

Childhood and Adolescent Hematology

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Hereditary Periodic Fever Syndromes

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The hereditary periodic fevers are a group of Mendelian disorders characterized by seemingly unprovoked fever and localized inflammation. Recent data indicate that these illnesses represent inborn errors in the regulation of innate immunity. Pylrin, the protein mutated in familial Mediterranean fever, defines an N-terminal domain found in a large family of proteins involved in inflammation and apoptosis. Through this domain pyrin may play a role in the regulation of interleukin (IL)-1 β , nuclear factor (NF)- κ B, and leukocyte apoptosis. Cryopyrin/NALP3, another protein in this family, is mutated in three other hereditary febrile syndromes and participates in the inflammasome, a newly recognized macromolecular complex crucial to IL-1 β activation. Somewhat unexpectedly, mutations in

the 55 kDa receptor for tumor necrosis factor also give rise to a dominantly inherited periodic fever syndrome, rather than immunodeficiency, a finding that has stimulated important investigations into both pathogenesis and treatment. Finally, the discovery of the genetic basis of the hyperimmunoglobulinemia D with periodic fever syndrome suggests an as yet incompletely understood connection between the mevalonate pathway and the regulation of cytokine production. These insights extend our understanding of the regulation of innate immunity in man, while providing the conceptual basis for the rational design of targeted therapies, both for the hereditary periodic fevers themselves and other inflammatory disorders as well.

The hereditary periodic fever syndromes are a group of disorders characterized by recurrent episodes or, in some cases, fluctuating degrees of fever and localized inflammation,^{1,2} initially described as affecting primarily the serosal and synovial surfaces and the skin, but now recognized to include a somewhat broader distribution of affected tissues. The prototypic and probably most common hereditary periodic fever syndrome is familial Mediterranean fever (FMF). The hereditary periodic fevers now also include the tumor necrosis factor (TNF) receptor-associated periodic syndrome (TRAPS), the hyperimmunoglobulinemia D with periodic fever syndrome (HIDS), and three different clinical disorders all caused by mutations in *CIAS1*, which

encodes a protein denoted cryopyrin or NALP3.

The hereditary periodic fevers differ from autoimmune diseases such as systemic lupus erythematosus and rheumatoid arthritis in that they lack high-titer autoantibodies or antigen-specific T-cells; they are termed autoinflammatory diseases (**Table 1**) to highlight this distinction.² A number of other illnesses have subsequently been included under the autoinflammatory rubric, including Mendelian disorders such as Blau syndrome, as well as conditions with a more complex mode of inheritance, such as Behçet's disease. Recent advances in the genetics and molecular biology of the hereditary periodic fever syndromes have defined important new gene families and pathways in the regulation of innate immunity, thus substantiating the distinction from autoimmune disorders, which more directly affect the adaptive immune system. Insights into the pathogenesis of the hereditary periodic fevers have also catalyzed dramatic advances in targeted biologic therapies for some of these conditions.

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Familial Mediterranean Fever

FMF is a recessively-inherited disorder typically presenting in childhood or adolescence with 1- to 3-day episodes of fever often accompanied by severe abdominal pain, pleu-

Table 1. The systemic autoinflammatory diseases, a partial listing.

Syndromes	OMIM*	Inheritance	Genes or Risk Factors
Hereditary periodic fever syndromes			
Familial Mediterranean fever (FMF)	249100	Autosomal recessive	<i>MEFV</i>
TNF receptor–associated periodic syndrome (TRAPS)	142680	Autosomal dominant	<i>TNFRSF1A</i>
Hyperimmunoglobulinemia D with periodic fever syndrome (HIDS)	260920	Autosomal recessive	<i>MVK</i>
Familial cold autoinflammatory syndrome (FCAS)	120100	Autosomal dominant	<i>CIAS1/NALP3/PYPAF1</i>
Muckle-Wells syndrome (MWS)	191100	Autosomal dominant	<i>CIAS1/NALP3/PYPAF1</i>
Neonatal-onset multisystem inflammatory disease (NOMID)/ chronic infantile neurologic cutaneous and articular (CINCA) syndrome	607115	Sporadic, autosomal dominant	<i>CIAS1/NALP3/PYPAF1</i>
Idiopathic febrile syndromes			
Syndrome of periodic fever with aphthous stomatitis, pharyngitis, and cervical adenopathy (PFAPA)	—	Not usually familial	—
Systemic-onset juvenile idiopathic arthritis (SOJIA)	604302	Complex	IL-6, MIF polymorphisms
Adult-onset Still's disease	—	Not usually familial	—
Granulomatous disorders			
Crohn's disease	266600	Complex	<i>NOD2/CARD15, ABCB1</i> (Ala893), <i>MEFV</i> (?)
Chronic granulomatous synovitis with uveitis and cranial neuropathy (Blau syndrome)	186580	Autosomal dominant	<i>NOD2/CARD15</i>
Early onset sarcoidosis	609464	Sporadic, autosomal dominant	<i>NOD2/CARD15</i>
Pyogenic disorders			
Syndrome of pyogenic arthritis with pyoderma gangrenosum and acne (PAPA)	604416	Autosomal dominant	<i>PSTPIP1</i>
Chronic recurrent multifocal osteomyelitis (CRMO)	259680	Sporadic, autosomal recessive	<i>LPIN2</i> , when associated with congenital dyserythropoietic anemia (Majeed syndrome)
Synovitis, acne, pustulosis, hyperostosis, and osteitis syndrome (SAPHO)	—	Not usually familial	—
Hemophagocytic disorders			
Primary hemophagocytic lymphohistiocytosis	603553, 607624	Autosomal recessive	<i>PRF1, RAB27A</i>
Macrophage activation syndrome (MAS)	—	Not usually familial	Pediatric rheumatic diseases
Complement disorders			
Hereditary angioedema	106100	Autosomal dominant	<i>C1NH</i>
Vasculitic syndromes			
Behçet's disease	109650	Complex	<i>HLA-B51</i>

Other possible categories of autoinflammatory disease include metabolic disorders such as gout and pseudogout, storage diseases such as Gaucher's disease and Hermansky-Pudlak syndrome, and fibrosing disorders such as idiopathic pulmonary fibrosis.

