MEFV Mutations in IBD Patients: A Systematic Review and Meta-analysis

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INTRODUCTION

Crohn’s disease (CD), ulcerative colitis (UC) and inflammatory bowel disease unclassified (IBDU) – formerly known as indeterminate colitis (IC) – are disorders characterized by damaged epithelial barrier, defects in the innate immune responses as well as dysregulation of the adaptive immunity, collectively referred as inflammatory bowel diseases (IBD). Despite being heterogeneous, they present overlapping pathologic and clinical characteristics as they all affect the gastrointestinal tract in a chronic and relapsing disease pattern. Inflammatory bowel diseases pathogenesis is partly attributed to complex genetic susceptibility, as revealed by genome-wide association studies (GWAS) and meta-analysis of loci; however, well-established associations, as with NOD2 and IL23R genes, can only partly explain the genetic variance observed [1].

NLR family pyrin domain containing 3 (NLRP3) gene encodes cryopyrin, a protein component of inflammasome, a molecular platform regulating caspase-1 activation and interleukin (IL)-1β processing. Mutations in NLRP3 gene, may result in autoinflammatory disorders. Moreover, NLRP3 was proposed as a potential susceptibility gene for CD [2].

ABSTRACT

Background & Aims: Several studies have suggested that mutations in MEFV, the gene responsible for familial Mediterranean fever (FMF), are frequently detected in inflammatory bowel disease (IBD) patients. We aimed to provide further evidence regarding a potential correlation between MEFV gene mutations and IBD by identifying all relevant studies and analyzing their results.

Methods: EMBASE, PubMed/MEDLINE, and Google Scholar were used to identify all studies that published until January 2021 and reported MEFV mutation patterns in patients with ulcerative colitis (UC), Crohn’s disease (CD) and indeterminate colitis (IC) with or without a control group. The Newcastle-Ottawa quality assessment scale was used to appraise the quality of the included studies.

Results: Thirteen observational studies, including 937 patients and 977 controls, were analyzed. MEFV mutation rate in IBD patients was 0.238 (95%CI: 0.209-0.270; I²=95%); MEFV mutated alleles were more frequent in IBD patients when compared with controls (p=0.03 for UC, p=0.01 for CD and IC). Subgroup analysis indicated that MEFV mutations were increased in patients with IC when compared with UC and CD (I²=91%, p<0.001). Patients with extra-intestinal manifestations and pancolitis had 2.57 (95%CI 1.07-6.14; p=0.03) and 2.02 (95%CI: 1.01-4.04, P=0.049) odds ratios to carry MEFV mutant genotypes, respectively. Exon 10 mutations had the most serious impact. No source of heterogeneity was detected.

Conclusions: MEFV mutations are common in IBD and are linked with the presence of extra-intestinal manifestations and pancolitis. Further research to assess the clinical significance and evolutionary significance of MEFV mutations in IBD patients is warranted.

Key words: inflammatory bowel disease – familial Mediterranean fever – Pyrin – inflammasome – interleukin-1 beta – MEFV.

Abbreviations: CD: Crohn’s disease; FMF: familial Mediterranean fever; HWE: Hardy-Weinberg equilibrium; IBD: inflammatory bowel diseases; IBDU: IBD unclassified; IC: indeterminate colitis; OR: odds ratio; UC: ulcerative colitis.
Familial Mediterranean fever (FMF), the prototype of the hereditary autoinflammatory disorders resulting in seemingly unprovoked brief periodic activation of innate immunity presenting with episodes of fever, serositis and skin rash, is attributed to mutations in the MEFV gene. The SPRY domain of the protein pyrin, encoded by MEFV, has been reported to interact and modulate the activity of several inflammasome components including NALP3/cryopyrin, caspase-1, and, its substrate pro-IL-1β [3]. Pyrin also plays a role as an autophagy receptor contributing in selective autophagic degradation of key components of the inflammasome such as NLRP3 and pro-caspase 1 [4], while dysregulated autophagy has been strongly linked to IBD pathogenesis [5, 6]. Moreover, the pyrin inflammasome itself has evolved as an innate immune sensor to detect bacterial toxin-induced Rho guanosine triphosphatase inactivation, a phagocytosis suppression mechanism induced by pathogens, including intestinal microbes such as Clostridium difficile [7, 8]. Previously, we have reported high frequency of MEFV mutations in UC [9]. Moreover, we have demonstrated that FMF and UC share common molecular mechanisms in neutrophils supporting the autoinflammatory nature of UC [5].

Considering that both UC and FMF share common clinical and pathogenetic characteristics along with the co-existence of cryopyrin and pyrin, the products of NALP3 and MEFV, in the same signaling pathway, it would be reasonable to test the hypothesis that part of the susceptibility of UC and perhaps CD could be attributed to mutations in MEFV [10-12]. This hypothesis is supported by epidemiological data, since the clinical association between IB and FMF was firstly observed in non-Ashkenazi Jews twenty years ago followed by similar reports [13, 14]. Since then, several studies have investigated the frequency of MEFV mutations among IBD patients, some with contradictory results [9, 14-25].

As MEFV mutation pattern is non-uniform regarding distribution, phenotypic expression, neutrality and population genetics characteristics, evidence-based genetic data concerning the correlation of MEFV mutations, especially the most frequent ones (M694V, V726A, M680I, and E148Q) and IBD are emerging [26].

The present systematic review and meta-analysis was conducted with the aim of providing further evidence regarding a potential correlation between MEFV gene mutations and IBD by identifying all relevant studies and summarizing their results.

