

Interleukin-1 β and interleukin-1receptor antagonist polymorphisms in Egyptian children with febrile seizures

A case-control study

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Abstract

Febrile seizure is the most common seizure disorder of childhood. Of the pro-inflammatory cytokines, interleukin-1 is defined as the first endogenous pyrogen.

We designed this study to investigate single-nucleotide polymorphisms (SNPs) situated at positions -31 (C/T), and -511 (C/T) of interleukin-1beta (IL-1 β) gene promoter and interleukin-1receptor antagonist (IL-1RA) gene variable number of tandem repeats in intron 2 (VNTR); to determine whether these polymorphisms could be a marker of susceptibility to febrile seizures in Egyptian children and we also measured the serum level of IL-1 β to assess its relation to such polymorphisms.

This was a case-control study included 155 patients with febrile seizure, and matched with age, sex, ethnicity 155 healthy control subjects. IL-1 β promoter at positions -31 (C/T), -511 (C/T), and IL-1RA gene VNTR polymorphisms were genotyped by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), while the serum IL-1 β levels were measured by enzyme-linked immunosorbent assay (ELISA) method.

The frequency of the IL-1 β -511 TT genotype and T allele at the same position were observed to be increased in patients with febrile seizures (FS) compared with the control group (odds ratio [OR]: 3.96; 95% confidence interval [CI]: 1.68–9.5; $P=0.001$ for the TT genotype and OR: 1.65; 95% CI: 1.18–2.3; $P=0.003$ for the T allele, respectively). The IL-1 RA II/II homozygous variant and IL-1 RA allele II were overrepresented in patients with FS than control group (OR: 4.02; 95% CI: 1.78–9.15; $P=0.001$ and OR: 1.73; 95% CI: 1.24–2.4; $P=0.001$, respectively). We found a significant positive association between the IL-1 RA II/II genotype and susceptibility to FS in sporadic cases as did allele II at the same position (OR: 5.04; 95% CI: 2.1–12.5 for the IL-1 RA II/II genotype; $P=0.001$) and (OR: 1.94; 95% CI: 1.3–2.8 for the allele II; $P=0.001$, respectively). Carriers of the IL-1RA II/II homozygous variant and allele II had significantly higher serum levels of IL-1 β compared with those with other genotypes and alleles.

We demonstrate for the first time that the presence of a T allele or TT genotype at -511 of IL-1 β promoter and IL-1RA II/II genotype constitute risk factors for developing FS in Egyptian children.

Abbreviations: CI = confidence interval, EEG = Electroencephalography, ELISA = enzyme-linked immunosorbent assay, FS = febrile seizure, IL-1 α = interleukin-1alpha, IL-1 β = interleukin-1beta, IL-1RA = interleukin-1 receptor antagonist, OR = odds ratio, PCR = polymerase chain reaction, RFLP = restriction fragment length polymorphism, SNPs = single nucleotide polymorphisms, TNF- α = tumor necrosis factor- α .

Keywords: cytokines, febrile seizures, gene polymorphisms, interleukin-1 β

Editor: Victor Asensi.

Authors' contributions: SAM and HFA designed the study and performed data analysis. MANA submitted the manuscript. HAZ and HEI collected clinical data. MMF, HGA, AAE, and AMK conceived of the study and coordinated the sample collection. MSH, ARA, and BRI participated in the design of the study and, performed the statistical analysis. MEH, MAAF, and SSAE wrote the discussion and helped to draft the manuscript. MAN, MIAH, and AMS critically revised the manuscript and approve final version. HHG, RMN, and LAF performed laboratory analysis and genotyping. SFA interpreted EEG for all cases and approve final version. All authors read and approved all the manuscript.

Funding: No funds were available for the current research.

The authors declare that they have no conflict of interest.

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Medicine (2017) 96:11(e6370)

Received: 21 December 2016 / Received in final form: 17 February 2017 / Accepted: 20 February 2017

<http://dx.doi.org/10.1097/MD.00000000000006370>

1. Introduction

Febrile seizures (FS) comprise common convulsive disorders of early childhood, accounting for 30% of all seizures in children.^[1] The American Academy of Pediatrics (AAP) has defined FS as a seizure occurring in febrile children between the ages of 6 and 60 months who do not have an intracranial infection, metabolic disturbance, or history of a FS.^[2] Although they are considered as a benign seizure disorder, up to 7% of affected children develop epilepsy by adolescence.^[3] Complex interactions between immune-inflammatory process, genetic factors, and cytokine network activation are involved in FS pathogenesis.^[4]

