ASESSMENT OF SCN1A, SCN2A, EPM2A GENE MUTATUION IN DRAVET SYNDROME EPILEPSY SEIZURE DISORDER, IN HUMAN, TABRIZ, IRAN

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ABSTRACT
In this study we have analyzed 100 people. 40 patients and 60 control group had sleep disturbances. The genes SCN1A, SCN2A, EPM2A analyzed in terms of genetic mutations made. In this study, people who have genetic mutations were targeted, with dravet syndrome nervous disorders, seizure or epilepsy. In fact, of all people with seizure or epilepsy, 40 people had a genetic mutation in the genes SCN1A, SCN2A, EPM2A. Any genetic mutations in the target genes control group, did not show.

KEYWORDS: Genetic study, Seizure or Epilepsy, Mutation SCN1A, SCN2A, EPM2A, Real Time PCR, Neuro Genetics, Epigenetics, dravet syndrome.

INTRODUCTION
Today nervous disorders in humans, a lot of it is mental illness in humans. seizure or epilepsy disorders in humans stems from several factors. Factors such as malnutrition, polluted ecosystems to harmful chemical and physical factors, family problems and most important genetic changes. In this study, there are three important gene in human seizure or epilepsy disorders assessed. In this study, gene EPM2A meaningful and meaningless in terms of genetic mutations, were analyzed.
Epilepsy is a group of neurological diseases characterized by epileptic seizures.\cite{1,2} Epileptic seizures are episodes that can vary from brief and nearly undetectable to long periods of vigorous shaking.\cite{3} These episodes can result in physical injuries including occasionally broken bones.\cite{3} In epilepsy, seizures tend to recur, and have no immediate underlying cause.\cite{1} Isolated seizures that are provoked by a specific cause such as poisoning are not deemed to represent epilepsy.\cite{4} People with epilepsy in some areas of the world experience stigma due to the condition.\cite{3}

The cause of most cases of epilepsy is unknown, although some people develop epilepsy as the result of brain injury, stroke, brain tumors, infections of the brain, and birth defects.\cite{3} Known genetic mutations are directly linked to a small proportion of cases.\cite{5,6} Epileptic seizures are the result of excessive and abnormal nerve cell activity in the cortex of the brain.\cite{4} The diagnosis involves ruling out other conditions that might cause similar symptoms such as fainting and determining if another cause of seizures is present such as alcohol withdrawal or electrolyte problems. This may be partly done by imaging the brain and performing blood tests. Epilepsy can often be confirmed with an electroencephalogram (EEG), but a normal test does not rule out the condition.\cite{5}

Epilepsy that occurs as a result of other issues can be prevented.\cite{3} Seizures are controllable with medication in about 70% of cases.\cite{7} Inexpensive options are often available.\cite{3} In those whose seizures do not respond to medication, then surgery, neurostimulation, or dietary changes may be considered.\cite{8,9} Not all cases of epilepsy are life long, and many people improve to the point that treatment is no longer needed.\cite{3}