**METHODS**

**Literature Search**

A systematic literature review was conducted using EMBASE and PubMed/MEDLINE databases until January 2021 to identify all studies that reported MEFV mutation patterns in patients with UC, CD and IC and in healthy controls. The Google Scholar database was used as an additional pool of published data; moreover, we searched Google Library for unpublished dissertations and other unpublished work. An iterative search was performed until no additional publications could be traced. Lastly, the relevant protocol was submitted to the PROSPERO database (ID: 185247). No funding was received. No competing interests were declared by the authors.

**Studies’ Selection**

The review was independently conducted by 3 authors (V.P., C.A. and P.S.) using a search strategy that included the terms [inflammatory bowel disease] AND [familial Mediterranean fever]; [inflammatory bowel disease] AND [FMF]; [inflammatory bowel disease] AND [MEFV]; [ulcerative colitis] AND [familial Mediterranean fever]; [ulcerative colitis] AND [FMF]; [ulcerative colitis] AND [MEFV]; [crohn] AND [familial Mediterranean fever]; [crohn] AND [FMF]; [crohn] AND [MEFV]; [indeterminate colitis] AND [familial Mediterranean fever]; [indeterminate colitis] AND [FMF]; [indeterminate colitis] AND [MEFV]; a third author (KR) was responsible for resolving any discordance. No software was used for study retrieval. Sources of financial support were traced where possible.

**Outcome Measures**

The present study was conducted in accordance with the PRISMA (Preferred Reporting Items for Systematic reviews and Meta-Analyses) guidelines to formulate the basis of pre-specified eligibility criteria using the PICO (P - Populations/People/Patient/Problem: Patients suffering from any type of IBD, namely UC, CD or IC and healthy individuals (controls), I - Intervention(s): MEFV gene mutations, C - Comparison: i) prevalence of mutant MEFV alleles and/or genotypes among patients suffering from UC compared with other forms of IBD; ii) prevalence of mutant MEFV alleles and/or genotypes among patients suffering from CD compared with other forms of IBD; iii) prevalence of mutant MEFV alleles and/or genotypes among patients suffering from IC compared with other forms of IBD; iv) distribution of MEFV mutated alleles and/or genotypes among IBD patients and healthy individuals (controls); v) distribution of mutant MEFV alleles and/or genotypes among IBD patients with and without stricturing IBD; vi) distribution of mutant MEFV alleles and/or genotypes among IBD patients with and without inflammatory IBD; vii) prevalence of mutant MEFV alleles and/or genotypes among IBD patients and controls; viii) distribution of MEFV mutations among IBD patients and controls; ix) distribution of the M680I MEFV mutation among IBD patients and controls; x) distribution of the E148Q MEFV mutations among IBD patients and controls; xi) distribution of the V726A MEFV mutation among IBD patients and controls; xii) distribution of the exon 10 MEFV mutation among IBD patients and controls; xiii) distribution of MEFV mutations among IBD patients with and without extra-intestinal manifestations; xiv) distribution of MEFV mutations among IBD patients with and without pancolitis; xv) distribution of MEFV mutations among IBD patients with and without fistulizing IBD; O—Outcome: OR; percentage) worksheet and search strategy [27]. The AMSTAR checklist was used to confirm the high quality of the present meta-analysis [28].

Eligible studies were all that: 1) were written in English; 2) were either case-control or cross-sectional studies; 3) included pediatric or adult patients with any type of IBD, namely UC, CD and IC with or without a control group consisting of healthy
individuals; 4) included patients with no clinically overt FMF, based on Tel-Hashomer and Livneh criteria for the diagnosis of the disease [29, 30]; 5) reported any effect estimates for A) frequency of MEFV mutant (mt) and/or wild-type (wt) alleles and/or genotypes in IBD patients, analytically provided for i) UC, CD and IC patients, ii) presence of extra-intestinal manifestations, iii) endoscopic documentation of pancolitis, iv) MEFV mutational pattern regarding at least the four commonest mutations (EI48Q, V726A, M680I, M694V), and v) inflammatory, stricturing, and fistulizing form disease, as well as B) odds ratios (ORs) of presence of MEFV mt and/or wt alleles and/or genotypes between IBD patients and controls; and 6) were not duplicates of newer versions.

Data Extraction
A structured data collection form was used to extract the following data from each study: title of the study, name of the first author, year of publication, country where the study was conducted, type of IBD, number of patients, enrollment of infants, number of controls, adjustment for consanguinity, MEFV mt alleles and/or genotypes in patients and MEFV mt alleles and/or genotypes in controls. The data extraction process was independently carried out by three authors (V.P., C.A. and P.S.); a fourth author (K.R.) was responsible for cross-checking in case of any discordance. All data are available upon request.

Quality Assessment of the Studies
The Newcastle-Ottawa quality assessment scale (NOS) was used to appraise the quality of the included case-control studies in three grouping items, namely the selection (identification and recruitment of participants), the comparability between the two groups and either the ascertainment of the exposure (for case-control studies) or outcome of interest (for cross-sectional studies). For cross-sectional studies, a modified version of NOS was used [31]. In detail, selection item was given a maximum of 4 stars for case-control studies and 5 stars for cross-sectional studies, comparability item a maximum of 2 stars and exposure (for case-control studies) / outcome of interest (for cross-sectional studies) item a maximum of 3 stars. The quality assessment of the studies was independently carried out by three authors (V.P., C.A. and P.S.); a fourth author (K.R.) was responsible for cross-checking in case of any discordance. Kappa statistics were used for the evaluation of inter-rater agreement in the case of NOS. GRADE assessment was used for evaluation of evidence certainty rating risk of bias, imprecision, inconsistency, indirectness, publication bias, and effect size for every endpoint [32].

