	4 (66.7%)	1 (100%)	0 (0.0%)	180 (89.1%)	0.002
	Drug resistant	46 (23.2%)	0 (0.0%)	2 (33.3%)	0 (0.0%)	1 (100%)	22 (10.9%)	

The statistical analyses were done using the Fisher exact test. An alpha of 0.05 was adopted in all analyses.

The mean age at the first seizure of the patients with epilepsy included in our study was approximately 15 years, close to a unique Brazilian study (16.6 years) in the Southern region (22). In the Argentine study, the median age at the onset of active epilepsy was 10.9 years for male patients and 16.9 years for female patients (21). According to a meta-analysis study (3), the incidence of epilepsy is higher in the youngest and oldest age groups.

In the same way, our study and Caprara et al. (22) presented a White race predominancy (40.1 and 64.2%, respectively). No previous Brazilian studies have evaluated ethnicity in patients with epilepsy. However, Szaflarski et al. (25) evaluated patients with epilepsy in Minnesota (USA), and 70.0% were categorized as White. These authors hypothesized that other ethnic minorities have more limited access to healthcare.

Further, our study observed that febrile seizure was present in 7.9% of evaluated patients with epilepsy and their age at the first seizure was ∼2.6 years. Only one study in Brazil has observed the same rate; however, the authors have evaluated only patients ≥ 15 years old (22). Another Brazilian study has observed an average age of the first childhood febrile seizure of 1.6 (26) in children aged 0–5 years. Shrestha et al. (27) have analyzed the clinical characteristics of children with febrile seizures in a hospital in Nepal and found that most children (72.8%) presented their first episode of seizure below 24 months of age.

Regarding seizure classification, focal seizures were present in almost 65.0% of our patients, according to the previous Brazilian (28), American (29), and European (30) epidemiological studies. Focal seizures are the predominant type worldwide, constituting up to two-thirds of epilepsies cases (24). According to our study, González et al. (23) observed that 63.34% of patients with epilepsy presented with focal seizures. In contrast, the Argentine study has shown that seizures were generalized in 37 (58.0%) and focal in 24 (38.0%) patients with epilepsy (21). In a previous meta-analysis with 222 studies, between 1985 and 2017, the epilepsies of unknown etiology and those with generalized seizures had the highest prevalence rate (3).

Epilepsy has a variety of etiologies, ranging from genetic, metabolic, infectious, structural, immune, and unknown (31). Our study is the second that evaluated the variety of etiologies in Brazilian patients with epilepsy. The predominant etiology of epilepsy in our study was structural and unknown. A previous Brazilian study (22) has found structural and unknown etiology in 29.8 and 44.4% of the patients, respectively. Interestingly, the unknown etiology type worldwide is most prevalent, up to 50.0% (4, 24).

It was recently reported in an American study that 25–30% of new-onset seizures are provoked or secondary (32). Our study presented approximately 67.0% of the cases with infection seizures. The discrepancy might be because our referral hospital is in a low-income region, where infectious conditions, such as neurocysticercosis, human immunodeficiency virus, meningitis, and encephalitis are predominant diseases. In addition, our hospital is a tertiary center and referral center for traumatic brain injury.

Monotherapy was the most common treatment type in our evaluated patients. It was previously reported that about 50.0% of patients with epilepsy respond well to the initial monotherapy (33), while in another half, more than one antiepileptic drug is necessary (34). In our data, drug responsiveness occurred in most of the patients. Kwan et al. (35) have defined drug-resistant

epilepsy "as the failure of adequate trials of two tolerated and appropriately chosen and used antiepileptic drugs (whether as monotherapies or in combination) to achieve sustained seizure freedom" and drug-responsive epilepsy as "in which the patient receiving the current antiepileptic drug regimen has been seizure-free for a minimum of three times the longest preintervention interseizure interval or 12 months, whichever is longer." Kawn and Brodie (33) have studied 470 patients with epilepsy, of which ∼50% of them responded promptly to the first monotherapy. Of the unresponsive remainder, the therapeutic strategy was to substitute or add another antiepileptic drug. Of these, only 26.0% were seizure-free, meaning that approximately 30.0% of patients will be drug-resistant. The treatment is generally pharmacological, and about 20–30% of patients are refractory to medications and thus become potential surgical candidates (36). Therefore, we achieved a successful treatment plan for those patients who are seizure-free, epilepsy controlled, and responded well to the medication.

CONCLUSIONS

Our epilepsy prevalence rate was lower than other studies in the Southeast Region of Brazil, which reinforces the need for an adequate epidemiological assessment of epilepsy incidence in Southeast Brazil. Regarding the variables studied: sex, ethnicity, age of patients, age at onset of seizures, presence of childhood febrile seizures, type of epileptic seizure, type of treatment (mono or polytherapy), as well as drug resistance or not, our data are similar to the national and international literature. However, concerning the etiology of the seizures, our data diverged to the predominance of structural causes, whereas in studies from Brazil and the world, the unknown cause is predominant. These differences can be attributed to the characteristics of region studied by the high prevalence of neurocysticercosis and a referral center for traumatic brain injury.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of São Francisco University, Bragança Paulista, São Paulo, Brazil (approval #28258920.7.0000.5514). Written informed consent for participation was not provided by the participants' legal guardians/next of kin because: only medical record review.

AUTHOR CONTRIBUTIONS

RB, FM, and MO wrote the manuscript. RB and CO performed the clinical research. FM performed all statistical analyses and designed the figures. MO designed the research. All authors have read and approved the final manuscript.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2022.822537/full#supplementary-material

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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5. CAPÍTULO 3: artigo em fase final de elaboração.

Título do artigo: *Single-base gene variants in MIR-146a and SCN1A related to the epileptogenic process in drug-responsive temporal lobe epilepsy*

Autores: Renata Parissi Buainain[1,2], André Rodrigues Sodré[1,2], Jéssica Silva dos Santos[1,2],

Luciano de Souza Queiroz[3], Paulo Henrique Pires de Aguiar[1,2,4], Fernando Augusto Lima Marson[1,2], Manoela Marques Ortega[1,2*]

O objetivo deste trabalho foi avaliar a associação das SNVs rs2910164 no gene *MIR-146a* e rs2298771 e rs3812718 no gene *SCN1A* em pacientes com epilepsia do lobo temporal farmacorresistentes e farmacorresponsivos em uma amostra da população brasileira, por ausência de estudos nesta população. Concluímos que os genótipos rs2910164-CC no *miR-146a* e/ou rs2298771-AA e no gene *SCN1A* exercem influência significativa no risco para a doença responsiva, provavelmente devido a uma regulação positiva da via NF-kB e perda de função do gene *SCN1A*.

Single-base gene variants in MIR-146a and SCN1A related to the epileptogenic process in drug-responsive temporal lobe epilepsy

Renata Parissi Buainain[1,2], André Rodrigues Sodré[1,2], Jéssica Silva dos Santos[1,2],

Luciano de Souza Queiroz[3], Carlos Tadeu Parisi de Oliveira[1,2,4], Paulo Henrique Pires de Aguiar[1,2,5], Fernando Augusto Lima Marson[1,2], Manoela Marques Ortega[1,2*]

[1] Laboratory of Cell and Molecular Tumor Biology and Bioactive Compounds, São Francisco University, Bragança Paulista, São Paulo, Brazil

[2] Laboratory of Human and Medical Genetics, São Francisco University, Bragança Paulista, São Paulo, Brazil

[3] Department of Pathology, Faculty of Medical Science, University of Campinas, Campinas, São Paulo, Brazil

[4] São Francisco University Hospital, Bragança Paulista, São Paulo, Brazil

[5] Department of Neurosurgery, Hospital Santa Paula, São Paulo, São Paulo, Brazil

*Corresponding author

Manoela Marques Ortega, Bsc, Msc, PhD

Laboratory of Cell and Molecular Tumor Biology and Bioactive Compounds / Laboratory of Human and Medical Genetics

Post Graduate Program in Health Science

São Francisco University

Avenida São Francisco de Assis, 218

Jardim São José

Bragança Paulista, São Paulo, Brazil, 12916-900

Phone +55 11 2454 8471

email: manoela.ortega@usf.edu.br

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

ABSTRACT

Objective

The drug-resistant temporal lobe epilepsy (TLE) has recently been associated to single nucleotide variants (SNVs) in microRNA(miR)-146a (rs2910164) and *SCN1A* (rs2298771 and rs3812718). Moreover, there are no studies displaying association between these SNVs and susceptibility to drug-resistant and drug-responsive TLE in Brazil.

Methods

DNA samples from 120 patients with TLE (55 drug-responsive and 65 drug-resistant) and 240 healthy individuals were evaluated by real-time-PCR. The *MIR-146A* and *SCN1A* relative expression was performed by quantitative-PCR. Fisher or X^2 and odds ratio (OR:95%CI) evaluated the difference between groups and risk, respectively.

Results

For rs2910164, an increased frequency of the variant CC was observed in patients compared to controls (15.00% vs. 5.42%, *p-value*=0.009) with a 2.99 (95%CI=1.36-6.60) risk. Also, a higher CC genotype frequency was observed in drug-responsive patients compared to controls (21.81% vs. 5.42%, *p-value*=0.003) with 4.08 (95%CI=1.66-10.04) risk. Moreover, drug-resistant patients harboring GC (*p-value*=0.05) or CC (*p-value*=0.04) genotypes presented lower *MIR146A* expression compared to GG patients. For rs2298771, an increased frequency of wild-type AA versus AG+GG was observed in patients compared to controls (42.50% vs. 26.67%, *p-value*=0.004) with 2.03 (95%CI=1.28-3.22) risk; nonetheless, a higher A allele frequency was observed in patients (68.30% vs. 58.50%, *p-value*=0.014). Drug-responsive patients also presented a higher frequency of AA (47.27% vs. 26.67%, *p-value*=0.005) versus AG+GG with a 2.47 (95%CI=1.35-4.50) risk and also A allele frequency (77.00% vs. 58.50%, *p-value*=0.035) with a 1.65 (95%CI=1.06-2.58) risk compared to controls. Further, *SCN1A* level was significantly higher in AG (*p-value*=0.004) and GG (*p-value*=0.0002) than in AA patients. Patients harboring combined genotypes rs2910164-CC and rs2298771-AA (7.5% vs. 1.7%) showed an OR=4.76 (95%CI=1.30-21.62) versus controls. Drug-responsive patients also presented higher risk for combined rs2910164-CC and rs2298771-AA (9.1% vs. 1.7%; OR=5.849 (95%CI=1.21-30.57). No association was observed for SNV rs3812718 and patients.

