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COLLEGE OF SCIENCE

ANALYSIS OF SCN1A , SCN2A AND GABRG2 POLYMORPHISMS AS GENETIC RISK FACTORS FOR FEBRILE SEIZURES IN AL-SAMAWA CITY

A THESIS

SUBMITTED TO THE COUNCIL OF THE COLLEGE OF SCIENCE, MUTHANNA UNIVERSITY, IN PARTIAL FULFILLMENT OF REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN BIOLOGY / ZOOLOGY

BY

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2017 **1438**

بسم الله الرحمن الرحيم

﴿ إِنَّا فَتَحْنَا لَكَ فَتْحًا مُّبِينًا ﴿١﴾ ﴾

صدق الله العلي العظيم

(سورة الفتح- آية1)

Declaration

I certify that this thesis entitled **" Analysis of SCN1A , SCN2A and GABRG2 Polymorphisms As Genetic Risk Factors for Febrile Seizures in AL-Samawa city "** was prepared by " Maha sultan Ali " under my Supervision in the Department of Biology ,college of Science , Muthanna University in partial fulfillment of the requirements for the degree of Master of Science in Biology / Zoology .

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/ / 2017

Recommendation of Head department in view of the available recommendation , I forward this thesis for debate by the examining committee .

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/ / 2017

إقرار المقوم اللغوي

اشهد إني قد قومت هذه الرسالة والموسومة بعنوان **(Analysis of SCN1A , SCN2A and GABRG2 Polymorphisms As Genetic Risk Factors for Febrile Seizures in AL-Samawa city)** وقد اجرت الطالبة **(مها سلطان علي)** جميع التصحيحات المطلوبة , قسم علوم الحياة / كلية العلوم / جامعة المثنى .

التوقيع :

الاسم :

الدرجة العلمية :

التاريخ : / / 2017

Certification

We are certified as an examining committee , that we have read the thesis entitled " **Analysis of SCN1A , SCN2A and GABRG2 Polymorphisms As Genetic Risk Factors for Febrile Seizures in AL-Samawa city** " and examined the student " Maha sultan Ali " in 16/7/2017 , and found that the thesis meets the standard for the degree of Master of Science in Biology / Zoology .

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Dedication

To

My love who is my greatest inspiration …

"my mother"

To

The source of giving and hope …" my father "

To

Those who are my best support …" my brother and my sisters "

To

My dears …" my daughter and my husband"

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And sorry to those I forgot ....

Maha ...

1.1 Introduction

Epilepsy is a neurological condition characterized by recurrent seizures. Clusters of nerve cells, or neurons, in the brain sometimes signal abnormally and cause a person to have seizures. Epilepsy is one of the most common neurological disorders worldwide, affecting 50 million persons. Every year, approximately 50,000 new cases of epilepsy are diagnosed in children and adolescents under the age of 18. Epilepsy affects every child differently depending on age, types of seizures and response to treatment and can have a major effect on a child's development. Epilepsy begins with childhood where 60% of cases and most of the clinically significant aspects of the disease occurred during childhood (Neville , 1997).

Febrile convulsions (FCs) or febrile seizures (FSs) are seizures that related with fever as high as 38.5 ^{o}C during childhood with an approximate rate of (3 - 5) % without any infection within the central nervous system (CNS) (Moreno and Furtner, 2009). They most commonly occur in children between the ages of 6 months and 5 years with the high incidence of 18 months (Moghaddam *et al*., 2016). FSs tend to occur in families. In large families, the febrile seizure susceptibility trait is inherited by the autosomal dominant pattern . In a child with FS, the risk of FS is 10% for the sibling and almost 50% for the sibling if a parent has FS as well (Noah and Afify ,2014) .

Accumulating of epidemiological evidence indicate that FSs are the most common recognized previous for epilepsy in childhood, although the precise risk of developing epilepsy after febrile seizures is unclear. Following a first FSs , 2–4% of children will experience at least one unprovoked seizure, a risk four times that in the overall of population (Verity and Golding , 1991) and most of these children will subsequently develop epilepsy. Between (13- 19) % of children with afebrile seizures will have had one or more previous FS. Factors that consistently increase the risk for developing unprovoked seizures (epilepsy) following FSs, including a family history of epilepsy, complex features, and the presence of early onset neurodevelopmental abnormalities. These factors are different to the risk factors associated with recurrent FS, thereby supporting the argument that FS are distinct from unprovoked seizures (Trinka *et al.*, 2002) .United States and Western Europe, they occur in (2 - 4) % of all children (Shinnar and Glauser , 2002). Febrile convulsion happens more commonly in boys than in girls (Moghaddam *et al*., 2016).

In fact, FSs of children may involved a complex interaction between the immune inflammatory process, cytokine activation, and genetic factors (Chou *et al.*, 2003) . Modern molecular genetic studies have disclosed that mutations of the voltage gated sodium channel genes of α 1-subunit (*SCN1A*) ,α 2-subunit (*SCN2A*) (Escayg *et al.*, 2001), and the γ-aminobutyric acid (*GABA*) receptor genes of γ 2-subunit (*GABRG2*) are related with febrile convulsion (Tan *et al.* , 2012) . Voltage-gated sodium channel is heteromeric protein that is consists of one alpha and one or more beta subunits. The alpha subunit is responsible for channel functions and the beta-1 subunits regulate the channel kinetics . SCNA gene family consists of (9) genes (SCN1A, SCN2A, etc) and encodes the alpha subunit (Namazi *et al.* , 2015).

The pathogenesis of FSs remains obscure. Possible causes include viral infection of the CNS and lowered threshold for seizures in the presence of fever. An alteration in gamma-amino butyric acid (GABA)-ergic neurotransmission has been implicated as an etiologic factor. Neuronal inhibition in the mammalian brain is largely mediated by the binding of GABA to heteromeric GABA receptors . GABR, a ligand-gated Cl^- channel, functions as a tetramer consisting of α, β, γ and π subunits. Each subunit has many subtypes, and the main GABR in the CNS consists of α 1 , β 2, and γ 2 subunits. The genes encoding GABR subunits represent high-ranking candidates for idiopathic generalized epilepsy susceptibility genes because of the widespread distribution of GABRs in the CNS (Macdonald and Olsen, 1994).

Febrile seizures are commonly benign but can cause scare to the parents (Khaled *et al.* , 2014) **,** It should be differentiate from epilepsy, which is characterized by recurrent non-convulsion . A febrile seizure is an episode related with fever but without evidence of intracranial infection or defined cause . Some drugs are commonly utilized to treat febrile seizures (both antipyretics and anticonvulsants) that they are not very active in preventing the disease (Moghaddam *et al.*, 2016) .

Electroencephalography (EEG) and neuroimaging are of limited value and treatment with antiepileptic medications is rarely indicated (Farrell and Goldman ,2011). Genetic studies of complex diseases such as FSs are difficult to approach because of the uncertainty of polygenic traits. Single-nucleotide polymorphisms are the most abundant types of DNA sequence variation in the human genome (Tsai *et al.*, 2002). It is a single base pair on the DNA that varies from person to person.

Single nucleotide polymorphisms are markers that may provide a new way to identify complex gene-related diseases such as FSs (Noah and Afify , 2014).

1.2 Aims of the study:

The objectives of the study as the following :

1- Determine the various genotypes of *SCN1A*, *SCN2A* and *GABRG2* genes in febrile seizures (FSs) in children and comparing them with control persons using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique.

2- Molecular detection of *SCN1A*, *SCN2A* and *GABRG2* genes and comparison of febrile seizure genes in males and females .

3- Find out the relationship between biochemical tests and febrile seizure genes.

Chapter One
Introduction

Chapter Two
Literature Review

Chapter Three
Materials and Methods

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Chapter five
Conclusions and Recommendations

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Results
and
Discussion

References

3.1 Materials

3.1.1 Instruments

The necessary instruments and apparatuses a available in the laboratory are worthily used for preparing the appropriate experiments in this study , table (3-1).

Table 3.1 Instruments and their company and origin

No.	Instruments	Company	Origin
1-	Refrigerator	Concord	Lebanon
2-	Digital camera	Samsung	Korea
3-	PCR Thermocycler	Bio rad	Singapore
4-	uv -trans illuminator	Bio Source	India
5-	Gel & clot activator tube	AFCO	Jorden
6-	EDTA k3 tube	AFCO	Jorden
7-	Gel electrophoresis	Advance mupid - one	Japan
8-	Plain tube	Sun	Jorden
9-	Reflotron plus	Roche	Germany
10-	Centrifuge	Hettich	Germany
11-	Micropipettes and Tips (different volumes)	Eppendorf	Germany
12-	High speed Cold Centrifuge	Eppendorf	Germany
13-	Water bath	Kottermann	Germany
14-	Incubator	Memmert	Germany
15-	Spectrophotometer	Cecil	England
16-	Eppendorf tubes	Sigma	England
17-	Disposable syringes 3 cc	Changzhou Kangfulai Medical	China
18-	Ice box	Hengguan	China
19-	Vortex	CYAN	Belgium

3.1.2 Biochemical Kits

The Biochemical Kits that are used in the study illustrated in table (3-2) .

Table 3.2 Biochemical Kits and their company and origin

No.	Type of Kits	Company	Origin
1-	Calcium Kit	Randox	England
2-	Reflotron K+	Roche	Germany
3-	Reflotron creatinine	Roche	Germany
4-	Reflotron Glucose	Roche	Germany
5-	Sodium rapid Kit	Human	Germany
6-	Chlorid liquicolor	Human	Germany
7-	Phosphorus liquirapid	Human	Germany

3.1.3 Molecular Kits

In the table (3-3), the chemical materials that are used in this study with their companies and countries of origin are listed :

Table 3.3 The molecular kits and their company and origin

No.	Kit	Catalog no.	Company	Country
1-	Quick-gDNA™ MiniPrep Genomic DNA Extraction Kit	2E021-10	Zymoresearch	USA
2-	Hot start master mix	#1501	Bioland scientific LLC	USA
3-	ScrFI restriction enzyme	0111604	New England biolabs	USA
4-	ApoI restriction enzyme	0081509	New England biolabs	USA
5-	PvuII restriction enzyme	0151604	New England biolabs	USA

3.1.4 Primers

The gene primers which are used in REFLP-PCR for detection , , as following **,** table (3-4) :-

Table 3.4 PCR Primers , restriction enzyme and their restriction site sequence

Primer	Sequence 5' ⟶ 3'		PCR product size	R.E.	Restriction site sequence	References
SCN1A	F	5′-TGCACAAAGGAGTAGCTTATG-3′	336 bp	PvuII	↓ 5′…CAG CTG…3′ 3′….GTC GAC…5′ ↑	(Lakhan *et al* , 2009)
	R	5′-AGTCAAGATCTTTCCCAATTTCAG-3′				
SCN2A	F	5′-AATCACCTTTTATTCTAATGGTC-3′	400 bp	ScrFI	↓ 5′….CC NGG….3′ 3′….GG NCC….5′ ↑	(Lakhan *et al* , 2009)
	R	5′-CAGTGAAGGCAACTTACTAAGA-3′				
GABRG 2	F	5′-GAGTGCCAATTACAATTGCAAAA-3′	122 bp	ApoI	↓ ′5….R AATTY….3′ ′3….YTTAA R…5′ ↑	(Chou *et al*, 2003)
	R	5′-AATCAGAAAGACTGTAGGTGAGG-3′				

- Y= T or C
- R= A or G
- N= A,C,G or T

3.1.5 Chemicals

The chemical and biological materials used are listed below in table (3-5) below:

Table 3.5 Chemical materials with their company and origin

No.	Chemical	Company and Origin
1-	Ethanol (96-100)%	BDH (UK)
2-	LE-Agarose DSBIO	BioBasic (Canada)
3-	TBE buffer (008) DSBIO	BioBasic (Canada)
4-	Loading dye	Bioneer (Korea)
5-	Ehidium Bromide (2E0216-08)	BioBasic (Canada)
6-	Proteinase k (4A0116)	BioBasic (Canada)
7-	Free nuclease water	Bioneer (Korea)
8-	Ladder	Eurx (Poland)

3.2 Methods

3.2.1 Patients and Blood sampling

Blood was obtained from 50 children of ages ranging from "6 months to 6 years" voluntary to the Emergency Room whose visit to the Female and Children Educational Hospital in the AL-Samawah city during the period from December 2015 to February 2016 . An informed consent was obtained from the parent(s) of each child before commencement of the study. Fifty children whose suffered febrile seizure were included in the study . An age and sex matched group of fifty children with fever but without FSs were the controls . Diagnosis of FS and its sub classification to simple and complex FS was done according to the guidelines of the International League Against Epilepsy (Engel , 2006) , and divided into 2 portions: 1 ml of whole blood is collected into tubes containing EDTA (ethylene demine tetra acetic acid) , kept at –20 ºC, for genomic DNA extraction . And 2 ml in to gel tubes to obtained serum are separated immediately for chemical tests .

3.2.2 Exclusion criteria:

Children with the following criteria are excluded from the study:

- Afebrile seizures.
- Febrile seizures in children older than 6years.
- Epileptiform EEG traits.
- Evidence of metabolic disorder that might be the etiologic factor underlying seizures.
- Evidence of intracranial infection.
- Any cardiac, pulmonary or renal diseases.

All children were subjected to the following :-

1. The complete history- take with special emphasis on

a) The character of seizures (type -duration- number of previous fits).
b) Clinical features suggest CNS infection, e.g. meningeal irritation.
c) History of other neurological illness.
d) Pre-natal history.
e) Clinical patient history , e.g. jaundice, cerebral palsy or metabolic disease .

2. Biochemical tests, that included blood sugar , creatinine , calcium , sodium , phosphorus , chloride , potassium.

3 .2.3 The Biochemical tests

3.2.3.1 Spectrophotometer Apparatus

In this apparatus , determination of a particular element was based on the absorbance of the light beam intensity emitted at a wavelength characteristic for a given element .

A) Calcium test

Ca^{+2} Kit enables cloroimetric determination in alkaline medium.

Calcium ions+ O-cresolphthalein complex $\longrightarrow$ a violet complex

Kit Components:

Reagent 1 (standard) : Ca^{2+} , 2.50 mmol/l .

Reagent 2 (buffer) : 2-amino -2- methyl- propan-1- ol , 3.50 mol/l .

Reagent 3(chromogen) : 1)O-cresolphthalein complexone , 0.16 mol /l .

2) 8-Hydroxyquinoline , 6.89 mol / l .

3) Hydrochloric acid , 150 mol / l .

Randox kit and clinical chemistry in diagnosis and treatment by Zilva.Pannall.Mayne

Working steps of Ca^{+2} test

The methods of Kit Ca^{+2} was according to the manual procedures of kit as following:

- Added of the reagents:- (0.5 ml of R2 with 0.5 ml of R3) ml of reagents in (sample , blank and standard) tubes and after that added 25 µl from stander reagent 1 in the standers tube
- Added 25 µl of serum in the sample tube. Then mix , reading the absorbance of sample and standard against the reagent blank after 5 to 50 minutes and calculation :
  - Concentration=(A sample /A standard) * 2.50 (mmol/l) , (wavelength : 570 nm against reagent blank)

B) phosphorus test

Phosphate reacts with molybdate in strong acidic medium to form a complex . The absorbance of this complex in the near UV was directly proportional to the phosphate concentration . Reaction principle (simplified) :

$$7\ H_3PO_4 + 12\ (Mo_7O_{24})^{6-} + 51\ H^+ \longrightarrow 7\ [P\ (Mo_{12}O_{40})]^{3-} + 36\ H_2O$$

Kit component

RGT : 1) Ammoniumheptamolybdate , 0.3 mmol/l

2) Sulphuric acid (pH < 1.0) , 160 mmol/l

3)Detergent , 1 %

4)Activator and stabilizers

STD : phosphorus , 3.2 mmol/l

Working steps of P test

The methods of Kit P was according to the manual procedures of kit as following:-

- Added of the RGT (1000 µl) in (sample , blank and standard) tubes .Then add 10 µl of samples (serum) in the sample tube , After that make mix incubate at least 1 minute at room temperature . Measure the absorbance of the sample and STD against the reagent blank within 60 minute (A) and calculation :
- Concentration= (A sample /A STD) * 3.2 (mmol/l)
- (wavelength : 340 nm against reagent blank)

C) Chloride kit

Chloride ions react with a mercury (ll)-2,4,6-tri-(2-pyridyl)-s-triazine (TPTZ) complex to form mercury(ll) –chloride . The liberated TPTZ reacts with iron (ll) ions yielding a blue coloured complex . The resulting absorbance change at 590 nm is directly proportional to the amount of chloride ions in the sample .

Kit components

RGT(colour reagent): 1) 2,4,6-tri-(2-pyridyl)-s-triazine (TPTZ)

(partially as mercury(ll) complex) , 0.986 mmol/l

2) Iron (ll) sulphate , 0.53 mmol/l

STD (standard) : chloride (Cl^-) , 100 mmol/l

Working steps of Cl^- test

The methods of Kit Cl^- was according to the manual procedures of kit as following and we used Semi – micro method :-

- Pre-dilute STD and the sample 1 + 50 with distilled water ,e.g. 20 µl STD / sample + 1000 µl dist. Water after that me added 20 µl of serum in the sample tube, After that mix , incubate for 5 minutes in the dark and measure the absorbance of sample (A_{sample}) and STD (A_{STD}) within 60 minutes against the reagent blank and calculation :

  Concentration (C) =(A_{sample} /A_{STD}) * 100 (mmol/l)

  (wavelength : 590 nm against reagent blank)
- Mix , incubate for 5 minutes in the dark and measure the absorbance of sample (A_{sample}) and STD (A_{STD}) within 60 minutes against the reagent blank and calculation :

  Concentration (C) =(A_{sample} /A_{STD}) * 100 (mmol/l)

  (wavelength : 590 nm against reagent blank)

D) Sodium Test

Sodium is precipitated with Mg – uranyl acetate ; the uranyl ions remaining is suspension from a yellow – brown complex with thioglycolic acid . The difference between reagent blank (without precipitation of sodium) and analysis is proportional to the sodium concentration .

Kit components

PREC (precipitating solution) : 1) Uranyl acetate , 19 mmol/l .

