Hereditary autoinflammatory syndromes
- with emphasis on hyper-IgD and periodic fever syndrome -

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een wetenschappelijke proeve op het gebied van de
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Proefschrift

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Cover: Black bantam rooster (left) and Sebright bantam rooster (right), demonstrating the motto by William Harvey (see opposite). For more details, see appendix III. Picture courtesy of Sebright og Bantamklubben, Denmark (http://www.fjerkrae.dk/sebright_&_bantamklubben.htm).

It is even so - Nature is nowhere accustomed more openly to display her secret mysteries than in cases where she shows traces of her workings apart from the beaten path; nor is there any better way to advance the proper practice of medicine than to give our minds to the discovery of the usual law of Nature by careful investigation of rarer forms of disease. For it has been found in almost all things, that what they contain of useful or applicable is hardly perceived unless we are deprived of them, or they become deranged in some way.

William Harvey (1578-1657)

In a letter written six weeks before his death to a Dutch physician, Johannes Vlackveld of Haarlem, who had asked his advice about an unusual presentation of disease in one of his patients, 1657.

Willis R. (ed.): *The Works of William Harvey*. London: Sydenham Society, 1847, p.616
Harvey W. *The Circulation of the Blood*. Everyman's Library 262. London, England: J.M.Dent &Sons;1966:209-210.

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Outline of thesis

The publication of this thesis occurs exactly twenty years after the first description of the hyper-immunoglobulinaemia D (IgD) and periodic fever syndrome (HIDS) was published [1], and five years after the discovery of the genetic background of this autoinflammatory syndrome [2,3]. This last event was the final confirmation for the existence of HIDS as a distinct entity and has improved diagnosis. But at the same time it raised numerous questions on the pathogenesis – how can a genetic metabolic defect result in periodic inflammation? – which called for further investigation. The current thesis is filled with our exploits into the field of HIDS and auto-inflammation in the past four years. These will be briefly outlined here.

This thesis starts off with a clinical review on the current knowledge of familial auto-inflammatory syndromes (**chapter 1**). This offers an introduction to these rare hereditary disorders for clinicians.

The next four chapters deal with issues involving diagnosis of HIDS. The questions that we asked ourselves were:
- What are the genotypes found in a cohort of HIDS patients, and can we develop an effective molecular testing strategy for diagnosis? (**chapter 2**)

- What is the role of mevalonate kinase in HIDS, and can the subgroup of HIDS patients without mevalonate kinase deficiency be distinguished clinically? (**chapter 3**)

- Is the clinical phenotype of mevalonate kinase deficiency truly a continuous spectrum between mevalonic aciduria and classical type HIDS? (**chapter 4**)

- What are the characteristics of the acute phase response during inflammatory attacks in HIDS, and can these be used to discriminate between (serious bacterial) infection and a HIDS attack? (**chapter 5**)

More fundamental research questions prompted the following two studies:
- Why are classical type HIDS families, especially those carrying a V377I mutation in the *MVK* gene, clustered in the Netherlands? (**chapter 6**)

- What is the effect of an excessive amount of mevalonic acid on the function of peripheral blood mononuclear cells in classical type HIDS patients as well as in healthy controls? (**chapter 7**)

In the search for an effective treatment of the inflammatory attacks in HIDS, we performed two clinical trials to study the effect of two medications on the frequency, intensity and duration of these HIDS attacks:
- Thalidomide, a drug known to inhibit production of several cytokines, beneficial in a large variety of inflammatory disorders (**chapter 8**)

- Simvastatin, an inhibitor of HMG-CoA reductase which reduces the formation of mevalonic acid (**chapter 9**).

In **chapter 10**, the scope is broadened from HIDS to all hereditary autoinflammatory syndromes, to try and answer the question: would more rigorous and unlimited genetic screening allow further classification of patients with periodic fever attacks, who could not be diagnosed previously? An additional question raised here is whether combinations of mutations from different autoinflammatory syndromes occur regularly, as is suggested by some.

We take a closer look at TNF-receptor associated periodic syndrome (TRAPS) in the subsequent three chapters in the form of clinical observations:

- Diagnosis of two patients with TRAPS, after a delay of more than 40 years – and what is the effect of short course etanercept, a soluble TNF-receptor preparation, in the treatment of the inflammatory attacks? (**chapter 11**).

- Could the cessation of inflammatory attacks in a TRAPS patient who developed amyloidosis be related to her renal insufficiency? (**chapter 12**)

- How to treat a patient with severe TRAPS, refractory to etanercept? (**chapter 13**)

Chapter 14 is a clinical review on the subject of abdominal pain in familial Mediterranean fever (FMF), in which we try to answer the question: what is the best approach and treatment for abdominal attacks in FMF?

The thesis ends with a general summary of the research described and a look at future perspectives.

Familial Autoinflammatory Syndromes

Anna Simon, Jos W.M. van der Meer, Joost P.H. Drenth

Definition

The familial auto-inflammatory syndromes, otherwise known as hereditary periodic fever syndromes, form a group of rare disorders with a common phenotype of lifelong recurrent inflammatory episodes with fever, usually accompanied by other inflammatory symptoms such as abdominal pain, diarrhea, rash or arthralgia, and separated by symptom-free intervals [4]. In between the fever episodes, patients feel healthy and can generally function normally. Routine laboratory investigations during a fever attack invariably reveal a severe acute phase response with a high sedimentation rate, leukocytosis and high concentrations of acute phase proteins such as C-reactive protein (CRP), serum amyloid A (SAA) and several proinflammatory cytokines.The fever episodes most often arise without an obvious trigger, although some patients note a relation with physical stimulus (exposure to cold), emotional stress, or with the menstrual cycle in women. They resolve spontaneously after a number of days or weeks. Patients with periodic fever very often go undiagnosed for years. That represents a high level of discouragement and frustration for patients and physicians because in many instances elaborate clinical investigations fail to substantiate the diagnosis [5,6]. The term "auto-inflammatory", coined by McDermott et al. in 1999 for these disorders [7], adequately describes the phenotype of recurrent acute inflammatory responses. It is preferable to "auto-immune" in these cases, since autoimmune phenomena are not found.

