FACULTY OF MEDICINE AND HEALTH SCIENCES

GENETIC, CLINICAL AND TREATMENT HETEROGENEITY IN FAMILIAL MEDITERRANEAN FEVER

A NARRATIVE REVIEW OF LITERATURE

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Abstract

Introduction: Autoinflammatory diseases (AIDs) are caused by genetic disturbances resulting in exaggerated innate immune responses characterized by recurrent fever attacks and systemic inflammation. Familial Mediterranean Fever (FMF) is the most common autoinflammatory disease in the world and is caused by mutations in the *MEFV* gene that encodes the pyrin protein. Activation of pyrin, due to mutations in this *MEFV* gene, will trigger an inflammasome-dependent pathway resulting in excessive release of IL-1β, which gives rise to FMF pathogenesis. Although the genetic and the cytokine basis of FMF is known, there is considerable genetic, clinical and treatment heterogeneity in this disease, which is the subject of this thesis.

Methods: First, a quantitative approach was used to determine the scope of the thesis. Many papers were consulted to get an overview of the complexity of FMF available in literature. Thereafter, a qualitative approach focused on the selection of the different aspects of FMF. Recent as well as older research was used, to create an overview of the genetic, clinical and therapeutic heterogeneity within FMF. PubMed, Google Scholar and BioRxiv were utilized to select the different articles.

Results: The wide genetic heterogeneity of FMF is being elaborated, with more in-depth discussion of pathogenic and benign mutations as well as variants of unknown significance. Genotype-phenotype correlations for the most common mutations in the MEFV gene are displayed and this gives an overview of the influence of certain mutations on the clinical picture of FMF patients. Various complications, of which secondary AA amyloidosis is the most feared, are discussed leading to the need of adequate treatment to prevent these complications. Colchicine and anti-IL-1 therapy are profoundly discussed with focus on the efficacy, working mechanism, safety and interactions.

Discussion and future perspectives: Considering the genetic, clinical and treatment heterogeneity within FMF patients, the M694V mutation in the *MEFV* gene appears to be the most severe mutation resulting in more colchicine resistance, higher risk for several complications and extensive clinical manifestations. Anti-IL-1 therapy is an essential alternative for colchicine resistant or intolerant patients. However, further research is required to grasp the complexity of the exact pyrin mechanisms, to pave the way for new more selective treatment modalities.

Nederlandse samenvatting

Inleiding: Auto-inflammatoire ziekten (AIZ) worden veroorzaakt door genetische verstoringen die resulteren in overdreven aangeboren immuunreacties, gekenmerkt door wederkerende koortsaanvallen en systemische inflammatie. Familiaire Mediterrane Koorts (FMK) is de meest voorkomende autoinflammatoire ziekte ter wereld en wordt veroorzaakt door mutaties in het MEFV-gen dat codeert voor het pyrine-eiwit. Activering van pyrine, als gevolg van mutaties in dit MEFV-gen, brengt een inflammasoom-afhankelijke pathway op gang die leidt tot overmatige afgifte van IL-1β, waardoor de pathogenese van FMF ontstaat. Hoewel de genetische en cytokine basis van FMF bekend is, is er aanzienlijke genetische, klinische en behandelingsheterogeniteit bij deze ziekte, wat tevens het onderwerp is van deze thesis.

Methodologie: Eerst werd een kwantitatieve benadering gebruikt om de reikwijdte van de thesis te bepalen. Daarbij werden vele artikelen geraadpleegd om een overzicht te bekomen van de complexiteit van FMF in de literatuur. Daarna werd een kwalitatieve benadering gebruikt om de verschillende aspecten van FMF te selecteren. Zowel recent als ouder onderzoek werd verzamelt om een overzicht te creëren van de genetische, klinische en therapeutische heterogeniteit binnen FMF. PubMed, Google Scholar en BioRxiv werden gebruikt om de verschillende artikelen te selecteren.

Resultaten: De brede genetische heterogeniteit van FMF wordt uitgewerkt, met meer diepgaande bespreking van pathogene en goedaardige mutaties en varianten van onbekende betekenis (VUS). Genotype-fenotype correlaties voor de meest voorkomende mutaties in het MEFV-gen worden weergegeven en dit geeft een overzicht van de invloed van bepaalde mutaties op het klinische beeld van FMF-patiënten. Verschillende complicaties, waarvan secundaire AA amyloïdose de meest gevreesde is, worden besproken, wat leidt tot de noodzaak van adequate behandeling om deze complicaties te voorkomen. Colchicine en anti-IL-1 therapie worden diepgaand besproken met aandacht voor de werkzaamheid, het werkingsmechanisme, de veiligheid en de interacties.

Discussie en toekomstperspectieven: Gezien de genetische, klinische en behandelingsheterogeniteit binnen FMF-patiënten, blijkt de M694V-mutatie in het MEFV-gen de ernstigste mutatie te zijn die leidt tot meer colchicine-resistentie, een hoger risico op verschillende complicaties en uitgebreide klinische manifestaties. Anti-IL-1 therapie is een essentieel alternatief voor colchicine resistente of intolerante patiënten. Er is echter verder onderzoek nodig om de complexiteit van de exacte pyrinemechanismen te doorgronden, om de weg te effenen voor nieuwe, selectievere behandelingsmodaliteiten.

1. Introduction

1.1 Autoinflammatory Diseases (AIDs)

Autoinflammatory diseases (AIDs), also named periodic fever syndromes, are a heterogeneous group of systemic inflammatory diseases caused by genetic disturbances in genes encoding essential regulatory molecules of innate immunity. (1) Back in 1999, the term 'autoinflammation' was introduced for the diseases Familial Mediterranean Fever (FMF) and TNF Receptor Associated Periodic Syndrome (TRAPS). Autoinflammation was preferred over 'autoimmune' because autoantibodies or self-reactive T cells are not common features in these diseases.(2) The main dysfunctional character in AIDs is the innate immune system causing recurrent episodes of fever and systemic inflammation, particularly in infancy or childhood.(3)

Over the years, a variety of new AIDs emerged such as mevalonate kinase deficiency (MKD), cryopyrin-associated periodic fever syndromes (CAPS), pyrin-associated autoinflammation with neutrophilic dermatosis (PAAND) and pyogenic arthritis, pyoderma gangrenosum, and acne (PAPA). All these different AIDs show mutations in genes encoding proteins that regulate the innate immune system. All mutations identified thus far that can be linked to AIDs were given a classification in the Infevers database (infevers.umai-montpellier.fr/). The different classifications of this registry are the following: not classified, likely benign, likely malign, Variant of Unknown Significance (VUS), benign, malign/pathogenic and unsolved. Moreover, all of these mutations are given a status: to be validated, provisional, unsolved and validated. Validated malign mutations are certainly disease-causing mutations which means these mutations will have a high risk of leading to an AID. Validated benign mutations are certainly not-disease-causing mutations but they can still lead to a mild disease course if combined with malign mutations. It is important to mention these benign mutations because unlike the malign mutations, intensive follow-up is not needed within these patients. As mentioned above, other mutations are labelled as Variants of Unknown Significance (VUS) for which the association with disease risk is unclear. Especially the pathogenic mutations lead to dysfunctions in the innate immune system causing inflammation through disturbing different signalling pathways.

The main signalling pathways affected in AIDs are inflammasomes in Inflammasomopathies, Nuclear factor kappa B in Relopathies and Interferons in Interferonopathies.(4) Familial Mediterranean Fever (FMF), part of the inflammasomopathies, is the most common inherited AID in the world and will be the focus of this thesis.

1.2 Familial Mediterranean Fever

Familial Mediterranean Fever is the most frequent hereditary autoinflammatory disease in the world. FMF is specifically characterized by recurrent attacks of fever, abdominal pain, arthralgia, arthritis, serositis, erysipelas like erythema and long-term renal complications (renal amyloidosis).(5) In the following segments, the different aspects of FMF will be briefly discussed. To begin with, the prevalence and genetic aspects will be elaborated, followed by different methods to diagnose FMF. Lastly, the clinical manifestations, common as well as uncommon, will be discussed.

1.2.1 Epidemiology

The prevalence of FMF is highest in the Turkish, Jewish, Armenian and Arabic population. However, partially due to people moving from high-risk countries, FMF also occurs in other parts of the world such as Europe (especially Greece and Italy), North America and Japan (Figure 1).(6) Turkey has one of the highest FMF prevalences in the world with a peak of 1% in Central Anatolia.(7) However, the overall prevalence in endemic countries - Armenia, Turkey and Israel - is approximately 1/1000.(8)

The most prevalent mutations found in the *MEFV* gene responsible for FMF are very ancient mutations that appeared approximately 2500 years ago in Mesopotamia. From there on, sailors from the Middle East brought these mutations to Spain and North Africa or they were carried over by land immigration during the Muslim conquest of these regions. Armenia has a direct connection with Turkey, hence it is likely that FMF spread through neighbouring interactions between these two countries. Figure 1 indicates the possible migration patterns of FMF through history.(9)

Figure 1. Prevalence and spreading of FMF. The circles on the world map indicate the prevalence of FMF. The bigger the circles, the more FMF patients in that region. Arrows indicate FMF spreading. Red arrows show the migration in the ancient world (Middle-East to Europe). The yellow arrow shows the Silk Road (migration of FMF to Japan). Black arrows show the migration of FMF to the new world (US) in modern times. The figure is adapted from Ben-Chetrit et al.(9)

1.2.2 Genetics

FMF is caused by gain-of-function mutations in the *MEFV* gene, which is coding for a 781-amino-acid protein called pyrin that has regulatory functions in the innate immune system (see further in section 1.3). The human *MEFV* gene is located on chromosome 16 (16p13.3) and consists of 10 exons of which exon 2 and 10 contain the majority of variations.(10) The most common FMF-associated mutations (M694V, M680I, V726A and M694I) are located on exon

10 and account for approximately 75% of *MEFV* gene mutations in the Mediterranean area.(6) In the results, the emphasis will be on how these different mutations can cause a variety of clinical pictures that are seen in FMF. The most common FMF mutations will be explored in depth, as well as other certified pathogenic mutations according to the Infevers database. However, before going into detail about the genetics, other FMF aspects will be discussed to understand the implications that these mutations can have on FMF pathogenesis.

1.2.3 Diagnosis of FMF

Diagnosis of FMF is based on clinical features supported by family history, ethnic origin and genetic analysis of the *MEFV* gene.(11) These factors can help differentiate FMF from autoimmune diseases, systemic Juvenile Inflammation Arthritis and Inflammatory Bowel Disease, all of which have certain features that can mimic the onset of FMF attacks.(12) Early diagnosis is essential since FMF not only has an acute but also a chronic inflammatory state.(13) Patients suffering from AIDs can have similar symptoms which makes it very challenging to differentiate them when only clinical features are considered. Therefore, genetic analyses gained more attention over the years as a supporting factor for clinical features.

Through the years, three different classification criteria have been developed, described in table 1. Firstly, the Tel Hashomer criteria were composed through clinical observations in Israeli adults and were later revised by Livney by incorporating supporting criteria and excluding amyloidosis.(14) Second, the Yalcinkaya-Ozen criteria were created because of the lack of diagnostic information about children with FMF. Both Tel Hashomer and Yalcinkaya-Ozen criteria are invaluable tools to distinguish FMF based on clinical manifestations, however, they are restricted to clinical experience and therefore prone to individual interpretations. As a consequence, genetic confirmation could have a supporting role for the clinical manifestations. Hence, the Eurofever/PRINTO criteria were established as a third classification system including ethnicity, clinical manifestations and genotype.(14) Sag et al. studied the sensitivity and specificity of the new criteria (Eurofever/PRINTO). They concluded that the Eurofever classification was more sensitive but less specific and lead to more misclassifications compared to the Tel-Hashomer and Yalcinkaya-Ozen criteria.(15) Shinar et al. compiled different guidelines of genetic testing which will be discussed more in depth in the results (4.1.4).(16) Laboratory findings such as increases in CRP, SAA, immunoglobulins and neutrophil leucocytosis during an acute attack can support the interpretation of clinical FMF features.(12) However, it is important to mention that these findings are not very specific for FMF.

Table 1: Three classification systems for FMF diagnosis.

Tel-Hashomer criteria	*Yalcinkaya-Ozen criteria*	*Eurofever/PRINTO*
Major criteria 1) Recurrent febrile episodes accompanied by serositis (peritonitis, synovitis or pleuritis) 2) AA amyloidosis without predisposing disease 3) Good response to colchicine* treatment **Minor criteria** 1) Recurrent febrile episodes 2) Erysipelas-like erythema 3) FMF diagnosis in first-degree relative **≥ 2 major OR 1 major and 2 minor**	1) Fever (axillary temperature > 38°C)** 2) Abdominal pain** 3) Chest pain** 4) Arthritis** 5) Family history of FMF **≥ 2 criteria**	Presence of **confirmatory*** MEFV genotype and at least 1 among the following 1) Duration of episode 1-3 days 2) Arthritis 3) Chest pain 4) Abdominal pain Presence of **not confirmatory**** MEFV genotype and at least 2 among the following 1) Duration of episode 1-3 days 2) Arthritis 3) Chest pain 4) Abdominal pain

* Colchicine is an anti-inflammatory drug that is used to treat FMF
** 6-72h duration and ≥ 3 attacks of the same type
*** Pathogenic or likely pathogenic
**** Compound heterozygous for one pathogenic and one VUS or biallelic VUS or heterozygous for one pathogenic *MEFV* variant

Tel-Hashomer and Yalcinkaya-Ozen criteria use clinical criteria along with family history and treatment response. Tel-Hashomer defines FMF as exhibiting either 2 major criteria or 1 major and 1 minor criterium. Yalcinkaya-Ozen defines FMF as exhibiting at least 2 of its criteria. The Eurofever/PRINTO classification includes genetics and defines FMF as a combination of genetics with at least 1 or 2 clinical features, depending on the status of the genetic *MEFV* mutation observed in these patients. The specific clinical manifestations presented in this table will be profoundly discussed in the section below (1.2.4).

1.2.4 Clinical features of FMF

FMF attacks are characterized by sudden, recurrent episodes of spiking fever, serositis, arthritis and high inflammatory reactants usually spontaneously resolving in one to four days. The patients are usually asymptomatic between these recurrent attacks.(8) However, even though the period between the attacks is asymptomatic, there can be persistent inflammation. Persistent inflammation is a dangerous feature of FMF which sometimes includes continued elevated inflammatory reactants such as elevated CRP and serum amyloid A (SAA) levels in attack-free periods, as well as fatigue and weight loss. Additionally, growth retardation, anaemia, amyloidosis, decreased bone density and infertility are associated with persistent inflammation. Babaoglu et al. studied the different predictors for persistent inflammation as these patients often need more intensive treatment since it is accompanied by a severe disease course. The different independent predictors for persistent inflammation are the following: history of exertional leg pain, inflammatory comorbidities (Spondyloarthropathies and Inflammatory bowel disease), M694V homozygosity, colchicine resistance, lower education levels and musculoskeletal attack dominance.(17)

FMF patients usually describe some provoking triggers for FMF attacks such as menses, infections, emotional stress, certain drugs or exposure to cold.(6) The onset of these FMF attacks is in 90% of the FMF patients before the age of 20 years. Onset above the age of 40 is also documented and is associated with a milder disease evolution.(6) In the next paragraphs, the common clinical features will be discussed, whereas the rarer complications/features, will be discussed in the results.

