Due to the autosomal recessive mode of transmission, FMF was believed to be caused by a loss-of-function mutation of pyrin. However, pyrin knockout mice develop normally and do not exhibit an inflammatory phenotype. A further challenge to the loss-of-function theory is given by the fact that some individuals display the disease despite being heterozygous for one single mutation [26,27]. Moreover, asymptomatic carriers of MEFV mutations can have elevated acute-phase reactants [28]. Homozygous pyrin "knockin" mice harbouring mutant human B30.2 domains, but not pyrin-deficient, exhibited spontaneous inflammation similar to but more severe than human FMF [29]. Caspase-1 was constitutively activated in knockin macrophages, and active IL-1β was secreted after LPS stimulation, as observed in FMF patients. The inflammatory phenotype of knockin mice was reversed after crossing with IL-1 receptor-deficient or adaptor molecule ASC-deficient mice but not with NLRP3-deficient mice. These pivotal studies provide the final evidence for an ASC-dependent, NLRP3-independent inflammasome in which gain-of-function pyrin mutations cause FMF [29].

The clinical consequence of the dosage effect of MEFV variants was described in children with periodic fevers, in which the prevalence of FMF-related clinical manifestations was significantly correlated to the number and pathogenicity of MEFV variants carried by the patients [30]. Similarly, the degree to which IL-1β is over-secreted from FMF monocytes after LPS stimulation was proportional to the number and pathogenicity of MEFV variants carried by the patients [31].

Dependence from RhoA makes the pyrin inflammasome distinct from other inflammasomes that are activated by pattern-recognition receptors: it does not directly interact with PAMPs and DAMPs. Instead, it indirectly senses and responds to pathogen virulence factors that modify RhoA, acting as a molecular "guard" that senses alterations in the homeostasis of a cell [32].

The crucial role of the pyrin inflammasome in the response to pathogens inducing toxin release (such as *Y. pestis*) led to the fascinating hypothesis of a possible selective advantage for individual carriers of MEFV causative during plague times [33] (Figure 2C). In fact, the *Yersinia pestis* virulence factor, called YopM, stimulates the PKN-1/2-mediated phosphorylation of pyrin and thereby the inhibition of pyrin inflammasome reducing IL-1β secretion in response to infection [34]. In turn, MEFV pathogenic variants attenuate the pyrin–YopM interaction, thus interfering with the YopM-induced interleukin-1β suppression (Figure 2C). Leukocytes from FMF patients release heightened IL-1β, specifically in response to *Y. pestis*, as compared to healthy controls. *Y. pestis*-infected knockin mice for pathogenic MEFV variants exhibit IL-1-dependent increased survival relative to wild-type knockin mice. Thus, MEFV pathogenic mutations confer heightened resistance to *Y. pestis* [34].

IL-1β

Interleukin-1 (IL-1) is one of the most potent pro-inflammatory cytokines. Two distinct ligands (IL-1α and IL-1β) bind the IL-1 type 1 receptor (IL-1R1) inducing a pro-inflammatory cascade leading to the production of mediators, such as prostaglandins, cytokines, and chemokines [35]. The IL-1α precursor is constitutively present in most epithelial cells and is fully active. On the contrary, IL-1β is synthesised as an inactive precursor, only after activation of the cells, typically after the stimulation of toll-like receptors. The activation of IL-1β is contingent on proteolytic cleavage by caspase-1 [36]. IL-1 is able to induce the myeloid differentiation primary response gene 88(MyD88), and therefore the translocation of active NF-κB to the nucleus. NF-κB-dependent genes, such as NLRP3, pro-IL-1β, pro-IL-18, and IL-6, are the mediators in this process. The central role of IL-1 in the innate inflammatory processes, and, therefore, in autoinflammatory diseases, explains the clinical success of IL-1 blocking agents in treating such conditions [37,38].

