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SHORT COMMUNICATION

High Frequency of Inherited Variants in the *MEFV* Gene in Acute Lymphocytic Leukemia

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Abstract In the present study, we aimed to determine the frequency of inherited variants in the *MEFV* (*Me*diterranean *FeV*er), the gene responsible for familial Mediterranean fever (FMF), gene in patients with acute lymphocytic leukemia (ALL). The eight *MEFV* gene variants (M694I, M694V, M680I (G/C-A), V726A, R761H, E148Q and P369S) were detected in 36 patients with ALL and 65 healthy controls; none had own and/or family history compatible with FMF. We identified 11 heterozygous inherited variants in the *MEFV* gene in both ALL patients and controls. The mean overall frequency of inherited variants in the *MEFV* gene rate was higher in ALL patients than healthy controls ($P = 0.040$). It is interesting to note that M680I/0 is predominant variant in patients with ALL. In addition, E148Q variant frequency was also significantly higher in the patient group than the controls ($P = 0.012$). In conclusion, overall frequency of inherited variants in the *MEFV* gene was found to be higher in patients with ALL. Based on the present data, it is difficult to reach a definitive conclusion regarding the possibility that inherited variants in the *MEFV* gene could represent a causative role in ALL. However, the data of our study may provide some new insights in understanding of individual genetic differences in susceptibility to these neoplasms. Further investigations are needed to determine the actual role of inherited variants in the *MEFV* gene in pathogenesis of ALL.

Keywords *MEFV* gene · Acute lymphocytic leukemia · Interleukin-1β · Nuclear factor-κB

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Introduction

Familial Mediterranean fever (FMF) (OMIM 249100) is an autosomal recessive autoinflammatory syndrome occurring in populations originating from the Mediterranean basin, mainly Turks, Sephardic Jews, Levantine Arabs, Druze and Armenians [1–3]. Patients suffer from recurrent self-limited inflammatory febrile attacks, abdominal, chest or joint pain. FMF is caused by mutations in *MEFV* (*Me*diterranean *FeV*er) gene (Chr.16p13.3; GenBank NM_000243; OMIM 608107), a 10-exon gene encoded on the short arm of the chromosome 16. The Infevers database (http://fmf.igh.cnrs.fr/ISSAID/infevers) reports more than 200 variants and polymorphism in this gene. A subset of inherited variants in the *MEFV* gene (usually, E148Q in exon 2 and M694V, M680I, M694I, and V726A in exon 10) may

account for as many as 80% of FMF cases in classically affected populations [4].

MEFV complementary DNA is a member of a family of highly conserved genes that regulate embryonic development, hematopoiesis, oncogenesis, and inflammation [5–8]. Firstly, *MEFV* expression was reported in peripheral blood leucocytes and a colorectal adenocarcinoma cell line, SW480 [9]. Subsequent investigations showed that *MEFV* is expressed primarily in neutrophils, eosinophils, and monocytes [6]. In experimental studies, the monocytic cell lines U937 and THP-1 also express *MEFV* [10]. Moreover, in $CD34^+$ hematopoietic stem cells, the expression of *MEFV* is suggested at the myelocyte stage during granulocyte differentiation [10]. *MEFV* gene encodes a 781 amino acid protein known as *pyrin* (alternatively, *marenostrin*) [9, 11, 12]. Inherited variants in the *MEFV* gene prevent the formation of normal pyrin protein as well as changes in either its localization or interaction with other proteins [13]. Full-length pyrin contains five different domains including the PYRIN domain, the B30.2 domain, the B-Box zinc finger domain, the bZIP transcription factor basic domain and coiled-coil domain [14–17]. A critical insight into the normal function of pyrin came from the recognition of the PYRIN domain [10, 15]. The PYRIN domain can bind indirectly to at least two proteins important in inflammation: pro-caspase-1 and the inhibitor of nuclear factor-κB (NF-κB) kinase complex [17–19]. Thus, the production of interleukin-1β (IL-1β) is inhibited and normal apoptosis is allowed. On the other hand, pyrin may be able to modify the NF-κB pathway and apoptosis independently of IL-1β [20–23]. Therefore, any variant in the *MEFV* gene prevents the formation of normal pyrin protein, and it may lead to postponed apoptosis and inflammation due to the reduced ability of pyrin to control NF-κB and IL-1β activation [24, 25].