*Online Mendelian Inheritance in Man, an online catalog of genetic disorders available at <http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM>

ris, monoarticular arthritis, or an erythematous rash on the ankle or foot known as erysipeloid erythema (**Table 2**). During attacks there is usually neutrophilia and a brisk acute-phase response, and histologically there is a massive (sterile) influx of polymorphonuclear leukocytes into the affected anatomic compartment(s). Between attacks, patients feel well, although biochemical evidence for inflammation may persist. Among some FMF patients, systemic amyloidosis develops due to the deposition of a misfolded fragment of serum amyloid A (SAA), one of the acute-phase proteins. FMF is most common among individuals of Jewish, Armenian, Turkish, Arab, and Italian ancestry, but, with the availability of genetic testing, cases have been documented worldwide. Treatment with daily oral colchicine markedly reduces the frequency and severity of FMF attacks,

and usually prevents the development of amyloidosis,¹ and thus with early diagnosis the prognosis of FMF is good.

The gene for FMF, *MEFV*, is located on human chromosome 16p (**Table 3**) and was independently identified by two positional cloning consortia in 1997.^{3,4} *MEFV* is comprised of 10 exons, spanning approximately 15 kb of genomic DNA. All four of the initial FMF-associated mutations in *MEFV* were in exon 10, and even now, with over 55 mutations having been identified (available at <http://fmf.igh.cnrs.fr/infevers>), exon 10 remains the major site of mutations, with a smaller cluster in exon 2. Nearly all of the known FMF-associated mutations encode conservative missense changes. This suggests that the disease phenotype may require some level of residual protein function and that the episodic nature of FMF may be due to as yet

Table 2. Clinical features of the hereditary periodic fever syndromes.

Clinical Feature	FMF	TRAPS	HIDS	FCAS	MWS	NOMID/CINCA
Usual ethnicity	Turkish, Armenian, Arab, Jewish, Italian	Any ethnicity	Dutch, other Northern European	Mostly European	Mostly European	Any ethnicity
Duration of attacks	12-72 hours	Days to weeks	3-7 days	12-24 hours	2-3 days	Continuous, with flares
Abdominal	Sterile peritonitis, constipation	Peritonitis, diarrhea or constipation	Severe pain, vomiting, diarrhea, rarely peritonitis	Nausea	Abdominal pain	Not common
Pleural	Common	Common	Rare	Not seen	Rare	Rare
Arthropathy	Monoarthritis, rarely protracted arthritis in knee or hip	Arthritis in large joints, arthralgia	Arthralgia, symmetric polyarthritis			
Polyarthralgia	Polyarthralgia, oligoarthritis, clubbing	Epiphyseal overgrowth, contractures, intermittent or chronic arthritis, clubbing				
Cutaneous	Erysipeloid erythema on lower leg, ankle, foot	Migratory rash, underlying myalgia	Diffuse maculopapular rash, urticaria	Cold-induced urticarial rash	Urticaria-like rash	Urticaria-like rash
Ocular	Rare	Conjunctivitis, periorbital edema	Uncommon	Conjunctivitis	Conjunctivitis, episcleritis	Uveitis, conjunctivitis, progressive vision loss
Neurologic	Rarely aseptic meningitis	Controversial	Headache	Headache	Sensorineural deafness	Sensorineural deafness, chronic aseptic meningitis, mental retardation, headache
Lymphatic	Splenomegaly, occasional lymphadenopathy	Splenomegaly, occasional lymphadenopathy	Cervical adenopathy in children	Not seen	Rare	Hepatosplenomegaly, adenopathy
Vasculitis	Henoch-Schönlein purpura (HSP), polyarteritis nodosa	HSP, lymphocytic vasculitis	Cutaneous vasculitis common, rarely HSP	Not seen	Not seen	Occasional
Systemic amyloidosis	Risk depends on <i>MEFV</i> and <i>SAA</i> genotypes; more common in Middle East	Occurs in ~10%; risk increased with cysteine mutations	Rare	Rare	Occurs in ~25%	May develop in some patients, usually in adulthood

Abbreviations: FMF, familial Mediterranean fever; TRAPS, tumor necrosis factor receptor-associated periodic syndrome; HIDS, hyperimmunoglobulinemia D with periodic fever syndrome; FCAS, familial cold autoinflammatory syndrome; MWS, Muckle-Wells syndrome; NOMID/CINCA, neonatal-onset multisystem inflammatory disease/chronic infantile neurologic cutaneous and articular syndrome

uncharacterized environmental perturbations of the variant protein. Carrier frequencies for FMF mutations of 1:3 to 1:5 have been observed in several Mediterranean and Middle Eastern populations,⁵⁻⁸ raising the possibility of heterozygote selection. Intra-genic convergence of single nucleotide polymorphism haplotypes strongly suggests independent ancient ancestral origins for ethnically diverse modern-day carriers of at least four different common mutations.^{4,9} Although genetic testing may help confirm the diagnosis of FMF, particularly in countries in which the disease is uncommon, there are substantial numbers of patients with the clinical picture of FMF who have only one identifiable mutation, and even some patients with no identifiable mutations in *MEFV*, and thus clinical judgment remains important in establishing the diagnosis.¹