4. Genetics and Genotype–Phenotype Correlations

The MEFV gene is made up of 10 exons and is localised on chromosome 16p13.3. To date, more than 300 variants have been identified and reported in the INFEVERS database (Infevers, Sarrauste de Menthiere et al., http://fmf.igh.cnrs.fr/ISSAID/infevers/index.php (accessed 30 April 2023) (Figure 3); however, the relative frequencies and pathogenicity are not known for most of them. The hot spots of the FMF-causing MEFV variants were found on exon 2, at position 148, and on exon 10, at positions 680 and 694 [39,40]. In Turkey, the most frequent mutations are M694V, E148Q, M680I, and V726A [41]. In the Israeli community, the common mutation for non-Ashkenazi Jews is M694V (76.8%) [42] and E148Q for Ashkenazi Jews [43], whilst V726A is the most encountered mutation among Arabs. These last three variants represent probably the most ancient MEFV mutations, and it is calculated that their appearance in the Middle East (Mesopotamia) could be dated to more than 2500 years ago [44]. The hypothesis is that migrations of a few families around the Mediterranean basin during the centuries led to a founder effect. Further evidence for this phenomenon comes from a study on an isolated Jewish community in Palma de Mallorca, the "Chuetas", formed by 18 families in whom more than 60 FMF patients have been diagnosed, and their genotypes overlapped with those observed in North African Jews [45].

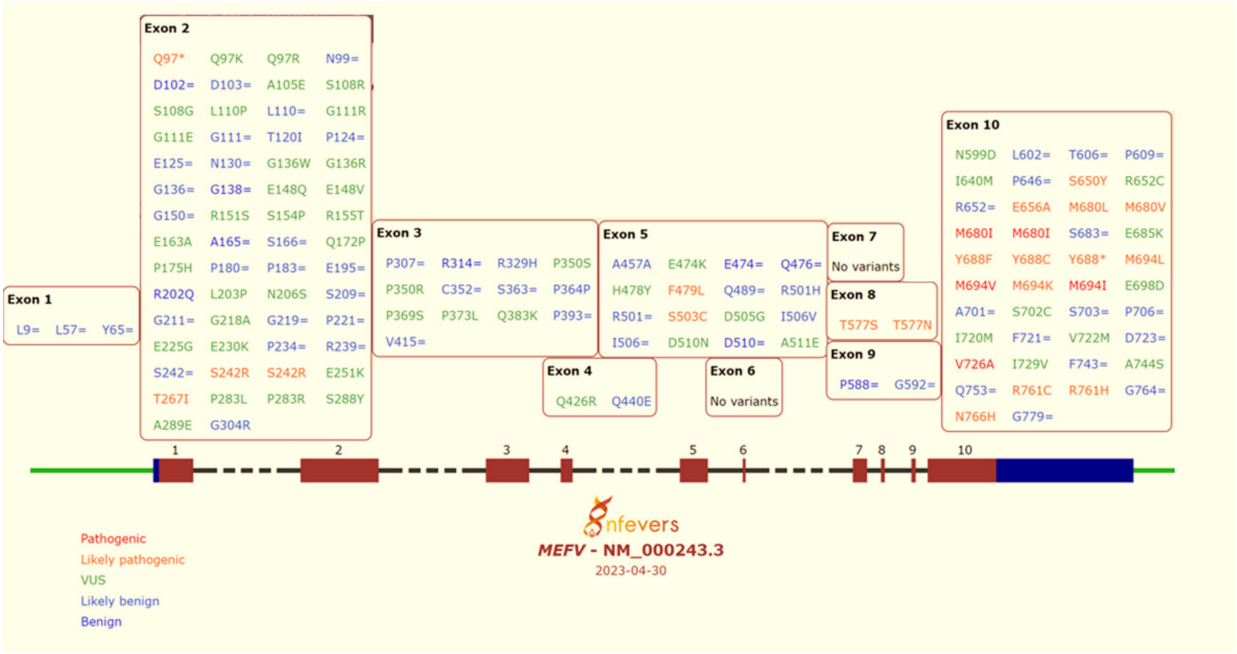

Figure 3. MEFV mutational spectrum based on the free source INFEVER online database. In red and orange are marked, respectively, pathogenic and likely pathogenic mutations; in green are marked VUS (variance of unknown significance); and in light blue and blue are marked likely benign and benign variants. Hot spots for pathogenic mutations are found on exons 2 and 10.