Data Synthesis
Data synthesis was performed using Revman 5.3 software from the Cochrane Collaboration (London, United Kingdom) and MedCalc Statistical Software version 19.1 (MedCalc Software bv, Ostend, Belgium; https://www.medcalc.org; 2019). Effect estimates, expressed as A) frequency of MEFV mt alleles and/or genotypes in IBD patients, analytically provided for i) UC, CD and IC, ii) presence of extra-intestinal manifestations, iii) presence of pancolitis, iv) MEFV mutational pattern, and v) inflammatory, stricturing and fistulizing form disease, as well as B) ORs of presence of MEFV mutations between IBD patients and controls, were extracted from each study and combined together using the random-effects, generic inverse variance method of DerSimonian and Laird, which assigned the weight of each study in the pooled analysis inversely to its variance [33]. Random-effects model allows generalizing common effect size beyond the (narrowly defined) population included in the analysis [34]. Tabulation of characteristics in Excel sheets and comparison against the planned groups for each synthesis was performed.

Conventional meta-analytic techniques rely on the assumption that effect size estimates from different studies are independent. This assumption is violated when studies produce several estimates based on the same individuals, as here. A commonly used strategy is to use the same meta-analytic procedures that would have been used if the effect size data were independent, ignoring the fact that some of the effect size estimates are not independent. Generally, this strategy provides inflated type I error rates when testing the significance of the moderators [35]; However, it may not be too misleading if relatively few studies report more than one effect size. Moreover, under realistic conditions it can lead to conservative results for tests of the difference between average effects of different types, which may be sufficient for rough inferences [36]. It has been supported that, in case that multiple subgroup analyses are performed simultaneously, the significance of within-subgroup treatment effects was adjusted for multiplicity according to the Benjamini-Hochberg correction [37, 38]. Hereby, in case that subgroups share common controls, the mixed effects model analysis was preferred using the Comprehensive Meta Analysis software (Version 3.3.070); for that purpose, a random effects model is used to combine studies within each subgroup, while a fixed effects model is used to combine subgroups and yield the overall effect. The study-to-study variance (τ²) is not assumed to be the same for all subgroups; therefore, τ² is computed within subgroups and not pooled across subgroups.

Statistical Analysis
Analysis of publication bias (PB) was performed through funnel plot with trim-and-fill analysis. Conventional funnel plots used to assess for potential PB in meta-analyses are inaccurate for meta-analyses of proportion studies with low proportion outcomes. Funnel plots of study size against log of odds may be a more accurate way of assessing for PB in these studies [39]. In case of imputed studies, the Egger’s test, the Begg and Mazumdar’s rank correlation test and the Classic failsafe-N test was further introduced using Comprehensive Meta Analysis software (Version 3.3.070).

Heterogeneity was approached using Q test and I² statistic; CI of I² statistics was computed using either the formula ±1.96•0.5•√([(Ln(Q)-Ln(df))/((2Q)−(2•df−1)))²] for Q>df+1 or ±1.96/[(2•df−1)•(1−{(1/3•(df−1)))³}] for Q≤df+1, where df denotes degrees of freedom [38]. Q test P-value <0.10 was indicative of a statistically significant result [40]; furthermore, a value of I²≤25% was indicative of insignificant heterogeneity, 26-50% of low heterogeneity, 51-75% of moderate heterogeneity and >75% of high heterogeneity [38, 40]. Analysis of heterogeneity was performed through two separate meta-regressions focusing
separately on study characteristics and quality assessment; multivariate analysis as well as subgroup analysis followed in case of univariate p<0.2 [38]. Effect estimates were log-transformed in case of deviation from normality documented by the use of both Kolmogorov-Smirnov and Shapiro-Wilk tests. To avoid collinearity, the minimum acceptable tolerance was set to 0.6. Multivariate analysis was omitted in cases that the available studies were less than 10. Subgroup analysis of proportions using mixed effects model was performed using logit event rate, namely \( 1/(1+e^{-\theta}) \) as effect measure. Sensitivity analysis was performed when appropriate. Meta-regressions were performed using SPSS 20.0 software (IBM Corp®). Missing values were not imputed so as to avoid any additional source of bias.

**Population Genetics Analysis**

Based on observed frequencies of total mt genotypes \( (q^2_{obs}+2pq_{obs}) \) and observed homozygous mt genotypes \( (q^2_{obs}) \) among both patients and controls, observed heterozygous wt alleles \( (2pq_{obs}) \) were initially computed; pobs and qobs denote observed wt and mt alleles, respectively. These data were used to test the application of the Hardy-Weinberg equilibrium (HWE), stating that allele and genotype frequencies in a population will remain constant from a certain generation to next one in the absence of disturbing factors, with the use of the on-line tool https://wpcalc.com/en/equilibrium-hardy-weinberg/#response which computes expected wt and mt genotypes \( (p^2_{exp} + q^2_{exp} + 2pq_{exp}) \) as well as the corresponding chi-squared value; \( p \)-value was extracted from chi-squared value with one degree of freedom, since variation is applicable only for either \( p \) or \( q \), as their sum is always 1. Moreover, inbreeding coefficient \( F \) was computed using the formula \( (2pq_{obs} - 2pq_{exp})/2pq_{obs} \); a positive \( F \) meaning fewer heterozygotes than expected, indicates inbreeding while negative \( F \), meaning more heterozygotes than expected, indicates excess outbreeding. Pairwise differences based \( [F_{ST}] \) values as derived from Analysis of Molecular Variance (AMOVA), standard diversity indices as gene diversity and expected homozygosity in a population at equilibrium between drift and mutation, deviation from HWE as described by Guo and Thompson, Ecens-Watterson homozygosity test, and Chakraborty's test of selective neutrality were computed using Arlequin 3.5.2.2. [41].

