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Recent insights in Pyrin inflammasome activation: identifying potential novel therapeutic

approaches in Pyrin-associated autoinflammatory syndromes

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Abstract

Pyrin is a cytosolic protein encoded by the MEFV gene, predominantly expressed in innate immune cells. Upon activation it forms an inflammasome; a multimolecular complex that enables the activation and secretion of IL-1 β and IL-18. In addition, the pyrin inflammasome activates Gasdermin D leading to pyroptosis; a highly pro-inflammatory cell death. Four autoinflammatory syndromes are associated Pyrin inflammasome dysregulation: Familial Mediterranean Fever, with Hyper IgD syndrome/Mevalonate kinase deficiency, Pyrin-Associated Autoinflammation with Neutrophilic Dermatosis, and Pyogenic arthritis, pyoderma gangrenosum, and acne syndrome. In this review, we discuss recent advances in understanding the molecular mechanisms regulating the two-step model of Pyrin inflammasome activation. Based on these insights we discuss current pharmacological options and identify a series of existing molecules with therapeutic potential for the treatment of Pyrinassociated autoinflammatory syndromes.

Keywords: Autoinflammatory syndromes, FMF, Pyrin, inflammasome, pharmacological therapy

1. Introduction

Hereditary autoinflammatory syndromes are a group of rare genetic diseases that are clinically characterized by persistent or recurrent fever and that are accompanied by an array of inflammatory symptoms. These autoinflammatory diseases are caused by mutations in genes encoding proteins that control the activation of innate immune cells [1]. Therefore, while autoimmune diseases are caused by dysregulation of the adaptive immune system, autoinflammatory diseases result from an inappropriate activation of innate immune cells without significant levels of autoantibodies or autoreactive T-cells [2, 3]. An immunological hallmark of multiple autoinflammatory diseases is overproduction of interleukin (IL)-1 β [4, 5]. This pro-inflammatory cytokine is expressed by hematopoietic cells as a pro-IL-1 β precursor protein that cannot bind the IL-1 receptor and needs to be converted into its bio-active form through intracellular cleavage by the cysteine protease caspase-1 [5]. To do so, caspase-1 itself requires activation, which occurs following the assembly of several cytosolic proteins into a complex termed the inflammasome. Given their central role in the production of mature IL-1 β , several types of inflammasomes have been implicated in the development of autoinflammatory diseases [1].

Familial Mediterranean fever (FMF), the most common autoinflammatory syndrome worldwide, is caused by mutations in the gene that codes for the Pyrin protein [2]. Pyrin is one of the cytosolic receptor proteins that upon activation can form an inflammasome capable of producing IL-1β. Recently, several studies have shed light on the mechanisms of how mutations in Pyrin caused overt systemic inflammation and inflammasome activation in FMF patients. In addition, three other autoinflammatory syndromes were linked to inappropriate activation of the Pyrin inflammasome: Pyrin associated autoinflammation with neutrophilic dermatosis (PAAND), pyogenic arthritis, pyoderma gangrenosum and acne syndrome (PAPA) and hyper IgD syndrome/mevalonate kinase deficiency (HIDS/MVK). The introduction of IL-1 blocking drugs in the treatment of these Pyrin inflammasome-associated autoinflammatory syndromes has proven very effective [3, 4], but many challenges remain to optimise therapy for these disease as an orally available, low cost drug with low toxicity is still lacking.

Here, we give an overview of recent advances related to Pyrin inflammasome regulation and activation, followed by a discussion on the potential pharmacological targets for Pyrin inflammasome-associated autoinflammatory syndromes that arise from these new insights.

2. Activation of the Pyrin inflammasome

Pyrin is a large cytosolic protein that is encoded by the *MEFV* gene located on chromosome 16 [5, 6]. It is predominantly expressed in neutrophils, monocytes, and dendritic cells, but not in lymphocytes [7]. Pyrin expression is upregulated after exposure to pathogen- associated molecular patterns (PAMPs) such as LPS as well as a variety of inflammatory mediators including IFN γ , TNF alpha, IL-4 and IL-10 [7-10]. The human Pyrin protein is composed of five domains, each of which has a distinct role in the interaction with proteins that cooperate with Pyrin in regulating cytokine secretion, cell death, and cytoskeletal signaling [11]. The N-terminal PYD-domain is separated by an interlinking domain from the bZIP transcription factor, B-box and α -helical coiled-coil domains (figure 1). Finally, the C-terminal end of human Pyrin contains a B30.2 domain which however is absent in murine Pyrin [12].