The modification to the methionine residue at position 694 was described as a high penetrance mutation on the discovery of the pyrin gene in 1997. In addition, despite the disease being typically considered recessive, some patients with the classical FMF phenotype were reported to have a seemingly dominant transmission [46].

Indeed, as far as phenotype–genotype correlations are concerned, several observations showed that a more severe phenotype, with high fever, splenomegaly, and musculoskeletal manifestations is usually associated with high penetrance mutations [47,48], such as M694V, which also seems to confer a less favourable response to colchicine [49]. On the other hand, the low penetrance variant E148Q has been suggested to have an aggravating effect: dominant transmission when allelic to M694I with a second wild-type allele and

amyloidosis when allelic to V726A with a second mutated allele [50]. The mild phenotype or incomplete penetrance has also been described in patients with K695R or P369S [40].

In the past, twin studies proved full concordance between monozygotic twins and a 30% concordance rate between dizygotic twins, with some degree of clinical variability [51]. However, in addition to the MEFV gene, some other genetic loci may impact the pathogenesis of the disease as modifiers, such as MICA (major histocompatibility complex class I chain-related gene A) [52] and polymorphisms of the SAA1 (serum amyloid A1) gene [53].

5. Clinical Features

The clinical presentation of FMF can be variable, likely depending on its genetic heterogeneity and environmental factors. However, the clinical picture is usually very suggestive of the underlying disease. It is typically characterised by recurrent episodes of fever and systemic inflammation with (pleural and abdominal) serositis and arthritis. Starting in childhood, patients present short-lasting, self-resolving attacks of fever and abdominal, chest, or joint pain with systemic inflammation [54]. Periodicity is not strict, and episodes may occur from once a week up to once every three to four months or more in untreated patients. These events are usually very disabling and in clear contrast with complete well-being in attack-free periods [55].

Several triggers have been identified for the attacks, such as stressful events [56], cold exposure [57], and the menstrual cycle in pubertal and post-pubertal females [58]. A prodrome was found to be a common manifestation of FMF, experienced by about 50% of the patients. Most commonly, it entails a sensation of general malaise and discomfort, including neurological manifestations such as headache [59] or abdominal pain.

In 90% of cases, the disease onset is in childhood, where 65% of patients are less than 10 years old [60]. A young age of onset (<2 years) is associated with a more severe course of illness, higher penetrance mutations [61], and a more pronounced delay in diagnosis [62]. Fewer studies have investigated adult-onset FMF, but it has been reported to have a mean age of clinical onset of 32.5 years, milder symptoms, and fewer or no disease complications [63]. For these reasons, adults suspected to have FMF may also benefit from different diagnostic tools compared to paediatric populations [64].

Disease flares in FMF are typically associated with an elevation in the acute phase response (C-reactive protein, CRP, and serum amyloid A, SAA). Sometimes, high CRP and SAA levels are also found during the attack-free periods, indicating a still active disease and a strong risk factor for the development of amyloidosis due to chronic inflammation [65]. Another recently described, yet not characterised, player in the development of proteinuria and amyloidosis in FMF is high serum levels of galectin-3 (gal-3). Gal-3 is a b-galactoside-binding lectin highly expressed in innate immune cells and involved in the pro-inflammatory and pro-fibrotic pathways [66]. Galectin-3 could be used in the future as a prognostic biomarker for the development of renal damage in FMF and other conditions [67].

5.1. Fever

A fever is present in 96% of inflammatory episodes, ranging from 38 °C to 40 °C [68]. It appears suddenly and lasts from 12 to 72 h. The typical cycle displays a spontaneous and fast rising in temperature followed by a plateau and rapid decrease [1]. In young children, a fever may represent the unique disease manifestation at onset, with the subsequent development of a typical clinical presentation (including serositis) over the next 2.9 $\pm$ 2.2 years [61].