As of 2013 about 22 million people have epilepsy.\cite{10} Nearly 80% of cases occur in the developing world.\cite{3} In 2013 it resulted in 116,000 deaths up from 112,000 deaths in 1990.\cite{11} Epilepsy becomes more common as people age.\cite{12,13} In the developed world, onset of new cases occurs most frequently in babies and the elderly.\cite{14} In the developing world onset is more common in older children and young adults, due to differences in the frequency of the underlying causes.\cite{15} About 5–10% of people will have an unprovoked seizure by the age of 80,\cite{16} and the chance of experiencing a second seizure is between 40 and 50%.\cite{17} In many areas of the world those with epilepsy either have restrictions placed on their ability to drive or are not permitted to drive until they are free of seizures for a specific length of time.\cite{18} The word epilepsy is from Ancient Greek: ἐπιλαμβάνειν "to seize, possess, or afflict".\cite{19}
Dravet syndrome spectrum disorders are rare genetic epileptic encephalopathies (dysfunction of the brain) with onset during the first year of life in an otherwise healthy infant. There is a spectrum of severity ranging from no clinical symptoms, to simple febrile seizures, and extending to Dravet syndrome, which is the most severe. Mutations of the SCN1A gene cause 79% of diagnosed cases of Dravet syndrome. Frequently referred to as a sodium channelopathy, this intractable (uncontrollable) epilepsy is characterized by unilateral (one-sided) clonic or tonic clonic (grand mal) seizures that may be prolonged (> 5 minutes) or progress to status epilepticus (>30 minutes). Myoclonic seizures, often called myoclonic jerks, are common but not always present. Over time seizures present without fever, illness or heat triggers. Seizures are frequent and resistant to treatment. The first seizure is often associated with vaccine administration at six months of age. Infants eventually develop other seizure types including atypical absence, eyelid myoclonia and non-convulsive seizures. Multiple drug therapy is necessary for acceptable seizure control. Some anti-epileptic drugs exacerbate seizures and should be avoided. In most cases, surgery is not indicated. The initial EEG is normal but within the second or third year of life, brief generalized spike, polyspike, or polyspike-wave paroxysms appear. MRI and metabolic studies are normal. Developmental delays appear to varying degrees in most patients by age two years and ataxia (abnormal gait) is common. Appropriate and aggressive seizure management, and implementation of global therapies are necessary to improve the outcome of children affected with Dravet syndrome spectrum disorders.

**Signs & Symptoms**

Febrile seizures (FS, FS+): childhood seizures that occur only in association with fever:

- Onset between ages 3 months and 6 years
- Seizures only with fever usually higher than 38° C (without evidence of CNS infection)
- Gene mutations associated with febrile seizures: FEB1, FEB2, FEB4, SCN1B, SCN1A, GABAA and GABRG2
- FEB4 may be the most common gene mutation in FS
- Treatment with drugs is not usually necessary unless seizures are prolonged, child typically outgrows prior to adolescence. Development is normal.
- 2008 International League Against Epilepsy (ILAE) classification for Febrile Seizures: seizure disorder that is not traditionally given the diagnosis of epilepsy. Febrile Seizures+ (FS+) is classified under Genetic and Developmental Epilepsy Syndromes. ILAE’s 2010 Revised Terminology and Concepts for Organization of Seizures and Epilepsies: Report
of the ILAE Commission on Classification and Terminology, 2005–2009 groups FS under “Electroclinical syndromes and other epilepsies” and further, “Conditions with epileptic seizures that are traditionally not diagnosed as a form of epilepsy per se”. In the same report, ILAE suggests that FS+ should be grouped under the term “Electroclinical syndromes and other epilepsies”, which are arranged by age. The age of onset for FS+ in this report is defined as childhood. Both FS and FS+ are considered genetic (inherited) conditions. Children with FS+ may have prolonged seizures with fever or atypical seizures outside of generalized tonic clonic seizures during fever. Development is typically normal and child usually outgrows seizures by adolescence.[20]

Genetic epilepsy with febrile seizures plus (GEFS+): refers to a family rather than an individual:
- Onset in infancy or early childhood (before 36 months)
- Often presents as severe or atypical febrile seizure (FS+)
- Occasional tonic, clonic, myoclonic, or absence seizures
- Usually responsive to medication, seizures remit by late childhood or early adolescence
- Good prognosis for cognitive development in most cases; however, spectrum of intellectual ability exists
- Offspring of affected parent, siblings, or identical twins may express different phenotypes implying genetic mosaicism (parent may carry mutated genes in DNA other than blood including egg or sperm), environmental, or multi-genetic variability
- In the 2008 International League Against Epilepsy (ILAE) classification revisions “Genetic” replaces “Generalized” Epilepsy with Febrile Seizure-Plus (GEFS+) and is classified under “Genetic and Developmental Epilepsy Syndromes”. No further information regarding GEFS+ is listed in ILAE’s 2010 Revised Terminology and Concepts for Organization of Seizures and Epilepsies: Report of the ILAE Commission on Classification and Terminology, 2005–2009.
- GEFS+ does not fit the current understanding of a syndrome as disorder with relative phenotypic homogeneity that can be recognized on an individual level.
- GEFS+ is now termed its “own cluster” or “phenomenon” and is listed under Genetic and Developmental Epilepsy syndromes.