Fig. 1 Model of Pyrin inflammasome activation. Schematic representation of the two-step activation process of the Pyrin inflammasome. The first step involves dephosphorylation of Pyrin and detachment of the chaperone protein 14-3-3. The second step requires a conformational change of Pyrin, facilitated by microtubules. This change exposes the PYD domain of Pyrin,

enabling its interaction with the adapter protein ASC and subsequent assembly of the Pyrin inflammasome. Created with BioRender.com

Ample evidence indicates that Pyrin can form an inflammasome that activates caspase-1, resulting in cleavage and activation of Gasdermin D (GSDMD) and the pro-inflammatory cytokines IL-1 β and IL-18 (figure 1) [13]. It is increasingly being appreciated that activation of Pyrin and subsequent formation of the Pyrin inflammasome requires two independent steps [14-16]. The first step involves dephosphorylation of Pyrin and the second step consists of Pyrin unfolding by microtubules, thereby allowing interaction with ASC and caspase-1 to form the full inflammasome complex.

The first step in Pyrin activation relates to its ancient function in fighting bacterial pathogens by detecting RhoA GTPase inhibition [17]. RhoA GTPase is a prominent factor in the regulation of cytoskeletal dynamics, transcription, cell cycle progression, and cell transformation [18, 19]. Activation of RhoA GTPase is facilitated by a switch from its inactive GDP-bound to active GTP-bound state. This RhoA activation depends on its association with the cellular plasma membrane [15, 16, 20], for which the isoprenoid geranylgeranyl pyrophosphate is necessary [21, 22]. Several bacterial toxins such as C3 toxin from Clostridium botulinum, TcdA and TcdB toxins derived from Clostridium difficile and IbpA_Fic1 from Pseudomonas spp. covalently modify RhoA to render it inactive [17, 21, 23]. Although Pyrin senses this bacterial-inflicted RhoA inhibition, it does not directly interact with RhoA. Instead, bacterial inactivation of RhoA reduces Pyrin phosphorylation [17]. Phosphorylation of Pyrin at residues Ser-208 and Ser-242 in the interlinking domain is required for its binding to the chaperone protein 14-3-3, which keeps Pyrin in an inactive state [23-25]. RhoA GTPase activates phosphokinase 1/2 (PKN1/2), which are essential to maintain Pyrin phosphorylation [21]. Inhibition of RhoA by bacterial toxins or by its detachment from the membrane leads to PKN1/2 inactivation. This, in turn, leads to dephosphorylated Ser-208 and Ser-242 residues and detachment of 14-3-3 from Pyrin (figure 2) [16, 21, 26-28].

While the above described Pyrin dephosphorylation and release of its 14-3-3 chaperone is the first step to activate the Pyrin inflammasome, full Pyrin activation requires a second independent step, which involves a change in its three-dimensional structure [29]. While binding of 14-3-3 keeps Pyrin in a steric

conformation preventing interaction with inflammasome components, the release of 14-3-3 is not sufficient to allow Pyrin inflammasome assembly. With respect to the latter, an additional Pyrin unfolding step is essential to fully expose the PYD domain, enabling the binding of an adapter protein called apoptosis-associated speck-like protein with a caspase-recruitment domain (ASC) [23, 29, 30]. Defects in microtubules have been implicated in several inflammatory diseases [31]. Furtermore, microtubules appear critical in Pyrin inflammasome activation, particularly in unmasking the PYD domain to enable the Pyrin interaction with ASC [23, 32]. Indeed, activated Pyrin associates with microtubules and colocalizes with actin filaments [33, 34]. Moreover, microtubule-targeting disrupting and destabilizing drugs including nocodazole, paclitaxel, BAL27862 and high dose colchicine can inhibit Pyrin-mediated IL-1 β production [15, 23]. The role of the cytoskeleton in the second step of Pyrin inflammasome activation is further underscored by its interaction with proline-serine-threonine phosphatase interacting protein 1 (PSTPIP1). PSTPIP1 is a cytoskeleton-organizing protein that binds to the B-box domain of Pyrin. The binding of PSTPIP1 to Pyrin's B-box unmasks the N-terminal PYD domain of Pyrin and leads to interaction and dimerization with ASC through homotypic PYD-PYD interactions [33, 35, 36]. Furthermore, the PSTPIP1-facilitated Pyrin-ASC interaction is regulated by the C-terminal receptor domain B30.2 domain of Pyrin [29, 37-39]. Indeed, β2 microglobulin (β2MG) has recently been shown to be a ligand for B30.2 domain that thereby triggers the binding of Pyrin with PSTPIP1 and the subsequent recruitment of ASC [40]. Also, Magnotti et al. recently showed that steroid hormone catabolites activate the Pyrin inflammasome in a B30.2-dependent way, despite that these metabolites did not directly bind the B30.2 domain [29]. Taken together, these studies suggest that the PYD-PYD interaction with ASC that constitutes the second step in Pyrin inflammasome assembly is regulated by several ligands, either directly or through indirect interaction with the B30.2 domain.