Pro-inflammatory and anti-inflammatory cytokines play a pivotal role in the regulation of febrile response during infections.^[5] Among these cytokines, interleukin-1 (IL-1) is defined as the first endogenous pyrogen, because it was originally discovered with function of inducing fever in experimental models and humans.^[6] The IL-1 cytokine family consists of IL-1 alpha (IL-1 α), IL-1 beta (IL-1 β), and IL-1 receptor antagonist (IL-1RA), all of which bind the IL-1 receptor (IL-1R). IL-1 β is mostly secreted but IL-1 α is predominantly membrane-bound. IL-1 β is more closely related to IL-1RA than it is to IL-1 α .^[7] Experimental animal models suggest a proconvulsive effect for IL-1 β while its naturally occurring IL-1RA has a powerful anticonvulsant action.^[8] In these models, endogenous IL-1 β has been shown to contribute to the development of hyperthermia-induced seizure^[9] which may suggest similar properties of this cytokine in children with FS.

Previous studies revealed increased plasma and cerebrospinal fluid (CSF) levels of some inflammatory cytokines in children with febrile seizures.^[10–12] Straussberg et al^[10] reported an increased production of IL-1 β , IL-6, IL-10, and TNF- α cytokines by LPS-stimulated mononuclear cells from 13 children with history of FS compared with 11 controls. Despite these studies, the association of raised IL-1 β levels with FS does not constitute proof of cause. One way to clarify this issue of causation involves a genetic approach. The IL-1 family accounts for 3 genes: IL-1 α , IL-1 β , and their inhibitor, the IL-1 RA. All 3 genes are located in the long arm of chromosome 2. In IL-1 β gene, 3 bi-allelic polymorphisms have been identified and all result from C to T transitions at positions (–31, –511, or +3954).^[13] The IL-1 β -511 single nucleotide polymorphisms (SNP) have been reported to enhance gene transcription leading to an increased expression of the encoded protein.^[14]

Previous studies demonstrated that cytokine genes SNPs could be, at least in part, an aetiopathogenetic factor in the manifestation of FS, particularly in sporadic cases.^[15–18] A few studies in the literature concerned the association of IL-1 β gene polymorphisms with febrile seizures and the susceptibility to febrile seizures.^[18–20]

We designed this study to investigate SNPs situated at positions –31 (C/T), and –511 (C/T) of IL-1 β gene promoter and IL-1RA gene variable number of tandem repeats in intron 2 (VNTR); to determine whether these polymorphisms could be a marker of susceptibility to febrile seizures in Egyptian children and we also measured the serum level of IL-1 β to assess its relation to such polymorphisms.

2. Methods

This was a prospective case-control study performed in Zagazig University Children Hospital, and outpatient clinics in the same hospital from March 2015 to January 2017. This study was approved by the ethical committee of Zagazig University, Egypt

and written informed consent from parents of all the children was provided in accordance with the Declaration of Helsinki.

One hundred and fifty-five children; who had manifested febrile seizures as diagnosed in the Department of Pediatrics in the same hospital, were included in this study. The age of the patients ranged from 6 to 72 months (median, 29 months). Diagnosis of febrile seizures followed the criteria established by The American Academy of Pediatrics (AAP).^[21] We defined simple FSs as generalized on onset, lasting less than 15 minutes, and do not occur more than once in 24 hours followed by a brief postictal period, whereas complex FSs last more than 15 minutes, have focal features with multiple recurrences within 24 hours and associated with postictal neurological abnormalities.^[21] The electroencephalogram (EEG) was normal for all patients with FS or showed mild non-specific abnormalities.

2.1. Exclusion criteria

Patients with FS beginning at the age of 6 years or later, evidence of intracranial infections, afebrile seizure, epileptiform EEG traits, or metabolic disturbance.

One hundred and fifty-five healthy children, of comparable age and sex; without a history of febrile or afebrile seizures or any neurologic disorders; were enrolled as a control group while attending the outpatient clinics for routine care. Patients and control subjects belonged to the same ethnic group: African Caucasian. All patients and control children were subjected to proper history taking, thorough clinical and detailed neurological examination.

2.2. Blood sampling

Venous blood samples were collected from all patients within 30 minutes of the time of FS onset, and divided into 2 portions: 2 mL of whole blood was collected into tubes containing EDTA, for genomic DNA extraction. Sera were separated immediately from the remaining part of samples and stored at –20°C till the time of analysis. Control samples were collected and similarly analyzed.

2.3. Genomic DNA extraction

Genomic DNA extracted by using the DNA Blood Kit according to the manufacturer's instruction (MBI Fermentas, Germany). DNA was stored at –20°C before genotyping.

