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LIST OF ABBREVIATIONS

χ^2	:	Chi square test statistics
53BP1	:	53 binding protein 1
5mC	:	5 methylcytosine
Ac	:	Acetylated lysine
AED	:	Antiepileptic drugs
asRNAs	:	Antisense RNAs
ATP	:	Adenosine triphosphate
BD	:	Bromodomains
BET	:	Bromodomain and extra terminal
BRD2	:	Bromodomain -containing protein 2
BRDT	:	Bromodomain -containing protein testis-specific
CAE	:	Childhood absence epilepsy
CASR	:	Calcium sensing receptor
CI	:	Confidence Interval
COBRA	:	Combined bisulfite restriction analysis
CpG	:	Cytosine-phosphate-Guanine
CTM	:	C-terminal motif
CX36	:	Connexin-36
dCTP	:	Deoxycytidine triphosphate
dGTP	:	Deoxyguanosine triphosphate
DNMT	:	Deoxyribonucleic acid methyltransferase
dNTP	:	Deoxyribonucleotide triphosphate
DSBs	:	Double-strand breaks
dsDNA	:	Double-stranded DNA
dTTP	:	Deoxythymidine triphosphate
EC	:	Electrochemical
EDTA	:	Ethylene diamine tetraacetic acid
EEG	:	Electroencephalogram
EFHC1	:	EF-hand domain containing 1
ELISA	:	Enzyme-Linked Immunosorbent Assay
eRNAs	:	Enhancer RNAs
ES	:	Embryonic stem

ABSTRACT

Background: Juvenile myoclonic epilepsy (JME) is the most prevalent among the idiopathic generalized epilepsy syndromes (IGE); it starts during late childhood and early adolescence and is a lifelong disorder. *BRD2* is a gene encoding the bromodomain-containing protein 2. *BRD2* has been linked to JME and was identified as a possible JME susceptibility gene**.** Alterations in DNA methylation in *BRD2* may play a role in the development and maintenance of JME. This study assessed the association of DNA status of three CpG sites at the *BRD2* gene promoter with JME in a sample of 88 Egyptian participants categorized into 3 groups: 30 patients with JME compared with 30 age matched patients with other forms of IGE and 28 age matched apparently healthy volunteers. This study followed up a conflict results between two previous reports highlighting *BRD2* promoter methylation in Caucasian patients with JME**.**

Methodology: Full clinical examination and an electroencephalogram were done for all cases. Determinations of DNA percent methylation of three CpG sites at the *BRD2* gene promoter were done using bisulfite-pyrosequencing DNA methylation analysis.

Results: The overall methylation percentage of the three CpGs at the *BRD2* gene promoter ranged from ……..

Conclusion: *BRD2* promoter hypermethylation in a sample of Egyptian patients with JME was significantly different from healthy control but not different from other forms of IGE patients. This may be explained by the effect of environmental factors on DNA methylation as epilepsy patients exposed to stressful conditions caused by seizures. Moreover, antiepileptic drugs may be another cause for DNA hypermethylation in the epileptic patients.

Keywords: Methylation status, *BRD2*, Pyrosequencing, Juvenile myoclonic epilepsy.

1. INTRODUCTION

Epilepsy is considered one of the most prevalent neurological diseases. People of different ages, races, social levels, and geographical areas can be affected by epilepsy (Beghi, 2020). About 50 million people worldwide are impacted by it (Levite & Goldberg, 2021).

The International League Against Epilepsy (ILAE) has divided epilepsy into three categories based on its onset as generalized onset, focal onset and unknown onset. Idiopathic generalized epilepsies (IGE) are a well-known and prevalent subtype of generalized epilepsies. The IGEs include four well-established epilepsy syndromes: juvenile myoclonic epilepsy (JME), childhood absence epilepsy (CAE), juvenile absence epilepsy (JAE) and generalized tonic–clonic seizures alone (GTCSA) (Scheffer et al., 2017).

Juvenile myoclonic epilepsy (JME) is the most prevalent among the idiopathic generalized epilepsy syndromes; it starts during late childhood and early adolescence and is a lifelong disorder. It accounts for between 23 and 37% of all idiopathic generalized epilepsy cases and 5% to 10% of all epilepsy cases (Gilsoul et al., 2019; Kazis et al., 2021; Shilkina et al., 2021). Genetic factors play a major role in JME etiology with a wide genetic heterogeneity and complex inheritance (Schulz et al., 2019). Myoclonic seizures are required for the diagnosis of JME and are described as transient, usually bilateral jerking motions of the arms while maintaining consciousness (Amrutkar & Riel-Romero, 2023).