Fever

The recurrent fever in FMF usually has a sudden start, reaches temperatures of 38°- 40° C and is present in more than 96% of inflammatory attacks of FMF patients.(12) The duration of the fever varies from 12 hours to 72 hours. This specific pattern can help to differentiate between other autoinflammatory diseases such as Mevalonate kinase deficiency (MVD) that is characterized by a duration of fever ranging from 3 to 7 days.(6) On top of that, Kallinich et al. compiled some important questions, presented in table 2, to discriminate different patterns of recurrent fever manifestations.(18) In rare occasions, recurrent fever can be the only manifestation of FMF during childhood.(19)

Table 2: Important questions to help differentiate different recurrent fever patterns (18)

1. At what age did symptoms first appear?
2. What is the duration of the individual fever episodes?
3. What other symptoms are associated with the fever episodes?
4. What is the time interval between episodes (duration, variable or fixed intervals)?
5. What can trigger or alleviate a fever episode?
6. How have symptoms developed over time?
7. Which treatments have been used and what was the response?
8. Is there a family history; does the patient originate from a certain ethnicity?

Abdominal pain

Acute abdominal pain or peritonitis is another very common manifestation present in 90% of FMF patients. (20) This acute pain can appear in a specific part of the abdomen or it can even be described as a pain covering large parts of the abdomen. The abdominal pain in FMF is often indistinguishable of other causes of acute abdominal pain, such as appendicitis or cholecystitis, especially when the pain is located in the lower quadrant. Therefore, wrongly diagnosed patients tend to undergo many unnecessary surgical procedures, which secondarily might lead to peritoneal involvement (adhesions) resulting in small bowel obstructions.(21) Recurrent peritonitis is also linked to infertility, which will be further discussed in section 4.2.2 within the results.(8) The abdominal attacks are clinically accompanied by rebound tenderness, rigidity of the abdominal muscles and reduced bowel sounds.(22) Generally, all the signs of peritonitis during an attack reside after 12 to 72 hours, however, a post-attack diarrhoea is possible. Patients often suffer from constipation during the abdominal attacks.(8)

Musculoskeletal manifestation

Arthritis and arthralgia are the most common musculoskeletal manifestations in FMF patients. Arthritis is occurring in approximately 50% of the patients. This monoarticular inflammation generally presents itself in the lower limb such as the knee or hip and is more frequently reported among children. Arthritis is characterized by a sudden onset of red, swollen, painful joints which reaches its peak in day one or day three and resolves within 1 week without sequelae.(19) However, in 2-5% of the patients, a protracted arthritic attack is found, which

brings destruction of the joint unlike the typical monoarticular inflammation in FMF.(8) Arthritis or arthralgia, more common in homozygous M694V mutations, might be triggered by minor trauma, long walking or standing. Myalgia can be spontaneous or exercise-induced and is accompanied by fever and serositis. The most common form of myalgia is the standing myalgia in the calf muscles.(23)

Chest pain

30-50% of FMF patients suffer from chest pain due to serosal lung inflammation (lung peritonitis). This is usually unilateral and it causes typical signs such as sudden onset of painful breathing, higher respiratory rate and reduced air entry in the affected part of the lung. Chest pain can occur simultaneously with abdomen peritonitis and resolves within 12-48 hours.(22)

Skin involvement

The most common skin involvement of FMF is an erysipelas-like-lesion (ELE) that mainly affects the lower extremity such as the knee, ankle region and dorsum of the foot. ELE is typically accompanied by a swollen, painful, 5-7cm, sharply bordered red lesion that resides after 2 days. Generally, ELE is associated with a febrile episode.(8)

1.3 The pyrin protein

After discussing the different clinical aspects of FMF, there is a need for understanding the mechanisms that cause the variety of clinical manifestations. To begin with, the pyrin protein will be the focus in the following segment as it is essential for the comprehension of FMF pathogenesis.

As mentioned earlier, FMF is caused by mutations in the *MEFV* gene, which encodes for a 781-amino-acid protein called pyrin. Pyrin, part of a subgroup of pattern recognition receptors (PRRs) present in the cytosol of the cell, mostly appears in cells of the innate immune system such as neutrophils, macrophages, dendritic cells and eosinophils. PRRs are responsible for the sensing of different micro-organisms by recognising PAMPs (pathogen associated molecular patterns) of invading pathogens and/or DAMPs (damage associated molecular patterns) released by the host upon infection.(24) Several of these PRRs, including pyrin, induce the formation of multiprotein complexes called inflammasomes. Formation and subsequent activation of these inflammasomes contributes to driving several inflammatory processes.

The pyrin protein will be gradually discussed in the following segments. Firstly, the different pyrin domains will be emphasized which each have their important function and interaction partners. Thereafter, the focus lies on the process of the formation of the pyrin inflammasome and the consequences of its activation. Lastly, the role of the pyrin inflammasome in the

pathogenesis of FMF will be further elaborated. In addition, a possible evolutionary link between the pathogen *Yersinia pestis*, famous for causing the plague, and the high prevalence of FMF in the Mediterranean area will be discussed.

1.3.1 Pyrin domains

The pyrin protein consists of 5 different domains: PYD (pyrin domain), bZIP (transcription domain), B-box (zinc finger), CC (alfa helical coiled coiled) and the C-terminal B30.2 domain shown in figure 2. (25) The pyrin domains each have their individual function and interaction partners which will be progressively discussed in the following paragraphs (1.3.2 to 1.3.4).

Firstly, the **PYD-domain** (1-95) at the N-terminal end is part of the so-called death domain fold (DDF) domains. These DDF domains are very important for the innate immune system to assemble signalling platforms as a response to pathogen or danger signals.(26) The PYD domain interacts with the corresponding PYD domain of apoptosis-associated speck-like protein containing a CARD (ASC) which leads to the assembly of an inflammasome and caspase-1 activation. Through caspase-1, inflammatory cascades are activated resulting in the release of pro-inflammatory cytokines which will be thoroughly discussed in section 1.3.4.

According to various studies, due to the presence of the **bZIP transcription factor domain** (266-280) and two overlapping nuclear localization functions, a nuclear function of pyrin was suggested.(27-29) This hypothesis was further supported by a study showing that pyrin might have an interaction with the transcription factor NF-κB with its N-terminal fragment. However, later studies found that pyrin is mainly located in the cytosol and therefore contradicted the nuclear function of pyrin. Thus far, the cytosolic versus nuclear specific functions of pyrin remain unknown and further research is needed to define these functions.(25) The serine residues S208 and S242 are located in between the bZIP domain and the PYD domain and play a significant role in pyrin inflammasome (in)activation which will be discussed in the following section (1.3.2).

The **alfa-helical coiled domain** and the **B-box domain** are responsible for the oligomerization of pyrin and thus assist in assembling the inflammasome complex and in activating caspase-1. PSTPIP1, a protein important for cytoskeletal organization, is known for interacting with both of these domains, consequently enabling the PYD to interact with ASC.(30)

Lastly, the pyrin protein consists of a C-terminal **B30.2 domain** which is especially relevant because most mutations of FMF patients originate in this domain. Several studies showed that the B30.2 domain is capable of interacting with caspase-1. Thus, both the PYD-domain and the B30.2 domain play important roles in the caspase-1 cascade. Since the B30.2 domain contains a major part of the mutations leading to FMF, these mutations might have an effect on the interaction between B30.2 and caspase-1. (25)

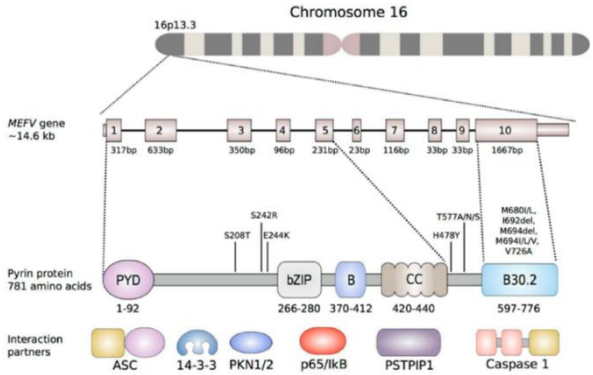


Figure 2. Different domains of pyrin and their interaction partners. Genomic location, exon structure, protein domains, some FMF-associated mutations, and interaction partners of the human pyrin protein are depicted. This figure is adapted from Schnappauf et al. (25)

1.3.2 Pathogen-induced activation of the pyrin inflammasome

After discussing the domains of pyrin with its interaction partners, the mechanisms resulting in pyrin inflammasome activation or inactivation need to be elaborated. In general, the pyrin inflammasome is activated upon sensing Ras homolog family member A (RhoA) inactivation as a consequence of pathogen actions. This sensing mechanism triggers a dephosphorylation of pyrin leading to its activation, as phosphorylated pyrin is kept in an inactive state (figure 3). RhoA is a member of the small GTPase family and is activated by an association with guanosine triphosphate (GTP). The exchange between guanosine diphosphate (GDP) and GTP is promoted by guanine nucleotide exchange factors (GEF) and counteracted by GTPase-activating proteins (GAP) and guanine nucleotide-dissociation inhibitors (GID). RhoA is an important regulator of cytoskeletal rearrangement, cell migration and the cell cycle.(31) Several bacterial toxins such as TcdA and TcdB (*Clostridium difficile*), VopS (*Vibrio parahaemolyticus*), YopE and YopT inhibit RhoA inducing activation of the pyrin inflammasome. YopE and YopT are effector proteins injected into the host cell by *Yersinia pestis*, a gram-negative bacterium that caused the famous plague. The connection between pyrin (in)activation and *Y. pestis* is discussed more in depth in section 1.3.3.

In summary, through the inhibition of RhoA, due to a variety of bacterial toxins, pyrin dephosphorylation is achieved, inducing the formation of the pyrin inflammasome. As seen in figure 3, RhoA activates serine/threonine-protein kinases PKN1/2 resulting in phosphorylation of the serine residues S208 and S424 of pyrin. When pyrin is in the phosphorylated form, the phosphoserine binding proteins 14-3-3ε and 14-3-3τ bind to pyrin. The particular binding of 14-3-3 proteins to the phosphorylated serine residues prevents the formation of an inflammasome. In contrast, RhoA inhibition leads to PKN inactivation resulting in the inability of PKN1/2 to phosphorylate S208 and S242. 14-3-3ε and 14-3-3τ proteins are therefore unable to bind the unphosphorylated serine residues leading to the assembly of a pyrin inflammasome. (25) In conclusion, bacteria inhibit RhoA for their own benefit to suppress leukocyte cytoskeletal rearrangements but this simultaneously leads to the formation of the pyrin inflammasome which activates different pathways of the innate immune system.(32, 33)

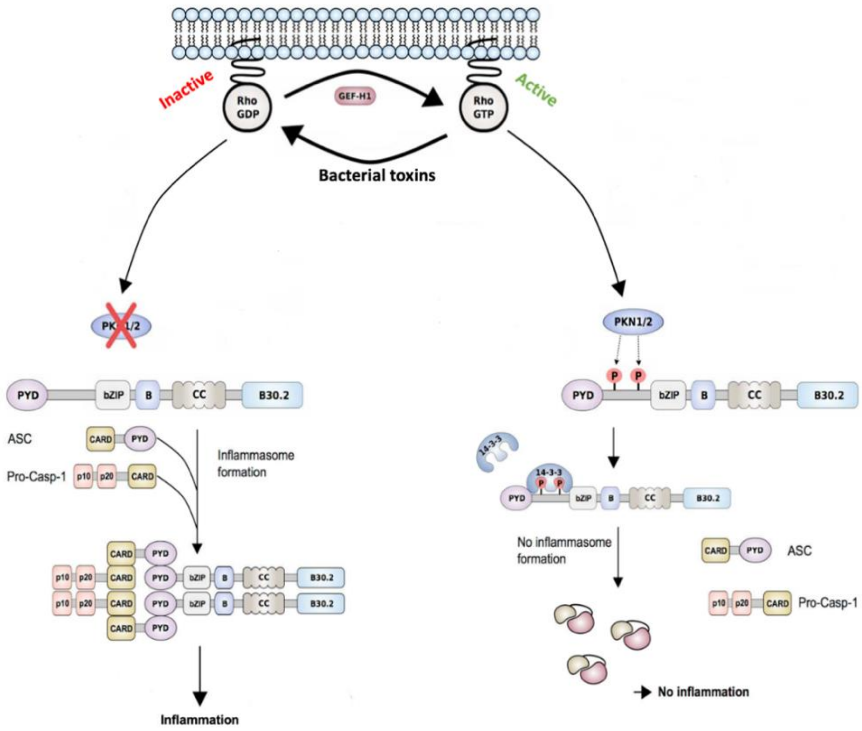


Figure 3. Activation and inactivation of the pyrin inflammasome. Activation of RhoA by GEF leads to phosphorylation of the Pyrin S204 and S242 residues by PKN1/2. Subsequently, 14-3-3ε binds the phosphorylated serines and thereby prevents inflammasome formation. Bacterial toxins inactivate RhoA leading to decreased pyrin phosphorylation and formation of the pyrin inflammasome. This is established through the binding of the PYD-domain of pyrin to ASC which binds to procaspase-1 resulting in a cascade that induces inflammation. Figure is inspired by Schnappauf et al. (25)

1.3.3 The evolutionary link between pathogen-induced pyrin inflammasome activation and FMF

As briefly quoted in the previous segment, the effector proteins YopE and YopT, secreted by *Yersinia pestis*, lead to the activation of the pyrin inflammasome. However, *Y. pestis* secretes two more effector proteins, YopM and YopJ, that have an inhibiting function on the pyrin inflammasome. Considering 4 of the 7 effector proteins of *Yersinia pestis* affect the pyrin inflammasome and the fact that it causes the plague, it could point to a link between the plague and FMF pathogenesis. In the next paragraphs, this hypothesis will be further explored.

Three pandemics of the *Yersinia pestis*-instigated bubonic plague happened throughout history with devastating consequences for the affected countries. The first pandemic, the plague of Justinian, erupted in Egypt in the year 541 and spread rapidly across different countries surrounding the Mediterranean Sea.(34) Interestingly, the epidemiology of FMF largely corresponds with the area affected by this outbreak (see figure 1). Therefore, this raises the question whether there is a link between the plague and FMF evolution in these countries.

Y. pestis can, with the use of Type 3 Secretion System (T3SS) inject different Yop effectors proteins into the host cell including YopE, YopT, YopM and YopJ. Those 4 Yop effector proteins all have an effect on the pyrin inflammasome pathway (Figure 4).(35) YopT (indicated in figure 4 as number 1) is a cysteine protein that acts by cleaving GTPases leading to detachment from the host membranes and thus inactivation of RhoA. YopE (number 2) works as a GAP that increases the conversion of RhoA GTP (active form) to RhoA GDP (inactive form). In contrast, YopM (number 3) is known for inhibiting the pyrin inflammasome with the

cooperation of YopJ and is therefore essential for the virulence of *Y. pestis*.(35, 36) The absence of pyrin inflammasome formation results in no inflammatory reaction of the host to the invading pathogens. YopM hijacks host kinases such as PKN (also known as PRK) and ribosome S6 kinase (RSK) to keep the serine residues S208 and S242 phosphorylated and consequently promotes the binding of 14-3-3 proteins. This process is especially important in the pathogenesis of the plague. However, pyrin is only phosphorylated by RSK when YopM is present in the cell which means that YopM forms a linkage between pyrin and RSKs. Interestingly, the linkage between YopM and pyrin is substantially reduced in the presence of mutations in the B30.2 domain in FMF patients resulting in the inability of 14-3-3 proteins to bind to the phosphorylated serine residues and thus leading to the formation of the pyrin inflammasome. (25) In other words, mutations in the B30.2 domain interrupt *Y. pestis* virulence since YopM cannot execute its inhibiting function on the pyrin inflammasome formation. In consideration that FMF mutations have a protective effect on the plague, Park et al. concluded that the plague could have played an important role in selecting for mutations that cause FMF.(37) As such, selective pressures of the plague in the Mediterranean area are suggested to have driven the selection of *MEFV* gain-of-function variants encoding pyrin mutants that produce excessive levels of IL-1β (pro-inflammatory cytokine).