**Publication Bias Analysis**

To approach publication bias several tests were introduced: i) visual inspection of the funnel plot for asymmetry, ii) trim-and-fill analysis for imputed studies, iii) Egger's test, iii) Begg and Mazumdar’s rank correlation test based on Kendall’s tau with continuity correction yielded to a non-significant result (p=0.43 and p=0.30, consequently), while the Classic fail-safe N indicated that the number of missing studies needed to bring alpha >0.05 was 417.

No publication bias was detected. In detail, the funnel plot was not indicative of lack of symmetry and trim-and-fill analysis produced no imputed data points when random-effects model was used (Supplementary file, Fig. 1). Moreover, the Egger's test as well as the Begg and Mazumdar's rank correlation test based on Kendall's tau with continuity correction yielded to a non-significant result (p=0.43 and p=0.30, consequently), while the Classic fail-safe N indicated that the number of missing studies needed to bring alpha >0.05 was 417. Aiming to estimate possible confounding factors, meta-regressions were used suggesting that MEFV mutation frequency among IBD patients remains comparable, independently of reported IBD type, age, adjustment for consanguinity, and study quality assessment. None of these parameters was independently correlated with effect estimate. Similarly, no statistically significant result was revealed from meta-regression carried out regarding quality assessment items (Supplementary file, Table I). As event rate (MEFV mutation frequency) did not follow normal distribution (Kolmogorov-Smirnov p=0.045, Shapiro-Wilk p=0.031), log-transformed effect estimates were alternatively preferred (Kolmogorov-Smirnov p=0.200, Shapiro-Wilk p=0.159).

**Frequency of MEFV Mutations in IBD Patients**

Using the formula \( 1 / (1 + e^{\theta p}) \) on collective data derived from all 13 included studies, as presented in Supplementary file, Fig. 2, the frequency of MEFV mutation carriers among IBD patients was computed to be 0.238 (95%CI: 0.209-0.270); \( I2=95\% \). Of note, the mean frequency of MEFV mutations among FMF patients and controls is 78.5% and 15.5%, respectively, as can be deduced from already published data (Supplementary file, Fig. 3) [26]. Compared with controls, IBD patients had a 3.10 OR (95%CI: 1.68-5.72; p<0.001) to carry MEFV mutation alleles; moderate heterogeneity (\( I^2=70\% \); p<0.001) was observed (see Fig. 2 for subgroup analysis). A sensitivity analysis excluding E148Q mutation indicated that when compared with controls, IBD patients had a 3.39

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**RESULTS**

**Quality Assessment and Heterogeneity Analysis**

Five hundred and thirty-seven potentially relevant publications were identified through a thorough search of the literature; no unpublished data of interest were detected. Removal of duplicates and critical appraisal of title, abstract and full text of the remaining publications was performed. Finally, thirteen observational studies, including 937 patients (404 with UC, 514 with CD and 19 with IC) and 977 controls, contributing 3828 MEFV alleles combined to 1914 genotypes, were included in the meta-analysis (Fig. 1).

The overall inter-rater agreement for NOS was almost perfect (kappa=0.94; 95%CI: 0.86-1.00). In detail, kappa was 0.89 (95%CI: 0.77-1.00), 1.00, and 1.00 for selection, comparability, and exposure respectively. The relevant data did not reveal any profound quality handicap. All details concerning characteristics and quality assessment of the studies are presented in Table I. Allelic and genotypic data for both patients and controls are exhibited for each study in Table II. GRADE assessment of evidence certainty for each endpoint is provided in Table III.

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**Meta-regression Analysis**

Concerning study characteristics, four parameters were incorporated as independent in a meta-regression model: i) inclusion of CD endpoint, ii) inclusion of IC patients, iii) inclusion of adults, and iv) adjustment for consanguinity. Similarly, a second meta-regression model evaluated quality assessment items. In both models, event rate (MEFV mutation frequency) was used as dependent variable; Kolmogorov-Smirnov and Shapiro-Wilk tests were used to evaluate distributions of effect estimates.

**Frequency of MEFV Mutations in IBD Patients**

Using the formula \( 1 / (1 + e^{\theta p}) \) on collective data derived from all 13 included studies, as presented in Supplementary file, Fig. 2, the frequency of MEFV mutation carriers among IBD patients was computed to be 0.238 (95%CI: 0.209-0.270); \( I2=95\% \). Of note, the mean frequency of MEFV mutations among FMF patients and controls is 78.5% and 15.5%, respectively, as can be deduced from already published data (Supplementary file, Fig. 3) [26]. Compared with controls, IBD patients had a 3.10 OR (95%CI: 1.68-5.72; p<0.001) to carry MEFV mutation alleles; moderate heterogeneity (\( I^2=70\% \); p<0.001) was observed (see Fig. 2 for subgroup analysis). A sensitivity analysis excluding E148Q mutation indicated that when compared with controls, IBD patients had a 3.39
(95% CI: 1.93–5.94; p < 0.001) OR to carry any of the remaining exon 10 MEFV mt alleles; I² was low (12%; p = 0.34). In detail, exon 10 MEFV mt alleles were more prevalent in UC patients (OR: 4.79; 95% CI: 2.00–11.45; p < 0.001 with I² = 70%; p = 0.10), CD patients (OR: 5.55; 95% CI: 2.58–11.93; p < 0.001 with I² = 0%; p < 0.88), and IC patients (OR: 12.22; 95% CI: 3.62–41.26; p < 0.001) (Supplementary file, Figs 4A and 4B). A relevant summary table is provided (Supplementary file, Table II); exclusion of E148Q results in higher effect measure as well as lower inconsistency in all cases.