Significance

The rs2910164-CC and/or rs2298771-AA genotypes are exerting significant risk influence on responsive disease, probably due to an upregulated NF-kB and *SCN1A*-loss-of-function.

Key words

Temporal lobe epilepsy (TLE), drug-resistant and drug-responsive, single nucleotide variants (SNVs), *MIR-146a* and *SCN1A* genes.

Key points

- For SNV rs2910164 (*MiR-146a*), a significantly increased frequency of variant genotype (CC) was observed in drug-responsive TLE patients.
- *MIR-146A* relative expression level was lower in drug-resistant compared to drug-responsive patients for GC and CC genotypes versus GG for SNV rs2910164, indicating increased NF-kB inflammation process in drug-resistant patients harboring GC or CC genotypes. These results confirm our previous publication.
- For rs2298771 in the *SCN1A* gene, increased frequency of wild-type AA versus AG+GG was observed in drug-responsive TLE patients.
- *SCN1A* relative expression level was significantly higher in AG and GG versus AA genotype for SNV rs2298771, indicating that the wild-type genotype of the *SCN1A*, which encodes the NaV1.1 sodium channel alpha subunit, might result in truncation of the protein presenting a lower expression, with haploinsufficiency of NaV1.1.
- No association was observed for SNV rs3812718 in the *SCN1A* gene and Brazilian TLE patients, even when patients were divided into drug-responsive and drug-resistant groups.
- Alone or combined genotypes for SNVs rs2910164-CC and rs2298771-AA seem to be risk factors for Brazilian drug-responsive TLE patients; however, a larger number of patients needs to be evaluated to confirm our results.

INTRODUCTION

Epilepsy, a chronic disease of the central nervous system, affects individuals of all ages[1] with an average world incidence of around 5.4 per 1,000 individuals, being the average world lifetime incidence of approximately 7.0 per 1,000[2]. The World Health Organization[3] estimates 50 million people diagnosed with epilepsy in the world and 4 million in Brazil.[4] Still, about 66% of the epilepsy cases are temporal lobe epilepsy (TLE), classified as focal epilepsy.[5] It is the most common epileptic syndrome in adults, being 40% drug-resistant seizures.[6]

More than half of epilepsies cases present a genetic basis[7] and a complex inheritance pattern.[8] The single nucleotide variants (SNVs) alter amino acids of protein-coding genes and can drastically influence protein function and play a vital role in the pathophysiology of diseases such as epilepsy.[9] SNVs at microRNA (miR)-146a[11, 12] and voltage-gated sodium channel *SCN1A* (Sodium Voltage-Gated Channel Alpha Subunit 1)[10] have been recently related to TLE.

MiRs are small non-coding molecules that bind to mRNA and prevent its translation[13] and recent studies have observed miRs related to epilepsy.[12] The miR-146a is upregulated in human astrocytes in epileptogenesis[14] and it regulates the inflammatory process through nuclear factor-kappa B (NF-kB) signaling.[15] The SNV n.60G>C (rs2910164) located at mature sequence of miR-146a has been studied in Italian,[16] Chinese,[17,18] and Brazilian[11] population with contradictory results. In addition, only one Chinese and one Brazilian study have evaluated TLE patients.[11,18]

Epileptic seizure susceptibility versus multidrug-resistance has recently been related to the different genotypes from SNVs in the *SCN1A* gene, including SNV c.3184A>G (rs2298771)[19-25] and SNV IVS5N+5G>A (rs3812718).[19, 22, 26-28] There is only one study that has evaluated the SNV rs3812718 and risk to epilepsy not multidrug-resistant related in patients with TLE.[29]

In our best acknowledgment, this is the first study showing the association of SNV rs2910164 in the *MIR-146a* and SNVs rs2298771 and rs3812718 in the *SCN1A* gene and the susceptibility to drug-resistant and drug-responsive TLE in Brazilian population.

MATERIALS AND METHODS

Research Ethics Committee

This study was approved by the Ethic Committee of São Francisco University (approval #45723615.0.0000.5514).

Patient's Selection

The selection of patients with epilepsy was performed from an electronic medical record system at the hospital. Thus, a total of 70 patients with TLE were selected, being 55 drug-responsive and 15 drug-resistant. For the TLE diagnosis and seizure classification it was evaluated the personal and family history of epilepsy, clinical and neurological physical examination, electroencephalogram and/or video-electroencephalogram, magnetic resonance imaging or computed tomography.

In addition, the study comprised 50 samples of human drug-resistant TLE tissues obtained from surgical amygdalohippocampectomy patients between January 2015 to December 2018. All epilepsy paraffin-embedded tissues were donated from Prof. Dr. Luciano de Souza Queiroz, Department of Pathology, University of Campinas, São Paulo, Brazil. Moreover, those samples were used for a previous publication by our group.[11] In the present study, 50 samples were included in the group of drug-resistant TLE, totalizing 65 patients.

The patients' inclusion criteria were based on the International League Against Epilepsy.[30-33]

In addition, 240 healthy blood donor individuals with no personal and family history of epileptic seizures from Blood Center of São Francisco University were selected for the control group. Of the 240 samples, 66 were used in a previous publication by our group.[11]

Also, 1,171 healthy blood donor individuals from Brazilian genomic variants (ABraOM), a repository containing genomic variants of Brazilian population with a total of 77,236,632 variants (September 2020)[34], were added as a second control population for the studied SNVs.

DNA samples and SNVs identification

Ten milliliters of peripheral venous blood were collected from 55 drug-responsive, 15 drug-resistant patients, and all healthy control individuals. Further, genomic DNA samples for

genotyping were isolated using lithium chloride extraction.[35] Genomic DNAs were previously isolated from 50 drug-resistant patients´ tissues using a phenol/chloroform-based protocol.[11]

The *MIR-146a* (rs2910164) and *SCN1A* (rs2298771 and rs3812718) genotypes were identified using real-time polymerase chain reaction (RT-PCR) performed on StepOne RT-PCR (Applied Biosystems®, USA) using standard TaqMan® genotyping assay (rs2910164: C_15946974_10; rs2298771: C_11748767_20; rs3812718: C_25982233_10) according to the manufacturer's instructions.

Quantitative real-time polymerase chain reaction

Total RNA was isolated using Trizol® Reagent (Invitrogen™ from peripheral blood or paraffin-embedded epileptic tissue samples [rs2910164: GG n=12; GC n=10; CC n=7; rs2298771: TT n=10; CT n=9; CC n=6], according to the manufacturer's instructions. *MIR-146a* (assay 000468), and U6 (assay 001973) complementary DNA (cDNA) were synthesized from total RNA according to the TaqMan® real-time assays protocol (Applied Biosystems®). The relative expression of each target was quantified by the delta cycle threshold (ΔΔCt) method.[36] Each sample was examined in triplicate and the raw data were presented as the relative quantity of the target, normalized by U6. The expression value of each gene was represented in arbitrary units (AUs). For *SCN1A* analyses, cDNA conversion from total RNA was performed using High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems®, USA). Each sample was examined in triplicate and the expression of each gene was normalized by the control gene glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) and calculated by applying the 2-DDCt method. The expression value of the *SCN1A* gene was represented in AUs. Primer sequences used for amplification by quantitative polymerase chain reaction (PCR) with the SYBRGreen dye (Applied Biosystems®, USA) are as follows: *SCN1A* (forward) 5′-AGGCTGGAATATCTTTGACGG-3′ and (reverse) 5′-GCCAACTTGAAAACTCGCAG -3′; *GAPDH* (forward) 5′-CCACTTGATTTTGGAGGGAT-3′ and (reverse) 5′-GCACCGTCAAGGCTGAGAAC-3′.

Statistical Analyses

The Hardy-Weinberg equilibrium (HWE) was tested using the Chi-square test (χ^2). Differences between groups were analyzed by Chi-square (χ^2) or Fisher Exact test. For all statistical tests, significance is two-sided and achieved when *p-values* were less than 0.05. All tests were

performed using the Statistical Package for the Social Sciences (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp.).

RESULTS

Patients and healthy controls

The study's groups comprised 120 patients with TLE, 55 patients diagnosed as drug-responsive (22 male; 33 females; mean age: 45.96 years) and 65 patients as drug-resistant (27 male; 38 females; mean age: 51.18 years), and 240 healthy individuals as controls (112 female; 128 male; mean age: 43.65 years). In brief, there was no difference between control and patients' groups regarding sex (*p-value*=0.082). Besides that, the mean age of patients was higher in the drug-resistant than in the drug-responsive group (*p-value*<0.001) (Table 1).

The average of seizure onset age was earlier in the drug-resistant than in the drug-responsive group (11.09 vs. 15 years; *p-value*=0.004). The average of Caucasian patients was higher than 70% in both TLE groups (*p-value*=0.002). The side of epileptiform paroxysms were similar in both TLE groups (*p-value*=0.066) (Table 1). However, it was observed brain tissue injury was significant in the drug-resistant than in the drug-responsive group (90.80% vs. 40.70%; *p-value*<0.001) (Table 1).

The drug-resistant group used anti-epileptic drugs polytherapy in 96.90% of the cases while the drug-responsive used monotherapy in 72.20% of the cases (*p-value*<0.001) (Table 1).