2) Magnesium acetate , 140 mmol/l .

RGT (colour reagent) : 1)Ammonium thioglycolate , 550 mmol/l .

2)Ammonia , 550 mmol/l

STD (standard) : sodium (Na^+) , 150 mmol/l .

Working steps of Na^+ test

The methods of Kit Na^+ was according to the manual procedures of kit as following and it is used Semi – micro method :-

The process of this method that me add of the PREC (1000 µl) in (sample and standard) tubes and added 20 µl from STD in the stander tube .then added 20 µl of serum in the sample tube , Now me must add (20 µl) from clear supernatant in the stander and sample tubes , After that me must Add (20 µl) from PREC in the blank tube and Mix , after 5-30 minutes , measure absorbance of reagent blank , the standard (A_{STD}) and the sample (A_{sample}) against distilled water and calculation:

$$C = (A_{sample} / A_{STD}) * 150 \text{ (mmol/l(}$$

wavelength : Hg 405 nm against reagent blank

3.2.3.2 Reflotron plus

The Reflotron plus is an in vitro diagnostic device designed for quantitative determination of clinical chemistry using parameters using Reflotron test reagent strips . It works on the principle of reflectance photometry and ensures rapid and reliable results while being easy to use . An incorporated plasma separating system in the Test strips make it possible to use whole blood as well as plasma and serum . A reflectance measurement is recorded based on the colour change on the test strip .

A) potassium test

1- **Test principle**

After application to the test strip , the sample flows into the reaction zone . in a reagent film consisting of two phases the potassium diffuses from the aqueous into the organic phase and is complexed bu valinomycin . to balance the charge , a PH indicator in the organic phase give off a proton , yielding an anion . A strong acid competing with the indicator and forming a colourless anion permits an optimal change in reflectance .

K^+ + valinomycin + Ind – H [valinomycin – K] $^+$ [Ind] $^-$ + H^+

K+ + valinomycin + A – H ⇌ [valinomycin – K] $^+$ [A] $^-$ + H^+

Ind – H :indicator ; A-H : acid

At a temperature of 37 ºC the couler intensity produced is measured at 642 nm and the potassium concentration is displayed after about 90 sec in mval / l or mmol / l depending on how the instrument has been set .

2 - Components per test

valinomycin (Streptomyces spec.) 33 µg ; 4- [(2,6 dibromo-4-nitrophenyl) azo] – 2 –octadecyloxy -1- naphthol 18.1 µg ; 2,4,6,8, – tetranitro -5- octadecyloxy -1- naphthol 5.3 µg ; buffer .

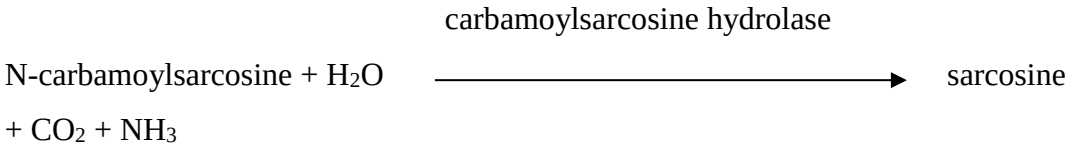
B) Creatinine test

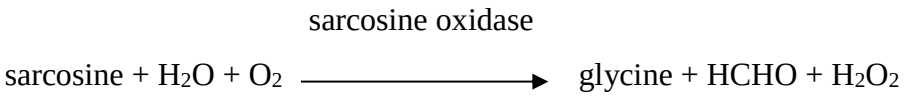
1- **Test principle**

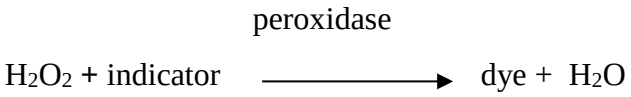
After application to the test strip , the sample flows into the reaction zone , in the case of blood after separating of the erythrocytes from the plasma . In a reaction catalyzed by creatinine iminohydrolase creatinine is hydrolyzed to N – methylhydantoin , with release of ammonia . In further reaction steps , hydrogen peroxide is formed that reacts with an indicator to form a blue dye which is proportional to the creatinine concentration in the sample :

$$\text{Creatinine} + H_2O \xrightarrow{\text{creatinine iminohydrolase}} \text{N–methylhydantoin} + NH_3$$

$$\text{N–methylhydantoin} + 2H_2O + \text{ATP} \xrightarrow{\text{N–methylhydantoinase}} \text{Ncarbamoylsarcosine} + \text{ADP} + P_i$$







At a temperature of 37 °C the formed dye is measure at 642 nm and the creatinine concentration displayed after about 120 sec in mg/dl or µmol/l depending on how the instrument has been set .

2 - Components per test

Creatinine iminohydrolase **(** Corynbacterium lilium) ≥ 1.38 U ; N–methylhydantoinase (E. coli rec.) ≥ 0.108 U ; carbamoylsarcosine hydrolase (E. coli rec.) ≥ 0.439 U ; sarcosine oxidase (E. coli rec.) ≥ 0.475 U ; peroxidase (horseradish) ≥ 2.29 U ; ascorbate oxidase (zucchini) ≥ 0.285 U ; ATP : 82.8 µg ; 2-(3,5-di-tert-butyl-4-hydroxyphenyl) -4- (5) – (9-julolidino)-5- (4) –methyl –(1 H) –imidazole , hydrochloride (indicator) : 19.5 µg ; buffer .

C) Glucose test

1- **Test principle**

After application to the test strip , the sample flows into the reaction zone , where , in the case of blood samples , the separation of the erythrocytes from the plasma occurs. D-glucose is oxidized to δ-D-gluconolactone by atmospheric oxygen in the presence of glucose oxidase (GOD) . The resulting hydrogen peroxide oxidizes an indicator of the presence of peroxidase (POD) . The dye formed in this manner is proportional to the glucose concentration of the sample :

GOD

$$\text{Glucose} + O_2 \longrightarrow \delta\text{-D-gluconolactone} + H_2O_2$$

$$H_2O_2 + \text{indicator} \xrightarrow{\text{POD}} \text{dye} + H_2O$$

The glucose concentration (proportional to the formed dye) is measured kinetically at a wavelength of 642 nm and 37 C , and is displayed after 125 sec in mg/dL or mmol/L .

2 - Components per test

GOD (Aspergillus niger) ≥ 3.2 U ; POD (horseradish) ≥ 3.2 U ; 3,3',5,5'-tetramethylbenzidine (indicator) 72.6 µg ; buffer .

Testing procedure (for three tests by Reflotron plus)

- Additional material required: Reflotron instrument ; Reflotron pipette and pipette , controls , usual laboratory equipment for collecting blood . Before carrying out a test , Switch on the instrument .
- When the display shows " ready " , remove a test strip from the container .
- Upward the strip , taking care not to bend it .
- Using the Reflotron pipette , draw sample material in to the pipette [avoiding bubbles] and apply as a drop to the centre of the yellow application zone – being careful not to touch the application zone with the pipette tip required volume of specimen for application 30 µl . With the sliding cover or flap open , place the test strip on to the guide within 15 sec and slide forward horizontally until it lock into place . Closing the sliding cover or flap .

3.2.4 Molecular Methods

3.2.4.1 Genomic DNA Extraction

1. Added 400 µl of Genomic Lysis Buffer to 100 µl of whole blood (4:1). and mixed completely by vortex 4-6 seconds, letting stand for 5-10 minutes at room temperature , then added 20µl of proteinase K and put it in water path for 30 minutes . And transfer the mixture to a Zymo-Spin™ Column in a Collection Tube. Centrifuge at 10,000 rpm for one minute. Discarding the Collection Tube with the flow through

2. Transfer the Zymo-Spin™ Column to a new Collection Tube. Added 200 µl of DNA Pre-Wash Buffer to the spin column. Centrifuging at 10,000 rpm for one minute. After that me added 500 µl of g-DNA Wash Buffer to the spin column. Centrifuge at 10,000 rpm for one minute.Then Transfer the spin column to a cleaned microcentrifuge tube. Add ≥50 µl DNA Elution Buffer or water to the spin column. Incubating 2-5 minutes at room temperature and then centrifuging at top speed for 30 seconds to elute the DNA. The eluted DNA can be used immediately for molecular based applications or stored ≤-20ºC for future use.

3.2.4.2 Genomic DNA investigation

The extracted genomic DNA is checked by using Gel electrophoresis. The products of each sample are analyzed by using agarose gel electrophoresis method as following steps:

1- Agarose gel (1%) was prepared in using 1X TBE and dissolving in water bath at 100 °C for 15 minutes, after that, left to cool 50°C . Then added 3µL of ethidium bromide stain in to the agarose gel solution.

2- Agarose gel solution is poured in tray after fixed the comb in proper position after that, left to solidified for 15 minutes at room temperature, then the comb is removed gently from the tray and 10µl of the product are added in to each comb well and 5ul of loading dye in one well.

3- The gel tray is fixed in electrophoresis chamber. Then electric current is performed at 100 volt for 40 min .

4- The products are visualized by using U.V.

3.2.4.3 Molecular detection of genes

Conventional- PCR technique is performed for detection important pathogen factor genes in febrile convulsion in children between the ages from six months to six years.

3.2.4.4 Conventional-PCR master mix reaction preparation

PCR master mix reaction is prepared by using (2x hot start master mix) and this master mix done according to company instructions as showing in table (3-6):

Table 3.6 PCR Master mix reaction and their volume

Conventional -PCR Master mix	**Final volume**
2x Hot start master mix	12.5 µl
Forward primer (10 Pmol/ µl)	0.5 µl
Reverse primer (10 Pmol/ µl)	0.5 µl
Template (0.1-1 µg for single copy)	1 µl
Nuclease free water	10.5 µl
Total volume	25 µl

After that, these PCR master mix reaction components that mentioned above, placed in standard PCR tubes which containing the Quick-gDNA™ MiniPrep Kit that containing all other components needed to PCR reaction such as (Hot start *T*aq DNA polymerase, standard Hot start *T*aq reaction buffer , dNTPs , tracking dye and

stabilizer). Then the tube is placed in vortex centrifuge for (1) minutes ,to be transferred in PCR thermo cycler .

3.2.4.5 Conventional-PCR Thermocycler Program

PCR thermocycler program are done by using convention PCR thermocycler system . See table (3-6) , (3-7) , (3-8) :

Table 3.7 PCR Thermocycler Program for SCN1A , (Lakhan *et al* , 2009).

PCR step	Temp.	Time	Repeat
Initial Denaturation	95°C	5 min	1
Denaturation	94°C	30 s	30 cycles
Annealing	57°C	30 s	
Extension	72°C	30 s	
Final extension	72°C	7 min	1
Hold	4 °C	Forever	-

Table 3.8 PCR Thermocycler Program for SCN2A , (Lakhan *et al* , 2009).

PCR step	Temp.	Time	Repeat
Initial Denaturation	95°C	5 min	1
Denaturation	94°C	30 s	30 cycles
Annealing	60°C	30 s	
Extension	72°C	30 s	
Final extension	72°C	7 min.	1
Hold	4 °C	Forever	-

Table 3.9 PCR Thermocycler Program for GABRG2 **,** (Chou *et al* , 2003) .

PCR step	Temp.	Time	Repeat
Initial Denaturation	95°C	5 min	1
Denaturation	94°C	30 s	35 cycles
Annealing	55°C	30 s	
Extension	72°C	45 s,	
Final extension	72°C	7 min	1
Hold	4 °C	Forever	-

3.2.4.6 Conventional-PCR Product Analysis

The PCR products was analyzed by agarose gel electrophoresis following steps:

1- Agarose gel (1%) is prepared in using 1X TBE and dissolving in water bath at 100 °C for 15 minutes, after that, left to cool 50°C.

2- Then (3µL) of ethidium bromide stain are added into agarose gel solution.

3- Agarose gel solution is poured in tray after fixed the comb in proper position after that, leaving to solidified for 15 minutes at room temperature, then the comb was removed gently from the tray and 10µl of PCR product were added in to each comb well and 5ul of (100bp Ladder) in one well.

4- The gel tray is fixed in electrophoresis chamber and fill by 1X TBE buffer. Then electric current is performed at 100 volt for 20 min then 50 volt for 60 min .

5- PCR products are visualized by using ultraviolet transilluminator.

3.2.4.7 REFLP- PCR Master Mix Preparation

Polymerase chain reaction-restriction fragment length polymorphism (RFLP-PCR) master mix is prepared for detection SCN1A , SCN2A and GABRG2 genes mutation in blood samples of Febrile convulsion cases in children (6 months to six years) by using restriction endonuclease (*PvuII*) that digestion of the (336) bp PCR product of SCN1A gene, (Recognition sequence $\begin{matrix}5'\text{...CAG^CTG...}3'\\3'\text{....GTC^GAC...}5'\end{matrix}$) , restriction endonuclease (*ScrFI*) that digest of the (400) bp PCR product of SCN2A gene , (Recognition sequence $\begin{matrix}5'\text{....CC^ NGG....}3'\\3'\text{....GG ^NCC....}5'\end{matrix}$) and restriction endonuclease(*Apo*I) that digest of the (122) bp PCR product of GABRG2 gene , (Recognition sequence $\begin{matrix}'5\text{....R^ AATTY....}3'\\'3\text{....YTTAA^ R...}5'\end{matrix}$) , this master mix done according to company instructions as following , table (3-9) :

Table 3.10 REFLP-PCR Master mix and their volume

REFLP-PCR Master mix	Volume
PCR product	1 µl
Restriction enzyme buffer 10X	5 µl
Restriction enzyme	1 µl
Free nuclease water	43 µl
Total volume	50 µl

This master mix is placed in micro vortex centrifuge at 3000 rpm for 2 minutes, to be transferred into incubation at 37°C for 5-15 minute . After that, REFLP-PCR product is analyzed by agarose gel electrophoresis methods that are mention in PCR product analysis.

3.2.5 Statistical analysis

In this thesis , several statistical tests are used to find the significant differences among the studied parameters of patients children with febrile seizures (FSs) or between the studied parameters of patients children with febrile seizures (FSs) and control group at ($P < 0.05$) level of significance.

The biochemical tests means are compared using the least significant difference (LSD) at ($P < 0.05$) level of significance, and the results expressed as Mean ± SD.

Chi-square ($\chi 2$) test is used to compare Genotypes and allele frequencies among the two groups. For all tests a probability (p) less than 0.05 is considered significant. Data processed and analyze by using statistical program social science (SPSS 22) .

Abstract

Febrile seizure (FS) is a common disease occur in children aged from 5 months to 6 years whose suffering from high temperature(more than 39 ^{o}C) . They represent the majority of childhood seizures with an incidence of 3 - 5% of children . It's is closely associated with heredity and family history, and there is a risk of future epilepsy (complex type). A total of 50 children with febrile seizures (25 males & 25 females) and 50 normal control (25 males & 25 females) subjects are included in the study during the period from December 2015 to March 2016 who visit to the emergency department at the Children's Hospital in Samawaa city . PCR-RFLP is used to identify the A/G polymorphisms of the *SCN1A* gene (rs2298771) c.3184 ,G/A polymorphisms of the *SCN2A* (rs17183814) c.56 gene and C/T polymorphisms of the *GABRG2* (rs211037,Asn196Asn) gene . Genotypes and allelic frequencies for the *SCN1A*, *SCN2A* and *GABRG2* genes polymorphisms in both groups are compared. The study determines some biochemical tests (glucose, creatinine, potassium) were performed by Reflotron , (sodium, phosphorus, chloride and calcium) by a Spectrophotometer. Results show that the *SCN1A*-A/G gene, *SCN2A*-G/A in both groups were not significantly different. However, these two gene genes responsible for epilepsy and their presence in the febrile seizure may help to stimulate seizures but are not responsible for febrile seizure . But, the number of individuals with the *GABRG2*-C/C genotype in patients with febrile seizures is significantly that's greater compare with control subjects (p=0.0243) and the *GABRG2*-C allele frequency in patients with febrile seizures is significantly which compared with control subjects (p=0.0172). The study showed increase in glucose and phosphorus levels, decrease in sodium and chloride levels, normal value in serum K , Ca and creatinine levels in children with febrile seizure compare with control. It's concluded that there is no relationship between *SCN1A*, *SCN2A* and *GABRG2* genes and some biochemical tests in males and females with febrile seizures and it's suggest that the *GABRG2* gene might be one of the susceptibility factors for febrile seizures .

