Association of Genetic Polymorphism of \textit{Pre-MicroRNA-146a rs2910164} and Serum High-Mobility Group Box 1 With Febrile Seizures in Egyptian Children


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What is This?
Association of Genetic Polymorphism of Pre-MicroRNA-146a rs2910164 and Serum High-Mobility Group Box 1 With Febrile Seizures in Egyptian Children

Marianne Samir Makboul Issac, MD¹, Marian Girgis, MD², Mervat Haroun, MD², and Amal Shalaby, MSc²

Abstract
Interaction between immune-inflammatory process and genetic factors might be implicated in the pathogenesis of febrile seizures. Pre-microRNA (miR)-146a rs2910164 polymorphism is postulated to modulate expression of miR-146a whose anti-inflammatory role involves regulation of high-mobility group box 1. Our aim is to examine whether rs2910164 polymorphism influences serum high-mobility group box 1 levels and whether an association exists between both and febrile seizures. The study included 136 children, divided into 4 groups. Real-time polymerase chain reaction was used for detection of rs2910164 polymorphism and high-mobility group box 1 was measured using enzyme-linked immunosorbent assay. High-mobility group box 1 levels were higher in febrile seizure patients compared to the other groups. Rs2910164 polymorphism was not associated with increased risk of febrile seizures. Rs2910164 polymorphism might be accompanied by an upregulation of the proinflammatory process as it might be associated with an increase in high-mobility group box 1 and leukocytic count.

Keywords
miRNA-146a, NC_000005.10, high-mobility group box 1, febrile convulsions

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Febrile seizures are the most common form of childhood seizures, occurring in 2% to 5% of children younger than age 6 years.¹,² The pathogenesis of febrile convulsions remains obscure. In fact, febrile seizures of children involve a complex interaction between the immune-inflammatory process, cytokine activation, and genetic factors.³ Experimental and clinical studies point to the role of astrocytes as a major source and/or targets of proepileptogenic inflammatory signaling, such as the interleukin-1, high-mobility group box 1, and Toll-like receptor signaling pathways.⁴,⁵

High-mobility group box 1 protein is a highly conserved non-histone chromosomal protein,⁶ involved in maintenance of nucleosome structure and regulation of gene transcription.⁷ It can be released from activated or injured neurons in the central nervous system, thus acting as an endogenous inflammatory mediator.⁷ Regulation of its secretion is dependent on various processes such as phosphorylation by calcium-dependent protein kinase C.⁸ It was suggested that high-mobility group box 1 signaling in cortical cells may contribute to lower membrane thresholds and mediate rapid changes in neuronal excitability.⁹

Recent studies have pointed to the critical role of micro-ribonucleic acids (microRNAs) in several biological processes, including negative feedback loops that modulate inflammatory signalling of the central nervous system.¹⁰,¹¹ Micro-RNAs represent a family of small (22-24 nucleotides), endogenous non-coding RNAs, which post-transcriptionally regulate target gene expression by binding complementary sequences in the 3’-untranslated region of target messenger RNAs (mRNAs), either through translational inhibition or destabilization of target messenger RNAs.¹² In particular, microRNA-146a has been associated with the regulation of Toll-like and interleukin-1 receptors signaling.¹³,¹⁴ There is still little information regarding function of microRNA-146a that is expressed in human brain in astrocytes.¹⁴ MicroRNA-146a has been shown to be

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upregulated in experimental models of epilepsy, as well as in human temporal lobe epilepsy.15 It is speculated that single-nucleotide polymorphisms located at microRNA-binding sites are likely to affect the expression of the microRNAs and may therefore contribute to an individual’s susceptibility to various complex diseases. A G/C polymorphism (rs2910164, NC_000005.10) has been identified in the microRNA-146a precursor, pre-microRNA-146a gene on chromosome 5q34, which results in a change from a G:U pair to a C:U mismatch in its stem region,16 which modulates expression of mature microRNA-146a.17 There is a substantial body of evidence that there is a relationship between expression of high-mobility group box 1 in brain cells and pre-microRNA-146a gene polymorphism and their expected interaction to mediate epileptogenesis. The aim of the present study is to examine whether rs2910164, NC_000005.10 polymorphism influences serum high-mobility group box 1 levels and investigate the possible association of serum high-mobility group box 1 and rs2910164, NC_000005.10 polymorphism with risk factors and clinical parameters of febrile seizures comparing a group of children with febrile seizures and controls.

