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Increased Frequency of Mutations in the Gene Responsible for Familial Mediterranean Fever (*MEFV)* in a Cohort of Patients with Ulcerative Colitis: Evidence for a Potential Disease-Modifying Effect?

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The *MEFV* gene, responsible for familial Mediterranean fever (FMF), is involved in inflammatory reactions through altered leukocyte apoptosis, secretion of interleukin (IL)-1β, and activation of the NF-κB pathway. Ulcerative Colitis (UC) and FMF are both characterized by a recurrent pattern of presentation with periods of remission and flares associated with neutrophilic infiltration at the site of injury. The aim of this study was to investigate the possible correlation between UC and *MEFV* gene alterations. Twenty-five consecutive, first-diagnosed and untreated UC patients, 28 control patients with rheumatoid arthritis, and 65 normal individuals were analyzed. Nonisotopic RNase Cleavage Assay (NIRCA) was applied as a first-step mutational screening method of exons 10 and 2 of *MEFV* gene; direct sequencing was subsequently performed to confirm the results. *MEFV* mutations were identified in 7 (3 M694V/0, 2 M680I/0, 1 E148Q/E148Q, and 1 A744S/0) out of 25 UC patients versus 1 (M694V/0) out of 28 rheumatoid arthritis patients ($P = .0199$) and 1 (M694V/0) out of 65 healthy controls ($P = .0004$). Four out of 7 patients with *MEFV* mutations had inflammatory arthritis, a clinical finding that was not observed in the 18 UC patients with unmutated *MEFV* ($P = .0028$). Patients with UC almost universally carried the T A C G *MEFV* exon 2 haplotype in contrast with normal individuals ($P < .0001$) and FMF patients ($P = .0310$). In conclusion the increased frequency of mutations of *MEFV* in UC patients, especially in those with episodic arthritis, suggests a possible modifying effect of *MEFV* in the disease process and its localization within the joint. The difference in distribution of *MEFV* exon 2 haplotypes between UC patients and both FMF patients and normal individuals, suggests that UC patients constitute a genetically distinct population. Larger, longitudinal studies are needed to confirm these initial findings.

KEY WORDS: *MEFV*; FMF; ulcerative colitis; extraintestinal manifestations.

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Familial Mediterranean fever (FMF) is the prototype of the periodic inflammatory clinical syndromes, characterized by acute episodes of fever and inflammation painful in the abdomen, chest and joints (1). Although there are established criteria for the diagnosis of FMF, an unexplained clinical heterogeneity is not uncommon (1). In 1997 the gene linked to FMF, called *MEFV,* was cloned and some of the mutations associated with the disease were identified (2, 3). To date, >50 mutations have been described and the autosomal recessive inheritance of the disease has been established (4). Molecular analysis of *MEFV* is a useful tool in clinical practice, mainly in the atypical forms of the disease (4). However, many clinically typical FMF patients are heterozygous or no disease-associated mutations are found (4–6).

The protein encoded by the *MEFV* gene, termed *pyrin* or *marenostrin,* includes a PYRIN domain (PYD), which has been already analyzed crystallographically (7). However, the precise function of the entire pyrin protein is still unknown (2, 3, 8). Experiments performed on animal models and cell lines, as well as the presumed structure of pyrin and the presence of certain protein domains, suggest that the wild type of the protein has anti-inflammatory properties (8–10). Pyrin presumably participates in a complex interplay with the PYD protein superfamily, lipopolysaccharide (LPS) via Toll-like receptor family and procaspase-1 activation, and is implicated in the homeostatic control of inflammation through leukocyte apoptosis, IL-1β, and NF-κB pathway activation (8, 9, 11).

Ulcerative colitis (UC) is an inflammatory disease of the rectal and colonic mucosa characterized by periodicity and relapse of clinical features in combination with neutrophil infiltration at the site of injury during the active phase (12). Although the pathogenesis of the disease is still unclear, environmental, immune, and genetic factors have been implicated (13). UC is more common in family members of affected individuals and a certain ethnic groups, such as Jews, raising the possibility for a genetic basis of the disease (14). Identification of the spectrum of genes involved in the pathogenesis of UC could provide important insights regarding the nature of the environmental and immunological factors that trigger and drive the disease process (13). The absence, however, of a simple Mendelian pattern of inheritance suggests that multiple genes and their expression products contribute to a person's risk to the disease (15). Today, the genetics of UC is an area of increased interest.