Table 1. Clinical variables of the patients with temporal lobe epilepsy and 240 healthy control individuals enrolled in the study.

Markers	Groups	Patients		Controls n (%)	*p*-value
		Drug-resistant n (%)	Drug-responsive n (%)		
Sex	Female	38 (58.50)	33 (60.00)	112 (46.70)	0.082[a]
	Male	27 (41.50)	22 (40.00)	128 (53.30)	
Age (years)*		51.18 (48.25-54.26)	45.96 (40.38-51.46)	43.65 (42.12-45.27)	<0.001[c]
Race	White	51 (78.50)	39 (70.90)	197 (82.10)	0.002[b]
	Pardos (Admixed race)	13 (20.00)	4 (7.30)	20 (8.30)	
	Black	1 (1.50)	10 (18.20)	21 (8.80)	
	Asian	0 (0.00)	2 (3.60)	2 (0.80)	
Age of onset (years)**		11.09 (9.13-13.10)	15 (12-19)	NA	0.004
Electroencephalogram	Not specifed	2 (3.10)	5 (9.10)	NA	0.066[b]
	Normal	0 (0.00)	5 (9.10)	NA	
	Bilateral temporal	12 (18.50)	8 (14.50)	NA	
	Right temporal	22 (33.80)	18 (32.70)	NA	
	Left temporal	29 (44.60)	19 (34.50)	NA	
Lesion	Yes	59 (90.80)	22 (40.70)	NA	<0.001[a]
	No	6 (9.20)	32 (59.30)	NA	
Therapy	Monotherapy	2 (3.10)	39 (72.20)	NA	<0.001[b]
	Polytherapy	63 (96.90)	15 (27.80)	NA	

[a], Chi-square test; [b], Fisher's exact test; [c], T-test; [d], Mann-Whitney test. *, mean (95%CI); **, median (95%CI). NA, not applicable.

Genotype and allele frequency distribution for SNV rs2910164 at *MIR-146a*

For rs2910164, patient samples were in HWE (*p-value*=0.842), contrasting with control samples (*p-value*=0.015). Moreover, when patients were divided into two groups, drug-resistant (*p-value*=0.510) and drug-responsive (*p-value*=0.126) TLE samples remained in HWE (Table 2). Similar frequencies of wild-type homozygous (GG) (40.83% vs. 44.17%) and heterozygous (GC) genotypes (44.17% vs. 50.41%, *p-value*=0.915) were observed in patients and controls, respectively. In contrast, a significantly increased frequency of variant (CC) was observed in patients compared to controls (15.00% vs. 5.42%, *p-value*=0.009) with a 2.99 (95%CI=1.36-6.60) chance for TLE. In addition, when combined genotypes GG+GC were compared to CC alone, a significantly higher CC frequency was observed in TLE patients (15.00% vs. 5.42%, *p-value*=0.004) and higher chance for disease [3.08 (95%CI=1.45-6.53)] (Table 2).

Similar frequencies of genotypes were observed in drug-resistant patients and controls (*p-value*>0.05); although an increase in CC genotype was observed in drug-resistant patients compared to controls (9.23% vs. 5.42%, *p-value*=0.209), with an increase of 1.96 (95%CI=0.68-5.65) in the chance to be classified as patient (Table 2).

Interestingly, a significant CC genotype was also observed in drug-responsive TLE patients compared to controls (21.81% vs. 5.42%, *p-value*=0.003) with 4.08 (95%CI=1.66-10.04) chance for drug-responsive TLE. Still, when combined genotypes GG+GC were compared to CC alone in the drug-responsive group, a significantly higher CC frequency was observed in patients (21.81% vs. 5.42%, *p-value*<0.001) with increased drug-responsive TLE [4.87(95%CI=2.08-11.40)] (Table 2).

The frequencies of C (37.08% vs. 30.62%) and G (62.92% vs. 69.38.62%) alleles were similar in patients and controls (*p-value*=0.098), respectively (Table 2), as well as for drug-resistant and drug-responsive versus controls (*p-value*>0.05) (Table 2).

The comparative association of the variant rs2910164 in *MIR-146a* and susceptibility to TLE and controls are summarized in Tables 2.

Table 2. Comparative association of the variant rs2910164 in *MIR-146a* and susceptibility to temporal lobe epilepsy and controls.

Genotypes	**Patients n (%)[a]**	**Controls n (%)[b]**	***p-value***	**OR (95%CI)**
GG	49 (40.83)	106 (44.17)		Reference
GC	53 (44.17)	121 (50.41)	0.915*	0.948 (0.59-1.51)
CC	**18 (15.00)**	**13 (5.42)**	**0.009***	**2.995 (1.36-6.60)**
GG+CC	67 (55.83)	119 (49.58)		Reference
GC	53 (44.17)	121 (50.42)	0.316*	0.78 (0.50-1.21)
GG+GC	102 (85.00)	227 (94.58)		Reference
CC	**18 (15.00)**	**13 (5.42)**	**0.004***	**3.08 (1.45-6.53)**
GC+CC	71 (59.17)	134 (55.83)		Reference
GG	49 (40.83)	106 (44.17)	0.625*	1.15 (0.73-1.79)
Allele C	89 (37.08)	147 (30.62)	0.098	1.33 (0.96-1.85)
Allele G	151 (62.92)	333 (69.38)		Reference
Genotypes	**Drug-resistant n (%)[c]**	**Controls n (%)**	***p-value***	**OR (95%CI)**
GG	25 (38.46)	106 (44.17)		Reference
GC	34 (52.30)	121 (50.41)	0.553*	1.19 (0.67-2.13)
CC	6 (9.23)	13 (5.42)	0.209*	1.96 (0.68-5.65)
GG+CC	31 (47.69)	119 (49.58)		Reference
GC	34 (52.30)	121 (50.42)	0.896*	1.08 (0.62-1.87)
GG+GC	59 (90.77)	227 (94.58)		Reference
CC	6 (9.23)	13 (5.42)	0.401*	1.78 (0.65-4.87)
GC+CC	40 (61.54)	134 (55.83)		Reference
GG	25 (38.46)	106 (44.17)	0.495*	1.23 (0.72-2.22)
Allele C	46 (35.38)	147 (30.62)	0.353*	1.24 (0.83-1.87)
Allele G	84 (64.62)	333 (69.38)		Reference
Genotypes	**Drug-responsive n (%)[d]**	**Controls n (%)**	***p-value***	**OR (95%CI)**
GG	24 (43.63)	106 (44.17)		Reference
GC	19 (34.54)	121 (50.41)	0.352*	0.69 (0.36-1.34)
CC	**12 (21.81)**	**13 (5.42)**	**0.003***	**4.08 (1.66-10.04)**
GG+CC	36 (65.45)	119 (49.58)		Reference
GC	**19 (34.54)**	**121 (50.42)**	**0.048***	**0.52 (0.28-0.96)**
GG+GC	43 (78.19)	227 (94.58)		Reference
CC	**12 (21.81)**	**13 (5.42)**	**<0.001***	**4.87 (2.08-11.40)**
GC+CC	31 (56.36)	134 (55.83)		Reference
GG	24 (43.63)	106 (44.17)	0.937*	1.22 (0.56-1.84)
Allele C	43 (39.09)	147 (30.62)	0.109*	1.45 (0.95-2.23)
Allele G	67 (60.91)	333 (69.38)		Reference

*, Chi-square with Yates correction; OR, odds ratio; 95%CI, 95% confidence interval. *P*-value for Hardy-Weinberg equilibrium: a) 0.842; b) 0.015; c) 0.510; and d) 0.126. Values below 0.05 indicate that the sample is out of Hardy-Weinberg equilibrium.

Genotype and allele frequency distribution for SNVs rs2298771 and rs3812718 at *SCN1A* gene

For rs2298771, control samples were not in HWE (*p-value*<0.001), contrasting with TLE samples (*p-value*=0.105), which remain in HWE after division into two groups, drug-resistant (*p-value*=0.070) and drug-responsive samples (*p-value*=0.830) (Table 3).

Similar frequencies of heterozygous (AG) (51.67% vs. 63.75%) and variant (GG) (5.83% vs. 9.58%) genotypes were observed in patients and controls, respectively (*p-value*=0.681). An increase on wild-type homozygous (AA) genotype was observed in patients compared to controls (42.50% vs. 26.67%, *p-value*=0.059) with 2.62 (95%CI=1.04-6.59) chance for disease (Table 3). In fact, when combined genotypes AG+GG were compared to AA alone, a significantly higher AA frequency was observed in TLE patients (42.50% vs. 26.67%, *p-value*=0.004) and a higher chance for disease [2.03 (95%CI=1.28-3.22)] (Table 3).

The G (31.70% vs. 41.50%) allele frequency was similar in patients and controls. In contrast, the A (68.30% vs. 58.50%, *p-value*=0.014) allele frequency was higher in patients compared to controls (Table 3).

Similar frequencies of genotypes/alleles were observed in drug-resistant patients and controls (*p-value*>0.05); although an increase in AA genotype was observed in drug-resistant patients compared to controls (38.46% vs. 26.67%, *p-value*=0.088) with 1.72 (95%CI=0.97-3.06) chance for disease (Table 3).

Similar frequencies of genotypes were observed in drug-responsive patients and controls (*p-value*>0.05); although an increase in AA genotype was observed in the drug-responsive patients compared to controls (47.27% vs. 26.67%, *p-value*=0.218) with 2.32 (95%CI=0.69-10.14) chance for a responsive disease (Table 3). In fact, when combined genotypes AG+GG were compared to AA alone, a significantly higher AA frequency was observed in TLE patients (47.27% vs. 26.67%, *p-value*=0.005) with a higher chance for responsiveness TLE [2.47 (95%CI=1.35-4.50)] (Table 3).