At the moment, four distinct subtypes of hereditary periodic fever are recognized, if we take a genetical basis for the classification. Despite the common phenotype described above, these subtypes can often be differentiated clinically by a number of specific characteristics; in particular the mode of inheritance, age of onset, average duration of the fever episodes and the fever-free interval, geographical region of origin of the patients family, and occurrence of long-term complications like amyloidosis or deafness (*table 1; figure 1*).

Since the end of the 1990s, when three of these subtypes were genetically characterized (the fourth followed in 2001), they have obtained a more definite foothold in medicine, because for the first time a positive diagnosis became possible instead of a *diagnosis per exclusionem*. However, there still remains a significant number of patients with periodic fever phenotypes who do not fit into this classification. It is probable that there are more (genetic) defects that can lead to periodic fever, which have not yet been recognized. Another disappointing aspect is the lack of efficacious therapy for these disorders. Hopefully, a further characterization of the distinct pathogenetic mechanisms in the near future will give us a clue on treatment of these debilitating disorders.

In this chapter, we will describe the six major subtypes of familial auto-inflammatory syndromes: Familial Mediterranean fever (FMF), the hyper-IgD and periodic fever syndrome (HIDS), the TNF-receptor associated periodic syndrome (TRAPS), Familial cold autoinflammatory syndrome (FCAS), Muckle-Wells syndrome (MWS) and Chronic infantile neurological cutaneous and articular syndrome (CINCA) or the neonatal onset multisystemic inflammatory disease (NOMID) as it is known in Anglo-Saxon literature.

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	FMF	Mevalonate kinase deficiency			TRAPS	Cryopyrin associated periodic syndromes		
		classic HIDS	mevalonic aciduria	variant HIDS		FCAS	MWS	CINCA/NOMID
mode of inheritance	aut. recessive	aut. recessive	aut. recessive	?	aut. dominant	aut. dominant	aut. dominant	aut. dominant
age at onset (yr)	<20	<1	<1	<10	<20	<1	<20	<1
duration attack (days)*	<2	4-6	4-5	6-8	>7-14	<2	1-2	?
cutaneous involvement	erysipelas-like erythema	maculo-papular rash	morbilliform rash	maculo-papular rash	migratory rash, overlying area of myalgia	cold-induced urticaria-like lesions	urticaria-like lesions	urticaria-like lesions
musculo-skeletal involvement	monoarthritis common	arthralgia, occasional oligoarthritis	arthralgia common	arthralgia	severe myalgia common; occasional frank monoarthritis	arthralgia common; occasional mild myalgia	arthralgia common; lancing limb pain, arthritis can occur	epiphyseal bone formation
abdominal involvement	sterile peritonitis common	splenomegaly, severe pain common	splenomegaly, pain may occur	may occur	severe pain very common	none	may occur	hepatospleno-megaly
eye involvement	uncommon	uncommon	uncommon	uncommon	conjunctivitis and/or periorbital edema very common	conjunctivitis	conjunctivitis, sometimes optic nerve elevation	papilledema with possible loss of vision, uveitis
distinguishing clinical symptoms	erysipelas-like erythema	prominent cervical lymphadeno-pathy	dysmorphic features, neurological symptoms	lymphadeno-pathy may occur	migratory nature of myalgia and rash, periorbital edema	cold-induced urticaria-like lesions	sensorineural hearing loss	chronic aseptic meningitis, sensorineural hearing loss, arthropathy
gene involved	MEFV	MVK	MVK	?	TNFRSF1A	CIAS1	CIAS1	CIAS1
protein involved	Pyrin (marenostrin)	Mevalonate kinase	Mevalonate kinase	?	type 1 TNF-receptor	Cryopyrin	Cryopyrin	Cryopyrin

Table 1. Differential diagnosis of familial auto-inflammatory syndromes.