Similarly, when compared with controls, IBD patients had a 2.64 OR (95% CI: 1.48–4.70; p = 0.001) to carry MEFV mt genotypes; moderate heterogeneity (I² = 66%; p = 0.002) was observed (see Supplementary file, Fig. 5 for subgroup analysis); the relevant OR for FMF patients is 24.1, as deduced from published data [26]. Consequently, IBD patients carry MEFV mutations more frequently than healthy individuals.

**MEFV Mutations and IBD Phenotypes**

Given the phenotypic variation that characterizes IBD, we sought to elucidate whether MEFV mutations were unequally distributed among different IBD types. Accordingly, we demonstrated that the frequency of MEFV mutation carriers was 0.253 (95% CI: 0.209–0.303; I² = 78%), 0.211 (95% CI: 0.175–0.253; I² = 80%), and 0.889 (95% CI: 0.646–0.973; I² = 0%), in UC, CD and IC patients respectively (Supplementary file, Figure 2). Of interest, subgroup analysis indicated that MEFV mutations were increased in patients with IC when compared with UC and CD (I² = 91%, p < 0.001).

Collectively, these observations imply that IC is characterized by the highest MEFV mutational load among all IBD types.

**MEFV Mutations and IBD Clinical Manifestations**

Next, we try to investigate whether various IBD clinical manifestations may be associated with MEFV mutational burden. We noticed that IBD patients with extra-intestinal manifestations had a 2.57 OR (95% CI: 1.07–6.14; p = 0.03) to carry MEFV mt genotypes, when compared with IBD patients lacking extra-intestinal manifestations. Low to moderate heterogeneity (I² = 47%; p = 0.10) was observed (Fig. 3A).

As far as disease pattern is referred, IBD patients suffering from pancolitis had a 2.02 OR (95% CI: 1.01–4.04; p = 0.049) to carry MEFV mt genotypes compared with IBD patients presenting localized disease patterns. No heterogeneity (I² = 0%; p = 0.92) was observed (Fig. 3B). Moreover, subgroup analysis indicated comparable frequency of MEFV mt genotypes between strictureting and fistulizing types of IBD. In detail, when compared with other forms of IBD, strictureting and fistulizing forms had an OR of 2.66 (95% CI: 0.50–14.24, p = 0.25) and 1.20 (95% CI: 0.63–2.29, p = 0.58), respectively (Supplementary file, Fig. 6). Taken together, autoinflammatory responses due to altered pyrin are associated to disease severity by the means of extra-intestinal manifestations and pancolitis.

**Mutations of MEFV exon 10 prevail in IBD patients**

Given that pyrin may contribute to IBD pathophysiology and considering the different penetrance of various MEFV mutations, we aimed to further analyze the distribution of MEFV mutational spectrum among IBD patients in order to investigate their relationship with the disease.

Subgroup analysis within the four available MEFV mutations (M680I, M694V, V726A and E148Q) indicated unequal distribution among IBD patients (I² = 84%; p < 0.001) (Fig. 4).
Table I. Eligible studies (characteristics and quality assessment)

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<th>Study, year, reference</th>
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<th>Adults</th>
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<th>Controls</th>
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<th>ME(F)(^V) mutated alleles (genotypes) in patients</th>
<th>ME(F)(^V) mutated alleles (genotypes) in controls</th>
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<th>NOS comparability</th>
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<td>UC vs controls</td>
<td>No</td>
<td>Yes</td>
<td>139 (UC)</td>
<td>(31)</td>
<td>200</td>
<td>53 (52)</td>
<td></td>
<td>****</td>
<td>**</td>
<td>**</td>
</tr>
<tr>
<td>Akyuz, 2013, [22]</td>
<td>Turkey</td>
<td>Case-control</td>
<td>UC vs controls</td>
<td>No</td>
<td>Yes</td>
<td>75 (UC)</td>
<td>(15)</td>
<td>101</td>
<td>12 (10)</td>
<td></td>
<td>****</td>
<td>**</td>
<td>**</td>
</tr>
<tr>
<td>Beser, 2014, [23]</td>
<td>Turkey</td>
<td>Cross-sectional</td>
<td>UC</td>
<td>No</td>
<td>Yes</td>
<td>35 (UC)</td>
<td>(11)</td>
<td>(1) (3)</td>
<td>(3)</td>
<td></td>
<td>*</td>
<td>*</td>
<td>***</td>
</tr>
<tr>
<td>Salah, 2016, [24]</td>
<td>Egypt</td>
<td>Case-control</td>
<td>UC vs controls</td>
<td>No</td>
<td>Yes</td>
<td>17 (UC)</td>
<td>(16)</td>
<td>1/42</td>
<td>17 (16)</td>
<td></td>
<td>****</td>
<td>**</td>
<td>**</td>
</tr>
<tr>
<td>Saito, 2019, [25]</td>
<td>Japan</td>
<td>Cross-sectional</td>
<td>IC</td>
<td>No</td>
<td>Yes</td>
<td>1 (UC)</td>
<td>(0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td></td>
<td>**</td>
<td>**</td>
<td>**</td>
</tr>
</tbody>
</table>

\(^1\)Extra-intestinal manifestations; \(^2\)Newcastle-Ottawa scale; \(^3\)for case-control studies; for cross-sectional studies.
MEFV mutations in IBD patients

In detail, compared to controls, IBD patients had a 6.54 (95%CI: 2.73-15.69; p<0.001) and 4.54 (95%CI: 1.33-15.50; p=0.02) OR to carry exon 10 M694V and M680I mutations respectively; however, the same cannot be documented regarding V726A (OR: 1.75, 95%CI: 0.24-12.65; p=0.58), nor exon 2 E148Q mutation (OR: 0.91, 95%CI: 0.58-1.43; p=0.68) (Fig. 4).

