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# Association of *IL6* single nucleotide polymorphisms with febrile seizures

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ABSTRACT

Febrile seizures (FSs) are the most common convulsive event in children. Inflammatory elements and genetics have major roles in their pathogenesis. As of the importance of interleukin-6 (IL-6) in FS, this study was performed to assess *IL6* single nucleotide polymorphisms (SNPs) in a group of patients with FS.
*IL6* gene (−174 and +565) SNPs were studied on genomic DNAs of 90 children with FS, using PCR-SSP method. The results were compared to 139 healthy individuals.
The presence of the G allele or the GG genotype at +565 position reduced risk of FS, while the A allele at +565 position of the promoter regions was a constituted risk factor for developing FS.
This study could support the idea that *IL6* SNPs play a role in the pathogenesis of FS.


## 1. Introduction

Febrile seizures (FSs) are the most common convulsive event in children with a prevalence of 2–5%. The etiology of FS is still unclear. A positive family history of febrile seizure is the most important risk factor, and the more relatives affected, the greater the risk is [1,2]. Twin and family studies suggest that genetic factors may have an important effect on predisposition of FS. This underlying genetic predisposition, along with the association of fever with the seizures, implies the possibility of the involvement of some gene or genes for the regulation of pro-inflammatory and anti-inflammatory cytokines [Interleukin (IL)-1, IL-6 and tumor necrosis factor (TNF)] which cast as an important player in the regulation of febrile responses [3,1,4].

Previous studies revealed increased levels in plasma and cerebrospinal fluid of some inflammatory cytokines in patients with FS. Straussberg et al. reported an increase in the production of IL-1b, IL-6, TNF-a, and IL-10 cytokines by lipopolysaccharide-stimulated mononuclear cells from individuals of 13 FS patients and 11 control without any history of FS, but the secretion of IL-6 and IL-10 in response to lipopolysaccharide was higher in those with a previous history of convulsions [5]. Nur et al. genotyped 92 patients and 98 healthy controls for their polymorphisms, using polymerase chain reaction restriction fragment length polymorphism. They found that the frequencies of −174 G alleles and of the −174 and −572 GG genotypes were significantly higher in patients than in controls. The −174 GG genotype frequency was significantly higher in children with a family history of FS [2]. Hu et al. performed a study on 9 children with FS and 21 with severe acute encephalitis. In multivariate analysis, IL-6 was significantly increased in the plasma of the FS patients compared to those with severe acute encephalitis, suggesting that IL-6 is activated during the acute stage of a FS. A lower plasma IL-6 concentration was significantly associated with severe acute encephalitis [6].

This study was performed to determine the associations between SNPs of the *IL6* gene and FS in a group of Iranian patients.

## 2. Patients and methods

### 2.1. Participants

Ninety children with confirmed diagnosis of FS who were referred to the Department of Pediatric Neurology and Pediatric Emergency Service of the Children's Medical Center Hospital, the Pediatrics Center of

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**Table 1**
Affected relatives in probands with familial FSs.

| Affected relatives with FS | Number of probands (total = 21) |
|---|---|
| A parent | 3 |
| A parent and a sibling | 0 |
| A parent and an uncle/aunt/grandfather | 0 |
| Sibling(s) | 4 |
| Sibling(s) and an uncle/aunt/cousin/grandfather | 0 |
| Uncle(s)/aunt(s) | 11 |
| Other | 3 |

**Table 2**
Demographic data of patients with simple and complex FS.

| | Simple FS (total = 45) | Complex FS (total = 45) |
|---|---|---|
| Gender (M/F) | 28/17 | 23/22 |
| Onset age of FS (months) | | |
| Mean | 38.88 | 23.1 |
| Median | 24 | 19 |

Excellence in Tehran, Iran, were enrolled in this study. The results of the study on the patient group were compared to 139 control individuals from the same region [7] which was not in Hardy–Weinberg equation (Chi-square = 27.686, p = 0.0001).

Family histories were obtained through the pediatricians' interviews with parents. Twenty-one FS patients had a positive family history of FSs in near relatives (familial FS group; Table 1). Informed consent was obtained from the parents of each individual before blood sampling, and the study was approved by the Ethics Committee of Tehran University of Medical Sciences.