5.2. Abdominal Manifestations

Abdominal pain is extremely frequent during fever episodes, being reported in 94% of patients [6], and is secondary to a sterile inflammation in the peritoneum. Usually, the pain is severe and induces the patient into an antalgic position and bed rest. Sometimes, it may mimic an acute abdomen with rebound tenderness, reduced peristalsis, and distension and rigidity of abdominal muscles. Radiographic features may reveal small air-fluid levels

and can wrongly guide caregivers toward an explorative laparoscopy and possibly an unnecessary appendicectomy or cholecystectomy. Indeed, appendectomy in FMF patients was found to be much higher than the reported rate in the general population (40% vs. 12–25%), whilst the number of non-inflamed appendectomies was much higher (80% vs. 20%) [69]. Most of the time, peritonitis is completely resolved within 2–3 days without sequelae. Constipation is often observed during the episode, while diarrhoea occurs in 10–20% of episodes. In addition, the described vomiting rate is approximately 30% in children [30]. Possible long-term complications consequent to repeated bouts of peritoneal inflammation are abdominal adhesions, leading to sub-occlusion, and infertility, which were frequently observed in the pre-colchicine era [70].

A palpable enlarged spleen is found according to different series variably in 10–60% of patients [30,55]. Splenomegaly not related to amyloidosis can be detected using ultrasonography and is usually the direct result of bowel sterile inflammation, which also causes abdominal micro-lymphadenopathy [71].

5.3. Pleurisy and Pericarditis

Pleural serositis is also for chest pain, which manifests with dyspnoea and bilateral respiratory and pleural auscultatory friction sounds in the involved site of the pleura [72]. The frequency changes in diverse study groups and ranges between 20 and 60%. An additional X-ray finding is a transient small pleural effusion, which resolves within 48 h after the episode [60].

Pericarditis is rare but more frequent than in the general population (around 7 per 1000 cases vs. 0.5) [73] and may present with retrosternal pain and ST abnormalities on electrocardiogram. It usually occurs years after the diagnosis, even though uniquely, it could be its first sign [74]. Interestingly, idiopathic recurrent acute pericarditis (IRAP) can be seen in both autoinflammatory (such as FMF and TRAPS) and autoimmune conditions (systemic lupus erythematosus, rheumatoid arthritis, progressive systemic sclerosis, and others) suggesting a mixed pathogenesis involving both innate and adaptive immune systems [75].

5.4. Musculoskeletal Symptoms

Joint manifestations are observed in 50% of cases and manifest as transient arthralgia or mono/oligoarthritis. Recurrent monoarthritis is the most common presentation, and it usually affects the knee, hip, and/or ankle [76]. The arthritis is typically associated with a robust inflammatory reaction with redness and swelling in the involved joint. Arthrocentesis results in an aseptic exudate with a high number of inflammatory cells. Synovitis usually resolves after 24–48 h, with the same evolution of abdominal or chest attacks with no sequelae. However, progressively destructive arthritis has been described in the literature in some cases [77]. A less common manifestation of joint involvement in FMF is spondyloarthropathy, which is often HLA–B27 negative.

Myalgia may be associated with FMF, ranging from spontaneous generalised self-resolving muscle ache, exertional leg pain and, less commonly, protracted febrile myalgia. Exertional leg pain has been characterised as less intense post-exercise acidification on the muscle of FMF patients as compared to controls [78]. Protracted febrile myalgia syndrome (PFMS) is a rare complication of FMF, which cannot be prevented with colchicine. PFMS is a long-lasting (4–6 weeks), intense, and disabling muscle pain, usually in the lower limbs, associated with high-grade fever and high inflammatory parameters, but normal muscle enzymes and non-specific inflammatory changes in the EMG [1]. A high signal intensity distributed around myofascicles in the inflamed muscles can be detected using MRI [79]. It requires high-dosage cortisone treatment or anti-Il1 agents [80].

5.5. Other Manifestations

As for other serosal membranes, inflammation of the tunica vaginalis producing orchitis may be another event occurring in FMF patients. The frequency of acute scrotum