The G (33.10% vs. 41.50%) and A (66.90% vs. 58.50%) alleles frequencies were similar in drug-resistant patients and controls (*p-value*=0.103). Also, the G (33.00% vs. 41.50%) allele frequency was similar in drug-responsive patients and controls. In contrast, the A (77.00% vs. 58.50%, *p-value*=0.035) allele frequency had a significant increase in drug-responsive patients compared to controls (Table 3).

Table 3. Comparative association of the variant rs2298771 in *SCN1A* gene and susceptibility to temporal lobe epilepsy and controls.

Genotypes	**Patients n (%)[a]**	**Controls n (%)[b]**	***p-value***	**OR (95%CI)**
AA	51 (42.50)	64 (26.67)	0.059**	2.62 (1.04-6.59)
AG	62 (51.67)	153 (63.75)	0.681**	1.33 (0.54-3.26)
GG	7 (5.83)	23 (9.58)		Reference
AA+GG	58 (48.33)	87 (36.25)		Reference
AG	62 (51.67)	153 (63.75)	0.037**	0.61 (0.39-0.95)
AA+AG	113 (94.17)	217 (90.42)		Reference
GG	7 (5.83)	23 (9.58)	0.314**	0.58 (0.24-1.40)
AG+GG	69 (57.50)	176 (73.33)		Reference
AA	**51 (42.50)**	**64 (26.67)**	**0.004****	**2.03 (1.28-3.22)**
Allele G	76 (31.70)	199 (41.50)		Reference
Allele A	**164 (68.30)**	**281 (58.50)**	**0.014****	**1.53 (1.10-2.12)**
Genotypes	**Drug-resistant n (%)[c]**	**Controls n (%)**	***p-value***	**OR (95%CI)**
AA	25 (38.46)	64 (26.67)	0.131*	2.97 (0.79-16.81)
AG	37 (56.92)	153 (63.75)	0.479*	1.85 (0.53-6.51)
GG	3 (4.62)	23 (9.58)		Reference
AA+GG	28 (43.08)	87 (36.25)		Reference
AG	37 (56.92)	153 (63.75)	0.388**	0.75 (0.42-1.37)
AA+AG	62 (95.38)	217 (90.42)		Reference
GG	3 (4.62)	23 (9.58)	0.305*	0.458 (0.08-1.59)
AG+GG	40 (61.54)	176 (73.33)		Reference
AA	25 (38.46)	64 (26.67)	0.088**	1.72 (0.97-3.06)
Allele G	43 (33.10)	199 (41.50)		Reference
Allele A	87 (66.90)	281 (58.50)	0.103**	1.43 (0.95-2.16)
Genotypes	**Drug-responsive n (%)[d]**	**Controls n (%)**	***p-value***	**OR (95%CI)**
AA	26 (47.27)	64 (26.67)	0.218*	2.32 (0.69-10.14)
AG	25 (45.45)	153 (63.75)	>0.999*	0.94 (0.29-4.05)
GG	4 (7.27)	23 (9.58)		Reference
AA+GG	30 (54.54)	87 (36.25)		Reference
AG	25 (45.45)	153 (63.75)	0.019**	0.47 (0.26-0.86)
AA+AG	51 (92.72)	217 (90.42)		Reference
GG	4 (7.27)	23 (9.58)	0.816*	0.74 (0.18-2.30)
AG+GG	29 (52.72)	176 (73.33)		Reference
AA	**26 (47.27)**	**64 (26.67)**	**0.005****	**2.47 (1.35-4.50)**
Allele G	33 (33.00)	199 (41.50)		Reference
Allele A	**77 (77.00)**	**281 (58.50)**	**0.035****	**1.65 (1.06-2.58)**

*, Fisher's exact test; **, Chi-square with Yates correction; OR, odds ratio; 95%CI, 95% confidence interval. *P*-value for the Hardy-Weinberg equilibrium: a) 0.105; b) <0.001; c) 0.070; and d) 0.830. Values below 0.05 indicate that the sample is out of Hardy-Weinberg equilibrium.

For rs3812718, control samples were not in HWE (*p-value*=0.026), contrasting with TLE samples (*p-value*=1.00) and the genotypes/alleles prevalence were similar in patients and controls (*p-value*>0.05) (Table 4).

Table 4. Comparative association of the variant rs3812718 in *SCN1A* gene and susceptibility to temporal lobe epilepsy and controls.

Genotypes	**Patients n (%)[a]**	**Controls n (%)[b]**	***p-value***	**OR (95%CI)**
GG	30 (25.00)	40 (16.67)	0.286*	1.50 (0.79-2.86)
GA	60 (50.00)	140 (58.33)	0.667*	0.86 (0.50-1.46)
AA	30 (25.00)	60 (25.00)		Reference
GG+AA	60 (50.00)	100 (41.67)		Reference
GA	60 (50.00)	140 (58.33)	0.165*	0.71 (0.46-1.11)
GG+GA	90 (75.00)	180 (75.00)		Reference
AA	30 (25.00)	60 (25.00)	0.897*	1.00 (0.60-1.66_
GA+AA	90 (75.00)	200 (83.33)		Reference
GG	30 (25.00)	40 (16.67)	0.081*	1.67 (0.98-2.85)
Allele A	120 (50.00)	260 (54.20)		Reference
Allele G	120 (50.00)	220 (45.80)	0.329*	1.18 (0.87-1.61)
Genotypes	**Drug-resistant n (%)[c]**	**Controls n (%)**	***p-value***	**OR (95%CI)**
GG	17 (26.15)	40 (16.67)	0.161*	1.96 (0.86-4.48)
GA	35 (53.85)	140 (58.33)	0.824*	1.15 (0.57-2.34)
AA	13 (20.00)	60 (25.00)		Reference
GG+AA	30 (46.15)	100 (41.67)		Reference
GA	35 (53.85)	140 (58.33)	0.612*	0.83 (0.48-1.45)
GG+GA	52 (80.00)	180 (75.00)		Reference
AA	13 (20.00)	60 (25.00)	0.500*	0.75 (0.38-1.47)
GA+AA	48 (73.85)	200 (83.33)		Reference
GG	17 (26.15)	40 (16.67)	0.119*	1.34 (0.91-1.97)
Allele A	61 (46.90)	260 (54.20)		Reference
Allele G	69 (53.10)	220 (45.80)	0.171*	1.34 (0.91-1.97)
Genotypes	**Drug-responsive n (%)[d]**	**Controls n (%)**	***p-value***	**OR (CI95%)**
GG	13 (23.64)	40 (16.67)	0.909*	1.15 (0.50-2.62)
GA	25 (45.45)	140 (58.33)	0.254*	0.630 (0.32-1.25)
AA	17 (30.91)	60 (25.00)		Reference
GG+AA	30 (54.55)	100 (41.67)		Reference
GA	25 (45.45)	140 (58.33)	0.113*	0.60 (0.33-1.07)
GG+GA	38 (69.09)	180 (75.00)		Reference
AA	17 (30.91)	60 (25.00)	0.466*	1.34 (0.71-2.55)
GA+AA	42 (76.36)	200 (83.33)		Reference
GG	13 (23.64)	40 (16.67)	0.310	1.55 (0.76-3.14)
Allele A	59 (53.60)	260 (54.20)		Reference
Allele G	51 (46.40)	220 (45.80)	0.996	1.02 (0.67-1.55)

*, Chi-square with Yates correction; OR, odds ratio; 95%CI, 95% confidence interval. *P*-value for Hardy-Weinberg equilibrium: a) 1.000; b) 0.026 c) 0.808; and d) 0.816. Values below 0.05 indicate that the sample is out of Hardy-Weinberg equilibrium.

The comparative association of the variants rs2298771 and rs3812718 in *SCN1A* and susceptibility to TLE and controls are summarized in Tables 3 and 4.

Genotype and allele frequency distributions for SNVs rs2910164, rs2298771 and rs3812718 using ABraOM controls

ABraOM control samples were in HWE for all studied SNVs (*p-value*>0.05) (Supplementary Tables 1, 2 and 3).

For rs2910164 the frequencies of heterozygous genotype (CC) (15.00% vs. 9.65%, *p-value*=0.043) and allele C (37.80% vs. 29.97%, *p-value*=0.028) were superior in patients with ETL compared to controls with risk for the disease [1.89 (95%CI=1.06-3.37); 1.38 (95%CI=1.04-1.82, respectively] (Supplementary Table 1). In addition, drug-responsive patients also presented higher CC [(21.81% vs. 9.65%, *p-value*=0.015; 2.58 (95%CI=1.25-5.30)] and allele C [(39.09% vs. 29.97%, *p-value*=0.05; 1.50 (95%CI=1.01-2.22)] frequencies/risk compared to controls (Supplementary Table 1).

For rs2298771 the frequencies of heterozygous genotype (AG) (51.67% vs. 40.40%, *p-value*=0.027) was superior in patients with ETL compared to controls with risk for the disease [2.42 (95%CI=1.08-5.41)] (Supplementary Table 1). In addition, drug-resistant patients also presented higher AG frequency [(56.92% vs. 40.40%, *p-value*=0.041; 3.36 (95%CI=1.04-17.30)] compared to control group. However, drug-responsive patients, the genotypes/alleles prevalence were similar in patients and controls (Supplementary Table 2).

The results observed when AbraOM control was evaluated were similar to the results using our control group for rs2910164; however, for rs2298771, drug-resistant presented higher risk than drug-responsive patients.

Combined Genotypes frequency distribution for SNVs rs2910164 and rs2298771

Further, it was performed an evaluating of combined risk genotypes for SNVs rs2910164 and rs2298771, considering: (1) patients with TLE and control groups; (2) drug-resistant TLE patients and control groups; (3) drug-responsive TLE patients and control groups. In the patients' group, individuals harboring combined genotypes rs2910164 (CC) and rs2298771 (AA) (7.5% vs. 1.7%) showed an OR=4.76 (95%CI=1.30 to 21.62). When drug-responsive patients were compared to control group, combined genotypes rs2910164 (CC) and rs2298771 (AA) (9.1% vs. 1.7%) showed an OR=5.849 (95%CI=1.21 to 30.57) (Table 5).