Epigenetics refer to hereditary and dynamically reversible modifications of a chromatin without changing its DNA's base pair sequence, that produce a change in phenotype without altering in genotype (Choi & Han, 2021).

Neuroepigenetics are a relatively recent emerging field in neurobiology that examines the epigenetic mechanism in different post-mitotic neurons, both throughout time and in response to external environmental stimuli. Changes in these neuroepigenetic mechanisms have been linked to numerous neurodevelopmental, psychiatric, and neurodegenerative diseases (Cholewa-Waclaw et al., 2016).

The bromodomain and extra terminal [BET] family of bromodomain -containing proteins contains four members; BRD2, BRD3, BRD4 and BRDT. The BET family of proteins are epigenetic readers that bind to acetylated histones to regulate transcription of genes (Patel et al., 2021). Bromodomain-containing proteins play a significant role in brain-

derived neurotrophic factor expression and affect neuroplasticity. *BRD2* is a gene encoding the bromodomain-containing protein 2 (McCarthy et al., 2020). *BRD2* has been linked to JME and was identified as a possible JME susceptibility gene (Greenberg et al., 2000; Thakran et al., 2020).

DNA methylation, as one of the most important epigenetic mechanisms, is known to have a significant role in brain function and behavior, and dysregulated DNA methylation is a cause of many diseases (Cholewa-Waclaw et al., 2016). DNA hypermethylation of *BRD2* with subsequent gene silencing is associated with epileptogenesis and recurrent seizures. Alterations in DNA methylation in *BRD2* may play a role in the development and maintenance of JME (Pathak et al., 2018).

DNA methylation can be analyzed using three main methods including bisulfite conversion, methylation-sensitive restriction enzymes, and affinity enrichment-based approaches (Martisova et al., 2021). Pyrosequencing, one of the methods of bisulfite conversion approache, is a sequencing by synthesis (SBS) technique which is a reasonably cheap and fast method for sequencing short segments of DNA (Elkins, 2022).

To the best of our knowledge, no published data are available for studying the association of DNA methylation of the promoter region of *BRD2* and juvenile myoclonic epilepsy among Egyptian patients. Hence, this study was designed to test whether DNA methylation of four Cytosine-phosphate-Guanine (CpG) sites at the *BRD2* gene promoter is related to the JME phenotype in a sample of Egyptian patients compared to both, other Egyptian patients suffering from other forms of idiopathic generalized epilepsy and to apparently healthy controls. This might offer a potential for JME therapeutics targeting *BRD2*.

1.1 Aim of the work

The aim of the present study was to assess the association of DNA methylation status of four CpG sites at the *BRD2* gene promoter with juvenile myoclonic epilepsy in a sample of Egyptian patients as a possible seizure susceptibility motif.

2. REVIEW OF LITEREATURE

2.1. Epilepsy

2.1.1. Definition

Epilepsy is defined by any of the following conditions, according to the International League Against Epilepsy (ILAE): (1) a minimum of two unprovoked (or reflex) seizures occurring more than 24 hours apart; (2) one unprovoked (or reflex) seizure and a probability of further seizures similar to the general recurrence risk (at least 60%) after two unprovoked seizures, occurring over the next 10 years; and (3) a diagnosis of an epilepsy syndrome (Robert S. Fisher et al., 2014; McWilliam & Al Khalili, 2021).

2.1.2. Epidemiology

Epilepsy is considered one of the most prevalent neurological diseases. People of different ages, races, social levels, and geographical areas can be affected by epilepsy (Beghi, 2020). About 50 million people worldwide are impacted by it (Levite & Goldberg, 2021). In a systematic review and meta-analysis of incidence studies by Fiest et al. (2017), the worldwide annual incidence rate of epilepsy was 61.4 per 100,000 persons. The incidence was higher in low/middle-income countries than in high-income countries. The overall lifetime prevalence of epilepsy was 760 per 100,000 persons. There was no difference in the prevalence of epilepsy by age group or gender. The highest prevalent types were generalized seizures and epilepsy of unknown etiology (Fiest et al., 2017; Beghi, 2020).

In Egypt, the prevalence of epilepsy was estimated in different areas such as Fayoum, Qena, Al Kharga and Al-Manial Island. The lifetime prevalence of epilepsy was in the range of 690 to 1200 per 100,000 persons of the population, while the prevalence of active epilepsy was in the range of 510 to 580 per 100,000 persons of the population (Hashem et al., 2015; Farghaly et al., 2018; Fawi, Abbas, & Gamea, 2020; Abdel-Whahed, Shaheen, Thabet, & Hassan, 2022).