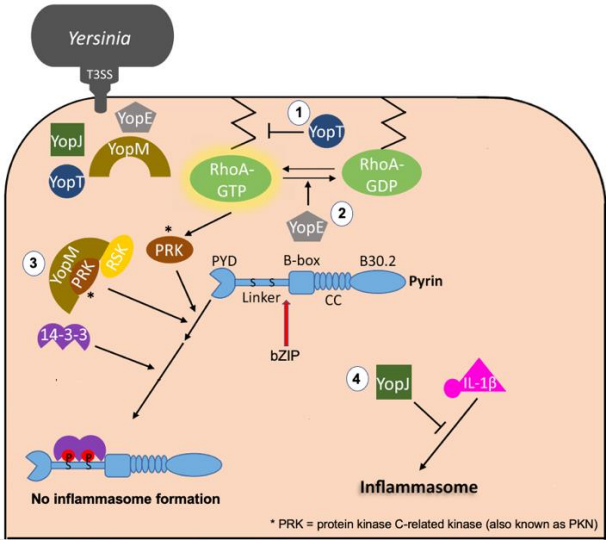


Figure 4. Yop effector proteins with their effect on the pyrin inflammasome pathway. Both YopT (1) and YopE (2) inhibit RhoA activation and they thereby provide a signal for Pyrin dephosphorylation. However, YopM (3) stimulates PRK and RSK mediated phosphorylation of the serine residues S208 and S242 resulting in the absence of the formation of the pyrin inflammasome. YopJ (4) inactivates important regulators of the NF-κB pathway and therefore reduces the amount of IL-1β release resulting in an anti-inflammatory effect. IL-1β, which we will thoroughly discuss in section 1.4, is a pro-inflammatory cytokine generated by the pyrin inflammasome that acts as an important driver for FMF. Figure is inspired by Malik et al. (36)

1.3.4 The pyrin inflammasome as the molecular driver of FMF

In the previous sections, the (in)activation of the pyrin inflammasome by a variety of bacterial toxins was discussed, in which RhoA activation is inhibited resulting in the dephosphorylation of pyrin. Subsequently, an interaction between the PYD domains of pyrin and ASC is established leading to the formation of the pyrin inflammasome. In this segment, the formation of the pyrin inflammasome will be profoundly discussed as it is essential to understand the pathogenesis of FMF. In other words, the question whether the inappropriate activation of the

pyrin inflammasome manages to cause an autoinflammatory disease like FMF will be elaborated in the following paragraphs.

Inflammasomes are multiprotein complexes, present in the cytosol of immune cells, that mediate the activation of inflammatory caspases. Each inflammasome has a unique PRR for the recognition of PAMPs and/or DAMPs.(38) Inflammasomes consist of a cytosolic PRR sensor component (either part of the NLR family such as NLRP1, NLRP3 and NLRC4, or pyrin, or absent in Melanoma 2 (AIM2)), an adapter protein (ASC) and an effector caspase (for example caspase-1).(39) ASC consists of two death fold domains: the pyrin domain (PYD) and the caspase recruitment domain (CARD). These domains help ASC to connect the inflammasome sensor to caspase-1.(38) More specifically, the inflammasome sensor pyrin uses its PYD domain to create an interaction with the N-terminal PYD of ASC resulting in an PYD-PYD interaction. Subsequently, the CARD-CARD interaction between ASC and pro-caspase-1 provides the autoproteolytic activation of caspase-1 (figure 5). (25)

Caspases, a family of intracellular cysteine proteases, are key components in different types of cell death such as apoptosis, necroptosis and pyroptosis.(40) Caspase-1 induces two mechanisms (figure 5). First, it cleaves pro-IL-1β and pro-IL-18 to their mature forms IL-1β and IL-18. Second, caspase-1 cleaves gasdermin D (GSDMD), which is a key event triggering pyroptosis.(41) GSDMD is composed of an N and C-terminal domain. The C-terminal domain has an autoinhibiting function which keeps the cytotoxic effects of the N-terminal domain inhibited. When the N-terminal domain, or pore forming domain (PFD), gets cleaved from the C-terminal by caspase-1, it can form pores in the cell membranes through oligomerization resulting in pyroptosis.(42) Pyroptosis leads to cell swelling, plasma membrane lysis, chromatin fragmentation and release of the intracellular proinflammatory contents.

A variety of different inflammasomes with many different activating triggers exist. Although their activation proceeds in different ways, it always leads to activation of inflammatory caspases. A dysregulation in inflammasome activation, due to a mutation in the encoding gene, can lead to excessive stimulation of the inflammatory caspases which in their turn results in different AIDs. To date, non-infectious endogenous activating triggers for the pyrin inflammasome are largely unknown. However, a recent study showed that low doses of steroid catabolites could trigger the formation of the pyrin inflammasome without activating the pyrin dephosphorylation. Nevertheless, high doses of steroid catabolites induce pyrin dephosphorylation as well as pyrin inflammasome activation which means there exists a coupling mechanism between these two steps. In essence, steroid catabolites can induce autoinflammation through the pyrin inflammasome which could explain some provoking triggers for FMF attacks, such as menses or emotional stress, during which these steroid catabolites fluctuate.(43)

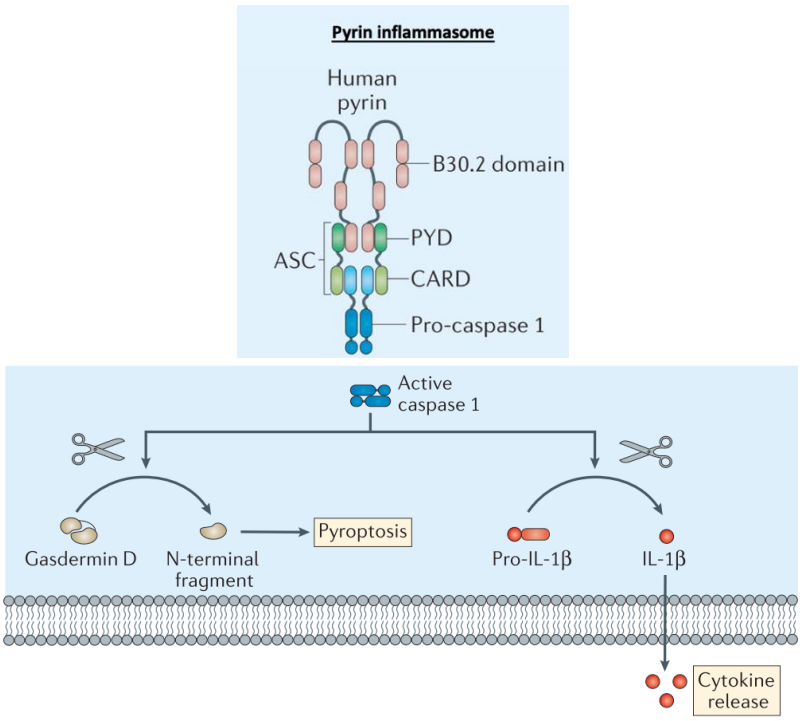


Figure 5. The pyrin inflammasome as molecular driver of FMF. The pyrin inflammasome with its different domains (discussed in 1.3.1) is presented in the top half of the figure. The adapter protein (ASC) containing a CARD interacts with the CARD of pro-caspase-1 leading to its activation. Active pro-caspase-1 leads to the activation of caspase-1. On the one hand, active caspase-1 cleaves Gasdermin D to induce pyroptosis, on the other hand it cleaves pro-IL-1β to its mature form IL-1β resulting in the release of different pro-inflammatory cytokines. This figure is inspired by Broz et al. (38)

1.4 Inflammasome-generated cytokine IL-1β as driver of FMF

In summary, the pyrin inflammasome activation will initiate the release of IL-1β and will promote pyroptosis causing the different clinical FMF manifestations. In the next sequence, the reason behind why excessive IL-1β leads to the specific clinical presentation of FMF will be addressed.

The IL-1 family consists of 4 main members: IL-1α, IL-1β, IL-33 and IL-R1A (only inhibitory member). IL-1β is matured by cleavage of pro-IL-1β mediated by caspase-1 activation as discussed above. Synthesis and processing of IL-1β is triggered by two important signals. On the one hand, there is 'priming' to allow the transcription of the IL1B gene. On the other hand, there is the activation signal that comes from the activation of inflammasomes and the cleavage of pro-IL-1β by caspase-1. IL-1β has different ways to leave the cell when synthesized such as shedding of microvesicles from the plasma membrane, direct release through transporters, exocytosis of secretory lysosomes and more importantly in this case, pyroptosis. Pyroptosis induces pores in the cell membrane that enables IL-1β and even pro-IL-1β to leave the cell. Additionally, sodium and water enter the cell through these pores. If the activation signal and the GSDMD induced pores are low, membrane fusion can eventually patch the pores. However, when the activation signal and the GSDMD induced pores are high, the sodium and water will enter the cell and induce membrane rupture.(44, 45)

IL-1β can bind to two different IL-1 receptors being IL-1R1 and IL-1R2. IL-1R1 is part of the TIR/IL-1 family which means it has a TIR domain. The binding of IL-1β to IL-1R1 induces a structural change which leads to the binding of the coreceptor IL-1R3 to IL-1R1. IL-1R3 is necessary for activation of the transmission signal. Interestingly, binding of IL-1β to IL-1R2 will not induce an activation signal since IL-1R2 does not have a TIR domain. Thus, IL-1R2 is described as a decoy receptor. The trimeric signalling complex consisting of IL-1β, IL-1R1 and IL-1R3 recruits Myd88 resulting in a cascade illustrated in figure 6. As a result, NF-κB translocates from the cytosol to the nucleus where it activates NF-κB dependent genes.(44) Through these pathways IL-1β induces the expression and synthesis of cyclo-oxygenase-2 (COX-2), phospho lipase A2 and nitric oxide synthase (iNOS). As a result, the production of prostaglandin E2, platelet activating factor and NO leads to local and systemic responses.(46)

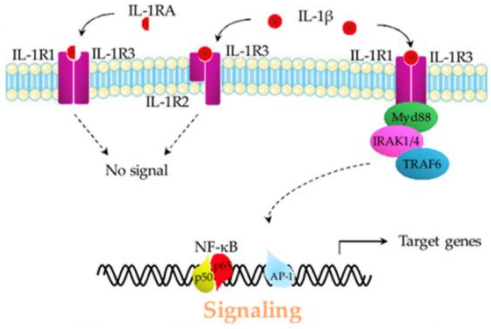


Figure 6. Signalling pathway of IL-1 and activation of NF-κB dependent genes. IL-1RA, part of the IL-1 family, is the only inhibitory cytokine. Subsequently, when it binds to the IL-1R1 receptor no signal is produced. Only the binding of IL-1β to IL-1R1 can result in a structural change leading to the binding of IL-1R3 forming a trimeric signalling complex (IL-1β/IL-1R1/IL-1R3). This leads to a cascade which activates NF-κB dependent genes. This figure is adapted from Rébé (2020) (44)

Continuous IL-1β inflammation can lead to exaggerated immune responses by inducing suppression of Treg cell responses. In addition, IL-1β works as a counteract of TGF-β induced FOXP3 expression in CD4 T cells. Subsequently, the differentiation of Tregs gets inhibited, and IL-1β drives the switch form CD4 T cells to Th17 instead.(47) Moreover, IL-1β can lead to Th17 mediated immunopathology and as a consequence, this Th17 polarisation is also seen in FMF. Th17 cells secrete IL-17 among other pro-inflammatory cytokines including IL-6, TNF, IL-22 and IL-21 which promotes inflammation and cartilage/bone destruction when expressed chronically.(48) IL-17 is an important cytokine in the pathogeneses of spondyloarthropathy and IBD. Therefore, there is a development of seronegative spondyloarthropathy (mainly sacroiliitis) in approximately 3% of the FMF cases.(49) In addition, IL-1β increases the expression of different adhesion molecules. This, in combination with different chemokines, leads to the promotion of infiltration of inflammatory and immunocompetent cells. Additionally, IL-1β is an important factor in angiogenesis where it can lead to induction of the formation of blood vessels.(46) IL-1β also produces different metalloproteases and inhibits proteoglycan and type II collagen resulting in the breakdown of articular cartilage. Moreover, IL-1β has an (in)direct stimulatory effect on osteoclast production that can lead to the progression of arthritis. (50) Due to the high amounts of IL-1β causing inflammation, the production of SAA is significantly higher, leading to more risk of developing AA amyloidosis (resulting in renal insufficiency), the most severe complication of FMF.(51)

The importance of IL-1β as a driver for inflammation was first recognized by a disease in infants characterized by a loss-of-function mutation in the IL-1 receptor antagonist (IL-R1A). This condition, deficiency of interleukine-1 antagonist (DIRA), is accompanied by an exaggerated number of infiltrating neutrophils resulting in systemic sterile inflammation with effects on the skin, joints and bone.(52) Normally, IL-R1A works as an inhibitor for the IL-1 receptor type 1 (IL-R1) which is present on many different cell types. Therefore, without this inhibition, IL-1β can cause systemic inflammation. Fortunately, the systemic inflammation and fatal outcome in DIRA is reversed by daily use of IL-1 inhibitors such as anakinra.(52) In the case of FMF, most *MEFV* variants – associated with exaggerated IL-1β production – do not interfere with the production of IL-R1A, which could explain the periodic character. FMF attacks mostly resolve within 2 to 3 days. This so called 'hyperinflammatory state' can be reached by an overdrive of the innate immune system due to a specific trigger causing inflammatory responses responsible for the symptoms of FMF during the attack-period. However, in between attacks, the FMF patient returns to a normal state without inflammation. Unlike this hyperinflammatory state, there are cases that describe a continuous inflammatory state within FMF. This 'autonomous inflammatory state' is characterized by a continuous release of IL-1β production and therefore also results in consistent inflammation between the attacks.(49)

In conclusion, IL-1β plays a major role in the emergence of symptoms in FMF patients. Due to a study conducted by Sharma et al., it is known that aberrant caspase-1 activation promoted the maturation and release of IL-1β in a mouse model of FMF.(53) Therefore, together with therapeutic interventions to be discussed further in section 4.3, IL-1β is believed to be the ultimate driver and the most important factor of the pathogenesis of FMF.

1.5 Treatment of FMF

Since 1972, colchicine, also used for the treatment of gout, is considered to be the standard therapy for adult and paediatric FMF patients. Colchicine reduces the frequency of attacks and can effectively prevent secondary AA amyloidosis which is the severest complication of FMF.(54) As soon as the FMF diagnosis is made, treatment with colchicine should be started.(55) However, colchicine resistance is documented in some patients leading to a growing need for new therapies. Since IL-1β is seen as the ultimate driver of FMF, as discussed in the previous section, IL-1 antagonists (canakinumab and anakinra) were introduced and are proven effective in the treatment of FMF in cases of colchicine resistance or intolerance.(6) In the results, the different treatment options, along with the genetics and complications of FMF will be thoroughly discussed, to create an overview of the efficacy of these treatments for the variety of FMF patients.

2. Research question

The pyrin inflammasome and consequently the excessive release of IL-1β play a central role in the development of the various clinical manifestations of FMF. Therefore, anti-IL-1 therapy was a major breakthrough to control excessive IL-1β responses and thus improving the quality of life in these patients. However, despite the causative role of IL-1β in FMF, the clinical picture and therapeutic responses of various FMF patients can be very different in individual patients. The aim of this paper will be to elaborate on these differences and to discuss their potential causes.