UC and FMF share common clinical and biological features. Abdominal inflammation with infiltration by neutrophils—as the main cellular population—at the site of injury, periodicity and relapse of clinical features, as well as the involvement of joints are some examples (1, 12, 13) Recent data suggest that abnormal regulation of apoptosis along with the involvement of IL-1β and NF-κB pathway could be implicated in both UC and FMF (8, 9, 13, 15). Epidemiologic data in non-Askhenazi Jews indicate that inflammatory bowel disease (IBD)—Crohn's disease plus UC—is more common and severe in patients with FMF (14), raising the possibility of a modifying effect of the *MEFV* gene in the expression of certain inflammatory diseases. Furthermore, no significant association of *MEFV* mutations and Crohn's disease was revealed in recent studies (16, 17). However, in UC patients without signs of FMF and a free familial history, the frequency of *MEFV* alterations is unknown. All these observations prompted us to study a group of consecutive UC patients, exploring a possible association between UC and the *MEFV* gene.

METHODS

Study Subjects

The study population consisted of 25 unrelated and naive UC patients diagnosed between October 2002 and March 2005 at the University Hospital of Alexandroupolis, Greece. For the purposes of this study we included 3 control groups. First, because the incidence of individuals heterozygous for *MEFV* mutations is to date unknown in Greece, we included 65 randomly chosen healthy individuals with no FMF history, inhabiting the same geographical region as the UC patients (northeastern Greece); this number should be adequate to examine the incidence of the *MEFV* mutations in our population. In addition, 28 patients with rheumatoid arthritis were also included in the study, serving as an internal disease control group for a disease with a nonepisodic pattern of inflammation. Finally, 15 patients (12 heterozygotes and 3 homozygotes), contributing 18 *MEFV* mutated chromosomes, have been included for *MEFV* exon 2 haplotype analysis. Mean age and gender ratio (shown in Table 1) are similar between UC patients and healthy individuals ($P = .294$ for age, $P = .814$ for gender ratio), patients with rheumatoid arthritis ($P = .525$ for age, $P = .785$ for gender ratio), and FMF patients ($P = 1.000$ for age, $P = 1.000$ for gender ratio).

Diagnosis of UC was assessed according to established clinical guidelines and criteria based on endoscopic, radiologic, and histopathologic examination (12, 18). Demographic and routine clinical data, including presentation and location of UC, disease-related complications, and prescription data, were recorded by interview at the time of enrollment.

TABLE 1. DEMOGRAPHIC DATA IN UC PATIENTS VERSUS HEALTHY AND RHEUMATOID ARTHRITIS CONTROLS

	N	*Gender (M/F)*	*Age (mean ± 9 5% CL)*
UC Patients	25	11/14	41.04 ± 8.50
Healthy controls	65	32/33	45.80 ± 11.26
Rheumatoid arthritis controls	28	11/17	41.64 ± 12.03
FMF controls	15	7/8	40.89 ± 10.93

Abbreviations: M, male; F, female; CL, confidence limit; UC, ulcerative colitis.

TABLE 2. CLINICAL AND GENOTYPIC DATA OF UC PATIENTS HETEROZYGOUS FOR *MEFV Mutations*

Patient	*Gender Age*	*Disease Location*	*Extra-Intestinal Manifestations*	*MEFV Genotype*	*MEFVexon 2 Haplotypes**
1	M/19	Rectosigmoiditis	No	M694V/0	TACG/TACG
2	M/50	Left-sided colitis	Knee and wrist arthritis	M694V/0	TACG/TACG
3	F/56	Left-sided colitis	No	E148Q/E148Q	TACG/TACG
4	M/42	Left-sided colitis	Knee arthritis	M680I/0	TACG/TACG
5	F/37	Left-sided colitis	No	M694V/0	TACG/TACG
6	M/47	Pancolitis	Knee and wrist arthritis	M680I/0	CGAG/CGAA
7	F/28	Rectosigmoiditis	Knee and wrist arthritis	A744S/0	TACG/TACG

*The TACG/TACG haplotype pattern was similar between 7 UC/*MEFV* positive patients and 18 UC/*MEFV* negative.

