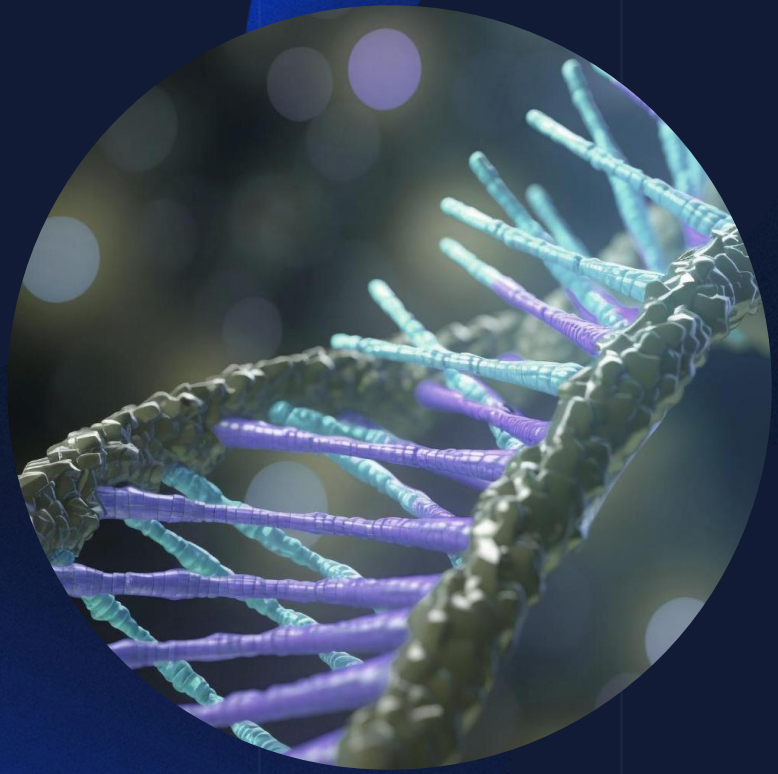
Study of DNA methylation status of four CpG sites at the bromodomain-containing protein 2 (*BRD2*) gene promoter using pyrosequencing in Egyptian juvenile myoclonic epileptic patients