First, an in-depth discussion of the different FMF mutations will give significance to the genetic heterogeneity within FMF. The Infevers database established different mutations linked to FMF and gave them a classification as briefly discussed in the introduction. In the results section, the most important classified mutations will be presented and discussed in order to reveal how these different mutations are more likely to cause an aggressive form of FMF or rather a mild disease course.

Second, the complications of FMF, with special attention for the most important complication being secondary AA amyloidosis, will be discussed alongside the discussion of the most important mutations resulting in FMF with their implications on the clinical picture. To end, the aim is to correlate the various FMF mutations with treatment responses and focus on efficacy, working mechanism and use of colchicine and anti-IL-1 therapy in FMF patients. Taken together, discussing the impact of various different FMF-related Pyrin mutations on the clinical manifestation and therapy responsiveness will shed light on the importance for genetic diagnosis in FMF in order to identify the most appropriate therapy for these patients.

3. Methodology

The methods used for the research consisted of a quantitative and qualitative approach. Initially, a quantitative approach was utilized to determine the scope of the thesis. The aim was to find as many papers as possible to get an overview of the different aspects of FMF, without assessing the quality or usefulness of the papers in the first place. Thereafter, a more qualitative approach was utilized in which the focus lied on the different aspects independently. The aim was to verify which articles were useful based on criteria that will be discussed down below. Since FMF was introduced in the twentieth century, many articles were published over the years, presenting the molecular pathways and clinical manifestations of FMF. In order to clarify the chronological path, some of the older articles describing these pathways are included in this paper.

In contrary to experimental research, the goal for this research is not to find new ways of therapies for FMF, but to create an overview of the different treatment modalities already available. Therefore, many papers were consulted to create a detailed overview. It is unrealistic to discuss all the aspects of FMF and AIDs in a detailed way, therefore in the results section, 3 different aspects will be profoundly discussed: the variety of disease-causing mutations in the MEFV gene, the complications caused by FMF and lastly the treatment of FMF. Due to this approach, the focus to select certain articles was mainly based on relevance for the paper.

The main instruments used to select articles were online databases, specifically: PubMed, Google Scholar and BioRxiv. A few of the Mesh terms used in the process include Familial Mediterranean Fever, pyrin, inflammasomes, recurrent fever, abdominal pain, interleukin 1 beta, arthritis, colchicine and Hereditary Autoinflammatory Diseases. Additionally, through the search, the Infevers database was found, in which a list of all different mutations exists that may or certainly have a link to FMF. After consideration, only the validated mutations were included in the paper. If all the non-validated or unsolved mutations were included, it would take the thesis too far. Moreover, 9 papers were consulted that were present in the Infevers database through the ‘details’ section of the different mutations. The strategy to determine the final articles used for the paper, is presented in the figure below.

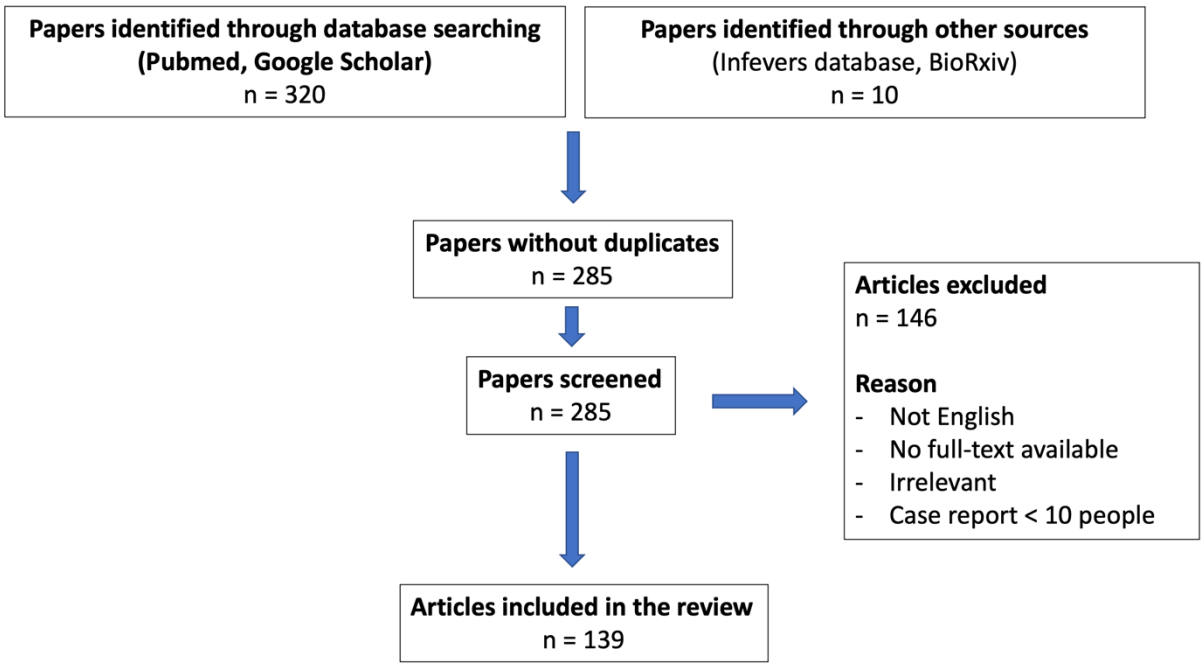


Figure 7: Overview of the strategy for the search of final articles. Through various databases, a total of 330 articles were selected. After removing the duplicates, 285 articles remained that were screened considering several factors of exclusion.

Firstly, the duplicates were removed, then the different exclusion criteria were applied such as no full-text available, irrelevant and articles not written in English. The clinical aspects of FMF were searched independently of each other, without any timeline restriction to get an idea of all different clinical aspects discussed over the years. However, for the in-depth discussion of the molecular pathways (pyrin, pyrin inflammasome, IL-1β), the search period was mostly narrowed down to more recent literature from 2019 to 2022 because of the constant evolutions in these pathways. In addition, through the references of these recent articles, older papers were found, that gave an overview of the molecular pathways, published before 2019. In this way it was possible to create a historical overview.

Some studies about the different treatment modalities and complications consisted of case reports, which made it challenging to draw conclusions from these articles. However, these case reports do highlight the variety of complications and symptoms FMF has to offer.

4. Results

4.1 Genetic heterogeneity in FMF

To date there are 391 sequence variants known in the MEFV gene. All the sequence variants have been given a classification in the Infevers database: not classified, variants of uncertain significance (VUS), unsolved, likely benign, likely pathogenic, benign and pathogenic. In table 3, all the sequence variants are ordered per location and classification showing the distribution of the various mutations. In addition, table 9 in the annex shows a profound overview of all validated mutations exhibited in a more detailed manner containing the sequence names. A few important and common mutations will be deliberated in the section below. Firstly, the most common and most important mutations (pathogenic, VUS and benign) will be discussed. Thereafter, a brief notion of the importance of genetic testing will be provided. In the section 'clinical heterogeneity', the genotype-phenotype correlations will be discussed, where the linkage between mutations and clinical manifestations becomes more tangible.

Table 3: Number of sequence variants ordered per location and per classification

Location	Classification						
	Not classified	*VUS*	*Unsolved*	*Likely benign*	*Benign*	*Likely pathogenic*	*Pathogenic*
5-flanking	9	-	-	-	-	-	-
5UT	1	1	-	-	-	-	-
Exon 1	4	2	3	4	1	4	-
Exon 2	8	50	5	42	4	12	-
Intron 2	-	-	-	4	-	-	-
Exon 3	4	13	5	11	1	7	-
Intron 3	1	-	-	4	-	-	-
Exon 4	1	3	-	1	-	-	-
Intron 4	1	-	-	2	-	-	-
Exon 5	2	11	4	9	3	4	-
Intron 5	-	-	-	5	-	-	-
Intron 6	-	-	-	2	-	1	-
Exon 7	-	3	1	-	-	-	-
Intron 7	1	-	-	1	-	-	-
Exon 8	-	-	-	1	-	4	-
Intron 8	-	-	1	5	-	-	-
Exon 9	1	2	-	2	1	-	-
Intron 9	-	-	1	3	-	-	-
Exon 10	9	30	16	24	-	25	5
3UT	-	-	1	5	-	-	-
TOTAL #	**42**	**115**	**37**	**125**	**10**	**57**	**5**

In this table, the 391 *MEFV* sequence variants from the Infevers database are ordered per classification and per location. This overview gives an insight in the distribution of different mutations in the MEFV gene leading to a better comprehension of the pathogenic or more benign locus. As seen above, most of the pathogenic mutations (pathogenic + likely pathogenic) are clustered in exon 10. In contrast, likely benign and benign mutations are more clustered in exon 2.

Before profoundly discussing several important mutations, a brief notion of the inheritance pattern of FMF is needed. In most cases, FMF is believed to arise from gain-of-function missense mutations. However, recent literature suggested the existence of pathogenic MEFV hypermorphic mutations, in which the mutation leads to an increased level of activity with a gene dosage effect. In general, the inheritance pattern of FMF is recessive, but several studies have shown that even one *MEFV* mutation can suffice to cause FMF in clinically diagnosed patients.(56) To explain these differences, several reasons have been proposed. In case of only 1 mutation leading to FMF, the second mutation could have been missed due to incomplete genetic screening, large deletions, location in an intron and mosaicism. Interestingly, mutations at amino acid position 577 of pyrin cause a dominant FMF inheritance pattern, suggesting a crucial role for T577 in pyrin's function.(57) In addition, Rwoczenio et al. reported a novel mutation p.P373L that also caused a dominant inheritance pattern of FMF.(58)

4.1.1 Pathogenic mutations

Pathogenic mutations are defined as variants responsible for causing disease. The mutations indicated on figure 8 with the red frame are validated pathogenic mutations in the *MEFV* gene according to the Infevers database. In this first section, a number of characteristics of these exon 10 mutations will be discussed.

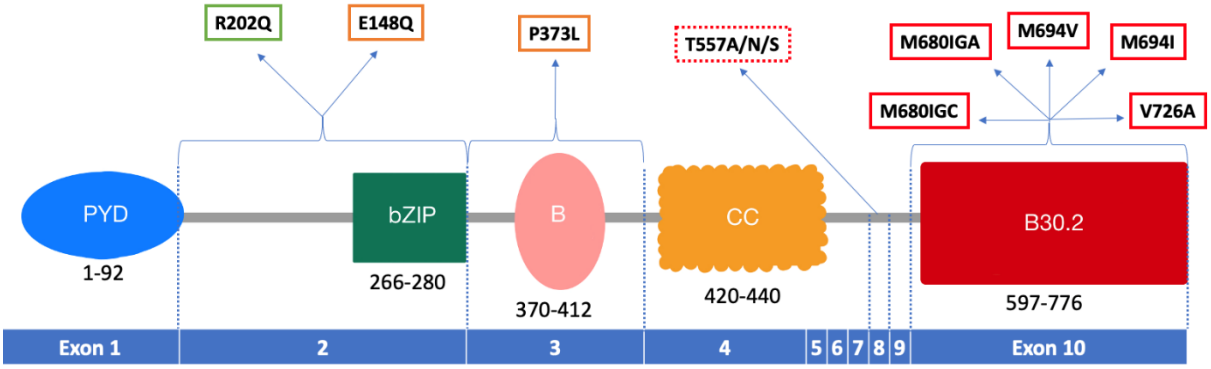


Figure 8. Pyrin domains with distribution of important mutations in the MEFV gene. The exon 10 mutations with a red frame are all validated pathogenic mutations according to Infevers database. R202Q, with the green frame, is a certified benign mutation. E148Q and P373L in orange are validated variants of unknown significance and T577 mutations in the dashed box, located on exon 8, are classified as likely pathogenic. In the sections below, their relation to the FMF disease course and manifestations will be discussed more in depth. Figure is inspired by Accetturo et al. (59)

Concerning prevalence of these different mutations, Ozdemir et al. conducted a large study comprising 3340 patients in the region of Anatolia, where the prevalence of some of the mutations in Fig 8 was as follows: M694V (43.12%), E148Q (20.18%), M680I(G/C) (15.00%) and V726A (11.32%).(60) Recently, an even larger study, involving over 27,000 patients, presented different outcomes across Turkey: M694V (29.47%), E148Q (18.27%), R202Q

(17.90%), M680I (10.61%), and V726A (10.14%). Although the patients are not evenly distributed according to all different regions, this study does seem to provide a general perspective of FMF prevalence in Turkey.(61) In both studies, the M694V mutation was the most prevalent. Similarly, it was also identified as the most common MEFV mutation among the Iranian population.(62) Conversely, the M680I mutation is more commonly seen in Armenians, whereas M694I and V726A are frequently seen in Arab populations.(63) Nevertheless, it is important to note that mutation prevalence can vary between different studies due to various reasons. Firstly, there can be differences in mutation identification or sample sizes. Secondly, different ethnicities of study populations strongly affect the frequency of mutations.(64) To end, allele frequencies can also drastically differ by region within the same country. For instance, the V726A mutation is much more prevalent in the North East Turkey than in Central Anatolia. These differences may be caused by interactions between different ethnic and cultural groups over the years, leading to a wide genetic diversity.(7)

Concerning pathogenic penetrance, Touitou et al. concluded early in 2001 that homozygosity of the highly prevalent M694V mutation has a very high penetrance and correlates with a severe FMF disease course.(65) Indeed, M694V is known as the most pathogenic MEFV mutation as it is associated with higher risk of joint and skin involvement and a higher risk of developing secondary amyloidosis, especially when it presents itself in a homozygous state.(66) Pathogenic M694V mutations can also occur associated with other mutations to form compound heterozygotes such as M694V/R202Q and M694V/E148Q. In conclusion, patients who carry homozygous, heterozygous or compound heterozygous mutations with M694V should endure a good follow-up due to the high probability of a severe disease course.(14)

Besides M694V, also M694I and both genetic forms of M680I mutations are linked with a severe disease course especially in homozygous state. However, in a heterozygous form such as M680I/E148Q and even M694V/E148Q, it is a predictor of a mild disease course with late symptom onset.(67) Finally, V726A is a validated pathogenic mutation located on exon 10 that is prevalent in Arabs, Ashkenazi and Iraqi Jews.(68). Yet, despite being pathogenic, it is frequently associated together with E148Q and thus, perceived as a mutation causing mild FMF.(69) In addition, Kriegshauser et al. described a correlation between late-onset of the disease and a homozygous V726A/V726A mutation. (67) Moreover, fever and abdominal pain are less common in patients with V726A mutations. (10) A more in-depth genotype-phenotype correlations of these exon 10 pathogenic FMF mutations will be discussed in section 4.2.1.

4.1.2 VUS, important for the disease course?

Variants of unknown significances are genetic variations for which the effects on the organism are not yet completely revealed. E148Q, a VUS located on exon 2, is a very prevalent mutation in the Mediterranean basin and therefore one of the most important VUS in the *MEFV* gene. Touitou et al. described it as a functional polymorphism since it may affect the patient's phenotype, although it is usually correlated with a mild disease course. To this day, it is still highly debated whether E148Q is indeed a functional polymorphism or rather a disease-causing mutation.(65) On the one hand, the allele frequency is very high in the general population which would suggest that E148Q is a benign polymorphism. On the other hand, some studies show that patients carrying homozygous E148Q variants appear to have mild FMF manifestations.(70) In the paragraphs below, a few studies are displayed, which attempted to grasp the complexity of the E148Q mutation in the *MEFV* gene.