Fig. 2 Phosphorylation of Pyrin. RhoA GTPase activates PKN1/2, which is essential for Pyrin phosphorylation. Bacterialinduced inhibition of RhoA GTPase reduces Pyrin phosphorylation, leading to the detachment of the chaperone protein 14-3-3 and subsequent activation of Pyrin inflammasome assembly. Created with BioRender.com

After establishing Pyrin-ASC binding, a caspase recruitment (CARD) domain in ASC further enables the recruitment of caspase-1 through its own CARD domain [33, 41]. Within this assembled inflammasome complex, caspase-1 auto-activates by oligomerization [42]. Auto-processing first generates a caspase-1 tetramer composed of two p10 and two p20 subunits. However, further autoprocessing releases the p20 subunit and terminates its activity. Interestingly, it was proposed that the B30.2 domain of Pyrin can bind to the p20 subunit of caspase-1, thereby creating a negative feedback loop that inhibits IL-1 β production [38].

3. The Pyrin inflammasome-associated autoinflammatory syndromes

With the discovery of the Pyrin molecule, the evolving understanding of inflammasome activation and the rapid development in genetic analyses, such as Next Generation Sequencing and Whole Genome Sequencing, four monogenic auto-inflammatory syndromes have been identified in which the Pyrin inflammasome plays a central role. Each syndrome however interferes with Pyrin inflammasome activation at a different level.

3.1 Familial Mediterranean Fever

FMF is the most prevalent monogenic autoinflammatory syndrome with an estimated 100.000 patients affected worldwide [43]. The disease concentrates in populations origination around the Mediterranean basin and Armenia. FMF is clinically characterized by recurrent attacks of spiking fever [44]. Fevers typically last 12 hours to 3 days and are accompanied by serositis, such as peritonitis, pleuritis, pericarditis, and arthritis [45]. The majority of FMF patients experience their first attack in the first decade of life, although late onset has been described [46]. Most patients indicate that physical or emotional stress provokes an attack. In between attacks, the majority of patients are symptom-free. If left untreated, up to three-quarter of patients will eventually develop secondary AA amyloidosis, which can lead to end-stage kidney disease [47, 48].

FMF is caused by missense mutations in *MEFV* exon 10, encoding the B30.2 domain of Pyrin [6, 46]. Mendelian transmission of the disease occurs mostly in an autosomal recessive mode. However, in up to 30% of clinically diagnosed FMF patients, genetic analysis only reveals a single *MEFV* pathogenic variant [49, 50]. It's worth noting that there is an ongoing debate regarding heterozygous carriers of a pathogenic *MEFV* mutation, particularly p.M694V, as this mutation has also been associated with PAPA (pyogenic arthritis, pyoderma gangrenosum and acne) [51]. FMF-associated Pyrin mutations do not interfere with phosphorylation of the Pyrin molecule. Nevertheless, the Pyrin activation threshold is lower in monocytes from FMF patients than in healthy control monocytes after stimulation with RhoA-inhibiting bacterial toxins [16, 21, 28]. Interestingly, Van Gorp et al. showed that inhibition of microtubules prevents Pyrin inflammasome activation in Peripheral Blood Mononuclear Cells (PBMCs) from healthy donors but does not do so in PBMCs from FMF patients [15]. Considering that microtubule interactions with the B30.2 domain of Pyrin are essential for ASC binding to the Pyrin PYD domain, this observation suggests that FMF mutations disable the inhibitory action of the B30.2 domain in preventing the PYD-mediated Pyrin interaction to ASC and thereby promote this second step in Pyrin inflammasome activation.