The study was approved by the Institutional Review Board and informed consent was obtained from all participating subjects.

***MEFV* Exons 10 and 2 Mutation Analysis.** Mutational analysis was carried out at the DNA level. Exons 10 and 2 of the *MEFV* gene were analyzed for mutations; these sequences contain the vast majority and the main disease-related mutations (4–6). Nonisotopic RNase cleavage assay (NIRCA) analysis was used as first screening method to detect mutations of *MEFV* as previously described (5, 19). Briefly, mutations are detected by ribonuclease cleavage of both strands of duplex RNA obtained by in vitro transcription of polymerase chain reaction (PCR) products containing opposable T7 and SP6 phage promoters. NIRCA was based on previously described protocols (5) and the experimental conditions were carried out according to the instructions of MutationScreener Kit (Ambion, Austin, TX, USA). Nested amplification of exons 10 and 2 sequences provides 797- and 745-bp PCR products, respectively and the positions and sequences of primers, as well as the PCR conditions for NIRCA analysis have been previously described (5).

Sequencing. For direct sequencing analysis, Lark Technologies sequencing service was used (http://www.lark.com/).

Statistical Analysis. Fisher's exact test was used for comparison between discrete parameters. Student's t-test for unpaired samples was used for comparison between quantitative parameters when allowed by the number of cases (>10) and the normality tests (Kolmogorov–Smirnov test, Lilliefors test). The level of statistical significance was set to $P = .05$.

Population Genetics Analysis. The Arlequin 2.0 software (Genetics and Biometry Laboratory, University of Geneva, Switzerland, 2000) was used for the calculation of gene diversity (the probability that 2 randomly chosen haplotypes are different in a sample), expected homozygosity (the probability that an individual is homozygous for any haplotype in a sample), observed homozygosity (the true ratio of homozygous individuals for any haplotype in a sample), and for the performance of Ewens-Watterson neutrality test ($P > .05$ reflects deviation from neutrality indicating favorable or harmful genes).

RESULTS

Mutational Analysis

NIRCA revealed a characteristic positive digestion pattern of *MEFV* gene, corresponding to certain mutations in 7 out of 25 UC patients (28%). Direct sequencing confirmed the mutational pattern of NIRCA and identified 2 patients with M680I/0, 3 with M694V/0, 1 with E148Q/E148Q, and 1 with A744S/0. Only 1 out of 65 normal individuals was positive after NIRCA analysis in exon 10 and direct sequencing revealed M694V/0 mutation ($P = .0004$ compared to UC patients). Similarly, 1 out of 28 control patients with rheumatoid arthritis revealed again a M694V/0 mutation ($P = .0199$ compared to UC patients).

Clinical Profile of UC Patients Carrying *MEFV* Mutations

Seven out of 25 UC patients had been identified to carry *MEFV* mutations; none had a history compatible with FMF. Clinical details including age, gender, disease location, and extra-intestinal manifestations along with genotype at *MEFV* locus in these patients are described in Table 2. Four out of 7 UC patients carrying *MEFV* mutations also developed arthritis. In 2, monoarthritis of the knee was the presenting symptom, 1 year before the onset of intestinal disease. In contrast, none of the remaining 18 UC patients, who lacked *MEFV* mutations, had a similar clinical profile ($P = .0028$).

MEFV Exon 2 Haplotype Analysis

All 50 chromosomes from UC patients and 130 chromosomes from normal individuals of the study, as well as 18 unrelated chromosomes from FMF patients, were analyzed for a cluster of 4 single nucleotide polymorphisms, which constitute the *MEFV* exon 2 haplotype (Table 3) and are linked with *MEFV* mutations (20). As a first step, NIRCA was applied and negative results confirmed the wild-type haplotypes (T A C G). NIRCA-positive results were analyzed further by direct sequencing.