* duration may be variable; here given is a typical duration; aut. = autosomal. Adapted from Hull et al. [8]
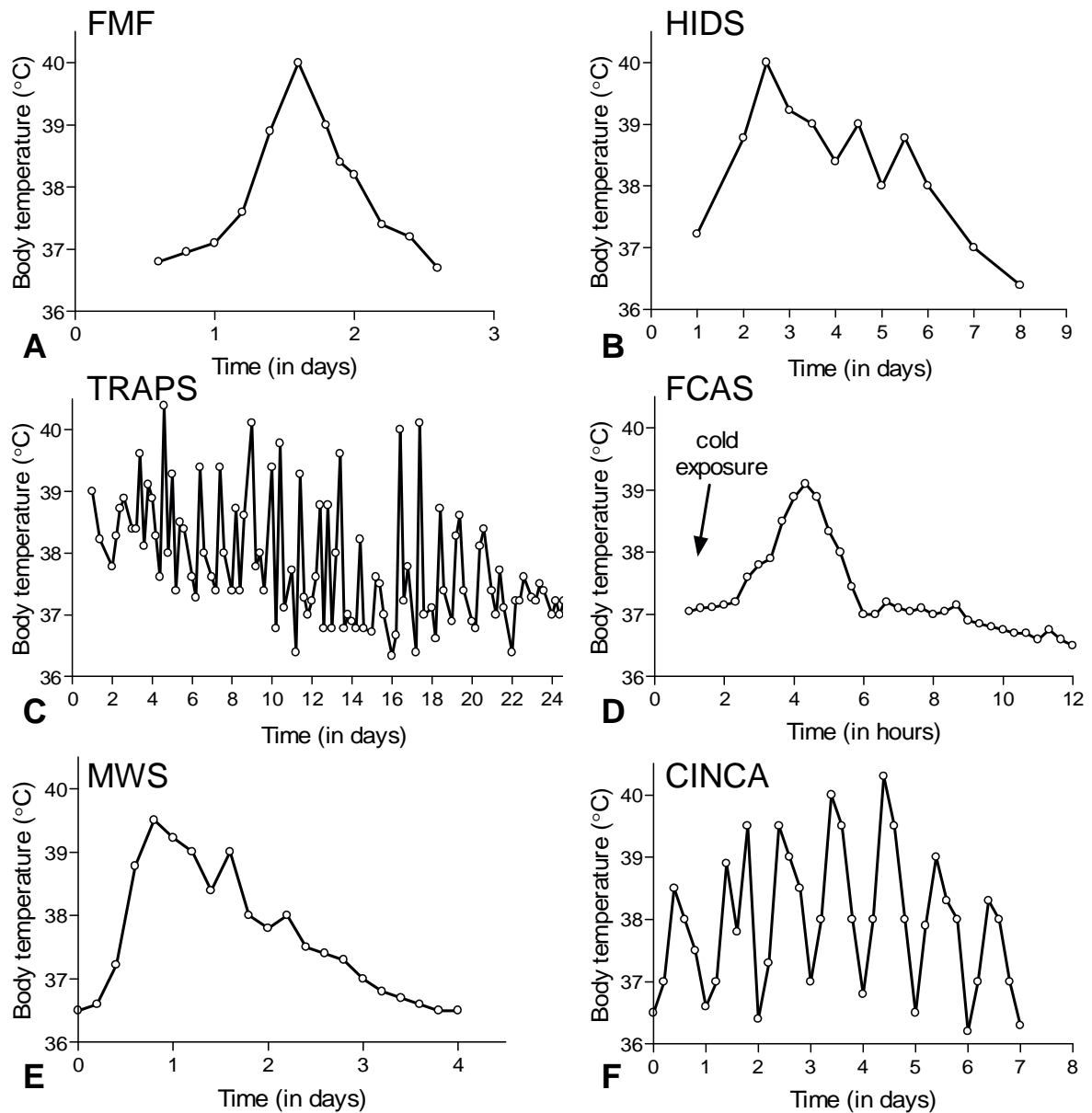
Figure 1. Characteristic patterns of body temperature during inflammatory attacks in the familial auto-inflammatory syndromes. However, there is considerable interindividual variability for each syndrome and and even for the individual patient, the fever pattern may vary greatly from episode to episode. Please note the different time scales on the X axes.

Recently, it has been proposed to include a number of other syndromes in the group of autoinflammatory disorders. These include Crohn's disease, Blau syndrome and also the inherited syndrome "Pyogenic sterile Arthritis, Pyoderma gangrenosum and Acne" (PAPA), which has been genetically characterised as well [9]. We will discuss PAPA and Blau syndrome briefly at the end of the chapter.

Differential diagnosis

When do we consider the diagnosis periodic fever? When a patient has had recurrent fever episodes for more than two years, it is increasingly unlikely that these are caused by an infection or a malignant disorder. The differential diagnosis at that time may include numerous inflammatory disorders such as juvenile rheumatoid arthritis, adult-onset Still's disease, inflammatory bowel disease, Blau syndrome, Schnitzler syndrome and Behçet's disease, besides the hereditary periodic fever syndromes *(table 2)*. Since the hereditary syndromes are rare (except for Familial Mediterranean fever in people with a distinct ethnical background), the more common diagnoses need to be sought for first.

Table 2. Differential diagnosis of periodic fever

Hereditary – see table 1
Non-hereditary
infectious:
hidden infectious focus (e.g. aorta-enteral fistula, Caroli syndrome)
recurrent reinfection (e.g. chronic meningococcemia; host defense defect)
specific infection (e.g. Whipple's disease, malaria)
non-infectious inflammatory disorders:
adult onset Still's disease
juvenile chronic rheumatoid arthritis
periodic fever, aphtous stomatitis, pharyngitis and adenitis (PFAPA)
Schnitzler syndrome
Behçet syndrome
Crohn's disease
sarcoidosis
extrinsic alveolitis
humidifier lung, polymere fume fever
neoplastic:
lymphoma (Hodgkin's disease, angioimmunoblastic lymphoma (AILD))
solid tumour (pheochromocytoma, myxoma, colon carcinoma)
vascular:
recurrent pulmonary embolism
hypothalamic
psychogenic periodic fever [10]
factitious / fraudulent

The mainstay of the diagnosis of hereditary periodic fever is clinical assessment with a detailed medical history (which includes family history) and preferably at least once observation of the patient during a fever episode, as physical examination of the patient in a remission period is seldom abnormal. This clinical assessment will often yield enough information for the first differential diagnosis of the specific familial autoinflammatory syndromes *(table 1)*. The next step is to try for a more definite, genetic diagnosis. A specific diagnosis within the group of familial autoinflammatory syndromes is important as it will yield information on prognosis, treatment and genetic counselling.

Familial Mediterranean Fever

Epidemiology

Familial Mediterranean Fever (FMF, MIM 249100) is the most prevalent disorder among the hereditary autoinflammatory syndromes, with more than 10,000 patients affected worldwide. It occurs mostly in people originating from the Mediterranean basin, including Armenians, Sephardic Jews, Arabs and Turks. FMF is an autosomal recessively inherited disorder. Most families reported with an apparent autosomal dominant inheritance pattern of FMF [11] represent examples of pseudodominant inheritance due to consanguinity and the high carrier frequency of FMF mutations in certain populations [11-13]; at least three families do seem to show a true dominant inheritance even after extensive genetic analysis [13].