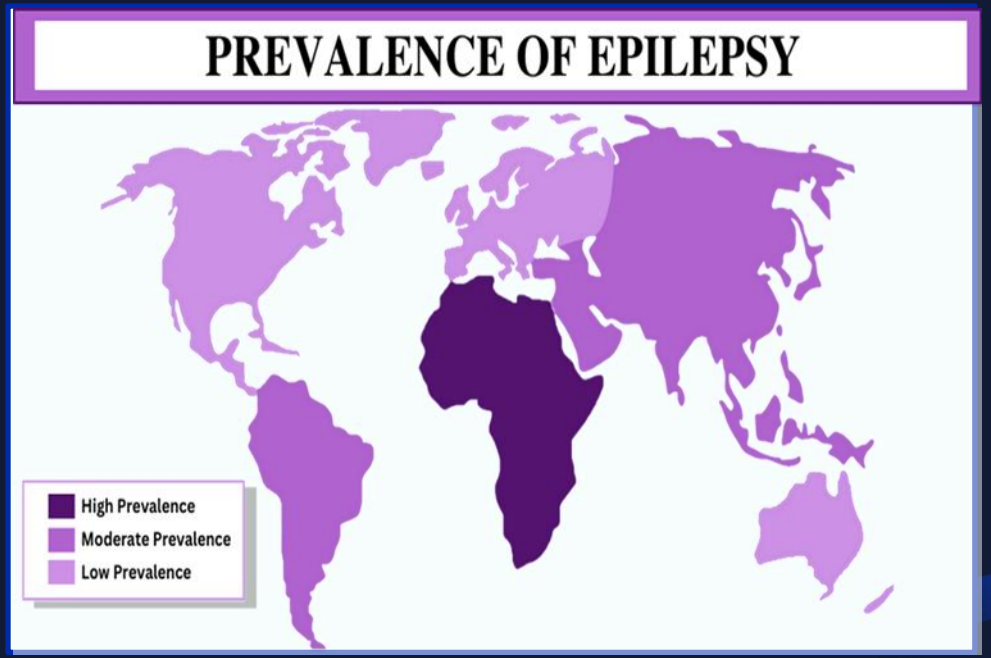
Introduction

Epilepsy

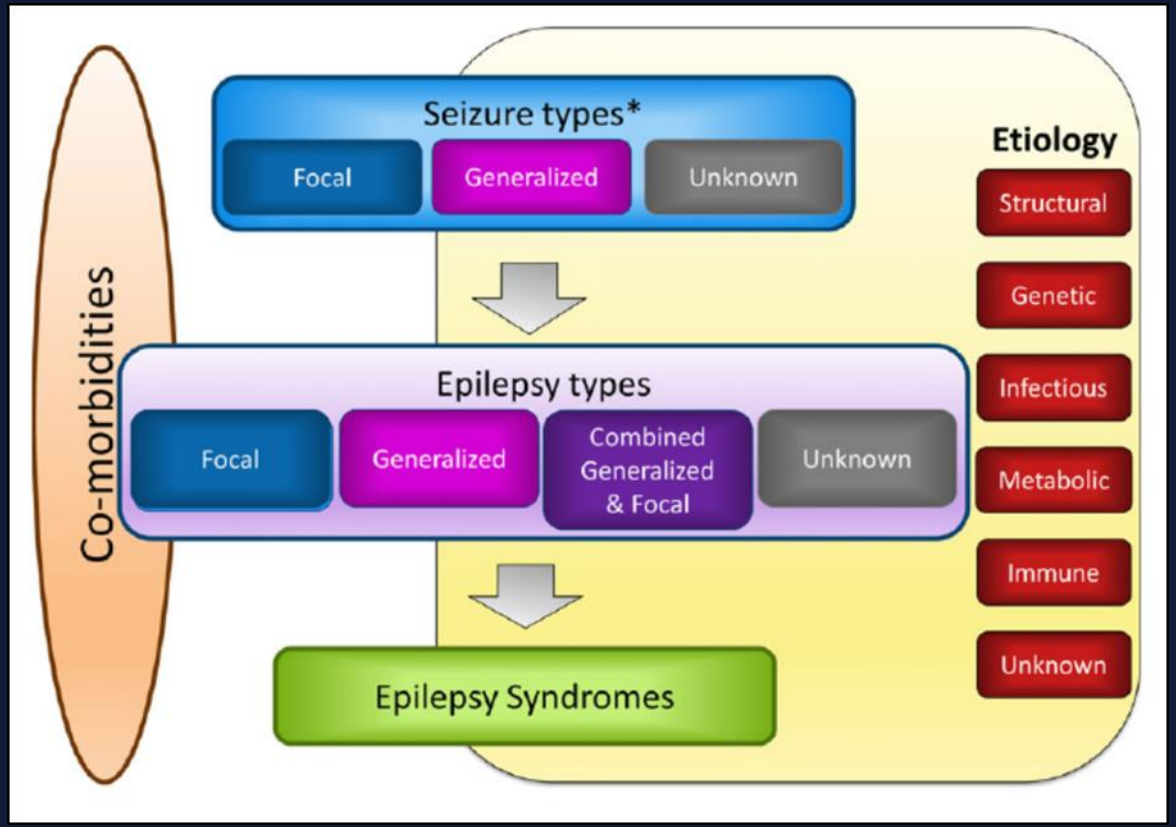
- Epilepsy is considered one of the most prevalent neurological diseases.
- People of different ages, races, social levels, and geographical areas can be affected by epilepsy.
- About 50 million people worldwide are impacted by it.

EPILEPSY PREVALENCE

The prevalence is higher in low/middle-income countries than in high-income countries.



classification of Epilepsy according to ILAE



Idiopathic generalized epilepsies (IGE)

- They are a well-known and prevalent subtype of generalized epilepsies and include four well-established epilepsy syndromes:
- Juvenile myoclonic epilepsy (JME),
- Childhood absence epilepsy (CAE),
- Juvenile absence epilepsy (JAE)
- Generalized tonic–clonic seizures alone (GTCSA)

Juvenile Myoclonic Epilepsy

- It is the most prevalent among the idiopathic generalized epilepsy syndromes.

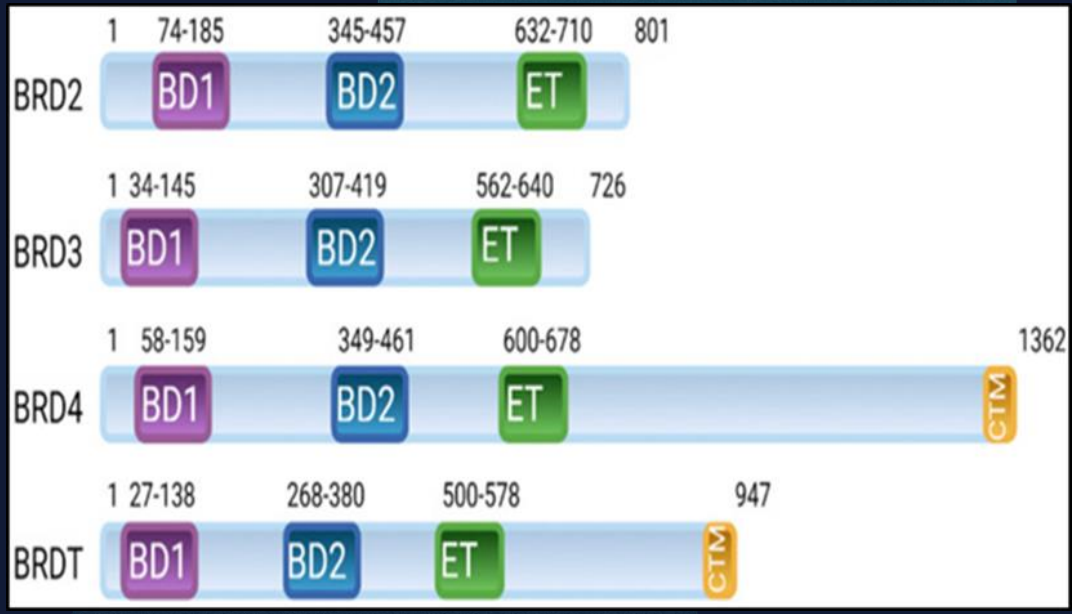
- It accounts for between 23 and 37% of all IGE cases and 5% to 10% of all epilepsy cases.