2.4. Genotyping

All subjects were genotyped for IL-1 β promoter polymorphisms at positions –31 and –511 by polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) as previously described.^[18,22] For the polymorphisms at position –31, we used the sense primer 5'-AGAAGCTTCCACCAATACTC-3' and antisense primer 5'-AGCACCTAGTTGTAAGGAAG-3'. For the polymorphism at position –511 of IL-1 β , the primers 5'-GCCTGAACCCTGCATACCGT-3' and 5'-GCCAATAGCCCTCCCTGTCT-3'; were used. Polymerase chain reaction-based genotyping of IL-1RA (variable number of tandem repeats in intron 2) was performed using the primers 5'-CTCAGCAACACTCCTAT-3' and 5'-TCCTGGTCTGCAGGTAA-3' as described before.^[18,22] Polymorphisms of IL-1RA were identified as IL-1RN1 (4 repeats; 410 bp), IL-1RN2 (2 repeats; 240 bp), IL-1 RN3 (5 repeats; 500 bp), IL-1RN4 (3 repeats; 325 bp), and IL-1 RN5 (6 repeats; 595 bp).

Table 1
Baseline clinical and laboratory data of the studied patients and control groups.

	Patients n = 155	Controls n = 155	P
Age, mo	29 (6–72)	30 (6–71)	>0.05*
Sex (male/female)	85/70	82/73	>0.05†
Type of febrile seizure:			
Simple	113	—	—
Complex	42	—	—
Age at onset of febrile seizure, mo	13 (6–40)	—	—
Seizure duration, min	7 (1–28)	—	—
Family history:			
Febrile seizure	59	—	—
Epilepsy	16	—	—
Serum IL-1β, pg/mL	9.4 ± 1.3	2.5 ± 1.2	<0.01‡

Data are presented as median (range) or mean ± SD. P value <0.05 indicates a significant difference. IL-1β = interleukin-1β. * Mann–Whitney U test. † Chi-squared test. ‡ Student t test.

2.5. Measurement of serum IL-1β levels

Serum IL-1β was estimated using an enzyme-linked immunosorbent assay (ELISA) (kit provided by CLBkit -pelikine Netherland) according to the manufacturer’s instructions by using standard curve. Assay range: 2 to 400 pg/mL and sensitivity: ≤1 pg/mL.

2.6. Statistical analysis

IL-1β genotype and allele frequencies in FS patients and healthy controls were tested for Hardy–Weinberg equilibrium and deviations between observed and expected frequencies were tested for significance using Pearson chi-squared and Fisher exact tests. Odds ratios (OR) and 95% confidence intervals [95% (CI)] were calculated as a measure of the association between IL-1β (–31, –511) or IL-1RA (VNTR) gene polymorphisms and FS. The Student t test and analysis of variance (ANOVA) were used to compare numeric variables within groups. P value <0.01 was considered to be statistically significant. All data were analyzed using Statistical Package for Social Sciences version 22.0 (IBM Corp., Armonk, NY) and the Epi Info statistical software (version 6.2, World Health Organization, Geneva, Switzerland).

3. Results

This study included 155 patients with FS (113 patients had simple FS and 42 patients with complex FS, their age ranged from 6 months to 6 years [median 29 months], 85 were men and 70 were women) and 155 healthy control subjects whose characteristics are listed in Table 1. The control group was age, sex, and ethnicity matched to children with FS. The mean age at the onset of FS was 13 months (range, 6–40 months). The average duration of the seizures was 7 minutes (range, 1–28 minutes). Within the group with complex FS, 26 patients had experienced 2 or more seizures, 9 patients had experienced focal seizures, and 7 patients had experienced prolonged seizure lasting longer than 20 minutes. Fifty-nine patients had a family history of FS, and 16 patients had a family history of epilepsy. Our data revealed that patients with FS had increased serum levels of IL-1β than did

Table 2
Distribution of IL-1β and IL-1RA genotypes, alleles in patients with febrile seizures (FS) and the control group.

Genotype	Patient group		Control group		OR (95% CI)	P
	n (155)	%	n (155)	%		
IL-1β (–31)	CC	36 (23)	39 (25)			0.84
	CT	96 (62)	91 (59)			
	TT	23 (15)	25 (16)			
Alleles	C	168 (54)	169 (55)			0.93
	T	142 (46)	141 (45)			
	IL-1β (–511)	CC	42 (27)	59 (38)	Referent	
Alleles	CT	82 (53)	85 (55)			3.96 (1.68–9.5) 0.001
	TT	31 (20)	11 (7)			
	C	166 (53.5)	203 (65)			
T	144 (46.5)	107 (35)				
IL-1RA	I/I	40 (26)	58 (37)	Referent		0.22
	I/II	79 (51)	84 (55)			
	II/II	36 (23)	13 (8)			
Alleles	I	159 (51)	200 (64.5)			0.58 (0.4–0.8) 0.001
	II	151 (49)	110 (35.5)			

Values in parentheses are percentages or data are presented as mean ± SD. P value <0.05 indicates a significant difference. Chi-squared test. CI = 95% confidence interval, IL-1β = interleukin-1beta, IL-1RA = interleukin-1 receptor antagonist, OR = odds ratio.

healthy control subjects (9.4 ± 1.3 pg/mL vs. 2.5 ± 1.2 pg/mL; P < 0.01 respectively); see Table 1.