Etiology

In 1997, two groups independently traced the genetic background of familial Mediterranean fever to a hitherto unknown gene on the short arm of chromosome 16, which was dubbed *MEFV* gene. At least 67 disease-linked mutations in the *MEFV* gene have been described so far, most of which are clustered in the tenth exon of this gene. Most of the mutations are missense mutations, leading to a single amino acid change in the protein (*figure 2*).

There are 6 common mutations, accounting for almost 99% of all FMF chromosomes; these are M694V (occurring in 20 to 65 percent of cases, depending on the population examined [14]), V726A (in 7 to 35 percent), M680I, M694I, V694I and E148Q. For the first three mutations mentioned here, a founder effect has been established [15], pointing to common ancestors at least 2500 years ago. The high frequency of the mutated *MEFV* gene in more than one Middle Eastern population has led to the hypothesis that heterozygous carriers have an as yet unknown advantage, possibly a heightened (inflammatory) resistance to an endemic pathogen of the Mediterranean basin [15]. Such a pathogen has not been identified so far.
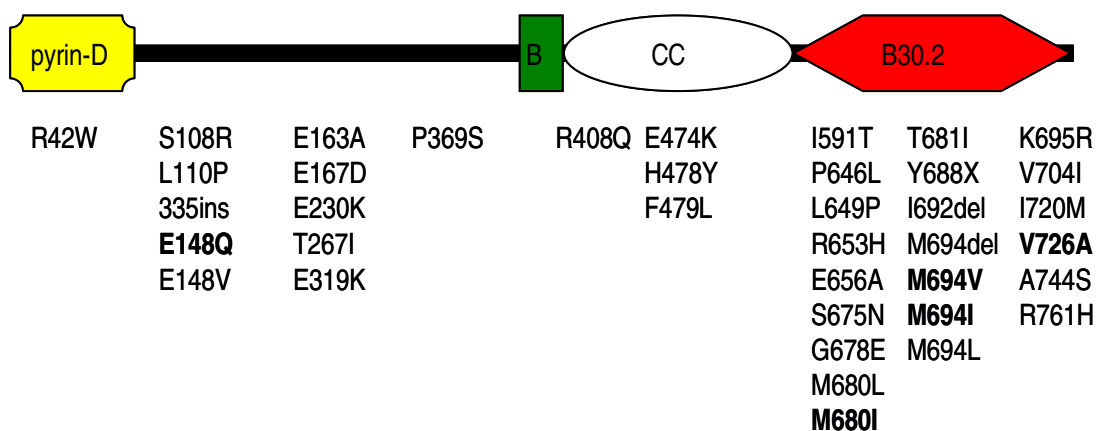

Figure 2. Schematic representation of pyrin (marenostrin) protein, with 4 conserved domains, including a pyrin domain (yellow), a coiled-coil domain (white) and a B30.2 domain (red). Indicated are the mutations as found in FMF, with the 5 most common missense mutations in bold type.

The *MEFV* gene encodes for a protein of 781 amino acids known as pyrin or marenostrin, which is mainly expressed as a cytoplasmic protein in mature neutrophils and monocytes [16], in association with microtubuli [17]. The expression of pyrin is effectively stimulated by inflammatory mediators such as interferon-γ and tumor necrosis factor [18]. The pyrin domain is shared by a number of proteins involved in apoptosis and inflammation and is a member of the six-helix bundle death-domain superfamily that includes death domains, death effector domains, and caspase recruitment domains. Pyrin binds specifically to other proteins that contain a pyrin domain. There is increasing evidence for a function of pyrin as mediator of apoptosis [19]. Another hypothesis on pathogenesis of FMF implicates a specific inhibitor of chemotaxis, a serine protease that inactivates the complement factor C5a and interleukin 8, which can be found in peritoneal and synovial fluids [19-21]. It is thought that this inhibitor acts to prevent inappropriate inflammation [20]. Activity of this C5a-inhibitor was greatly reduced in serosal fluid from FMF patients [19,21], which might contribute to the development of a severe inflammatory response after a rather innocuous stimulus. A relationship between pyrin and C5a-inhibitor is hypothetical and has not been established so far.

Clinical features

In approximately 90 percent of patients, symptoms start before the age of 20 years [22]. The inflammatory attacks of FMF usually last only 1 to 3 days, and frequency of the attacks can be very variable, though 2 to 4 weeks is the most common interval (*figure 1*). Fever is the main feature of the attacks in FMF, and is usually accompanied by symptoms of serositis (either peritonitis, pleuritis or synovitis). Abdominal pain of one or two days' duration occurs in 95 percent of patients [23], varying in severity from severe peritonitis resembling acute abdomen to only mild abdominal pain without overt peritonitis [24]. Arthritis is often confined to one large joint, such as the knee, ankle or wrist, and may also be the sole manifestation of an attack. Chronic destructive arthritis and migratory polyarthritis are rare. Chest pain due to pleuritis is usually unilateral, and associated with diminished breathing sounds, a friction rub or transient pleural effusion. Skin involvement occurs in approximately 30 percent of patients, most often as an erysipelas-like skin lesions on the shins or feet (*figure 3*) [25]. Other, more uncommon, symptoms are pericarditis, occurring in less than 1 percent [26], acute scrotal swelling and tenderness [27], aseptic meningitis and severe protracted myalgia, especially of the legs.

Figure 3. Erysipelas like eruption in a patient with a FMF attack. Picture courtesy of Professor A. Livneh, Heller Institute of Medical Research, Tel Hashomer, Israel

Recurrent attacks of peritonitis may lead to intraabdominal or pelvic adhesions, resulting in complications such as small bowel obstruction or reduced fertility in female patients. Another serious long-term complication of FMF is AA amyloidosis, which is found primarily in the kidneys, but also in the gastrointestinal tract, liver and spleen, and eventually in the heart, testes and thyroid, leading to organ dysfunction. The prevalence of amyloidosis is variable, especially according to the population, but generally high in untreated patients. It is common among Sephardic Jews but rare in Ashkenazi Jews [28].