Consistent with the biology of FMF, *MEFV* is expressed predominantly in granulocytes, monocytes, dendritic cells, and in fibroblasts derived from skin, peritoneum, and synovium.¹⁰⁻¹² It encodes a full-length 781 aa protein denoted pyrin or marenostrin. In transfection experiments full-length pyrin is exclusively cytoplasmic, while endogenous pyrin is cytoplasmic in monocytes but is predominantly nuclear in granulocytes, dendritic cells, and synovial fibroblasts.^{12,13}

Although *sui generis* at the time pyrin was discovered, the domain encoded by exon 1 is now recognized as the prototype for a 92 aa motif, the PYRIN domain, that is found in 20 human proteins involved in the regulation of inflammation and apoptosis.¹⁴⁻¹⁷ This motif has a six alpha-helix structure similar to that of death domains, death effector domains, and caspase-recruitment domains, a configuration known to mediate homotypic interactions. Through cognate PYRIN-PYRIN interactions with the bipartite adaptor protein ASC (apoptosis-associated speck-like protein with a caspase recruitment domain, which is comprised of an N-terminal PYRIN domain and a C-terminal caspase recruitment domain),

Table 3. Genetics and pathophysiology of the hereditary periodic fever syndromes.

Disease	Chromosome	Gene	Protein	Proposed Pathophysiology
Familial Mediterranean fever (FMF)	16p13.3	<i>MEFV</i>	Pyrin (also known as marenostrin)	Disorder in the regulation of interleukin (IL)-1 β activation; possible abnormalities in nuclear factor (NF- κ B) activation, leukocyte apoptosis, as well
TNF receptor–associated periodic syndrome (TRAPS)	12p13	<i>TNFRSF1A</i>	TNFRSF1A (TNFR1, p55, CD120a)	Impaired ectodomain cleavage of p55 receptor with stimulation; impaired trafficking of mutant receptors possibly causing constitutive NF- κ B activation; impaired ligand-binding (resulting in preferential stimulation through p75?); impaired tumor necrosis factor (TNF)-stimulated leukocyte apoptosis
Hyperimmunoglobulinemia D with periodic fever syndrome (HIDS)	12q24	<i>MVK</i>	Mevalonate kinase (MK)	Accumulation of mevalonic acid, the substrate of the MK enzyme or deficiency in isoprenoids (products of the mevalonate pathway). Phenotype unlikely to be due to excessive IgD or cholesterol deficiency (another product of the mevalonate pathway).
The cryopyrinopathies (familial cold urticaria, Muckle-Wells, neonatal-onset multisystem inflammatory disease)	1q44	<i>CIAS1</i> (also known as <i>NALP3</i> , <i>PYPAF1</i> , <i>CATERPILLER 1.1</i>)	Cryopyrin (or NALP3, PYPAF1, CATERPILLER 1.1)	Activating mutations in the cryopyrin/NALP3 protein, leading to increased activity of the inflammasome, and consequently excessive production of interleukin (IL)-1 β ; mutant cryopyrin shows increased sensitivity to activation by muramyl dipeptide.

pyrin acts as an upstream regulator of interleukin (IL)-1 β activation.^{18,19} Pyrin appears to have both inhibitory and potentiating effects on IL-1 β production depending on experimental conditions, and thus the role of wildtype human pyrin in the IL-1 pathway remains controversial. There is also evidence that pyrin plays a role in regulating nuclear factor (NF)- κ B activation and apoptosis, at least in part through its interactions with ASC.¹⁸⁻²² Perhaps reflecting the relevance of these PYRIN domain–mediated interactions to the development of autoinflammatory disease, mutations in exon 1 are extremely rare in FMF.

In contrast, mutations in the C-terminal B30.2/rfp/SPRY domain of pyrin, encoded by exon 10, predominate in FMF. This motif is found in proteins with a variety of different functions, and is thought to mediate protein-protein interactions. Some authors have suggested the possibility that the B30.2/rfp/SPRY domain of pyrin might be an intracellular domain that binds pathogens, similar to the leucine-rich repeat domain of cryopyrin/NALP3 (*vide infra*).^{19,23} This hypothesis is based on (1) the large number of FMF-associated mutations in this domain; (2) the evidence for heterozygote selection for exon 10 mutations in Mediterranean populations; (3) data suggesting selection for specific B30.2/rfp/SPRY sequences in primate evolution;²³ and (4) evidence that the B30.2/rfp/SPRY domain of another protein, TRIM5a, blocks infection with certain retroviruses, and has undergone positive selection in primate evolution similar to pyrin.^{24,25} Nevertheless, at the time of this writing there is no direct experimental evidence that the C-terminal domain of pyrin binds pathogen-derived molecules.