There is a little information about relationship between inherited variants in the *MEFV* gene and hematologic neoplasms. In more recent pilot studies [26, 27], analysis of the most common inherited variants in the *MEFV* gene in some hematologic neoplasms, such as acute lymphocytic leukemia (ALL) [27], showed an unexpectedly high frequency of inherited variants in the gene. Since sample size is small in this study [27], we aimed to investigate the actual frequency of inherited variants in the *MEFV* gene in patients with ALL.

Patients and Methods

The study included 36 patients with ALL (30 men, 6 women) and 65 healthy controls (40 men and 25 women). None of the subjects included had a personal or family history of FMF. The study was conducted according to the recommendations set forth by the Declaration of Helsinki on Biomedical Research Involving Human Subjects. Each subject gave his informed consent to the study, which was previously approved by our local ethical committee and institutional review board. All patients donated 2 ml of blood, collected in an EDTA tube. The eight inherited variants in the *MEFV* gene (M694I, M694V, M680I (G/C-A), V726A, R761H, E148Q and P369S) were detected by Dr. Zeydanli® FMF Type I PCR System (Ankara, Turkey) based 5′ nuclease assay method in ABI 7500 (Applied Biosystems, Foster City, CA, USA).

Statistical Analysis

Data were analyzed with SPSS 17.0 (SPSS Inc., USA) statistical software. Differences between the groups were investigated with the χ^2 test. A $P \leq 0.05$ was evaluated as statistically significant.

Results

The mean age of the patients with ALL and healthy controls was 23.92 ± 3.13 and 30.25 ± 10.62 years, respectively. Hematological characteristics and identified variants in the *MEFV* gene in patients with ALL were shown in Table 1. In healthy control group, we found 11 heterozygous variants. Analytical data concerning overall frequency of inherited variants in the *MEFV* gene between patients with ALL and comparisons with healthy controls are given in Table 2. M694I/0 and R761H/0 variants were not found in any of the groups. The difference in overall frequency of inherited variants in the *MEFV* gene between the patient group and the control group was statistically significant ($P = 0.040$). It is interesting to note that M680I/0 is predominant variant in patients with ALL. In addition, E148Q variant frequency was also significantly higher in the patient group than the controls ($P = 0.012$). The frequencies of the M694V, V726A and P369S variants were not statistically significant.

Discussion

In the present study, overall frequency of inherited variants in the *MEFV* gene was found to be higher in patients with ALL than healthy controls. Interestingly, M680I variant, which is commonly found in FMF patients and variant located within this hot spot is associated with more severe phenotype [28], was significantly higher in ALL patients than healthy controls; none had own and/or family history compatible with FMF. Conceptually, in individuals with

Table 1 Hematological characteristics and identified variants in *MEFV* gene in patients with acute lymphocytic leukemia (n = 36)

Patient no.	Age	Sex	Immunophenotype	Bone marrow blasts (%)	WBC ($\times 10^9$/l)	PLTS ($\times 10^9$/l)	Hb (g/dl)	Inherited variants in *MEFV* gene
1	23	M	Uncertain	90	7.840	113	5.3	E148Q/0[a]
2	22	M	T-cell	70	95.600	6.63	11	V726A/0
3	22	M	Precursor-T	80	41.100	26.1	8.6	E148Q/0
4	19	M	Uncertain	70	7.390	221	16	M680I/0
5	26	M	Precursor-B	90	203.000	274	11.9	M694V/0
6	18	F	Uncertain	95	12.500	128	7.6	E148Q/0
7	55	M	Uncertain	95	16.000	16	10.1	M694V/0
8	58	M	Precursor-B	90	435.000	14.500	7.9	P369S/0
9	23	M	T-cell	90	55.800	23.600	10.9	M680I/0
10	39	M	T-cell	40	44.200	285	12.6	M680I/0
11	40	M	B-ALL	70	9.970	238	15.4	M680I/0

F female, *M* male, *WHO* World Health Organization classification, *Hb* hemoglobin, *WBC* white blood cells, *PLTS* platelets

[a] 0 Variant indicates that the chromosome carries a mutation not determined in our study