SCN1A, SCN2A, SCN1B, GABAA, GABRD, GABRG2 gene mutations are associated with GEFS+ Epilepsy with mental retardation limited to females (EMRF):
• EMRF is a recently discovered disease that shares many Dravet syndrome features but occurs only in females. Scientists are working to understand the full spectrum of this disorder and to discover improved treatment.
• X-linked disorder affecting females and sparing males (father carries the gene but is unaffected, transmits it to female offspring).
• Caused by mutation in protocadherin 19 gene (PCDH19).
• Seizure onset in first three years.
• Multiple seizure types, including febrile seizures, drug resistant.
• Intellectual ability varies from normal to severely delayed, but most (67%) patients have borderline intellectual ability (typically a better prognosis vs. Dravet Syndrome). Autistic traits are common.

The following electroclinical syndromes are within the Dravet syndrome spectrum: Severe myoclonic epilepsy of infancy borderline (SMEB):

A variety of definitions for SMEB exist in the literature, and often SMEB is interchangeable with intractable childhood epilepsy with generalized tonic clonic Seizures (ICE-GTC).

Children with SMEB do not have myoclonic seizures or other clinical characteristics associated with Dravet syndrome.

• SMEB and ICE-GTC are associated with the same gene mutations as Dravet.
• syndrome, including SCN1A, SCN1B, SCN2A, GABRD, GABRG2. SCN1A is the predominant gene mutation responsible for disease.
• Onset before age one year.
• 69% have an SCN1A mutation.
• Seizures may be frequent, prolonged, and include status epilepticus or may present on the milder end of the spectrum.
• SMEB does not imply a more favorable cognitive prognosis.
• Not listed in ILAE classification system outside of DS.
• Intractable childhood epilepsy with generalized tonic-clonic seizures (ICE-GTC): ICE-GTC is distinguished by the absence of other generalized seizure types, specifically myoclonic seizures.[21]
• Onset before age one year.
Seizures are frequent, prolonged, GTC seizures evolve to status epilepticus frequently, fever and illness are triggers. Myoclonic seizures are not present.

Poor prognosis for cognitive development; distinction from DS is difficult; alternating hemi-convulsions and or myoclonic seizures suggest a DS diagnosis.

2008 ILAE classification system: not listed.

SCN1A mutation in 79% reported cases; GABAA and GABRG2 mutations are also reported.

Dravet syndrome

Dravet syndrome is considered the most severe of the SCN1A related epilepsies.

Onset before age one year

Up to 79% diagnosed have an SCN1A mutation

Initial EEGs are usually normal, but become abnormal over time – generally by age 4 years

Early seizures are often prolonged febrile seizures, or status epilepticus (>30 min)

Modest temperature elevation (low grade fever, warm bath, physical exertion), vaccines, illness, excitation, and light fractionation are common triggers

Hemi convulsive, myoclonic, GTC, absence, atypical absence seizures, and non-convulsive seizures with or without fever appear over time: “eye blink seizures” which may be myoclonic or atypical absence commonly appear in the toddler years; prolonged seizures, status epilepticus, and non-convulsive status epilepticus is common; seizures are treatment resistant and a multiple drug regimen is necessary for acceptable seizure control.

A seizure type “obtundation status”, implying non-convulsive seizures with impairment of consciousness with variable intensity lasting hours to days; EEG shows diffuse dysrhythmia of slow waves with focal and diffuse spikes.