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Abbreviation

Symbols	Description
VGSCs	Voltage gated sodium channels
FSs	febrile seizures
FC	Febrile Convulsion
NIH	National Institutes of Health
ILAE	International League Against Epilepsy
EEG	Electroencephalography
CNS	central nervous system
GABA	Gamma amino butyric acid
GABR	Gamma amino butyric receptor
GABRG2	Gamma aminobutyric acid receptor genes of γ 2-subunit
$GABA_A$	Gamma amino butyric acid receptor A
$GABA_B$	Gamma amino butyric acid receptor B
$GABA_c$	Gamma amino butyric acid receptor C
E/I	Excitation/Inhibition
PCR	Polymerase chain reaction
RFLP	Restriction fragment length polymorphism
CFS	cerebrospinal fluid
GS	Generalized seizures
AEDs	antiepileptic drugs
HHV6	human herpes virus 6
APs	action potentials
IL-1β	interleukin-1beta
Nav1.1	the α1 voltage-gated sodium channel

Nav1.2	the α2 voltage-gated sodium channel
Nav1.3	the α3 voltage-gated sodium channel
Nav1.4	the α4 voltage-gated sodium channel
Nav1.5	the α5 voltage-gated sodium channel
Nav1.8	the α6 voltage-gated sodium channel
Nav1.7	voltage-gated sodium channel type IV α subunit
α subunit	the major pore forming subunit of the voltage-gated sodium channel
β subunit	the auxiliary subunit of the voltage-gated sodium channel
SNPs	single nucleotide polymorphisms
GEFS+	generalized epilepsy with febrile seizures plus
SMEI	Myoclonic Epilepsy in infancy
TLE	Temporal lobe Epilepsy
PBAS	population-based association studies
Ig	immunoglobulin fold
P-loop	pore loop formed by reentrant loop between S5 and S6 segments
chr.	Chromosome
TTX	Tetrodotoxin , sodium channel pore blocker
TTX-R	Tetrodotoxin resistant
TTX-S	Tetrodotoxin sensitive
S1	transmembrane segment 1
S2	transmembrane segment 2
S3	transmembrane segment 3
S4	transmembrane segment 4
S5	transmembrane segment 5

S6	transmembrane segment 6
FSE	Febrile Status Epilepticus
SCN1A	Sodium voltage - gated channel alpha subunit 1
SCN2A	Sodium voltage - gated channel alpha subunit 2

Appendix A : Questionnaire paper used in the study

s	Name	Age	Sex	Resi.	Birth history		Neonatal jaundice	Trauma	Drug	Metabolic			Cong. abnormalities	Fever	Family History	Age at onset	Type of FS	Notes
					Natural	Birth asphyxia				Hypocalcaemia	Hypoglycemia	others						

Appendix B (1): Results of biochemical tests for febrile seizure cases of different age of females

Age	Glu (120-70) mg/dl	Crea (0.7-1.4) mg/dl	Na (136-145) mmol/l	Cl (95-105) mmol/l	P (1.3-2.3) mmol/l	Ca (2.1-2.5) mmol/l	K (3.6-5.0) mmol/l
36 m	116	0.3	148	114	1.6	1.3	5.7
24 m	69	0.4	143	106	1.2	1.2	4.7
24 m	83	0.3	143	110	1.1	2.6	4.7
60 m	94	0.4	142	110	1.1	2.6	4.6
12 m	87	0.4	136	107	1.9	2.3	4.9
9 m	120	0.4	144	110	1.7	2.4	5
24 m	83	0.4	145	105	1.7	2.2	4.5
60 m	249	0.2	141	105	1.1	2.3	4.4
16 m	80	0.4	145	106	2	2.3	4.1
18 m	106	0.4	137	106	2.8	2.2	3.9
36 m	118	0.4	141	106	2.1	2.7	5.9
17 m	96	0.3	139	98	2.1	2.4	3.9
48 m	211	0.2	139	105	1.7	2.5	4.7
36 m	136	0.3	137	104	2	2.4	3.8
12 m	116	0.3	135	105	1.7	2.3	4.2
36 m	166	0.3	131	105	1.9	2.2	4.2
29 m	92	0.4	131	96	2.1	2.3	4.6
24 m	90	0.2	142	102	2.1	2.7	5.6
17 m	149	0.3	137	102	1.5	2.4	4.1
12 m	96	0.4	136	103	1.6	2.3	5.6
24 m	125	0.2	133	105	1.6	2.3	4.6
10 m	190	0.3	139	110	1.8	2.4	4.8
17 m	136	0.2	147	107	1.7	2.4	5.2
18 m	74	0.4	152	128	1.7	2.6	4.2
17 m	246	0.4	147	123	1.4	2.6	5.1

Appendix B (2): Results of biochemical tests for febrile seizure cases of different age of males

Age	Glu (120-70) mg/dl	Crea (0.7-1.4) mg/dl	Na (135-145) mmol/l	Cl (95-105) mmol/l	P (1.3-2.3) mmol/l	Ca (2.1-2.5) mmol/l	K (3.6-5.0) mmol/l
24 m	119	0.3	149	102	1.5	2.3	4.3
9 m	143	0.4	143	103	1.7	2.3	4.4
28 m	121	0.3	144	115	1.7	2.9	5.5
9 m	81	0.3	144	107	2.4	2.5	3.9
24 m	102	0.4	143	109	1.9	2.5	4.2
12 m	158	0.3	153	121	1.8	2.5	5.1
36 m	89	0.3	141	108	1.4	2.7	4.4
12 m	103	0.3	145	114	1.6	2.6	5.2
48 m	102	0.3	136	96	1.4	2.3	3.5
12 m	99	0.3	138	103	2.5	2.2	4.1
17 m	158	0.4	143	99	1.8	2.6	5.3
36 m	78	0.3	144	105	1.8	2.5	4.5
48 m	194	0.4	143	100	2.1	2.3	5.4
24 m	98	0.3	148	102	1.8	2.5	5.6
53 m	123	0.4	145	103	3.5	2.4	4.1
12 m	95	0.2	143	102	2.5	2.5	4.3
12 m	81	0.2	133	102	1.6	2.3	3.8
12 m	123	0.4	142	101	1.3	2.4	4.4
17 m	82	0.4	139	103	2.1	2.3	4.2
48 m	128	0.4	134	104	1.4	2.4	4.2
41 m	72	0.4	143	102	2.4	2.4	4.1
12 m	152	0.3	130	96	1.8	2.4	3.9
17 m	93	0.4	133	99	1.6	2.5	4.5
6 m	75	0.3	138	98	1.7	2.6	4.1
29 m	117	0.4	134	102	1.4	2.3	3.7

Appendix C (1): Relationship between *SCN1A* , *SCN2A* and *GABRG2* genes polymorphism and Biochemical tests for males

GABRG2	SCN2A	SCN1A	Type of fs	Recurrent Seizure	First with fs	Family history with epilepsy	Family history with fs	Na	Cl	P	Ca	K	Crea	Glu	Age in month	sex	Patients sequenc
CT	GG	AG	Simple		+		-	138	98	1.7	2.6	4.1	0.3	75	6	M	p1
CC	AA	AG	Simple		+		+	143	103	1.7	2.3	4.4	0.4	143	9	M	P2
CC	GA	AG	Simple	+			-	144	107	2.4	2.5	3.9	0.3	81	9	M	P3
CC	AA	AG	Simple		+		+	153	121	1.8	2.5	5.1	0.3	158	12	M	P4
CC	GA	AA	Simple	+			+	133	102	1.6	2.3	3.8	0.2	81	12	M	P5
CC	AA	GG	Simple		+		-	142	101	1.3	2.4	4.4	0.4	123	12	M	P6
CT	AA	GG	Simple		+	+		138	103	2.5	2.2	4.1	0.3	99	12	M	P7
CC	GG	AG	Simple	+			+	143	102	2.5	2.5	4.3	0.2	95	12	M	P8
CC	AA	AA	Simple		+		-	130	96	1.8	2.4	3.9	0.3	152	12	M	P9
CT	GG	AG	Simple	+			-	145	114	1.6	2.6	5.2	0.3	103	12	M	P10
CC	AA	AG	Simple		+		+	143	99	1.8	2.6	5.3	0.4	158	17	M	P11
CC	AA	AA	Simple		+		-	139	103	2.1	2.3	4.2	0.4	82	17	M	P12
CC	AA	AG	Simple		+		+	133	99	1.6	2.5	4.5	0.4	93	17	M	P13
CT	GA	GG	simple		+		+	149	102	1.5	2.3	4.3	0.3	119	19	M	P14
TT	GA	GG	Simple		+		+	143	109	1.9	2.5	4.2	0.4	102	24	M	P15
CC	AA	AG	Simple		+		-	148	102	1.8	2.5	5.6	0.3	98	24	M	P16
CC	GA	AA	Simple	+			+	144	115	1.7	2.9	5.5	0.3	121	29	M	P17
TT	GA	AG	Simple		+		+	134	102	1.4	2.3	3.7	0.4	117	29	M	P18
CC	AA	GG	Simple		+		+	141	108	1.4	2.7	4.4	0.3	89	36	M	P19
CT	GA	GG	Simple		+		-	144	105	1.8	2.5	4.5	0.3	78	36	M	P20
TT	GA	AG	complex		+		-	143	102	2.4	2.4	4.1	0.4	72	41	M	P21
CC	GA	AA	Simple	+			+	136	96	1.4	2.3	3.5	0.3	102	48	M	P22
TT	GA	AG	Simple	+			-	143	100	2.1	2.3	5.4	0.4	194	48	M	P23
CC	GG	GG	Simple		+		+	134	104	1.4	2.4	4.2	0.4	128	48	M	P24
TT	GG	GG	Simple		+		+	145	103	3.5	2.4	4.1	0.4	123	53	M	P25

Appendix C (2): Relationship between *SCN1A* , *SCN2A* and *GABRG2* genes polymorphism and Biochemical tests for females

GABRG2	SCN2A	SCN1A	Type of fs	Recurrent Seizure	First with fs	Family history with epilepsy	Family history with fs	Na	Cl	P	Ca	K	Crea	Glu	Age in month	sex	Patients sequenc
CC	AA	AG	Simple	+			-	144	110	1.7	2.4	5	0.4	120	9	F	P1
CC	GG	GG	Simple		+		+	139	110	1.8	2.4	4.8	0.3	190	10	F	P2
TT	AA	AG	Simple		+		-	136	107	1.9	2.3	4.9	0.4	87	12	F	P3
CC	GA	AG	Simple		+		-	136	103	1.6	2.3	5.6	0.4	96	12	F	P4
CC	AA	AG	Simple	+			-	135	105	1.7	2.3	4.2	0.3	116	12	F	P5
CT	GA	GG	Simple	+			-	145	106	2	2.3	4.1	0.4	80	17	F	P6
CC	GG	GG	Simple		+		+	137	106	2.8	2.2	3.9	0.4	106	17	F	P7
TT	GA	AA	Simple		+		+	139	98	2.1	2.4	3.9	0.3	96	17	F	P8
CC	GG	AG	complex		+		+	147	107	1.7	2.4	5.2	0.2	136	17	F	P9
CC	AA	AG	Simple		+		+	147	123	1.4	2.6	5.1	0.4	246	17	F	P10
TT	GA	AA	Simple		+		+	137	102	1.5	2.4	4.1	0.3	149	17	F	P11
CC	GA	AG	Simple	+			-	152	128	1.7	2.6	4.2	0.4	74	18	F	P12
TT	GA	AA	Simple		+		-	145	105	1.7	2.2	4.5	0.4	83	24	F	P13
CC	GA	GG	Simple	+			-	143	106	1.2	1.2	4.7	0.4	69	24	F	P14
CC	AA	AA	Simple	+			+	142	102	2.1	2.7	5.6	0.2	90	24	F	P15
TT	GA	AG	Simple		+		+	133	105	1.6	2.3	4.6	0.2	125	24	F	P16
CT	AA	AG	Simple	+			-	143	110	1.1	2.6	4.7	0.3	83	24	F	P17
CT	GG	AG	Simple		+		+	131	96	2.1	2.3	4.6	0.4	92	29	F	P18
CC	GA	AG	Simple	+			+	141	106	2.1	2.7	5.9	0.4	118	36	F	P19
CT	GA	AA	Simple	+			-	131	105	1.9	2.2	4.2	0.3	166	36	F	P20
CT	GA	GG	Simple	+			+	148	114	1.6	1.3	5.7	0.3	116	36	F	P21
CC	GA	GG	Simple	+			+	137	104	2	2.4	3.8	0.3	136	36	F	P22
TT	GA	GG	Simple	+			-	139	105	1.7	2.5	4.7	0.2	211	48	F	P23
CC	AA	AG	Simple		+		+	141	105	1.1	2.3	4.4	0.2	249	60	F	P24
TT	AA	AG	Simple		+		-	142	110	1.1	2.6	4.6	0.4	94	72	F	P25

5.1 Conclusions

1. During febrile seizures mild disturbances electrolyte balance occur frequently. It has been suggested that changes in electrolyte balance , might predispose a child to convulsions during febrile illness .The weakness of the association between serum electrolytes changes and incident FSs suggests that alteration in serum electrolytes is unlikely to play a clinically significant role in causing seizures in patients with FSs. Therefore, routine measurement of serum electrolytes is not warranted in this group of seizure disorders .
2. The study suggests that the *GABRG2* gene might be one of the susceptibility factors for FSs.
3. No association is found between *SCN1A* and *SCN2A* genes polymorphisms in children with FSs.
4. We found that its no relationship between *SCN1A* , *SCN2A* and *GABRG2* genes and some biochemical tests in males and females with febrile seizures.

5.2 Recommendation

1. Preferably prospective studies with larger sample size are needed to clarify the role of *SCN1A* and *SCN2A* variants in the genetic susceptibility of FS as well as that of epilepsy in general.
2. More studies can looking on the analysis of GABRG2 RNA and protein in children with FSs and this study may support the basis for addition survey of *GABRG2* polymorphism .
3. To investigate relationship between *GABRG2* gene polymorphism and drug responsiveness in FSs patients .
4. Although most of the FSs are self-limiting, the definition of the basis of genetic predisposition for the development of recurrent FSs with poor prognosis, febrile status epilepticus, or epileptic syndromes following infantile FS will represent an important future target for researchers because of their important clinical implications.
5. Advanced molecular studies , such as gene sequencing , can be more effective in the detection of genes in FSs .

4.1 Distribution of patients according to demographic characteristics

The study included 50 patients with febrile convulsion FC (febrile seizure FS) , 48 (96 %) patients with Simple FS and 2 (4 %) patients with Complex FS , their age ranged from 5 to 72 months , (25 males and 25 females) . This results agree with the study of Khoshdel *et al*, (2012) who found 85% of the cases had simple febrile seizures , and 50 healthy control subjects whose characteristics are listed in table (4-1) . The control group were age and gender matched to children with FS.

The most affected age group corresponding to 13 to 36 months with 27 (54%) cases " 10 males (37.03 %) , 17 females (62.96 %)" , followed by the group of 5 to 12 months with fifteen (30%) cases, ending with the group of 37 to 72 months eight (16%), results agree with study by Alwan and Hussein , (2013) showed that an age of 12-24 months at initial seizure are associated with high risk group of recurrence of FSs . This finding is not consistent with the finding of Offringa *et al.* ,(1992) who found that the age below 30 months are associated with low risk of recurrence .

Twenty seven (54%) patients "fourteen males (54.5 %) , thirteen females (45.4 %)" had a family history of febrile seizures, twenty two (44%) patients "ten males (44.4 %) , twelve females (55.5 %)" without family history and one (2%) patient " one males (100%) " had a family history of epilepsy , this is inagreement with Millar ,(2006) and Herose *et al*., (2003) . The genetic component for febrile seizures is manifested by its positive relation with family history of febrile and afebrile seizures documented in several studies. The study by Choueiri *et al.*, (2001) showed that patients with febrile seizures were more likely to have a family history of febrile seizures (20%) than patients with epilepsy (1.1%). They did not have an increased frequency of consanguinity which favored an autosomal dominant rather than recessive inheritance.

A total of 30 (60%) " 17 (56.6%) males and 13(43.3%) females" of patients have a first FS seizures. A total of 20 (40 %)" 8(40 %) males and 12 (60 %) of patients had recurrent seizures . This result agree with study by Berg et al., (1997) as they are found in a total of 136 children (31.8%) experienced recurrent seizures: 73 (17.1%) have only 1 recurrence, 38 (8.9%) had 2 recurrences, and 25 (5.8%) had 3 or more recurrences .

Febrile convulsion is one of the most common seizure disturbances in children with an approximate rate of (3 - 5) % that associated with fever as high as 38.5ºC in children 6 months to 5 years without any infection within the central nervous system (CNS) or other factors explaining its incident. The pathogenesis of FSs remains obscure. Possible causes include viral infection of the CNS (Naoh and Afify , 2014) , lowered threshold for seizures in the presence of fever (Schuchmann *et al.* , 2006) and changes of neurotransmitter and trace elements in the biological fluids (Amiri *et al.* , 2010)**.** In fact, FSs of children may involve a complex interaction between the immune-inflammatory process, cytokine activation, and genetic factors (Naoh and Afify, 2014)**.**

Table 4.1 Distribution of patients according to demographic characteristics

Variable	Males (%)	Females (%)	Total (%)
Age in months			
(5-12)	(66.6)	(33.3)	(30.0)
(13-36)	(37.03)	((62.96)	(54.0)
(37-72)	(62.5)	(37.5)	(16.0)
Children presenting with a first FS	(56.6)	(43.3)	(60.0)
Children presenting with a recurrent seizure	(40.0)	(60.0)	(40.0)
family history with FS	(54.5)	(45.4)	(54.0)
family history without FS	(44.4)	(55.5)	(44.0)
family history with epilepsy	(100.0)	(0.0)	(2.0)
Type of Fs:			
Simple	(50.0)	(50.0)	(96.0)
Complex	(50.0)	(50.0)	(4.0)

4.2 The biochemical evaluations

Febrile Seizures are common in childhood and many physicians do extend the investigation for these benign events. There are some reports that suggest no routine laboratory examinations i.e., serum electrolytes are needed for patients with simple FSs (Afsharkhas and Tavasoli ,2014). We are made a predictive model to evaluated the probability of normal biochemical blood test results in children presenting with a seizure correlated with fever. The model were based on different collection of patient characteristics of the history and physical examination of 50 children (25 males , 25 females). The characteristics included gender, age, preceding history of febrile seizures, family history of febrile seizures and family history with epilepsy , Appendix A . We have measured the number of chemical tests "calcium, chloride , potassium , phosphorus ,sodium ,creatinine and glucose" in children with seizures correlated with fever , Appendix B (1), Appendix B (2) , addition to clinical data available from the history and physical examination of the patient . Of all 50 children with FSs and 50 children without FSs served as control whose underwent to all the tests , the resulting :

The mean values of Serum potassium in FS cases and controls were 4.55 ± 0.03 and 4.60 ± 0.02 respectively , mean values of Serum calcium in FS cases and controls were 2.38 ± 0.02 and 2.31 ± 0.01 respectively , mean values of Serum creatinine in FS cases and controls were 0.36 ± 0.001 and 0.34 ± 0.001 respectively.

In our study, a few patients had abnormal level of serum potassium, calcium levels but there were no significant differences between patients and control , table (4-2) . and there were no abnormalities in serum creatinine level. Similar to our Study Afsharkhas and Tavasoli , (2014) detected that among 291 patients with FSs, there were hypokalemia, and hypocalcemia in 4 and 16 cases, respectively and there were no abnormalities in serum creatinine level ,but there were no significant differences between patients with simple, complex, and recurrent febrile convulsions during study in renal Function in Children with Febrile Convulsions .

And agree with study done by Nickavar *et al.,(*2009) where 175 children were enrolled with a mean age group of 23 months were divided into three groups.1st with simple febrile seizures, 2nd with recurrent febrile seizures and the 3rd, as control. Serum calcium levels were 9.17, 8.97 and 9.32 respectively, with no difference of serum calcium levels in all three groups with an insignificant P value similar to

present study . Serum electrolyte values may be abnormal in children after a febrile seizure, but this should be manifested by physical examination and history taking (Mikati and Rahi , 2005).

