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ORIGINAL ARTICLE

Detection of Mediterranean fever gene mutations in Egyptian children with inflammatory bowel disease

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Abstract

Aim: The aim of the current study is to investigate the prevalence of familial Mediterranean fever gene (*MEFV*) mutations in a cohort of Egyptian children with inflammatory bowel disease (IBD), and to characterize familial Mediterranean fever (FMF)-IBD patients, helping better understanding of IBD pathogenesis.

Methods: The study enrolled 17 patients with ulcerative colitis (UC), 15 with Crohn's disease(CD), 10 with indeterminate colitis (IC) and 33 healthy children as controls. All cases and controls were tested for 12 FMF gene mutations by reverse hybridization after multiplex polymerase chain reaction amplification and DNA sampling.

Results: Eighty-eight percent of the IBD patients carried the mutations, with Sequence variant V627A being the commonest versus 42.4% of controls. No associations were found between *MEFV* gene mutations, and phenotypic characteristics of IBD patients.

Conclusion: IBD patients, in populations with a high background carrier rate of *MEFV* variants, should be screened for *MEFV* gene mutations, especially those diagnosed as indeterminate colitis. Testing larger numbers of healthy Egyptian children for *MEFV* gene mutation is important to further determine the allele frequency in Egypt.

Key words: disease, etiology and pathogenesis – human, immunogenetics, paediatric rheumatology, pediatric rheumatology, sasic sciences.

BACKGROUND

Inflammatory bowel diseases (IBD), including Crohn's disease (CD), ulcerative colitis (UC) and indeterminate colitis (IC), are immune-mediated disorders causing chronic relapsing inflammation of the gastrointestinal tract.[1] Etiology includes genetic and environmental factors; generating deregulated inflammatory cascade leading to mucosal injury.[2] The most common symptoms at presentation are abdominal pain, diarrhea, weight loss and fever, and in UC, bloody stools. Children and adolescents are at risk for significant growth retardation and growth failure.[3] UC is a disease of rectal and colonic mucosa,[4,5] whereas CD affects any region from the esophagus to anus, and its chronic inflammation is similar to that of UC.1,6 Several susceptibility gene loci for IBD have been identified, and termed IBD1–7. Among them, the caspase activation recruitment domain, family member 15 (*CARD15*) genes, encoding for nuclear oligomerization domain 2 protein (NOD2) on IBD-1 locus, was found to be strongly related to CD susceptibility.[6] Familial Mediterranean fever (FMF; OMIM 249100) is an auto-inflammatory disease characterized by episodes of abdominal pain, fever and arthralgia. The Mediterranean fever gene (*MEFV*) responsible for FMF is

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localized at 16p13.3 and encodes pyrin (marenostrin) protein.[7] IBD and FMF both have recurrent and periodic symptoms. Both the *MEFV* gene and the (NOD2/CARD15) are located on chromosome 16. Additionally, genetic products (pyrin and NOD2/CARD15), are structurally similar and belong to the same apoptosis-regulating protein family. They play a role in cytokine processing and inflammation. In some epidemiological studies, IBD was found to be more prevalent and severe in families with FMF, with a modifying effect of *MEFV* gene mutations on IBD clinical picture.[4,8,9] Studies on a Turkish population showed association between the *MEFV* gene variations and inflammatory bowel disease,[10–12] showing improvement of IBD patients, with *MEFV* gene mutations when colchicine was started.[13] Despite being one of the Mediterranean countries, no studies discussing the relation between IBD and *MEFV* gene mutation among Egyptian children have been published. The aim of this study was to investigate the prevalence of *MEFV* mutations in a cohort of Egyptian children with IBD, and to investigate the effects of these mutations on the clinical status of IBD, for better management and understanding of IBD pathogenesis.