Evaluation and management

There is no specific biologic marker for FMF available. During an inflammatory attack there will be a polymorphonuclear leukocytosis and serum markers of acute phase response are found to be elevated. These include serum amyloid A (SAA), C-reactive protein (CRP) and plasma fibrinogen. Proteinuria in patients with FMF is highly suggestive of amyloidosis [24].

FMF is a clinical diagnosis, and there is a set of validated diagnostic criteria (*table 3*) [29], with a reported sensitivity and specificity of 96-99%. However, this was validated in a population with a very high prevalence of FMF and low prevalence of the other auto-inflammatory disorders, and it is not known whether this also applies to other populations [4].

Since the location of FMF mutations is known, it is possible to establish a molecular diagnosis of FMF, but there are some limitations [4]. Genetic laboratories usually screen for the five most common mutations, and those that are more rare will be missed. Furthermore, *MEFV* mutations occur on both alleles in only 70 percent of typical cases [30], while in the remaining 30 percent only one or no mutation can be detected, even after sequencing. There is also evidence of reduced penetrance. Despite these limitations, molecular testing can be used as a confirmatory test in cases in which there is a high index of suspicion. Whether or not the results are positive, treatment with colchicine is warranted in symptomatic cases fulfilling the diagnostic criteria [31-34].

An issue of some importance in the management are the fertility problems encountered in FMF. For a variety of reasons, including peritoneal adhesions and ovulatory dysfunction, subfertility in females is not rare [35]. In males, subfertility due to azoospermia (sometimes secondary to testicular amyloidosis) or impairment of sperm penetration has been noted [36].

Table 3. Diagnostic criteria for diagnosis of FMF (Tel-Hashomer) [29]

Major criteria:
    Typical attacks with peritonitis (generalized)
    Typical attacks with pleuritis (unilateral) or pericarditis
    Typical attacks with monarthritis (hip, knee, ankle)
    Typical attacks with fever alone
    Incomplete abdominal attack
Minor criteria:
    Incomplete attacks involving chest pain
    Incomplete attacks involving monarthritis
    Exertional leg pain
    Favorable response to colchicine

Requirements for diagnosis of FMF are ≥1 major criteria or ≥2 minor criteria.
Typical attacks are defined as recurrent (≥3 of the same type), febrile (≥38℃) and short (between 12 hours and 3 days). Incomplete attacks are defined as painful and recurrent attacks not fulfilling the criteria for a typical attack.

Treatment

Colchicine is the first-line of treatment for patients with FMF. Its efficacy has been established in two controlled clinical trials in 1974 [33,34]. Colchicine will prevent inflammatory attacks in 60 percent of patients, and significantly reduces the number of attacks in another 20 to 30 percent [22]. The average dose in adults is 1 mg daily, but this may be increased up to 3 mg in cases where no response is seen at the low dose. This regimen is usually well tolerated, and gastrointestinal side effects including diarrhea or abdominal pain will generally resolve with dose reduction. More serious side effects, such as myopathy, neuropathy and leukopenia are rare, and occur mainly in patients with renal or liver impairment. During a fever attack, diclofenac (75 mg administered intramuscularly) may be used for pain relief. Other agents (e.g. reserpine, steroids) have limited efficacy.

Compliance with colchicine use is very important, since colchicine has been shown to prevent the occurrence of amyloidosis. Since the introduction of colchicine treatment, the incidence of amyloidosis in FMF has dropped dramatically, while in areas with a high prevalence of FMF where colchicine is not routinely available, such as Armenia, amyloidosis is still common.

Colchicine's main effect at the cellular level occurs by its interaction with tubulin at the microtubules, inhibiting motility and exostosis of intracellular granules. Furthermore, it has a powerful antimitotic effect by causing metaphase arrest. Therefore it has been speculated, in cases of infertility in patients treated with colchicine, that this medication causes azoospermia. However, colchicine does not have a significant adverse effect on sperm production or function [37]. Unfounded fear of teratogenic effects of colchicine often wrongly leads to cessation of this drug in young women who wish to get pregnant, with a subsequent increased frequency and severity of attacks, which enhances problems with fertility and pregnancy. Colchicine has proven safe even in early pregnancy and treatment should not be interrupted for this reason [35,38]; it can also be used while breast feeding [36].

Hyper-IgD syndrome

Epidemiology

The hyper-IgD syndrome (HIDS, MIM 260920) is also an autosomal recessively inherited disorder, but is far less prevalent than FMF. The International Hyper-IgD syndrome Registry, based at Nijmegen, the Netherlands, in which clinical information is actively collected from physicians worldwide, currently holds data on some 190 patients. More than 80% of these stem from Western Europe, 55% originate from the Netherlands and France. Almost all of the hyper-IgD patients are of Caucasian origin. These observations can at least in part be explained by a founder effect [39]. In the Netherlands the carrier frequency of a hyper-IgD mutation is estimated to be 1:350. There are equal numbers of men and women affected. Based on the genotype and phenotype we now distinguish classic HIDS and variant HIDS [40].

Etiology

The hyper-IgD syndrome in its classic form is caused by mutations in the gene encoding for the enzyme mevalonate kinase, located on the long arm of chromosome 12 [2,3,40]. Mevalonate kinase is part of the isoprenoid pathway; it is the next step after 3'5'-hydroxymethylglutaryl-coenzyme A (HMG-CoA) reductase, and phosphorylates mevalonic acid. The isoprenoid pathway has a number of very diverse endproducts, which include cholesterol, dolichol and ubiquinone. It also leads to isoprenylation of proteins, which is a post-translational modification directing these proteins, such as Rho and Ras, to the cell membrane [41].