The mean values of Serum Glucose in FS cases and controls were 118.28 ± 1.02 and 95.58 ± 0.79 respectively with a $L.S.D_{0.05}$ value being 8.444 , it is statistically higher significant ,table (4-2) . It's agreement with studies by Kiviranta *et al.*, (1995) who studied the effects of convulsion and fever on the CSF and blood glucose concentrations in four different groups of children: febrile and non-febrile children, with and without convulsions . They found the concentration of glucose in the blood was significantly higher in febrile children . Their results show that hyperglycaemia in febrile convulsions is not explained just by a stress reaction, evoked by the seizure, as it has been hypothesized earlier, but by the influence of increased body temperature as well . Hyperglycaemia is a common phenomenon associated with febrile convulsion , but It is important to recognize this phenomenon to prevent an incorrect diagnosis of diabetes mellitus. and inappropriate treatment with insulin . A blood glucose determination, although not routinely needed, should be obtained if the child has a prolonged period of postictal obtundation .

The mean values of Serum sodium in FSs cases and controls were 140.76 ± 1.25 and 147.32 ± 1.19 respectively with a $L.S.D_{0.05}$ value being 1.082 , it is significant lower compared with control ,table (4-2) . This results agreement with Hugen et al. (1995) in a prospective study of 69 children with febrile convulsions, serum sodium levels were often lower than normal (52% had levels < 135 mmol/l) . Fever plays an important role to cause disturbances in fluid and electrolyte balance. Hyponatraemia has been thought to enhance the susceptibility to seizures associated with febrile illnesses in childhood. The lower the serum sodium level , the higher the probability of a repeat convulsion.

The mean values of Serum phosphorus in FS cases and controls were 1.80 ± 0.02 and 1.55 ± 0.01 respectively with a $L.S.D_{0.05}$ value being 0.177, , it is significant higher compared with control , table (4-2) , we not found any reference .
In our study the mean values of Serum Chloride in FSs cases and controls are 105.48 ± 0.95 and 112.76 ± 1.00 respectively with a $L.S.D_{0.05}$ value being 2.709, it is significant lower compared with control ,table (4-2) ,it isn't found any reference . During acute febrile diseases mild disturbances of water and electrolyte balance occur

frequently. It has been suggested that changes in electrolyte balance , might predispose a child to convulsions during febrile illness (Kiviranta *et al.* , 1996). The weakness of the association between serum electrolytes changes and incident FSs suggests that alteration in serum electrolytes is unlikely to play a clinically significant role in causing seizures in patients with FSs. Therefore, routine measurement of serum electrolytes is not warranted in this group of seizure disorders (Nickavar *et al.* , 2009) .

Table 4.2 The average results of chemical tests for patients with febrile seizure and control (means± standard error)

Biochemical tests	Febrile seizure (means± SE)	Control (means± SE)
Glu (120-70) mg/dl	118.28 ± 1.02 a	95.58 ± 0.79 b
L.S.D$_{0.05}$	8.444	
Crea (0.7-1.4) mg/dl	0.36 ± 0.001	0.34 ± 0.001
L.S.D$_{0.05}$	N.S	
K (3.6-5.0) mmol/l	4.55 ± 0.03	4.60 ± 0.02
L.S.D$_{0.05}$	N.S	
Ca (2.1-2.5) mmol/l	2.38 ± 0.02	2.31 ± 0.01
L.S.D$_{0.05}$	N.S	
P (1.3-2.3) mmol/l	1.80 ± 0.02 a	1.55 ± 0.01 b
L.S.D$_{0.05}$	0.177	
Cl (95-105) mmol/l	105.48 ± 0.95 b	112.76 ± 1.00 a
L.S.D$_{0.05}$	2.709	
Na (135-145) mmol/l	140.76 ± 1.25 b	147.32 ± 1.19 a
L.S.D$_{0.05}$	1.082	

[a,b] Means within columns with no common superscript differ significantly (p<0.05) .N.S: non - significant

4.3 Genetic Study:

4.3.1 Genetic Variation of *SCN1A* , *SCN2A* and *GABRG2* genes

The Genomic DNA extraction to polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) assay was detected on gel electrophoresis. All these steps were performed for analysis of genetic variation of *SCN1A* , *SCN2A* and *GABRG2* genes.

4.3.2 Genomic DNA Extraction:

The genomic DNA is extracted from whole blood samples to analysis the *SCN1A* , *SCN2A* and *GABRG2* genotypes for patients with FS and control , Fig. (4-1) .

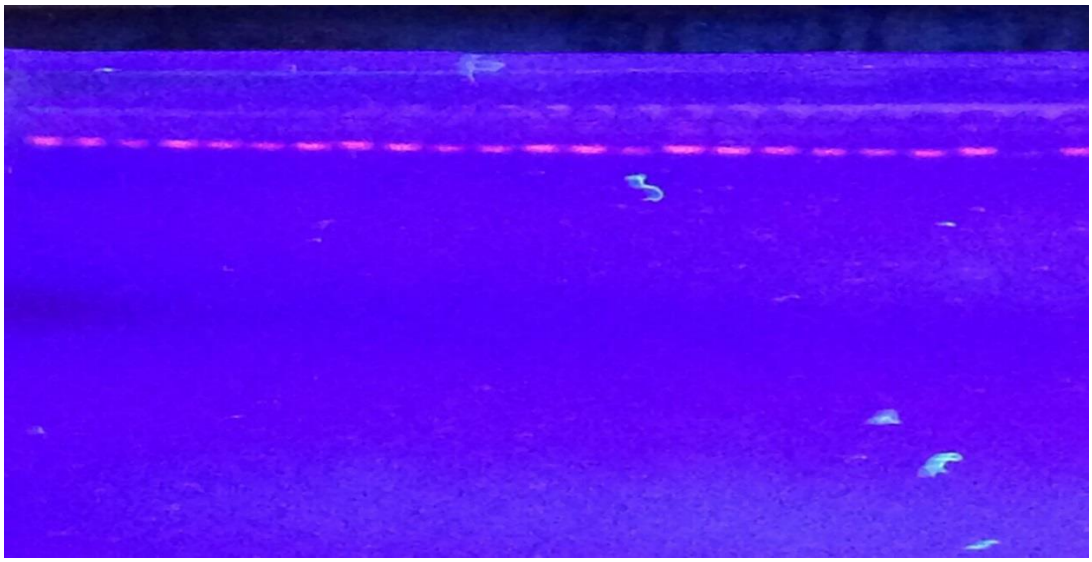


Figure 4.1 Ethidium Bromide-Stained of genomic DNA extraction for patients with FS . On (1%) agarose gel at 100 voltage for 40 min .

4.3.3 DNA Amplification

4.3.3.1 voltage-gated sodium channel α-subunit type I *(SCN1A)*

The products of successful binding between the extracted DNA and specific primers for *SCN1A* gene were detected by gel electrophoresis analysis using DNA marker and the products size was 336 bp for both patients and control groups, and the result was positive for two group , Fig. (4-2).

Figure 4.2 Ethidium Bromide-Stained of PCR Amplified 336 bp of *SCN1A* gene. Lane (M):DNA molecular size marker , Lane (1-8) for FS patients, Lane (9-10) for control group. On (1%) agarose gel at 100 voltage for 20 min then 50 voltage for 60 min .

4.3.3.2 Voltage-gated sodium channel α-subunit type II *(SCN2A)*

The products of successful binding between the extracted DNA and specific primers for *SCN2A* gene were detected by gel electrophoresis analysis using DNA marker and the products size was 400 bp for both patients and control groups, the result was positive for two group , Fig. (4 -3).

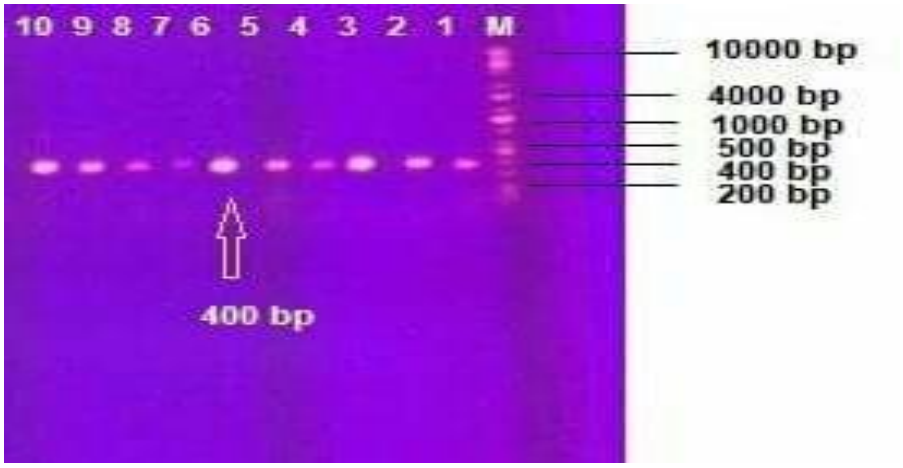


Figure 4.3 Ethidium Bromide-Stained of PCR Amplified 400 bp of *SCN2A* gene. Lane (M): DNA molecular size marker , Lane (1-8) for FS patients, Lane(9-10) for control group. On (1%) agarose gel at 100 voltage for 20 min then 50 voltage for 60 min .

4.3.3.3 Gamma-aminobutyric acid type A receptor gamma2 subunit (*GABRG2*)

The products of successful binding between the extracted DNA and specific primers for *GABRG2* gene are detected by gel electrophoresis analysis using DNA marker and the products size is 122 bp for both patients and control groups, the result was positive for two group , Fig. (4- 4).

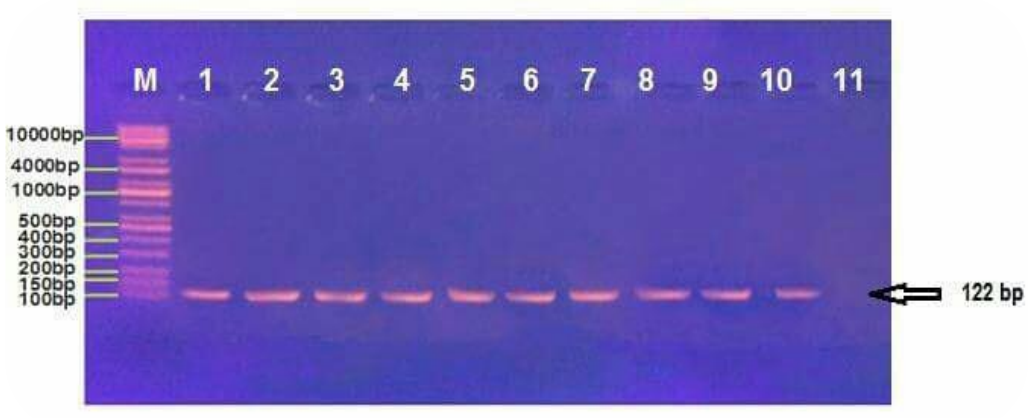


Figure 4.4 Ethidium Bromide-Stained of PCR Amplified 122 bp of *GABRG2* gene. Lane (M): DNA molecular size marker , Lane (1-8) for FS patients, Lane (9-10) for control group. On (1%) agarose gel at 100 voltage for 20 min then 50 voltage for 60 min .

4.4 Detection of Gene Polymorphisms

4.4.1 Detection of *SCN1A* A→G Polymorphisms :

Genotyping is performed using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique . After amplification , PCR products are digested using specific restriction Endonucleases . The *SCN1A* c.3184 A→G polymorphism is identified by loss of the *Pvu*II restriction site AA (168 bp) , AG (168 /145 bp) and GG (145 bp) , However, < 100 bp fragments are not seen on the gel . The PCR products are directly analyzed on 1% agarose gel by electrophoresis, and each allele is recognized according to its size. Allelic frequencies were expressed as a percentage of the total number of alleles, fig.(4 - 5 ,(4 - 6) . This results agree with the reports of other studies such as the study of Lakhan *et al.* ,(2009) .

Figure 4.5 Ethidium Bromide-Stained Agarose Gel of PCR – RFLP Amplified 336 bp of SCN1A Gene for febrile seizure (females) group . Lane (M): DNA molecular size marker , lane (8,11) = Homozygous AA genotype (168bp), Lane(1,3,4,59,10,12)= Heterozygous AG genotype (168,145bp), Lane (2,6,7) = Homozygous GG genotype (145 bp). On 1% agarose gel at 100 voltages for 10 min then at 50 voltages 45 min .

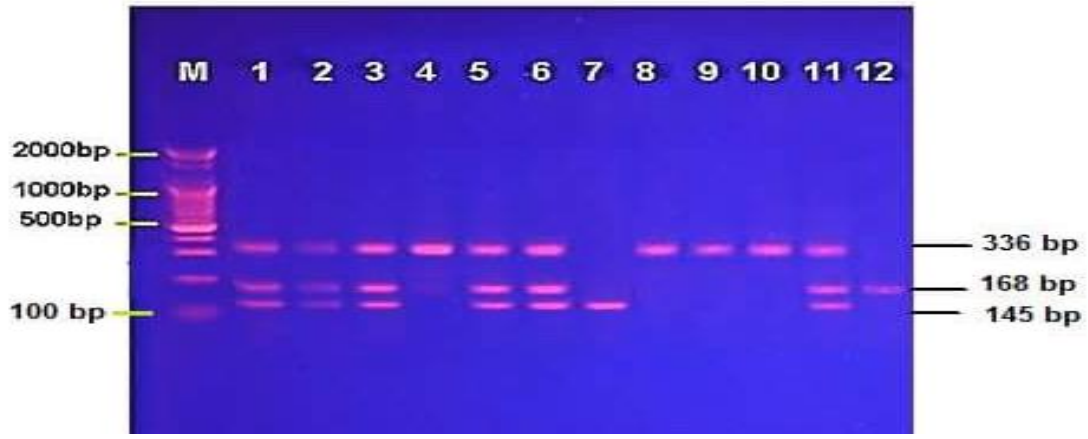


Figure 4.6 Ethidium Bromide-Stained Agarose Gel of PCR – RFLP Amplified 336 bp of SCN1A Gene for febrile seizure (males) group. Lane (M): DNA molecular size marker , Lane (12) = Homozygous AA genotype (168bp), Lane(1,2,3,5,6,11)= Heterozygous AG genotype (168,145bp) , Lane (7) = Homozygous GG genotype (145 bp). On 1% agarose gel at 100voltages for 10 min then at 50 voltages 45 min.

The genotype proportions and allele frequencies for *SCN1A* c.3184 A→G in both patient with FS and control were not significantly different , table (4-3) . The most common genotype for *SCN1A* c.3184 A→G gene in FS is A/G heterozygote . Proportions of A homozygote, A/G heterozygote, and G homozygote for *SCN1A* c.3184 A→G are as follows: in patients with fs , 20 % , 50 %, and 30 %, respectively; and in control , 24 %, 62 %, and 14 %, respectively , The allele A and G frequencies for *SCN1A* c.3184 A→G in patients with FS is 45% and 55%, respectively; and in control 55 % and 45%, respectively , (table 4-3).

The frequency of AG genotype of *SCN1A* c.3184 A→G polymorphism was not significantly in patients with FS *vs.* healthy controls. Our results agreement with study by Chou *et al.* ,(2003) as they are found that genotypes and allelic frequencies for the SCN1A gene polymorphisms in 104 Taiwanese children with FSs and 83 normal control were not significantly different , And a agreement with study by Zhang *et al.* ,(2010) , they are found that there was no statistically significant difference in either allele or genotype frequency of any of the SNPs of *SCN1A* studied between epilepsy patients with and without FS, and between epilepsy patients with FS and controls . A recent study in Caucasians found an association between the single nucleotide polymorphism (SNP) of SCN1A, IVS5N +5 G>A (rs3812718), and febrile seizures (FS) (Zhang *et al.* ,2010). Because SCN1A mutations are recognized as the most common cause of the rare mendelian syndrome of generalized epilepsy with FS plus (GEFS+) (Mulley *et al.*, 2005), the sodium channel gene is considered a

potential candidate for the more common, presumably polygenic, forms of FS. Specifically, the single nucleotide polymorphism (SNP) of SCN1A, IVS5N +5 G>A (rs3812718), is believed to be a candidate functional variant because it alters the proportions of the neonatal and adult transcripts of the gene (Heinzen *et al.*, 2007)

Recent studies provided evidence that mutations in *SCN1A* represent the most frequent cause of generalized epilepsy with febrile seizures plus an autosomal-dominant epilepsy syndrome. *SCN1A* mutations alter channel inactivation, resulting in persistent inward sodium current. It is unknown if polymorphisms in those genes involved in familial epilepsies also contribute to the pathogenesis of FSs. These suggest that the *SCN1A* gene might not be one of the susceptibility factors for FSs. Pure FSs and febrile convulsions associated with idiopathic generalized epilepsy may not share a common genetic etiology. Initiation and propagation of seizures is due to misfiring of neurons in the brain, and >300 mutations in *SCN1A* gene have thus far been identified in epilepsy and other neurological disorders (Lossin , 2008) . Lakhen *et al.* , (2009) found an association of *SCN1A* c.3184 A→G polymorphism with overall susceptibility to epilepsy.

Table 3.4 Genotypes and allele frequencies of *SCN1A* c.3184 A/G Polymorphisms in children with febrile seizures and normal control subjects

	Control No. (%) (n.=50)	febrile seizure No. (%) (n.=50)	P value*
Genotype			
AA	12 (24.00)	10 (20.00)	0.0112
AG	31(62.00)	25 (50.00)	
GG	7 (14.00)	15 (30.00)	
Allelic frequency**			
A	55 (55.00)	45 (45.00)	0.00792
G	45(45.00)	55 (55.00)	

* P- value were calculated by X2 test .