Supplementary Table 1. Comparative association of the variant rs2910164 in *MIR-146a* and susceptibility to temporal lobe epilepsy and ABraOM controls.

Genotypes	**Patients n (%)[a]**	**Controls n (%)[b]**	***p-value***	**OR (95%CI)**
GG	49 (40.83)	582 (49.70)		Reference
GC	53 (44.17)	476 (40.65)	0.213	1.32 (0.88-1.99)
CC	**18 (15.00)**	**113 (9.65)**	**0.043**	**1.89 (1.06-3.37)**
GG+CC	67 (55.83)	695 (59.35)		Reference
GC	53 (44.17)	476 (40.65)	0.517	1.16 (0.79-1.69)
GG+GC	102 (85.00)	1,058 (90.35)		Reference
CC	18 (15.00)	113 (9.65)	0.090	1.65 (0.37-2.83)
GC+CC	71 (59.17)	589 (50.30)		Reference
GG	49 (40.83)	582 (49.70)	0.079	0.70 (0.48-1.02)
Allele C	**89 (37.08)**	**702 (29.97)**	**0.028**	**1.38 (1.04-1.82)**
Allele G	151 (62.92)	1,640 (70.03)		Reference
Genotypes	**Drug-resistant n (%)[c]**	**Controls n (%)**	***p-value***	**OR (95%CI)**
GG	25 (38.46)	582 (49.70)		Reference
GC	34 (52.30)	476 (40.65)	0.078	1.66 (0.98-2.83)
CC	6 (9.23)	113 (9.65)	0.836	1.24 (0.50-3.08)
GG+CC	31 (47.69)	695 (59.35)		Reference
GC	34 (52.30)	476 (40.65)	0.084	1.60 (0.97-2.64)
GG+GC	59 (90.77)	1,058 (90.35)		Reference
CC	6 (9.23)	113 (9.65)	0.917	0.95 (0.40-2.25)
GC+CC	40 (61.54)	589 (50.30)		Reference
GG	25 (38.46)	582 (49.70)	0.102	0.63 (0.38-1.06)
Allele C	46 (35.38)	702 (29.97)	0.227	1.28 (0.88-1.85)
Allele G	84 (64.62)	1,640 (70.03)		Reference
Genotypes	**Drug-responsive n (%)[d]**	**Controls n (%)**	***p-value***	**OR (95%CI)**
GG	24 (43.63)	582 (49.70)		Reference
GC	19 (34.54)	476 (40.65)	0.958	0.97 (0.52-1.79)
CC	**12 (21.81)**	**113 (9.65)**	**0.015**	**2.58 (1.25-5.30)**
GG+CC	36 (65.45)	695 (59.35)		Reference
GC	19 (34.54)	476 (40.65)	0.447	0.77 (0.44-1.36)
GG+GC	43 (78.19)	1,058 (90.35)		Reference
CC	**12 (21.81)**	**113 (9.65)**	**0.007**	**2.61 (1.34-5.10)**
GC+CC	31 (56.36)	589 (50.30)		Reference
GG	24 (43.63)	582 (49.70)	0.459	0.78 (0.45-1.35)
Allele C	**43 (39.09)**	**702 (29.97)**	**0.05**	**1.50 (1.01-2.22)**
Allele G	67 (60.91)	1,640 (70.03)		Reference

*, Chi-square with Yates correction; OR, odds ratio; 95%CI, 95% confidence interval. *P*-value for Hardy-Weinberg equilibrium: a) 0.842; b) 0.555; c) 0.510 and d) 0.126. Values below 0.05 indicate that the sample is out of Hardy-Weinberg equilibrium.

Supplementary Table 2. Comparative association of the variant rs2298771 in *SCN1A* gene and susceptibility to temporal lobe epilepsy and ABraOM controls.

Genotypes	**Patients n (%)[a]**	**Controls n (%)[b]**	***p-value***	**OR (95%CI)**
AA	51 (42.50)	569 (48.60)	0.298	1.65 (0.73-3.72)
AG	**62 (51.67)**	**473 (40.40)**	**0.027**	**2.42 (1.08-5.41)**
GG	7 (5.83)	129 (11.00)		Reference
AA+GG	58 (48.33)	698 (59.60)		Reference
AG	**62 (51.67)**	**473 (40.40)**	**0.022**	**1.58 (1.08-2.30)**
AA+AG	113 (94.17)	1,042 (89.00)		Reference
GG	7 (5.83)	129 (11.00)	0.109	0.50 (0.23-1.10)
AG+GG	69 (57.50)	602 (51.40)		Reference
AA	51 (42.50)	569 (48.60)	0.240	0.78 (0.54-1.14)
Allele G	76 (31.70)	731 (68.79)		Reference
Allele A	164 (68.30)	1,611 (31.31)	0.943	0.98 (0.74-1.30)
Genotypes	**Drug-resistant n (%)[c]**	**Controls n (%)**	***p-value***	**OR (95%CI)**
AA	25 (38.46)	569 (48.60)	0.438	1.89 (0.56-9.92)
AG	**37 (56.92)**	**473 (40.40)**	**0.041**	**3.36 (1.04-17.30)**
GG	3 (4.62)	129 (11.00)		Reference
AA+GG	28 (43.08)	698 (59.60)		Reference
AG	**37 (56.92)**	**473 (40.40)**	**0.012**	**1.95 (1.18-3.23)**
AA+AG	62 (95.38)	1,042 (89.00)		Reference
GG	3 (4.62)	129 (11.00)	0.136	0.39 (0.08-1.23)
AG+GG	40 (61.54)	602 (51.40)		Reference
AA	25 (38.46)	569 (48.60)	0.143	0.66 (0.40-1.10)
Allele G	43 (33.10)	731 (68.79)		Reference
Allele A	87 (66.90)	1,611 (31.31)	0.727	0.92 (0.63-1.34)
Genotypes	**Drug-responsive n (%)[d]**	**Controls n (%)**	***p-value***	**OR (95%CI)**
AA	26 (47.27)	569 (48.60)	0.665	1.47 (0.50-5.91)
AG	25 (45.45)	473 (40.40)	0.464	1.70 (0.57-6.86)
GG	4 (7.27)	129 (11.00)		Reference
AA+GG	30 (54.54)	698 (59.60)		Reference
AG	25 (45.45)	473 (40.40)	0.455	1.23 (0.71-2.12)
AA+AG	51 (92.72)	1,042 (89.00)		Reference
GG	4 (7.27)	129 (11.00)	0.537	0.63 (0.16-1.77)
AG+GG	29 (52.72)	602 (51.40)		Reference
AA	26 (47.27)	569 (48.60)	0.958	0.95 (0.55-1.63)
Allele G	33 (33.00)	731 (68.79)		Reference
Allele A	77 (77.00)	1,611 (31.31)	0.871	1.06 (0.70-1.61)

*, Fisher's exact test; **, Chi-square with Yates correction; OR, odds ratio; 95%CI, 95% confidence interval. *P*-value for the Hardy-Weinberg equilibrium: a) 0.105; b) 0.127; c) 0.070 and d) 0.830. Values below 0.05 indicate that the sample is out of Hardy-Weinberg equilibrium.

Supplementary Table 3. Comparative association of the variant rs3812718 in *SCN1A* gene and susceptibility to temporal lobe epilepsy and ABraOM controls.

Genotypes	**Patients n (%)[a]**	**Controls n (%)[b]**	***p-value***	**OR (95%CI)**
GG	30 (25.00)	245 (20.90)	0.144	1.54 (0.90-2.62)
GA	60 (50.00)	549 (46.90)	0.211	1.37 (0.87-2.17)
AA	30 (25.00)	377 (32.20)		Reference
GG+AA	60 (50.00)	622 (53.10)		Reference
GA	60 (50.00)	549 (46.90)	0.579	1.13 (0.78-1.65)
GG+GA	90 (75.00)	794 (67.80)		Reference
AA	30 (25.00)	377 (32.20)	0.131	0.70 (0.46-1.08)
GA+AA	90 (75.00)	926 (79.00)		Reference
GG	30 (25.00)	245 (21.00)	0.357	1.26 (0.81-1.95)
Allele A	120 (50.00)	1,303 (50.66)		Reference
Allele G	120 (50.00)	1,269 (49.34)	0.898	1.03 (0.79-1.34)
Genotypes	**Drug-resistant n (%)[c]**	**Controls n (%)**	***p-value***	**OR (95%CI)**
GG	17 (26.15)	245 (20.90)	0.090	2.01 (0.96-4.22)
GA	35 (53.85)	549 (46.90)	0.084	1.85 (0.97-3.54)
AA	13 (20.00)	377 (32.20)		Reference
GG+AA	30 (46.15)	622 (53.10)		Reference
GA	35 (53.85)	549 (46.90)	0.334	1.32 (0.80-2.18)
GG+GA	52 (80.00)	794 (67.80)		Reference
AA	13 (20.00)	377 (32.20)	0.055	0.53 (0.28-0.98)
GA+AA	48 (73.85)	926 (79.00)		Reference
GG	17 (26.15)	245 (21.00)	0.396	1.34 (0.76-2.37)
Allele A	61 (46.90)	1,303 (50.66)		Reference
Allele G	69 (53.10)	1,269 (49.34)	0.458	1.16 (0.82-1.65)
Genotypes	**Drug-responsive n (%)[d]**	**Controls n (%)**	***p-value***	**OR (CI95%)**
GG	13 (23.64)	245 (20.90)	0.810	1.18 (0.56-2.47)
GA	25 (45.45)	549 (46.90)	0.897	1.01 (0.54-1.90)
AA	17 (30.91)	377 (32.20)		Reference
GG+AA	30 (54.55)	622 (53.10)		Reference
GA	25 (45.45)	549 (46.90)	0.945	0.94 (0.55-1.63)
GG+GA	38 (69.09)	794 (67.80)		Reference
AA	17 (30.91)	377 (32.20)	0.959	0.94 (0.53-1.69)
GA+AA	42 (76.36)	926 (79.00)		Reference
GG	13 (23.64)	245 (21.00)	0.754	1.17 (0.62-2.21)
Allele A	59 (53.60)	1,303 (50.66)		Reference
Allele G	51 (46.40)	1,269 (49.34)	0.607	0.89 (0.60-1.30)

*, Chi-square with Yates correction; OR, odds ratio; 95%CI, 95% confidence interval. *P*-value for Hardy-Weinberg equilibrium: a) 1.000; b) 0.228; c) 0.808 and d) 0.816. Values below 0.05 indicate that the sample is out of Hardy-Weinberg equilibrium.