Patients with classic hyper-IgD syndrome are most often compound heterozygotes for two different missense mutations in the mevalonate kinase gene (*figure 4*). Two mutations (leading to a valine-to-isoleucine change, V377I, and to a isoleucine-tyrosine change, I268T) together account for more than 85% of patients described so far [41,42]. The mutations lead to a constantly diminished activity of mevalonate kinase to about 5-15% of normal, which appears to be further reduced during a fever attack [43].

Because of this reduced enzyme activity, its substrate mevalonic acid can accumulate in serum and urine. This accumulation of mevalonic acid is predominantly observed during the fever episodes. There does not appear to be a dramatic shortage of a specific end-product; concentrations of cholesterol,
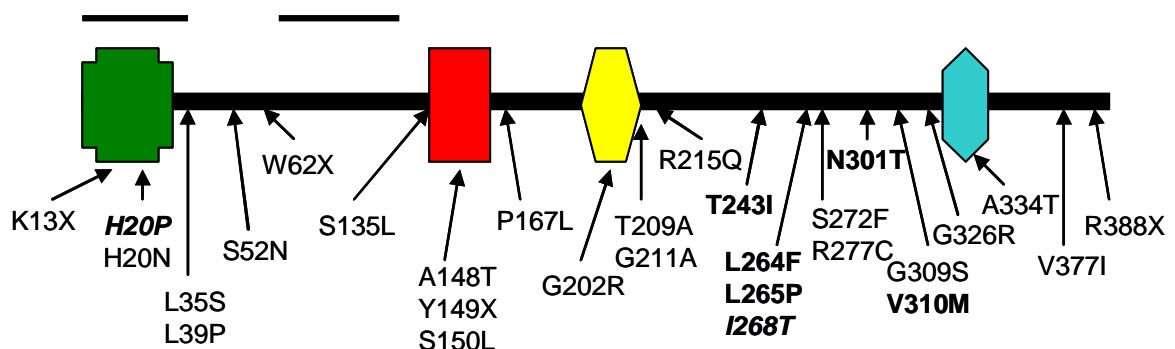
Figure 4. Mevalonate kinase, with four conserved domains represented by colored boxes. Indicated are the missense mutations, nonsense mutations and two deletions which have been identified in mevalonate kinase deficiency. In bold, mutations found in mevalonic aciduria patients, in bold and italic mutations found in both classical HIDS and mevalonic aciduria.

ubiquinone and dolichol in patients are normal to low-normal. How this metabolic defect in the isoprenoid pathway leads to the inflammatory phenotype of the hyper-IgD syndrome is still unknown. The cause of the characteristic high serum concentrations of immunoglobulin D in this syndrome, which have led to its name, are also still unexplained.

Another syndrome was already linked to mutations in the mevalonate kinase gene before the hyper-IgD syndrome [44]. Mevalonic aciduria patients carry specific mutations which cause a more severe reduction of mevalonate kinase enzyme activity, often to undetectable limits. These patients constantly accumulate large amounts of mevalonic acid, and often have more than thousand times as much mevalonic acid in the urine than hyper-IgD patients [45]. They also have a more severe phenotype, which will be described later. Evidence is emerging that mevalonic aciduria and the hyper-IgD syndrome are not two sides of a dichotomy, but rather two extremes of a continuous spectrum of disease related to mevalonate kinase deficiency.

Not all patients with HIDS appear to have an increased urinary excretion of mevalonic acid and a gene defect of mevalonate kinase. We have designated these patients as variant HIDS (*table 1*) [40].

Figure 5. Facial erythematous macules and papules in a HIDS patient during an attack

Figure 6. Petechiae on the leg of a HIDS patient during a febrile attack.

Figure 7. Aphthous ulceration detected on the tongue in a patient with HIDS. Picture courtesy of Dr. K. Antila, North Carelian Central Hospital, Joensuu, Finland.

Clinical features

Ninety percent of patients with classic HIDS will experience the first fever episode in the first year of life [46]. These episodes tend to be most frequent in childhood and adolescence, and the high fever may result in febrile seizures, especially in young children. Vaccination, minor trauma, surgery and physical or emotional stress are factors which seem able to provoke a fever episode, though often a

triggering factor is not obvious. The fever episode often starts with cold chills and a sharp rise in body temperature [4]. It is almost always accompanied by (cervical) lymphadenopathy and abdominal pain with vomiting and diarrhea. Other frequent symptoms are headache, myalgia and arthralgia. Apart from the lymphadenopathy, physical signs frequently consist of splenomegaly and a skin rash with erythematous macules and papules (*figure* 5) or petechia (*figure* 6) [47]. Sometimes there are also signs of frank arthritis, mostly of large joints, and hepatomegaly. About forty percent of patients report painful aphthous ulcers in the mouth, vagina or on the scrotum (*figure 7*). The fever will disappear spontaneously after 3 to 5 days, though it may take longer before the symptoms of joints or skin disappear completely. These inflammatory attacks occur on average once every 4 to 6 weeks although this may be very variable from patient to patient or in an individual patient (*figure 1*). The phenotype of variant HIDS differs slightly from and is milder than that of classic HIDS [40].

Patients with mevalonic aciduria, the metabolic disorder which is also caused by mevalonate kinase gene mutations, experience similar inflammatory episodes as hyper-IgD patients, but these are often of lesser importance in comparison with the severity of the rest of the phenotype. This phenotype consists of psychomotor retardation, ataxia, failure to thrive, cataracts and dysmorphic facies, and patients usually die in early childhood [45].