TNF Receptor–Associated Periodic Syndrome

TRAPS is a dominantly-inherited disorder caused by mutations in the 55 kDa TNF receptor (TNFRSF1A), which is encoded on human chromosome 12p.²⁶ Several years be-

fore the discovery of these TNF receptor mutations, a dominantly inherited periodic fever syndrome that later proved to be TRAPS was described in a family of Irish and Scottish ancestry whose illness had been termed familial Hibernian fever. Subsequent studies have demonstrated a very broad population distribution of patients with periodic fever and TNF receptor mutations, and hence the more ethnically neutral TRAPS terminology is usually preferred. To be consistent with the original description of TRAPS, mutations in the *TNFRSF1A* gene in the appropriate clinical setting are required to make the diagnosis, although there remain a number of patients with TRAPS-like illnesses who do not have identifiable mutations.¹

As is the case for FMF, TRAPS presents in childhood or adolescence, but in TRAPS the duration of attacks tends to be more variable, ranging from short episodes of one to two days to flares lasting weeks at a time and, in some cases, patients experience nearly continuous, fluctuating symptoms. In addition to fever, common clinical features of TRAPS include abdominal pain (due to sterile peritonitis), pleurisy, periorbital edema, a migratory erythematous skin rash with underlying myalgia, arthralgia or frank arthritis, and scrotal pain. Marked leukocytosis and elevation of acute-phase reactants is usually seen during attacks, and often persists into the intercritical period. Systemic amyloidosis may occur in up to 10% of patients, with an increased risk observed in patients with mutations involving cysteine residues.²⁷ Although the attacks of TRAPS can often be debilitating, amyloidosis appears to be the major factor limiting longevity.¹

The p55 TNF receptor protein is 455 aa in length, including a 29 aa leader, a 182 aa extracellular domain, a 21 aa transmembrane domain, and a 223 aa intracellular region that includes a death domain. The extracellular domain is, in turn, divided into four cysteine-rich subdomains, each of which contains three disulfide-bonded pairs of cys-

teines that constrain the three-dimensional folding of the protein. To date all of the over 45 known TRAPS mutations (<http://fmf.igh.cnrs.fr/infevers>) reside in the extracellular domain of the protein, and most are in the first two cysteine-rich subdomains, with about half being missense substitutions at cysteine residues that disrupt normal disulfide bonding. To date no patients have been identified with mutations in the transmembrane or intracellular domains, with null mutations, or with mutations in the p75 TNF receptor, which is encoded on chromosome 1p.

The pathophysiology of TRAPS is complex. Initial studies of a family with the C52F *TNFRSF1A* mutation indicated a defect in activation-induced ectodomain shedding of p55 (but not p75) receptors.²⁶ Since receptor shedding is thought to play a homeostatic role both by limiting available cell surface TNF receptors and by creating a pool of potentially antagonistic soluble receptors, it was attractive to hypothesize that the “shedding defect” observed in TRAPS patients might explain their autoinflammatory phenotype. Subsequent studies indicate that, while plausible, this hypothesis cannot fully explain the TRAPS phenotype, since the shedding defect is seen in patients with some, but not all, p55 mutations.²⁶⁻²⁹ Moreover, recent studies indicate a number of other functional abnormalities in mutant p55 receptors, including altered intracellular trafficking,³⁰ impaired TNF binding,³⁰ and a defect in TNF-induced leukocyte apoptosis.³¹

Patients with TRAPS generally do not respond to treatment with colchicine, which is highly effective in FMF, and, although they are responsive to high-dose corticosteroids, side effects are often limiting. The recognition of the role of the TNF-pathway has led to the introduction of etanercept, the soluble p75 TNFR:Fc fusion protein, in the treatment of TRAPS. Etanercept is effective in reducing, although usually not totally eliminating, clinical and laboratory evidence of inflammation in TRAPS,³²⁻³⁴ a result that is consistent with a role for both TNF-dependent and -independent pathways in the pathogenesis of TRAPS.

Hyperimmunoglobulinemia D with Periodic Fever Syndrome

HIDS is a recessively inherited periodic fever syndrome first described in the Netherlands and still diagnosed most often in Northern Europeans.³⁵ Attacks often begin during the first year of life, lasting 3-7 days, and are sometimes triggered by childhood immunizations. Concomitant findings may include headache, abdominal pain, prominent cervical lymphadenopathy, polyarthralgia or polyarticular arthritis, diffuse maculopapular rash, and aphthous ulcerations.³⁶ As in FMF and TRAPS, HIDS attacks are accompanied by leukocytosis and elevated acute-phase reactants. Serum polyclonal IgD levels are also elevated in most HIDS patients, regardless of whether they are experiencing an attack, but do not correlate with disease severity, either over time for any given patient or when comparing patients. Increased serum IgD levels have also been observed

in the minority of patients with FMF³⁷ and TRAPS,^{38,39} but the magnitude is usually much less in these latter two conditions. In contrast with FMF or TRAPS, systemic amyloidosis is extremely rare in HIDS, although it has been reported.⁴⁰

In 1999 two groups, using complementing positional and functional approaches, identified HIDS-associated mutations in *MVK*, an eleven-exon gene on chromosome 12q that encodes the mevalonate kinase (MK) enzyme.^{41,42} MK catalyzes the conversion of mevalonic acid to 5-phosphomevalonic acid in the synthesis of a number of important molecules, including cholesterol, vitamin D, bile acids, steroid hormones, and nonsterol isoprenoids. HIDS-associated mutations leave some residual enzymatic activity, while more profound mutations in *MVK* that totally ablate enzymatic activity cause mevalonic aciduria, a rare condition manifesting not only periodic fevers but also a number of other severe developmental abnormalities. Currently over 35 HIDS-associated mutations have been described (<http://fmf.igh.cnrs.fr/infevers>), and they are distributed throughout the 396 aa MK protein. The mutations associated with mevalonic aciduria cluster around the active sites of the enzyme.^{43,44} Recognition of the molecular genetic basis of HIDS permits the use of genetic testing and/or screening for urinary mevalonate (during attacks) to help establish the diagnosis. This is especially helpful in the minority of patients with borderline or normal serum IgD levels.