The T A C G haplotype (Table 4) was by far the most common, compared to other haplotypes, mainly represented by C G A (G/A), when patients with UC are compared with normal individuals ($P < .0001$) and FMF patients ($P = .0310$). The statistically significant difference in distribution of *MEFV* exon 2 haplotypes between

TABLE 3. *MEFV* HAPLOTYPE ANALYSIS: CHARACTERISTICS OF SNPS CONSTITUTING *MEFV* EXON 2 HAPLOTYPES

SNP ID Number	*MEFV* c-DNA *Nucleotide**	*MEFV* exon 2 *Residue*†	*SNP Type*	*Nucleotide in Major Allele*‡	*Nucleotide in Minor Alleles*
224225	306	102	Synonymous	T	C
224224	414	138	Synonymous	A	G
224223	495	165	Synonymous	C	A
224222	605	202	Nonsynonymous	G	A

*The reference sequence and version number of MEFV cDNA used is NM_000243.1 (GenBank).
†Available: http://www.ensembl.org/Homo_sapiens/protview?transcript = ENST00000219596&db = core.
‡Based on the frequency in Greek population; most frequent allele is considered as the wild-type allele; the less frequent allele is considered as the mutant one.

UC patients and both FMF patients and normal individuals (Table 4), suggests that UC patients might constitute a distinct population, unrelated to FMF patients (Table 5). Interestingly, the 7 UC/*MEFV*-positive patients presented similar haplotypic pattern ($P = .611$) with the rest UC/*MEFV*-negative patients (Tables 2 and 4).

DISCUSSION

This study reports for the first time that 28% of unselected, consecutive patients with UC carry mutations of the *MEFV* gene. These preliminary data raise several intriguing questions regarding disease pathogenesis: Do the observed genetic alterations constitute a primary genetic event or not? Does *MEFV* serve as a modifying gene in the complex inflammatory reactions in this disease? Is there an overlap in some cases between UC with atypical cases of FMF? What is the role of mutations in *MEFV* gene in the development of inflammation within the joint?

M694V and M680I mutations are commonly found in FMF patients and mutations located within these hot spots are associated with more severe phenotypes. In contrast, E148Q homozygosity is associated with a milder phenotype (4). Interestingly, there are occasional M694V or M680I heterozygous FMF patients with severe clinical manifestations in the absence of other genetic alterations of *MEFV* (4–6).The statistically significant predominance of these strong heterozygous mutations in UC patients—in comparison to the group of 65 healthy individuals—is provocative. As in the case of heterozygous M694V or M680I FMF patients, it is reasonable to speculate that other genes, modifier loci, and epigenetic factors may also involved in UC patients (8, 10). In view of previous findings demonstrating the presence of an enhanced acute phase response among FMF carriers (21), it is reasonable to assume that altered pyrin in *MEFV* heterozygous UC patients may modify the inflammatory response. Furthermore, in Mediterranean populations other than Greeks, the

TABLE 4. DISTRIBUTION OF *MEFV* HAPLOTYPES IN CHROMOSOMES OF PATIENTS WITH UC, PATIENTS WITH FMF, AND NORMAL INDIVIDUALS ALONG WITH THE RELEVANT POPULATION GENETICS DATA

SNP ID Number				*UC patients chromosomes*	*FMF patients chromosomes*	*Normal individuals chromosomes*
224225	*224224*	*224223*	*224222*			
T	A	C	G	45 (90.00%)	12 (66.66%)	86 (66.15%)
C	G	A	A	2 (4.00%)	2 (11.11%)	17 (13.08%)
C	G	A	G	2 (4.00%)	1 (5.66%)	17 (13.08%)
C	A	C	A	1 (2.00%)	0	1 (0.77%)
C	A	C	G	0	2 (11.11%)	1 (0.77%)
T	A	C	A	0	0	4 (3.08%)
C	A	A	A	0	0	1 (0.77%)
C	G	C	A	0	0	1 (0.77%)
T	A	A	G	0	1 (5.66%)	1 (0.77%)
C	A	A	G	0	0	1 (0.77%)
Total				50	18	130
Gene diversity				0.1902 ± 0.0734	0.5556 ± 0.1304	0.5309 ± 0.0466
Observed $F*$				0.814	0.475	0.473
Expected F†				0.511	0.349	0.289
E-W test P				.945	.890	.930

*Observed homozygosity.
†Expected homozygosity.
Abbreviations: E-W test, Ewens–Watterson neutrality test; FMF, familial Mediterranean fever; UC, ulcerative colitis.

