European Review for Medical and Pharmacological Sciences 2014; 18: 657-660

The prevalence of Familial Mediterranean Fever common gene mutations in patients with simple febrile seizures

F. ÖZEN, N. KOÇAK[1], S. KELEKÇI[2], I.H. YILDIRIM[3], G. HACIMUTO, Ö. ÖZDEMIR[4]

Department of Medical Genetics, Medeniyet University, Goztepe Training and Research Hospital, Turkey
[1]Department of Medical Genetics, Selçuk University, Selçuklu Medical Faculty, Turkey
[2]Department of Pediatrics, Dicle University Medical Faculty, Turkey
[3]Department of Medical Genetics, Dicle University Veterinary Medicine, Turkey
[4]Department of Medical Genetics, Çanakkale 18 Mart University Medical Faculty, Turkey

Abstract. – BACKGROUND: Febrile seizures (FS) represent the most common form of childhood seizures that occurs in 2-5 % of the children younger than 6 years. There have been many recent reports on the molecular genetic and pathogenesis of FC. It has been recognized that there is significant genetic component for susceptibility of FC with different reported mutation. FEB1, FEB2, FEB4, SCNA1, SCNA2, GABRG2 and IL-1β are related to with febrile convulsions (FCs). Interleukin 1β (IL-1β) is a cytokine that contributes to febrile inflammatory responses. There are conflicting results on increasing this cytokine in serum during FC.

AIM: The determine the association between mutations of MEFV gene product pyrine and febrile seizures.

PATIENTS AND METHODS: The study was carried out on 104 children that were diagnosed as FS and 96 healthy children. MEFV gene mutations were detected and analyzed with PyroMark Q24. PCR was performed using the PyroMark PCR Kit and pyrosequencing reaction was conducted on instrument instructions.

RESULTS: M694V is the most common mutation in our patient group and we found a significant association between MEFV gene mutations and FSs. Of 104 patients, 68 were heterozygotes for any mutation and 10 patients were compound. 17.7% of control group were heterozygotes for any studied mutation.Statistical analyses showed that there was strongly significant statistical difference between results obtained from FS and control group ($X = 46.20$, $p < 0.0001$).

CONCLUSIONS: MEFV gene mutations, especially M694V mutation, are positively associated with FSs.

Key words:

Febrile seizures (FS), MEFV, Pyrine, IL-1 , M694V.

Introduction

Febrile seizures (FSs) are the most commonest form of convulsions occuring between the age of 3 months and 5 years[1]. The incidence of FSs is between 2-5%[1,2]. These type of convulsions are associated with a rapidly raising temperature that reaches 39°C or higher without evidence of intracranial infection or other defineable causes like acute electrolyte imbalance[2]. FSs result from the combination of genetic and environmental factors. The exact mechanism of inheritance is still unclear[1,3,4]. They tend to occur in families so, children who have febrile seizure more often tend to have FSs in close relatives[2,4]. Several genes like-FEB1, FEB2, FEB4, SCNA1, SCNA2, GABRG2 and IL-1β have been reported to be associated with febrile convulsions (FCs)[3,5].

IL-1β is one of the proinflammatory cytokines acting as a pyrogens, causing fever when administered into the lateral cerebral ventricules or peripherally[6,5]. Additionaly, fever of any cause induce IL-1β synthesis in brain microglia and the released IL-1β binds to the type 1 receptors that are sensitive to seizures, leading to enhanced neuronal excitability and decreased seizure treshold[6,7]. Dube et al[7] have shown the requirement of IL-1β expression and presence of IL-1RI (type 1 receptor) in an experimental model. In this study, IL-1β receptor deficient mice were resistant to experimental FS and high IL-1β doses induced seizure. Serdaroglu et al[8] also showed a positive correlation between IL-1β -511 and IL1 receptor antagonist intron 2 variable tandem repeat polymorphism and FS. Kira et al[9] have found an association between IL-1β promotor polymormphism and FS while Chou et al[10] have found a correlation between IL-Ra allele I and FS.