Juvenile Myoclonic Epilepsy

- It starts during late childhood and early adolescence and is a lifelong disorder.
- Genetic factors play a major role in JME etiology with a wide genetic heterogeneity and complex inheritance.

The bromodomain and extra terminal [BET] family of bromodomain -containing proteins

- It contains four members; BRD2, BRD3, BRD4 and BRDT.

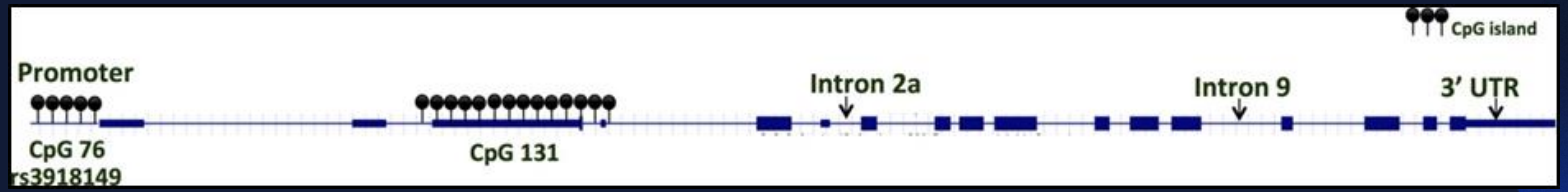


- They are epigenetic readers that bind to acetylated histones to regulate transcription of genes.
- They play a significant role in brain-derived neurotrophic factor expression and affect neuroplasticity.

BRD2

- *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.



Epigenetics

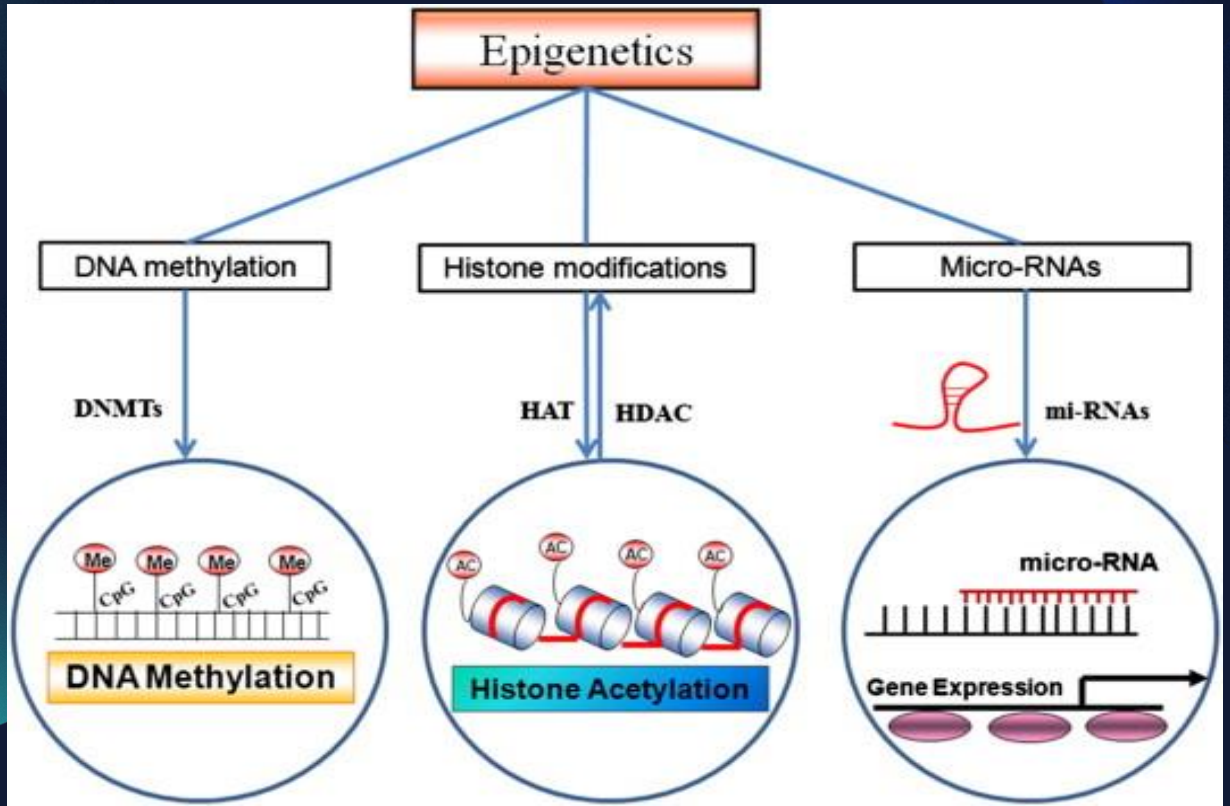
Epigenetics refers to

- Hereditary and dynamically reversible modifications of a chromatin

- Without changing its DNA base pair sequence,

- Producing a change in phenotype without altering in genotype.