Subjects and Methods

Subjects
This is a case-control study conducted on 136 children recruited from Abo-ElReesh Children’s Hospital at Cairo University, Cairo, Egypt, during July 2012 to March 2013. The children were divided into 4 groups. Group 1 included 50 children with febrile seizures recruited from the outpatient clinic for routine vaccination or follow-up. Group 2 included 51 children with febrile illness without convulsions, who were recruited from the inpatient depart- }

Methods

Blood Sampling
Five milliliters of blood was collected within 30 minutes of the time of seizure; 2 mL of blood was collected in a tube containing ethylenediaminetetraacetate as an anticoagulant for subsequent DNA extraction and stored at –20°C, whereas 3 mL of blood was transferred into plain tubes, allowed to clot for at least 30 minutes, and centrifuged at 1000 × g for 15 minutes. Serum was then aliquoted and stored frozen at –20°C for subsequent measurement of high-mobility group box 1 and routine laboratory investigations.

Genotyping of pre-microRNA-146a rs2910164, NC_000005.10 polymorphism by TaqMan real-time polymerase chain reaction.20 Genomic DNA was isolated using the Qiaqen DNA Blood Mini Kit (Qiagen, Inc, Hilden, Germany), according to the manufacturer’s instructions. DNA concentration was measured using the Nanodrop ND-1000 (NanoDrop Technologies, Inc, Thermo Fisher Scientific, DE). For analysis of rs2910164, NC_000005.10, real-time polymerase chain reaction (PCR) allelic discrimination was performed on Step-One Real-Time PCR (Applied Biosystems, Foster City, CA) using standard TaqMan genotyping assays according to the manufacturer’s instructions. In brief, probes, primers, and TaqMan universal PCR Master Mix were obtained from Applied Biosystems. A reaction solution of 25 µL contained 1.25 µL TaqMan Genotyping Assay mix (C__15946974_10), (consisting of 20X Mix of unlabeled PCR primers and TaqMan minor groove binder probes, FAM™ and VIC® dye-labeled), 12.5 µL of TaqMan Universal PCR Master Mix (2X), 25 ng of genomic DNA and water was added to complete total volume to 25 µL. The PCR consisted of pre-PCR read at 60°C for 30 seconds, holding stage at 95°C for 10 minutes, 50 cycles of denaturing at 92°C for 15 seconds, annealing 60°C for 1 minute 30 seconds and post-PCR read at 60°C for 30 seconds. After PCR amplification, an endpoint plate read was performed using an Applied Biosystems real-time PCR system. The Sequence Detection System Software uses the fluorescence measurements made during the plate read to plot fluorescence values based on the signals from each well. The plotted fluorescence signals indicate which alleles are in each sample.

Measurement of Serum High-Mobility Group Box 1 level
High-mobility group box 1 was quantitatively measured by a sandwich enzyme-linked immunosorbent assay technique21 using...
commercially available kits according to the manufacturer’s instructions (WKEA Med Supplies Corp, Changchun, China). A standard curve is constructed by plotting absorbance values against concentrations of standards. The concentrations of unknown samples were determined using this standard curve.

### Statistical Analysis

Data management and analysis were performed using Statistical Package for Social Sciences, version 17. Data were statistically described in terms of mean ± standard deviation, median (range), or frequencies (number of cases) and percentages when appropriate. Comparison of numerical variables between more than 2 groups was done using 1-way analysis of variance with post hoc multiple 2-group comparisons in normal data, and the Kruskal-Wallis test with post hoc multiple 2-group comparisons in non-normal data. The observed genotype frequencies were compared with those expected under Hardy-Weinberg equilibrium using a chi-squared (X²) test. Association between qualitative data was done using the chi-square test. To measure the strength of association between numeric variables, Spearman’s correlation coefficients were used. The receiver operating characteristic curve displays the relationship between sensitivity and specificity; it was used to find the cutoff value that will provide the best prediction of febrile seizures. To study the independent effect of the different predictors of febrile seizures, a stepwise logistic regression analysis was performed. All P values are 2-sided. P values <.05 were considered significant.22

### Results

#### Characteristics of the Studied Groups

The demographic data of the studied subjects and results of laboratory investigations are summarized in Table 1. There was a statistically significant increase in serum high-mobility group box 1 level in group 1 patients when compared with groups 3 and 4, P ≤ .001. However, the level was higher in group 1 versus group 2 patients, but the difference was not statistically significant. The detailed characteristics of the 50 patients with febrile seizures are summarized in Table 2.