According to a study conducted by Touitou et al., E148Q results in no symptoms in nearly half of the FMF patients when it presents itself in a homozygous state.(65) Conversely, when associated with exon 10 mutations such as M694V or M680I in a compound heterozygous form, there is an increased level of clinical symptoms. Stella et al. showed that E148Q and R761H, despite being low penetrance alleles, can cause symptomatic FMF especially in Southern Italy.(71) Patients with only a E148Q mutation usually have an older age at disease onset, lower FMF family history, and lower colchicine dose requirements than patients with M694V mutations or compound heterozygous mutations consisting of E148Q and exon 10 mutations.(72) From the findings above, it seems that E148Q in the homozygous form causes a mild form of FMF, however when accompanied with other FMF causing mutations, it can demonstrate an increase in FMF manifestations. In support of this hypothesis, Eyal et al. investigated the penetrance of E148Q together with M694V in comparison to a M694V/M694V genotype. Interestingly, the disease penetrance of M694V/E148Q was 17 times higher than M694V/M694V genotype. Thus, these studies concluded that E148Q is a functional polymorphism that affects the phenotype of the FMF patient when combined with M694V mutations.(73) However, in sharp contrast, in a study by Tirosh et al. the clinical phenotypes of FMF patients with E148Q heterozygosity, M694V heterozygosity, E148Q/M694V compound heterozygosity and M694V homozygosity were compared. This study suggested that E148Q heterozygosity is highly unlikely to aggravate the disease course in M694V-associated FMF patients. Tirosh displayed three possible explanations for this phenomenon: firstly, because the prevalence of E148Q mutations is very high, even in healthy controls, it indicates that E148Q alone is not sufficient to be a disease-causing mutation. Secondly, patients in the E148Q group displayed a milder phenotype. Lastly, the coexistence of the M694V and E148Q

mutation did not aggravate the phenotype, it rather attenuated the disease course since there was a slight decrease in rates of abdominal pain and exertional leg pain.(74)

Many patients in Japan have more atypical FMF compared to Mediterranean patients, which could be justified by the high prevalence of exon 2 variants as these are often genetic polymorphisms found in genetically healthy people. A study conducted by Miyashita in Japan on the role of the E148Q mutation in combination with M694I variants provided support for the functional polymorphism hypothesis for E148Q. The patients in this study with compound heterozygous E148Q and M694I variants displayed typical FMF manifestations. In contrast, the patients only carrying the M694I mutation or carrying heterozygous or homozygous E148Q variants displayed no disease manifestations. Miyashita et al. concluded that the patients carrying heterozygous M694I mutation, may have a worse disease outcome when the E148Q mutation is present. (70) Another study situated in Japan indicated that a group of FMF patients with heterozygote E148Q and other variants are clinically more severe compared to those with heterozygous E148Q mutations alone. In other words, patients who were heterozygous for E148Q along with other non-exon 10 and therefore non-pathogenic MEFV alleles demonstrated a higher risk of expressing the FMF phenotype compared with those with heterozygous E148Q alone.(75) In conclusion, these studies suggested that modifying genetic factors such as the E148Q variant are helping or are in some cases even necessary to coexist with exon 10 or other mutations to develop FMF.(70)

Overall, the disease-causing ability of the E148Q is highly disputed. On the one hand, E148Q homozygosity seems to correlate with milder disease course or even asymptomatic patients. Then again, E148Q could have aggravating effects on the symptomatology of FMF patients when it coexists with exon 10 mutations.

4.1.3 R202Q, a rather benign or malign mutation?

According to the Infevers database, R202Q is a validated benign mutation located on exon 2, resulting in the classification of R202Q as not disease-causing. Indeed, R202Q can present itself in a homozygous state in a healthy population. However, the high incidence of the R202Q mutation in FMF regions and its frequent association with clinical symptoms indicate a risk for FMF disease.(63, 72) Then again, this frequent association could also be an effect of co-occurring malign mutations in these FMF patients. In the paragraphs below, a few studies are discussed, suggesting that R202Q is rather a pathogenic/malign mutation.

A number of studies have been conducted to investigate if R202Q has an additive effect on FMF severity when coexisting with other mutations. Ozturk et al. discovered that R202Q in a heterozygous state had no effect on the clinical spectrum of FMF. Conversely, when it presented itself in combination with other pathogenic mutations, the clinical spectrum became

apparent. (76) Kandur et al. supported this finding. In his study, two study groups were compared: R202Q/M694V and M694V/-. He detected a higher severity disease score in the R202Q compound group, and on top of that, increased symptom severity within patients carrying the R202Q alteration, particularly when combined with a M694V pathogenic mutation. (77) Similarly, Comak et al. found that R202Q even in heterozygous form appear to be associated with an inflammatory phenotype. Moreover, in a Greek study, Giagliis et al. implied that R202Q homozygosity is linked to a severe disease course.(78) Similarly, in Turkey, Yigit et al. found a possible correlation between homozygous R202Q as a pathogenic mutation for FMF.(79) Specifically, Yigit et al. indicated that R202Q, when occurring in a homozygous state, is more prevalent in patients with FMF than in healthy controls suggesting a disease-causing trend in homozygous form.(79)

Thus, although there is still a lot unknown about the R202Q alteration, numerous studies show that R202Q might rather be a disease-causing mutation than a benign polymorphism. As such, R202Q homozygosity as well as R202Q heterozygosity seems to have a more severe disease course in FMF patients. Still, further research such as comprehensive prospective studies are needed to help find the exact correlation between R202Q mutations and clinical manifestations.(80)

4.1.4 The importance of genetic testing

As apparent from the above described variety in FMF causing mutations, genetic analyses are needed to support clinical diagnosis of FMF. Clinical phenotype is still the most important tool to diagnose FMF, however, molecular verification can attribute in establishing an earlier diagnosis in suspected cases. Shinar et al. compiled different guidelines of genetic testing since genetics are a crucial supporting factor for the diagnosis of FMF (16). In the table below, a summary of the guidelines is compiled.

Table 4: Summary of the guidelines composed by Shinar et al. (16)

- If there are two pathogenic variants (homozygous or compound heterozygous) found in the ***MEFV*** gene, FMF diagnosis is confirmed.
- If the patients are compound heterozygous with one pathogenic variant and a variant of uncertain significance (VUS), this could be in line with the clinical diagnosis of FMF.
- If there are two VUS (homozygous or compound heterozygous) found in the ***MEFV*** gene, diagnosis relies on the clinical criteria.
- If one pathogenic or VUS variant or even no variants are found in the patient, consider the possibility of other periodic fever syndromes.
- If there are variants found on a complex allele, parental testing is recommended.

These guidelines could provide very useful information about FMF patients to accurately diagnose the disease, without fully relying on only clinical manifestations. Clinical manifestations can sometimes be deceiving since some mutations in the *MEFV* gene cause very mild FMF, however, there can be continuous inflammation where treatment is required. Accurately assessing pathogenicity of specific variants is still a challenge leading to several inconsistencies in the classification of variants. Indeed, 30% of variants in the *MEFV* gene remain unclassified or classified as a VUS, which elevates the need for more specific genetic assessment of FMF mutations, because it is vital to detect a severe disease course early enough to prevent AA amyloidosis. As a result, physicians frequently need to deal with inconclusive genetic results leading to impaired diagnosis.(59) Genetic testing has become very specific and very sensitive due to the introduction of next generation sequencing (NGS), but it is not possible to use it for every suspected patient due to its higher cost.(61) But still, NGS seems to be a very important tool for finding rare and/or new mutations and thus appears to be essential for early diagnosis and treatment in suspected FMF patients.(81)

4.2 Clinical heterogeneity in FMF

4.2.1 Genotype-phenotype relations

On the website of the EUROFEVER project (www.printo.it/eurofever) the genotype-phenotype correlation of the *MEFV* gene is displayed with different examples from different FMF patients. Specifically, a web-based collection of genotype-phenotype correlation has been established and continues to be updated.(82) Table 5 shows a comprehensive summary of these genotype-phenotype relations concerning clinical manifestations in patients carrying the most common FMF mutations.

Multiple studies have shown that M694V mutations are associated with a more severe disease course such as earlier onset of secondary AA amyloidosis (most severe complication of FMF) and arthritis.(83) Grossman et al. concluded in a comparative study that the rate of FMF attacks, overall frequency of chronic manifestations, higher dose of colchicine, frequency of arthritis attacks is more prevalent in the study group with homozygous M694V mutations.(84) Moreover, in a study conducted by Balta et al., the most common allele in patients with development of amyloidosis was M694V. In addition, there is a higher risk of an appendectomy, proteinuria and colchicine resistance in homozygous M694V mutations.(85) In a retrospective study Balci et al. investigated comorbidities of FMF in 2000 genetically confirmed patients. Correspondingly with the findings above, the mutation M694V had a high risk of appendectomy (38%), renal amyloidosis (87%) and chronic kidney disease (50%). Other pathogenic mutations such as M680I had a much lower percentage of prevalence for appendectomy (10%), renal amyloidosis (8%) or chronic kidney disease (7%).(86) Dundar et al. conducted a

large study with a cohort of more than 27.000 people across Turkey to investigate the variant profiles in the Turkish population and to correlate the complex relationship between the *MEFV* gene and clinical FMF symptoms.(61). Nearly half of the patients carrying M694V had fever and M694V also increased the probability of developing arthritis.(61) To conclude, FMF patients who carry a homozygous M694V genotype, in comparison to FMF patients carrying other genotypes, have a higher disease severity, need higher doses of colchicine, have a poor response to colchicine and more chronic FMF associated morbidity/comorbidity resulting in poor quality of life and higher PRAS scores.(84) PRAS scores are used to determine the severity of FMF for paediatric patients through assessing following factors: onset of disease, frequency of attacks on admission, arthritis severity, erysipelas-like erythema and dosage of colchicine.(87)

Besides M694V, a retrospective study conducted by Ozturk et al., consisting of a very large FMF series of children, showed that many exon 10 homozygous or combined heterozygous mutations caused an earlier disease onset and more severe disease outcome. The most important physical features of FMF such as abdominal pain, fever and arthritis were more common with the group of patients carrying homozygous or compound heterozygous exon 10 mutations.(88) Although V726A is usually associated with a mild disease course and, thus, considered as the mildest mutation out of the pathogenic exon 10 mutations, the results involving V726A show great variability. Specifically, some studies report that homozygous or compound heterozygous V726A mutations cause a more severe disease course than M680I mutations.(11) In contrast, V726A is known to be associated with late-onset FMF. Indeed, in a large study of 10,740 patients conducted in Armenia, the V726A/V726A genotype was among the genotypes associated with late-onset FMF as well as mild disease severity. These features usually go together, as FMF patients under 40 years old have significantly higher rates of fever, chest pain, skin involvement and a more severe disease course overall. However, abdominal pain was found to be more frequent in the group of patients over 40 years.(67)

Although mutations in exon 10 often correlate with a severe disease progression, the coexistence of MEFV exon 2 variants and exon 10 variants are also suggested to have additional effects on the phenotype and inflammation in FMF patients as discussed in section 4.1.2. and as seen in table 5 below.(77)

Table 5: Genotype-phenotype correlations in compound M694V heterozygous mutations

Mutations		Frequent clinical manifestations and characteristics	Source(s)
Exon 10	**Exon 10**		
M694V	M694V	Recurrent fever episodes, arthritis, proteinuria, abdominal pain, myalgia, chest pain, erysipelas-like erythema	(61) (82) (83) (84) (85) (89)
	V726A	Higher risk for fever attacks and abdominal pain, myalgia, vomiting, chest pain, diarrhoea	(82, 90, 91)
	M680IGC	Recurrent fever episodes, abdominal pain, myalgia, oligoarthritis, chest pain	(82, 91)
	M680IGA	Recurrent fever episodes, abdominal pain, chest pain, constipation	(82, 91)
	M694I	Recurrent fever episodes, abdominal pain, arthralgia, headache, chest pain	(82)
	Exon 2		
	E148Q	Recurrent fever episodes, possibly slight decrease in rates of abdominal pain and leg pain in paediatric patients	(74)
	R202Q	Recurrent fever episodes, higher rates ELE, proteinuria and arthritis	(92) (82)

4.2.2 Complications of FMF

FMF has a wide heterogeneity of manifestations and complications of which the development of secondary AA amyloidosis is the most severe. Before discussing the different treatment modalities, we go more in depth in these wide range of complications to understand the importance of prevention strategies through treating FMF with drugs such as colchicine and anti-IL-1 biologicals.

4.2.2.1 Secondary AA Amyloidosis

Secondary AA amyloidosis is a rare complication that can develop following a long-term inflammatory disorder. Due to chronic inflammation, extracellular deposition of AA amyloid fibrils (misfolded proteins) can lead to abnormalities in the kidney, liver, spleen, intestines, adrenals and heart. Papa et al. described amyloid fibrils as follows: 'Amyloid is an amorphous and insoluble proteolytic resistant material derived from the spontaneous aggregation of fibrils composed of twisted protofilaments.'(93) Amyloid precursors or misfolded proteins, compose these protofilaments. In AA amyloidosis, a soluble apolipoprotein (SAA) encoded by the SAA1 gene, is the amyloid precursor. SAA is an acute-phase reactant synthesised by hepatocytes, macrophages, and endothelial cells through regulation of pro-inflammatory cytokines such as TNF, IL-1β and IL-6. Any condition that is characterized by chronic inflammation has a risk of developing amyloidosis.(93) However, FMF is the most significant cause of secondary amyloidosis in the world. Other causes of secondary AA amyloidosis are presented in table 6.

Imaging is a very important tool to detect amyloid deposits in the body in a non-invasive manner. Specifically, total body SAP scintigraphy is an imaging method to detect amyloid

deposits all over the body through binding to all types of amyloid deposition (figure 9). Subsequently, it can be used as follow-up and as a diagnostic tool for amyloid depositions. However, there are a few downsides of this imaging method: firstly, the correlation between the quantity of amyloid deposits and the loss of organ function is limited. SAP scintigraphy also fails to detect amyloid depositions in very small parts of the body such as the gastrointestinal tract, skin, and nerves. Lastly, SAP scintigraphy seems to be unable to identify amyloid deposits in heart and lungs because of the blood movements in these parts. Although cardiac amyloidosis is very challenging to diagnose, new imaging techniques such as 2-dimensional Doppler echocardiography and cardiac magnetic resonance (CMR) are utilized to evaluate the grade of cardiac amyloidosis.(93) Histological evaluation is essential and required for the diagnosis of amyloidosis. However, a negative histology does not exclude the possibility of amyloidosis in a patient. Today, the presence of a green fluorescent birefringence when viewing a Congo red marked tissue biopsy with cross polarized light is considered to be the golden standard for the diagnosis of amyloidosis (figure 9).(93)

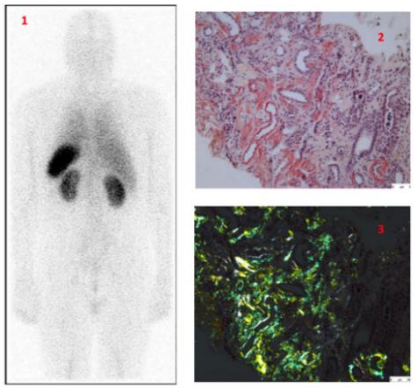


Figure 9: **The use of SAP scintigraphy and histology for the diagnosis of secondary amyloidosis**. 1) The SAP scintigraphy shows amyloid deposits in the kidney, liver and spleen of this patient. 2) Renal biopsy tissue stained with Congo red containing amyloid deposits. 3) The appearance of green fluorescent birefringence under cross polarized light. Figure adapted from Papa et al. (93)

The most common initial manifestation of secondary AA amyloidosis is proteinuria. However, a wide-ranging nephrotic syndrome (oedema, weight gain, fatigue, loss of appetite, proteinuria) may occur as the first sign of amyloidosis as well. Amyloid deposits in spleen, liver, gastrointestinal wall and heart can cause acute manifestations resulting in dangerous consequences.(93) For example, cardiac deposition may lead to heart failure or death even before renal failure. (94) Nevertheless, these manifestations are extremely rare. Adequate management is essential to control and prevent secondary AA amyloidosis. Firstly, it is important to assess the cause of the amyloidosis since it affects the way of treatment. In this case, FMF induces the chronic inflammation and thus results in the development of amyloidosis. Supplementary to the treatment of FMF with colchicine or anti-IL-1 agents (see below), maintenance of the kidney function is vital when amyloidosis is already present. As discussed above, kidney involvement is by far the most common manifestation of secondary AA amyloidosis.(93) In table 6 below, the most important aspects amyloidosis in FMF are displayed.