The three main epigenetic mechanisms

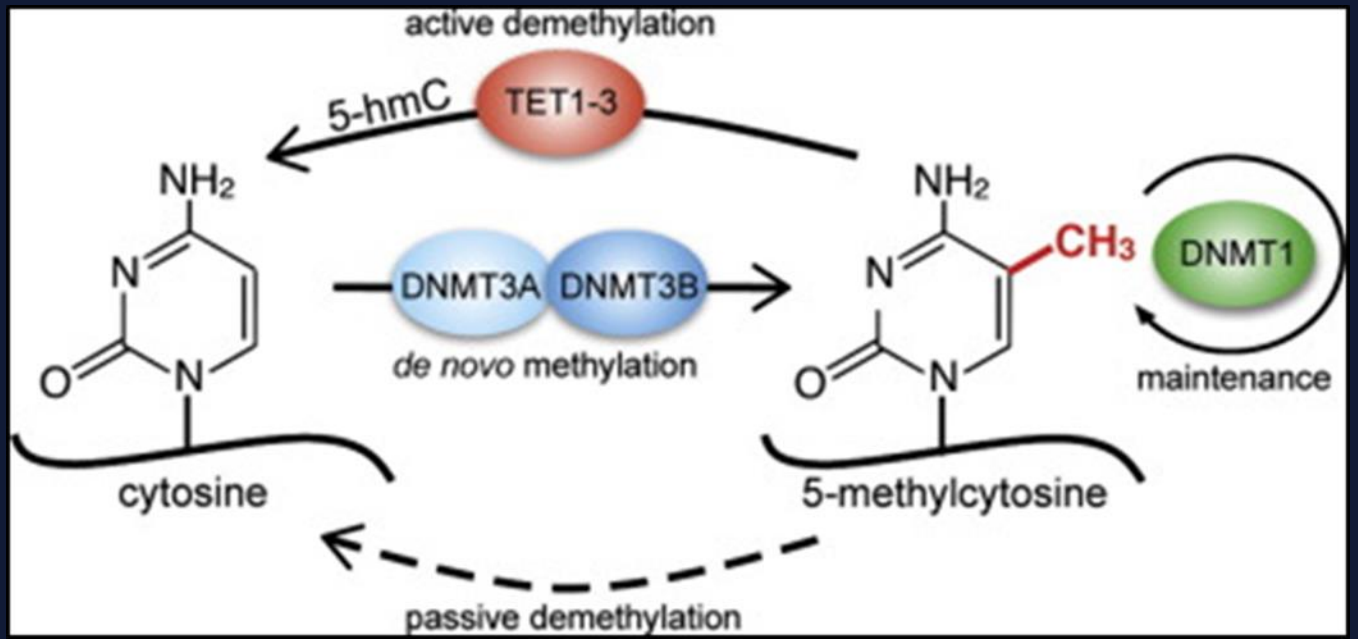


DNA methylation

- It is one of the most important epigenetic mechanisms.
- It is known to have a significant role in brain function and behavior, and dysregulated DNA methylation is a cause of many diseases.
- DNA hypermethylation of *BRD2* with subsequent gene silencing is supposed to be associated with epileptogenesis and recurrent seizures.

DNA methylation

- Alterations in DNA methylation in *BRD2* may play a role in the development and maintenance of JME.

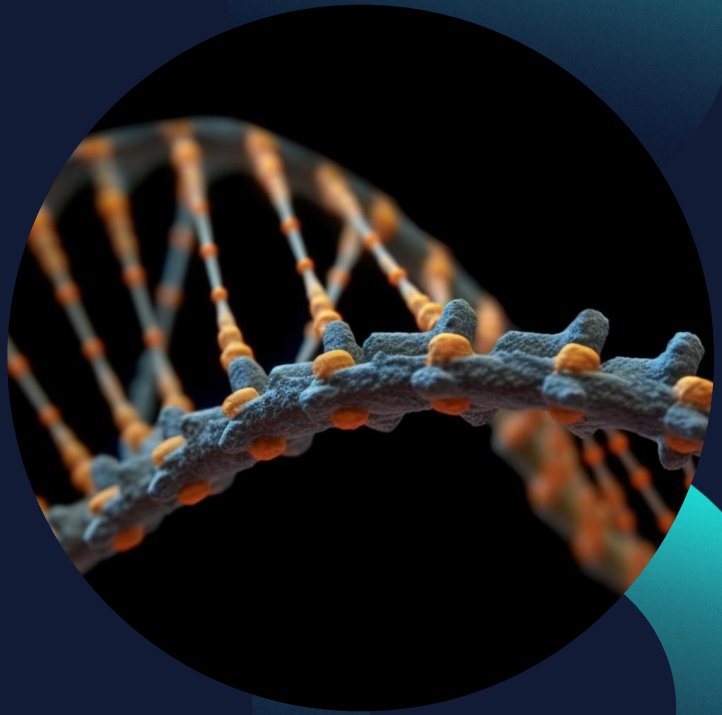


Methods of detection of DNA methylation

DNA methylation can be analyzed using three main methods:

- Bisulfite conversion which include Pyrosequencing.
- Methylation-sensitive restriction enzymes.
- Affinity enrichment-based approaches.