3.2 Hyper IgD syndrome / Mevalonate kinase deficiency

Hyper IgD syndrome / Mevalonate kinase deficiency (HIDS/MVK) is an autosomal recessive disease that has an onset in early childhood. Patients suffer from recurrent attacks of fever accompanied by an

array of symptoms, including painful cervical lymphadenopathy, colitis, abdominal pain and arthralgias [52]. There is a marked heterogeneity between individuals in the frequency and severity of attacks [53, 54]. Symptoms persist for 3-7 days [55]. Typically, parents recall a first attack of their children after a childhood vaccination, which is a strong trigger for an attack [56]. In 1999, two international research groups independently identified the *MVK* gene, located on the long arm of chromosome 12, as the gene that causes HIDS/MVK [57, 58]. *MVK* encodes for mevalonate kinase, an enzyme in the isoprenoid pathway in which cholesterol is produced in addition to a number of nonsterol isoprenoids [59]. Mutation sin *MVK* lead to a reduced but not absent mevalonate kinase enzymatic activity. The production of geranylgeranyl pyrophophate is one of the non-sterol isoprenoids that is impaired in patients with HIDS/MVK. Park *et al.* showed that the geranylgeranylation of RhoA is essential for its membrane localization and its ability to activate PKN 1/2. In PMBCs from HIDS/MVK patients the deficiency of geranylgeranyl pyrophosphate activates the Pyrin inflammasome through reduced Pyrin phosphorylation by PKN 1/2 [21].

3.3 Pyrin-Associated Autoinflammation with Neutrophilic Dermatosis

PAAND is an autosomal dominant disease caused by mutations in the *MEFV* gene and was first reported in 2016 [26]. Currently less than 20 patients have been described in the literature. The disease has a childhood onset and is mostly characterized by chronic systemic inflammation with fever, elevated acute phase reactants, arthralgia and myalgia and neutrophilic dermatosis (e.g. acne, pyoderma gangrenosum) [60]. In contrast to FMF, the *MEFV* mutations causing PAAND are not situated in the B30.2 domain but in the binding site of 14-3-3. The PAAND-associated mutations restrict 14-3-3 binding to Pyrin. Since binding 14-3-3 is essential for Pyrin's autoinhibited conformation [61], development of PAAND might be driven by a constitutively active first step of Pyrin inflammasome activation.

3.4 PSTPIP1-associated and other Pyrin-inflammasome related autoinflammatory diseases

Pyogenic arthritis, pyoderma gangrenosum and acne (PAPA) syndrome is an autosomal dominant autoinflammatory disorder that has been described in only a few families. It presents with recurrent episodes of severe pyogenic inflammation in the skin and joints. The classic clinical triad consists of severely scarring cystic acne, recurrent destructive pyogenic arthritis, and sterile ulcerative skin lesions (pyoderma gangrenosum). Two missense mutations in the coiled-coil domain of the cytoskeleton binding protein PSTPIP1 have been identified in patients with PAPA syndrome [9]. Binding of PSTPIP1 to Pyrin unmasks the latter's PYD domain and thereby facilitates ASC binding. The mutated PSTPIP1 proteins in PAPA patients were shown to have a higher binding affinity to Pyrin than wild-type PSTPIP1 [13, 33, 36], which could lower the threshold for Pyrin inflammasome formation in PAPA patients. Supporting the recent studies indicating elevated serum IL-18 levels were associated with PAPA syndrome [62].

In addition to PAPA syndrome, mutations in PSTPIP1 have also been linked to another rare autoinflammatory disorder known as PSTPIP1-associated myeloid-related proteinemia inflammatory (PAMI) syndrome [63]. PAMI syndrome is triggered by two deleterious mutations (p.E250K and p.E257K). In addition to the primary clinical features observed in PAPA syndrome, PAMI is characterized by neutropenia, hepatosplenomegaly, and elevated concentrations of myeloid-related protein 8 (MRP8) and MRP12 [63, 64]. Much like PAPA syndrome, mutations in PSTPIP1 result in an increased affinity for Pyrin, which in subsequently triggers inflammasome activation and excessive production and release of the proinflammatory cytokines IL-1 β and IL-18 [63, 65].

In addition, a novel *MEFV* variant was recently identified in three patients, triggering Pyrin inflammasome assembly and activation [66]. Intriguingly, these patients exhibited a unique presentation, as they did not manifest recurrent fevers but rather experienced recurrent chest and abdominal pains [66]. Notably, the phenotype in these cases was effectively managed with colchicine treatment [66].

4. Pharmacological targets in Pyrin associated autoinflammatory syndromes

The current concept of Pyrin inflammasome activation and the recent insights into the pathophysiology of Pyrin associated autoinflammatory syndromes provide a basis for understanding the mode of actions of current pharmacological therapies. In addition, these insights also hint at potential new therapeutic targets (figure 3).