Epilepsy is a complex nosographic entity since the recurrence of seizures, the underlying etiology, and the adverse effects of treatment have a severe impact on the neurologic, cognitive,

psychosocial, and social quality of life of those who are affected (Beghi, 2020).

People with epilepsy have a mortality rate two to three times higher than that of the general population (Suna & Suna, 2021).

2.1.3. Classification

The International League Against Epilepsy (ILAE) classifications for epilepsies and seizures have undergone numerous updates over the past few decades to reflect the significant advancements made in diagnosis and understanding of the etiology of epilepsies and seizures as well as to correct some of the issues of the terminology from the 1980 original taxonomy (Rosenow et al., 2020).

The ILAE epilepsies classification (2017) is a multi-level classification, made to classify epilepsy in various clinical settings **(Figure 2.1)** (Scheffer et al., 2017; Wirrell, 2021).

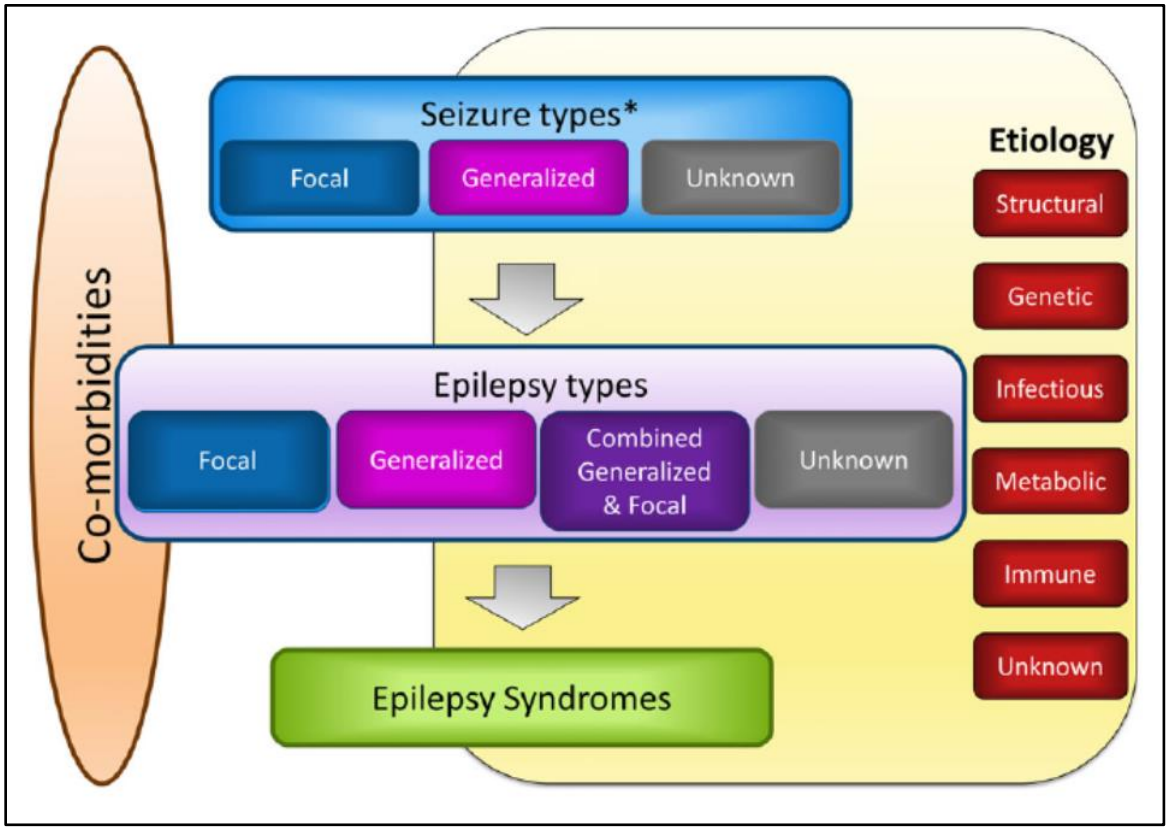

Figure (2.1): Framework for classification of the epilepsies. *Denotes onset of seizure (Scheffer et al., 2017).

Seizure type

The seizure type serves as the starting point for the epilepsy classification. There are three types of seizures: those with unknown onset, generalised onset, and focal onset. Seizures with a focal onset are those that start in networks that are confined to one hemisphere. Generalized onset seizures are those that start somewhere within, and rapidly engaging, bilaterally distributed networks (Robert S Fisher et al., 2017).