TABLE 5. MUTATIONAL ANALYSIS OF FMF PATIENTS IN CORRELATION WITH HAPLOTYPIC DATA

Haplotype	*E148Q*	*E167D*	*T267I*	*M680I*	*M694V*	*K695R*	*V726A*	*Total*
CACG		2						2
TACG	3			2	4	1	2	12
TAAG		1						1
CGAG					1			1
CGAA			1	1				2
Total	3	3	1	3	5	1	2	18

most common FMF mutations have been associated with the C G A A/G haplotype (20). These mutations have been associated with the T A C G haplotype in Greek population (Table 5) and this controversy might reflect a distinct founder effect.

Previous studies have suggested that IBD (Crohn's disease and UC) is particularly frequent and severe in FMF families (14). Moreover, Crohn's disease is more prevalent in FMF than in the general population and patients with both Crohn's disease and FMF have more frequent attacks (22). Although not the primary focus of this study, these findings indicate that the frequency of *MEFV* mutations should also be explored in patients with Crohn's disease.

The possibility of disease overlap with UC in some cases of atypical FMF has been raised previously in the literature (23). For example, inflammatory arthritis is a common clinical finding shared by both FMF and UC. Indeed, 4 of 7 UC patients carrying mutations in *MEFV*—but none of the remaining 18 UC patients who lacked the mutated *MEFV* gene—developed arthritis. Similarly in Crohn's disease extraintestinal manifestations have been also linked with the presence of *MEFV* mutations (16).

More studies, particularly in populations where FMF and UC are frequently observed, may elucidate the potential involvement of *MEFV*. Recent findings indicate that several *MEFV* transcripts are expressed both under basal condition and upon stimulation with LPS in primary human synovial fibroblasts (24). Of interest, a prominent splice isoform that lacks the C-terminal domain that is highly mutated in FMF was also detected in these cells. In contrast to native pyrin that is nuclear in its localization, these recombinant forms are cytoplasmic. The existence of various pyrin isoforms with potential proinflammatory functions within the joint may explain the localization of the attack in this site. To this end, the increased frequency of *MEFV* in patients with UC and episodic arthritis is certainly provocative especially in view of the lower frequency of these mutations in patients with chronic, nonepisodic arthritis such as the rheumatoid arthritis. However, these data need to be confirmed in larger studies.

Although we have not screened genetically the pedigrees of UC/*MEFV* patients, the family history of UC relatives for FMF manifestations was negative. This observation, along with the absence of diagnostic criteria for FMF and the haplotypic pattern characterized by limited gene diversity and increased homozygosity of patients with UC (see Table 4), suggests that UC patients might constitute a distinct population where the mutated pyrin can modify the expression of a broad array of genetic or epigenetic factors also involved in the pathogenesis of UC. *MEFV* exon-2-haplotype–associated UC may also reflect an as yet unknown susceptibility allele.

Recent studies indicated a complex interplay between molecules containing PYD and/or caspase recruitment domain (CARD) in innate immunity control and many families of these proteins have been classified (8, 10, 11). These proteins are implicated–directly or indirectly—in this complicated cross-talk. Pyrin, apoptosis-associated speck-like protein (which contains a CARD), NALPs (NACHT-LRR-PYD–containing proteins), nucleotide-binding oligomerization domain-containing proteins (NODs), and Toll-like receptors (TLRs) are some of members of this family (8–11, 15). TLR and NOD proteins have been recently shown to mediate the innate immune response to bacterial products and members of these protein families are genetically associated with susceptibility to IBD (15). The possible modifying effect of pyrin in this complex cross-talk remains to be further elucidated. Longitudinal studies involving larger populations are needed to further document the role of pyrin in the homeostatic control of inflammation both under normal conditions and in disease states.