** Allelic frequency of A = AA + 1/2 AG

Allelic frequency of G = GG + 1/2 AG

4.4.2 Detection of *SCN2A* c.56 G→A Polymorphisms:

Genotyping is performed using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique .After amplification, PCR products are digested using specific restriction Endonucleases . The *SCN2A* c.56 G→A polymorphism was identified by loss of the *Scr*FI restriction site (G allele 178, 130, 64 and 28 bp ;and A allele 206,130 and 64 bp) . However, < 100 bp fragments are not seen on the gel . The PCR products are directly analyzed on 1% agarose gel by electrophoresis, and each allele is recognized according to its size. Allelic frequencies were expressed as a percentage of the total number of alleles , Fig.(4 -7) , (4- 8). these results agree with the reports of other studies such as the study of Lakhan *et al.*, (2009) .

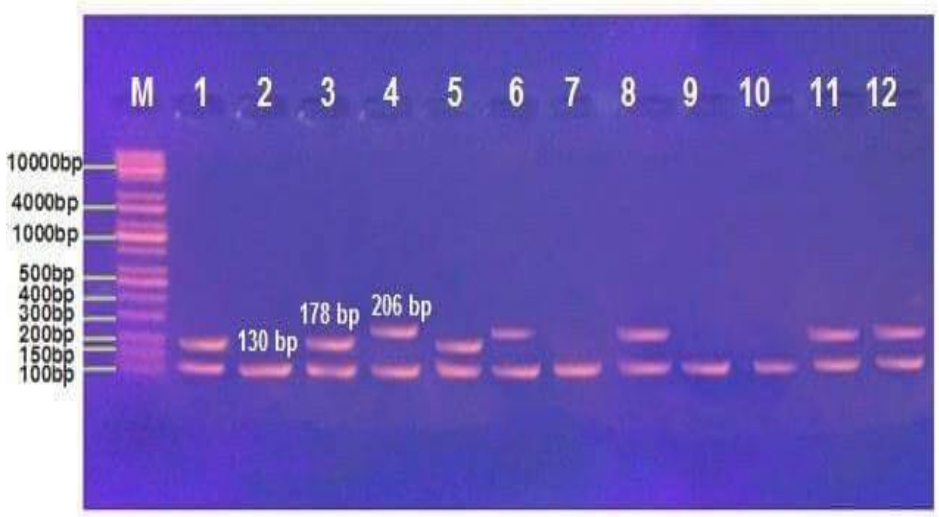


Figure 4.7 Ethidium Bromide-Stained Agarose Gel of PCR – RFLP Amplified 400 bp of *SCN2A* Gene for febrile seizure (females) group . Lane (M): DNA molecular size marker , Lane(1,3,5) = Homozygous GG genotype (178 bp), Lane (4,6,8,11,12) = Heterozygous GA genotype (206 , 178 bp), lane (2,9,10) = Homozygous AA genotype (130 bp). On 1% agarose gel at 100 voltages for 10 min then at 50 voltages 45 min .

Figure 4.8 Ethidium Bromide-Stained Agarose Gel of PCR – RFLP Amplified 400 bp of *SCN2A* Gene for febrile seizure (males) group. Lane (M): DNA molecular size marker , Lane(1,8,10) = Homozygous GG genotype (178 bp), Lane (2,3,4,5,6,7,9) = Heterozygous GA genotype (206/178 bp) . On 1% agarose gel at 50 voltages for 10 min then at 100 voltages 45 min.

4.4.3 Distribution of *SCN2A* c.56 G→A polymorphisms; genotype and allele frequencies in febrile seizure patients *vs.* healthy controls

The genotype proportions and allele frequencies for *SCN2A c.*56 G→A in both patient with FS and control are not significantly different. The most common genotype for *SCN2A c.*56 G→A gene in patient with FS is AG heterozygote. Proportions of A homozygote, A/G heterozygote, and G homozygote for *SCN2A c.*56 G→A are as follows: in patients with FS , 36 %, 46 %, and 18%, respectively; and in control , 24 %, 56 %, and 20 %, respectively. The allele A and G frequencies for *SCN2A c.*56 G→A in patients with FSs is 59 % and 41 %, respectively; and in control , 52 % and 48 % respectively , table 4-4.

we observed no significant differences in genotypic in patients with FSs and control, but we observed higher significant in allelic frequency between the patients with FSs and healthy control for the *SCN2A c.*56 G/A gene polymorphism ,table (4-4). Results agree with a study by Nakayama *et al.* ,(2002) as they are found there are no significant differences in genotype or allele frequencies of the R19K polymorphism between 93 Japanese patients with FS, 35 Japanese patients with FS associated with afebrile seizures including GEFS+, and 100 control subjects, so they are failed to provide evidence supporting a causal relation between the *SCN2A*

mutation /polymorphism and FS or FS associated with afebrile seizures including GEFS+ in the Japanese population. As the causal relation between *SCN2A* 56 G/A , *SCN1A* c.3184 A/G polymorphisms and FS associated with afebrile seizures or idiopathic generalized epilepsy has not been proven genetically, identification or confirmation of the association in different populations would be important in establishing a role for the *SCN1A* and *SCN2A* gene in the development of seizures (Lakhan *et al.* 2009).

Table 4.4 Genotype and allele frequencies of *SCN2A* c.56 G/A Polymorphisms in children with febrile seizure patients and control

	Control No. (%) (n.=50)	febrile seizure No. (%) (n.=50)	P value*
Genotype			
AA	12 (24.00)	18 (36.00)	
AG	28 (56.00)	23(46.00)	0.0122
GG	10 (20.00)	9 (18.00)	
Allelic			
frequency**	52 (52.00)	59 (59.00)	0.00752
A	48 (48.00)	41 (41.00)	
G			

* P- value were calculated by X2 test .

** Allelic frequency of A = AA + 1/2 AG

Allelic frequency of G = GG + 1/2 AG

4.4.4 Detection of *GABRG2* (SNP211037, Asn196Asn) C/T Polymorphisms:

Genotyping is performed using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique . After amplification, PCR products are digested using specific restriction Endonucleases . *GABRG2* (SNP211037, Asn196Asn) was identified by loss of the *Apo*I restriction site CC (102 bp) , CT (102,122 bp) and TT (122 bp). The PCR products are directly analyzed on 1% agarose gel by electrophoresis, and each allele is recognized according to its size.

Allelic frequencies were expressed as a percentage of the total number of alleles. The nucleotide change from C to T at 588 position in the *GABRG2* gene creates a restriction site for the *Apo*I restriction enzyme . The wildtype allele of *GABRG2* is C, and the mutant is T. The C588T substitution generates a *Apo*I restriction recognition sequence, and restriction digestion gives fragments of the following sizes: 122+ 102+ 20 bp (C allele) and 102 + 20 bp (T allele), Fig. (4-9) , (4-10) . However, < 100 bp fragments are not seen on the gel . These results agree with the reports of other studies such as the study of Chou *et al.*, (2003).

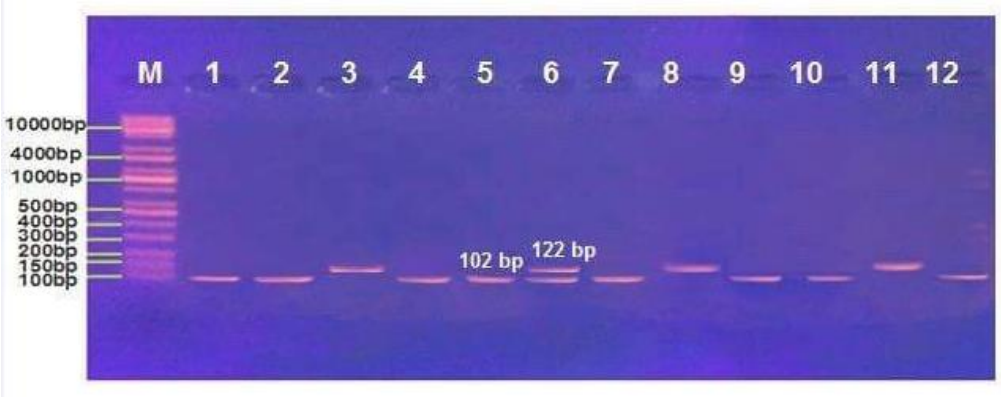


Figure 4.9 Ethidium Bromide-Stained Agarose Gel of PCR – RFLP Amplified 122 bp of GABRG2 Gene for febrile seizure (females) group . Lane (M): DNA molecular size marker , lane (1,2,4,57,9,10,12) = Homozygous CC genotype (102 bp) , Lane (6) = Heterozygous CT genotype (102/122 bp), Lane(3,8,11) = Homozygous TT genotype (122 bp) . On 1% agarose gel at 100 voltages for 10 min then at 50 voltages 45 min.

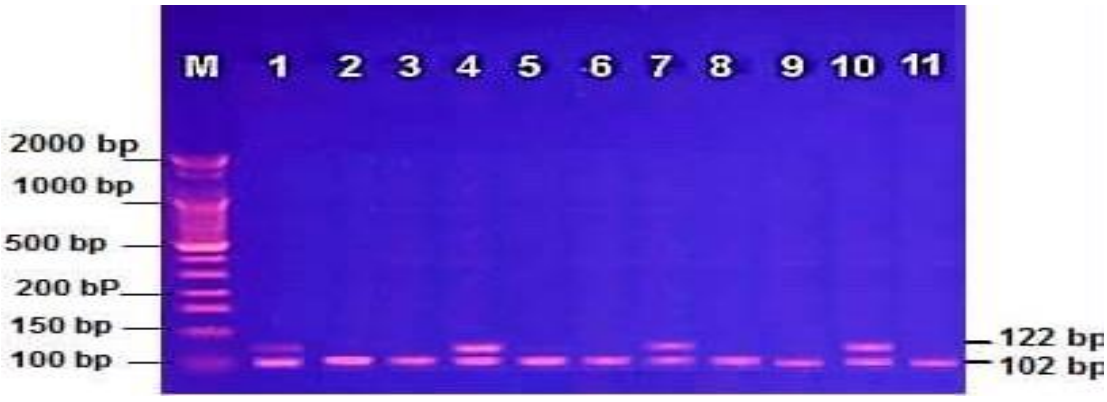


Figure 4.10 Ethidium Bromide-Stained Agarose Gel of PCR – RFLP Amplified 122 bp of GABRG2 Gene for febrile seizure (males) group . Lane (M): DNA molecular size marker , lane (2,35,6,8,9,11) = Homozygous CC genotype (102 bp) , Lane (1,4,7,10) = Heterozygous CT genotype (102/122 bp) . On 1% agarose gel at 100 voltages for 10 min then at 50 voltages 45 min.

4.4.5 Distribution of *GABRG2* (SNP211037, Asn196Asn) C→T polymorphisms ; genotype and allele frequencies in febrile seizure patients *vs.* healthy controls

The genotype proportions and allele frequencies for *GABRG2* (SNP211037) in both patient with FSs and control are significantly different , (table 4-5). The most common genotype for *GABRG2* (SNP211037) gene in patient is C homozygote. Proportions of C homozygote, C/T heterozygote, and T homozygote for *GABRG2* (SNP211037) were as follows: in patients with FS , 56 %, 20 %, and 24 %, respectively; and in control , 52 %, 30 %, and 18 %, respectively. The allele C and T frequencies for *GABRG*2 (SNP211037) in patients with FS is 66 % and 34 %, respectively; and in control , 65 % and 35 %, respectively . It is observed significant differences in genotypic in patients with FSs more than control group , but it is observed higher significant in allelic frequency between the patients with FS and healthy controls for the *GABRG2* (SNP211037, Asn196Asn) C/T Polymorphism ,table (4-5).

Results agree with study of Chou *et al.* (2003) as they are found that FSs are associated with the *GABRG2* (SNP211037)- C allele had a higher incidence of febrile seizures. And agree with study by Abdel Salam *et al.* ,(2012) as they are found that the frequency of CC genotype of *GABRG2* gene is significantly higher in children with simple FS compared to healthy children ($p \leq 0.0001$) . This evidence indicates that the *GABRG2* (SNP211037)-C allele is a candidate genetic marker for FSs.

FSs are an age-specific disease, and remit spontaneously without treatment . *GABRG2* (SNP211037) gene cluster on 5q33 chromosome in children with FSs. Developmental changes in GABR *per se* have been well characterized (Ganguly *et al.* , 2001) . In the adult CNS, GABA is the primary inhibitory neurotransmitter. Early in development, however, GABAergic synaptic transmission is excitatory and can exert widespread trophic effects. During the postnatal period, GABAergic responses undergo a switch from being excitatory to inhibitory. The decreased seizure susceptibility of the mature brain may be related to postnatal segregation of GABA-sensitive networks (Moshe *et al.*, 1994) . Some studies previously demonstrated that the GABA concentration in the cerebrospinal fluid of children with recurrent FSs is lower than that in control subjects and suggested that an immature GABAergic system underlies FSs (Knight *et al.* , 1985) .

The threshold for FSs is considered to depend on the activity of the GABAergic system; low activity of the GABAergic system allows FSs to occur easily. Hyperthermia-induced seizures in experimental animals have been used to study the mechanism of FSs , and glutamate is known to play an important role in the induction of hyperthermia-induced seizures . Arias et al. (1992) report that glutamate decarboxylase activity is suppressed by hyperthermia in newborn rats (Arias et al. , 1992). The susceptibility to hyperthermia-induced seizures is higher in developing than in adult animals, similar to the case of human FSs (Morimoto *et al.* , 1995).

The GABAergic system in developing animals is immature comparison to the excitatory system . The genetic susceptibility to FSs seems to involve multiple genes in most instances . Additionally, a mutation in the *GABRG2* gene has been identified in individuals with FSs either with or without childhood absence epilepsy . In the polygenic inheritance of the FSs, therefore, a large number of genes might be involved, and a given single gene might have only a very small impact on the disease. The present study suggests that *GABRG2* gene might be one of the susceptibility factors for FSs (Chou *et al.* , 2003).

Table 4.5 : Genotypes and allele frequencies of GABRG2 (SNP211037). Polymorphisms in children with febrile seizures and normal control subjects

	Control No. (%) (n.= 50)	febrile seizure No. (%) (n.= 50)	P value*
Genotype			
CC	25 (50.00)	28(56.00)	0.0243
CT	15 (30.00)	10 (20.00)	
TT	10 (20.00)	12 (24.00)	
Allelic frequency**			
C	65 (65.00)	66 (66.00)	0.0172
T	35 (33.00)	34 (34.00)	

* P- value were calculated by X2 test

** Allelic frequency of C = CC + 1/2 CT

Allelic frequency of T = TT + 1/2 CT

4.5 Relationship between *SCN1A* , *SCN2A* and *GABRG2* genes polymorphism and Biochemical tests

It's observed that some patients have A/G genotype (12 males and 13 females) for *SCN1A* gene , they have family history (6 males and 6 females) , and they have a first FS seizures (8 males and 8 females) , patients have recurrent seizures (4 males and 5 females) .

Their biochemical tests are : all normal creatinine levels , abnormal glucose levels (3 males and 4 females) , abnormal potassium levels (3 males and 4 females) , abnormal calcium levels (2 males and 4 females) , abnormal phosphorus levels (1 males and 3 females) , abnormal chloride levels (1 males and 7 females) , abnormal sodium levels (3 males and 4 females).

While it's observed that some patients have G/G genotype (7 males and 7 females) for *SCN1A* gene , they have family history (5 males and 4 females) and family history with epilepsy (1 boy) , and they had a first FS seizures (7 males and 2 females) , they had recurrent seizures (5 females) .

Their biochemical tests are : all normal creatinine levels , abnormal glucose levels (4 males and 4 females) , normal potassium levels , abnormal calcium levels (1 males) , abnormal phosphorus levels (1 males and 1 females) , abnormal chloride levels (1 males and 3 females) , abnormal sodium levels (1 males). Some patients have A/A genotype (5 males and 5 females) for *SCN1A* gene , they have family history (3 males and 3 females) , and patients had a first FS seizures (2 males and 3 females) , patients had recurrent seizures (3 males and 2 females) .

Their biochemical tests are : all normal creatinine levels , abnormal glucose levels (1 boy and 2 females) , abnormal potassium levels (1 males and 1 females) , abnormal calcium levels (1 boy and 1 girl) , normal phosphorus levels, abnormal chloride levels (1 males) abnormal sodium levels (1 boy and 1 girl).

The patients have G/A genotype (10 males and 13 females) for *SCN2A* gene , they have family history (6 males and 6 females) , and patients have a first FS seizures (5 males and 5 females) , patients have recurrent seizures (5 males and 8 females) .

Their biochemical tests are : all normal creatinine levels , abnormal glucose levels (2 males and 6 females) , abnormal potassium levels (2 males and 3 females) ,

abnormal calcium levels (1 males and 4 females) , abnormal phosphorus levels (2 males) , abnormal chloride levels (3 males and 4 females) , abnormal sodium levels (3 males and 4 females).

While it's observed that some patients have G/G genotype (5 males and 4 females) for *SCN2A* gene , they have family history (3 males and 4 females), and they had a first FS seizures (3 males and 4 females) , patients had recurrent seizures (2 boy) .

Their biochemical tests are : all normal creatinine levels , abnormal glucose levels (2 males and 2 females) , abnormal potassium levels (1 males), abnormal calcium levels (2 males) , abnormal phosphorus levels (2 males and 1 girl) , abnormal chloride levels (1 boy and 3 females) , abnormal sodium levels (1 boy and 2 females). Some patients have A/A genotype (10 males and 8 females) for *SCN2A* gene , they have family history (5 males and 2 females) , family history with epilepsy (1 boy) and patients have a first FS seizures (10 males and 4 females) , patients have recurrent seizures (4 females) .

Their biochemical tests are : all normal creatinine levels , abnormal glucose levels (5 males and 2 females) , abnormal potassium levels (3 males and 2 females) , abnormal calcium levels (2 males and 4 females) , abnormal phosphorus levels (1 males and 3 females), abnormal chloride levels (2 males and 5 females), abnormal sodium levels (4 males and 1 girl).

It's observed that some patients have C/C genotype (15 males and 13 females) for *GABRG2* gene , they have family history (10 males and 8 females) , and they had a first FS seizures (9 males and 6 females) , patients have recurrent seizures (6 males and 7 females) .

Their biochemical tests are : all normal creatinine levels , abnormal glucose levels (7 males and 6 females) , abnormal potassium levels (4 males and 5 females) , abnormal calcium levels (3 males and 5 females) , abnormal phosphorus levels (2 males and 3 females) , abnormal chloride levels (4 males and 8 females) , abnormal sodium levels (6 males and 3 females).

