# *Aquaporin 4 Gene Polymorphism in Children With Febrile Seizure*

## *Febril Nöbet Geçiren Çocuklarda Akuaporin 4 Gen Polimorfizmi*

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**Abstract:** Febrile seizure (FS) has a genetic background. The purpose of this study is to search aquaporin-4 (AQP4) gene polymorphisms in children with FS and to explore their effect on the clinical features of FS. This prospective multicenter, case-control included 122 patients with febrile seizure and matched with age, sex 136 healthy control children. A real-time hybridization method was used to analyze and detect the rs1058424 (A/T) and rs3763043 (C/T) single-nucleotide polymorphisms (SNPs). In patient group, the frequencies of rs3763043 (A/T) genotypes AA, AT and TT were 48.4%, 42.6%, and 9% respectively, compared with 44,9%, 46,3% and 8,8% respectively, in control group. The results showed that the frequencies of rs1058424 (C/T) genotypes CC, CT, and TT 61,5%, 34,4% and 4,1% respectively in children with febrile seizures, compared 64%, 29,4% and 6,6% respectively in controls (p >0.05). The majority of children experienced simple type FS and their first FS. The statistical analysis showed that wild-type genotype was more common in children with simple FS. There was no statistically significant effect of the SNPs on the features of FS, such as family history, number of seizure or duration. The data obtained from molecular analysis show a lack of association between the rs1058424 (A/T) and rs3763043 (C/T) SNPs and FS in children. This is the first research conducted to examine the relationship between AQP4 and FS.
**Key Words:** febrile seizure, aquaporin-4, gene, polymorphism

Carman KB, Tuncel T, Calik M, Karal Y, Isikay S, Kocak O, Ozcelik A, Yazar AS, Nuhoglu C, Sag C, Kilic O, Dinleyici M, Yimenicioglu S, Ekici A, Perk P, Tosun A, Isik I, Yarar C, Karacan H, Atay E, Dinleyici EC. 2019, Aquaporin 4 Gene Polymorphism in Children With Febrile Seizure, *Osmangazi Journal of Medicine*, 41(2): 132-140, **Doi:** 10.20515/otd.469491

**Özet:** Febril nöbeti genetik temeli mevcuttur. Çalışmamızın amacı febril nöbet geçiren çocuklarda akuaporin 4 gen polimorfizminin ve febril nöbet kliniği üzerine olan etkilerinin araştırılmasıdır. Çok merkezli, prospektif olarak yürütülen bu çalışmaya febril nöbet geçiren 122 hasta ve kontrol grubu olarak 136 sağlıklı çocuk alınmıştır. Real time hibridizasyon yöntemi ile rs1058424 (A/T) and rs3763043 (C/T) tek nükleotid polimorfizmleri (SNPs) araştırıldı. Yapılan analiz sonucu rs3763043 (A/T) genotip AA, AT ve TT sıklığı sırasıyla hasta grubunda % 48.4, %42.6, %9, kontrol grubunda %44,9, %46,3% ve %8,8 saptandı (p> 0.05). rs1058424 (C/T) genotip CC, CT, ve TT sıklıkları ise hasta çocuklarda %61.5, %34.4, %4.1 bulunurken bu oranlar kontrol grubunda %64, %29.4 ve %6.6 belirlendi (p> 0.05). Hastaların çoğunda febril nöbet basit tipteydi. İstatistiksel analiz normal genotip basit febril nöbet geçiren çocuklarda daha sık olduğu gösterdi. Polimorfizimle aile öyküsü, nöbet sayısı, süresi gibi nöbet özellikleri arasında istatiksel olarak anlamlı bir farklılık saptanmadı. Ulaştığımız veriler rs1058424 (A/T) ve rs3763043 (C/T) polimorfizmleri arasında bir ilişki olmadığını gösterdi ve bu konuda yapılan ilk araştırmadır.
**Anahtar Kelimeler:** febril nöbet, aquaporin-4, gen, polimorfizm

Çarman KB, Tuncel T, Çalık M, Karal Y, Işıkay S, Kocak O, Özçelik A, Yazar AS, Nuhoğlu C, Sag C, Kılıç Ö, Dinleyici M, Yimenicioğlu S, Ekici A, Perk P, Tosun A, Işık I, Yarar C, Karacan H, Atay E, Dinleyici EC. 2019, Febril Nöbet Geçiren Çocuklarda Akuaporin 4 Gen Polimorfizmi, *Osmangazi Tıp Dergisi,* 41(2):132-140, **Doi:** 10.20515/otd.469491

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## 1. Introduction

Febrile seizure (FS) is a common neurological disorder of childhood. FS is defined as a seizure event that is associated with fever in a child without a history of unprovoked seizure, central nervous system (CNS) infection, electrolyte imbalance, and/or other acute symptomatic events [1-4].