In vitro studies suggest that HIDS-mutant MK functions best at 30°C, with progressive decreases in function at 37°C and 39°C.⁴⁵ This observation may account for the association of HIDS attacks with immunizations and infections, and may also explain the increased urinary mevalonate levels observed during HIDS attacks. It is unlikely that IgD plays a primary role in the pathogenesis of HIDS, given the poor correlation of IgD levels with disease severity, and the identification of occasional patients with the HIDS phenotype and *MVK* mutations but persistently normal IgD levels.^{42,46,47} Diminished cholesterol biosynthesis also appears an unlikely mechanism of disease, since HIDS patients generally have low-normal serum cholesterol levels, and more severe disorders of cholesterol synthesis do not exhibit a HIDS-like phenotype. Instead, current thinking on the pathogenesis of HIDS focuses either on the accumulation of mevalonic acid or the shortage of isoprenoids. Favoring the latter hypothesis are in vitro data demonstrating accentuated IL-1 β secretion by leukocytes from HIDS patients that can be reversed by the addition of farnesol or geranyl-geraniol.⁴⁸ Possibly, the small guanoside triphosphate-binding proteins, which undergo farnesylation or geranylation, may be the link between the mevalonate pathway and the innate immune system.⁴⁷ There are currently no established therapies for HIDS, although TNF inhibition and statins are investigational.¹

The Cryopyrinopathies

The cryopyrinopathies are a spectrum of clinical disorders caused by mutations in *CIAS1*,⁴⁹⁻⁵² a nine-exon gene on chromosome 1q encoding a protein that has variously been denoted cryopyrin, NALP3, PYPAF1, or CATERPILLER 1.1. Although a number of overlap syndromes have been described, three relatively distinct clinical disorders are recognized: familial cold autoinflammatory syndrome (FCAS), Muckle-Wells syndrome (MWS), and neonatal-onset multisystem inflammatory disease (NOMID, also known as chronic infantile neurologic cutaneous and arthropathy [CINCA] syndrome). An urticaria-like rash is common to all of the cryopyrinopathies and is characterized histologically by infiltrates of lymphocytes and neutrophils rather than mast cells, indicating that it is not true urticaria. FCAS is characterized by episodes of fever, urticaria-like rash, and polyarthralgia, usually precipitated by generalized exposure to the cold and lasting about 12 hours. In MWS, attacks are not usually precipitated by cold exposure but include the same urticaria-like rash as well as fever, malaise, limb pain, and, at times, abdominal pain, conjunctivitis, and arthralgia. Sensorineural hearing loss and/or systemic AA amyloidosis may also develop. Patients with NOMID/CINCA, the most severe of the cryopyrin-

opathies, often exhibit nearly continuous disease activity that fluctuates in severity. In addition to the fevers, arthralgia, hearing loss, and amyloidosis seen in MWS, patients with NOMID/CINCA often develop a deforming arthropathy and central nervous system involvement that can include chronic aseptic meningitis, intellectual impairment, and loss of vision. Eosinophilia or coagulopathy can also be seen in some patients with NOMID/CINCA.

The full-length cryopyrin/NALP3 protein is 920 aa in length, and consists of three major domains: an N-terminal PYRIN domain (aa 13–83) similar to the N-terminal domain of pyrin, a central NACHT domain (aa 217–513) that appears to be important both in intra- and inter-molecular interactions, and a C-terminal tandem array of 7 leucine rich repeats (LRR, aa 697–920). Nearly all of the more than 40 known mutations in cryopyrin (<http://fmf.igh.cnrs.fr/infervers>) are missense substitutions in the NACHT domain, encoded by exon 3 of *CIAS1*.

Cryopyrin/NALP3 has been shown to play an important role in the regulation of IL-1 β activation through its participation in a macromolecular complex denoted the NALP3 inflammasome (Figure 1).⁵³ In addition to cryopyrin/NALP3, this complex includes ASC, caspase-1 (which cleaves pro-IL-1 β to produce biologically active IL-1 β), and Cardinal, a caspase-recruitment domain-containing protein. The NALP3 inflammasome is a potent activator of IL-1 β , and macrophages from patients with MWS spontaneously secrete active IL-1 β . Through its LRR, cryopyrin/NALP3 binds muramyl dipeptide,⁵⁴ a component of the bacterial cell wall, an event that increases inflammasome activity. Moreover, this process appears to be accentuated in the macrophages of patients with MWS.

The newly recognized role of cryopyrin/NALP3 in IL-1 β activation has provided the conceptual basis for a series of studies establishing an important role for IL-1 β inhibition in the cryopyrinopathies. In one protocol, pretreatment with the IL-1 β receptor antagonist anakinra effectively prevented clinical symptoms or acute-phase elevations in patients with FCAS exposed to cold temperatures.⁵⁵ Anakinra also induced a complete remission of clinical symptoms and biochemical changes in MWS.^{56,57} Most recently, in a series of 18 patients with NOMID/CINCA, anakinra doses of 1-2 mg/kg/d resulted in a resolution of uveitis, rash, and fever and a sig-