SUBJECTS AND METHODS

The study enrolled 42 unrelated IBD patients diagnosed as one of the three IBD types: UC, CD or IC, by standard radiologic, endoscopic and histological criteria according to Porto criteria (2005).[14] It also enrolled 33 healthy children with no family history or clinical manifestations suggestive of FMF or IBD (recruited from relatives of patients in general pediatric unit). The patients were followed up at the Tropical Pediatric Clinic, Cairo University Children's Hospital, from 2004 to 2011. This study was approved by the Cairo University Clinical Research Ethics Committee. Informed consents were obtained from parents of all participants. Only IBD patients with age from 6 months to 15 years at time of diagnosis were included in the study. Any child diagnosed as FMF before the onset of IBD was excluded. Demographic and routine clinical and laboratory characteristics of the patients were evaluated at the time of study enrollment. Data collected included: age at diagnosis, presenting manifestations, disease duration, duration of follow-up, gastrointestinal sites involved, extra-intestinal manifestations, therapy strategies and disease course. All patients and controls were screened for 12 common *MEFV* mutations.

DNA isolation

DNA was isolated using Invisorb Spin Blood Mini Kit (Invitek, Berlin, Germany) according to the manufacturer's instructions.

Reverse hybridization essay

Twelve mutations (E148Q [exon 2], P369S [exon 3], F479L[exon 5], M680I (G = C), M680I (G = A), 1692 del, M694V, M694I, K695R, V726A, A744S and R731H [exon 10]) of the *MEFV* gene were analyzed by reverse hybridization assay (FMF Strip Assay; Vienna Lab, Vienna, Austria) kit according to the manufacturer's instructions.[15]

Statistical analysis

Data were statistically described in terms of: mean ± standard deviation (± SD), median and range; or frequencies (number of cases) and percentages when appropriate. Comparison of numerical variables between the study groups was done using Mann–Whitney *U*-test for independent samples. For comparing categorical data, Chi-square (χ^2) test was performed. Exact test was used instead, when the expected frequency was <5. All statistical calculations were done using the computer program SPSS (Statistical Package for Social Science; SPSS Inc., Chicago, IL, USA) version 15 for Microsoft Windows.

RESULTS

The study included 42 Egyptian patients with IBD and 33 healthy children not related to patients. Nineteen patients were female and 23 were male (female-to-male ratio 1 : 1.2), with a mean age at diagnosis of 5.1 ± 3.7 years. The mean disease duration was 3.3 ± 1.7 years (range 0.5–7 years). Parents of 18 patients (42.9%) were consanguineous. The demographic and clinical characteristics of the study group are summarized in Table 1.

Of 42 patients with the IBD, 17 (40.4%) were diagnosed as UC, 15 (35.7%) as CD, and IC was present in 10 (23.8%). Forty-five percent of our study group presented with diarrhea, followed by hematochezia in 38.1%, skin rash in 2.4%, abdominal pain and failure to thrive, each, in 7.1% of the patients. Throughout the disease course, the frequency of recurrent abdominal pain, diarrhea and bleeding per rectum increased; occurring in 98.6%, 78.6% and 57.1% of patients, respectively.

Table 1 Demographic and clinical characteristics of current IBD patients

Variable	IBD patients (n = 42)
Gender n (%)	
Male	23 (54.7%)
Female	19 (45.3%)
Male: female ratio	1.2 : 1
Chronological age (years)	10.1 ± 4.2
Mean age at diagnosis (years)	5.1 ± 3.7
Mean follow-up duration (years)	3.3 ± 1.7
Positive consanguinity, n (%)	18 (42.9%)
IBD type, n (%)	
CD	15 (35.7%)
UC	17 (40.4%)
IC	10 (23.8%)
Type of CD, n (%)	
Inflammatory	12 (80%)
Stricturing	3 (20%)
Disease location, n (%)	
Ileocolon	12 (28.6%)
Pancololn	12 (28.6%)
Terminal ileum	2 (4.8%)
Rectosigmoid	9 (21.4%)
Patchy disease distribution	7 (16.8%)
Perianal disease, n (%)	1 (2.4%)
Extra-intestinal disease, n (%)	12 (33.5%)

Quantitative data are represented as mean ± SD, while qualitative data are represented as frequency (percentage); CD, Crohn's disease; IBD, inflammatory bowel diseases; IC, indeterminate colitis; UC, ulcerative colitis.