Several studies report that FS presents a complex multifactorial genetic background; in this context, some possible chromosomal locations and susceptibility genes have been put forward, but there is no exact consensus about a disease-related gene or a specific mutation linked with FS[5-7]. In addition, genetic studies have thus far focused on certain groups of FS patients in an effort to discover a new gene (or genes) that could be related to the disease [8].

Aquaporin-4 (AQP4) is the most abundant water channel found in the brain and spinal cord making AQP4 channel proteins primary components of CNS homeostasis [9-12]. AQP4 channel proteins react in response to osmotic dynamics and regulate water transport in astrocytes [13,14]. AQP4 channels also take part in regulating ion concentrations by fine-tuning the water concentration of the cell, emphasizing their role in the general homeostasis of the brain. AQP4 has been reported to be involved in many pathophysiologic conditions, such as neuromyelitis optica, Parkinson's disease, Alzheimer's disease, brain edema, traumatic brain injury, and epilepsy [9,10,15-19]. AQP4 is reported to cooperate with a K+ channel (Kir4.1), forming a water and potassium transport complex that may be involved in neuronal excitability that can account for proneness to seizures [15,20]. The AQP4 knockout mice model was originally generated by targeted gene disruption in 1997 [21]. Seizure susceptibility of AQP4 knockout mice was initially examined using pentylenetetrazol (PTZ), which is a $GABA_A$ antagonist. At 40 mg/kg PTZ (intraperitoneal injection [IP]), all wild-type mice exhibited seizure activity, whereas six of seven AQP4 knockout mice did not exhibit seizure activity. At 50 mg/kg PTZ (IP), both groups exhibited seizure activity; however, the latency to generalized (tonic-clonic) seizures was longer in AQP4 knockout mice [22].

The expression of AQP4 is inducible by cytokines [23]. Many studies about epilepsy have found that cytokines and immune cells display differences; they suggest that nervous factors are predominant in epileptogenesis. Interleukin-1ß (IL-1ß), tumor necrosis factor-α, and interleukin-6 (IL-6) are three important cytokines in the CNS [24]. Proinflammatory cytokine secretion is significantly reduced among AQP4 knockout mice [25]. Therefore, it is speculated that AQP4 may regulate inflammatory cytokine expression in FS.

The AQP4 protein encoded by the AQP4 gene, which is located in chromosome 18, consists of five exons and four introns and, through the alternative splicing mechanism of the AQP4 protein, has two isoforms [26]. Coding regions of AQP4 are highly conserved, but noncoding regions represent high sequence variation [20]. Coding nucleotide variants of AQP4 may influence conformational changes on protein structure, and this may alter proper-functioning AQP4 proteins; nevertheless, noncoding regions of the AQP4 gene are also thought to be involved in transcriptional and post-transcriptional regulation because of the binding affinity of transcriptional factors. In particular, the three-prime untranslated region (3'UTR) may be a target for miRNAs, which effect the expression patterns of a gene [26]. Wei et al [27] analyzed possible miRNA binding sites on AQP4 using TargetScan (http://www.targetscan.org), revealing that miR-323-3p has a putative binding site to single-nucleotide polymorphism (SNP) rs1058424. In this respect, we selected two AQP4 3'UTR SNPs (rs1058424 and rs376043) to investigate possible associations with FS.

## 2. Materials and Methods

### *Study Population*

This prospective multicenter study was performed in eight different cities between

March 1, 2017 and November 1, 2017. We selected these eight cities from different geographical areas of Turkey to ensure that the country is well represented. All centers enrolled all admitted cases. This study was approved by the Eskisehir Osmangazi University Local Ethical Committee. Written informed consent was obtained from the parents of all children.