These observations indicate that IBD is mainly linked to exon 10 M694V and M680I MEFV mutations.

**MEFV Mutations among IBD Patients and Evolutionary Effect**

Aiming to detect whether MEFV mutations are neutral or convey any evolutionary effect (either beneficial or harmful) in IBD phenotype, we have concentrated all appropriate allelic and genotypic data from included studies (Table II). Observed homozygous/total mt genotypes were 11/425 (2.6%) in IBD patients and 1/471 (0.2%) in controls, respectively; consequently, a strong deviation from the HWE was documented for IBD patients, but not for controls (exact test using a Markov chain p=0.001 and p=0.652, respectively).

Moreover, a positive inbreeding coefficient reflecting lack of heterozygosity was observed in IBD patients (0.126; p<0.001) but not for controls (-0.012, p=0.684). These observations suggested a potential evolutionary trend involving MEFV mutation in IBD phenotype.

Genetic differentiation and evolutionary tendency concerning MEFV mutations may vary among ethnic groups. Egyptians, presenting the highest frequency of MEFV mt alleles among both IBD patients and controls, constituted the most genetically distinct population as demonstrated by population pairwise $F_{ST}$ values.

### Table II. Population genetics analysis; Allelic and genotypic data

<table>
<thead>
<tr>
<th>Study</th>
<th>p</th>
<th>q</th>
<th>$p_d^*$</th>
<th>$q_d^*$</th>
<th>$2pq_d^*$</th>
<th>$p_r^*$</th>
<th>$q_r^*$</th>
<th>$2pq_r^*$</th>
<th>$\chi^2$</th>
<th>$P$</th>
<th>$M694V$</th>
<th>$M680I$</th>
<th>$V726A$</th>
<th>$E148Q$</th>
<th>Other†</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Giaglis, 2006</td>
<td>0.82</td>
<td>0.18</td>
<td>0.04</td>
<td>0.96</td>
<td>0.01</td>
<td>0.99</td>
<td>0.01</td>
<td>0.01</td>
<td>0.004</td>
<td>0.95</td>
<td>1/130</td>
<td>0/130</td>
<td>0/130</td>
<td>0/130</td>
<td>0/130</td>
<td>1/130</td>
</tr>
<tr>
<td>Renda, 2008†</td>
<td>0.98</td>
<td>0.02</td>
<td>81</td>
<td>0</td>
<td>3</td>
<td>81.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.00</td>
<td>1.00</td>
<td>0/144</td>
<td>0/144</td>
<td>0/144</td>
<td>0/144</td>
<td>0/144</td>
<td>0/144</td>
</tr>
<tr>
<td>Somi, 2013*</td>
<td>0.88</td>
<td>0.12</td>
<td>108</td>
<td>3</td>
<td>28</td>
<td>107.1</td>
<td>2.1</td>
<td>29.8</td>
<td>0.53</td>
<td>0.47</td>
<td>7/278</td>
<td>1/278</td>
<td>0/278</td>
<td>0/278</td>
<td>26/278</td>
<td>34/278</td>
</tr>
<tr>
<td>Akyuz, 2013</td>
<td>0.85</td>
<td>0.15</td>
<td>101</td>
<td>3</td>
<td>31</td>
<td>100.5</td>
<td>2.5</td>
<td>31.9</td>
<td>0.11</td>
<td>0.74</td>
<td>23/270</td>
<td>1/270</td>
<td>8/270</td>
<td>9/270</td>
<td>27/270</td>
<td>41/270</td>
</tr>
<tr>
<td>Somi, 2013†</td>
<td>0.94</td>
<td>0.06</td>
<td>89</td>
<td>0</td>
<td>12</td>
<td>89.4</td>
<td>0.4</td>
<td>11.3</td>
<td>0.40</td>
<td>0.53</td>
<td>3/202</td>
<td>1/202</td>
<td>1/202</td>
<td>1/202</td>
<td>7/202</td>
<td>12/202</td>
</tr>
<tr>
<td>Salah, 2014</td>
<td>0.48</td>
<td>0.52</td>
<td>5</td>
<td>4</td>
<td>33</td>
<td>11.0</td>
<td>10.0</td>
<td>21.0</td>
<td>13.76</td>
<td>&lt;0.001</td>
<td>5/84</td>
<td>1/84</td>
<td>16/84</td>
<td>10/84</td>
<td>2/84</td>
<td>44/84</td>
</tr>
<tr>
<td>Akyuz, 2013</td>
<td>0.79</td>
<td>0.21</td>
<td>19</td>
<td>0</td>
<td>14</td>
<td>20.5</td>
<td>1.5</td>
<td>11.0</td>
<td>2.39</td>
<td>0.12</td>
<td>1/66</td>
<td>1/66</td>
<td>4/66</td>
<td>8/66</td>
<td>0/66</td>
<td>14/66</td>
</tr>
</tbody>
</table>

†PTS, patients; ‡CTS controls; referring to Hardy-Weinberg equilibrium (df=1); §A744S, 692del.

Fig. 2. MEFV mt alleles in IBD patients vs controls.
Additionally, Italians and Greeks presented statistically significant difference concerning MEFV mt alleles between IBD patients and controls, being characterized by pronounced evolutionary tendency in relation to IBD phenotype, as
documented by Chakraborty’s test of selective neutrality (Supplementary file, Table III). Further analysis using AMOVA, demonstrated that 91.4% of the universal variation of MEFV mutations in IBD patients was distributed within individuals of the same population/ethnicity (Supplementary file, Table IV).