Infants are typically developing prior to seizure onset

Slowing in development noted by age 2 in most cases and regression of acquired skills and/or developmental delay usually appear in varying degrees; however, there are reports of normal development in children diagnosed with Dravet syndrome. Autistic spectrum disorder, attention deficit disorder, and other behavioral disorders are common as are sleep disturbances.[21]
- Painful orthopedic conditions may result from low motor tone, pronation, and abnormal gait.
- Neurologic symptoms such as ataxia or tremor appear over time.
- Mortality rate is up to 20% by age 20 years, due to accident or sudden unexpected death (SUDEP)
- The 2008 ILAE revision classifies DS as an epileptic encephalopathy with onset during the first year of life and of a fundamental genetic basis, most frequently a sodium channelopathy, and also perhaps a part of the GEFS+ spectrum. DS is also classified in the 2008 revision as a Genetic and Developmental Epilepsy syndrome. ILAE’s 2010 Revised Terminology and Concepts for Organization of Seizures and Epilepsies: Report of the ILAE Commission on Classification and Terminology, 2005–2009 groups under “Electroclinical syndromes and other epilepsies under the age “infancy”. This report defines Dravet syndrome as a genetic syndrome.

![Neuronal voltage-gated Na⁺ channel](image)

**Causes**

Mutations of the SCN1A gene cause 79% of diagnosed cases of Dravet syndrome. To date, more than 400 SCN1A mutations have been identified. The type or location of the mutation is not well correlated to severity of illness or cognitive outcome, however, de-novo mutations (mutation not passed from parent) are more likely to be associated with more severe disease and impaired cognition than mutations passed from a parent. The course of disease or clinical outcome in a child that has inherited an SCN1A mutation from a parent with a less severe
clinical presentation is not clear cut as multi-genetic variability has been reported in the literature and the spectrum of this genetic epilepsy is not completely elucidated.

Genetic and environment factors contributing to this spectrum are not fully understood.

**Affected Populations**

Dravet syndrome is a rare disorder with an incidence estimated between 1:20,000 and 1:40,000 representing about 7% of all severe epilepsies starting before the age of 3 years. Prevalence of SCN1A related seizure disorders, syndromes, and encephalopathies are currently difficult to estimate as the commercial availability of genetic testing is recent.

Febrile seizures affect 2-5% of children in North America and Europe, and 6-9% of children in Japan. Thirty five percent of first and 33% of recurrent febrile seizures had one or more complex features such as focal onset (affecting one side of the brain), duration 10 minutes or greater, or multiple seizures during the illness. Epidemiologic studies of GEFS+ are lacking; no comprehensive epidemiology reports are found in the literature.[22]

**Related Disorders**

More common diagnoses such as trauma, hypoxia, sequelae of meningitis or hemorrhage, infectious or autoimmune cerebritis, vasculitis, paraneoplastic syndrome, toxins (including drug withdrawal), endocrinopathy, endocrinopathy pyridoxine-dependent seizures and B6-related epilepsies, folinic acid-responsive seizures, inborn errors of metabolism, including mitochondrial dysfunction, and glucose transporter type 1 deficiency should be ruled out before less obvious genetic mutations are implicated as cause of seizure activity.

If there is a family history of epilepsy including benign familial neonatal seizures, benign familial infantile seizures, benign childhood epilepsy with centrotemporal spikes, childhood occipital epilepsy, absence epilepsies it is worthwhile to determine if child has one of these epilepsy types.

SCN1A mutations have also been reported in children diagnosed with a cryptogenic generalized epilepsy and cryptogenic focal epilepsy.
Diagnosis

Dravet syndrome often presents around age six months, near the time of six month vaccinations. The vaccine, a cold or a fever, are triggers for seizures. If a child presents with a seizure around the time of 6 month vaccination and has a subsequent seizure (especially if seizures prolonged) with or without a trigger prior to the first birthday, SCN1A gene testing may be indicated.[22]

Consider SCN1A testing to help clinical diagnosing of Dravet syndrome when it is not certain and medical history reveals:

I: Infant onset (<12 months); initial development, EEG, MRI, metabolic studies normal; subsequent developmental delays.

C: Clonic hemi-convulsions (one-sided seizure) common; modest temperature elevation and illness are seizure triggers.

E: Episodes may be frequent, prolonged, and are treatment resistant.