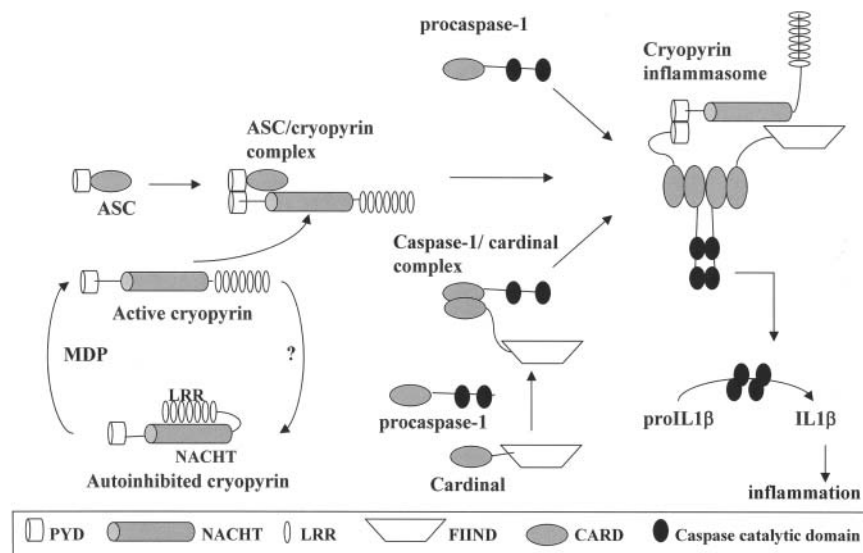


Figure 1. Activation of interleukin (IL)-1 β through the cryopyrin/NALP3 inflammasome. On the lower left, the major domain structure of cryopyrin is depicted. Interaction of cryopyrin with muramyl dipeptide (MDP) is thought to activate cryopyrin and allow it to interact with the other components of the inflammasome. In the upper left, activated cryopyrin binds ASC through cognate PYRIN-PYRIN domain interactions. ASC, in turn, binds caspase-1 through homotypic interactions of their caspase recruitment (CARD) domains (upper right). This complex then binds Cardinal, which has recruited a second caspase-1 molecule. The full assembly of this macromolecular complex, the inflammasome, induces proximity of the catalytic domains of the two caspase-1 molecules, leading to autocatalysis. The released catalytic domains are then available to activate pro-IL-1 β to its biologically active form, which mediates fever and inflammation. Abbreviations: PYD, PYRIN domain; NACHT, the nucleotide-binding domain of cryopyrin; LRR, leucine-rich repeat; FIIND, a domain of Cardinal that interacts with cryopyrin; CARD, caspase-recruitment domain.

nificant decline in cerebrospinal fluid pressure.⁵⁸ These results will not only dramatically improve the quality of life and prognosis of patients with a potentially devastating disease, but will provide powerful support for a model that places cryopyrin/NALP3 at a critical juncture in the regulation of IL-1 β activation.