Corresponding Author: Filiz Özen, MD; e-mail: dr.fozen@hotmail.com

IL-1β is generated from the cleavage of pro-IL-1β by caspase-1. Full length pyrin, product of Mediterranean fever (MEFV) gene, competes with caspase-1 for binding to ASC (Apoptosis-associated Speck-like Protein containing a caspase activation and recruitment domain: CARD), a known caspase-1 activator[11]. And this leads to the prevention of formation of active form of IL-1β[11]. In addition, pyrine has an important role in NF-κB transcription factor activation that modulates the transcription of inflammatory genes[12].

The objective of the present study was to investigate the association between mutations of MEFV gene product pyrine and febrile seizures.

Patients and Methods

The blood samples were collected from 104 children that were diagnosed as FS and age/sex matched 96 healthy children (HC). Mean age of FC patients was 6.58±4.01 and HC was 7.22± 4.79 years. Genomic DNA was isolated using the QIAamp DNA Mini Kit (Qiagen, Maryland, Germany) and used as template in PCR reactions. PCR was performed using the PyroMark PCR Kit (Qiagen, Maryland, Germany). Following 15 min of denaturation at 95°C the DNA was amplified for 45 cycles with the following conditions: 15 sec at 95°C, 30 sec at 60°C and 30 sec at 72°C. Prior to pyrosequencing, PCR product is biotinylated and bound to Streptavidin-coated sepharose beads which are captured with vacuum tool. After washing and denaturating steps single-stranded DNA is generated. This template DNA is released into the Pyrosequencing reaction plate containing the sequencing primer, and after primer annealing, the plate is placed into the PyroMark instrument. Pyrosequencing reaction was conducted on the PyroMark Q24 instrument according to the manufacturer's instructions (Qiagen, Maryland, Germany). PyroMark Q24MDx software used for analysis of our results.

Statistical analysis

Descriptive statistics were expressed as count and percent. C*hi* square test was used to test the significance of the distribution of mutations between FS patients and HC. Statistical significance levels were considered as 5% and SPSS ver. 16 (SPSS Inc., Chicago, IL, USA) statistical program was used for all statistical computations.

Results

All the mutations obtained from the febrile convulsion patients and healthy subjects were heterozigotes mutations. Of 104 patients, 68 (65.38%) were heterozygotes for any mutation and 10 (0.9%) patients were compound heterozygotes (4 patients for E148Q/M694V and 6 patients for M694V/V726A). 17.7% of control group were heterozygotes for any studied mutation. The gentotype frequencies of studied MEFV polymorphisms are shown in Table I.

Table I. Genotype frequencies of patient and control group.

Genotype	Control group N=96	Patient group N=104	Genotype frequencies of controls	Genotype frequencies of patients
E148Q				
EE	86	88	0.90	0.85
EQ	10	16	0.10	0.15
QQ	0	0	0.00	0.00
M680I				
MM	93	94	0.97	0.90
MI	3	10	0.03	0.10
II	0	0	0.00	0.00
M694V				
MM	94	64	0.98	0.62
MV	2	40	0.02	0.38
VV	0	0	0.00	0.00
V726A				
VV	94	92	0.98	0.88
VA	2	12	0.02	0.12
AA	0	0	0.00	0.00

Table II. Statistical evaluation of patient and control group.

Genotype	Control group N=96	Patient group N=104	OR (95% CI)	*p* value
E148Q				
EE	86	88	1.564 (0.672-3.637)	0.297
EQ	10	16		
M680I				
MM	93	94	3.298 (0.880-12.366)	0.063
MI	3	10		
M694V				
MM	94	64	29.375 (6.855-125-884)	**0.000**
MV	2	40		
V726A				
VV	94	92	6.130 (1.335-28.150)	**0.009**
VA	2	12		

Statistical analyses showed that there was strongly significant statistical difference between results obtained from FS and control group (X = 46.20, $p < 0.0001$). The main difference derived from the M694V mutation (X = 39.62, $p < 0.0001$). Additionaly, there was a significant correlation between V726A mutation (X = 6.82; $p = 0.009$) and a poor correlation between M680I mutation (X = 3.44, $p = 0.064$). However, the number of E148Q mutations was found higher but there was not any statistical correlation between FS and control group. The results of statistical analyses are given in Table II. The distribution of the genotypes in the control population was in Hardy-Weinberg equilibrium.