TABLE 6: SECONDARY AA AMYLOIDOSIS IN FMF

CLINICAL MANIFESTATIONS	*Kidney*	proteinuria, nephrotic syndrome, renal failure
	Liver and spleen	acute atraumatic organ rupture, hepatomegaly
	Gastrointestinal system	acute obstruction or bleeding, recurrent abdominal pain, malabsorption with chronic diarrhoea
	Heart	heart failure
PROGNOSTIC FACTORS	*Negative* ➢ High levels serum creatinine/proteinuria ➢ Cardiac involvement ➢ Amyloid deposits in liver ➢ Crohn disease or chronic sepsis ➢ Older age at time of diagnosis	*Positive* ➢ Periodic fever syndrome ➢ Regression amyloid deposits on SAP scintigraphy
TREATMENT	Supporting care for the affected organs (mostly kidney) Colchicine, anti-IL-1β therapy (anakinra, canakinumab)	
OTHER CAUSES NOT RELATED TO FMF	Chronic infections, Conditions predisposing to chronic infections, Immunodeficiency, Neoplasia, Inflammatory Arthritis, Systemic vasculitis, Periodic fevers, Inflammatory bowel syndrome	

4.2.2.2 Other rarer complications

In this next section, the other rarer complications of FMF will be discussed. In table 7, the genotype-phenotype correlations for possible complications of FMF are composed. Not all complications have been linked to a specific mutation, however, M694V mutations have been correlated to many rare and severe complications.

FMF is associated with **infertility**. On the one hand, regarding male infertility, progressive FMF and acute orchitis (mainly in prepubertal boys) can cause an aggravation of testicular function, affecting spermatogenesis. Moreover, in a study conducted by Kaya Aksoy et al., half of the FMF patients had abnormal sperm parameters. The sperm mobility as well as the overall sperm count was decreased while having an FMF attack under colchicine therapy. Optimal colchicine dose is essential to reduce the rate of FMF attacks and prevent amyloid deposits in the testicles since it is linked with azoospermia. On the other hand, infertility is also present in female FMF patients, however, it is rather linked to oligomenorrhea.(95) Moreover, in untreated or poorly controlled patients, abdominopelvic adhesions due to recurrent peritonitis, and ovarian impairment due to amyloid depositions can affect fertility.(96) Furthermore, because of a misdiagnosis of acute abdominal attacks in FMF patients prior to their diagnosis, more unnecessary laparotomies are performed since appendicitis is often expected. Subsequently, multiple surgical procedures can cause these adhesions and thus impaired fertility.(97) In conclusion, it is very important to correctly assess acute abdomen manifestations as part of FMF, and consequently treat these patients with colchicine, instead of an operation. It is also very important to identify the potential risk factors for infertility in FMF patients. Specifically, FMF disease onset before 20 years and colchicine nonresponse are the main predictors for infertility in FMF patients.(98)

In line with the section above, **appendectomy history** is very common in FMF patients. Many FMF patients tend to undergo numerous surgical procedures due to the wrongful diagnosis of abdominal FMF attacks. For patients with a history of appendectomy, higher doses of colchicine or even anti-IL-1 agents are required because of a more severe disease.(99)

Protracted febrile myalgia syndrome (PFMS) is a rare manifestation of FMF characterized by severe myalgia lasting up to 6 weeks. It is accompanied by fever, abdominal pain, arthritis/arthralgia and diarrhoea. However, the muscle enzymes and muscle biopsies are normal.(100) Interestingly, PFMS may even be the initial sign of FMF which makes it a challenging diagnosis. In line with the sections above, homozygous M694V mutations result in more joint involvement and might be a risk factor for PFMS.(101) Colchicine is not recommended for the treatment of PFMS, however, prednisolone (1mg/kg up to 6 weeks) is used to suppress the symptoms in these patients. In mild cases, NSAIDs can also be utilized to treat PFMS. Furthermore, in case of NSAID and corticosteroid unresponsiveness, anti-IL-1 therapy may be suggested as a treatment option.(102)

Children with FMF seem to have more **sleep disturbances** than their healthy peers. High numbers of FMF attacks and leg pain were associated with poor quality of sleep including sleep anxiety, sleep-onset delay, and night wakings. Thus, managing sleep problems in children is important.(103) Other neurological symptoms seem to be more prevalent in children with FMF. Recurrent headaches and febrile seizures were reported to be more common in this population where febrile seizures especially appeared during FMF attacks. Additionally, ADHD is frequently diagnosed in children with FMF that may be explained by the neuro-immune hypothesis through inflammatory cytokines that pass the blood-brain barrier.(104) In general, neurological manifestations are more common in patients who carry the M694V mutation.(105)

Pericarditis, a rare manifestation of FMF, is characterized by retrosternal chest pain and is diagnosed through use of electrocardiography, chest X-ray and echocardiogram. NSAIDs are essential in treating this manifestation as well as colchicine which can reduce the likelihood of recurrence of pericarditis.(94) (106)

Several monogenic AIDs including FMF can show **ocular involvement**. The following ocular conditions are associated with FMF (ranging from more frequent, to less frequent): progressive reduction of choroidal thickness, retinal vasculitis, uveitis and corneal ectasia.(107)

Monoarthritis is a very common feature of FMF, however, in 5% of FMF patients **sacroiliitis** can develop. These patients generally present themselves with unilateral or bilateral sacroiliitis, esthesitis and inflammatory neck/back pain. Especially the mutation M694V is associated with musculoskeletal symptoms. In line with the nature of the musculoskeletal features, this type of sacroiliitis can often be misdiagnosed as spondyloarthritis (SpA).

However, most patients are HLA-B27 negative, which can be an indication to further investigate the possibility of an underlying FMF disease. SpA can also be present in FMF patients as a comorbidity. In general, sacroiliitis occurs in the adult population, although, sacroiliitis in paediatric FMF patients has also been reported. Unfortunately, there is no standard treatment for FMF complicated by sacroiliitis. Nonetheless, NSAIDs, methotrexate and biologicals (for example tocilizumab, adalimumab) appear to be effective in these patients.(108)

Liver involvement is a rare complication occurring in FMF patients. FMF seems to play a role in development of increased liver enzymes, non-alcoholic fatty liver disease (NAFLD) and cirrhosis. IL-1 production plays a major role in the progression of chronic liver disease causing inflammation and pyroptosis. Thus, IL-1 inhibition appeared to be beneficial for reversing liver injury although only investigated in murine models.(109)

Table 7: Genotype-complication correlations in compound M694V heterozygous mutations

Mutations		Possible complications	Source(s)
Exon 10	**Exon 10**		
M694V	M694V	Higher risk for appendectomy, renal amyloidosis, chronic kidney disease, infertility	(61) (82) (83) (84) (85) (89)
	V726A	unknown	(82, 90, 91)
	M680IGC	Rare renal amyloidosis, flexion contractures, peritoneal adhesions	(82, 91)
	M680IGA	unknown	(82, 91)
	M694I	Peritoneal adhesions	(82)
	Exon 2		
	E148Q	unknown	(74)
	R202Q	Chronic periodontitis, secondary amyloidosis	(92) (82)

4.3 Therapeutic heterogeneity in FMF

In this section, the available treatment strategies for FMF patients will be profoundly discussed. Firstly, colchicine in all its different aspects, including working mechanism and particularly colchicine resistance will be elaborated. Colchicine is the most important treatment modality to prevent the development of complications discussed in section 4.2.2, especially secondary AA amyloidosis. Thereafter, IL-1 inhibition will be discussed which is utilized when colchicine is contra-indicated or unresponsive. The three anti-IL-1 agents (anakinra, canakinumab and rilonacept), their specific working mechanism and their safety will be elaborated. In the last section, a comprehensive table is assembled to collect all the information given in section 4.3. Additionally, a proposed therapeutic algorithm is established to help physicians guide their patient through the treatment aspect of FMF. (figure 12, 4.3.3)

4.3.1 Colchicine

There are three main reasons to start colchicine therapy early in children diagnosed with FMF. Firstly, to protect them against future febrile attacks. Secondly, to avoid any potential unnecessary medical interventions (appendectomy, laparotomy etcetera). Lastly, to protect them from secondary AA amyloidosis.(110) In this following segment, the efficacy and working mechanism of colchicine will be discussed, while briefly referring to section 1.3.2 of the introduction, in relation to preventing future attacks and secondary AA amyloidosis.

Colchicine is a life-long therapy, administered in the following dosing scheme:

- Children 4-6 years of age: 0,3-1,8 mg/day
- Children between 6 and 12 years: 0,9-1,8 mg/day
- Children > 12 years: 1,2-2,4 mg/day
- Children with renal amyloidosis or persistent symptoms: 1,5-2,0 mg/day
- Adults: 1.2-2.4 mg/day

4.3.1.1 Efficacy and working mechanism

Colchicine is considered to be the standard therapy for adult and paediatric FMF patients to prevent FMF attacks as well as prevent development of amyloid deposits. The main excretion route of colchicine is the hepatobiliary excretion and with the faeces. 15-30% is excreted in urine within the first 24 hours.(110) Furthermore, it is mainly metabolized by the liver through the CYP3A4 pathway. Colchicine has a half-life of approximately 4 hours.(111)

Colchicine is a tricyclic alkaloid that has the capacity to bind β-tubulin and to form soluble colchicine-tubulin complexes leading to inhibition of self-assembly and polymerization of microtubules; as seen in figure 10. Colchicine modulates the production of chemokines and prostanoids, inhibits neutrophil chemotaxis and reduces expression of adhesion molecules resulting in decrease of inflammation.(112, 113) As illustrated in figure 10, colchicine can prevent inflammation in FMF patients through direct stimulation of RhoA. Colchicine executes this function by releasing and stimulating GEF from depolymerized microtubules. In addition, colchicine inhibits different intracellular signalling pathways such as NF-κB and caspase-1 and therefore, diminishes the innate immune response. As a result, colchicine acts as a powerful anti-inflammatory agent through different pharmacological effects. (114)

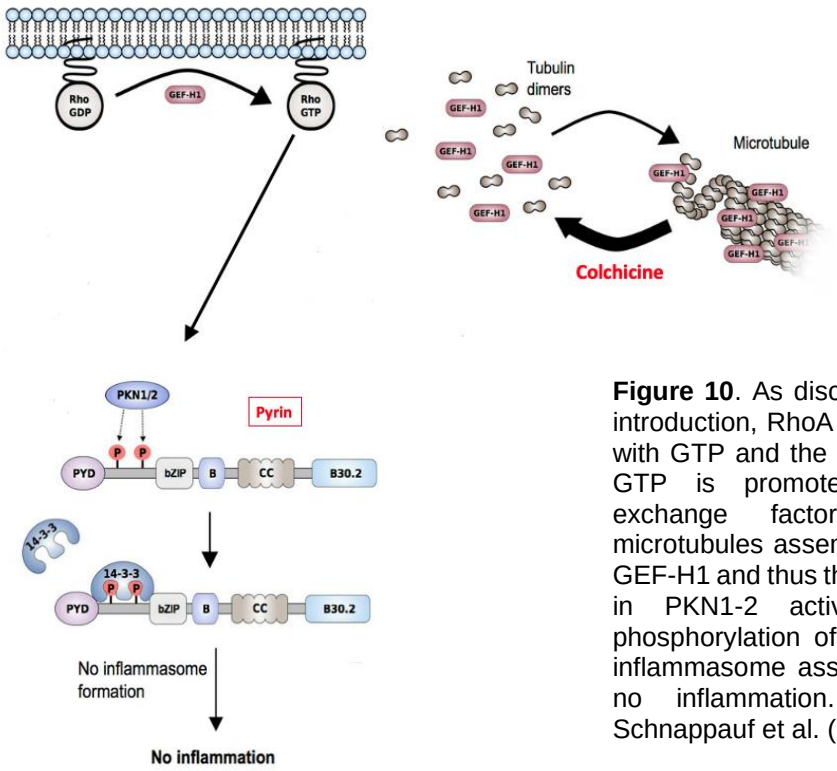


Figure 10. As discussed in section 1.3.2 in the introduction, RhoA is activated when associated with GTP and the exchange between GDP and GTP is promoted by guanine nucleotide exchange factors (GEF). Inhibition of microtubules assembly enhances the release of GEF-H1 and thus the activation of RhoA resulting in PKN1-2 activity which leads to the phosphorylation of pyrin. As a result, no pyrin inflammasome assembly is achieved and thus, no inflammation. Figure is inspired by Schnappauf et al. (25)

Before moving on to the part about safety and interactions, colchicine compliance needs to be discussed, since it logically has a massive impact on the efficacy of colchicine treatment. Thus, due to the narrow therapeutic window of colchicine, it is very important that the compliance and dosage is appropriately adjusted. As a consequence, close monitoring and surveillance is needed. Furthermore, compliance is also essential to reach the full effect of colchicine, whereas noncompliance can lead to more FMF attacks. For instance, when a patient just one time forgets to take his dose of colchicine, an FMF attack can occur in the next few days.(115) However, it is rather difficult to check the compliance of patients since there is no reliable detection method to evaluate colchicine levels in the body.(116)

Interestingly, according to several studies, full compliance is not very common. Especially for toddlers and new-borns, who have to swallow these bitter tablets (considering no alternative is widely available), it can tamper with compliance rates within this population.(115) In the adult population, non-compliance is also rather common. Tekgöz et al. investigated a few reasons or explanations for this non-adherence. Patients seem to be non-adherent due to concerns regarding overuse of colchicine and thus potential toxic effects. Interestingly, patients with concomitant diseases seem to have more adherence than patients without. The concerns of having worse outcomes may be an explanation for this trend. Reminding the patient to take the medication with the help of a partner, a parent or an alarm could increase compliance.(117) In conclusion, as a physician, it is vital to take these elements into consideration when treating FMF patients and thus regularly question patients about their adherence and discuss the importance of the effectivity of colchicine.

4.3.1.2 Safety and interactions

Most frequent adverse effects are gastrointestinal problems such as loose stools, vomiting, diarrhoea and frequent bowel movements. Diarrhoea is certainly the most common side-effect, which can be handled by slightly reducing the dose (which also decreases the effectivity of colchicine) or adding antimotility agents. Furthermore, abnormality in liver function tests, myopathy and leukopenia are described as side-effects of colchicine.(118) Colchicine neuromyopathy is also documented after chronic daily colchicine use, especially in patients with impaired renal function in which the dosage is not adjusted.(116) Because of the most common adverse effects, which are gastrointestinal problems and liver toxicity, patients can develop colchicine intolerance. Additionally, anaemia can be seen in these intolerant patients because colchicine seems to interfere with vitamin B12 and iron absorption. Colchicine intolerance is defined as not being able to increase the dosage to reach the optimal level of effectivity of colchicine due to its side-effects. As a result, it increases rates of complications and ongoing attacks caused by this suboptimal dosing.(118) Therefore, alternative treatment modalities such as anti-IL-1 agents are needed to control persistent inflammation and prevent the development of severe complications.