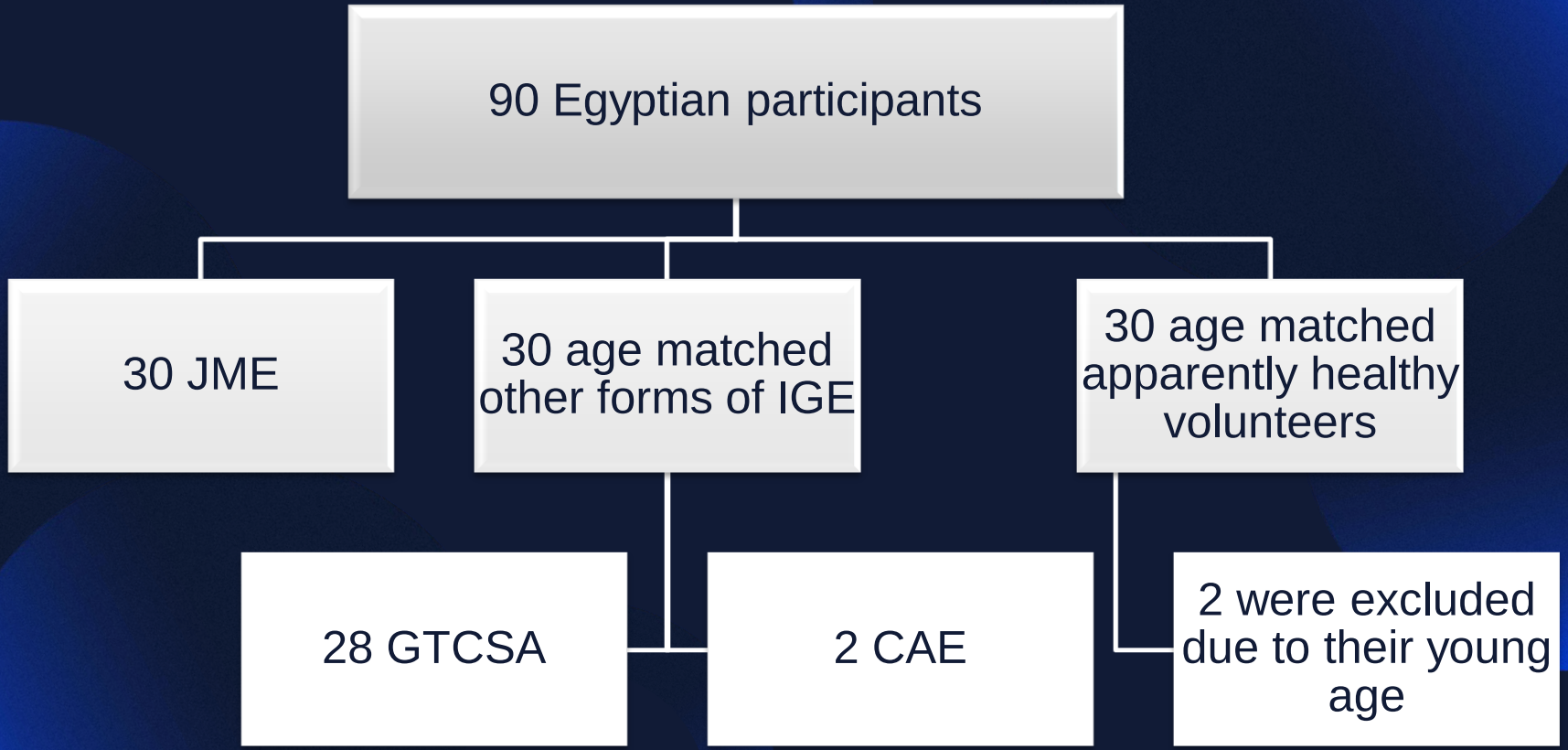
Aim of the Work



The present work aims 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.