Table 5. Comparative association of the SNVs rs2910164 in the miR-146a and rs2298771 in *SCN1A* gene between patients with temporal lobe epilepsy and healthy individuals.

rs2910164 and rs2298771 combination	**Patients n (%)**	**Controls n (%)**	***p-value***	**OR (95%CI)**
CC+AA	**9 (7.5%)**	**4 (1.7%)**	**0.016***	**4.761 (1.30-21.62)**
Other	111 (92.5%)	236 (98.3%)		Reference
CC or CG + AA or AG	67 (55.8%)	120 (50.0%)	0.351**	1.264 (0.81-1.96)
Other	53 (44.2%)	120 (50.0%)		Reference
Genotypes	**Drug-resistant n (%)[c]**	**Controls n (%)**	***p-value***	**OR (95%CI)**
CC+AA	4 (6.2%)	4 (1.7%)	0.133*	3.846 (0.70-21.28)
Other	61 (93.8%)	236 (98.3%)		Reference
CC or CG + AA or AG	38 (58.5%)	120 (50.0%)	0.285**	1.407 (0.81-2.45)
Other	27 (41.5%)	120 (50.0%)		Reference
Genotypes	**Drug-responsive n (%)[d]**	**Controls n (%)**	***p-value***	**OR (95%CI)**
CC+AA	**5 (9.1%)**	**4 (1.7%)**	**0.014***	**5.849 (1.21-30.57)**
Other	50 (90.9%))	236 (98.3%)		Reference
CC or CG + AA or AG	29 (52.7%)	120 (50.0%)	0.830**	1.115 (0.62-2.01)
Other	26 (47.3%)	120 (50.0%)		Reference

*, Fisher's exact test; **, Chi-square with Yates correction; OR, odds ratio; 95%CI, 95% confidence interval. Values below 0.05 indicate that the sample is out of Hardy-Weinberg equilibrium.

MIR-146A and *SCN1A* quantification considering SNVs rs2910164 and rs2298771

The *MIR-146A* relative expression levels in TLE patients' samples were lower in GC (1.02 vs. 1.63, *p-value*=0.23] and CC (1.03 vs. 1.63, *p-value*=0.20) compared to GG genotype (Figure 1A). Moreover, when patients were divided into drug-resistant and drug-responsive the *MIR-146A* relative expression level was lower in drug-resistant compared to drug-responsive patients for GG (1.3 vs. 2.0, *p-value*=0.28) and it was significantly lower for GC (1.6 vs. 0.1, *p-value*=0.049) and CC (1.8 vs. 0.6, *p-value*=0.039) genotypes (Figure 1B), indicating increased NF-kB inflammation process in drug-resistant patients harboring GC or CC genotypes for SNV rs2910164.

In contrast, the *SCN1A* relative expression levels in TLE patients' samples were significantly higher in AG [2.09 vs. 1.10, *p-value*=0.004] and GG (3.19 vs. 1.10, *p-value*=0.002) compared to AA genotype (Figure 2A). The results may indicate a *SCN1A* loss-of-function in patients with ELT harboring AA genotypes for SNV rs2298771.

In addition, when patients were divided into drug-resistant and drug-responsive, there was no difference in the *SCN1A* expression level for AA (1.1 vs. 1.1, *p-value*=0.89), AG (2.6 vs. 1.7; *p-value*=0.11) and GG (2.6 vs. 3.8, *p-value*=0.24) genotypes (Figure 2B).

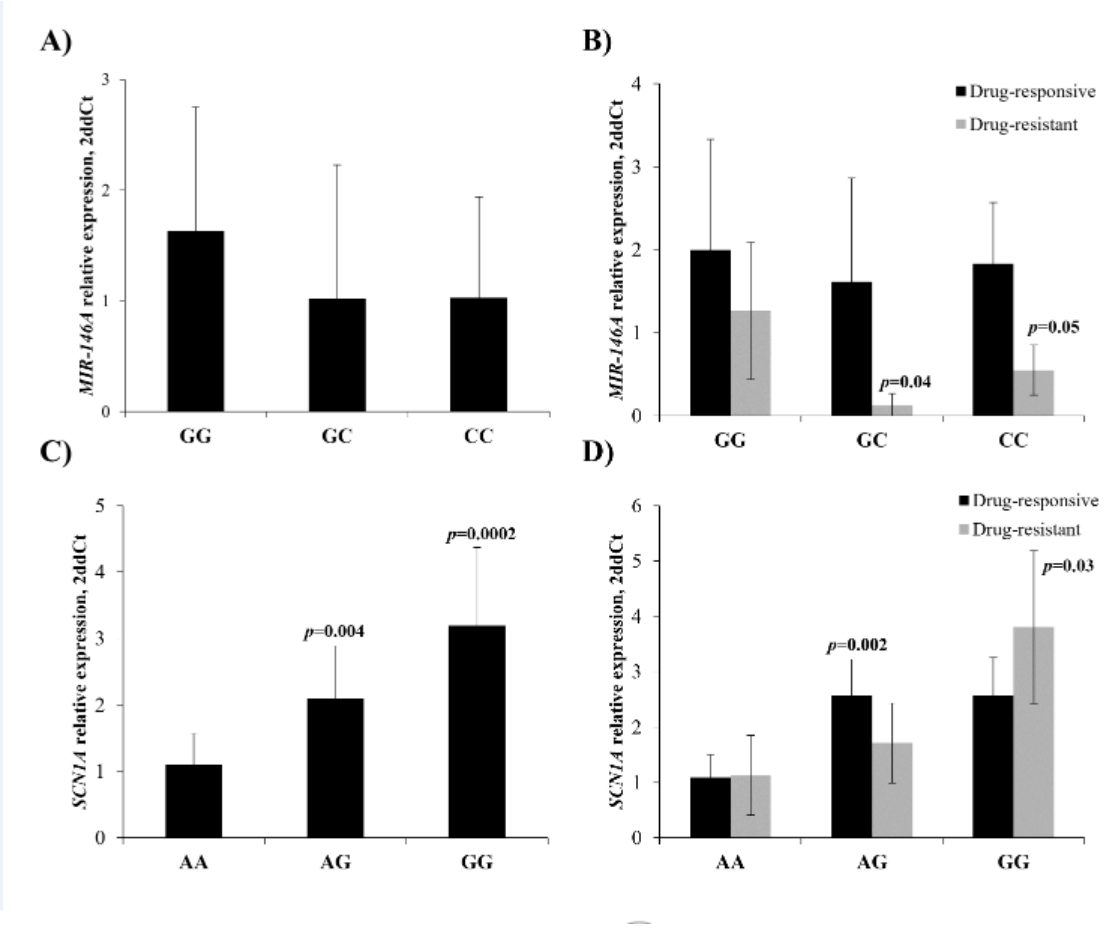

FIGURE 1. (A) Real-time quantitative polymerase chain reaction (RT-PCR) for *MIR-146A* expression was evaluated in peripheral blood from patients with temporal lobe epilepsy (TLE). The expression values for each genotype for SNV rs2910164 [GG (n=12); GC (n=10); CC (n=7)] were evaluated by *T*-Test to compare the expression levels of each group (2-DDCt). (B) RT-PCR for *MIR-146A* expression was evaluated in peripheral blood from patients with drug-responsive and drug-resistant TLE. The expression values for each genotype for SNV rs2910164 [drug-responsive: GG (n=6); GC (n=6); CC (n=4); drug-resistant: GG (n=6); GC (n=4); CC (n=3] were evaluated by *T*-Test to compare the expression levels of each group (2-DDCt). (C) RT-PCR for *SCN1A* expression was evaluated in patient epileptogenic tissues or peripheral blood from patients with TLE. The expression values for each genotype for SNV rs2298771 [AA (n=10); AG (n=9); GG (n=6)] were evaluated by T-Test to compare the expression levels of each group (2-DDCt). (D) RT-PCR for *SCN1A* expression was evaluated in patient epileptogenic tissues or peripheral blood from patients with drug-responsive and drug-resistant TLE. The expression values for each genotype for SNV rs2298771 [drug-responsive: AA (n=6); AG (n=4); GG (n=3); drug-resistant: AA (n=4); AG (n=5); GG (n=3] were evaluated by *T*-Test to compare the expression levels of each group (2-DDCt).

The *MiR-146a* and *SCN1A* relative expression values for each evaluated sample are presented at Supplementary Table 4.

Supplementary Table 4. *MiR-146a* and *SCN1A* relative expression values for each evaluated sample.