Fig. 3 Pharmacological targets in Pyrin associated autoinflammatory syndromes. Overview of potential pharmacological treatments and drug targets for Pyrin-associated autoinflammatory syndromes. Inhibition of IL-1 β and IL-18 release using anti-IL-1/IL-18 agents, such as Anakinra, Canakinumab and Tadekinig alfa, has shown efficacy in treating various syndromes. Additionally, targeting specific pathways involved in Pyrin activation and inflammation, such as RhoA activation with geranylgeraniol, PKN1/2 activation with bryostatin-1 or arachidonic acid, caspase-1 inhibition with Belnacasan, and gasdermin D-mediated pyroptosis with Disulfiram, hold promise as potential therapeutic strategies. Created with BioRender.com

4.1 Current therapies

4.1.1 Colchicine

Colchicine is a lipophilic alkaloid compound found in a variety of plants. The name derives from the *Colchicum autumnale*, a poisonous plant of the lily family [67]. The seeds of this plant are used as a treatment for joint pain and swelling for over three and a half millennia [68]. In the seventies of the last century colchicine was found to prevent attacks in FMF patients [69-71]. Since then it remains the first-line treatment in FMF. It is cheap, readily available, well-tolerated by most patients and it prevents attacks and development of amyloidosis. Adult patients typically need 1 to 1.5 mg of colchicine per day to prevent attacks. When necessary the dose can be increased to 3 mg per day, although many patients

will experience diarrhoea at higher doses. However, an estimated 5–10% of FMF patients are resistant to colchicine and continue to experience inflammatory attacks despite being given the maximal tolerated dose [72, 73].

Colchicine forms a tubulin–colchicine complex causing microtubule depolymerization. By inhibiting microtubule formation, colchicine exerts anti-inflammatory, anti-mitotic and anti-fibrotic activities [74]. However, colchicine effects on cellular function are dose dependent. At nanogram/ml concentrations, colchicine only causes actin re-organization while leaving microtubules intact [75]. At higher concentration the cytoskeleton is disrupted. Colchicine has been shown to interact with the Pyrin inflammasome at different levels of Pyrin activation. First, the binding of tubulin and depolymerisation of microtubules results in the release of the RhoA activator guanine-nucleotide-exchange factor (GEF)-H1, which is inactive when bound to microtubules [76]. In PBMCs from FMF patients, low concentrations of colchicine (10 ng/ml) could reverse the dephosphorylation of Pyrin induced by C3 toxin, inhibiting IL-1ß release. Second, at much higher concentration (>399 ng/ml) colchicine also disrupted microtubule network and prevented the binding of ASC to Pyrin [23]. Interestingly, Van Gorp et al. showed that colchicine at a concentration of 399 ng/ml as well as the unrelated microtubule polymerization inhibitor CYT997, impaired TcdB-induced IL-1ß secretion in PBMCs from healthy donors at the level of inflammasome formation, as TcdB-induced ASC speck formation was similarly impaired in these cells. This demonstrated that microtubules are necessary for the second step in Pyrin inflammasome activation after its dephosphorylation. However, in contrast, microtubule-destabilizing compounds did not prevent TcdB-induced ASC speck formation in PBMCs from FMF patients [15]. As this indicates that FMF mutations lift the obligatory requirement for microtubules in Pyrin inflammasome activation, this observation suggests that colchicine rather exerts its beneficial effects in FMF by impairing the first step of Pyrin inflammasome activation i.e. the process that facilitates its dephosphorylation. This is further underlined by the observation that serum colchicine levels in FMF patients were found to be 750 ± 380 pg/mL, which is below the level needed in vitro for affecting the cytoskeleton [11]. Similarly, although there is some colchicine accumulation within mononuclear cells, its intracellular concentrations are likely below the concentrations needed to prevent Pyrin-ASC interactions [77].

This proposed mode of action of colchicine could also explain why colchicine is not effective in HIDS/MVK, PAAND, and PAPA syndrome. In HIDS/MVK colchicine cannot activate RhoA that is not localized to the cell membrane through geranylgeranylation [21]. In PAAND the mutations in Pyrin are the cause of dephosphorylation independent of the RhoA activity. Also, in PAPA syndrome, there is a normal Pyrin molecule and the pathogenic process of PAPA downstream of the phosphorylation step.

4.1.2 IL-1 blocking drugs

The inflammatory symptoms in Pyrin inflammasome-associated autoinflammatory syndromes are mediated by IL-1 β released from innate immune cells. Blocking IL-1 β in systemic circulation is therefore an attractive therapeutic strategy in all four diseases, which can be achieved using three available anti-IL-1 agents: Anakinra, Canakinumab and Rilonacept [4].