Differentiation of the spectrum of SCN1A related epilepsies and overlapping epilepsy syndromes is difficult. Hattori et al developed a scored Dravet syndrome prediction tool for infants less than 12 months:

- An age of onset of febrile seizure < 7 months
- Total number of seizures > 5
• Prolonged seizures lasting more than 10 minutes were regarded as significant risk factors for DS. Other factors highly predictive of DS were hemi-convulsions, partial seizures, myoclonic seizures and hot water – induced seizures.

**Standard Therapies**

**Treatment**

The following drugs should be avoided in children with a diagnosis of Dravet syndrome OR in patients with a confirmed SCN1A gene mutation because they are likely to WORSEN seizures.

Phenytoin (Dilantin), fosphenytoin (Cerebyx, Prodimantin), carbamazepine (Tegretol), oxcarbazapine (Trileptal), lamotrigine (Lamictal), vigabatrin (Sabril), rufinamide (Banzel) and tiagabine (Gabitril). Phenytoin or fosphenytoin are likely to be administered in the emergency department during a prolonged seizure – avoid these drugs in emergency management of children with Dravet syndrome.

Parents should be provided with instructions from the neurologist on how to treat fever and should have a written protocol for emergency management of prolonged seizures (> 5 minutes) including instructions for parents, paramedics, and emergency department staff regarding avoiding phenytoin and fosphenytoin. EEG monitoring in the emergency room setting may be necessary to determine if the child is in non-convulsive status epilepticus.

Dravet syndrome is extremely resistant to treatment. In clinical studies, the most well documented and proven combination of drugs for Dravet syndrome include valproic acid, clobazam, and stiripentol (Diacomit®). Clobazam has been available for more than 25 years outside of the US and was FDA approved in 2011 under the brand name Onfi®. Stiripentol is not FDA approved in the US, but may be obtained by permission from the FDA through an Expanded Access Investigational New Drug Application (IND). A physician must contact FDA in order to get approval for an IND. Please visit www.ICE-Epilepsy.org for more information about obtaining stiripentol. Information on Expanded Access INDs may be found at www.fda.gov. Topirimate (Topamax), zonisamide (Zonegran), levetiracetam (Keppra), and the ketogenic diet also have evidence of efficacy in this syndrome. Use of more than one drug is necessary to get adequate control of seizures in most cases. Felbamate (Felbatol), ethosuximide (Zarontin), and bromides have been useful for controlling certain seizure types. DS often leaves children cognitively and developmentally impaired. Developmental
assessments should begin as early as possible and be repeated regularly. Early implementation of global therapies is essential. Children with DS should receive physical, occupational, speech, and social/play therapies and an enriched environment is encouraged.\[23\]

Expressive and receptive language is often impaired in children with DS and early intervention with speech therapy optimizes potential for improvement.

Pronation of feet often goes unnoticed leading to painful orthopedic conditions by adolescence including ataxia and gait abnormalities. Physical therapy and preventative orthotics may correct or prevent these problems. Low muscle tone is prevalent.

Chronic infection, low humoral immunity, growth, nutrition, and sleep disorders are common. Dysautonomias have been associated and are being studied.

Autistic spectrum, attention deficit hyperactivity disorders (ADHD) and other behavioral disorders, familial autism, as well as headache and psychiatric disorders have been linked to SCN1A, SCN2A and SCN3A mutations.\[23\]

**MATERIALS AND METHODS**

In this study, 40 patients with dravet syndrome and 60 healthy controls were studied. Peripheral blood samples from patients and parents with written permission control was prepared. After separation of serum, using Real Time-PCR technique of RNA molecules were collected. To isolate Neuroglial cells erythrocytes were precipitated from hydroxyethyl starch.
(HES) was used. At this stage, HES solution in ratio of 1:5 with the peripheral blood of patients and controls were mixed. After 70 minutes of incubation at room temperature, the supernatant was removed and centrifuged for 20 min at 500 Gera. The cells sediment with PBS (phosphate buffered saline), pipetazh and slowly soluble carbohydrate ratio of 1:2 on ficole (Ficol) was poured in the 680G was centrifuged for 15 minutes. Mono nuclear Neuroglial cells also are included, has a lower density than ficole and soon which they are based. The remaining erythrocytes has a molecular weight greater than ficole and deposited in test tubes.