Found some patients have C/T genotype (5 males and 5 females) for *GABRG2* gene , they have family history (1 boy and 2 females) , and they have a first FS seizures (4 males and 1 girl) , patients had recurrent seizures (1 boy and 4 females) .

Their biochemical tests are : all normal creatinine levels , abnormal glucose levels (1 females) , abnormal potassium levels (1 males) , abnormal calcium levels (2 males and 1 females) , abnormal phosphorus levels (1 females) , abnormal chloride levels (1 boy and 3 females) , abnormal sodium levels (1 boy and 3 females).

Finally , some patients have T/T genotype (5 males and 7 females) for *GABRG2* gene , they have family history (3 males and 3 females) , and they had a first FS seizures (4 males and 6 females) , patients had recurrent seizures (1 males and 1 females) .

Their biochemical tests are : all normal creatinine levels , abnormal glucose levels (2 males and 3 females) , abnormal potassium levels (1 males) , abnormal calcium levels (1 females) , abnormal phosphorus levels (2 males and1 females) , abnormal chloride levels (1 males and 2 females) , abnormal sodium levels (1 males and 1 females).

Its no relationship between *SCN1A* , *SCN2A* and *GABRG2* genes and some biochemical tests in males and females with febrile seizures , Appendix C (1) , (2) .

2.1 Epilepsy

Epilepsy is a common chronic neurological condition that is distinguished by recurrent unprovoked seizures. Two most common seizure types are generalized seizures (GS) affecting adults and febrile seizures (FS) affecting children (Ponnala *et al.* ,2012). Children with febrile seizures seem to be at increase risk of developing epilepsy . Many of population-based studies report a risk of 2-7% of unprovoked seizures compared to 0.5-2.0% in children without febrile seizure (Vestergaard *et al.* , 2007) . In incidence cohorts of epilepsy preceding febrile seizures are reported in 13-21% .This does not necessarily mean that febrile seizures increase the risk of developing epilepsy (Visser ,2011).

Febrile seizures have been the first semblance of an underlying seizure disorder. This is corroborative by the fact that a family history of epilepsy increases the risk of subsequent unprovoked seizures. However, Indicators found that especially prolonged and focal febrile seizures can cause brain damage. This brain damage might reinforce subsequent unprovoked seizures. Retrospective studies especially linked a history of prolonged febrile seizures in early childhood to temporal lobe epilepsy (McClelland *et al.*, 2011). Approximately 70% of all patients with epilepsy lack an obvious extraneous cause and genetics is presumed to be the predominant factor underlying the disorder. Most epilepsy phenotypes result from interactions between genes and ecological factors. The genetic variation could affect the aetiology , diagnosis and consequences of epilepsies to varying degrees in different individuals, , including responsiveness to antiepileptic drugs (AEDs) (Lakhan *et al.*, 2009) . Voltage-gated sodium channels, fundamental for action potential of generation, also have a main role in membrane excitability. The genes coding for channel components are considered to be a major class of genes related with various epilepsy phenotypes. The voltage-gated sodium ion channels consist of α and β subunits. Each α subunit is associated with one or more β subunits to form functional voltage-gated ion channels. Defects in subunits of sodium channels make them susceptible to slow inactivation, i.e. membrane remains depolarized for a longer time that can result in epileptogenesis and propagation of seizures (Alekov *et al.*, 2000).

In fact, altered sodium channel transcript levels in human epilepsy have been found in brain tissues, suggesting a potential role for sodium channels in the pathophysiology of epilepsy (Lombardo *et al.*,1996). Several single nucleotide polymorphisms (SNPs) in the sodium channel genes have been described so far, but

only a few including *SCN1A* p. Thr1067Ala or c.3184 A→G (rs2298771) , SNPs (AGACAGTTGTATGTCCAATCATACA[A/G]CAGAAATTGGGAAAGATCTTG ACTA) , Allele origin : G(germline)/A(germline) and *SCN2A* p.Arg19Lys or c.56G→A (rs17183814),SNPs (CCTGACAGCTTCCGCTTCTTTACCA[A/G]GG AATCCCTTGCTGCTATTGAACAA) , Allele origin : G(germline)/A(germline) gene polymorphisms are found to have functional significance in different neurological disorders (Lossin , 2008). In a proband of the Japanese family with missense mutations in *SCN2A* gene, the patient had partial epilepsy after febrile seizures (FS) ; along with other genetic variants identified, the *SCN2A* c.56 G→A variant was also observed. Although it is not a disease-causing mutation, it is believed that this variant in *SCN2A* gene along with other genes could have modified the phenotype of the individual affected in that family (Ito *et al.* , 2004).

This polymorphism causes amino acid substitution (Arg19Lys) in a cytoplasmic part of the channel and this Arg19 is a moderately conserved residue. Moreover , this SCN2A c.56 G→A variant that codes for lysine is significantly more frequent in patients with FS related with afebrile seizures including generalized epilepsy with febrile seizures (GEFS)1 than in controls (Lakhan *et al.*, 2009). Along with other genetic variants, c.*SCN2A* 56 G→A allele may be a possible modifying factor for epilepsy susceptibility and therapeutic response. Defects in *SCN1A* are a cause of severe myoclonic epilepsy in infancy, GEFS plus type 2 and intractable childhood epilepsy with generalized tonic- clonic seizures (Fujiwara *et al.*, 2003). As the causal relation between *SCN2A* 56 G→A, *SCN1A* c.3184 A→G polymorphisms and FSs associated with afebrile seizures or idiopathic generalized epilepsy has not been proven genetically, identification or confirmation of the association in different populations would be important in establishing a role for the *SCN1A* and *SCN2A* gene in the development of seizures (Lakhan *et al.* ,2009).

GABA is the major inhibitory neurotransmitter in the mammlian brain, where it acts at GABA-A receptors, which are ligand-gated chloride channels. GABA-A receptors are pentameric . Mutations in this gene have been associated with epilepsy and febrile seizures. Multiple transcript variants encoding different isoforms have been identified for this gene (Balan *et al,* 2013). SNPs for GABRG2 (rs211037) (CTGAGTGC CAA T TACAATTGCACAA[C/T] TTTC CAAT GGA TGAA CACT CCTGCC) , Functional Consequence: synonymous codon .

2.2 Febrile seizure : General consideration and historical notes

Febrile seizure is the most prevalent type of seizures during childhood with an incidence 3-5 % . It is the age-dependent and appear between 6 months and 6 years of age, and are precipitated by a rapid rise in temperature to 39 ^{O}C or more because of a viral fever, Uri, acute otitis media, etc. in the early course of the fever. There may be a family history of febrile seizures of the parents or siblings (Krishna and Devendra , 2012) .

Febrile seizures are subdivided into three categories : simple , complex and febrile status spilepticus. Simple febrile seizures last for less than (15 min) , are generalized (without a focal component), and appear once in a 24-hour period, whereas complex febrile seizures are prolonged (15 min) , or are focal, or happen more than once in 24 hours (Alwan and Hussein, 2013).

Febrile Status Epilepticus (FSE) , this case is characterized by prolonged FS lasting more than (30 min) in duration . The peak age is between (12 – 24) months, and this condition is very uncommon after 5 years (Nordli *et al.* , 2013) . Most FSs are look benign (the type simple), but one-third are complex with a prolonged duration (<15 min) and are related with a risk of subsequent epilepsy (Mashimo *et al*., 2010) . Although the childhood febrile seizures stage in most cases are benign and limiting in impact, testifying the appearance of such attacks are terrible experience for all family (Capovilla *et al.* ,2009) .The risk of developing epilepsy is approximately 1% in children with febrile seizures. Although, febrile seizures generally have a good diagnosis, they may denote serious latent acute infectious diseases such as sepsis or bacterial meningitis. So, each child must be neatly checked up and suitably investigated to find whether there are related causes for the fever or not . Infectious diseases are important reasons of mortality and morbidity in children (Farrell and Goldman ,2011) . FSs are result from the combination of genetic and environmental factors (Özen *et al.* ,2014).

Different familial studies and twin studies have indicated that genetic predisposition may participate significantly to the etiology of FS, and recent genetic studies have shown that at least nine loci are responsible for FS .Voltage - gated sodium channels are critical for the initiation and propagation of action potentials in neurons (Mashimo *et al.*, 2010) . The accurate mechanism of inheritance is still unclear (Özen *et al.* ,2014). The risk of recurrence increases when there is a family

history of febrile seizures and , in some but not all studies, when there is a family history of afebrile seizures and when the child has a neurologic defect (Alwan and Hussein , 2013).

A preliminary assessment should define whether features of a complex seizure are present and identify the source of fever. Routine blood tests, neuroimaging, and electroencephalography are not recommended, and lumbar puncture is no longer recommended in patients with uncomplicated febrile seizures. After a first febrile seizure, doctors should reassure parents about the low risk of long-term effects, including neurologic consequences, epilepsy, and death. But, there is a (15 to 70) % risk of recurrence in the first two years after a first febrile seizure. This risk is increased in children who under the age of 18 months and those with a lower fever, short duration of fever before seizure start, or a family history of febrile seizures. Continuous or intermittent antiepileptic or antipyretic medication is not recommended for the barring of recurrent febrile seizures (Graves *et al.*, 2012). Electroencephalography (EEG) and neuroimaging are of limited value , and treatment with antiepileptic medications is rarely indicated (Farrell and Goldman ,2011).

2.3 Definition

The International League Against Epilepsy (ILAE) defines a febrile seizure as **"**a seizure in association with a febrile disease in the absence of a central nervous system infection or acute electrolyte imbalance in children older than 1 month of age without prior a febrile seizures **"** (Shinnar and Glauser , 2013). A child with the diagnosis of FS cannot have a history of neonatal seizures, a previous unprovoked seizure or meet criteria for other acute symptomatic seizures . The minimum age of the ILAE definition is younger than the limit proposed formerly by the National Institutes of Health (NIH). The NIH Consensus Conference definition of FS is an episode usually appearing between three months and five years of age, related with fever, but without evidence of intracranial infection or know reason (John and Syndi , 2013) .

Seizures with fever in children who have suffered a previous non - FC are excluded. This definition excludes seizures that escort meningitis, electrolyte imbalance or toxic encephalopathy. The temperature related with the febrile disease must be greater than 38.4°C, although the temperature may not be apparent until after the seizure . Prior epidemiologic studies have used either 1 month or 3 months as

the youngest age of appearance, while no specific upper age limit was employed (Nelson and Ellenberg ,1978) . Seizures in these instances may carry a more ominous prognosis than the benign course of FS to the effects of associated illness .This condition has been described since the time of Hippocrates. It was initially thought to be due to teething. This may have been considered as the etiology because it happens most frequently in toddlers. In the Nineteenth century, febrile seizures were felt to be a form of epilepsy triggered by fever. but now understand that febrile seizures are an age dependent response of the immature brain to a febrile illness. (Kundu *et al.* ,2010) .

2.4 Signs and Symptoms of Febrile Seizure

Febrile seizure symptoms vary. In mild cases, the child's eyes may roll or his or her limbs may become rigid (stiff). During a febrile seizure, children are unable to respond (i.e., unresponsive) and may lose consciousness. If the child is standing, he or she will fall.

Other symptoms of febrile seizures include the following:

- Breathing difficulty (e.g., apnea; the child may turn bluish in color)
- Contraction of the muscles of the face, limbs, and trunk
- Involuntary moaning, crying, and/or passing of urine
- Shaking
- Twitching
- Vomiting

After a period of time (usually a few seconds to a few minutes), the muscles relax, causing rhythmic jerking. Once the seizure is over, the child may be sleepy or confused (Stanley , 2008).

2.5 Incidence

FSs have a peak incidence at eighteen months of age and are most common between six months and five years . Most FSs are simple with approximately (20-30) % being complex . The distribution of a first FS duration can be described using a two population model, one with short seizure duration and the other with long seizure duration, with the cut-off at approximately 10 minutes . Approximately 5% of FS will last ≥30 minutes . No correlation has been identified between duration of the first FS and duration of the second FS . In spite of it has been observed that a recurrent febrile seizure is probably to be prolonged if the initial FS was prolonged . By definition, a febrile disease is required for a child to have a FS. Children with FS have higher temperatures with disease compared to febrile controls . The rapid onset of fever was previously thought to be precipitating factor of FS , but this is no longer thought to be true . Gender predominance of FS has also been studied. There are studies that conclude a higher incidence of FS in males . Males have continuous shown as having a higher frequency of FS (male to female ratio, 1.1:1 to 2:1) (Shinnar, 2002) , and others showed no significant difference based on gender (John and Syndi , 2013).

Incidence has a wide variation amongst different population groups being as low as 2-4% in Caucasians and as high as 15.3% in Africa , and affecting between 2 - 4% of children in the United States and Western Europe, 9 - 10% of children in Japan (Shinnar and Glauser, 2013) . Data from developing countries are limited possibly because they may be very difficult to differentiate simple FSs from seizures related with CNS infection (AL-Zwaini , 2007) . The Iraqi League Against Epilepsy (IqLAE) is a national non-govermental organization of the ILAE consist of medical personnel who are involved in the diagnosis, treatment and research in the field of Epileptology and composed of Neurologists, Neurophysiologists, Paediatricians, Paediatric neurologists, Neurosurgeons and Psychiatrists may contribute to assistance in relation of febrile seizure . Common causes of febrile seizure in the tropics include malaria , pneumonia urinary tract infection, septicemia and viral infections (Jarrett *et al.* ,2012). FSs appear in the state of a febrile disease, which could cause seasonal variation . In Japan a study of FS showed two peaks of incidence, November to January and June to August, which agree with the peaks of viral upper respiratory infections and gastrointestinal infections respectively . A study performed in Italy,

which examined at 188 first FS, found that there is a high significant in FS from 6 PM to 11:59 PM and a seasonal peak in January . There are many studies have supported the conclusion that FS have a peak in the winter and end of the summer (John and Syndi, 2013) .

2.6 Morbidity and Mortality

The morbidity and mortality related with febrile seizures are very few (Shinnar and Glauser, 2013). Several studies have shown no any evidence of persistent motor deficits next febrile seizures or febrile status epilepticus. No reports of acute impairment of cognitive abilities have been noted next febrile seizures, even in series limited to status epilepticus (Verity *et al.,* 1993). Three large studies have shown that cognitive abilities and school performance of children with febrile seizures were comparable to those of controls. Prolonged febrile seizures do not show to be related with adverse cognitive outcomes . No deaths are notify from the National Collaborative Perinatal Project (Shinnar and Glauser ,2013) .

2.7 Pathophysiology

Although the mechanism of FS remains unclear , animal models are informative (Dube and Brewster ,2009). First, high brain temperature change many neuronal functions, including several temperature-sensitive ion channels (Shibasaki *et al.* ,2007) . This impact neuronal firing and increases the probability of generating massive neuronal activity, i.e., seizures. Also , an inflammatory process including secretion of cytokine in the periphery and in the brain is known to be a part of the mechanism. Second, it is to show that fever and hyperthermia participate common mechanisms in provoking seizures: the fever-promoting pyrogen interleukin-1β contributes to fever generation and conversely, fever leads to the synthesis of this cytokine in the hippocampus (Cartmell *et al.* , 1999).

In addition, interleukin-1β (IL-1β) has been shown to increase neuronal excitability, acting via both glutamate and GABA (Vezzani and Granata , 2005). In vivo, these actions of interleukin-1β enhance the actions of seizure-provoking agents. The importance of endogenous interleukin-1β in the appearance of FS is promoted by studies in mice that lacked the receptor for this cytokine. Fever of specific infectious etiologies, specifically human herpes virus 6 (HHV6), might influence the probability

of generation of FS (Dube *et al.,* 2005). Third, hyperthermia-induced hyperventilation and alkalosis have been suggest as a essential element of FS generation in that alkalosis of the brain provokes neuronal excitability (Shinnar , associated with acute alkalosis, including prolonged crying and pyloric stenosis of infants, are not associated with the generation of seizures (Dube and Brewster ,2009) .

2.8 Etiology

2.8.1 pathogenesis

A fever episode is a prerequisite for a febrile seizure. For a long time the raise in temperature of the brain has held solely responsible for the seizure to occur . Elevated brain temperature changes many neuronal functions and can effect in neuronal excitability (Mizunuma *et al.* ,2009). The lower of body temperature during the initial FS, the greater is the risk of recurrences , so that each degree of increase in body temperature during the first seizure reduces the recurrence risk by 18% (Tarkka , 2003).

In the previous decades however, evidence of both clinical and experimental studies has emerged that components of the immune response are directly involved in the pathogenesis of febrile seizures, next to causing an increase in body temperature. Studies have specifically focussed on the pro-inflammatory cytokine interleukin-1 beta(IL-1 β). For example, in an experimental setting an exaggerated IL-1β production was shown in leukocytes of children with febrile seizures and in brain tissue of a rat model (Heida and Moshe ,2009). As well it is discovered that children with specific genetic alterations in the interleukin system are more liable to febrile seizures. Cytokines are supposed to lead to an increased body temperature as well as independently to an increased neuronal excitability. The above may explain why antipyretic therapy is not protective for recurrences of febrile seizures whereas GABA-ergic drugs do prevent the seizures . It may also explain why the same elevated in temperature does not always result in febrile seizures in susceptible children (Visser , 2011).

2.8.2 Risk factors

Three critical factors influence the chance to develop a FS : age, fever and genetic predisposition. As it is described in the definition of the NIH and ILAE, age and fever are always crucial. FS specially happens at an age of specific developmental period . Fever is mostly dependent on an infection: different viral and bacterial infections have been related with FS (Millichap , 2006), however a warm bath can also cause a FS. Furthermore , vaccinations are also a risk factor to develop FS, since fever appears frequently after vaccination (Hessel , 2010).

Febrile seizures frequently recur. Although febrile seizure is ordinarily appeared as single, isolated incidents, the reoccurrence rate is 30% overall , and increases to 50% if the initial febrile seizure appears in a child under one year of age . Those who experience a second febrile seizure, the risk of recurrence increases 2-fold .Predictors of recurrent febrile seizures include: a history of focal, prolonged, and multiple seizures , Influenza A viral infection , family history of febrile seizures , onset of febrile seizure <12 months of age , temperature <40°C (<104 °F) at time of seizure , and a history of complex, initial febrile seizures . A low proportion (2-4%) of children who experience at least one febrile seizure event , go on to develop recurrent a febrile seizures (epilepsy) (Jones and Jacobsen, 2007).