### 2.2. Genotyping

DNA was extracted from peripheral blood. Cytokine genotyping was performed, using polymerase chain reaction with sequence-specific primers (PCR-SSP assay kit, Heidelberg University). The method was previously explained in details [7]. Briefly, the gene was amplified using a Tedane Flexigene thermal cycler (Roche). Thereafter, the availability of the PCR products was assessed using 2% agarose gel electrophoresis. The gel was placed on a UV trans-illuminator, and a digital

**Table 3**
Alleles and genotype frequencies in patients with FS and controls.

| Gene polymorphism | Alleles/genotypes | Patients (n = 90) N% | Controls (n = 139) N% | Odds ratio (95% CI) | P-value |
|---|---|---|---|---|---|
| *FS and controls* | | | | | |
| *IL6* (−174) | C | 55(36.2) | 101(36.3) | 0.99 (0.64–1.53) | 0.94 |
| | G | 97(63.8) | 177(63.7) | 1.01(0.65–1.55) | 0.94 |
| | CC | 5(6.75) | 4(2.9) | 2.45(0.55–11.27) | 0.28 |
| | CG | 42(56.75) | 93(66.9) | 0.65(0.35–1.21) | 0.18 |
| | GG | 27(36.5) | 42(30.2) | 1.33(0.7–2.51) | 0.43 |
| *IL6* (+565) | A | 43(28.3) | 50(18) | 1.8(1.1–2.95) | 0.01 |
| | G | 109(71.7) | 228(82) | 0.56(0.34–0.91) | 0.01 |
| | AA | 4(5.4) | 4(2.9) | 1.93(0.39–9.51) | 0.45 |
| | GA | 33(44.6) | 42(30.2) | 1.86(0.99–3.48) | 0.052 |
| | GG | 37(50) | 93(66.9) | 0.49(0.27–0.92) | 0.023 |
| *Simple FS and controls* | | | | | |
| *IL6* (−174) | C | 29(36.7) | 101(36.3) | 1.02(0.59–1.76) | 0.94 |
| | G | 50(63.3) | 177(63.7) | 0.98(0.57–1.71) | 0.94 |
| | CC | 2(5.1) | 4(2.9) | 1.82(0.22–12.25) | 0.61 |
| | CG | 24(61.5) | 93(66.9) | 0.79(0.36–1.79) | 0.66 |
| | GG | 13(33.3) | 42(30.2) | 1.15(0.5–2.62) | 0.86 |
| *IL6* (+565) | A | 25(31.6) | 50(18) | 2.11(1.15–3.85) | 0.01 |
| | G | 54(68.4) | 228(82) | 0.47(0.26–0.87) | 0.01 |
| | AA | 1(2.6) | 4(2.9) | 0.89(–) | 1 |
| | GA | 22(56.4) | 42(30.2) | 2.99(1.36–6.61) | 0.004 |
| | GG | 16(41) | 93(66.9) | 0.34(0.16–0.76) | 0.006 |
| *Complex FS and controls* | | | | | |
| *IL6* (−174) | C | 26(35.6) | 101(36.3) | 0.97(0.55–1.71) | 0.98 |
| | G | 47(64.4) | 177(63.7) | 1.03(0.58–1.83) | 0.98 |
| | CC | 3(8.6) | 4(2.9) | 3.16(0.53–17.91) | 0.14 |
| | CG | 18(51.4) | 93(66.9) | 0.52(0.23–1.18) | 0.13 |
| | GG | 14(40) | 42(30.2) | 1.54(0.67–3.54) | 0.36 |
| *IL6* (+565) | A | 18(24.7) | 50(18) | 1.49(0.77–2.87) | 0.26 |
| | G | 55(75.3) | 228(82) | 0.67(0.35–1.3) | 0.26 |
| | AA | 3(8.6) | 4(2.9) | 3.16(0.53–17.9) | 0.14 |
| | GA | 11(31.4) | 42(30.2) | 1.06(0.44–2.52) | 0.94 |
| | GG | 21(60) | 93(66.9) | 0.74(0.32–1.7) | 0.57 |
| Complex and simple | | | | | |
| | Alleles/genotypes | Complex FS | Simple FS | OR (95% CI) | P |
| *IL6* (−174) | C | 26(35.6) | 29(36.7) | 0.95(0.47–1.95) | 0.97 |
| | G | 47(64.4) | 50(63.3) | 1.05(0.51–2.15) | 0.97 |
| | CC | 3(8.6) | 2(5.1) | 1.73(0.22–16.03) | 0.66 |
| | CG | 18(51.4) | 24(61.5) | 0.66(0.24–1.84) | 0.52 |
| | GG | 14(40) | 13(33.3) | 1.33(0.46–3.84) | 0.72 |
| *IL6* (+565) | A | 18(24.7) | 25(31.6) | 0.71(0.33–1.53) | 0.44 |
| | G | 55(75.3) | 54(68.4) | 1.41(0.65–3.07) | 0.44 |
| | AA | 3(8.6) | 1(2.6) | 3.56(0.3–93.56) | 0.34 |
| | GA | 11(31.4) | 22(56.4) | 0.35(0.12–1.02) | 0.054 |
| | GG | 21(60) | 16(41) | 2.16(0.77–6.1) | 0.16 |

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