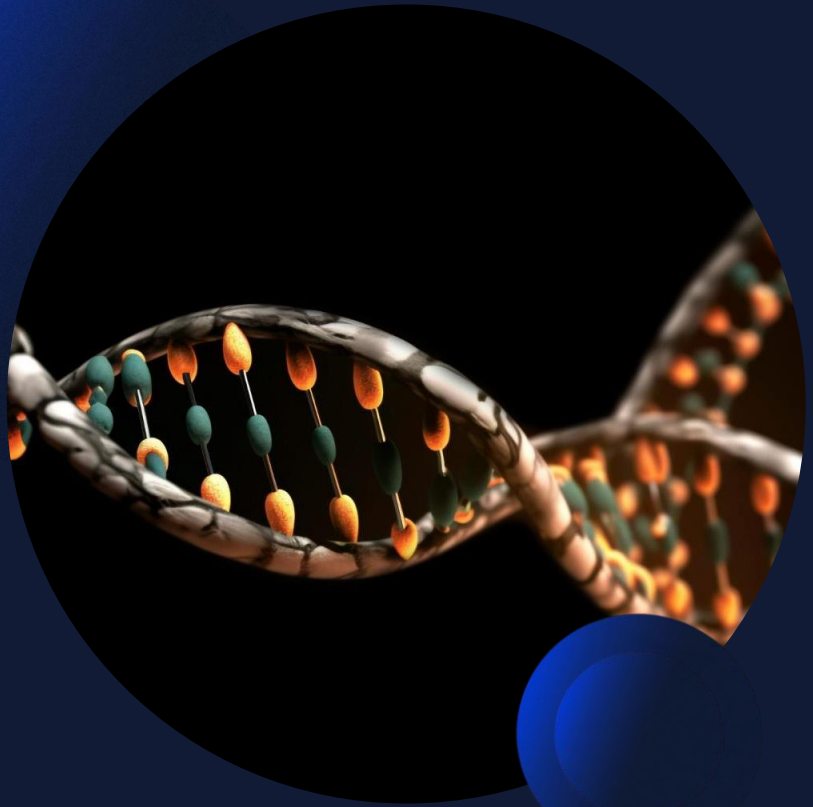
Subjects


90 Egyptian participants
30 JME
30 age matched other forms of IGE
30 age matched apparently healthy volunteers
28 GTCSA
2 CAE
2 were excluded due to their young age

Patients were recruited from the epilepsy clinic in El-Hadara University Hospital and diagnosed in accordance with the International League Against epilepsy.

Exclusion criteria

Patients having known neurological diseases other than epilepsy were excluded..



methods

To all patients the following were done:

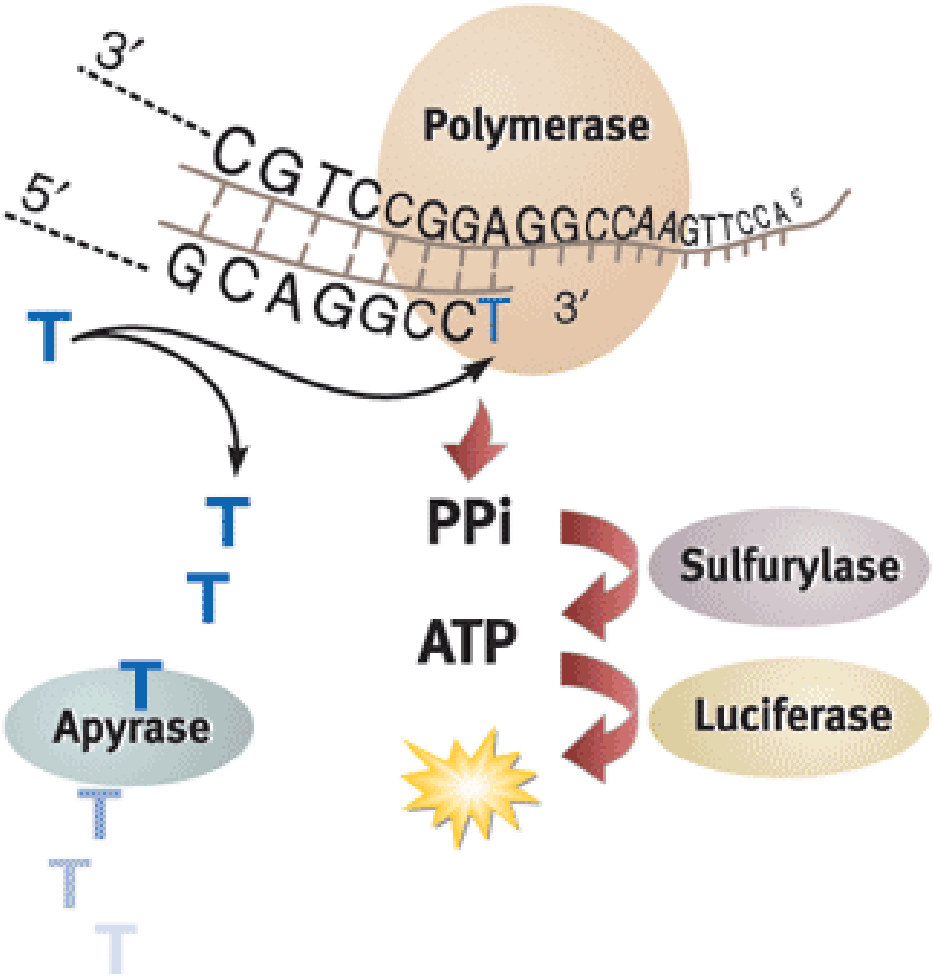
- Medical history taking
- Neurological examination