From 2005 onwards, case reports and case series indicated that both Anakinra and Canakinumab were effective in suppressing inflammation in patients with colchicine resistant FMF [78, 79], HIDS/MVK [80], PAAND [60], and PAPA [81]. In a landmark placebo-controlled trial published in 2018, Canakinumab strongly reduced the number of attacks and subclinical inflammation in both colchicine resistant FMF patients and HIDS/MVK [82]. Anakinra and Canakinumab are FDA and EMA approved for the treatment of FMF and HIDS/MVK and recommended by international guidelines [83].

4.2 Potential new pharmacological treatments or drug targets and current drugs in development

4.2.1 Membrane bound RhoA activation: Geranylgeraniol

Recruitment of small GTPases to the cell membrane is governed by prenylation, a posttranslational process consisting of the attachment of hydrophobic isoprenoids to the C-terminal CAAX motif of the protein [84]. The isoprenoid geranylgeranyl pyrophosphate is necessary for tethering RhoA to cell membranes [21, 22]. This geranylgeranyl pyrophosphate facilitated membrane localization of RhoA is essential for its ability to maintain Pyrin in an inactive phosphorylated state [21]. Since HIDS/MVK mutations lead to an impaired production of geranylgeranyl pyrophosphate, exogenous supplementation 11 of geranylgeranyl may be of potential benefit for HIDS/MVK patients. Geranylgeraniol (GGOH) is a precursor of geranylgeranyl pyrophosphate that can be found in a variety of plants. For instance, extracts of annatto seeds contain up to 80% of GGOH. GGOH is also commercially available as a food supplement [85] and its dietary supplementation seems to have a favourable safety profile [86]. Interestingly, pre-clinical data show that GGOH can counteract side-effects of statins and bisphosphonate that both inhibit the isoprenoid pathway (table 1) [87, 88]. Moreover, in cells derived from HIDS/MVK patients GGOH can rescue geranylgeranylation of RhoA [89]. Therefore, exogenous GGOH supplementation may correct the deficient endogenous isoprenoid metabolism in HIDS/MVK patients. However, to our knowledge, thus far no HIDS/MVK patients have been treated with GGOH supplementation.

4.2.2 PKN 1/2 activation: bryostatin-1 and arachidonic acid

Since phosphorylation of Pyrin by PKN1/2 is essential for 14-3-3 proteins to keep Pyrin in its inactive state, pharmacological activation of PKN1/2 could raise the Pyrin activation threshold in Pyrin inflammasome-associated autoinflammatory syndromes. Bryostatin-1 can bind to PKN1/2 at nanomolar concentrations and can thereby enhance its kinase activity [90]. PKN1/2 influence a variety of intracellular processes and its activity has been linked to several diseases [91]. While clinical studies investigating the effect of bryostatin-1 in a variety of cancers, HIV and Alzheimer's disease were unsuccessful, these studies showed that bryostatin-1 is safe and well tolerated [92, 93]. Therefore, bryostatin-1 is an oral drug that remains an attractive candidate for treatment of both FMF and HIDS. In this respect, bryostatin-1 was found to inhibit IL-1 β secretion of LPS-treated PBMCs of FMF patients as well as from LPS-treated BMDMs from mice that express a human FMF-associated PyrinV726A mutant. Moreover, bryostatin-1 substantially decreased the LPS-induced IL-1 β release in PBMCs of HIDS/MVK patients [21]. However, despite these promising in vitro observations using FMF and HIDS/MVK PBMCs, so far there are no reports of patients with autoinflammatory syndromes treated with bryostatin-1.

Immediately after the PKN enzyme was isolated in 1994, unsaturated fatty acids such as arachidonic acids were identified as potential activators [94]. The C-terminal part of the molecule contains a lipid responsive domain that is critically involved in the control of the catalytic activity and activation [95, 96]. Arachidonic acid is a polyunsaturated omega-6 fatty acid and part of the human diet [97]. Arachidonic acid has shown to activate PKN1/2 in vitro and increase binding of 14-3-3 to Pyrin in BMDMs from mice baring a FMF mutation similar to bryostatin-1 [21]. Since arachidonic acid is readily available as food supplements, it is an attractive candidate as a low cost and safe pharmacological intervention.