Distribution of IL-1β genotypes, alleles, and serum IL-1β levels in patients with FS and control group are summarized in Table 2. Both groups were in Hardy–Weinberg equilibrium, with no significant chi-squared values for the observed and expected genotype frequencies.

The frequency of the IL-1β-511 TT genotype and T allele at the same position were observed to be increased in patients with FS compared with the control group (OR: 3.96; 95% CI: 1.68–9.5; P = 0.001 for the TT genotype and OR: 1.65; 95% CI: 1.18–2.3; P = 0.003 for the T allele, respectively); see Table 2. On the other hand, no significant difference was found between the 2 groups in the genotype distribution or allele frequency at –31 position of IL-1β promoter (P > 0.05); see Table 2.

IL-1 RA (VNTR) polymorphisms in patients with FS and healthy controls are summarized in Table 2. In our study, we have observed just allele I (4 repeats; 410 bp) and allele II (2 repeats; 240 bp) for IL-1 RA.

The IL-1 RA II/II homozygous variant were more frequent in patients with FS than in control group (23%, OR: 4.02; 95% CI: 1.78–9.15; P = 0.001); see Table 2. Of note, we found a significant increase in the frequency of the IL-1 RA allele II among FS patients (49%, OR: 1.73; 95% CI: 1.24–2.4; P = 0.001); where a concomitant significant decrease in the frequency of the IL-1 RA allele I was observed compared with the control group (51%, OR: 0.58; 95% CI: 0.4–0.8); see Table 2.

When patients with familial and sporadic febrile seizures were evaluated separately, we found a significant positive association between the IL-1 RA II/II genotype and susceptibility to febrile seizures in sporadic cases as did allele II at the same position (OR: 5.04; 95% CI: 2.1–12.5 for the IL-1 RA II/II genotype; P = 0.001) and (OR: 1.94; 95% CI: 1.3–2.8 for the allele II; P = 0.001, respectively); see Table 3. Meanwhile, no significant differences were observed between the 2 groups in genotype distribution or

Table 3**Comparison of genotype and allele frequency of IL-1 β and IL-1RA gene polymorphisms between familial and sporadic febrile seizure patients and control subjects.**

Genotype		Familial cases n=59 (%)	Sporadic n=96 (%)	Controls n=155 (%)	OR (95% CI)	P
IL-1 β (-31)	CC	16 (27)	20 (21)	39 (25)		0.88
	CT	35 (60)	61 (64)	91 (59)		
	TT	8 (13)	15 (15)	25 (16)		
Alleles	C	67 (57)	101 (53)	169 (55)		0.67
	T	51 (43)	91 (47)	141 (45)		
IL-1 β (-511)	CC	16 (27)	26 (27)	59 (38)		0.34
	CT	30 (51)	52 (54)	85 (55)		
	TT	13 (22)	18 (19)	11 (7)		
Alleles	C	62 (53)	104 (54)	203 (65)		0.52
	T	56 (47)	88 (46)	107 (35)		
IL-1RA	I/I	17 (29)	23 (24)	58 (37)		0.001
	I/II	32 (54)	47 (49)	84 (55)	5.04 (2.1–12.5)	
	II/II	10 (17)	26 (27)*	13 (8)		
Alleles	I	66 (56)	93 (48)	200 (64.5)		0.001
	II	52 (44)	99 (52)*	110 (35.5)	1.94 (1.3–2.8)	

Values in parentheses are percentages.

CI=95% confidence interval, IL-1 β =interleukin-1beta, IL-1RA=interleukin-1Receptor antagonist, OR=odds ratio.

* P value <0.05 indicates a significant difference. Chi-squared test.

allele frequencies at the -31 and -511 positions of IL-1 β promoter (All $P > 0.05$); see Table 3.

However, we could not find any significant association between studied IL-1 β /IL-1 RA polymorphisms and seizure duration, type of seizure, or family history of epilepsy among our patients (all $P > 0.05$). No significant differences in genotype distribution or allele frequencies were observed between patients with simple or complex febrile seizures ($P > 0.05$).