FS is defined as a seizure event that is associated with fever in a child without a history of unprovoked seizure, CNS infection, electrolyte imbalance, and/or other acute symptomatic events. FS is classified as "simple" if the seizure is generalized, occurred only once within 24 hours, and lasted less than 15 minutes. FS is classified as "complex" if the seizure was focal, recurred within 24 hours, or persisted for more than 15 minutes [1,2,4].

The previous medical history and demographical features of all children were recorded, and the previous seizure histories and family histories were evaluated. Detailed physical examinations, including neurological examinations, were noted. Daycare attendance was also inquired about and noted. Finally, the final diagnosis, presence of hospitalization, duration between the onset of fever and seizures, white blood cell counts (WBC, as $mm^3$), and C-reactive protein (CRP, mg/Dl) levels were recorded.

One hundred and thirty-six healthy children, of comparable age and sex and without a history of febrile or afebrile seizures or any neurological disorders, were enrolled as a control group.

***Genetics***

Blood samples were provided by participants, using ethylenediaminetetraacetic acid (ETDA) tubes. DNA extraction was done using Quick Gene DNA Whole Blood Kit S (Kurabo, Japan). First, 30 µl proteinase K, 200 µl blood, and 250 µl lysis buffer were added to Eppendorf tubes. After two minutes of incubation at 56°C, 250µl ethanol was added. The mix was transferred to filtered tubes, after the tubes were vortexed. Washing was done three times with 750µl wash buffer, supplied by the kit. Before five minutes of incubation at room temperature, 100µl of elution buffer was added. Finally, the DNA samples, fixed to the filter, were transferred to Eppendorf tubes with a Quick Gene Mini 80 device (Kurabo, Japan). Quantitation of the extracted DNA was then completed (Nanodrop, ThermoScientific).

The real-time hybridization method was used to analyze and detect rs1058424 (A/T) and rs3763043 (C/T) SNPs (Roche Light Cycler 480II, Switzerland). The primer-probes are listed in Table 1. The PCR mix was prepared using Diagen Mastermix AXI-1 (Ankara/Turkey). The PCR protocol began with pre-denaturation at 95°C for 30 seconds. Then, pre-denaturation at 95°C for one second, 55°C for 10 seconds, and 72°C for 5 seconds was followed for 45 cycles. After the PCR cycles, the melting-curve step was started at 95°C for 1 second and 35°C for 1 minute. The melting curve was produced by increasing the temperature by 1°C for every 0,14 second and taking a reading. Then, the samples were cooled to 40°C for 1 minute.

**Table 1.**
Primer probe list

| Hyb qPCR | 5' Label | 5-Sekans-3 | 3' Label |
|---|---|---|---|
| rs3763043 Primer-Forward | - | CTTTGCCTCTGCCTCTACA | - |
| rs3763043 Primer-Reverse | - | CCGTGTGTCAAGATTTGGT | - |
| rs3763043 Sensor | - | ACCAATATTCCAGTAGAGAAGGAATAAGCT | FAM |
| rs3763043 Anchor | CY5 | TAGACGTGTCTTTGAGTTCTGTCAGGCAAGACTTA | Phosphate |
| rs1058424 Primer-Reverse | - | CTTTGCCTCTGCCTCTACA | - |
| rs1058424 Primer-Forward | - | CCGTGTGTCAAGATTTGGT | - |
| RS1058424 Sensor | CY5 | GTCCCACATTACCTTGGGCA | Phosphate |
| RS1058424 Anchor | - | GGATTTCTGAATATTCTTTTCAAATTTCTGGGCTT | Fitc |

### *Statistical Methods*

Statistical analyses were performed using SPSS version 10.0 software (SPSS, Inc, Chicago, IL). The data of FS duration, temperature and WBC that appears to follow normal distribution was analyzed using *One-Way ANOVA* and the results were expressed as mean and standart deviation (SD). The p value of less than 0.05 was considered significant.

The categorized variables were analyzed with Chi-square test and it is considered significant if p value less than 0.05.

## 3. Results

### *Patient Demographics and Clinical Features of FS*

One hundred twenty-two children (67 boys and 55 girls) aged between 2 and 60 months, who experienced FS and were admitted to emergency units from 8 cities between March 1, 2017 and November 1, 2017, were evaluated. The control group was composed of 136 children (72 boys and 64 girls).