4.2.3 Caspase-1 inhibition: Belnacasan

Belnacasan (VX-765) is a peptidomimetic reversible inhibitor of the protease that is central to inflammasome functions [98]. Belnacasan is an orally active prodrug, being converted in the body to the active drug VRT-043198 (*O*-desethyl-belnacasan) [99]. In phase I and II clinical trials in Rheumatoid Arthritis patients, it exhibited significant anti-inflammatory effects with good pharmacokinetics profile [100]. It also had positive outcomes for treatment of epilepsy and psoriasis in mice and was announced to undergo clinical trial [99]. Since caspase-1 is the principal enzyme that cleaves pro-IL-1 β , it may be effective in all four of Pyrin inflammasome associated syndromes. The safety of VX-765 for long-term treatment in humans still needs to be determined. Hitherto, belnacasan has not been investigated in patients with autoinflammatory diseases.

4.2.4 Gasdermin D mediated pyroptosis: Disulfiram

Besides cleaving the precursor forms of both IL-1 β and IL-18, inflammasome-activated caspase-1 also cleaves the latent pore-forming protein GSDMD [101, 102]. The N-terminal fragment of cleaved GSDMD oligomerizes and inserts into the inner leaflet of the plasma membrane to form pores [101, 103]. This leads to an inflammatory cell death mode termed pyroptosis, which accelerates innate immune responses by spreading danger signals in the extracellular environment in addition to the maturated forms of IL-1 β and IL-18 [17, 104, 105]. A crucial involvement of GSDMD-mediated pyroptosis in FMF pathogenesis was suggested by a study showing that GSDMD deficiency prevented

autoinflammation in mice expressing an FMF-associated Pyrin^{V726A} mutant [106]. GSDMD deficiency prevented IL-1β circulation in these Pyrin^{V726A}-expressing mice, consistent with a role for pyroptosis in the release of this inflammasome-generated cytokine. In addition, GSDMD was recently shown to mediate release of S100A8/9 proteins that in the same mouse model contributed to exacerbating the autoinflammatory pathology [107]. These findings suggest that pharmacological GSDMD targeting could have dual beneficial effects in FMF pathogenesis. While to our knowledge GSDMD inhibitors have not been evaluated in clinical trials, several compounds such as necrosulfonamide, disulfiram and dimethyl fumarate have been identified that can effectively block GSDMD-mediated pyroptosis [108]. In this respect, disulfiram is an FDA-approved drug against alcohol addiction [109], underscoring its potential safe and tolerable use in Pyrin associated autoinflammatory diseases. However, to our knowledge GSDMD inhibitors have not been tried for potential use in Pyrin associated autoinflammatory diseases.

4.2.4 IL-18 blocking drugs

Since PSTPIP1 related autoinflammatory diseases are marked by and excessive release of IL-18 [62], there is a potential interest in exploring drugs that specifically target IL-18. Several IL-18 inhibitors are already in various stages of development, including Tadekinig alfa, a recombinant human IL-18 binding protein (rhIL-18bp), and neutralizing IL-18 antibodies [110-114]. Tadekinig alfa has proven effective in treating other rare inflammatory diseases, such as X-linked inhibitor of apoptosis deficiency and Adult-onset Still's disease [111, 112]. Additionally, anti-IL-18 agents have demonstrated the ability to reduce disease severity in a mouse model of colitis [113]. These therapeutic findings underscore the potentialsignificance of IL-18 targeting in the context of PSTPIP1-related diseases. In addition, it is possible that combination therapies targeting both IL-1 and IL-18 might be beneficial in Pyrin-related autoinflammatory diseases characterized by elevated IL-18 expression. For instance, a patient with an NLRC4-related inflammasomopathy that was refractory to Anakinra could be treated with additional Tadekinig alfa supplementation [115].

Table I: Overview of data regarding the potential new pharmacological treatments and drug targets

In vitro data	In vivo data	Clinical data

GGOH can rescue	In vivo, GGOH has	
geranylgeranylation of	demonstrated to be	
RhoA in fibroblasts of	effective against	
MKD patients [89].	Bisphosphonate-Related	
	Osteonecrosis of the Jaw	
	and statin-induced	
	skeletal muscle fatigue,	
	both inhibitors of the	
	isoprenoid pathway,	
	without side effects [88,	
	116]. Preece et. al.	
	demonstrated a	
	favourable safety profile	
	[86].	
Bryostatin-1 decreases		Bryostatin-1 has shown
IL-1 β release in isolated		to be safe and well
PBMCs of MVK and		tolerated in cancer, HIV
FMF patients when co-		and Alzheimer's disease
treated with Pyrin		patients [92, 93].
inflammasome stimuli		
[21].		
Arachidonic acid		
activates PKN1/2 in vitro		
leading to an increased		
binding of 14-3-3 to		
Pyrin in BMDMs from		
	GGOHcanrescuegeranylgeranylationofRhoA infibroblastsofMKD patients [89].IMKD patients [89].IBryostatin-1decreasesIL-1β release in isolatedPBMCsPBMCsofMVKPBMCsofMVKandFMF patientswheninflammasomestimuli[21].acidArachidonicacidindingof14-3-3bindingof14-3-3Pyrinin BMDMs <from< td=""></from<>	GGOH can rescue In vivo, GGOH has geranylgeranylation demonstrated to be RhoA in fibroblasts of effective against MKD patients [89]. Bisphosphonate-Related Osteonecrosis of the Jaw and statin-induced keletal muscle fatigue, both inhibitors of the isoprenoid pathway, without side effects [88, 116]. Preece et. al. favourable safety profile isourable safety profile isourable safety profile al. fBryostatin-1 decreases [86]. isourable safety profile al. fL-1β release in isolated isourable safety profile juli juli