Twelve (33.5%) patients had extra-intestinal manifestations, including arthritis, oral ulcers, skin rash, in the form of erythema nodosum, testicular affection and autoimmune hepatitis, occurring in 43.8%, 18.7%, 18.7%, 12.5% and 6.3% of patients, respectively.

During follow-up, none of our patients had proteinuria or signs of amyloidosis in bowel biopsies.

Regarding treatment at the time of study enrollment, 40 patients (95%) were on mesalamine, nine (21.4%) on corticosteroids, 24 (61.9%) on azathioprine and two were on biological treatment (infliximab), either in combined or as solitary treatment regimens.

Several *MEFV* gene mutations were detected in 37 (88.1%) of the total studied patients. Thirty-one (83.8%) of the positive patients carried heterozygous mutations, four (10.8%) with homozygous gene mutations and two (5.4%) with a compound heterozygous mutation.

Of the four IBD patients carrying homozygous mutations, three were diagnosed as IC and one as UC. The types of gene mutations in them were; M680I gene mutation in two patients, V726A mutation in one patient and M694V mutation in another patient.

Among gene mutations detected in IBD patients, V726A mutation was the most frequent, with an allelic frequency of 16 alleles (19%), followed by M680I, E148Q, M694V and I692del gene mutations, with an allelic frequency of 11, 10, 6 and 1 allele respectively as shown in (Table 2).

Out of 33 controls, 14 carried heterozygous gene mutations, with E148Q being the most frequent mutation, found in seven (50%) of mutation-positive controls (Table 2).

A significant difference was found between *MEFV* gene mutations, detected in 37/42 (88.1%) IBD patients, and mutations in controls, detected in 14/33 (42.4%) patient, with a P-value < 0.001 (Table 2).

Among IBD patients with *MEFV* positive mutations, four (10.9%) had a third 'autoimmune' disease, two had type I diabetes mellitus (DM), one had celiac disease and one had systemic lupus erythematous (SLE).

Although extra-intestinal diseases were more common among *MEFV*-positive IBD patients, being detected in 11 out of 37 (29.7%) patients compared to one (20%) out of five IBD patients without mutations, no statistically significant difference was found between them (P = 0.651).

Distribution of gene mutations among IBD patients with EIM (extra-intestinal manifestations) follows exactly the same pattern as all gene-positive IBD patients, with V726A being the most frequent mutation with an allelic frequency of 25%. These mutations were homozygous (M680I) mutation in one patient, compound heterozygous in two patients (V726A, E148Q& I692del and E148Q&M694V), while the remaining eight were heterozygous as shown in Table 3.

For statistical purposes, IBD patients were classified according to the presence or absence of gene mutations into: group I (with detected gene mutation, n = 37) and group II (without gene mutation, n = 5). No statistically significant difference was detected between the two groups in all demographic and laboratory aspects; as shown in Table 4.

Regarding prognosis, only one patient, with CD and *MEFV* gene mutation, died at the age of 10 years from sepsis and severe protein-losing enteropathy. This patient had a severe course of CD for 1.5 years; with failure to achieve remission in spite of intensive immune-suppressive therapy and enteral feeding.

Of the three IC patients with homozygous *MEFV* gene mutations, one patient with M680I mutation improved on colchicine treatment for 3 months, and

Table 2 *MEFV* gene mutations in IBD patients and controls

	IBD patients (n = 42)	Controls (n = 33)	*P*-value			
Wild-type, (mutation+)	5 (11.9%)	19 (57.6%)				
Presence of *MEFV* gene mutations	37 (88.1%)	14 (42.4%)	< 0.001			
	IBD patients with mutations n = 37/42	UC n = 16/17	CD n = 11/15	IC n = 10/10	Controls (n = 33)	*P*-value
Heterozygous for one mutation	**31 (73.8%)**				**14 (42.4%)**	
p.V726A/−	13 (30.9%)	8 (47%)	3 (20%)	2 (20%)	4 (12.1%)	0.06
p.E148Q/−	8 (19%)	3 (17.6%)	3 (20%)	2 (20%)	8 (24.2%)	0.757
p.M680I (G/A)/−	7 (16.6%)	3 (17.6%)	2 (13.3%)	2 (20%)	1 (3%)	0.07
p.M694V/−	3 (7.1%)	1 (5.8%)	1 (6.6%)	1 (10%)	1 (3%)	0.29
Homozygous for one mutation	**4 (9.5%)**					
M680I	2 (4.7%)			1 (10%)		
V627A	1 (2.3%)			1 (10%)		
M694V	1 (2.3%)	1 (5.8%)		1 (10%)		
Compound two mutations	**1 (2.3%)**					
E148Q/M694V			1 (6.6%)			
Compound three mutations	**1 (2.3%)**					
E148Q/V627A/I694del			1 (6.6%)			