#### Frequency of pre-microRNA-146a rs2910164, NC_000005.10 Genotypes in the Studied Groups

The genotypic and allelic frequencies of pre-microRNA-146a rs2910164, NC_000005.10 did not show deviation from Hardy-Weinberg equilibrium and were not statistically significantly different when compared between the studied groups as shown in Table 3.

### Table 1. Characteristics and Laboratory Investigations of the Studied Subjects.23

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group 1 Febrile seizures (n = 50)</th>
<th>Group 2 Febrile illness (n = 51)</th>
<th>Group 3 Afebrile seizures (n = 10)</th>
<th>Group 4 Healthy control (n = 25)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (mo)</td>
<td>11.0 (6.0-50.0)</td>
<td>15.0 (6.0-51.0)</td>
<td>26.5 (6.0-50.0)</td>
<td>9.0 (6.0-49.0)</td>
<td>.491</td>
</tr>
<tr>
<td>Male/female ratio</td>
<td>27/23</td>
<td>26/25</td>
<td>5/5</td>
<td>12/13</td>
<td>.967</td>
</tr>
<tr>
<td>Body temperature (°C)</td>
<td>39.0 ± 0.4a</td>
<td>39.0 ± 0.4a</td>
<td>37.0 ± 0.1b</td>
<td>37.0 ± 0.2b</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Serum high-mobility group box 1 (ng/mL)</td>
<td>52.8 ± 5.0 (5.0-543.0)</td>
<td>33.0 ± 4.0 (4.5-423.5)</td>
<td>23.3 ± 2.0 (16.5-38.5)</td>
<td>32.0 ± 2.0 (3.5-61.0)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>White blood cell count (cells/mm³)</td>
<td>7729 ± 3266a</td>
<td>7786 ± 3022a</td>
<td>5690 ± 2009ab</td>
<td>5672 ± 2401b</td>
<td>.005</td>
</tr>
<tr>
<td>Calcium (mg/dL)</td>
<td>10.1 ± 0.6a</td>
<td>10.1 ± 0.6a</td>
<td>8.7 ± 0.8b</td>
<td>9.5 ± 0.8b</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

*Quantitative data are represented as mean ± standard deviation, except age and serum high-mobility group box 1 are represented as median (range). Qualitative data as male/female ratio is represented as frequency. Numbers carrying different subscript letters are statistically significantly different.

### Table 2. Characteristics of Children With Febrile Seizures.23

<table>
<thead>
<tr>
<th>Variable</th>
<th>Children with febrile seizures (n = 50)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Family history of febrile seizures</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>9 (18)</td>
</tr>
<tr>
<td>Negative</td>
<td>41 (82)</td>
</tr>
<tr>
<td>Character of first presenting seizure</td>
<td></td>
</tr>
<tr>
<td>Generalized tonic-clonic</td>
<td>48 (96)</td>
</tr>
<tr>
<td>Myoclonic</td>
<td>2 (4)</td>
</tr>
<tr>
<td>Duration of seizure</td>
<td></td>
</tr>
<tr>
<td>&lt;15 min</td>
<td>45 (90)</td>
</tr>
<tr>
<td>≥15 min</td>
<td>5 (10)</td>
</tr>
<tr>
<td>History of febrile seizures attacks</td>
<td></td>
</tr>
<tr>
<td>First attack</td>
<td>44 (88)</td>
</tr>
<tr>
<td>Recurrent attacks</td>
<td>6 (12)</td>
</tr>
<tr>
<td>Type of febrile seizures</td>
<td></td>
</tr>
<tr>
<td>Typical</td>
<td>39 (78)</td>
</tr>
<tr>
<td>Atypical</td>
<td>11 (22)</td>
</tr>
<tr>
<td>Age of first attack</td>
<td>(in nonrecurrent seizure patients, n = 44)</td>
</tr>
<tr>
<td>6-12 mo</td>
<td>24 (48)</td>
</tr>
<tr>
<td>&gt;12 mo</td>
<td>20 (40)</td>
</tr>
<tr>
<td>(in recurrent seizure patients, n = 6)</td>
<td></td>
</tr>
<tr>
<td>6-12 mo</td>
<td>6 (12)</td>
</tr>
</tbody>
</table>