Interestingly, we observed that FS patients with the IL-1 RA II/II genotype had significantly higher serum IL-1 β levels (13.7 ± 1.4 pg/mL) compared with those with I/II genotype (4.2 ± 1.3 pg/mL) and I/I genotype (1.7 ± 0.9 pg/mL); $P < 0.01$, see Table 4. The IL-1 RA allele II was also associated with increased mean serum IL-1 β levels (11.3 ± 1.5 pg/mL) versus (2.8 ± 0.9 pg/mL) for the allele I among studied patients ($P < 0.01$); see Table 4. Our data revealed no significant association between IL-1 β genotypes or alleles and serum IL-1 β levels in patients with FS ($P > 0.05$); see Table 4.

Table 4**Association of IL-1 β /IL-1RA genotypes and alleles with serum IL-1 β levels in patients with febrile seizures.**

Gene polymorphism	Genotype/Alleles	Serum IL-1 β (pg/mL)	P
IL-1 β (-31)	CC	7.8 \pm 1.4	0.43*
	CT	5.9 \pm 0.7	
	TT	6.3 \pm 1.2	
Alleles	C	6.5 \pm 1.1	0.67 [†]
	T	5.7 \pm 1.3	
IL-1 β (-511)	CC	3.5 \pm 0.8	0.28*
	CT	5.4 \pm 1.2	
	TT	4.3 \pm 0.9	
Alleles	C	5.2 \pm 0.6	0.55 [†]
	T	7.4 \pm 1.0	
IL-1RA	I/I	1.7 \pm 0.9	<0.01*
	I/II	4.2 \pm 1.3	
	II/II	13.7 \pm 1.4	
Alleles	I	2.8 \pm 0.9	<0.01 [†]
	II	11.3 \pm 1.5	

IL-1 β =interleukin-1beta, IL-1RA=interleukin-1 receptor antagonist.Data are presented as mean \pm SD.

P value <0.01 indicates a significant difference.

* Calculated by ANOVA test.

[†] Student t test.

4. Discussion

FS is the most common seizure disorder of childhood.^[23] Despite their prevalence, the pathophysiology of FS remains obscure. Pro-inflammatory cytokines, including IL-1 β are key modulators involved in host response to infection and induction of fever.^[24] Production of these cytokines seems to be affected by SNPs within the coding region of the cytokine gene.^[25] In experimental seizure models, exogenously applied IL-1 β prolonged and worsened both electrographic and behavioral seizure activity while the depression of IL-1 β activity suppressed them.^[26] Because of potential immune-modulatory effects of IL-1 β and its importance as a major pro-inflammatory cytokine, IL-1 β gene SNPs may affect individual susceptibility to FSs.

In the present study, the IL-1 β -511 TT genotype and T allele were overrepresented in patients with FS compared with the control group. In addition, we observed that homozygous individuals with the TT genotype had a 3.9-fold higher risk for developing FS, thus revealing that patients were more susceptible to FS. Our results are in accordance with Serdaroglu et al^[19] who studied IL-1 β promoter SNPs on genomic DNAs of 90 Turkish children with febrile seizures, compared with 106 age-matched healthy subjects. They reported that the presence of the T allele or the TT genotype at -511 position of the IL-1 β promoter constituted risk factor for developing FS in Turkish children. An earlier study by Virta et al^[27] demonstrated an allelic association

between the IL-1 β -511 T allele and febrile seizures in Finnish children, as they discovered an increased risk for FS in carriers of T allele.

In our study, no significant relationship was evident between genotype distribution or allele frequency at -31 position of IL-1 β promoter and susceptibility to FS among studied patients. In the same line, a recent study by Ozen et al^[28] have demonstrated a poor correlation of T/C substitution at the -31 position of IL-1 β promoter and the risk for FS in Turkish children.

By contrast, previous studies^[18,20,29,30] reported that IL-1 β gene polymorphisms is not a useful marker for prediction of the susceptibility to FS in Turkish, Iranian, Taiwanese, and German children; respectively.

Discrepancy between our study and previously published studies may be explained by the differences in age group; study design or geographic and ethnic variations, or by gene-gene or gene-environmental interaction. Genetic predisposition to FSs seems to be polygenic, with many variants in multiple gene loci, playing an important role.