**Fig. 3.** MEFV mt genotypes (designated as “events”) in IBD patients in relation with (A) extra-intestinal manifestations (EM) and (B) pattern of pancolitis.

**Fig. 4.** MEFV mutation spectrum in IBD patients and controls.
Collectively, population genetics data regarding MEFV mutations imply an evolutionary role in the disease phenotype.

**DISCUSSION**

To the best of our knowledge, this is the first systematic review and meta-analysis concerning the relation between MEFV mutations and IBD. In the present study, we have demonstrated that there is a mere three-fold increased OR of IBD patients to carry MEFV mutations, mostly M694V and M680I, when compared with healthy controls. Moreover, we have shown that MEFV mutations are more frequent in IBD patients who present extra-intestinal manifestations and in severe forms of UC patients suffered from pancolitis. Considering the increased frequency of MEFV mutations in IBD patients, when compared with controls, it would be tempting to suggest a possible direct pathogenic role for pyrin linking autoinflammation and IL-1β with IBD. However, based on the concept of the omnigenic model, we may also assume that MEFV could in fact be implicated in the IBD regulatory network, thus contributing at least slightly to the disease phenotype; therefore, MEFV mutation carriage state could represent a modifier factor of IBD phenotype [17, 42, 43]. The omnigenic model attributes complex human traits to a small number of “core” genes with biological reference to the given trait, as well as a larger number of “peripheral” genes, which are associated with the regulators and associated pathways of the “core” genes, far outnumber “core” genes, and contribute much more to the heritability of the entity [43].

Our study demonstrates a mean 28.5% frequency of MEFV mutations among IBD patients, while the mean frequency of MEFV mutations among FMF patients and controls is 78.5% and 15.5%, respectively, as can be deduced from already published data [26]. Moreover, we observed that IBD patients present a 2.64 increased OR to carry MEFV mutations in comparison to controls; similarly, based on published data, FMF patients had a 24.1 OR to carry MEFV mutations [26]. The above-mentioned data indicate that the presence of MEFV mutations in IBD is not uncommon implying that altered pyrin could probably play a crucial role in the pathophysiology of the disease. Indeed, pyrin has been demonstrated to promote intestinal barrier integrity and prevent colon inflammation and tumorigenesis in an animal model of induced colitis [44]. Additionally, several experimental and clinical studies have indicated that NLRP3 inflammasome containing pyrin domain is involved in the pathogenesis of colitis, suggesting both harmful and protective effects [45].

Worth mentioning, among IBD subtypes, UC patients performed the closely association with MEFV mutations. It could be argued that some IBD patients are in fact FMF patients with some colonic inflammation at a moment of disease evolution; this might be supported by the overlap observed between FMF and UC (0.6%), as well as FMF and CD (1.2%) [46]. However, whether these cases represent real IBD or merely FMF-associated intestinal mucosal inflammation, or a distinct MEFV gene-related enteroctilosis mimicking IBD is unclear [25]. Friedrich et al. [47] recently described the identification of a distinct histopathological and cellular feature (pathotype) in IBD that is defined by high neutrophil infiltration, activation of fibroblasts and vascular remodeling at sites of deep ulceration. By demonstrating that activated fibroblasts in the ulcer bed display neutrophil-chemoattractant properties that are IL-1R, but not TNF, dependent, the authors provided a biological rationale for IL-1 signaling blockade in ulcerating disease [47]. In this context, what is considered to be IC might constitute such a distinct, IL-1-related, entity due to defective pyrin. This is keeping with the results of the present meta-analysis, which suggest that searching for MEFV mutations is a rational approach in patients suffering from IC, or even refractory IBD. Accordingly, therapeutic trial with colchicine or IL-1 inhibitors, such as Anakinra, could be considered in this group of IBD patients carrying MEFV mutations, and especially the highly penetrant mutations of exon 10 [25, 48].

One of the most interesting findings of this study was the association of IBD manifestations and severity with MEFV mutational load. Compared with IBD patients without extra-intestinal manifestations, IBD patients with extra-intestinal manifestations had a 2.57 OR to carry MEFV mt genotypes. Additionally, compared with IBD patients without pancolonic pattern, IBD patients with pancolitis had marginally comparable OR to carry MEFV mt genotypes. The fact that no heterogeneity is observed regarding the increased OR for an IBD patient with pancolitis to carry MEFV mutations further supports the possibility that this maybe a true phenomenon. These observations enhance the potent implication of pyrin in the development of the clinical spectrum of IBD therefore suggesting a pathogenic role for IL-1β in IBD [5]. In this context, IL-1β inhibition has been shown to ameliorate sacroiliitis attacks in UC patients [5]. Consistently, clinical trials targeting IL-1β in UC pancolitis are currently in progress today [49]. Functional genetics could substantially facilitate the assessment of clinical implication of IBD-associated genetic variation, thus contributing to the urgent need for redefining of this entity [50, 51]; hopefully, these latter have been the topics of two recently published studies [52, 53].

The most predominant MEFV mutations are M694V and M680I located in exon 10, as supported by the statistically significant increase in related OR concerning presence of these mutations in IBD patients when compared with normal controls. As has been already documented, M694V and M680I mutations substantially affect pyrin function reflecting severe FMF phenotype, especially in homozygotic or double heterozygotic state [54, 55]. However, the impact of other pyrin mutations located outside MEFV exon 10 and 2 on IBD phenotype cannot be excluded, as studies included in the present meta-analysis lack relevant data [56].