Colchicine is a lifelong therapy. Thus, it is important to review the effect of colchicine on pregnancy outcomes. Disease course of FMF during pregnancy is variable. Generally, in most patients, pregnancy improves FMF disease course which is in strict contrast with the fact that menstruation (elevated hormone production) is one of the possible provoking triggers for an FMF attack. Nevertheless, FMF attacks during pregnancy can cause serious harm, such as uterine contractions as well as spontaneous abortion and preterm delivery.(96) The use of colchicine during pregnancy has led to controversy because some animal studies suggested foetal harm possibly due to its anti-mitotic properties. Therefore, several observational studies have been published which focus on patients with FMF during pregnancy. In conclusion, colchicine did not increase miscarriage in FMF patients, it rather seemed to work protective due to preventing peritoneal adhesions which can induce spontaneous abortions. The risk of foetal abnormalities was not higher in the population that took colchicine during pregnancy. However, it is important to note that no randomized controlled studies have been published which could potentially confound these results. The FDA classified colchicine as a category C drug, which means that foetal risk cannot be excluded, however, it is not entirely contraindicated.(119, 120)

Colchicine adverse effects, although tampering with the compliance, are considered to be rather mild. However, acute colchicine intoxication is an exception. Acute colchicine intoxication can lead to bone marrow hypoplasia, myocardial depression, acute respiratory distress syndrome and acute kidney failure. Symptoms of colchicine toxicity include nausea,

vomiting, diarrhoea, burning sensations in throat and stomach, dehydration, hypotension, shock, confusion, and neuropathy. Bone-marrow deficiency and a mortality rate of 10% occurs with doses between 0,5-0,8 mg/kg. After ingestion of more than 0,8 mg/kg, mortality rates are nearly 100%. If a colchicine intoxication is suspected, the patient should be taken to the hospital immediately, where activated charcoal is given and the stomach gets emptied. Additionally, symptomatic cardiovascular, pulmonary, and renal support is required.(110, 113)

Because of the narrow-therapeutic marge of colchicine, the possibility of an extreme intoxication and the lifelong treatment, interactions with other drugs becomes more impactful. Colchicine is metabolized by the cytochrome P450 3A4 system in the liver. Additionally, colchicine concentrations are regulated through the P-glycoprotein pump (P-gp). As a result, interactions with medication that inhibit CYP3A4 enzymes or P-gp could drastically increase blood levels of colchicine in FMF patients resulting in an increased risk of acute colchicine intoxication. When giving following frequently used medications/substances, this effect has to be taken into account: clarithromycin (macrolide), cyclosporin, grapefruit juice, ketoconazole and verapamil/diltiazem (calcium channel blockers).(110, 115) These effects are especially impactful when renal function is already impaired, for example in the elderly. In contrast, inductors of CYP3A4 enzymes or P-gp such as phenytoin, carbamazepine and rifampicin, could reduce the concentration of colchicine leading to an increase of FMF symptoms resulting in impaired disease control.

4.3.1.3 Colchicine resistance

Colchicine is effective in 85-90% of FMF patients. Nevertheless, 5-10% of the patients do not respond to colchicine treatment and approximately 5% of FMF patients are colchicine intolerant. It is essential to assess if the compliance of colchicine treatment is ideal since non-adherence can mimic colchicine unresponsiveness. Moreover, the following factors must be considered before colchicine resistance is confirmed: verify the correct diagnosis of FMF, eliminate common causes of fever/pain, ensuring colchicine tolerance or look for other causes of inflammation.(116) Importantly, certain FMF mutations have an influence on the efficacy of colchicine. In table 8, the various mutations and their effect on colchicine is displayed.

Nevertheless, colchicine resistance is described by numerous authors in various ways. Colchicine resistance can be defined in the following conditions: patient receives maximal tolerated dose of colchicine and there is ongoing disease activity or persistent elevation of the acute-phase reactants CRP and SAA between attacks.(115) Others describe it as having 2 or more FMF attacks per month despite taking the maximum tolerated dose.(121) The European League Against Rheumatism (EULAR) describes colchicine resistance as having $\geq$ 1 FMF attack per month and elevated acute-phase reactants despite receiving the maximal dosage.(122) Due to the lack of consensus revolving around colchicine resistance, it is

currently best as a physician to observe and document the frequency of FMF attacks and severity of inflammatory episodes by closely following and re-evaluating the acute-phase reactants for a period of 6 months. As discussed above, educating the patient on the disease and efficacy of treatment is still vital for optimal drug compliance.(123)

Furthermore, it is important to note the difference between colchicine resistance and colchicine intolerance. Colchicine intolerant patients complain about abdominal discomfort and pain, vomiting and diarrhoea. Therefore, it can tamper with the compliance of the treatment resulting in a decrease of effectivity and more ongoing attacks and complications. In comparison to colchicine intolerance, to explain colchicine resistance or unresponsiveness, there is need for elaborating on the mechanisms of colchicine's function. Clinical drug responses can be explained by numerous reasons: environmental factors, genetic factors, various proteins and enzymes essential for metabolization and transportation.(124)

The pharmacokinetics of colchicine are defined by three important proteins. Firstly, tubulin plays an essential role as a kind of colchicine receptor. Secondly, intestinal and hepatic CYP3A4 has a function in the bio-transformation of colchicine. Lastly, the multidrug resistant transporter MDR1/P-glycoprotein (P-gp) plays a role in the distribution and excretion of colchicine. Within colchicine resistant patients, there appear to be disturbances in each or one of these protein pathways. Furthermore, the genes that encode these three proteins could be associated with the existence of colchicine unresponsiveness. For example, genetic polymorphisms in the MDR1 gene appear to have an influence on the P-gp expression and functions.(124) Specifically, the C3435T variant (polymorphism) within the MDR1 gene seems to be correlating with decreased P-gp levels, MDR1 activity and drug response. In addition, this variant is demonstrated to be more common in colchicine refractory patients. However, the specific effect of these polymorphisms on colchicine cellular content has not been clarified. Further research is needed to elaborate the exact relation between these polymorphisms and colchicine.(121, 125) Nonetheless, mutations in the MEFV gene also seem to influence colchicine resistance, as listed in table 8. In numerous studies, patients with homozygous M694V genotype not only have a more severe disease course, but also a less favourable response to colchicine treatment resulting in a higher dosage and thus increased side effects.(126)

Table 8: Genotype-treatment correlations in compound M694V heterozygous mutations

Mutations		Treatment response	Source(s)
Exon 10	**Exon 10**		
M694V	M694V	Require higher dose colchicine + unfavorable response to colchicine	(61) (82) (83) (84) (85) (89)
	V726A	Complete or partial response to colchicine. Requires higher dosage of colchicine.	(82, 90, 91)
	M680IGC	Response to colchicine partial or complete. Requires higher dosage of colchicine.	(82, 91)
	M680IGA	Good response to colchicine. Requires higher dosage of colchicine.	(82, 91)
	M694I	Partial or incomplete response to colchicine	(82)
	Exon 2		
	E148Q	Response to colchicine partial or complete.	(74)
	R202Q	Good response to colchicine	(92) (82)

4.3.1.4 Intravenous (IV) colchicine, an alternative?

Due to colchicine resistance, IV colchicine is being investigated as an alternative for colchicine resistant paediatric FMF patients. Tal et al. concluded that IV colchicine is a safe and efficient treatment option for these patients. The unavailability of anti-IL-1 agents in certain areas of the world, partly because of the very high cost, raises the need for new solutions such as IV colchicine in oral colchicine resistant patients.(127) Because of the narrow therapeutic spectrum of colchicine, administration of IV colchicine has to be under strict supervision. Furthermore, renal and hepatic disease, extrahepatic biliary obstruction or very low creatinine values are absolute contraindications. Monitoring of renal and liver function is also strongly recommended. Nevertheless, Grossman et al. showed that appropriate administration of IV colchicine seems to be a safe alternative, even on the long-term.(128)

4.3.2 Anti-IL-1 therapy

Anti-IL-1 therapy, introduced in 2006 as treatment for FMF, seems to be a worthy alternative for the use of colchicine. IL-1 inhibitors are indicated in the following cases: patients with colchicine resistance, colchicine intolerance or toxicity and severe complications or associated comorbidities.(116) In the following sections, the different anti-IL-1 agents and their specific working mechanism will be discussed. Thereafter, the different indications for IL-1 inhibitors will be elaborated. To end, the adverse effects and possible interactions will be discussed. So far, only anakinra and canakinumab are approved agents for clinical use in Europe, whereas rilonacept is only available in the United States.(129) The high cost of anti-IL-1 agents remains a drawback (seen in figure 11), in comparison to annual colchicine treatment that ranges from $5.500 to $7.000 in the United States (https://www.drugs.com/price-guide/colcrys) and from €165 to €328 in Europe, Belgium (www.bcfi.be).

4.3.2.1 Introduction to anti-IL-1 agents

Anakinra is a recombinant, non-glycosylated form of the IL-1Ra leading to the blockade of IL-1α and IL-1β activity. This IL-1α/IL-1β blocker prevents signal transduction because it is competing with IL-1β and IL-1α for the IL-1R1 binding. Anakinra has a short half-life of approximately 4 to 6 hours. The main dosage for adults is 100 mg subcutaneously (sc) daily and 1-2 mg/kg/d in children.(130, 131) Short half-life shows advantages as well as disadvantages. Advantages include lower risk in case of infection and preferred for short-term use (for example when there is diagnostic uncertainty).(125) However, daily injections could decrease compliance in comparison to canakinumab where only 1 injection every 6-8 weeks is necessary. Anakinra is acceptable from the age of 8 months and with a body weight of more than 10 kg and it improves quality of life and disease severity in FMF patients.(122)

Rilonacept works as a decoy receptor for IL-1α and IL-1β and thus strongly prevents IL-1 activity. In contrast to anakinra, this IL-1α/IL-1β/IL-1Ra blocker has a half-life of approximately 6,4 to 8,6 days and is used subcutaneously (sc) every week in a dosage of 160mg in adults.(130, 131) Very few studies have been conducted investigating the efficacy of rilonacept in FMF children in comparison to anakinra and canakinumab. The only randomized controlled trial conducted reported that 2 patients (out of 14 patients) had a complete response, 8 patients had a partial response and 2 patients had no response.(6)

Canakinumab, the first approved IL-1β blocker for FMF, is a human anti-IL-1β monoclonal antibody that specifically blocks IL-1β with high affinity resulting in a blockade of an inflammatory signalling cascade through preventing IL-1β to bind to its receptor. Canakinumab has a longer half-life than anakinra and rilonacept, approximately 21 to 28 days.(130) It is administered every 8 weeks with a dosage of 150mg subcutaneously (sc) for adults and 2mg/kg in children.(131) In unresponsive patients to anakinra after 12 weeks of therapy, canakinumab is a good option with less side-effects. A possible explanation could be that canakinumab only selectively inhibits IL-1β, rather than blocking the whole IL-1 pathway. (132)

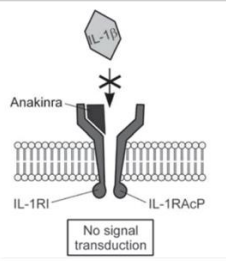
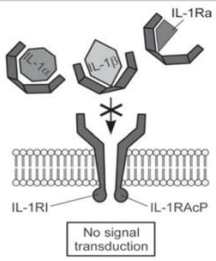
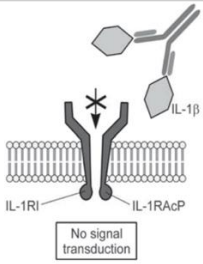
Anti-IL-1	Anakinra	Rilonacept	Canakinumab
Working mechanism			
Blockade	IL-1α, IL-1β	IL-1α, IL-1β, IL-1Ra	IL-1β selectively
Half-life	4-6 hours	6,4-8,6 days	21-28 days
Administration	Subcutaneously daily	Subcutaneously weekly	Subcutaneously every 8 weeks
Annual price	$ 65.780 (US) € 11.160 (B)	$ 261.600 (US) / (B)	$ 117.000 (US) € 70.382 (B)

Figure 11. Comparison of the different therapeutic anti-IL-1 drugs. Mechanism, specificity, half-life, administration and price of canakinumab, anakinra and rilonacept are compared. The annual prices were calculated for the United States (US) (www.drugs.com) and Belgium (B) (www.bcfi.be). Rilonacept is only available in the United States. Figure is inspired by Moll et al. (131)

4.3.2.2 Indications for the use of IL-1 inhibitors in FMF

In a large Cochrane review of treatment modalities in FMF, anakinra and canakinumab seemed to be effective in colchicine-intolerant or colchicine-resistant patients. The use of rilonacept is not yet recommended since more studies are needed to conclude its effect on the disease course. In an RCT in Israel, anakinra was compared with a placebo consisting of the following outcomes: number of attacks, adverse drug reactions and acute phase response. In both number of attacks and acute phase response, anakinra was favoured. However, only CRP levels were in favour of anakinra, there was no evidence for a difference concerning SAA levels in these patients. Correspondingly, canakinumab was compared to a placebo, favouring canakinumab on attack frequency and acute phase reactants (both CRP and SAA).(133) Hence, for FMF patients who are resistant to colchicine treatment, IL-1 agents, particularly anakinra and canakinumab, are used as a safe and worthy substitute in most FMF cases. Moreover, in severe cases of colchicine intolerance, anti-IL-1 therapy can be used.(115)

In a large web-based registry of paediatric patients (HELIOS) treated with a biologic agent, the efficacy and safety of anti-IL-1 agents was investigated in colchicine resistant or intolerant FMF patients. In conclusion, anakinra and canakinumab were safe and effective agents for paediatric FMF patients due to following reasons: number of attacks and CRP levels decreased and remained under control, decreased severity of the attacks, decrease in organ systems involved and less abdominal pain. Interestingly, the most common mutation in these FMF patients were biallelic exon 10 mutations, once again emphasizing the importance of genetic testing as support for the clinical findings.(134) In relation to AA amyloidosis, there is still a growing need for research to specify the efficacy of IL-1 inhibitors preventing this complication. A few reports show that in the short-term IL-1 inhibitors had a good efficacy against the progression of AA amyloidosis. Nonetheless, colchicine remains the main treatment option. Only in cases of severe renal failure, where colchicine is contraindicated, anti-IL-1 therapy should be utilized to normalize inflammatory markers. Other indications for anti-IL-1 agents comprise colchicine unresponsiveness due to other inflammatory disease such as ankylosis spondylitis, inflammatory bowel disease and hidradenitis suppurativa. IL-1 inhibitors also seemed to be effective for paediatric FMF patients with failure to thrive.(116)

There is need for more prospective studies to draw conclusions over the superiority of one anti-IL-1 agent over another, since no comparative effectiveness assessment studies have been published.(135) Reasons for switching from canakinumab to anakinra is mostly due to the price difference and/or reimbursement conditions.(116) In general, cost is an important drawback of anti-IL-1 therapy in comparison with colchicine which is a way cheaper option than anti-IL-1 injections.(115) However, in paediatric patients, anakinra tends to be replaced by canakinumab possibly due to its preferable pharmacokinetic mechanisms. Moreover, local

injection-site reactions are more frequent in patients who take anakinra since its daily administration.(111) A different approach for using anti-IL-1 agents can be ‘on demand therapy’. Consequently, this gives the advantages of reducing the cost and adverse effects in comparison to continued treatment of IL-1 inhibitors. Secondly, the use of anakinra during prodromal period could increase the quality of life by reducing the symptoms of an imminent attack.(116) Lastly, on demand use of anti-IL-1 agents seems to be effective for adolescent women who have severe FMF attacks while on a menstrual period.(134)