2.8.3 Risk Factors for First Febrile Seizure

Many studies have examined risk factors related with a febrile seizure as two studies , in a 1993 case control population-based study, four factors were related with an increased risk of febrile seizures:

(1) The degree of the first- or second of relative with a history of febrile seizures (2) a neonatal nursery stay of >30 days, (3) developmental delay, or (4) attendance at day care. There was a 28% opportunity that suffer at least one febrile seizure for children with two of these factors (Bethune *et al.*, 1993) . second case-control study examined the issue of which children with a febrile illness were most likely to experience a febrile seizure using febrile controls matched for age, site of routine pediatric care, and date of visit (Berg *et al.* ,1992) . Significant independent risk factors, on a multivariable analysis, were the height of the temperature and a history of febrile seizures in a first- or higher-degree relative. Gastroenteritis as the

underlying disease appeared to have a significant inverse (ie, protective) association with febrile seizures (Kundu *et al.*, 2010).

2.8.4 Risk factors for febrile seizure recurrence

Risk factors for febrile seizure recurrence have been studied. Factors related with an increased risk are :

1-First febrile seizure in the first year of life (Stuijvenberg *et al.*, 1998) .

2- Relatively low temperature at the start of the seizure .

3-Frequent fever episodes .

4-Positive family history of febrile seizures .

A family history of epilepsy was related with recurrent febrile seizures to a lesser degree (Visser , 2011) .

2.8.5 Risk Factors for Subsequent Epilepsy

The risk factors for developing epilepsy after febrile seizures are summarized in . Following a single simple febrile seizure, the risk of developing epilepsy is not substantially different than the risk in the general population (Berg and Shinnar, 1996) . Data from five large cohorts of children with febrile seizures indicate that 2 to 10% of children who have febrile seizures will subsequently develop epilepsy . In each of these five large studies, the occurrence of a family history of epilepsy and the occurrence of a complex febrile seizure were related with an increased risk of subsequent epilepsy (Verity and Golding, 1991) .

The occurrence of multiple febrile seizures is related with a slight but statistically significant increased risk of subsequent epilepsy in two studies . The first study has found that children with a febrile seizure that occurred within 1 hour of a recognized fever (ie, at onset) have a higher risk for subsequent epilepsy than those children with a febrile seizure associated with longer fever duration. The second study have found that very prolonged febrile seizures (ie, febrile status epilepticus) are related with an increased risk of subsequent epilepsy above that of a complex febrile seizure that is less prolonged (Berg and Shinnar, 1996) . The number of complex features in a febrile seizure may possibly affect the risk of recurrence. Although one study finds that patients with two complex features (eg, prolonged and focal) may further

increased risk of subsequent epilepsy. Another study does not detect this association . A family history of febrile seizures, age at first febrile seizure, and the height of fever at first seizure are not related with a differential risk of developing epilepsy . The only common risk factor for both recurrent febrile seizures and subsequent epilepsy was duration of fever prior to the febrile seizure ; this may be a marker for overall seizure susceptibility (Berg *et al.*, 1997).

The types of epilepsy that develop are variable . In general, the types of epilepsy that appear in children with prior febrile seizures are varied and are not very different from those that appear in children without such a history. Febrile seizures can also be the initial manifestation of specific epilepsy syndromes, such as severe myoclonic epilepsy of infancy . It is controversial whether febrile seizures are simply an age-specific marker of future seizure susceptibility or have a causal relationship with the subsequent epilepsy (Shinnar , 1998). Two factors support the former, and not the latter, interpretation. There is no increased incidence of epilepsy in populations with a high cumulative incidence of febrile seizures (eg, 10% in Tokyo, Japan). Second, no evidence exists that treatment of febrile seizures alters the risk of subsequent epilepsy (Shinnar and Glauser, 2002) .

2.9 Genetic factors

Genetic risk factors have long been known to contribute significantly to the etiology of febrile seizures. It tends to appear in families and one of the major risk factors is a first degree relative (parent or sibling) with febrile seizures (Varma, 2002). Although a number of single gene mutations, such as those affecting GABA and sodium channels, have been identified, these gene mutations account for only 1% to 2% of occurrences of febrile seizures (Nakayama and Tadao , 2006) .

However, the exact mode of inheritance is unknown. It is thought that approximately 10-20% of siblings of children with febrile seizures will develop febrile seizures. The possibility for development of febrile seizures in children is high if one of the parents also has a history of febrile seizures (Varma, 2002). Twin and family studies have shown that FS have a heritable component of about 70% . Most studies support a polygenic or multifactorial mode of inheritance . However, there are rare families with a monogenic inheritance model . Although infantile FS are mostly

benign, patients have an increased risk for developing epilepsy later in their life (Kundu *et al.* ,2010).

FSs are complex and heterogeneous . A positive family history of febrile seizures is a definite risk factor for both a first febrile seizure and recurrent febrile seizures (Shinnar and Glauser ,2013).This is supported by the findings in twin and family studies which demonstrated an important genetic component in the etiology of FS (Wang *et al.* , 2014) . Human linkage studies identified ten familial FS loci . Five FSs genes have been identified in these FEB loci (*FEB3a: SCN1A; FEB3b: SCN9A; FEB4: GPR98* or *MASS/VLGR1* gene*; FEB6: IMPA2; FEB8: GABRG2)* (Hessel , 2010) , table (2-1) .The precise underlying pathogenic factor is still unclear which has hampered many aspects of the study of FS (Wang *et al.*, 2014) . Genetic factors appear to play an important role in determining FS susceptibility. About 25% of children who experiencing FS have a positive family history and concordance for FS is about 40–60% in monozygotic twins, but only 10–20% in dizygous twins (Hessel *et al.* , 2014) .

Table 2.1: FS loci and genes related with FS found by linkage studies (Hessel ,2010)

FS Linkage Studies	Locus	Gene
FEB1	8q13-q21	
FEB2	19p13.3	*CSNK1G2*
FEB3A	2q23-2q24	*SCN1A*
FEB3B	2q23-2q24	*SCN9A*
FEB4	5q14-15	*GPR98*
FEB5	6q22-24	
FEB6	18p11.2	*IMPA2*
FEB7	21q22	
FEB8	5q31.1-q33.1	*GABRG2*
FEB9	3p24.2-p23	
FEB10	3q26.2-q26.33	

Several genetic epilepsy syndromes can start with febrile seizures. These are Generalized Epilepsy with Febrile Seizures plus (GEFS+), Severe Myoclonic Epilepsy in Infancy (SMEI or Dravet's syndrome), and Temporal lobe Epilepsy (TLE) . The GEFS+ is an autosomal dominant syndrome with a highly variable phenotype. Onset is usually in early childhood and remission is usually in mid childhood. It is characterized by multiple febrile seizures, and several types of afebrile generalized seizures including generalized tonic-clonic , absence, myoclonic or a tonic and myoclonic astatic seizures with variable degrees of severity (Mikati and Rahi, 2005) .

2.10 Diagnosis

The diagnosis of febrile seizure is essentially clinical. Episodes should be classified into simple or complex febrile seizure (Siqueira and Luis , 2010). Seizures of any type are usually a manifestation of a number of underlying pathologic conditions to differentiate between them, careful history taking, physical examination, and laboratory work-up are usually required (Jones and Jacobsen , 2007) . Children with complex seizures and other differential diagnoses (such as focal epilepsy or new-onset acute symptomatic seizure) must always be considered, despite their rarity. Lumbar puncture is indicated with children in 18 months or younger with clinical signs suggesting meningitis or in particularly severe presentations. Blood glucose measurement is essential, and any further laboratory testing should be requested as necessary according to clinical condition and diagnostic hypothesis. Electroencephalography may show changes in various cases, and therefore there is no practical use. Neuroimaging is also of little value in the diagnosis of febrile seizures.The main differential diagnosis is CNS infection. (Siqueira and Luis , 2010).

2.11 Febrile Seizure Complications

In most cases, febrile seizures resolve without complications. If the child is standing, eating, or drinking when the seizure occurs, he or she may be injured by the fall, may choke, or may inhale fluid into the lungs. The child also may bite his or her tongue, lips, or inside of the cheek during a febrile seizure. Febrile seizures increase the risk for epilepsy slightly. However, more than 95% of children who have febrile seizures do not develop a seizure disorder. Febrile seizures do not increase the risk for brain damage or mental retardation. In rare cases, a condition called status

epilepticus can occur during a febrile seizure. **Status epilepticus is a medical emergency** in which a seizure lasts longer than 30 minutes or seizures recur without recovery for 30 minutes or longer. This condition is more common in children under the age of 1 year. Status epilepticus can cause brain damage and may be fatal (Stanley , 2008) .

2.12 Familial studies

Although no risk factors are identifiable with 50% of children with FS, a positive familial history of first-degree relatives can be considered as a more consistent risk factor for developing FS than were fever, concurrent infections, and prenatal and early-life exposure (Fetveit , 2008) (Hesdorffer *et al.* , 2012) . Familial susceptibility to FS can be expressed in terms of appearance and recurrence risk. The estimated appearance risk in siblings of cases with FS were calculated to be about 10% and 12%, respectively, in a clinic-based study of the Sophia Children's Hospital in Rotterdam and in a population-based study in southern Taiwan (Huang *et al.*, 1999) . The risk could increase up to 46% if other risk factors are present (van *et al.* ,1998*)* . In a PBAS (population-based association studies) in 1990 , Annegers et al. reported that a recurrence risk of 36% after an initial FS with children of a positive familial history (vs. a recurrence risk of 22% of children without a familial history) .

The recurrence risk of FS was higher, up to 42% with children in who the positive familial history was related with an early age at onset (Saghazadeh *et al.*, 2014) . Offringa et al. (1994) evidenced, through analysis of pooled data of two population-based studies and three clinic-based studies, that a familial history of seizures (either febrile or afebrile) was associated with increased recurrence hazard after the initial FS (1.49) and after the first recurrence (1.32) . Different studies have calculated and compared the pairwise and the proband-wise concordance in monozygotic and dizygotic twins in Japan, Denmark, Australia, the United States, and Norway. All these studies reported significantly higher concordance rates in monozygotic pairs than in dizygotic ones (range, 0.10 – 0.80 vs. 0.03 – 0.32) . Among 47 PBASs, evidences for the role of 16/36 investigated genes in the overlap between FS and epilepsy were obtained. These genes could be easily categorized into three coherent groups: (1) genes that have at least one positive association with both FS and epilepsy (SCN1A, CHRNA4, GABRG2, and IL-1 β), (2) genes that have no positive

associations with both FS and epilepsy [sodium channel voltage-gated type 1 β subunit (SCN1B), TNF- α , cholinergic receptor nicotinic β polypeptide 2 (CHRNB2), leucine-rich glioma inactivated 1 (LGI1), IL-1 α , SCN2A, and apolipoprotein E (APOE), and (3) genes that have a positive association with FS alone or epilepsy alone (IL- 1Ra, IL-6, BDNF, HCN2, and KCNQ2). If we excluded the third group of genes, the remaining groups can be linked to FS and epilepsy (Saghazadeh *et al.*, 2014).

2.13 Voltage gated sodium channels (VGSCs)

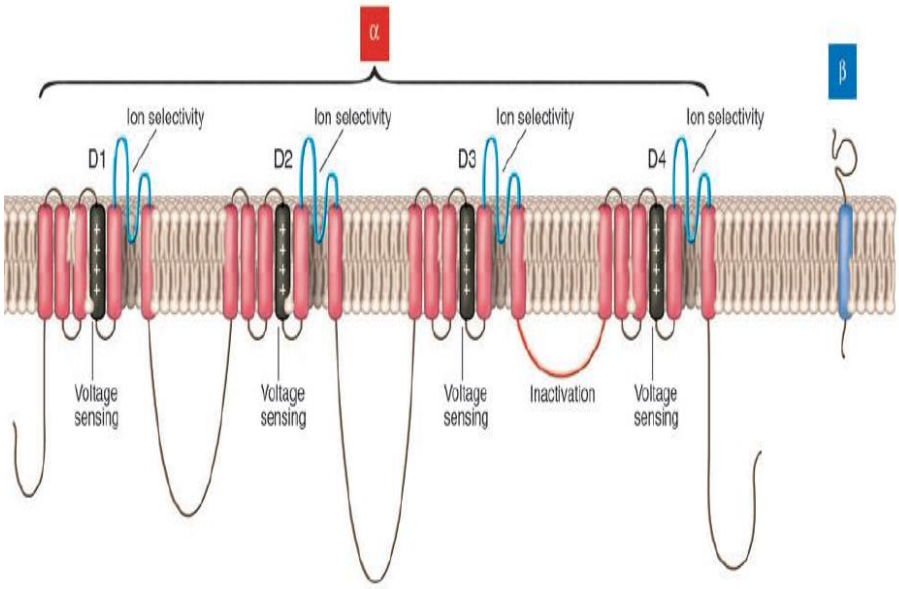
Voltage-gated sodium channels are essential for encoding the receptor potential to a series of action potentials and for conducting the action potentials along the axon , Neurons differ in the shape of their action potentials and also in the rate and regularity at which they fire action potentials (Fein , 2012) . Sodium channels are integral membrane proteins that containing 4 homologous domains each of which consists of (6) transmembrane segments (S1 to S6) that form voltage sensitive and ion selective pores (König , 2011) . Voltage-gated sodium channel is heteromeric protein that is consists of one alpha and one or more beta subunits . These alpha subunits are connected to each other via intracellular and extracellular loops . The sodium channel's voltage sensor is found in the S4 alpha helix in each of the four alpha subunit domains (I-IV) (Waxman , 2011) . Voltage sensor residues in S4 are either lysine or arginine that are arranged in an interval of every 3rd amino acid, allow only positively charged Na+ ions and only at an appropriate membrane potential to enter through the pore by an opening or otherwise closing the channel pore (König , 2011).

The molecular weight of the α subunits is about 220- 260 KDa and each voltage gated sodium channel is related with auxiliary beta subunits. Four different beta subunits (β1, β2, β3, β4) have been identified (John *et al.*, 2004) . Beta subunits are single transmembrane proteins with an intracellular and extracellular binding domain. Interestingly the extracellular domain of β- subunits is homologous to a V-set (Ig-related domains of non-Ig molecules are described as being V -like when they are have a pattern of β-strands : V and V related domains have about 65-75 amino acid residues between the conserved disulphide bond, and there are four β-strands in each β-sheet plus a short β-strand segment across the top of the domain, (König , 2011) of

Ig superfamily like adhesion molecules. Still not completely understood is what function β subunits fulfil to modulate VGSCs. It is hypothesized that β subunits interact with the cytoskeletal and the extracellular matrix via their V set Ig domain and thereby might play a role in stabilizing the Nav proteins in the membrane or help to even guide Nav polypeptides to membrane sites (John *et al.* , 2004). Co-expression of certain α subunits with a specific auxiliary β- subunit has been shown to modulate sodium channel properties (Cummins *et al.* , 2007) by changing activation / inactivation properties of VGSCs , fig.(2-1) A . For example, Nav1.2 co-expressed with β1A subunit leads to a 2.5 fold increase in sodium current density due to increased channel functionality (John *et al.*, 2004) . All nine pore-forming isoforms (Nav1.1 – Nav1.9) of the alpha subunits are encoded by different genes (Lampert *et al.* , 2010) .

In particular Nav1.1, Nav1.2, Nav1.3 and Nav.1.7 are closely structurally and functionally related and are encoded by a set of four genes clustered in the chromosomal locus chr. 2q24.3 , fig.(2-1) B . Each of the nine sodium channel isoforms has its own highly specific distribution pattern within the CNS and peripheral nervous system (Drenth and Waxman , 2007) . In humans, voltage gated sodium channels are highly conserved and show 75 % primary sequence homology of the transmembrane and extracellular domains (John *et al.* , 2004) . One can distinguish two general classes of voltage-gated sodium channels based on their sensitivity to tetrodotoxin (TTX) a potent neurotoxin that blocks action potentials in nerves by binding to the pore of voltage-gated sodium channel α-subunits. Not all α-subunits are sensitive to TTX, therefore we can distinguish between TTX sensitive (TTX-S) and TTX resistant (TTX-R) sodium channels (Fein , 2012). Channelopathies encompass a subset of genetic epilepsy syndromes with related mutations in genes encoding various voltage-gated and ligand-gated ion channels such as potassium and sodium channels (Alfred, 2005)

A



B

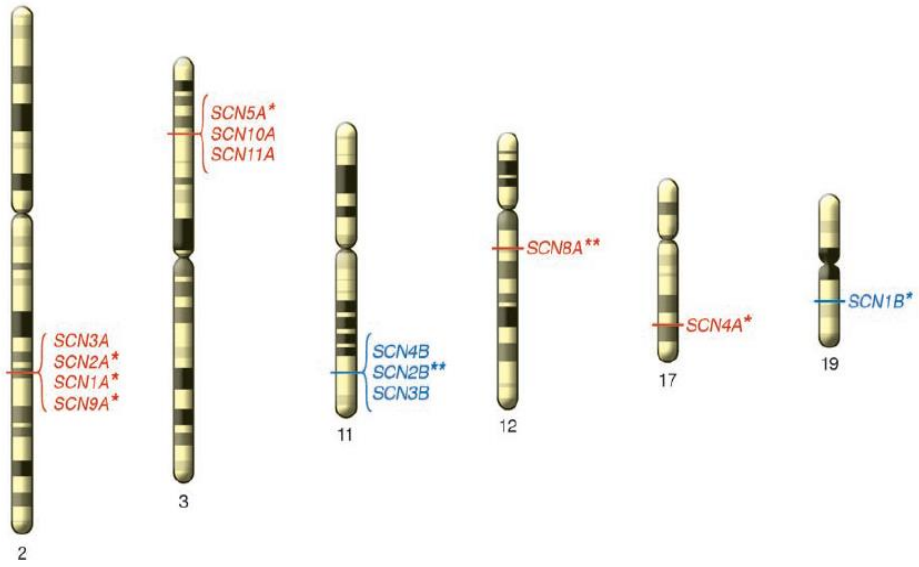


Figure 2.1
Structure and genomic location of human NaVChs. (A) Simple model representing transmembrane topology of α and β NaVCh subunits. Structural domains mediating key functional properties are labeled. (B) Chromosomal location of human genes encoding (red) and (blue) subunits across the genome. An asterisk next to the gene name indicates association with an inherited human disease. A double asterisk indicates association with murine phenotypes.