Discussion

In the present study, we found a significant association between MEFV gene mutations and FSs. The highest mutation frequency was observed in M694V substitution. Also Koksal et al[13] have found the same results, M694V has the highest mutation frequency. In a previous study carried out by Notarnicola et al[14] on FMF patients where expression of *MEFV* gene was determined, diminished expression of this gene was reported in M694V mutation carrier patients and control group. Also Ustek at al[15] have found the same results and concluded that decreased MEFV expression is associated with acute inflammation[14,15].

Evidence indicate an association between decreased MEFV gene expression and IL-1β formation. IL-1β is one of the pro-inflammatory cytokines that are postulated to be involved in the development of FSs[6-10]. Dube et al[7] have found that IL-1β receptor deficient mice were resistant to experimental FC. Additionally, they found that high IL-1β doses induced seizures only in IL-1β receptor expressing mice[7]. Feld et al[16] showed that pyrin seems to comprise a part of the inflammasome NLRP3 (NLR Family, Pyrin domain-containing 3), that regulates the level of IL-1β (interleukin 1-β) and thereby the degree of inflammation. Virta et al[17] reported an increased frequency of IL-1β-511 T in children with FS compared to healthy controls. Vezzani et al[18] reported that locally synthesized endogenous IL-1 RA reduces concentrations of IL-1β and limits the fever. Like IL-1RA regulation, MEFV gene product pyrin may play a role in formation and regulation of mature IL-1β. In this process, pro-IL-1β is converted to mature IL-1β by Caspase-1/Interleukin-1 converting enzyme[19]. ASC (apoptosis-associated speck-like protein containing a CARD) is a known caspase-1 activator that stimulates autocatalysis of caspase-1[20,21]. Pyrine competes with caspase-1 for binding to ASC and prevents the formation of mature IL-1β[11,12]. As described above, decreased expression of pyrine may be a reason of reduced formation of IL-1β and thus inflammation and seizures.

The other more frequent mutation found in FSs is E148Q. Mutation frequency of this polymorphism is high in control group too. In an expression study[14], patients with E148Q mutations had higher MEFV mRNA levels than did the other patients. In a study carried out on the parents of FMF patients[22], homozygote E148Q mutation was found. Somehow these mutation carriers had no symptom of FMF. As a result of this study, it was suggested that E148Q mutations was not found to

be the cause of FMF disease and inflammation; therefore, this mutation should be considered as a "sequence variant".

Conclusions

We suggest that MEFV gene mutations, especially M694V mutation, are positively associated with FSs. Further investigations of MEFV gene mutations and gene expression studies for MEFV and IL-1β level elevation are needed to confirm the present findings.