Epilepsy type

Epilepsy type makes up the second tier of the classification system for epilepsy. In addition to generalized epilepsies and focal epilepsies, the epilepsy type level now has a new category called "combined generalized and focal epilepsy." There is also an unknown category. Many epilepsies will include several types of seizures. The diagnosis is made by clinical basis, supported by characteristic electroencephalogram (EEG) findings (Scheffer et al., 2017; Thakran et al., 2020).

Epilepsy syndromes

Epilepsy syndrome diagnosis is the third level of the classification system for epilepsy. It describes a collection of characteristics including seizure types, EEG characteristics, and imaging features that frequently co-occur. Age at onset, age at remission (if applicable), diurnal variation, seizure triggers, and occasionally prognosis are age-dependent characteristics that are frequently present. Along with particular EEG and MRI abnormalities, it may also have characteristic comorbidities like intellectual and mental impairment. It may have specific etiology, prognosis, and treatment. There are many widely known syndromes, including Dravet syndrome, childhood absence epilepsy, West syndrome, and juvenile myoclonic epilepsy (JME) (Scheffer et al., 2017).

The 2022 International League Against Epilepsy classification has updated some epilepsy syndromes to incorporate current knowledge from new advances in imaging, genetic, and electroencephalographic studies (Hirsch et al., 2022; Riney et al., 2022; Specchio et al., 2022; Zuberi et al., 2022).

Idiopathic generalized epilepsies

Idiopathic generalized epilepsies (IGE) represent a well-known and prevalent subtype of generalized epilepsies. The IGE include four recognized epilepsy syndromes: generalized tonic-clonic seizures alone, childhood absence epilepsy, juvenile absence epilepsy, and juvenile myoclonic epilepsy. JME is the most prevalent of these IGE (between 23 and 37%) and is lifelong, with few antiepileptic drugs (AED) showing good efficiency and efficacy (Gilsoul, Grisar, Delgado-Escueta, de Nijs, & Lakaye, 2019; Hirsch et al., 2022).

2.1.4. Etiology

Etiologic categories include structural, genetic, infectious, metabolic, immunological, and an unknown group (**Figure 2.1**). A patient's epilepsy may have more than one etiology (Scheffer et al., 2017).

2.1.4.1. Structural etiology

A structural etiology is an anomaly that can be seen on structural neuroimaging. Infection, trauma, and stroke are examples of causes of acquired structural etiologies. Structural etiologies may also be of genetic origin, such as many malformations of cortical development (Scheffer et al., 2017).

2.1.4.2. Genetic etiology

A genetic etiology means a pathogenic mutation that significantly contributes to the patient's epilepsy. Genetic factors are involved in approximately 70 to 80 percent of the epilepsy cases, while the remaining 20 to 30 percent of cases have an acquired factor such as stroke, tumor, or head injury (Myers & Mefford, 2015; Thakran et al., 2020).

First, a family history of an autosomal dominant condition alone may be sufficient to establish a genetic origin. For instance, most families with benign familial neonatal epilepsy have mutations in either the *KCNQ2* or *KCNQ3* potassium channel genes **(Figure 2.2)** (Grinton et al., 2015). On the other hand, in the syndrome of autosomal dominant nocturnal frontal lobe epilepsy, the underlying mutation is known in only a small percentage of people (Tinuper et al., 2016).

Second, a genetic cause may be proposed by clinical research in patients with the same syndrome such as juvenile myoclonic epilepsy or childhood absence epilepsy. Evidence for a genetic cause comes from investigations such as Lennox's twin studies done in the 1950s and familial aggregation studies (Lennox, 1947, 1951; Scheffer et al., 2017).

Third, a molecular basis might have been discovered, implicating a single gene or copy number variant of major effect. Molecular genetics have led to identification of the causative mutation in numerous epilepsy genes such as Dravet syndrome in which more than eighty percent of patients have a pathogenic variant of *SCN1A* (Scheffer et al., 2017; Palmer, Howell, & Scheffer, 2021).

Environmental stimuli can still be present even though the cause is genetic. It is widely acknowledged that environmental factors can play a role in genetic epilepsy; for instance, many people with epilepsy are more prone to experience seizures when they are sleep deprived, stressed out, or ill (Scheffer et al., 2017).