	Allelic frequency of *MEFV* gene mutations				
	IBD patient alleles (n = 84)				Control alleles (n = 66)
	Total IBD patients with mutations n = 74/84	UC n = 32/34	CD n = 22/30	IC n = 20/20	
V726A	16 (19%)	8 (23.5%)	4 (13.3%)	4 (20%)	4 (6.1%)
E148Q	10 (11.9%)	3 (8.8%)	5 (16.6%)	2 (10%)	8 (12.1%)
M680I (G/A)	11 (13%)	5 (14.7%)	2 (6.6%)	4 (20%)	1 (1.5%)
M694V	6 (7.1%)	1 (2.9%)	2 (6.6%)	3 (15%)	1 (1.5%)
I694de	1 (1.2%)				
Total	**44 (52.3%)**				

CD, Crohn's disease; IC, indeterminate colitis; UC, ulcerative colitis; bold values indicate total number and percentage

Table 3 Allelic frequency of *MEFV* gene mutations in IBD patients with extra-intestinal manifestations

Gene mutation	IBD patients with extra-intestinal alleles (n = 24)
V627A	6 (25%)
E148Q	3 (12.5%)
M680I	2 (8.3%)
M694V	2 (8.3%)
I692del	1 (4.16%)

follow-up endoscopy was free. While the other two patients improved on colchicine together with azathioprine and mesalamine.

The patient with UC and homozygous M680I gene mutation developed four diagnostic criteria for SLE (nephritis, cerebritis, antinuclear anitbodies and anti-DNA positive), and was treated by low-dose steroids, azathioprine and mesalamine.

DISCUSSION

Both IBD and FMF have recurrent and periodic symptoms, with genetic and environmental factors underlying disease pathogenesis.[16] Mutations in both genes lead to abnormalities in regulation of apoptosis, cytokine processing and inflammation.[17] This may raise the possibility that abnormalities in one of the proteins, in

Table 4 Demographic and laboratory characteristics of IBD patients stratified by the presence of *MEFV* mutations

Variable	Group I (n = 37)	Group II (n = 5)	P-value
Gender ratio (M/F)	19/18	4/1	0.22
Disease type (no. of patients with UC/CD/IC)	16/11/10	1/4/0	0.08
Age at diagnosis (years)	5.11 ± 3.79	5.3 ± 2.97	0.75
Weight for age at diagnosis	19.78 ± 10.73	19.28 ± 8.04	0.90
Weight for age at the last visit	34.71 ± 18.88	29.7 ± 12.04	0.786
Height for age at diagnosis	111.6 ± 18.67	107.16 ± 28.78	0.771
Height for age at the last visit	129.15 ± 24.92	135 ± 19.82	0.726
Disease location	n (%)		Fisher exact test
Pancolonic	12 (31.7%)	0 (0%)	0.16
Recto-sigmoid	8 (21.6%)	1 (20%)	0.71
Ileo-colonic	9 (21.3%)	3 (60%)	0.13
Terminal ileum	3 (8.1%)	0 (0%)	0.67
Patchy distribution	6 (16.1%)	1 (20%)	0.61 (Fisher exact test)
CD behavior			
Inflammatory	8 (72.7%)	4 (100%)	
Stricturing	3 (27.3%)	0 (0%)	0.36
EIM (n, %)	11 (29.7%)	1 (20%)	0.65
Platelet count(/mm^3) at diagnosis	350 840 ± 127.9	377 000 ± 139.3	0.71
Albumin (g/dL) at diagnosis	3.381 ± 1.024	3.28 ± 1.049	0.923
Surgery+ (n, %)	3 (8.1%)	0 (0%)	0.67 (Fisher exact test)
Appendectomy (n, %)	3 (8.1%)	1 (20%)	0.41 (Fisher exact test)