References

1) Sadleir LG, Scheffer IE. Febril seizures. Br Med J 2007; 334: 307-311.
2) Al-Aulouni SF, Kodah IH. Febrile convulsions in children. Neurosciences 2000; 5: 151-155.
3) Iwasaki N, Nakayama J, Hamano K, Matsui A, Arinami T. Molecular genetics of febrile seizures. Epilepsia 2002; 43: 32-35.
4) Daoud A. Febrile convulsion: review and update. Jp Neurology 2004: 2: 9-14.
5) Nakayama J. Progress in searching for febrile seizure susceptibility genes. Brain Dev 2009; 31: 359-365.
6) Gatti S, Vezzani A, Bartfai T. Mechanisms of fever and febrile seizures: putative role of interleukin-1 system, In: Baram TZ, Shinnar S, eds. Febrile seizures. San Diego: Academic Press, 2002; pp. 169-188.
7) Dube C, VezzaniA, Behrens M, Bartfai T, Baram TZ. Interleukin-1 contributes to the generation of experimental febrile seizure. Ann Neurol 2005; 57: 152-155.
8) Serdaroglu G, Alpman A, Tosun A, Pehlivan S, Ozkinay F, Tekgul H, Gokben S. Febrile seizures: interleukin 1 and interleukin-1 receptor antagonist polymorphisms. Pediatr Neurol 2009; 40: 113-116.
9) Kira Ryutaro, Torisu H, Takemoto M, Nomura A, Sakai Y, Sanefuji M, Sakamoto K, Matsumoto S, Gondo K, Hara T. Genetic susceptibility to simple febrile seizures: interleukine-1 promoter polymorphisms are associated with sporadic cases. Neurosci Lett 2005; 384: 239-244.
10) Chou C, Lin WD, Wang CH, Tsai CH, Li TC, Tsai FJ. Interlekine (IL)-1 , IL-1 receptor antagonist, IL-6, IL-8, IL-10, and tumor necrosis factor gene polymorphisms in patients with febrile seizures. J Clin Lab Anal 2010; 24: 154-159.
11) Chae JJ, Komarow HD, Cheng J, Wood G, Raben N, Liu PP, Kastner DL. Targeted distruption of pyrin, the FMF protein, causes heightened sensitivity to endotoxin and a defect in macrophage apoptosis. Mol Cell 2003; 11: 591-604.
12) Masumoto J, Dowds TA, Schaner P, Chen FF, Ogura Y, Li M, Zhu L, Katsuyama T, Sagara J, Taniguchi S, Gumicio DL, Nunez G, Inohara N. ASC is an activating adaptor for NF- B and caspase-8-dependent apoptosis. Biochem Biophys Res Commun 2003; 303: 69-73.
13) Koksal B, Nur N, Sari M, Candan M, Acemo lu M, Kocak N, Ozen F, Ozdemir O. Clinical and Molecular analysis of common MEFV gene mutations in Familial Mediterranean Fever in Sivas population. Biologia 2009; 64/2: 388-393.
14) Notarnicola C, Didelot MN, Koné-Paut I, Seguret F, Demaille J, Touitou I. Reduced MEFV messenger RNA expression in patients with familial mediterranean fever. Arthritis Rheum 2002; 46: 2785-2793.
15) Ustek D, Ekmekci CG, Selçukbiricik F, Cakiris A, Oku B, Vural B, Yanar H, Taviloglu K, Ozbek U, Gül A. Association between reduced levels of MEFV Messenger RNA in peripheral blood leukocytes and acute inflammation. Arthritis Rheum 2007; 56: 345-350.
16) Feld O, Yahalom G, Livneh A. Neurologic and other systemic manifestations in FMF: Published and own experience. Best Pract Res Clin Rheumatol 2012; 26: 119-133.
17) Virta M, Hurme M, Helminen M. Increased frequency of interleukin-1 beta (-511) allele 2 in febrile seizures. Pediatr Neurol 2002; 26: 192-195.
18) Vezzani A, Moneta D, Richichi C, Perego C, De Simoni MG. Functional role of proinflammatory and anti-inflammatory cytokines in seizures. Adv Exp Med Biol 2004; 548: 123-133.
19) Thornberry NA, Bull HG, Calaycay JR, Chapman KT, Howard AD, Kostura MJ, Miller DK, Molineaux SM, Weinder JR, Aunins J. A novel heterodimeric cysteine protease is required for interleukine-1 beta processing in monocytes. Nature 1992; 356: 768-774.
20) Shiohara M, Taniguchi S, Masumoto J, Yasui K, Koike K, Komiyama A, Sagar J. ASC, which is composed of a a PYD and a CARD, is up-regulated by inflammation and apoptosis in human neutrophils. Biochem Biophys Res Commun 2002; 293: 1314-1318.
21) Stehlik C, Fiorentino L, Dorfleutner A, Bruey JM, Ariza EM, Sagara J, Reed JC. The PAAD/PYRIN-family protein ASC is a dual regulator of a conserved step in nuclear factor B activation pathways. J Exp Med 2002; 196: 1605-1615.
22) Ben-Chetrit E, Lerer I, Malamud E, Domingo C, Abeliovich D. The E148Q mutation in the MEFV gene: is it a disease-causing mutation or a sequence variant? Hum Mutat 2000; 15: 385-386.