	mice baring an FMF		
	mutation [21].		
Belnacasan	Belnacasan effectively	Belnacasan was	Caspase-1 inhibition
	suppressed the release of	efficiently converted to	exhibited significant anti-
	IL-1 β and IL-18 in	VRT-043198 when	inflammatory effects in
	cultures of PBMCs and	administered orally to	Rheumatoid Arthritis
	whole blood from healthy	mice and reduced disease	patients and had a good
	subjects stimulated with	severity in models of	pharmacokinetics profile
	bacterial products [99].	rheumatoid arthritis and	[100].
		skin inflammation [99].	
Disulfiram	Disulfiram inhibits	<i>In vivo</i> , Disulfiram	Disulfiram is safe and
	nigericin-induced IL-1β	protects mice from lethal	tolerable to use against
	secretion and pyroptosis	LPS-induced septic	alcohol addiction [109].
	in human THP-1 cells	shock by inhibiting	
	and BMDMs [117].	GSDMD pore formation	
		and has been tested in	
		many inflammatory	
		disorders [117, 118].	
Tadekinig alfa			Tadekinig alfa is
			effective in treating the
			inflammasomopathies X-
			linked inhibitor of
			apoptosis deficiency,
			Adult-onset Still's
			disease and NLRC4-
			related

		inflammasomopathies
		[111, 112, 115].
Anti-IL-18	Neutralization of IL-18	GSK1070806, an anti-IL-
	reduces disease severity	18 therapy, is currently
	in murine colitis [113].	undergoing clinical trials
		in the Behcet's disease
		population to assess its
		safety and tolerability
		[119].

4.3 Future perspectives

In addition to previously discussed existing compounds, that could be repurposed for the treatment of Pyrin inflammasome-associated autoinflammatory syndromes, more innovative therapies can hold promise for these patients. Gene therapy, initially proposed in 1972, offers a highly targeted approach by addressing the disease-causing genes, providing precise and personalized treatment [120]. The number of clinical trials involving gene therapy has seen a significant increase in the recent years, yielding promising results in the treatment of monogenic diseases, such as spinal muscular atrophy [121-123]. Given that the discussed Pyrin inflammasome-associated autoinflammatory syndromes are monogenic in nature, gene therapy holds significant promise for patient treatment. Another noteworthy approach involves small interfering RNA (siRNA), a class of double-stranded RNA molecules capable of interfering with the expression of specific genes [124]. Numerous siRNA-based therapeutics have already been extensively investigated for a variety of diseases and have the potential to target a wide array of genes of interest [125, 126]. The use of siRNA to selectively inhibit specific elements of the Pyrin pathway, such as Gasdermin D, holds promise as a potential treatment approach. Furthermore, hematopoietic stem cell transplantation (HSCT) can be used for addressing genetic disorders that affect hematopoiesis and immunity. The hematopoietic nature of FMF has been substantiated through experiments involving bone marrow transplantation in FMF mice [127]. Notably, HSCT has already been performed in several patients with PSTPIP1 mutations [64], underscoring its therapeutic potential in the realm of autoinflammatory diseases. All in all, these studies indicate that more innovative therapies show significant promise in targeting Pyrin related disorders.

5. Conclusion

The recent advances in the knowledge of Pyrin inflammasome activation and regulation has unravelled the pathogenetic mechanisms of four autoinflammatory syndromes. It also explains the efficacy of anti-IL-1 treatment in all four diseases and the efficacy of colchicine only in FMF. Based on the insights of Pyrin inflammasome we identify a variety of potential oral treatment options. Future research will indicate if these can successfully be added to the current therapeutic regimens.

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Author Contributions

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