*Qualitative data are represented as frequency (percentage).
Characteristics of Children With Febrile Seizures and Their Association With Serum High-Mobility Group Box 1 and pre-miRNA-146a rs2910164, NC_000005.10 Genotypes

There was no statistically significant difference in serum high-mobility group box 1 levels or frequency of pre-miRNA-146a rs2910164, NC_000005.10 genotypes when compared between patients with positive versus negative family history of febrile seizures, typical versus atypical febrile seizures, and first attack versus recurrent attacks, as summarized in Table 5. White blood cell count was higher in rs2910164 GC and CC genotype febrile seizure patients when compared to GG genotype patients, with a statistical significance encountered between GG and GC genotype febrile seizure patients as shown in Table 6.

Multivariate Logistic Regression Analysis

To detect the contribution of the following variables (rs2910164, NC_000005.10 genotypes, serum level of high-mobility group box 1, age, gender, positive family history, serum levels of sodium, potassium, calcium, creatinine, and blood urea nitrogen) to febrile seizures occurrence, taking healthy controls as febrile seizures–negative cases. The only variables that independently affected febrile seizures were serum levels of high-mobility group box 1 and calcium. A unit increase in high-mobility group box 1 will increase the odds of febrile seizures about 1.04 times after controlling the effect of calcium. A unit increase in calcium will increase the odds of febrile seizures about 3.53 times after controlling the effect of high-mobility group box 1.

Receiver Operating Characteristic Curve for High-Mobility Group Box 1 and Calcium Predicting Febrile Seizures Cases From Healthy Controls

Receiver operating characteristic curve analysis suggested that the optimum serum high-mobility group box 1 level cut-off points for febrile seizures was 34.8 ng/mL, and this level can be used in the prediction of febrile seizures from healthy controls with a sensitivity and specificity of 68% and 68%, respectively. Receiver operating characteristic curve analysis suggested that the optimum serum calcium level cut-off points...
for febrile seizures was 9.9 mg/dL, and this level can be used in the prediction of febrile seizures from healthy controls with a sensitivity and specificity of 76% and 64% respectively as shown in Figure 2.

**Correlations**

Serum high-mobility group box 1 correlated positively with each of body temperature (r = 0.280, P = .001) and serum calcium (r = 0.202, P = .018).

**Discussion**

Interaction between immune-inflammatory process and genetic factors might be implicated in the pathogenesis of febrile seizures. However, the precise mechanism and the contribution of each are not fully determined. To our knowledge, this is the first study evaluating the effect of a genetic variant in the pre-miRNA-146a gene on the risk of developing febrile seizures and investigating the association between pre-miRNA-146a polymorphism and serum high-mobility group box 1 levels.

Our results show that serum high-mobility group box 1 levels were significantly higher in febrile seizure patients than in the other groups, except for the febrile controls. Choi et al\textsuperscript{23} reported that serum high-mobility group box 1 levels were significantly higher in febrile seizure patients than in febrile controls. The discrepancy in results might be due to ethnic variation and differences in number of recruited children.

In our study, higher levels of high-mobility group box 1 were seen in febrile seizure patients with recurrent attacks when compared to patients in their first attack, those with positive family history when compared to those with negative family history, and those showing atypical febrile seizures compared to typical febrile seizures; however, the differences

### Table 4.

| Pre-miRNA-146a genotypes in febrile seizure patients (n = 39) |  |
|---|---|---|---|---|
| GG (n = 12) | GC (n = 16) | CC (n = 11) | P value |
| Serum high-mobility group box 1 level (ng/mL) | 41.3 (18.5-122) | 49.8 (5.5-543) | 119.5 (29-429) | .210 |

### Table 5.

| Pre-miRNA-146a genotypes in febrile illness patients (n = 30) |  |
|---|---|---|---|
| GG (n = 11) | GC (n = 12) | CC (n = 7) |
| Serum high-mobility group box 1 level (ng/mL) | 31.5 (7-85) | 57.8 (4.5-404.5) | 34 (27-73) | .646 |

| Pre-miRNA-146a genotypes in healthy controls (n = 25) |  |
|---|---|---|---|
| GG (n = 7) | GC (n = 12) | CC (n = 6) |
| Serum high-mobility group box 1 level (ng/mL) | 24.5 (3.5-26) | 32.0 (25.5-44.5) | 46.3 (35.5-61.0) | <.001 |

*Quantitative data are represented as median (range). Numbers carrying different subscript letters are statistically significantly different.