Sixty-seven out of 122 (54.9%) of the children were admitted after experiencing an FS episode for the first time. Of all the FS cases examined, 70.5% (86/122) were classified as "simple" FS and 29.5% (36/122) were classified as "complex" FS. The presence of a positive family history of febrile and/or afebrile seizures was 36.9% (45/122) of the children and 12.3% (15/122) of the children, respectively. Five children had a history of daycare attendance.

The mean measured peak body temperature was 38.21°C (±1.05) and ranged between 36.2°C and 41.1°C. The mean interval between the onset of fever and seizure was 6.17 ±8.35 hours. The final diagnosis was upper respiratory tract infections in a majority of children (67.2%).

The mean WBC count and CRP levels of the children in the study was measured as 12866 ± 5746/mm$^3$ and 6.65± 18.63 mg/L, respectively. Demographic and clinical features are summarized in Table 2.

**Table 2.**
Demographic and clinical findings about children with FS.

| | |
|---|---|
| **Gender (n, %)** | |
| **Boys** | 67 (54.9) |
| **Girls** | 55 (45.1) |
| **Age (months) mean ± SD** | 27.67 ± 16.37 |
| **Type of FS (n, %)** | |
| **Simple** | 86 (70.5) |
| **Complex** | 36 (29.5) |
| **First FS (n, %)** | 67 (54.9) |
| **Recurrent FS (n, %)** | 55 (45.1) |
| **Family history of FS (n, %)** | 63 (36.2) |
| **Family history of epilepsy (n, %)** | 18 (10.3) |
| **Cause of fever diagnosed** | |
| **URTI (n, %)** | 82 (67.2) |
| **LRTI (n, %)** | 26 (32.3) |
| **Other (n, %)** | 14 (11.5) |

*FS: febrile seizure; URTI: upper respiratory tract infection; LRTI: lower respiratory tract infection; SD: standard deviation*

***Genetic Analysis Results***

The results of the genetic analysis revealed that the rs3763043 polymorphism was detected as heterozygous in 36.88% of the children with FS and homozygous in 7.80% of the children. The results for the rs1058424 polymorphism were 28.37% and 6.38%, respectively. The statistical analyses showed that there was no statistically significant difference between the control and study groups (Table 3).

**Table 3.**
Analysis of the genotypes

| Gene polymorphism | Alleles / Genotypes | Patients (n=122), n% | Controls (n= 136), n% | Test value $\chi^2$; p |
|---|---|---|---|---|
| | A | 170 (69,7) | 185 (68) | |
| | T | 74 (30,3) | 87 (32) | |
| rs3763043 (A/T) | AA | 59 (48,4) | 61 (44,9) | |
| | AT | 52 (42,6) | 63 (46,3) | 0.370 ; 0.830 |
| | TT | 11 (9) | 12 (8,8) | |
| | C | 192 (78,7) | 214 (78,7) | |
| | T | 52 (21,3) | 58 (21,3) | |
| rs1058424 (C/T) | CC | 75 (61,5) | 87 (64) | |
| | CT | 42 (34,4) | 40 (29,4) | 1.545 ; 0.461 |
| | TT | 5 (4,1) | 9 (6,6) | |

The statistical analysis showed that wild-type genotype was more common in children with simple FS ($p<0.005$). There was no relationship between polymorphisms and gender, seizure recurrence, or family history of febrile/afebrile seizure (Table 4).

**Table 4.**
Relationship between sociodemographic features and genotype

| | rs3763043 | | | | Test value |
|---|---|---|---|---|---|
| | Wild n(%)* | Heterozygous n(%)* | Homozygous n(%)* | Total n(%)** | $\chi^2$; p |
| **Gender** | | | | | |
| Male | 30 (44.8) | 31 (46.3) | 6 (9.0) | 67 (54.9) | 0.859; 0.651 |
| Female | 29 (52.7) | 21 (38.2) | 5 (9.1) | 55 (45.1) | |
| **Consaguinity** | | | | | |
| Positive | 12 (44.4) | 12 (44.4) | 3 (11.1) | 27 (22.1) | 0.306; 0.858 |
| Negative | 47 (49.5) | 40 (42.1) | 8 (8.4) | 95 (77.9) | |
| **Family history of FS** | | | | | |
| Positive | 20 (44.4) | 21 (46.7) | 4 (8.9) | 45 (36.9) | 0.501; 0.778 |
| Negative | 39 (50.6) | 31 (40.3) | 7 (9.1) | 77 (63.1) | |
| **FS type** | | | | | |
| Simple | 16 (44.4) | 15 (41.7) | 5 (13.9) | 36 (29.5) | 1.518; 0.468 |
| Complex | 43 (50.0) | 37 (43.0) | 6 (7.0) | 86 (70.5) | |
| **FS number** | | | | | |
| 1 | 32 (47.8) | 28 (41.8) | 7 (10.4) | 67 (54.9) | 0.373; 0.830 |
| > 1 | 27 (49.1) | 24 (43.6) | 4 (7.3) | 55 (45.1) | |
| **Total** | **59 (48.4)** | **52 (42.6)** | **11 (9.0)** | **122 (100)** | |
| | | **rs 1058424** | | | **Test value** |