The long-term outcome in both classic and variant hyper-IgD syndrome is relatively benign. No cases of amyloidosis have been reported in the literature, nor has this occurred in the 190 patients in the International Nijmegen HIDS registry since its initiation in 1992 [4]. There is no excess mortality in this patient group. In many patients, the fever episodes occur less frequently and become less severe later in life. Joint destruction is extremely rare. Abdominal adhesions are more frequently seen, resulting from repeated abdominal inflammation and/or (unnecessary) diagnostic laparotomy because of suspected "acute abdomen".

Evaluation and management

HIDS is diagnosed on a combination of characteristic clinical findings and continuously elevated immunoglobulin D (IgD) concentrations (more than 100 IU/mL) (*table 4*). However, IgD values may be normal in very young patients (especially those less than three years old) [48] and persistently low levels have been reported in a small number of patients with typical clinical findings and the genotype for HIDS [3]. More than 80 percent of HIDS patients combine a high concentration of IgD with high IgA levels [48,49]. During fever attacks, a brisk acute-phase response is observed, which includes leukocytosis, high levels of SAA and CRP, and activation of the cytokine network [50]. The diagnosis of classic HIDS can be confirmed by DNA analysis of the mevalonate kinase gene. The best approach is to start with screening for the two most prevalent mutations, V377I and I268T. If this screening is negative but the clinical suspicion remains very high, sequencing of the entire gene can be considered. An alternative is the measurement of urinary mevalonic acid concentrations during an attack, which are slightly elevated. However, gas chromatography–mass spectroscopy is necessary to detect this slight increase [51]. The measurement of mevalonate kinase enzyme activity is complicated and time-consuming, and is mostly reserved for scientific research purposes.

Table 4. Diagnostic indicators of HIDS

At attacks
> Elevated ESR and leukocytosis
> Abrupt onset of fever (≥38.5°C)
> Recurrent attacks
> Lymphadenopathy (cervical)
> Abdominal distress: vomiting, diarrhoea, pain
> Skin manifestations (erythematous macules and papules)
> Arthralgias / Arthritis
> Splenomegaly

Constantly present
> Elevated IgD (≥ 100 U/mL) measured at 2 occasions with at least one month apart[a]
> Elevated IgA (≥2.6 g/L)
> *Classic HIDS:* mutations in mevalonate kinase gene
> *Classic HIDS*: decreased mevalonate kinase enzyme activity

[a](Extremely) high serum concentrations of IgD are characteristic but not obligatory.

Treatment

There is no efficacious therapy for the hyper-IgD syndrome. Some individual patients have been reported to have benefited from treatment with corticosteroid, colchicine, intravenous immune globulin or cyclosporin, but these results can not be repeated in the majority of patients [46]. Thalidomide did not have an effect on disease activity in a recent placebo-controlled trial [52]. Preliminary results of a double-blind placebo-controlled trial that we performed with the HMG-CoA reductase inhibitor simvastatin in a small group of HIDS patients indicate a beneficial effect of this drug, reducing the number of days illness by approximately 50 percent.

TNF-receptor associated periodic syndrome (TRAPS)

Epidemiology

TNF-receptor associated periodic syndrome (TRAPS, MIM142680) has an autosomal dominant inheritance pattern. It was originally described in a large family from Irish and Scottish descent as "Familial Hibernian Fever" [53]. It is primarily found in patients originating from north-western Europe, but has also been described in families from Australia, Mexico, Puerto Rico, Portugal, and the Czech Republic [54], and it is clear that any ethnic group may be affected. Other previous nomenclature for this syndrome include "autosomal dominant familial periodic fever" [55] and "familial perireticular amyloidosis" [56].

Etiology

Mutations are found in the gene for the type I TNF-receptor (TNFRSF1A), which is located on the short arm of chromosome 12 [7]. These are mainly single-nucleotide missense substitutions, located in exons 2, 3 and 4, which encode for the extracellular domain of TNFRSF1A. Many disrupt one of the highly conserved cysteine residues involved in extracellular disulfide bonds of the 55-kD protein (*figure 8*).

These mutations are supposed to be gain-of-function mutations, leading to increased TNF-α signalling through the TNF-receptor. TNF-α is a pleiotropic molecule, which induces cytokine secretion, activation of leukocytes, fever and cachexia [57]. Activation of the receptor by TNF-α causes cleavage and shedding of its extracellular part into the circulation, where it acts as an inhibitor of TNF-α. It has been suggested that the mutations in the TNFRSF1A found in TRAPS interfere with receptor shedding, leading to continuous TNF-α signaling and, hence, uncontrolled inflammation. An in vitro shedding defect has been demonstrated in the case of a number of TRAPS mutations, but not in all of them [7,58]. Also, serum concentrations of the shedded soluble TNFRSF1A in TRAPS patients during periods without symptoms are often found to be significantly reduced compared to normal subjects [54,58,59], but not in all cases. Thus, the hypothesis of reduced shedding, although attractive by its simplicity, is not supported as the sole cause of the fever attacks in TRAPS, and additional mechanisms seem to be at work.