The supernatant, which contained the mono nuclear cells was removed, and the 4600 Gera was centrifuged for 19 minutes. Finally, the sediment cell, the antibody and Neuroglial cells was added after 34 minutes incubation at 8 °C, the cell mixture was passed from pillar LSMACS. Then the cells were washed with PBS and attached to the column LSMACSS pam Stem cell culture medium containing the transcription genes SCN1A, SCN2A, EPM2A and were kept.[23]

To determine the purity of Neuroglial cells are extracted, flow cytometry was used. For this purpose, approximately 9-13 × 10^3 Neuroglial cells were transferred to 1.5ml Eppendorf tube and then was centrifuged at 5000 rpm for 7 minutes at time. Remove the supernatant culture medium and there maining sediment, 100μl of PBS buffer was added. After adding 5-10μl CD4^+ PE monoclonal antibody to the cell suspension for 60 min at 4°C, incubated and readimme diately by flow cytometry. For example, rather than control antibody Neuroglial cells PE, IgG1 negative control solution was used.

**Total RNA extraction procedure includes**

a) 1ml solution spilled Qiazolon cells, and slowly and carefully mixed and incubated at room temperature for 8 minutes. Then 400μl chloroform solution to target mix, then transfer the micro tubes were added, and the shaker well was mixed for 19 seconds. The present mix for 9 minutes at room temperature and then incubated for 16 min at 6°C an was centrifuged at 15100 rpm era. Remove the upper phase product were transferred to a new microtube and to the one times the volume of cold ethanol was added. The resulting mixture for 24 hours at -20°C were incubated.

b) Then for 45 min at 4°C an was centrifuged at 15000 rpm era. Remove the supernatant and the white precipitate, 1ml of cold 85% ethanol was added to separate the sediment from micro tubes were vortex well. The resulting mixture for 30 min at 6°C an by the time we
were centrifuged 15000 rpm. Ethanol and the sediment was removed and placed at room temperature until completely dry deposition. The precipitate was dissolved in 30μl sterile water and at a later stage, the concentration of extracted RNA was determined.

To assessment the quality of mi-RNAs, the RT-PCR technique was used. The cDNA synthesis in reverse transcription reaction (RT) kit (Fermentas K1622) and 1μl oligoprimers18 (dT) was performed. Following the PCR reaction 2μM dNTP, 1μg cDNA, Fermentas PCR buffer1X, 0 / 75μM Mg Cl2, 1.25 U / μL Tag DNA at 95°C for 4 min, 95°C for 30s, annealing temperature 58°C for 30s, and 72 °C for 30 seconds, 35 cycles were performed. Then 1.5% agarose gel, the PCR product was dumped in wells after electrophores is with ethidium bromide staining and colors were evaluated.

Figure 1: Schematic view of the band pattern on agarose gels SCN1A gene in multiplex PCR.
Figure 2: Schematic view of the band pattern on agarose gels SCN2A gene in multiplex PCR.

Figure 3: Schematic view of the nucleotide sequence of the mutated gene SCN1A in patients with Dravet syndrome.

Figure 4: Schematic view of the EEG epilepsy patients and the control group.
DISCUSSION AND CONCLUSION

According to the results of sequencing the genome of patients with dravet syndrome, and the genetic mutations SCN1A, SCN2A, EPM2A found that about 99% of patients with dravet syndrome, they have this genetic mutation. Patients with dravet syndrome, unusual and frightening images in the process of epilepsy, experience. Lot epigenetic factors involved in epilepsy. But the most prominent factor to induce dravet syndrome, mutations is SCN1A, SCN2A, and EPM2A. This gene can induce the birth and can also be induced in childhood and adulthood.

Figure 5: Schematic view of the band pattern on agarose gels epilepsy patients and the control group.
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