| | Wild n (%)* | Heterozygous n(%)* | Homozygous n(%)* | Total n(%)** | $\chi^2$; p |
|---|---|---|---|---|---|
| **Gender** | | | | | |
| Male | 40 (59.7) | 25 (37.3) | 2 (3.0) | 67 (54.9) | 0.652; 0.722 |
| Female | 34 (61.8) | 18 (32.7) | 3 (5.5) | 55 (45.1) | |
| **Consaguinity** | | | | | |
| Positive | 16 (59.3) | 9 (33.3) | 2 (7.4) | 27 (22.1) | 0.974; 0.615 |
| Negative | 58 (61.1) | 34 (35.8) | 3 (3.2) | 95 (77.9) | |
| **Family history of FS** | | | | | |
| Positive | 27 (60.0) | 17 (37.8) | 1 (2.2) | 45 (36.9) | 0.747; 0.688 |
| Negative | 47 (61.0) | 26 (33.8) | 4 (5.2) | 77 (63.1) | |
| **FS type** | | | | | |
| Simple | 17 (47.2) | 15 (41.7) | 4 (11.1) | 36 (29.5) | **8.245; 0.016** |
| Complex | 57 (66.3) | 28 (32.6) | 1 (1.2) | 86 (70.5) | |
| **FS number** | | | | | |
| 1 | 38 (56.7) | 26 (38.8) | 3 (4.5) | 67 (54.9) | 0.967; 0.617 |
| > 1 | 36 (65.5) | 17 (30.9) | 2 (3.6) | 55 (45.1) | |
| **Total** | **74 (60.7)** | **43 (35.2)** | **5 (4.1)** | **122 (100)** | |

**Percentage by rows*
*** Percentage by columns*

The results of present study show that neither rs3763043 nor rs1058424 affects the duration of FS. The mean measured peak body temperature of children with FS was 38.21°C (±1.05). The results revealed that peak mean temperature and WBC were not affected by rs3763043 or rs1058424 polymorphisms (Table 5).

**Table 5.**
Relationship between seizure and patient's characteristics and genotype

| Variable | Wild | rs3763043 Heterozygous | Homozygous | Test value *F*: p |
|---|---|---|---|---|
| | Mean, (±SD) | Mean (±SD) | Mean (±SD) | |
| **FS duration (minutes)** | 5.50 (8.15) | 6.23 (7.84) | 9.65 (11.37) | 1.046; 0.355 |
| **Variable** | **Wild** | **rs 1058424 Heterozygous** | **Homozygous** | **Test value *F*: p** |
| | **Mean, (±SD)** | **Mean (±SD)** | **Mean (±SD)** | |
| **FS duration (minutes)** | 5.14 (7.61) | 7.35 (8.67) | 11.0 (13.76) | 1.740; 0.181 |
| | **wild** | **rs3763043 Heterozygous** | **Homozygous** | **Test value *F*: p** |
| | **mean, (±SD)** | **mean (±SD)** | **mean (±SD)** | |
| **Temperature ($C^0$)** | 38.28 (1.07) | 38.25 (1.05) | 37.57 (0.69) | 2.066; 0.132 |
| | **wild** | **rs 1058424 Heterozygous** | **Homozygous** | **Test value *F*: p** |
| | **mean, (±SD)** | **mean (±SD)** | **mean (±SD)** | |