2.14 Mechanism of Action

Voltage gated sodium channels mainly contribute to action potential generation and propagation . Through their voltage sensor and ion-selective pores they allow the rapid influx of Na+ ions during the up stroke of an action potential (Cummins *et al.* , 2004) . Structural conformation of voltage gated sodium channels changes with changing membrane potential and can either be (a) open (active state), induced via membrane depolarization; (b) fast inactivated, which appears when the cytoplasmic loop binds to the pore of the VGSC to terminate the sodium flow and leads to (c) closed state: re-priming (recovery from inactivation), which appears while the membrane potential is repolarizing to get back to the resting membrane potential at –80mV (Cummins *et al.* , 2007) . This simplistic view of Nav1 open and closed states has been proven to not hold entirely true for all voltage gated sodium channels (Drenth and Waxman , 2007). Neuronal excitability depends , to a considerable extent , on sodium channel trafficking, distribution, and density, as well as the intrinsic properties of the channels themselves in terms of thresholds of activation and repriming characteristics (Waxman *et al.* , 2002). Naturally appearing mutations in both sodium channel α - and β - subunits have been implicated in various inherited disorders . (John *et al.* , 2004) , fig.(2-2) .

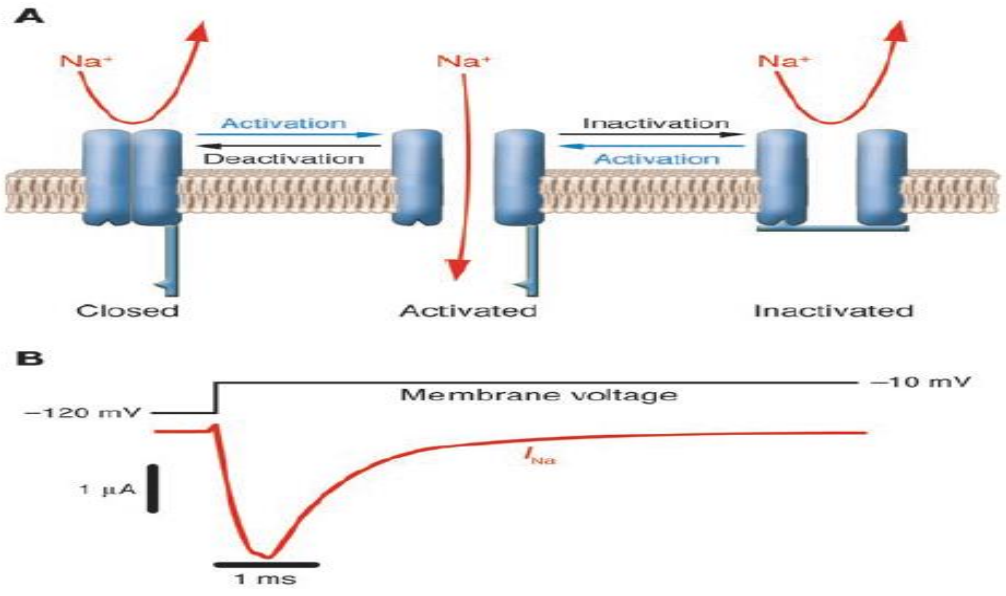


Figure 2.2 Functional properties of NaVChs. (**A**) Schematic representation of an NaVCh undergoing the main gating transitions. (**B**) Voltage-clamp recording of NaVCh activity in response to membrane depolarization. Downward deflection of the current trace (red) corresponds to inward movement of Na+.

2.15 Voltage-gated sodium channel *α* subunit

The voltage-dependent sodium channels are multimeric membrane proteins , forming a central pore in the membrane , which selectively conducts sodium ions . The pore consists of four homologous domains (D1-D4 a), each containing six transmembrane segments (S1-S6) . The *α* subunit is the major component of sodium channels , binding non covalently to the β1 or β3 subunit , and covalently to the β2 or β4 subunit. To form a functional channel , one *α* –subunit is sufficient , but by linking to the β- subunits , channel biophysics and trafficking are modulated . The *α* subunits are encoded by a series of homologous genes : SCN1A to SCN11A . Of them , mutations in *SCN1A* , *SCN2A* and SCN3A genes have been reported by some studies to be related with epilepsy (Eijkeelkamp *et al*, 2012) .

Genetic variation is a major factors of individual phenotypic diversity. Variants of low penetrance may influence in disease risk by causing small alterations in protein structure or gene expression. Combinations of these loci may act jointly to affect susceptibility to disease or resistance to drugs . The SCN1A , SCN2A and SCN3A genes, located at 2q24 , are widely expressed in the human brain (Ohashi and Tokunga, 2002) . In recent years several efforts have been made to identify the relation between polymorphisms and / or haplotypes of these genes , and AED dosage or responsiveness in epilepsy patients (Haerian *et al*, 2012) .

SCN1A is part of a group of sodium channel genes encoded on chromosome 2q24 that includes *SCN2A* and *SCN3A* . These voltage gated sodium channels are expressed in neurons and glia throughout the central and peripheral nervous system (Weiss *et al.* , 2003) . *SCN1A* encodes voltage-gated sodium channel alpha subunit. The alpha subunit of sodium channels forms the membrane pore. Most of *SCN1A* mutations cluster in the C-terminus and the pore loops in the first three domains of the protein , The pathophysiology of *SCN1A* mutations is a decrease in the activity of GABAergic inhibitory neurons (Mulley *et al.* ,2005) . Heterozygous mutations in the SCN1A gene, which encodes a voltage-gated sodium channel *α* I subunit (Nav1.1), are related with a broad range of childhood epilepsies, including generalized epilepsy with febrile seizures [Mendelian Inheritance] (Ogiwara *et al.* , 2013) .

Mutations in *SCN2A*, the gene encoding *α* 2 subunit of the neuronal sodium channel, are related with a assortment of epilepsies . Functional studies of *SCN2A* mutations appear that they can cause divergent biophysical defects in NaV1.2 and

weaken cell surface expressions .There is no regular relationship between genotype and phenotype (Shi *et al.* , 2012) .

2.16 Gamma- amino butyric acid (GABA)

In the CNS , GABA is the main inhibitory neurotransmitter that controls neuronal excitability and network interactions in the cerebral cortex of the brain (Kumari *et al.* , 2010) . It acts through three receptor classes: the ionotropic $GABA_A$, $GABA_C$ receptors and the metabotropic $GABA_B$ receptors. Among the three receptors, new findings highlight the significance of $GABA_A$ receptor heterogeneity for the concept of E/I (Excitation/Inhibition) balance and its relece to epilepsy (Fritschy , 2008) . Structurally $GABA_A$ receptors are pentameric chloride ion channels formed from different combinations of proteins encoded by α (α 1– α 6), β (β 1– β 3), γ (γ 1–γ 3); δ, ε, π, θ, and ρ (ρ1–ρ 3) subunit gene families. The α1 β 2 γ 2 subunit combination of $GABA_A$ receptor is most abundant in almost all regions of the brain (Christopher *et al.* , 2009) .

The genetic susceptibility to FSs look to include multiple genes in most cases (Chou *et al.* , 2003) . Some forms of family epilepsy may present as FSs and several of these disorders are caused by channelopathies such as neuronal sodium channels (Escayg *et al.*, 2000) or GABA receptors (Wallace *et al.* , 2001) . Thus, because of involvement important clinical features, FSs and these family epilepsies may participate a common genetic etiology. It is not known, however, whether polymorphisms in those genes include in familial epilepsies also contribute to the pathogenesis of FSs, because less than 3% of children with FSs adced to continual epilepsy (Baumann and Duffner, 2000) . Numerous steps in GABA synaptic function are relet to epileptogenesis: (a) GABA synthesis; (b) GABA release; (c) GABA transport; and (d) activation of receptors, subtypes A and B (Olsen and Avoli , 1997) . An alternation of GABAergic neurotransmission has been involved as an etiologic factor in epileptogenesis . Neuronal inhibition in the mammalian brain is largely interpose by the binding of GABA to heteromeric GABA Receptors (GABRs) (Olsen and Avoli, 1997) .

Dysfunction of genes are coding these subunits affects ion channel gating, expression, and trafficking of the GABA receptor to the cell surface. These genes are also believed to influence important drug targets substantial for the organization of neuronal activity in the brain (Chou *et al.*, 2007) . Genetic evidence for a potential

role of the GABAergic system in epileptogenesis has been acquired initially by the detection of various GABAR-G2 mutations identified in two families (Olsen and Avoli , 1997) . The phenotype in one of these families was described to be compatible with generalized epilepsy with FS plus (Baulac *et al.*, 2001) . In the second affected family, individuals predominantly had childhood absence epilepsy and FSs (Wallace *et al.* , 2001). Accordingly, these findings raise the question of whether genetic variation of the *GABRG2* gene confers susceptibility to the epileptogenesis of FSs. Genetic studies of complex diseases such as FSs are difficult to approach because of the uncertainty of polygenic traits.

2.17 Physiology of GABA-mediated inhibition

At the postsynaptic level, GABA mediates both rapid inhibition through $GABA_A$ receptors, which are membranes of the ligand-gated ion channel superfamily, and slow inhibition through GABA, receptors, which are members of the G-protein-linked receptor superfamily (Fig.2-3) . In both cases, GABA acts by rising membrane conductance for an ion having an equilibrium potential near or more negative than resting membrane potential. Thus, the neuron hyperpolarizes, thus preventing cell firing. The difference is that $GABA_A$ receptors act by generating a large $C1^-$ conductance, whereas $GABA_B$ receptors increase K+ conductance (Olsen and Avoli ,1997). In general , the effects of GABA are depressant . thus, the main role of GABA in the epileptic brain is that of exerting an "antiepileptic influence'' . However , in some cases GABAergic activity may be excitatory, depending on region circuitry [e.g., a GABA synapse can inhibit an inhibitory cell in a given neuronal circuit, which results in a disinhibitory effect].

Inhibitory GABAergic synapses can also play a synchronizing function in neuronal networks participatory in seizure generation . The cell membrane that carries GABA receptors, (Fig.2-3) , may be a nerve terminal instead of a cell body or dendrite. $GABA_B$ receptors indeed are localized to nerve endings, where they inhibit transmitter release (Thompson *et al* , 1993). As they are situated at excitatory terminals, their activation inhibits glutamate release; So, the overall effect is a decrease in excitation. However, when $GABA_B$ receptors are situated at inhibitory terminals (so-called autoreceptors), their activation will cause a decreased release of GABA, which may result in an excitatory influence . GABA, receptor-activated

chloride channels can depolarize and even cause firing of action potentials (APs) in some neurons . Depolarization may appear because in some circumstances $GABA_A$ receptor-activated channels become permeable to HCO,-, this anion has an equilibrium potential more positive than that of C1- (Staley *et al.* , 1995).

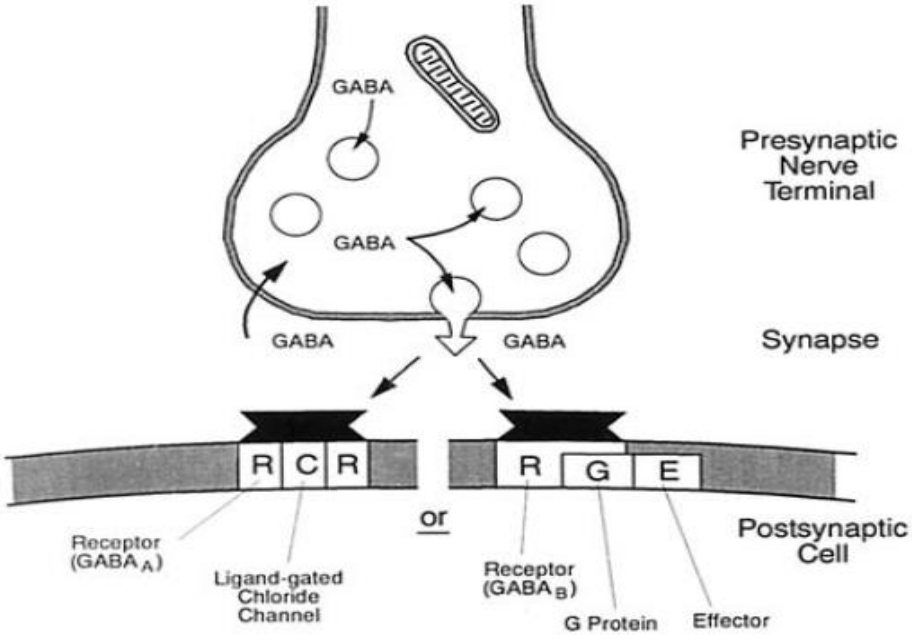


Figure 2.3 Schematic of synapse with $GABA_A$, and $GABA_B$ receptor. GABA is synthesized in the presynaptic nerve terminal and released upon stimulation. It enters the synaptic cleft, where it interacts with a receptor site on the postsynaptic cell membrane. Two types of receptors (R) inhibit the postsynaptic cell: $GABA_A$, receptors, which are ligand-gated chloride channels (C); and $GABA_B$ receptors, which regulate intracellular second messengers through G-proteins (G) coupled to various effector protein systems (E). Most cells have either type A or type B GABA receptors, but some have both. In addition, the postsynaptic cell may actually be a nerve terminal, in which case the GABA neuron inhibits the release of neurotransmitter from that second neuron, producing presynaptic inhibition of the third neuron that is postsynaptic to that nerve terminal (Chou *et al.* ,2003) .

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جمهورية العراق
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جامعــة المثنى
كليـــة العـــــلوم
قسم علوم الحياة

تحليل تغايرالاشكال للجينات SCN1A , SCN2A and GABRG2 كعوامل خطر وراثية للصرع الحراري في مدينة السماوة

رسالة مقدمة إلى مجلس كلية العلوم / جامعة المثنى كجزء من متطلبات نيل درجة الماجستير
علوم / علوم الحياة / علم الحيوان

من قبل

مها سلطان علي

بكالوريوس علوم حياة 2005

بإشراف

أ . م . د . ليث عبد الحسن محمد جواد

1438 2017

الخلاصة

الصرع الحراري هي نوبات تشنجية تحدث لدى الاطفال ما بين 6 شهور الى 5 سنوات من العمر نتيجة ارتفاع درجة الحرارة (اكثر من 39 ºم) ، وتعد التشنجات الحرارية اكثر انواع التشنجات انتشارا في الاطفال تبلغ نسبة حدوثها 3- 5% من الاطفال . ويرتبط التشنج الحموي بعلاقة وطيدة بالوراثة وتاريخ العائلة المرضي ، وان هناك خطر التعرض للصرع مستقبلا (النوع المعقد) .

وفي هذه الدراسة تم تشخيص تأثير ثلاث جينات في الصرع الحراري وعلاقتها مع بعض الاختبارات الكيميائية حيث جمعت 50 عينة (دم) من الاطفال المتعرضين لحالة الصرع الحراري خلال الفترة من شهر كانون الاول 2015 الى شهر آذار 2016 الذين يأتون لقسم الطوارئ في مستشفى الاطفال في محافظة المثنى ، وشملت الدراسة 25 عينة من الذكور و 25 عينة من الاناث . ($P\leq0.05$)

وتم اجراء الاختبارات الكيميائية لكل العينات التي شملت (الكلوكوز ، الكرياتينين ، البوتاسيوم) بواسطة جهاز Reflotron ، والاختبارات الكيميائية (الصوديوم ، الفسفور ، الكلورايد ، والكالسيوم) بواسطة جهاز Spectrophotometer .

اظهرت النتائج ارتفاع مستوى الكلوكوز والفسفور وانخفاض مستوى الصوديوم والكلور عند الاطفال المتعرضين للصرع الحراري ,الا ان مستوى الكرياتينين والكالسيوم والبوتاسيوم كان طبيعيا . ($P\leq0.05$)

وقد تم استخدام تقنية تقييد قطع طول تعدد الاشكال RFLP-PCR لتشخيص A/G للجين (c.3184)SCN1A , G/A للجين (c.56) SCN2A و C/T للجين GABRG2 (SNP211037)، حيث ظهر عدم وجود فروق معنوية في نسب التركيب الوراثي وتكرار ألاليلات للجينين (SCN1A , SCN2A) بين الاطفال المتعرضين للصرع الحراري والاطفال غير المتعرضين ، الا ان هذين الجينين من الجينات المسؤولة عن الصرع ووجودها في حالة الصرع الحراري قد يساعد على تحفيز النوبات الا انها ليست مسؤولة عن الصرع الحراري . ووجود فروق معنوية للاليل GABRG2 (SNP 211037)-CC (p =0.0243) وكذلك وجود فروق معنوية في تكرار الاليل GABRG2 (SNP 211037)-C (p =0.0172) بين الاطفال المتعرضين للصرع الحراري والاطفال غير المتعرضين لهذه الحالة ، وهذه النتائج تقترح ان جين GABRG2 هو احد الجينات المسؤولة عن الصرع الحراري . وكذلك لوحظ عدم وجود علاقة بين هذه الجينات والاختبارات الكيميائية بين الذكور والاناث .

الاقرار

نحن نشهد كأعضاء لجنة مناقشة قد اطلعنا على هذه الرسالة الموسومة ب (**تحليل تغايرالاشكال للجينات SCN1A , SCN2A and GABRG2 كعوامل خطر وراثية للصرع الحراري في مدينة السماوة**) وقد ناقشنا الطالبة (مها سلطان علي) في تاريخ 16 / 7 / 2017 ، ووجدنا بأن الرسالة تفي بمستوى الحصول على درجة الماجستير علوم / علوم حياة / علم الحيوان .

أ.د. حسن ريسان مبارك

(رئيسا)

جامعة ذي قار / كلية التربية للعلوم الصرفة

تاريخ : / / 2017

أ.م.د. علي مانع حسين

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عميد الكلية