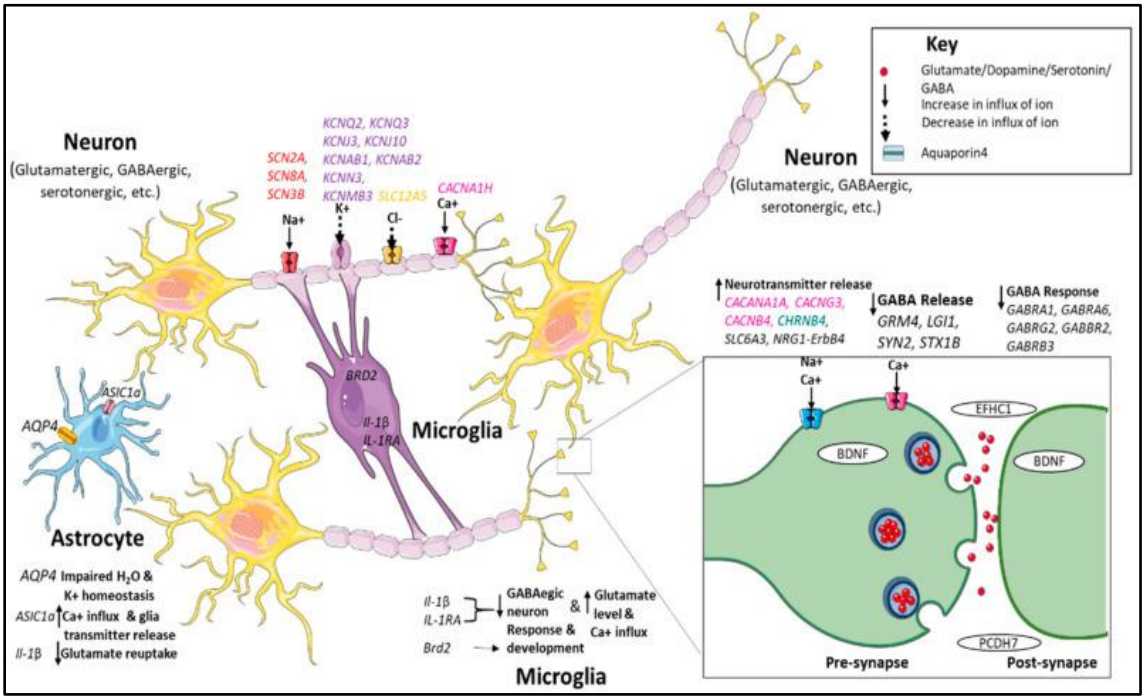

Figure (2.2): The common epilepsy associated genetic variants (Thakran et al., 2020)**.**

2.1.4.3. Infectious etiology

Common examples in particular parts of the world include tuberculosis, neurocysticercosis, HIV, cerebral malaria, subacute sclerosing panencephalitis, cerebral toxoplasmosis, and congenital infections such as cytomegalovirus and Zika virus (Vezzani et al., 2016; Scheffer et al., 2017; Hou et al., 2021).

2.1.4.4. Metabolic etiology

Metabolic epilepsy results from a known metabolic disease in which seizures are a primary symptom of the disease and metabolic dysfunction is both a cause and consequence of epilepsy. Several known metabolic disturbances are sufficient to cause epilepsies e.g. hypoglycemia, vascular dysfunction, hypoxia-ischemia in childhood, porphyria, aminoacidopathies, uremia, or pyridoxine-dependent seizures (Scheffer et al., 2017; Patel, 2018).

2.1.4.5. Immune etiology

The term "immune epilepsy" refers to epilepsy that arises directly from an immune disorder in which the main symptom is seizures. A number of immune epilepsies have been recently identified with specific presentations in both adults and children. Leucine-rich glioma protein1 (LGI-1), N-methyl-D-aspartate receptor (NMDA-R), and glutamic acid decarboxylase 65 (GAD 65) are the most prevalent immunoglobulin G (IgG) linked with autoimmune epilepsy (Scheffer et al., 2017; Husari & Dubey, 2021).

2.1.4.6. Unknown etiology

Unknown indicates that the epilepsy's underlying etiology is still unknown. One-third of all epilepsies are estimated to have unknown etiology (Dubey et al., 2017; Scheffer et al., 2017).

2.1.5. Comorbidities

A large number of the epilepsies are associated with comorbidities such as learning, behavioral, and psychological problems **(Figure 2.1 left hand vertical oval)**. These range in form and degree, from mild learning disabilities to intellectual disability, to psychiatric features like depression and autism spectrum disorders, to psychosocial issues. A complicated variety of comorbidities, such as motor deficits such cerebral palsy or deterioration in gait, movement

problems, scoliosis, sleep, and gastrointestinal disorders, may be present in cases of more severe epilepsies. Every patient with epilepsy should have the presence of comorbidities taken into consideration to enable early detection, diagnosis, and appropriate management (Scheffer et al., 2017).

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