Quantitative data are represented as mean ± SD, while qualitative data are represented as frequency (percentage). (Group I, patients with *MEFV* gene mutation; group II, patients without *MEFV* gene mutation; EIM, extra-intestinal manifestations; plt, platelets and +, resection of any part of colon).

related pathways, may affect the disease manifestation of the other.[18] Consequently, *MEFV* gene may affect expression of other inflammatory disorders, and their associated genes may trigger FMF.[19,20] Several studies not including any Egyptian study, detected the presence of association between *MEFV* gene variations and IBD.[10–12]

In the present study, we investigated the prevalence of *MEFV* gene mutations in 42 unrelated Egyptian children with IBD and 33 healthy controls, and the possible effects of these mutations on disease progress. *MEFV* gene mutations were detected in 37 (88.1%) of the IBD patients and 14 (42.4%) of the control group, with a statistically significant difference between the two groups ($P < 0.001$).

The high consanguinity among parents of our patients (42.9%) may be because most of our patients are inhabitants of rural areas, where consanguineous marriage is a part of their culture.

Detection of *MEFV* mutations in 88.1% of IBD patients in the present study was higher than their prevalence in other studies, ranging from 25.7%[12] to 19.1%.[21]

In our work, 94% of UC patients had genetic mutations, which is higher than other studies[7] where *MEFV* mutations were detected in 28% of UC patients.

Our results showed that 73% of CD patients had mutations. This is higher than the results of Fidder *et al.*[4] who identified seven patients with concomitant Crohn's disease and FMF (FMF-CD), out of 4978 FMF patients. The small number of patients included in our work together with the high consanguinity among parents of our patients, may be contributing factors to this marked difference in the results, but the low prevalence of IBD in Arab countries, together with the limited resources needed for *MEFV* gene detection in FMF patients, may explain the small sample size.[22]

The significant difference in *MEFV* mutations between patients and controls in our work ($P < 0.001$) does not coincide with the results of Karban *et al.*[9] who found that carriers of *MEFV* mutations are equally prevalent in CD patients and controls, and with Fidder *et al.*,[23] with no significant differences detected between *MEFV* mutations in CD patients and the general Israeli Jewish population.[24]

The presence of genetic mutations, among 42.4% of the control group, was quite surprising, being higher than expected from other studies,[25,26] where genetic mutations were detected in 10.4% and 18.4% of healthy controls, respectively. The very high rate of consanguineous marriage and recruitment of some of the controls from related families might explain this.

Among few studies discussing IC, one[12] detected *MEFV* gene mutation in 66.6% of patients; while in our work, all IC patients carried *MEFV* gene mutation; raising the possibility that FMF might be considered during follow-up of IC patient.

V726A was the most frequent mutation found in 15 (37.5%) of our *MEFV*-positive IBD patients, while M694V mutation was the fourth most common, found in five (12.5%) patients. This does not coincide with the results of some other studies,[7,12,23] where M694V, which is widely accepted to be a severe mutation, was the leading mutation. On the other hand, some studies[9,27] found E148Q to be the commonest mutation among IBD-FMF patients. This E148Q mutation was the second most common mutation detected in our patients, found in 10 (25%) patients. This variation among different studies may be related to different ethnic groups involved.

E148Q was the commonest mutation in controls, found in 50% of positive cases. This is in concordance with the commonest mutation detected in Egyptian healthy controls.[28] This is also in concordance with the results of other studies,[7,12] where E148Q heterozygous mutation was the commonest mutation among healthy controls.