IL-1 β is a pleiotropic cytokine that act as a critical molecular link between the immune and neuroendocrine systems.^[31] In the CNS, low levels of IL-1 β seem to exert a neuro-protective effect, but under certain pathological conditions, higher concentrations of IL-1 β may lead to neurotoxicity. Thus, it is associated with seizure susceptibility and epileptogenesis.^[32] In the current study, patients with FS had increased serum levels of IL-1 β compared with the control group in accordance with results from some previous studies^[10,12]; together with our findings, may support the pro-convulsive action of IL-1 β in FS. IL-1 β can evoke neuronal hyper-excitability by direct action on ionic currents or indirectly by inhibiting GABA (A) receptors function or enhancing extracellular glutamate concentrations. IL-1 β can also augment nitric oxide formation to raise seizure susceptibility.^[33] Nevertheless, we could not observe any significant association between studied IL-1 β promoter polymorphisms and serum IL-1 β levels as mentioned in some recent studies.^[28,34] Hall et al^[34] reported that the subjects carrying 1 or 2 copies of the T allele at position -31 of IL-1 β produce greater amounts of IL-1 β protein when compared with carriers of C allele. They suggested that this T/C substitution may take part in the increasing levels of IL-1 β and may be one of the causes of FS.^[28] Despite these findings, evidence of IL-1 β pro-conclusive role in febrile seizures and childhood epilepsy has yet to be shown.

Of the anti-inflammatory cytokines, the IL-1RA is structurally related to IL-1 α and IL-1 β and competes with these molecules for the occupation of IL-1 cell surface receptors.^[29] IL-1RA can block fever by competitive inhibition at the IL-1 receptor type I that blocks IL-1 β signaling, and thus fever.^[35] The IL-1 β bioactivity is under the genetic control of IL-1RA.^[36] Vezzani et al^[8] reported an increased threshold for seizure generation in transgenic mice overexpressing IL-1RA. Girard et al^[37] demonstrated that disequilibrium in IL-1 β /IL-1RA ratio could increase both inflammatory and neurotoxic potential of IL-1 β leading to brain damage and epilepsy. In this study, the IL1RA II/II genotype and allele II of variable tandem repeat polymorphism were more frequent among patients with FS than in control subjects. Of note, the IL1RA II/II homozygous variant and allele II were also associated with higher serum levels of IL-1 β among studied patients. On the other hand, we observed a significant negative association between IL1-RA allele I and febrile seizure suggesting that allele I was in some fashion protective against febrile seizures. Our

data confirm and extend the previous findings of Serdaroglu et al^[19] and Ozen et al^[28] who reported similar results in Turkish children with FS. The authors explained that IL-1 RA production may be decreased due to allele II and this may lead to an insufficient antagonistic effect of IL-1 RA to IL-1 β . This was supported by the in vitro study performed by Santtilla et al^[38] who demonstrated an elevated level of IL-1 β in the presence of allele II of IL-1 RA. They concluded that allele I is probably more efficient than allele II in increased gene expression of IL-1 RA. Our findings are different from those of Rasol et al^[39] who reported that the IL-1RA homozygous I/I and IL-1 β -511 CC genotype are associated with a higher susceptibility to febrile seizures in Upper Egypt. This discrepancy may be attributed to several ethnic groups in Upper Egypt. In our study, all the participants were African Caucasian chosen from the same town in Delta Egypt to allow for ethnic homogeneity. However, further measurements of serum IL-1 β and or IL-1RA levels and genotyping of other IL-1 gene cluster SNPs [such as IL-1 α , and IL-1R] should be examined in children with FSs to confirm our findings in different populations.

A positive family history of FSs is the most important risk factor, and the more relatives affected, the greater the risk.^[40] When patients with familial and sporadic febrile seizures were evaluated separately, we did not observe any significant association between IL-1 β or IL-1 RA polymorphisms and a positive family history of FSs among our patients, which was contrary to our expectations and to Serdaroglu et al^[19] who added that the IL-1 β -511T allele was observed to be more frequent in familial cases which comprised a risk factor for febrile seizure. Of note, we found a significant positive association between the IL-1 RA II/II genotype and allele II and susceptibility to FS in sporadic cases as they did. However, no significant relationship was evident between studied IL-1 β or IL-1 RA polymorphisms and seizure duration, type of seizure (simple or complex), family history of epilepsy among FS patients in accord with results from some previous studies.^[18,19,29] It is supposed that exposure to recurrent infections and other environmental factors; especially in developing countries, in addition to host defense response related to cytokines gene SNPs may be the cause of sporadic cases of FSs. In Caucasian population, the small number of studies concerning cytokines gene polymorphisms makes it difficult to express explicitly the hypotheses or concepts.

However, the small sample size was one of our limitations in the current study; we suggest that a multicenter approach is necessary to attain larger sample size. Furthermore, serum IL-1RA levels were not measured in our patients with FS, so the changes in the IL-1 β /IL-1R ratio were not estimated to better understand the status of the IL-1 system. CSF IL-1 β levels and interictal cytokine profile in patients with FSs was not measured which was another limitation in this study.