### Table 5.

| Pre-miRNA-146a genotypes in febrile seizures patients (n = 50) |  |
|---|---|---|---|---|
| GG (n = 39) | GC (n = 16) | CC (n = 11) | P value |
| Serum high-mobility group box 1 level (ng/mL) | 41.3 (18.5-122) | 49.8 (5.5-543) | 119.5 (29-429) | .210 |

*Quantitative data are represented as median (range), while qualitative data are represented as frequency (percentage).

\(b\) P value is for comparing GG versus GC+CC.
were statistically insignificant. These findings are in concordance with those of Choi et al., with the exception that febrile seizure patients with recurrent attacks showed lower levels of high-mobility group box 1 compared to those in their first attack. It was previously suggested that inflammatory responses in febrile seizures were accentuated by their repetition. In animal models, IL-1β was elevated chronically only in rats developing spontaneous limbic seizures after febrile status epilepticus.

Strong evidence supporting a signaling event by high-mobility group box 1, in a chronic epilepsy model, was discovered by Maroso and colleagues who demonstrated that blockade of high-mobility group box 1 markedly reduced seizure duration and frequency in rodent cortical neurons. The characterization of high-mobility group box 1/Toll-like receptor 4 interactions has led to the discovery of a cysteine residue at position 106 within high-mobility group box 1, which directly binds to Toll-like receptor 4 and induces cytokine release in cortical neurons leading to heightened excitability in the form of seizure activity.

It was reported that serum high-mobility group box 1 levels are elevated in patients with infection and/or systemic inflammatory response syndrome compared with healthy control individuals. In our study, serum levels of high-mobility group box 1 were significantly higher in febrile seizure patients and showed a positive correlation with body temperature. Our results, together with those of another study, suggest that active inflammation does occur and may play a common pathologic role in febrile seizures.

An interesting finding was the relationship between serum calcium and high-mobility group box 1. Our findings showed that both were positively correlated and were independent factors that contribute to the occurrence of febrile seizures. The exact relationship between them is yet to be determined, though previous studies reported the role of calcium in inflammation, the release of high-mobility group box 1 from cells, and pointed out that both play a key role in neuronal hyperexcitability. Perturbations in cellular calcium homeostasis are postulated to mediate the aberrant inflammation underlying organ dysfunction. Evidence is accumulating that production of high-mobility group box 1 is calcium dependent. Zhang et al. characterized that lipopolysaccharide-induced high-mobility group box 1 release is mediated by a calcium-dependent signaling cascade involving a calcium/calmodulin-dependent protein kinase IV, which phosphorylates high-mobility group box 1, an event that is required to facilitate its translocation from nucleus to cytoplasm. Sugaya et al. reported the relationship between the urinary bursting activities and the inflow of calcium into a neuronal cell. Papadimitriou described the relationship between the urinary concentrations of calcium and febrile convulsions.

Considering that microRNA-146a has been shown to critically modulate innate immunity, different genetic variations in microRNA precursors or targets might explain, at least in part, the well-orchestrated immune responses leading to inflammatory and immune processes in febrile convulsions. Our results show that G to C substitution in rs2910164, NC_000005.10 polymorphism has no contribution to febrile seizures. There were trends of higher high-mobility group box 1 levels in CC genotype compared with GG genotype. The

Table 6. Association of Body Temperature and White Blood Cell Count With Pre-microRNA-146a rs2910164, NC_000005.10 Genotypes in Febrile Seizure Patients.

<table>
<thead>
<tr>
<th>Pre-microRNA-146a genotypes in febrile seizure patients (n = 39)</th>
<th>GG (n = 12)</th>
<th>GC (n = 16)</th>
<th>CC (n = 11)</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body temperature (°C)</td>
<td>39 ± 0.3</td>
<td>38.9 ± 0.2</td>
<td>38.9 ± 0.4</td>
<td>.799</td>
</tr>
<tr>
<td>White blood cell count (cells/mm)$^3$</td>
<td>5758 ± 2478</td>
<td>9838 ± 3157</td>
<td>7873 ± 2500</td>
<td>.002</td>
</tr>
</tbody>
</table>

*Quantitative data are represented as mean ± standard deviation. Numbers carrying different subscript letters are statistically significantly different.