In the present study, distributions of the FMF gene mutations differ among patients with different IBD types. V726A was the most frequent mutation in UC, followed by M680I, which does not coincide with the results of Yurtcu *et al.*,[21] where E148Q was the most frequent mutation, and Giaglis *et al.*,[8] who found M694V and M680I to be the most frequent mutations.

As in the case of heterozygous FMF patients, we may speculate that other genes, modifier loci and epigenetic factors, may be also involved in UC patients.[28]

In our study, E148Q was the most frequent mutation in Crohn's disease patients, followed by V726A. This does not coincide with the results of Fidder *et al.*,[4] where M694V was the most frequent mutation. This may be due to the relatively high prevalence of M694V among Jewish FMF patients of North African background[4] (Table 5).

In the present study, four (10.9%) of the *MEFV* gene positive IBD patients had a third inflammatory condition. Interestingly, Fidder *et al.*[4] found 4/7 CD-FMF patients having a third inflammatory condition (two with ankylosing spondylitis, one with PAN (polyarteritis nodosa), one with MCTD (mixed connective tissue disease)), raising the possibility that different autoimmune diseases may share common genetic and immunological pathways.

The absence of amyloidosis and proteinuria among our IBD patients with detected *MEFV* mutation, coincides with the results of some other studies,[7,13,27] except Fidder *et al.*,[4] who found increased prevalence of persistent proteinuria among patients with FMF and Crohn's disease. This may be related to the low mean age and follow-up duration in the present study, and difference in type of *MEFV* genetic pattern.

The non-significant increase in frequency of *MEFV* gene mutations among IBD patients with extra-intestinal disease manifestations (27.9% *vs.* 20%; $P = 0.65$) is consistent with other studies.[9,12,27] On the other hand, Fidder *et al.*,[23] detected that extra-intestinal

Table 5 Different studies discussing the prevalence and modifying effect of *MEFV* gene mutations on different types of IBD patients

Study	IBD study group (*n*)	Mean age (years)	Distribution of patients according to IBD type			Number of patients with *MEFV* mutations				Commonest mutation
			CD	UC	IC	CD	UC	IC	EIM	
Turkey[21]	47	42.3	12	35	–	1	8	–	9	E148Q
Turkey[12]	33	13	14	16	3	4	1	2	5	M694V
Greece[7]	25	41	–	25	–	–	7	–	4	M694V
Israel[9]	209	23.6	209	–	–	47	–	–	12	E148Q
Israel[23]	105	28.2	105	–	–	18	–	–	11	E148Q
Salah *et al.* 2014 Egypt (the present work)	42	10.1	15	17	10	11	16	10	12	V627A

CD, Crohn's disease; IC, indeterminate colitis; UC, ulcerative colitis.

manifestations are about fourfold more frequent in CD patients with *MEFV* mutations. Also, Giaglis *et al.*[7] have shown that in UC patients with extra-intestinal symptoms, such as episodic arthritis, *MEFV* mutations modify disease pathogenesis.

The diversity in results between different studies is not surprising because discordant results are common among genetic investigations of complex diseases. Possible explanations for controversial results include clinical heterogeneity, ethnic differences, real genetic heterogeneity and small sample sizes.

Forty-two percent of healthy controls showed variants in the *MEFV* gene. Therefore, testing additional healthy Egyptian children from different governorates is important for further determination of the allele frequency, and to distinguish between single nucleotide polymorphisms and mutations.

A limitation of this study was the small and heterogeneous cohort of Egyptian IBD patients, making it difficult to interpret the effects of identified *MEFV* sequence variants on clinical findings and disease severity. Larger numbers of patients is needed in future studies, which is difficult due to the small number of IBD patients followed up in our center. Also, identification of other genes involved in IBD pathogenesis could provide important data about the nature of genetic factors triggering and driving the disease process.

In conclusion, in the present study there was a significant association between *MEFV* gene mutations and IBD in Egyptian children, especially in patients with indeterminate colitis. Sequence variant V726A was the most common in Egyptian patients with IBD.

Mutations were not associated with disease status. Frequent screening for *MEFV* gene mutations in IC patients is recommended.

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COMPETING INTERESTS

The authors declare that they have no potential conflict of interest.

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