5. Conclusion

We demonstrate for the first time that the presence of a T allele or TT genotype at -511 of IL-1 β promoter and IL-1RA II/II genotype constitute risk factors for developing febrile seizures in Egyptian children.

Finally, further large-scale studies on different ethnicities and more genetic information are needed to provide additional understanding of the possible role of IL-1 β and IL-1RA gene polymorphisms in the susceptibility to FS that may open up novel therapeutic targets.

Acknowledgments

The authors thank the staff of Pediatric Neurology and Outpatient Clinics in Zagazig University Children's Hospital for their collaboration in sampling as well as our patients who participated in the study.

References

- [1] Vestergaard M, Obel C, Henriksen TB, et al. The Danish National Hospital Register is a valuable study base for epidemiologic research in febrile seizures. *J Clin Epidemiol* 2006;59:61–6.
- [2] Steering Committee on Quality Improvement and Management; Subcommittee on Febrile Seizures American Academy of Pediatrics. Febrile seizures: clinical practice guideline for the long-term management of the child with simple febrile seizures. *Pediatrics* 2008;121:1281–6.
- [3] Vestergaard M, Pedersen CB, Sidenius P, et al. The long-term risk of epilepsy after febrile seizures in susceptible subgroups. *Am J Epidemiol* 2007;165:911–8.
- [4] Tsai F-J, Chou I, Hsieh Y-Y, et al. Interleukin-4 intron 3 polymorphism is not related to susceptibility to febrile seizures. *Pediatr Neurol* 2002;27:271–4.
- [5] Heida JG, Moshé SL, Pittman QJ. The role of interleukin-1beta in febrile seizures. *Brain Dev* 2009;31:388–93.
- [6] Jakeman KJ, Bird CR, Thorpe R, et al. Nature of the endogenous pyrogen (EP) induced by influenza viruses: lack of correlation between EP levels and content of the known pyrogenic cytokines, interleukin 1, interleukin 6 and tumour necrosis factor. *J Gen Virol* 1991;72(Pt 3):705–9.
- [7] Rijkers K, Majoie HJ, Hoogland G, et al. The role of interleukin-1 in seizures and epilepsy: a critical review. *Exp Neurol* 2009;216:258–71.
- [8] Vezzani A, Moneta D, Conti M, et al. Powerful anticonvulsant action of IL-1 receptor antagonist on intracerebral injection and astrocytic overexpression in mice. *Proc Natl Acad Sci USA* 2000;97:11534–9.
- [9] Dubizé C, Vezzani A, Behrens M, et al. Interleukin-1b contributes to the generation of experimental febrile seizures. *Ann Neurol* 2005;57:152–5.
- [10] Straussberg R, Amir J, Harel L, et al. Pro- and anti-inflammatory cytokines in children with febrile convulsions. *Pediatr Neurol* 2001;24:49e53.
- [11] Haspolat S, Mihçi E, Coskun M, et al. Interleukin-1beta, tumor necrosis factor-alpha, and nitrite levels in febrile seizures. *J Child Neurol* 2002;17:749–51.
- [12] Tütüncüoğlu S, Kütükçüler N, Kepe L, et al. Proinflammatory cytokines, prostaglandins and zinc in febrile convulsions. *Pediatr Int* 2001;43:235–9.
- [13] Wang P, Xia HH, Zhang JY, et al. Association of interleukin-1 gene polymorphisms with gastric cancer: a meta-analysis. *Int J Cancer* 2007;120:552–62.
- [14] Wen AQ, Wang J, Feng K, et al. Effects of haplotypes in the interleukin 1beta promoter on lipopolysaccharide-induced interleukin 1beta expression. *Shock* 2006;26:25–30.
- [15] Zare-shahabadi A, Soltani S, Ashrafi MR, et al. Association of IL4 single-nucleotide polymorphisms with febrile seizures. *J Child Neurol* 2015;30:423–8.
- [16] Azab SF, Abdalhadhy MA, Ali A, et al. Interleukin-6 gene polymorphisms in Egyptian children with febrile seizures: a case-control study. *Ital J Pediatr* 2016;42:31.
- [17] Shahrokhi A, Zare-Shahabadi A, Soltani S, et al. Association of TGFB, but not IL10, single nucleotide polymorphisms with febrile seizures. *Seizure* 2015;29:148–52.