| | | | | |
|---|---|---|---|---|
| **Temperature ($C^0$)** | 38.26 (1.11) | 38.13 (0.99) | 37.98 (0.50) | 0.324; 0.724 |
| | **wild** | **rs3763043 Heterozygous** | **Homozygous** | **Test value *F*: p** |
| | **mean, (±SD)** | **mean (±SD)** | **mean (±SD)** | |
| **WBC ($mm^3$)** | 12.21 (5.75) | 12.95 (4.69) | 16.05 (8.95) | 1.939; 0.149 |
| | **wild** | **rs1058424 Heterozygous** | **Homozygous** | **Test value *F*: p** |
| | **Mean, (±SD)** | **Mean (±SD)** | **Mean (±SD)** | |
| **WBC($mm^3$)** | 12.49 (5.44) | 12.79 (5.04) | 18.52 (11.60) | 2.642; 0.076 |

## 4. Discussion

In the present study, AQP4 gene polymorphisms were examined in children with FS. The results of the study reveal no association between FS and the rs1058424 (A/T) or rs3763043 (C/T) SNPs of the AQP4 gene. Although they have not yet been investigated in connection with FS, the two selected SNPs have been researched in relation to other neurological disorders. Burfeind et al. [18] reported that rs3763043 was associated with a more rapid cognitive decline after Alzheimer's disease diagnosis. Another study done in patients with traumatic brain injury revealed that the rs3763043 TT genotype was significantly more prevalent in poor outcomes of traumatic brain injury [19]. Heuser et al. [28] searched the AQP4 and Kir4.1 genes in patients with temporal lobe epilepsy, and the results showed that rs1058424 was associated with temporal lobe epilepsy.

AQP4 channels are the most ubiquitous water channels in the CNS. They are bidirectional water conduits that are highly concentrated in astrocytes [9,11,13]. The AQP4 has been implicated in the pathophysiology of many neurological diseases [9,10,18]. Although its role has previously been evaluated in epilepsy in both animal and human studies, the role of the AQP4 in FS has not yet been researched [28,29]. FS is associated with high levels of proinflammatory cytokines, especially IL-1ß and TNF-α [30,31]. Previous studied reported that polymorphism of these proinflammatory cytokines were associated with FS [6,30,31]. IL-1β is a potent activator of NFκB, which leads to increased AQP4 transcription [32]. When inactive, NFκB is located in the cytoplasm, sequestered by its inhibitory molecule, (IκB). IL-1β causes the increased degradation of IκB and nuclear translocation of NFκB, where it induces the transcription of target genes. A putative binding site for NFκB has been found near exon 1 of the AQP4 gene [23,33]. However, the upregulation of AQP4 by TNF-α has been confirmed in an epithelial cell line [13]. Despite these evidences, the role of AQP4 has not been searched previously.

Studies done with AQP4 knockout mice showed that a delayed resorption of brain water in the first four weeks postnatally. Normally, there is a rapid drop in brain water in this phase, from 87 to 79% [34]. This age corresponds with ages at which FS most often occurs [4,35].

A significant relationship between FS and mesial temporal sclerosis (MTS) has also been reported [36,37]. Biopsy samples of patients with MTS revealed the AQP4 to be significantly elevated [38]. In contrast to this finding, the results of a study done by Bebek et al. [39] showed that the relative expression levels of AQP4 did not differ significantly between MTS patients and healthy controls on tissue samples. However, they noted that two of their patients showed a very prominent decrease in AQP4 relative expression levels; these patients were both over 30 years old and

both had a history of FS and family history of FS. The results of an animal study revealed that the inhibition of AQP4 synthesis by acetazolamide decreased synthesis of IL-1ß, TNF-α, and IL-6 in the hippocampus of rats [40].

The results of present study showed that the most of children experienced simple type febrile seizure and the mean measured peak body temperature was 38.21°C. Although no significant association between rs3763043 or rs1058424 polymorphisms and seizure type and peak body temperature, these were compatible with literature [41].

Our study has some limitations: We searched only two SNPs and our results showed no significant differences between patients and controls. Other studies about SNPs might revealed in different result.

In conclusion, in present study, only two SNPs were researched. The genetic basis for FS may not be related to a single genetic variant but may be influenced by multiple genes acting synergistically with environmental factors to increase the likelihood of the disease developing. Genetic association studies offer a powerful approach for identifying the multiple and sometimes minor variables that modulate susceptibility to this common but complex disease.