E148Q MEFV mutation is regarded by INFEVERS database as of “uncertain significance” and evidence-based recommendations for genetic diagnosis of FMF report that “the E148Q variant is common and of unknown pathogenic significance; its presence as the only MEFV variant does not support the diagnosis of FMF” (GRADE B) [57]; however, that at least homozygosity as well as double heterozygosity (either in trans or in cis) have been reported to exert deleterious effect regarding FMF [58] or even perianal CD [15]. Therefore, to further consolidate our findings, we performed a sensitivity analysis excluding E148Q MEFV mutation and focusing on the
validated pathogenic exon 10 MEFV mutations. The resulted larger effect size and the diminished heterogeneity might be considered as evidence that these mutations play the most prominent role in affecting IBD phenotype.

Genome-wide association studies (GWAS) are more powerful to detect link between SNP variants and disease than meta-analyses. Luo et al. [59] performed whole genome sequencing in 4,280 IBD patients identifying a single new low-frequency (0.6%) missense risk variant in ADCY7 that doubles the risk of UC. However, the authors warn that their approach does not perfectly capture very rare variants, reporting 66% power for detection of 1% minor allele frequency (MAF) and OR 2, and 11% power for detection of 0.5% MAF and OR 1.5; this limitation was attributed to potential bias from both technical and population differences [59]. Considering that M694V and M680I MEFV mutations are both very rare among non-FMF patients and demonstrate significant ethnic differentiation, the GWAS study of Luo et al. [59] may well have failed to explain their contribution in the heritability of IBD [26, 60]. As has been stated, low frequency variants (0.5% < MAF < 5%) that increase disease risk two- to threefold without demonstrating clear Mendelian segregation, could contribute substantially to missing heritability [61].

Interestingly, neither publication bias, nor any other source of heterogeneity was detected except MEFV mutations distribution pattern between various ethnic groups, as already reported for Greeks and Turkish [26, 62-64]. Moreover, Salah et al. [24], observed a surprisingly high frequency of MEFV mutations among IBD patients; this might be explained by the increased frequency of MEFV mutations detected even in normal controls in Egypt (42.8%), partly attributed to the intense consanguinity reported especially at rural areas due to cultural habits encouraging consanguineous marriages followed by inbreeding [24,65]. The fact that inbreeding might contribute towards loss of heterozygosity for MEFV mutations (and, presumably, towards conservation of IBD phenotype) is further supported by the positive inbreeding coefficient among IBD patients, as documented in our results.

Furthermore, we tested whether MEFV mutations are in HWE. The HWE is a principle stating that both genotype and allele frequencies remain constant from one generation to the next within a population. However, different factors including de novo mutations, natural selection, nonrandom mating, genetic drift, and gene flow may disrupt HWE. The present study revealed deviation from the HWE for IBD patients but not for controls as far as MEFV mutations are concerned; the non-uniformity of deviation from HWE supports the possibility of a disease-related specific segregation; a similar deviation from HWE has been previously demonstrated in FMF patients [26]. In detail, the variation attributed to individuals was 91.4%, where only 8.6% of the reduction in heterozygosity was attributed to variation within or between population substructures. Whether unequal distribution of MEFV mutations among IBD patients and controls reflects gene flow, genetic drift, epigenetic changes, natural selection or patient selection bias remains to be answered [66]. Furthermore, MEFV mutations might reflect true evolutionary forces, presumably under the pressure of immune-inflammatory diseases as plague and cancer, towards which has been shown to be negatively correlated [67-70]. Of note, Park et al. [68] demonstrated that M694V, M680I, and V726A MEFV variants decrease the binding of Yersinia pestis virulence factor YopM. Furthermore, Isildak et al. [70] suggested signals of recent selection, hypothesizing that there might be an ongoing process conferring resistance to a broader spectrum of pathogens than Yersinia pestis, since no plague pandemic was observed for at least 400 years [70].

Our meta-analysis incorporated all the above-mentioned data, simultaneously performing quality assessment, publication bias analysis, subgroup analyses and meta-regressions. Though NOS cannot discriminate between studies of “high” and “low” quality, it constitutes a valuable tool for forming inclusion criteria for the meta-analysis, informing a sensitivity analysis or meta-regression, weighting studies, or highlighting areas of methodological quality poorly addressed by the included studies [71].

The main limitation of the present study might be that the data analyzed were derived from a limited number of case-control studies. Furthermore, the present study failed to incorporate unpublished data; lack of this kind of source might be linked to potential publication bias, despite the fact that no prejudiced correlation between MEFV gene mutations and IBD (positive or negative) prevailed in the literature. Such biases might not be traced in small-sized studies including less than 10 studies due to the fact that funnel plots, as well as any statistical tool used for the same purpose, are underpowered.

CONCLUSIONS

We argue that MEFV mutations are common in IBD patients and there is a three-fold increased OR of IBD patients to carry MEFV mutations when compared with healthy controls; highly penetrant mutations M694V and M680I mostly account for this susceptibility. MEFV mutations are linked with the presence of more severe forms of IBD such as patients suffering from extra-intestinal manifestations and/or pancolitis. Further evaluation is needed to assess the clinical significance and evolutionary potential of MEFV mutations in IBD patients, and to better clarify the role of pyrin in IBD pathogenesis, phenotype, and therapeutic perspectives.

Conflicts of interest: None to declare.

Authors’ contributions: V.P. conceived and designed the study. V.P., C.A., and P.S.: collected data. V.P. performed the statistical analysis. V.P., C.A., K.R., and P.S. analyzed data. V.P. and P.S. drafted the manuscript. All authors critically revised the manuscript, approved the final version to be published, and agreed to be accountable for all aspects of the work.

Supplementary material: To access the supplementary material visit the online version of the J Gastrointestin Liver Dis at http://dx.doi.org/

REFERENCES