SNV rs2910164 / *miR-146a*									
	Genotype	Samples	2-DDCt	Genotype	Samples	2-DDCt	Genotype	Samples	2-DDCt
Drug-responsive	GG	SE03	2.80	GC	SE01	0.77	CC	SE09	2.11
	GG	SE05	2.77	GC	SE08	1.39	CC	SE12	0.85
	GG	SE13	3.56	GC	SE52	1.22	CC	SE35	1.75
	GG	SE16	1.96	GC	SE14	1.85	CC	SE42	2.60
	GG	SE17	0.88	GC	SE41	0.47			
	GG	SE19	0.02	GC	SE43	3.97			
Drug-resistant	GG	SE02	0.66	GC	SE04	0.04	CC	SE07	0.70
	GG	SE11	1.07	GC	SE10	0.12	CC	SE18	0.75
	GG	SE15	1.78	GC	SE32	0.03	CC	SE33	0.21
	GG	SE29	2.56	GC	SE37	0.33			
	GG	SE30	1.25						
	GG	SE66	0.25						
SNV rs2298771 / *SCN1A*									
Drug-responsive	AA	SE09	1.34	AG	SE01	3.17	GG	SE08	2.21
	AA	SE12	1.13	AG	SE03	1.67	GG	SE25	2.16
	AA	SE13	0.45	AG	SE06	2.86	GG	SE70	3.36
	AA	SE14	0.71	AG	SE16	2.59			
	AA	SE17	1.57						
	AA	SE19	1.30						
Drug-resistant	AA	SE07	0.46	AG	SE02	0.78	GG	SE51	2.62
	AA	SE10	1.10	AG	SE04	2.45	GG	TE06	5.31
	AA	SE15	1.93	AG	SE11	1.35	GG	TE19	3.48
	AA	SE29	1.02	AG	SE30	1.54			
				AG	SE36	2.44			

DISCUSSION

In the present report, we performed a case-control study to analyze two potentially functional SNVs of the *MIR-146a* and *SCN1A* gene and the risk of epilepsy in a Brazilian cohort sample. Previously studies have suggested that decreased miR-146a expression may be associated with increased NF-kB inflammatory and susceptibility to the development of epilepsy[11, 12]. Moreover, *MIR-146A* was observed with increased expression level in human brain astrocytes, and it may inhibit target-genes related to epileptic inflammatory process.[37]

Only three previous studies have evaluated the SNV rs2910164 and TLE epileptogenesis. The first study has observed that rs2910164 variant in the pre-miR146a gene is unlikely to significantly influence the risk of developing TLE or its severity in Italian patients.[16] In the second study, Cui et al. (2015) have concluded that the rs2910164 variant was not associated with TLE epilepsy.[18] The third study from our group has suggested that the GC genotype for SNV rs2910164 appears to be associated with susceptibility to drug-resistant TLE in Brazilian patients, probably due to the decreased *miR146a* expression, favoring NF-kB pathway.[11] The CC genotype for SNV rs2910164 could also be related to susceptibility to drug-resistant TLE but there was a small number of CC patients. Thus, to confirm the results, we evaluated here a higher number of drug-resistant TLE patients. Same cohort (n=50) of drug-resistant patients evaluated previously was included in the present study. Additionally, more than 15 drug-resistant patients were also added in the present study. In Boschiero et al. (2020) it was identified only 3 drug-resistant patients harboring the CC genotype for SNV rs2910164;[11] here only more 3 drug-resistant patients were identified as CC genotype and no risk for TLE was observed, indicating that a bigger Brazilian cohort should be evaluate for SNV rs2910164. The hospital at Bragança Paulista (São Paulo, Brazil) is not known as high complexity reference and it is not prepared to receive many difficult-to-manage patients. Moreover, Brazilian population is extremely mixed being their origins mainly from Europeans, Amerindians, Africans, Levantines, and East Asians,[38, 39] explaining our contrasting results. Thus, the discrepancy in our results might be due to the ethnic variation and differences in number of recruited patients.

Another limitation of our study was that our control group was not at HWE. Therefore, we also evaluated our patients ‘group with AbraOM controls (healthy elderly Brazilian individuals) and the susceptibility for rs2910164 was similar. However, for rs2298771 drug-resistant patients

presented higher risk than drug-responsive patients, indicating the heterogeneity of the Brazilian population.

Corroborating with our previous publication,[11] *MIR-146A* expression level was lower in drug-resistant compared to drug-responsive patients for GC and CC genotypes, indicating increased NF-kB inflammation process in drug-resistant patients harboring GC or CC genotypes. Interestingly, when drug-responsive TLE patients were evaluated, the CC genotype was related to the disease susceptibility compared to control individuals with a 4-fold risk for responsiveness TLE. In fact, the *MIR-146A* expression level was lower in drug-responsive patients harboring CC variant, indicating increased NF-kB.

The α subunit of the voltage-gated sodium channel is a large protein of 2000 amino acids and its function is to generate a brief influx of sodium ions by transiently opening in response to neuronal membrane depolarization and closing within milliseconds.[40] Single amino acid substitutions can alter various components of channel function and deviate from normal channel function causing clinical consequences such as epilepsy.[41] Thus, our study evaluated the SNVs rs2298771 and rs3812718, two of the most common polymorphisms in intron and exon of the *SCN1A* gene, influencing the regulation of the gene expression and its structure and functionality, respectively; also, both SNVs are closely related to resistance to sodium channel blocking antiepileptic drugs.[19]

Two studies have found a correlation between the A allele and combined genotypes GA+AA for SNV rs2298771 and poor response to antiepileptic sodium channel blockers.[19, 22] In contrast, five studies have found no association for genotypes from SNV rs2298771 and sodium channel blockers metabolism or resistance.[20, 21, 23-25]

In our best knowledge, our study is the first one to evaluate the SNV rs2298771 in TLE patients. Interestingly, we found that both AA genotype and A allele present increased risk for the disease in drug-responsive patients. In fact, the *SCN1A* expression level was lower in drug-responsive patients harboring AA genotype. Our results seem indicate that the wild-type genotype for SNV rs2298771 in *SCN1A*, which encodes the NaV1.1 sodium channel alpha subunit, result in truncation of the protein presenting a lower expression, with haploinsufficiency of NaV1.1, as observed in *SCN1A* gene mutated in Dravet Syndrome[40, 42] as well as milder phenotypes associated with genetic epilepsy with febrile seizures plus.[43]

There are seven studies that have evaluated susceptibility for general epilepsy seizure and multidrug-resistance and the SNV rs3812718.[19, 20, 22, 23, 26-28] Only one study has analyzed TLE risk and the SNV rs3812718[23]. Thus, the authors have compared genotypes/alleles frequencies between South Indian Ancestry patients with mesial temporal lobe epilepsy with hippocampal sclerosis (mTLE-HS) and observed that AA genotype/A allele were overrepresented in these patients contributing to increased susceptibility to mTLE-HS[23]. Only one study has demonstrated association of the genotypes A allele (AA or AG genotype) for the SNV rs3812718 and the need to administer higher doses of AEDs than those with the GG genotype, whereas a correlation with multidrug resistance phenotype was not detectable.[26] Our present study demonstrated no susceptibility to TLE, drug-responsive or drug-resistant patients, for the SNV rs3812718.

Finally, our study identified that patients harboring combined genotypes rs2910164 (CC) in the miR-146a and rs2298771 (AA) in *SCN1A* showed almost 5-fold times risk for the disease, indicating combined effects of multiple independent SNPs that might contribute to the disease susceptibility. In addition, similar results were observed in drug-responsive patients with almost 6-fold times risk for the responsiveness TLE.

ABBREVIATIONS

Arbitrary Units (AUs); Confidence Interval (CI); Deoxyribonucleic Acid (DNA); Glyceraldehyde-3-phosphate Dehydrogenase (*GAPDH*); Hardy-Weinberg Equilibrium (HWE); Hippocampal Sclerosis (HS); Mesial Temporal Lobe Epilepsy (mTLE); Messenger RNA (mRNA); Micro Ribonucleic Acid (micro-RNA); Micro-RNA (*miR*)*;* Nuclear Factor-Kappa B (NF-kB); Odds Ratio (OR); Polymerase Chain Reaction (PCR); Real-Time Polymerase Chain Reaction (RT-PCR); Ribonucleic acid (RNA); Single Nucleotide Variants (SNVs); Sodium Voltage-Gated Channel Alpha Subunit 1 (*SCN1A*); Temporal Lobe Epilepsy (TLE); World Health Organization (WHO).

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AUTHORS' CONTRIBUTIONS

Conception and design: MMO; collection of tissue samples from patients who went to surgery: LSQ, PHPA, and CTPO; collection of blood samples from patients and healthy controls: RPB and ARS; acquisition of data: RPB, JSS and ARS; analyses and interpretation of data: MMO, FALM, RPB, JSS and ARS; statistical analyses: FALM; drafting of the manuscript: MMO and RPB; study supervision: MMO. All authors were involved in revision of the manuscript and have approved the final version.

CONFLICTS OF INTEREST STATMENT

None declared.

ETHICS APPROVAL STATEMENT

Ethic Committee of São Francisco University (approval #45723615.0.0000.5514).

PATIENT CONSENT STATEMENT

This study was approved by the Ethic Committee of São Francisco University (approval #45723615.0.0000.5514). Participants gave informed written consent.