References

- Kastner DL, Aksentijevich I. Intermittent and periodic arthritis syndromes. In: Koopman WJ, Moreland LW, eds. *Arthritis and Allied Conditions* (15th ed). Philadelphia: Lippincott Williams and Wilkins; 2005:1411-1461.
- Stojanov S, Kastner DL. Familial autoinflammatory diseases: genetics, pathogenesis and treatment. *Curr Opin Rheumatol*. 2005;17:586-599.
- French FMF Consortium. A candidate gene for familial Mediterranean fever. *Nat Genet*. 1997;17:25-31.
- International FMF Consortium. Ancient missense mutations in a new member of the *RoRet* gene family are likely to cause familial Mediterranean fever. *Cell*. 1997;90:797-807.
- Gershoni-Baruch R, Shinawi M, Leah K, Badarnah K, Brik R. Familial Mediterranean fever: prevalence, penetrance and genetic drift. *Eur J Hum Genet*. 2001;9:634-637.
- Kogan A, Shinar Y, Lidar M, et al. Common *MEFV* mutations among Jewish ethnic groups in Israel: high frequency of carrier and phenotype III states and absence of a perceptible biological advantage for the carrier state. *Am J Med Genet*. 2001;102:272-276.
- Stoffman N, Magal N, Shohat T, et al. Higher than expected carrier rates for familial Mediterranean fever in various Jewish ethnic groups. *Eur J Hum Genet*. 2000;8:307-310.
- Yilmaz E, Ozen S, Balci B, et al. Mutation frequency of Familial Mediterranean Fever and evidence for a high carrier rate in the Turkish population. *Eur J Hum Genet*. 2001;9:553-555.
- Aksentijevich I, Torosyan Y, Samuels J, et al. Mutation and haplotype studies of familial Mediterranean fever reveal new ancestral relationships and evidence for a high carrier frequency with reduced penetrance in the Ashkenazi Jewish population. *Am J Hum Genet*. 1999;64:949-962.
- Centola M, Wood G, Frucht DM, et al. The gene for familial Mediterranean fever, *MEFV*, is expressed in early leukocyte development and is regulated in response to inflammatory mediators. *Blood*. 2000;95:3223-3231.
- Matzner Y, Abedat S, Shapiro E, et al. Expression of the familial Mediterranean fever gene and activity of the C5a inhibitor in human primary fibroblast cultures. *Blood*. 2000;96:727-731.
- Diaz A, Hu C, Kastner DL, et al. Lipopolysaccharide-induced expression of multiple alternatively spliced *MEFV* transcripts in human synovial fibroblasts: a prominent splice isoform lacks the C-terminal domain that is highly mutated in familial Mediterranean fever. *Arthritis Rheum*. 2004;50:3679-3689.
- Mansfield E, Chae JJ, Komarow HD, et al. The familial Mediterranean fever protein, pyrin, associates with microtubules and colocalizes with actin filaments. *Blood*. 2001;98:851-859.
- Bertin J, DiStefano PS. The PYRIN domain: a novel motif found in apoptosis and inflammation proteins. *Cell Death Differ*. 2000;7:1273-1274.
- Martinon F, Hofmann K, Tschopp J. The pyrin domain: a possible member of the death domain-fold family implicated in apoptosis and inflammation. *Curr Biol*. 2001;11:R118-120.
- Pawlowski K, Pio F, Chu Z, Reed JC, Godzik A. PAAD - a new protein domain associated with apoptosis, cancer and autoimmune diseases. *Trends Biochem Sci*. 2001;26:85-87.
- Staub E, Dahl E, Rosenthal A. The DAPIN family: a novel domain links apoptotic and interferon response proteins. *Trends Biochem Sci*. 2001;26:83-85.
- Chae JJ, Komarow HD, Cheng J, et al. Targeted disruption of pyrin, the FMF protein, causes heightened sensitivity to endotoxin and a defect in macrophage apoptosis. *Mol Cell*. 2003;11:591-604.
- Yu JW, Wu J, Zhang Z, et al. Cryopyrin and pyrin activate caspase-1, but not NF- κ B, via ASC oligomerization. *Cell Death Differ*. 22 July 2005. [Epub ahead of print]
- Stehlik C, Fiorentino L, Dorfleutner A, et al. The PAAD/PYRIN-family protein ASC is a dual regulator of a conserved step in nuclear factor κ B activation pathways. *J Exp Med*. 2002;196:1605-1615.
- Dowds TA, Masumoto J, Chen FF, Ogura Y, Inohara N, Nunez G. Regulation of cryopyrin/Pypaf1 signaling by pyrin, the familial Mediterranean fever gene product. *Biochem Biophys Res Commun*. 2003;302:575-580.
- Masumoto J, Dowds TA, Schaner P, et al. ASC is an activating adaptor for NF- κ B and caspase-8-dependent apoptosis. *Biochem Biophys Res Commun*. 2003;303:69-73.
- Schaner P, Richards N, Wadhwa A, et al. Episodic evolution of pyrin in primates: human mutations recapitulate ancestral amino acid states. *Nat Genet*. 2001;27:318-321.
- Perron MJ, Stremmlau M, Song B, Ulm W, Mulligan RC, Sodroski J. TRIM5a mediates the postentry block to N-tropic murine leukemia viruses in human cells. *Proc Natl Acad Sci U S A*. 2004;101:11827-11832.
- Song B, Javanbakht H, Perron M, Park do H, Stremmlau M, Sodroski J. Retrovirus restriction by TRIM5a variants from Old World and New World primates. *J Virol*. 2005;79:3930-3937.
- McDermott MF, Aksentijevich I, Galon J, et al. Germline mutations in the extracellular domains of the 55 kDa TNF receptor, TNFR1, define a family of dominantly inherited autoinflammatory syndromes. *Cell*. 1999;97:133-144.
- Aksentijevich I, Galon J, Soares M, et al. The tumor-necrosis-factor receptor-associated periodic syndrome: new mutations in *TNFRSF1A*, ancestral origins, genotype-phenotype studies, and evidence for further genetic heterogeneity of periodic fevers. *Am J Hum Genet*. 2001;69:301-314.
- Nevala H, Karenko L, Stjernberg S, et al. A novel mutation in the third extracellular domain of the tumor necrosis factor receptor 1 in a Finnish family with autosomal-dominant recurrent fever. *Arthritis Rheum*. 2002;46:1061-1066.
- Aganna E, Hammond L, Hawkins PN, et al. Heterogeneity among patients with tumor necrosis factor receptor-associated periodic syndrome phenotypes. *Arthritis Rheum*. 2003;48:2632-2644.
- Todd I, Radford PM, Draper-Morgan KA, et al. Mutant forms of tumour necrosis factor receptor I that occur in TNF-receptor-associated periodic syndrome retain signalling functions but show abnormal behaviour. *Immunology*. 2004;113:65-79.
- Siebert S, Amos N, Fielding CA, et al. Reduced tumor necrosis factor signaling in primary human fibroblasts containing a tumor necrosis factor receptor superfamily 1A mutant. *Arthritis Rheum*. 2005;52:1287-1292.
- Hull KM, Drewe E, Aksentijevich I, et al. The TNF receptor-associated periodic syndrome (TRAPS): emerging concepts of an autoinflammatory disorder. *Medicine (Baltimore)*. 2002;81:349-368.
- Drewe E, McDermott EM, Powell PT, Isaacs JD, Powell RJ. Prospective study of anti-tumour necrosis factor receptor superfamily 1B fusion protein, and case study of anti-tumour necrosis factor receptor superfamily 1A fusion protein, in tumour necrosis factor receptor associated periodic syndrome (TRAPS): clinical and laboratory findings in a series of seven patients. *Rheumatology (Oxford)*. 2003;42:235-239.