Table 2 The comparison of the inherited variant frequency in *MEFV* gene between patients with ALL and normal controls

	n	Overall inherited variant frequency in *MEFV* gene	Heterozygote variant frequencies in *MEFV* gene						
			M694V/0	E148Q/0	M680I/0	V726A/0	M694I/0	R761H/0	P369S/0
ALL	36	0.153	0.028	0.042	0.055	0.013	0	0	0.013
Normal controls	65	0.084	0.038	0.015	0	0.023	0	0	0.007
χ^2		4.200	0.007	6.357	7.489	0.123	–	–	1.227
P		0.040	0.932	0.012	0.002	0.850	–	–	0.289

the wild-type *MEFV* gene, pyrin serves a key role in regulating the intensity of the inflammatory response. As mention above, any variant in the *MEFV* gene prevents the formation of normal pyrin protein, and it may lead to postponed apoptosis and inflammation due to the reduced ability of pyrin to control NF-κB and IL-1β activation [24, 25]. On the one hand, IL-1, a pleiotropic cytokine, participates in all phases of malignancy, including carcinogenesis, tumor growth, invasion, metastasis and patterns of interactions of the malignant cells with the host's immune system [29, 30]. Caspase-1, is a cysteine protease that cleaves the inactive precursor of IL-1β and also IL-18 and IL-33, and caspase-1 is also involved in apoptosis [31]. Depending on the stimulus, the activity of caspase-1 causes either cytokine secretion and inflammation or, alternatively, cell death, indicating the regulation of these events downstream of caspase-1 [31]. On the other hand, NF-κB is critical for the development of T and B lymphocytes and how NF-κB is activated in these cells is highly dependent on their developmental stage and the initiating signal [32, 33]. In this way, mice deficient for NF-κB have been generated the critical role of NF-κB as a survival factor from early lymphopoiesis [34] to latter stages of development and maturation of B and T cells [32, 33, 35–37]. As NF-κB plays an essential role in regulation of lymphocyte development, activation, proliferation and survival, deregulation of its signaling pathways may lead to inappropriate immune response and contributes to the growth and survival of malignant lymphocytes [38–41]. Meanwhile, most hematologic neoplasms harbour constitutive NF-κB activation and it can affect all six hallmarks of cancer through the transcriptional activation of genes associated with cell proliferation, angiogenesis, metastasis, tumor promotion, inflammation and suppression of apoptosis [32, 42–44]. NF-κB stimulates the expression of a number of antiapoptotic gene products such as FLICE inhibitory protein, c-inhibitor of apoptosis protein, survivin, B-cell leukemia/lymphoma 2 (Bcl-2), and Bcl-XL, underscoring its importance on cell death in hematologic malignancies [45, 46]. Consequently, although the molecular mechanism of NF-κB activation remains elusive at present, a constitutive NF-κB activity has been reported in ALL [47]. It seems likely that aberrant NF-κB activation due to defective pyrin functions may be related high frequency of inherited variants in the *MEFV* gene in ALL.

This study also has limitations. First, the sample size of our study is small. Second, we have not screened genetically the pedigrees of our patients carrying the inherited variants in the *MEFV* gene. But, the family history of relatives of these patients for FMF manifestations was negative. Likewise, as the diagnosis of FMF remains predominantly clinical [48], the possibility of disease overlap atypical FMF in some cases of ALL has been excluded. Third, our patients screened only eight *MEFV* variants; thus, rare or novel variants can be overlooked. Finally, prognostic value of the inherited variants in the *MEFV* gene in ALL is not one of the main aims for the present study.

In conclusion, overall frequency of inherited variants in the *MEFV* gene was found to be higher in patients with ALL. Based on the present data, it is difficult to reach a definitive conclusion regarding the possibility that inherited variants in the *MEFV* gene could represent a causative role in ALL. The data of our study may provide some new insights in understanding of individual genetic differences in susceptibility to these neoplasms. Although there is not a single underlying defect that could be targeted in ALL, to fully exploit the underlying mechanisms of the promoting role of the inherited variants in the *MEFV* gene will be important.

Acknowledgment

Conflict of Interest The authors of the present work have no interests, which might be perceived as posing a conflict or bias.

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