- [18] Haspolat S, Baysal Y, Duman O, et al. Interleukin-1alpha, interleukin-1beta, and interleukin-1Ra polymorphisms in febrile seizures. *J Child Neurol* 2005;20:565e8.
- [19] Serdaroglu G, Alpman A, Tosun A, et al. Febrile seizures: interleukin 1beta and interleukin-1 receptor antagonist polymorphisms. *Pediatr Neurol* 2009;40:113–6.
- [20] Soltani S, Zare-Shahabadi A, Shahrokhi A, et al. Association of Interleukin-1 gene cluster and Interleukin-1 receptor polymorphisms with febrile seizures. *J Child Neurol* 2016;31:673–7.
- [21] Chung S. Febrile seizures. *Korean J Pediatr* 2014;57:384–95.
- [22] Kira R, Torisu H, Takemoto M, et al. Genetic susceptibility to simple febrile seizures: Interleukin-1b promoter polymorphisms are associated with sporadic cases. *Neurosci Lett* 2005;384:239–44.
- [23] Wanigasinghe J. Febrile seizures. *Sri Lanka J Child Health* 2009;38:93e7.
- [24] Nakayama J, Arinami T. Molecular genetics of febrile seizures. *Epilepsy Res* 2006;70(Suppl 1):S190–8.
- [25] Morse HR, Olomolaiye OO, Wood NA, et al. Induced heteroduplex genotyping of TNF-alpha, IL-1beta, IL-6 and IL-10 polymorphisms associated with transcriptional regulation. *Cytokine* 1999;11:789–95.
- [26] Patel HC, Ross FM, Heenan LE, et al. Neurodegenerative actions of interleukin-1 in the rat brain are mediated through increases in seizure activity. *J Neurosci Res* 2006;83:385–91.
- [27] Virta M, Hurme M, Helminen M. Increased frequency of interleukin-1beta (-511) allele 2 in febrile seizures. *Pediatr Neurol* 2002;26:192–5.
- [28] Ozen F, Koçak N, Halil Yildirim I, et al. Does the imbalance between agonistic and antagonistic IL-1 play a role in progression of febrile convulsions? *Eur Rev Med Pharmacol Sci* 2016;20:120–4.
- [29] Chou IC, Lin WD, Wang CH, et al. Interleukin (IL)-1beta, IL-1 receptor antagonist, IL-6, IL-8, IL-10, and tumor necrosis factor alpha gene polymorphisms in patients with febrile seizures. *J Clin Lab Anal* 2010;24:154–9.
- [30] Tilgen N, Pfeiffer H, Cobilanschi J, et al. Association analysis between the human interleukin 1beta (-511) gene polymorphism and susceptibility to febrile convulsions. *Neurosci Lett* 2002;334:68–70.
- [31] Takeuchi O, Akira S. Pattern recognition receptors and inflammation. *Cell* 2010;140:805–20.
- [32] Allan SM, Tyrrell PJ, Rothwell NJ. Interleukin-1 and neuronal injury. *Nat Rev Immunol* 2005;5:629–40.
- [33] Zhu G, Okada M, Yoshida S, et al. Effects of interleukin-1beta on hippocampal glutamate and GABA releases associated with Ca2+ induced Ca2+ releasing systems. *Epilepsy Res* 2006;71:107–16.
- [34] Hall SK, Perregaux DG, Gabel CA, et al. Correlation of polymorphic variation in the promoter region of the interleukin-1 beta gene with secretion of interleukin-1 beta protein. *Arthritis Rheum* 2004;50:1976–83.
- [35] Cartmell T, Luheshi GN, Rothwell NJ. Brain sites of action of endogenous interleukin-1 in the febrile response to localized inflammation in the rat. *J Physiol* 1999;518(Pt 2):585–94.
- [36] Vamvakopoulos J, Green C, Metcalfe S. Genetic control of IL-1beta bioactivity through differential regulation of the IL-1 receptor antagonist. *Eur J Immunol* 2002;32:2988–96.
- [37] Girard S, Kadhim H, Larouche A, et al. Pro-inflammatory disequilibrium of the IL-1beta/IL-1Ra ratio in an experimental model of perinatal brain damages induced by lipopolysaccharide and hypoxia-ischemia. *Cytokine* 2008;43:54–62.
- [38] Santtila S, Savinainen K, Hurme M. Presence of the IL-1RA allele 2 (IL1RN*2) is associated with enhanced IL-1beta production in vitro. *Scand J Immunol* 1998;47:195–8.
- [39] Rasol HAA, Issac MSM, Ghaffar HA, et al. Interleukin-1 receptor antagonist and interleukin-1b-511 gene polymorphisms among Egyptian children with febrile seizures. *Comp Clin Pathol* 2014;23:419–25.
- [40] Berg AT, Shinnar S, Shapiro ED, et al. Risk factors for a first febrile seizure: a matched case-control study. *Epilepsia* 1995;36:334–41.