34. Hull KM, Shoham N, Chae JJ, Aksentijevich I, Kastner DL. The expanding spectrum of systemic autoinflammatory disorders and their rheumatic manifestations. *Curr Opin Rheumatol.* 2003;15:61-69.
35. Simon A, Mariman EC, van der Meer JW, Drenth JP. A founder effect in the hyperimmunoglobulinemia D and periodic fever syndrome. *Am J Med.* 2003;114:148-152.
36. Drenth JP, van der Meer JW. Hereditary periodic fever. *N Engl J Med.* 2001;345:1748-1757.
37. Medlej-Hashim M, Petit I, Adib S, et al. Familial Mediterranean fever: association of elevated IgD plasma levels with specific *MEFV* mutations. *Eur J Hum Genet.* 2001;9:849-854.
38. Simon A, van Deuren M, Tighe PJ, van der Meer JW, Drenth JP. Genetic analysis as a valuable key to diagnosis and treatment of periodic fever. *Arch Intern Med.* 2001;161:2491-2493.
39. Dode C, Andre M, Bienvenu T, et al. The enlarging clinical, genetic, and population spectrum of tumor necrosis factor receptor-associated periodic syndrome. *Arthritis Rheum.* 2002;46:2181-2188.
40. Obici L, Manno C, Muda AO, et al. First report of systemic reactive (AA) amyloidosis in a patient with the hyperimmunoglobulinemia D with periodic fever syndrome. *Arthritis Rheum.* 2004;50:2966-2969.
41. Drenth JP, Cuisset L, Grateau G, et al. Mutations in the gene encoding mevalonate kinase cause hyper-IgD and periodic fever syndrome. International Hyper-IgD Study Group. *Nat Genet.* 1999;22:178-181.
42. Houten SM, Kuis W, Duran M, et al. Mutations in *MVK*, encoding mevalonate kinase, cause hyperimmunoglobulinemia D and periodic fever syndrome. *Nat Genet.* 1999;22:175-177.
43. Cuisset L, Drenth JP, Simon A, et al. Molecular analysis of *MVK* mutations and enzymatic activity in hyper-IgD and periodic fever syndrome. *Eur J Hum Genet.* 2001;9:260-266.
44. Houten SM, Koster J, Romeijn GJ, et al. Organization of the mevalonate kinase (*MVK*) gene and identification of novel mutations causing mevalonic aciduria and hyperimmunoglobulinemia D and periodic fever syndrome. *Eur J Hum Genet.* 2001;9:253-259.
45. Houten SM, Frenkel J, Rijkers GT, Wanders RJ, Kuis W, Waterham HR. Temperature dependence of mutant mevalonate kinase activity as a pathogenic factor in hyper-IgD and periodic fever syndrome. *Hum Mol Genet.* 2002;11:3115-3124.
46. Saulsbury FT. Hyperimmunoglobulinemia D and periodic fever syndrome (HIDS) in a child with normal serum IgD, but increased serum IgA concentration. *J Pediatr.* 2003;143:127-129.
47. Takada K, Aksentijevich I, Mahadevan V, Dean JA, Kelley RI, Kastner DL. Favorable preliminary experience with etanercept in two patients with the hyperimmunoglobulinemia D and periodic fever syndrome. *Arthritis Rheum.* 2003;48:2645-2651.
48. Frenkel J, Rijkers GT, Mandey SH, et al. Lack of isoprenoid products raises ex vivo interleukin-1 β secretion in hyperimmunoglobulinemia D and periodic fever syndrome. *Arthritis Rheum.* 2002;46:2794-2803.
49. Hoffman HM, Mueller JL, Broide DH, Wanderer AA, Kolodner RD. Mutation of a new gene encoding a putative pyrin-like protein causes familial cold autoinflammatory syndrome and Muckle-Wells syndrome. *Nat Genet.* 2001;29:301-305.
50. Aganna E, Martinon F, Hawkins PN, et al. Association of mutations in the *NALP3/CIAS1/PYPAF1* gene with a broad phenotype including recurrent fever, cold sensitivity, sensorineural deafness, and AA amyloidosis. *Arthritis Rheum.* 2002;46:2445-2452.
51. Feldmann J, Prieur AM, Quartier P, et al. Chronic infantile neurological cutaneous and articular syndrome is caused by mutations in *CIAS1*, a gene highly expressed in polymorphonuclear cells and chondrocytes. *Am J Hum Genet.* 2002;71:198-203.
52. Aksentijevich I, Nowak M, Mallah M, et al. De novo *CIAS1* mutations, cytokine activation, and evidence for genetic heterogeneity in patients with neonatal-onset multisystem inflammatory disease (NOMID): a new member of the expanding family of pyrin-associated autoinflammatory diseases. *Arthritis Rheum.* 2002;46:3340-3348.
53. Agostini L, Martinon F, Burns K, McDermott MF, Hawkins PN, Tschopp J. NALP3 forms an IL-1 β -processing inflammasome with increased activity in Muckle-Wells autoinflammatory disorder. *Immunity.* 2004;20:319-325.
54. Martinon F, Agostini L, Meylan E, Tschopp J. Identification of bacterial muramyl dipeptide as activator of the NALP3/cryopyrin inflammasome. *Curr Biol.* 2004;14:1929-1934.
55. Hoffman HM, Rosengren S, Boyle DL, et al. Prevention of cold-associated acute inflammation in familial cold autoinflammatory syndrome by interleukin-1 receptor antagonist. *Lancet.* 2004;364:1779-1785.
56. Hawkins PN, Lachmann HJ, McDermott MF. Interleukin-1-receptor antagonist in the Muckle-Wells syndrome. *N Engl J Med.* 2003;348:2583-2584.
57. Hawkins PN, Lachmann HJ, Aganna E, McDermott MF. Spectrum of clinical features in Muckle-Wells syndrome and response to anakinra. *Arthritis Rheum.* 2004;50:607-612.
58. Dailey NJ, Aksentijevich I, Chae JJ, et al. Interleukin-1 receptor antagonist anakinra in the treatment of neonatal onset multisystem inflammatory disease. *Arthritis Rheum.* 2004;50:S440.