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ORCID

Renata Parissi Buainain

https://orcid.org/0000-0003-0244-4011

André Rodrigues Sodré

https://orcid.org/0000-0002-3969-5386

Jéssica Silva dos Santos

https://orcid.org/0000-0002-3733-6734

Luciano de Souza Queiroz

https://orcid.org/0000-0001-8648-6846;

Paulo Henrique Pires de Aguiar

https://orcid.org/0000-0002-9059-4978

Fernando Augusto Lima Marson

https://orcid.org/0000-0003-4955-4234

Manoela Marques Ortega

https://orcid.org/0000-0003-4609-7074

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6. CONCLUSÕES

- *miR146a* rs2910164 (GC) e rs57095329 (AA/GA/GG) são fatores de risco para a epilepsia farmacorresistente (Capítulo 1).
- Nossa taxa de prevalência de epilepsia foi menor do que outros estudos na região sudeste do Brasil (Capítulo 2).
- A etiologia estrutural predominou em nossa região em comparação com a causa desconhecida, que é mais frequente em outras regiões do Brasil e no mundo. As diferenças podem ser atribuídas à alta prevalência de neuroinfecção, principalmente neurocisticercose em nossa região e sermos um centro de referência para traumatismo cranioencefálico (Capítulo 2).
- Um aumento na frequência do genótipo homozigoto selvagem AA e do alelo A para a SNV rs2298771 foi observado no grupo de pacientes portadores de ELT e ELT responsiva quando comparados aos controles, com cerca de 2 vezes mais chance para desenvolver a doença e a doença de forma responsiva. No entanto, não se mostrou um risco para a resistência a fármacos (Capítulo 3).
- Pacientes com ELT portadores do genótipo AA para a SNV rs2298771 demonstraram uma expressão relativa diminuída do gene *SCN1A* em comparação a pacientes portadores dos genótipos AG e GG mas, não houve diferença estatística significativa da expressão deste gene entre os portadores de ELT farmacorresponsivos e farmacorresistentes quanto ao mesmo genótipo AA (Capítulo 3).
- A SNV rs3812718 não apresentou diferenças de frequências nos genótipos/alelos ou risco para ELT resistente ou responsiva a fármacos (Capítulo 3).
- Um aumento na frequência do genótipo homozigoto variante CC para a SNV rs2910164 foi observado no grupo de pacientes com ELT e pacientes com ELT farmacorresponsiva quando comparados aos controles com cerca de 3 e 4 vezes mais chance para a doença ELT e ELT farmacorresponsiva, respectivamente. A SNV rs2910164 não apresentou diferenças de frequências nos genótipos/alelos ou risco para ELT resistente a fármacos (Capítulo 3).
- Pacientes portadores dos genótipos GC e CC em comparação com pacientes portadores do genótipo GG para a SNV rs2910164, apresentaram expressão relativa diminuída do gene *MIR-146A*. Além disso, quando os pacientes foram divididos em resistentes e responsivos, o nível de expressão de *MIR-146A* foi menor em pacientes resistentes portadores do genótipo GG

em comparação com responsivos e significativamente menor para pacientes portadores dos genótipos GC e CC, indicando aumento do processo de inflamação pela via NF-kB em pacientes resistentes à fármacos e portadores dos genótipos GC ou CC (Capítulo 3).

- Ainda, pacientes diagnosticados com ELT responsivos a medicamentos e portadores dos genótipos combinados rs2910164 (CC) e rs2298771 (AA) apresentaram quase 6 vezes risco para a doença comparado com controles (Capítulo 3).

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ANEXOS

Anexo I – Parecer do CEP – Solicitação Inicial

Continua

UNIVERSIDADE SÃO FRANCISCO-SP

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: IDENTIFICAÇÃO DE VARIANTES GÊNICAS DE BASE ÚNICA NOS GENES SCN1A E MIR-146 ENVOLVIDOS COM O PROCESSO EPILEPTOGÊNICO EM PACIENTES COM EPILEPSIA

Pesquisador: Manoela Marques Ortega

Área Temática: Genética Humana:
(Trata-se de pesquisa envolvendo Genética Humana que não necessita de análise ética por parte da CONEP;);

Versão: 1

CAAE: 28258920.7.0000.5514

Instituição Proponente: Universidade São Francisco-SP

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 3.827.608

Apresentação do Projeto:
IDENTIFICAÇÃO DE VARIANTES GÊNICAS ENVOLVIDOS COM O PROCESSO EPILEPTOGÊNICO EM PACIENTES COM EPILEPSIA

Objetivo da Pesquisa:
Identificar os diferentes genótipos como fatores preditores de suscetibilidade para o desenvolvimento de epilepsia e como possíveis fatores de prognóstico

Avaliação dos Riscos e Benefícios:
Não há riscos para pacientes. Desconforto mínimo pela venopunção para coleta da amostra. Risco biológico para os pesquisadores no manuseio da amostra e seus produtos

Comentários e Considerações sobre a Pesquisa:
Pesquisa pertinente que ajudará a elucidar caracteres gênicos relacionados à epilepsia

Considerações sobre os Termos de apresentação obrigatória:
Termos e autorizações apropriados

Conclusões ou Pendências e Lista de Inadequações:
Projeto aprovado

Endereço: Av. São Francisco de Assis, 218, sala 35, prédio central
Bairro: Cidade Universitária **CEP:** 12.916-900
UF: SP **Município:** BRAGANCA PAULISTA
Telefone: (11)2454-8302 **E-mail:** comiteetica@usf.edu.br

Anexo I – Parecer do CEP – Solicitação Inicial

Continua

UNIVERSIDADE SÃO FRANCISCO-SP

Continuação do Parecer: 3.827.608

Considerações Finais a critério do CEP:
APÓS DISCUSSÃO EM REUNIÃO DO DIA 06/02/2020, O COLEGIADO DELIBEROU PELA APROVAÇÃO DO PROJETO DE PESQUISAS. APÓS A CONCLUSÃO DO PROJETO É OBRIGATÓRIO O ENVIO DO RELATÓRIO FINAL PARA ENCERRAMENTO DO PROJETO.

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_1488684.pdf	23/01/2020 17:13:30		Aceito
Declaração de Instituição e Infraestrutura	HUSF.pdf	23/01/2020 17:10:50	Renata Parissi Buainain	Aceito
Projeto Detalhado / Brochura Investigador	Projeto.docx	19/01/2020 11:03:49	Renata Parissi Buainain	Aceito
Folha de Rosto	Rosto.pdf	16/12/2019 18:27:43	Renata Parissi Buainain	Aceito
Outros	Confidencialidade.pdf	14/12/2019 09:13:10	Renata Parissi Buainain	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE.docx	14/12/2019 09:10:19	Renata Parissi Buainain	Aceito
Declaração de Pesquisadores	Pesquisador.pdf	14/12/2019 09:04:44	Renata Parissi Buainain	Aceito
Cronograma	Cronograma.docx	14/12/2019 08:59:46	Renata Parissi Buainain	Aceito

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

BRAGANCA PAULISTA, 07 de Fevereiro de 2020

Assinado por:
CARLOS EDUARDO PULZ ARAUJO
(Coordenador(a))

Endereço: Av. São Francisco de Assis, 218, sala 35, prédio central
Bairro: Cidade Universitária **CEP:** 12.916-900
UF: SP **Município:** BRAGANCA PAULISTA
Telefone: (11)2454-8302 **E-mail:** comiteetica@usf.edu.br

Anexo I – Parecer do CEP – Solicitação Inicial

Conclusão

UNIVERSIDADE SÃO FRANCISCO-SP

Continuação do Parecer: 3.827.608

Endereço: Av. São Francisco de Assis, 218, sala 35, prédio central
Bairro: Cidade Universitária **CEP:** 12.916-900
UF: SP **Município:** BRAGANCA PAULISTA
Telefone: (11)2454-8302 **E-mail:** comiteetica@usf.edu.br

Anexo II - Parecer do CEP – Inclusão de Novos Doadores

Continua

UNIVERSIDADE SÃO FRANCISCO-SP

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: IDENTIFICAÇÃO DE VARIANTES GÊNICAS DE BASE ÚNICA NOS GENES SCN1A E MIR-146 ENVOLVIDOS COM O PROCESSO EPILEPTOGÊNICO EM PACIENTES COM EPILEPSIA

Pesquisador: Manoela Marques Ortega

Área Temática: Genética Humana:
(Trata-se de pesquisa envolvendo Genética Humana que não necessita de análise ética por parte da CONEP;);

Versão: 1

CAAE: 28258920.7.0000.5514

Instituição Proponente: Universidade São Francisco-SP

Patrocinador Principal: Financiamento Próprio

DADOS DA NOTIFICAÇÃO

Tipo de Notificação: Outros

Detalhe: Solicitar a inclusão de novos doadores controles

Justificativa: Solicitar ao CEP a inclusão de novos doadores de sangue periférico do Hemonúcleo-

Data do Envio: 14/07/2021

Situação da Notificação: Parecer Consubstanciado Emitido

DADOS DO PARECER

Número do Parecer: 4.901.486

Apresentação da Notificação:

Inclusão de novos participantes (doadores)

Objetivo da Notificação:

Ajuste de amostra

Avaliação dos Riscos e Benefícios:

Sem riscos adicionais.

Comentários e Considerações sobre a Notificação:

Modificação justificada e pertinente

Endereço: Av. São Francisco de Assis, 218, sala 35, prédio central
Bairro: Cidade Universitária **CEP:** 12.916-900
UF: SP **Município:** BRAGANCA PAULISTA
Telefone: (11)2454-8302 **E-mail:** comiteetica@usf.edu.br

Anexo II - Parecer do CEP – Inclusão de Novos Doadores

Conclusão

UNIVERSIDADE SÃO FRANCISCO-SP

Continuação do Parecer: 4.901.486

Considerações sobre os Termos de apresentação obrigatória:
TCLE OK

Conclusões ou Pendências e Lista de Inadequações:
Aprovado

Considerações Finais a critério do CEP:
Após reunião do dia 05/08/2021 o colegiado deliberou pela aprovação da notificação do projeto de pesquisas.

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Outros	Adendo_CEP_hemonucleo.pdf	14/07/2021 09:52:32	Manoela Marques Ortega	Postado
Outros	TCLE_hemonucleo.pdf	14/07/2021 09:52:55	Manoela Marques Ortega	Postado
Outros	carta_Aut_DrEvaldo.pdf	14/07/2021 09:53:28	Manoela Marques Ortega	Postado

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

BRAGANCA PAULISTA, 12 de Agosto de 2021

Assinado por:
CARLOS EDUARDO PULZ ARAUJO
(Coordenador(a))

Endereço: Av. São Francisco de Assis, 218, sala 35, prédio central
Bairro: Cidade Universitária **CEP:** 12.916-900
UF: SP **Município:** BRAGANCA PAULISTA
Telefone: (11)2454-8302 **E-mail:** comiteetica@usf.edu.br