4.3.2.3 Safety and interactions

After the introduction of anakinra in 2002, many studies have been published to investigate its safety. Since biologicals have a high risk of exposing the patient to indolent infections such as reactivation of a latent tuberculosis infection, the same concerns were held for anakinra. Remarkably, anakinra did not cause more opportunistic infections, they are even quite rare.(52) The short half-life of anakinra may have an influence on this aspect as short-lived immunosuppressants usually give a lower risk in case of infection. Thus, in cases of high risk for infections, for instance post transplantation or chronic dialysis, anakinra is the preferred anti-IL-1 agent.(125) Most reported adverse effects of anakinra include injection-site reactions, severe skin reaction such as Drug Reaction with Eosinophilia and Systemic Symptoms (DRESS) (rare), leukopenia, headaches and urticarial rash. Although few cases have shown a healthy pregnancy under anakinra therapy, there is still a lack of studies regarding its use in pregnancy.(122) Indeed, Kharouf et al. concluded that anti-IL-1 therapy is not yet recommended during pregnancy due to limited research.(115)

The safety and efficacy of canakinumab has been studied in the CLUSTER trial (RCT study) where the most common adverse effect was the presence of infections.(125) Other adverse effects that have been described are injection site reactions, headache and abdominal pain.(136) In a study conducted by Yucel et al., canakinumab treatment was highly effective in cases of colchicine resistance or intolerance, low incidence of adverse effects and well-tolerated in paediatric FMF patients.(137) However, the results of the study only followed participants up to week 40. Therefore, Ozen et al. investigated the safety and efficacy of canakinumab from week 40 onwards. More than 90% of FMF patients had no FMF attacks or only one during a 72-week period. Furthermore, continuous treatment with canakinumab ensures a long-term control of the disease course partly due to low CRP levels reducing subclinical inflammation. Body weight seemed to be an important factor for the correct dosage of canakinumab since higher body weight required higher dosage to maintain the same effect. Importantly, the exact effect of canakinumab on development of amyloidosis is still unknown and therefore, continuation of colchicine is recommended.(138)

As stated above, rilonacept is poorly studied in FMF patients. Although rilonacept appeared to be a very promising biological, several adverse effects were reported, including injection-site reactions where patients sometimes needed to be hospitalized. Consequently, more attention is given to the other anti-IL-1 agents (anakinra and canakinumab).(111)

4.3.3 Algorithm for FMF treatment

In the figure below, an algorithm for the treatment of FMF patients is proposed to help physicians guide their patients through FMF therapy. In all confirmed FMF patients, colchicine treatment needs to be initiated. For most patients (85-90%), an adequate response to colchicine is obtained, and it can be given as a life-long treatment. However, in 5-10% of the patients, colchicine resistance can occur, where there is need for anti-IL-1 therapy to manage FMF within these patients. Similarly, colchicine intolerance (5%) leads to treatment with IL-1 inhibitors.

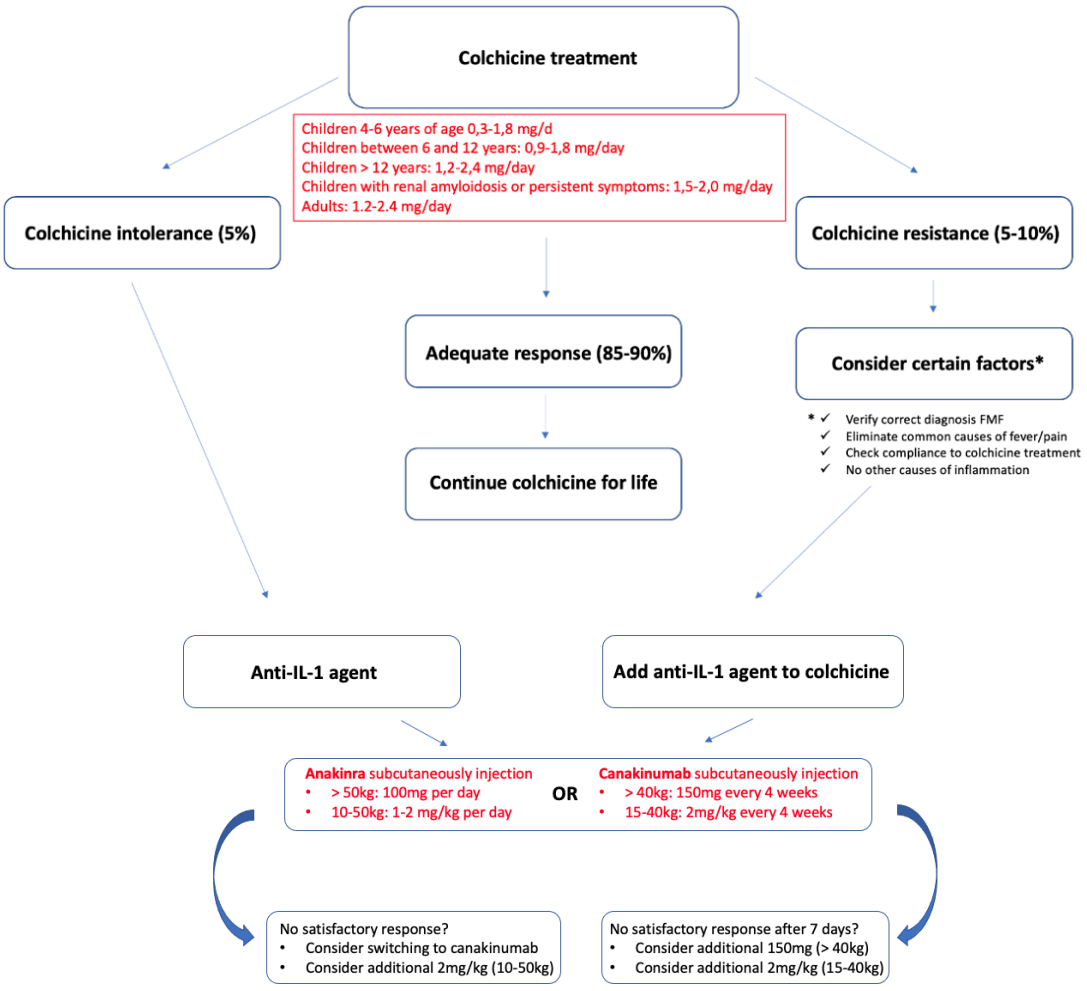


Figure 12. **Algorithm for FMF treatment**. The dosage for the different age groups is displayed in red. Figure is inspired by Hentgen et al. (116) (111)

4.3.4 Comprehensive table of the different treatment modalities

Treatment		Working mechanism/Effectivity	Safety	Recommended dose	
				Children	***Adults***
Colchicine		Inhibiting assembly and polymerization of microtubules Inhibiting neutrophil chemotaxis Inhibiting cyclo-oxygenase-2 activity Inhibiting TNFα synthesis Inhibiting pyrin inflammasome through RhoA activation	Gastrointestinal problems (loose stools, vomiting, diarrhoea and frequent bowel movements), abnormality in liver function tests, myopathy, leukopenia and neuromyopathy *BE AWARE*: interactions with following medication is possible: ketoconazole, cyclosporine, macrolide, grapefruit juice,	4-6y: 0.3-1.8 mg/d 6-12y: 0.9-1.8 mg/d > 12y: 1.2-2.4 mg/d Renal amyloidosis/persistent symptoms : 1.5-2.0 mg/d	1.2-2.4 mg/d
Anti-IL-1	***Anakinra***	Inhibiting binding of IL-1α and IL-1β to IL-1 receptor Can be used in case of colchicine resistance or intolerance	Injection-site reactions (common), severe skin reaction such as DRESS (rare), leukopenia, headache and urticarial rash	> 50kg: 100mg/d sc 10-50kg: 1-2 mg/kg/d	
	Canakinumab	Human anti-ILβ monoclonal antibody binds IL-1β resulting in inhibiting interaction with IL-1 receptor Can be used in case of colchicine resistance or intolerance	Infections (common), injection site reactions, headache and abdominal pain	> 40kg: 150mg sc every 4 weeks 15-40kg: 2mg/kg sc every 4 weeks	
	Rilonacept	Decoy receptor protein that binds IL-1α and IL-1β to hinder IL-1 activation	Very severe injection-site reactions	Loading dose of 4.4mg/kg Maintenance: 2.2mg/kg weekly	160mg weekly

5. Discussion and future perspectives

Familial Mediterranean Fever (FMF) is an auto-inflammatory syndrome caused by alterations in the *MEFV* gene, which are bundled and classified in the Infevers database. Within genetic analyses, the most common FMF-associated mutations are M694V, M680I, V726A and M694I located on exon 10 in the B30.2 domain of pyrin. M694V homozygous mutations in particular, are associated with a very severe FMF disease course including earlier onset of amyloidosis, colchicine resistance, higher rates of arthritis and FMF attacks and more chronic FMF associated morbidity. Several variants of unknown significance (VUS), such as the E148Q mutation, have been considered to have an additional effect on the phenotype when co-occurring with pathogenic exon 10 mutations. On top of that, the R202Q mutation, a certified benign mutation (Infevers database), seem to be a rather pathogenic mutation according to several studies discussed above. Therefore, the classification of certain mutations, although being validated, can be questioned as seen with the R202Q mutation. Insight in the exact influence of these mutations on the pyrin mechanism could enhance the ability to identify certain disease-causing mutations.

The main difficulty, while trying to assess the wide genetic spectrum of FMF, was the challenge and sometimes the impossibility to find certain mutations in the literature that are validated according to the Infevers database. Through this restriction, only the most common mutations on which there is literature available were discussed. Moreover, most studies have a small study population concerning these mutations which makes it hard to gain understanding in the exact influence of certain mutations. Recently, there are some extended studies (with well over 5000 participants) that do link some clinical manifestations to a certain mutation and establish these genotype-phenotype correlations.

Clinical manifestations remain invaluable tools to detect FMF, however, recent development concerning genetic diagnosis are gaining attention. Mild manifestations can mask the ongoing continuous inflammation resulting in a slow deterioration of the renal function due to the development of secondary AA amyloidosis. Consequently, NGS recently has been used to find rare and/or new mutations leading to early diagnosis and treatment of FMF patients.

Anti-IL-1 agents drastically improved diverse outcomes from FMF patients who are colchicine resistant or intolerant. However, this treatment modality requires patients to subcutaneously inject themselves, in case of anakinra every day, which can be perceived as uncomfortable as well as being the cause of injection-site reactions. Furthermore, the cost is an undeniable drawback of IL-1 inhibitors since annual price of the treatment can range from $60.000 to $200.000. In addition, by blocking an important component of the innate immune system, it inevitably comes with increased risk of infections. Additional adverse effects are headaches,

neutropenia, low platelet counts, abdominal pain and injection-site inflammation. Thus, upstream inhibition of the pyrin pathway could diminish these adverse effects since IL-1 could still drive host defences even when for example an inflammasome is inhibited. Therefore, small molecule inflammasome inhibitors, administered orally, could be the solution in the next years.(139) Either way, a better understanding of the molecular mechanisms of the pyrin inflammasome will lead to development of new treatment options for FMF patients.

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Annex

Table 9: List of all validated mutations in the MEFV gene according to Infevers database

Pathogenic or likely pathogenic		Benign or likely benign		Uncertain significance (VUS)	
Location	**Usual Name**	**Location**	**Usual Name**	**Location**	**Usual Name**
Exon 2	Q97X	Exon 1	L9L	5UT	-12C>G
Exon 2	S242R C > G	Exon 1	L57L	Exon 2	Q97K
Exon 2	S242R C > A	Exon 1	Y65Y	Exon 2	Q97R
Exon 2	T267I	Exon 2	N99N	Exon 2	A105E
Exon 2	F479L	Exon 2	**D102D**	Exon 2	S108R
Exon 2	S503C	Exon 2	D103D	Exon 2	S108G
Exon 8	T577N	Exon 2	L110L	Exon 2	L110P
Exon 8	T577SCG	Exon 2	G111G	Exon 2	G111R
Exon 10	S650Y	Exon 2	T1201	Exon 2	G111E
Exon 10	E656A	Exon 2	P124P	Exon 2	G136R
Exon 10	M680L	Exon 2	E125E	Exon 2	G136W
Exon 10	M680V	Exon 2	N130N	Exon 2	E148Q
Exon 10	**M680IGC**	Exon 2	G136G	Exon 2	E148V
Exon 10	**M680IGA**	Exon 2	**G138G**	Exon 2	R151S
Exon 10	Y688C	Exon 2	G150G	Exon 2	S154P
Exon 10	Y688F	Exon 2	**A165T**	Exon 2	R155T
Exon 10	Y688X	Exon 2	S166S	Exon 2	E136A
Exon 10	I692DEL	Exon 2	P180P	Exon 2	Q172P
Exon 10	**M694V**	Exon 2	P183P	Exon 2	P175H
Exon 10	M694L	Exon 2	E195E	Exon 2	L203P
Exon 10	M694DEL	Exon 2	**R202Q**	Exon 2	N206S
Exon 10	M694K	Exon 2	S209S	Exon 2	G218A
Exon 10	M694I	Exon 2	G211G	Exon 2	E225G
Exon 10	K695R	Exon 2	G219G	Exon 2	E230K
Exon 10	**V726A**	Exon 2	P221P	Exon 2	E251K
Exon 10	R761C	Exon 2	P234P	Exon 2	P283R
Exon 10	R761H	Exon 2	R239R	Exon 2	P283L
Exon 10	N766H	Exon 2	G304R	Exon 2	S288Y
		Intron 2	c.910+29C>T	Exon 2	A289E
		Intron 2	911-78T>C	Exon 3	P350S
		Intron 2	c.911-22T>G	Exon 3	P350R

Intron 2	c.911-12G>A	Exon 3	P369S
Exon 3	P307P	Exon 3	P373L
Exon 3	R314R	Exon 3	Q383K
Exon 3	R329H	Exon 4	Q426R
Exon 3	C352C	Exon 5	E474K
Exon 3	S363S	Exon 5	H478Y
Exon 3	P364P	Exon 5	D505G
Exon 3	P393P	Exon 5	D510N
Exon 3	V415V	Exon 5	A511E
Intron 3	1260+10C>T	Exon 10	N599D
Intron 3	c.1260+92G>A	Exon 10	I640M
Intron 3	1261-28A>G	Exon 10	R652C
Intron 3	1261-11T>G	Exon 10	E685K
Exon 4	Q440E	Exon 10	E698D
Intron 4	1356+44A>G	Exon 10	S702C
Intron 4	1356+98C>T	Exon 10	I720M
Exon 5	A457A	Exon 10	V722M
Exon 5	**E474E**	Exon 10	I729V
Exon 5	**Q476Q**	Exon 10	A744S
Exon 5	Q489Q		
Exon 5	R501H		
Exon 5	R510R		
Exon 5	I506V		
Exon 5	I506I		
Exon 5	**D510D**		
Intron 5	1587+18C>T		
Intron 5	c.1587+29G>T		
Intron 5	1587+33C>G		
Intron 5	c.1588-69G>A		
Intron 5	c.1588-17C>G		
Intron 5	c.1610+47A>T		
Intron 5	1610+96C>T		
Intron 7	1727-58T>C		
Intron 8	IVS8+8 C-T		
Intron 8	1760-30A>T		
Intron 8	1760-28T>A		
Intron 8	c.1760-5C>T		
Intron 8	1760-4G>A		

Exon 9	**P588P**
Exon 9	G592G
Intron 9	1792+39 G>A
Intron 9	IVS9+57C>T
Intron 9	1793-14A>G
Exon 10	L602L
Exon 10	T606T
Exon 10	P609P
Exon 10	P646P
Exon 10	R652R
Exon 10	S683S
Exon 10	A701A
Exon 10	S703S
Exon 10	P706P
Exon 10	F721F
Exon 10	D723D
Exon 10	F743F
Exon 10	Q753Q
Exon 10	G764G
Exon 10	G779G
3UT	*12T>C
3UT	*21C>G
3UT	c.*133G>A
3UT	c.*245G>A
3UT	c.*267G>A