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REFERENCES

1. Livneh A, Langevitz P, Zemer D, *et al.*: Criteria for the diagnosis of familial Mediterranean fever. Arthritis Rheum 40:1879–1885, 1997

2. The French FMF Consortium: A candidate gene for familial Mediterranean fever. Nat Genet 17:25–31, 1997
3. The International FMF Consortium: Ancient missense mutations in a new member of the RoRet gene family are likely to cause familial Mediterranean fever. Cell 90:797–807, 1997
4. Sarrauste de Menthiere C, Terriere S, Pugnere D, Ruiz M, Demaille J, Touitou I: INFEVERS: the Registry for FMF and hereditary inflammatory disorders mutations. Nucleic Acids Res 31:282–285, 2003
5. Ritis K, Giaglis S, Spathari N, *et al.*: Non-isotopic RNase cleavage assay for mutation detection in MEFV, the gene responsible for familial Mediterranean fever, in a cohort of Greek patients. Ann Rheum Dis 63:438–443, 2004
6. Konstantopoulos K, Kanta A, Deltas C, *et al.*: Familial Mediterranean fever associated pyrin mutations in Greece. Ann Rheum Dis 62:479–481, 2003
7. Hiller S, Kohl A, Fiorito F, Herrmann *et al.*: NMR structure of the apoptosis- and inflammation-related NALP1 pyrin domain. Structure (Camb) 11:1199–205, 2003
8. McDermott MF: A common pathway in periodic fever syndromes. Trends Immunol 25:457–460, 2004
9. Chae JJ, Komarow HD, Cheng J, *et al.*: Targeted disruption of pyrin, the FMF protein, causes heightened sensitivity to endotoxin and a defect in macrophage apoptosis. Mol Cell 11:591–604, 2003
10. McDermott MF, Aksentijevich I: The autoinflammatory syndromes. Curr Opin Allergy Clin Immunol 2:511–516, 2002
11. Stehlik C, Lee SH, Dorfleutner A, Stassinopoulos A, Sagara J, Reed JC: Apoptosis-associated speck-like protein containing acaspase recruitment domain is a regulator of procaspase-1 activation. J Immunol 171:6154–6163, 2003
12. Podolsky DK: Inflammatory bowel disease. N Engl J Med 347:417–429, 2002
13. Farrell RJ, Peppercorn MA: Ulcerative colitis. Lancet 359:331–340, 2002
14. Cattan D, Notarnicola C, Molinari N, Touitou I: Inflammatory bowel disease in non-Ashkenazi Jews with familial Mediterranean fever. Lancet 355:378–379, 2000
15. Lichtenberger GS, Flavell RA, Alexopoulou L: Innate immunity and apoptosis in IBD. Inflamm Bowel Dis 10(Suppl 1):58–62, 2004
16. Fidder H, Chowers Y, Ackerman Z, *et al.*: The familial Mediterranean fever (MEVF) gene as a modifier of Crohn's disease. Am J Gastroenterol 100:338–343, 2005
17. Karban A, Dagan E, Eliakim R, *et al.*: Prevalence and significance of mutations in the familial Mediterranean fever gene in patients with Crohn's disease. Genes Immun 6:134–139, 2005
18. Hommes DW, van Deventer SJ: Endoscopy in inflammatory bowel diseases. Gastroenterology 126:1561–1573, 2004
19. Goldrick MM, Kimball GR, Liu Q, Martin LA, Sommer SS, Tseng JY: NIRCA: a rapid robust method for screening for unknown point mutations. BioTechniques 21:106–112, 1996
20. Aldea A, Calafell F, Arostegui JI, *et al.*: The West Side Story: *MEFV* haplotype in Spanish FMF patients and controls, and evidence of high LD and a recombination "hotspot" at the MEFV locus. Hum Mutat 23:399–406, 2004
21. Tunca M, Kirkali G, Soyturk M, Akar S, Pepys MB, Hawkins PN: Acute phase response and evolution of familial Mediterranean fever. Lancet 353:1415, 1999
22. Fidder HH, Chowers Y, Lidar M, Sternberg M, Langevitz P, Livneh A: Crohn disease in patients with familial Mediterranean fever. Medicine (Baltimore) 81:411–416, 2002
23. Mor A, Gal R, Livneh A: Abdominal and digestive system associations of familial Mediterranean fever: Am J Gastroenterol 98:2594–2604, 2003
24. Diaz A, Hu C, Kastner DL, *et al.*: Lipopolysaccharide-induced expression of multiple alternatively spliced MEFV transcripts in human synovial fibroblasts. Arthritis Rheum 50:3679–3689, 2004