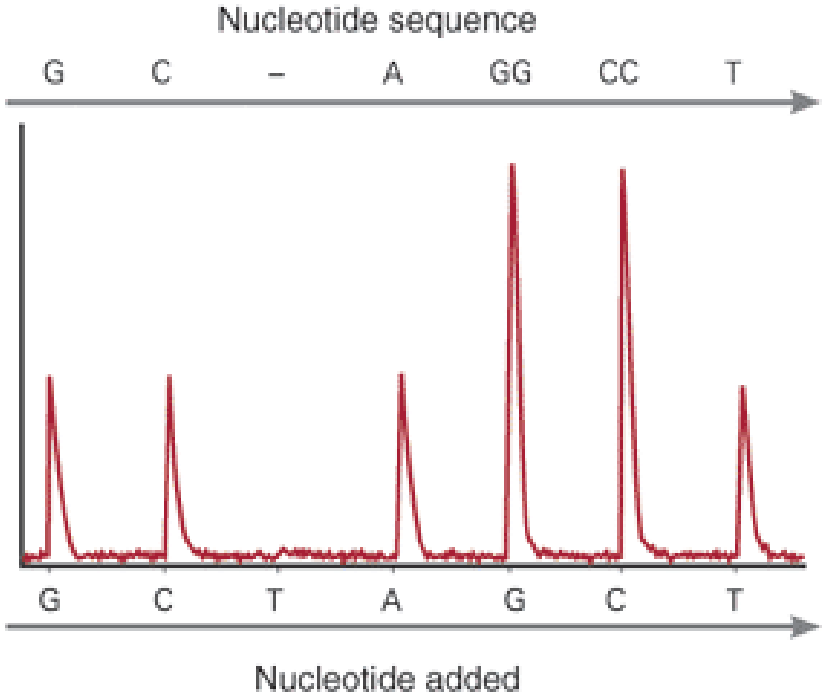
- An electroencephalogram (EEG).
- Pyrosequencing Analysis to all subjects.

Pyrosequencing principle

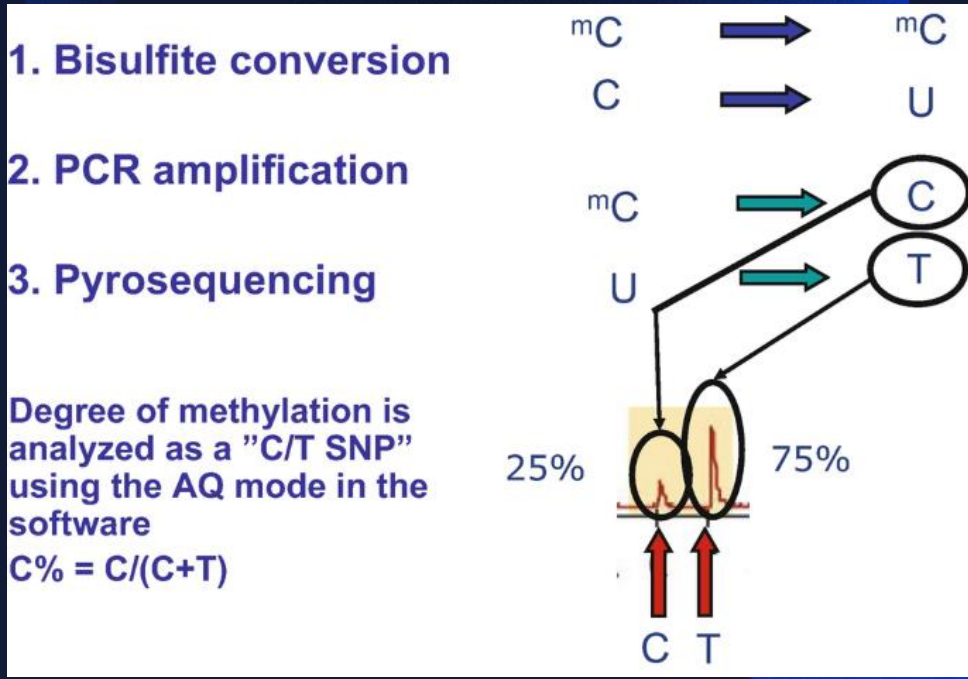
- Pyrosequencing is a DNA sequencing-by-synthesis technique.

- It starts with isolation of the PCR product with streptavidin beads and hybridization with a sequencing primer, and then sequencing.


3′
CGTCCGGAGGCCAAGTTCCA 5′
Polymerase
5′
GCAGGCCT 3′
T
T
T
T
T
T
Apyrase
PPi
ATP
Sulfurylase
Luciferase