## REFERENCES


1. Guidelines for epidemiologic studies on epilepsy. Commission on Epidemiology and Prognosis, International League Against Epilepsy. Epilepsia. 1993;34:592-6.
2. Shinnar S, Glauser TA. Febrile seizures. J Child Neurol. 2002;17 Suppl 1:S44-52.
3. Vestergaard M, Obel C, Henriksen TB, et al. The Danish National Hospital Register is a valuable study base for epidemiologic research in febrile seizures. Journal of clinical epidemiology. 2006;59:61-6.
4. Pediatrics AAo. Steering Committee on Quality Improvement and Management, Subcommittee on Febrile Seizures. Febrile seizures: clinical practice guideline for the long-term management of the child with simple febrile seizures. Pediatrics. 2008;121:1281-6.
5. Saghazadeh A, Mastrangelo M, Rezaei N. Genetic background of febrile seizures. Rev Neurosci. 2014;25:129-1.
6. Haspolat S, Mihci E, Coskun M, Gumuslu S, Ozben T, Yegin O. Interleukin-1beta, tumor necrosis factor-alpha, and nitrite levels in febrile seizures. J Child Neurol. 2002;17:749-1.
7. Camfield P, Camfield C. Febrile seizures and genetic epilepsy with febrile seizures plus (GEFS+). Epileptic Disord. 2015;17:124-3.
8. Mittal R. Recent advances in febrile seizures. Indian J Pediatr. 2014;81:909-6.
9. Iacovetta C, Rudloff E, Kirby R. The role of aquaporin 4 in the brain. Veterinary Clinical Pathology. 2012;41:32-4.
10. Assentoft M, Larsen BR, MacAulay N. Regulation and Function of AQP4 in the Central Nervous System. Neurochem Res. 2015;40:2615-7.
11. Nagelhus EA, Ottersen OP. Physiological roles of aquaporin-4 in brain. Physiol Rev. 2013;93:1543-2.
12. Agre P. Molecular physiology of water transport: aquaporin nomenclature workshop. Mammalian aquaporins. Biology of the Cell. 1997;89:255-5.
13. Hsu Y, Tran M, Linninger AA. Dynamic regulation of aquaporin-4 water channels in neurological disorders. Croat Med J. 2015;56:401-1.
14. Manley GT, Fujimura M, Ma T, Noshita N, Filiz F, Bollen AW et al. Aquaporin-4 deletion in mice reduces brain edema after acute water intoxication and ischemic stroke. Nature medicine. 2000;6:159-3.
15. Binder DK, Nagelhus EA, Ottersen OP. Aquaporin-4 and epilepsy. Glia. 2012;60:1203-4.
16. Matiello M, Schaefer-Klein J, Hebrink D, Kingsbury D, Atkinson E, Weinshenker BG. Genetic analysis of aquaporin-4 in neuromyelitis optica. Neurology. 2011;77:1149-5.
17. Hubbard JA, Szu JI, Yonan JM, Binder DK. Regulation of astrocyte glutamate transporter-1 (GLT1) and aquaporin-4 (AQP4) expression in a model of epilepsy. Experimental neurology. 2016;283:85-6.
18. Burfeind KG, Murchison CF, Westaway SK, Simon MJ, Erten-Lyons D, Kaye JA et al. The effects of noncoding aquaporin-4 single-nucleotide polymorphisms on cognition and functional progression of Alzheimer's disease. Alzheimer's & Dementia: Translational Research & Clinical Interventions. 2017;3:348-9.