Figure 2. Receiver operating characteristic curve of serum high-mobility group box 1 (HMGB1) level predicting febrile seizures cases from healthy controls; area under the receiver operating characteristic curve was 0.748 (95% confidence interval = 0.640-0.856). P value <.001. At a cut-off value of 34.8 ng/mL, sensitivity = 68%, whereas specificity = 68%. Receiver operating characteristic curve of serum calcium (Ca) level predicting febrile seizures cases from healthy controls; area under the receiver operating characteristic curve was 0.727 (95% confidence interval = 0.595-0.860), P value = .01. At a cut-off value of 9.9 mg/dL, sensitivity = 76%, whereas specificity = 64%. The red line represents the reference line (line at which the variable cannot predict febrile seizures), green line represents area for HMGB1, whereas the blue line represents area for calcium.
difference was statistically significant in healthy controls, whereas it did not reach statistical significance in febrile seizure patients. Moreover, the leukocytic count was lower in febrile seizure patients with GG genotype when compared with GC and CC genotype carriers, with a statistically significant difference between GG and GC carriers. The rs2910164, NC_000005.10 polymorphism was previously reported to lead to reduced expression of mature microRNA-146a,30,31 and the anti-inflammatory role of microRNA-146a is supported by its ability to regulate the interleukin-1β-induced release of several proinflammatory factors, such as interleukin-6, interleukin-8, and high-mobility group box 1.10 Putting all these findings together, it might be suggested that reduced expression of mature microRNA-146a in C allele carriers, which might result from rs2910164, NC_000005.10 polymorphism, leads to the reduction of microRNA-146a anti-inflammatory role and this was accompanied by an increase in high-mobility group box 1 and leukocytic count, which might be a reflection of upregulation of the proinflammatory process associated with this polymorphism. However, these speculations need to be confirmed in larger studies, both experimental and clinical, as these might be chance findings, because genotyping was performed in a relatively small number of the recruited groups.

Our study is not without limitations. One of these limitations is the relatively small number of epileptic children and also the availability of DNA samples for a group (n = 94) out of the total recruited children (n = 136). Although the majority of children were genotyped for rs2910164, NC_000005.10, detection of this polymorphism in the whole study subjects would have offered a better understanding of the association between rs2910164 NC_000005.10 polymorphism and serum high-mobility group box 1. We, thus, recommend performing this study in a larger cohort. The strengths include comparing serum high-mobility group box 1 level in an adequate number of febrile seizure children with control groups including healthy controls and pathologic controls, comprising febrile children and patients with afebrile seizures to investigate the differences in serum high-mobility group box 1 levels between the 4 groups of children. Moreover, blood sampling within 30 minutes of the time of seizure controlled variations due to sampling timing differences. The controls were adequately matched to the cases for age, gender, and ethnicity, and all patients as well as controls are Egyptians, living in the same geographical area to allow for background homogeneity.

In conclusion, there were trends of higher high-mobility group box 1 levels in febrile seizures compared to the other groups. High-mobility group box 1 levels correlated positively with serum calcium, and both were independent factors that contributed to the occurrence of febrile seizures. Rs2910164, NC_000005.10 polymorphism was not associated with increased risk of febrile seizures. There were trends of higher levels of high-mobility group box 1 levels in rs2910164, NC_000005.10 CC genotype compared with the GG genotype. Leukocytic count was lower in GG genotype febrile seizure patients when compared with GC genotype carriers. Rs2910164 polymorphism might be accompanied by an upregulation of the proinflammatory process as it might be associated with an increase in high-mobility group box 1 and leukocytic count. Whether an association exists between rs2910164, NC_000005.10 polymorphism and high-mobility group box 1 with risk of febrile seizures needs further studying in larger cohorts including subjects from different ethnic backgrounds, before definitive conclusions can be made.

Acknowledgments

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Author Contributions

MI, MG, and MH participated in study concept and design. MI critically revised the article for important intellectual content and analysis of data, performed the laboratory and molecular analysis, and prepared the manuscript. MG and MH provided clinical care to the patients, participated in the selection of the cases according to the diagnosis and revised the manuscript. AS followed up the patients, collected the specimens and patients’ data. All authors read and approved the final manuscript.

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Ethical Approval

The study was approved by the ethical committee at Abo-El Reesh Children’s Hospital, Cairo University, Cairo, Egypt.

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