Nucleotide sequence
G C – A GG CC T
G C T A G C T
Nucleotide added

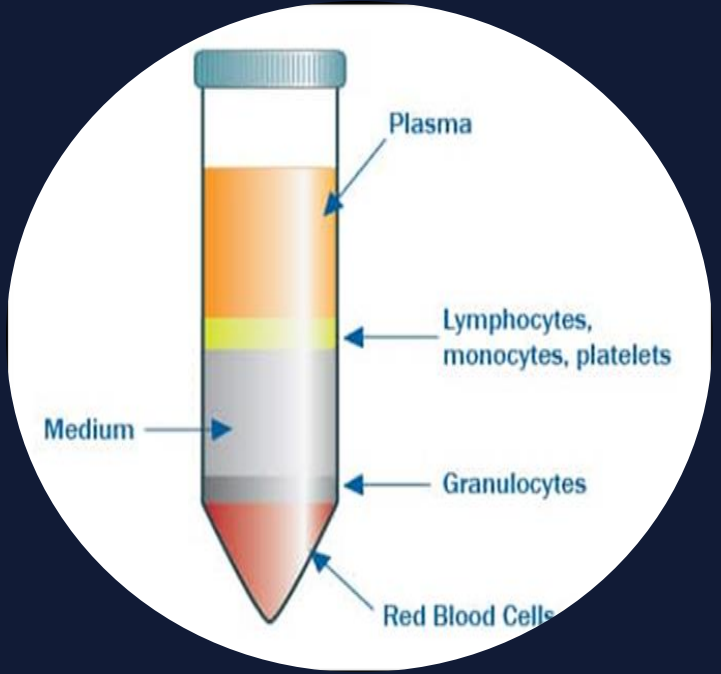
Methylation percentage is calculated from the ratio of heights of a cytosine peak (methylated signal) and the sum of cytosine and thymine peaks (methylated and unmethylated signal) for each cytosine in a CpG dinucleotide.

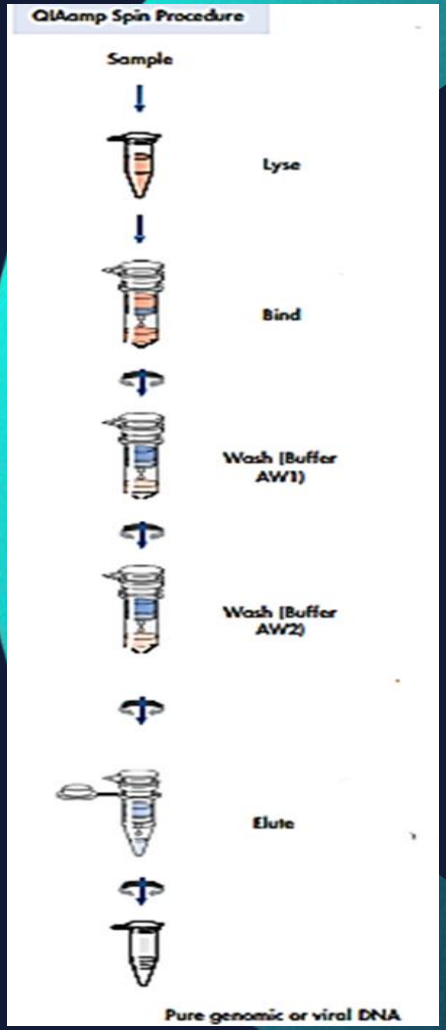


Procedural overview

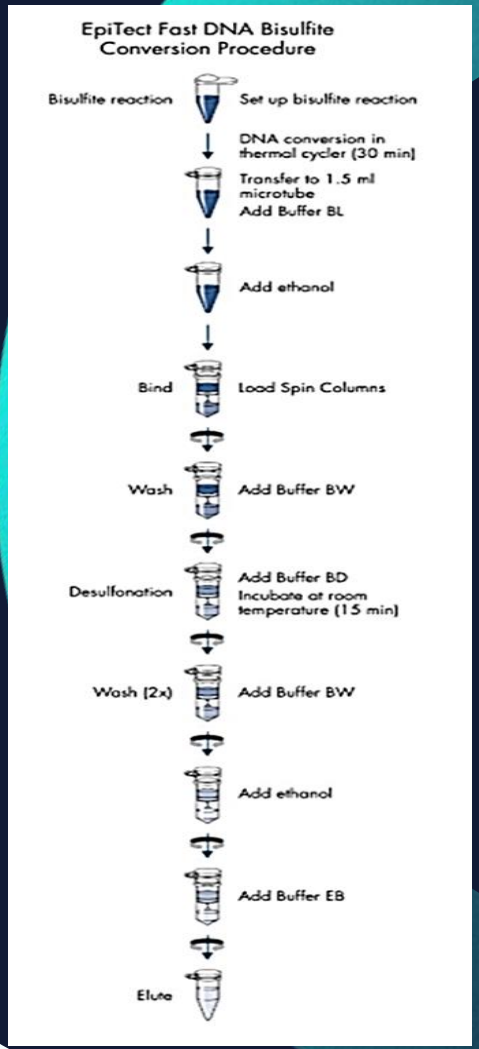
- a) About 5 ml peripheral venous blood samples were collected from the participants in EDTA vacutainer tubes.

- b) Peripheral blood mononuclear cells extraction using a density gradient medium (Ficoll) .





- c) Total DNA was extracted from blood mononuclear cells by QIAamp DNA Mini Kit.



- d) DNA bisulfite conversion using Epitect Fast DNA Bisulfite Kit.

e) PCR amplification by PyroMark PCR Kit and PyroMark custom assay kit.

Bands of PCR amplicon detected by 2% TBE agarose gel

- f) The detection of 4 CpG Methylation Status of BRD2 Gene Promotor in the amplified DNA was done according to manufacturer's instructions of PyroMark Gold Q24 reagents kit on PyroMark Q24 instrument and PyroMark Q24 software for analysis.

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Thanks!

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