19. Dardiotis E, Paterakis K, Tsivgoulis G, Tsintou M, Hadjigeorgiou GF, Dardioti M et al. AQP4 tag single nucleotide polymorphisms in patients with traumatic brain injury. Journal of neurotrauma. 2014;31:1920-6.
20. Sorani MD, Zador Z, Hurowitz E, Yan D, Giacomini KM, Manley GT. Novel variants in human Aquaporin-4 reduce cellular water permeability. Hum Mol Genet. 2008;17:2379-9.
21. Ma T, Yang B, Gillespie A, Carlson EJ, Epstein CJ, Verkman A. Generation and phenotype of a transgenic knockout mouse lacking the mercurial-insensitive water channel aquaporin-4. The Journal of clinical investigation. 1997;100:957-2.
22. Binder DK, Yao X, Zador Z, Sick TJ, Verkman AS, Manley GT. Increased seizure duration and slowed potassium kinetics in mice lacking aquaporin-4 water channels. Glia. 2006;53:631-6.
23. Ito H, Yamamoto N, Arima H, Hirate H, Morishima T, Umenishi F et al. Interleukin-1beta induces the expression of aquaporin-4 through a nuclear factor-kappaB pathway in rat astrocytes. J Neurochem. 2006;99:107-8.
24. Li G, Bauer S, Nowak M, et al. Cytokines and epilepsy. Seizure-European Journal of Epilepsy. 2011;20:249-6.
25. Li L, Zhang H, Varrin-Doyer M, Zamvil SS, Verkman A. Proinflammatory role of aquaporin-4 in autoimmune neuroinflammation. The FASEB Journal. 2011;25:1556-6.
26. Yang T-T, He Y, Xiang Y-J, Ao DH, Wang YY, Zhang Q et al. No association of AQP4 polymorphisms with neuromyelitis optica and multiple sclerosis. Translational Neuroscience. 2016;7:76-3.
27. Wei Q, Yanyu C, Rui L, Caixia L, Youming L, Jianhua H et al. Human aquaporin 4 gene polymorphisms in Chinese patients with neuromyelitis optica. J Neuroimmunol. 2014;274:192-6.
28. Heuser K, Nagelhus EA, Taubøll E, Indahl U, Berg PR, Lien S, et al. Variants of the genes encoding AQP4 and Kir4. 1 are associated with subgroups of patients with temporal lobe epilepsy. Epilepsy research. 2010;88:55-4.
29. Alvestad S, Hammer J, Hoddevik EH, Skare Ø, Sonnewald U, Amiry-Moghaddam M et al. Mislocalization of AQP4 precedes chronic seizures in the kainate model of temporal lobe epilepsy. Epilepsy research. 2013;105:30-1.
30. Serdaroglu G, Alpman A, Tosun A, Pehlivan S, Ozkinay F, Tekgül H, Gökben S. Febrile seizures: interleukin 1β and interleukin-1 receptor antagonist polymorphisms. Pediatric neurology. 2009;40:113-6.
31. Tutuncuoglu S, Kutukculer N, Kepe L, Coker C, Berdeli A, Tekgul H. Proinflammatory cytokines, prostaglandins and zinc in febrile convulsions. Pediatr Int. 2001;43:235-9.
32. Moynagh PN, Williams DC, O'Neill LA. Interleukin-1 activates transcription factor NFκ B in glial cells. Biochemical Journal. 1993;294:343-7.
33. Baldwin AS, Jr. The NF-kappa B and I kappa B proteins: new discoveries and insights. Annu Rev Immunol. 1996;14:649-3.
34. Yao X, Hrabětová S, Nicholson C, Manley GT. Aquaporin-4-deficient mice have increased extracellular space without tortuosity change. Journal of Neuroscience. 2008;28:5460-4.
35. Quinn R. Comparing rat's to human's age: how old is my rat in people years? Nutrition. 2005;21:775-7
36. Cendes F. Febrile seizures and mesial temporal sclerosis. Current opinion in neurology. 2004;17:161-4.
37. Waruiru C, Appleton R. Febrile seizures: an update. Archives of Disease in childhood. 2004;89:751-6.
38. Lee TS, Eid T, Mane S, Kim JH, Spencer DD, Ottersen OP et al. Aquaporin-4 is increased in the sclerotic hippocampus in human temporal lobe epilepsy. Acta neuropathologica. 2004;108:493-2.
39. Bebek N, Ozdemir O, Sayitoglu M, Hatırnaz O, Baykan B, Gürses C, et al. Expression analysis and clinical correlation of aquaporin 1 and 4 genes in human hippocampal sclerosis. J Clin Neurosci. 2013;20:1564-0.
40. Yu H, Qi G, Wang J, Chen L, Deng Z, Zhao YS et al. Aquaporin 4 inhibition decreased synthesis of cytokines by acetazolamide in the hippocampus of rats with pentrazol-induced chronic epilepsy. Genet Mol Res. 2016;15. gmr.15039012
41. Gontko-Romanowska K, Żaba Z, Panieński P, Steinborn B, Szemień M, Łukasik-Głębocka M et al. The assessment of risk factors for febrile seizures in children. Neurol Neurochir Pol. 2017;51(6):454-8