There are some general genotype-phenotype correlations, especially when mutations are grouped in cysteine and non-cysteine mutations. Non-cysteine mutations have overall a lower penetrance than cystein mutations, and amyloidosis is seen far more often in association with cystein mutations [58]. Two missense mutations in TNFRSF1A, P46L and R92Q, have a particularly low penetrance, and are found in approximately 1% of control chromosomes [58,58]. Especially R92Q has been observed in higher prevalence in for example a group of patients with arthritis. It is thought that the clinical manifestations of patients with an R92Q mutation depend on other so far unidentified modifying genes and/or environmental factors [58,58].
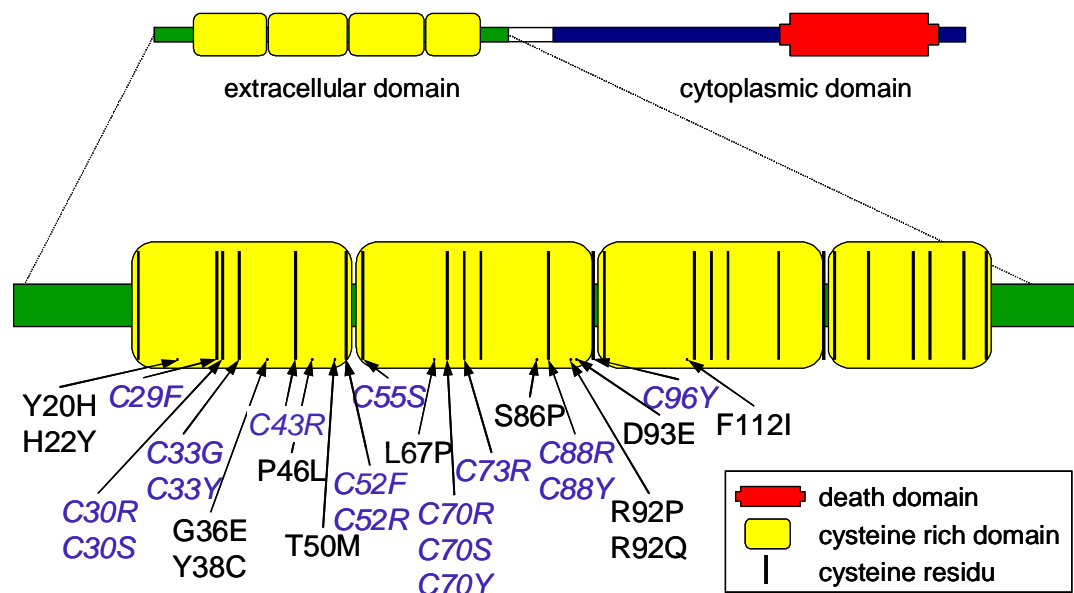

Figure 8. Schematic representation of the TNF-receptor type 1 protein (TNFRSF1A), depicting all mutations found in TRAPS up to this time (except for one intron mutation affecting a splice site). Mutations disrupting cysteine residues are in blue italics.
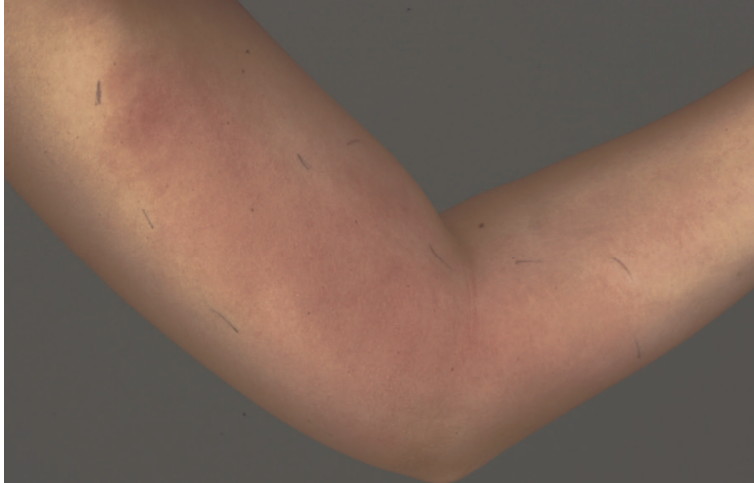
Figure 9.

Migrating erythematous rash during a TRAPS attack. Picture courtesy of Dr. T. Fiselier, University Medical Center St. Radboud, Nijmegen, The Netherlands.

Clinical features

The clinical features can vary much more between individual TRAPS patients than is generally seen in FMF or HIDS [54]. The age of onset can be very variable, even within the same family, with a range of 2 weeks to 53 years of age [54,60]. There is also a large variation in duration and frequency of the fever episodes in TRAPS. On average, attacks last 3 to 4 weeks and recur 2 to 6 times each year, but episodes may also be limited to a few days (*figure 1*). While the index patient through whom the diagnosis is made often displays well-defined inflammatory attacks, affected family members may suffer from less typical symptoms such as episodic symptoms of mild arthritis.

During inflammatory attacks, a high spiking fever can be accompanied by skin lesions, myalgia and arthralgia, abdominal distress and ocular symptoms. The most common cutaneous manifestation is a centrifugal migratory, erythematous patch, which may overlie a local area of myalgia (*figure 9*) [61], but urticaria-like plaques may also be seen. Myalgia is often located primarily in the thigh muscles, but may also migrate during the fever episode. It may effect limbs and torso, but also face and neck [54]. Arthralgia primarily affects large joints, including hips, knees and ankles. Frank synovitis is more rare, and when it does occur it is non-erosive, asymmetric and monoarticular [54]. Abdominal pain occurs in 92% of TRAPS patients during inflammatory attacks; other gastrointestinal symptoms often seen include vomiting and constipation. Ocular involvement is characteristic in TRAPS, and it may involve conjunctivitis, periorbital edema or periorbital pain, in one or both eyes. Severe uveitis and iritis has been described, and any TRAPS patient with ocular pain should be examined for these complications [54,61]. Other less frequently observed symptoms during fever attacks in TRAPS are chest pain, breathlessness, pericarditis and testicular and scrotal pain, which may be caused by inflammation of the tunica vaginalis [54,60]. It has been suggested from observation in one of the first families with TRAPS that this disorder is associated with an increased incidence of indirect inguinal hernias [59], but this has not been demonstrated in other patients. Lymphadenopathy is rare in TRAPS.

Reactive AA amyloidosis is the main determinant for the prognosis in TRAPS. It occurs in about 15-25% of patients [58,62], and generally leads to renal impairment. Amyloidosis in a patient with TRAPS places other affected family members at high risk for this complication. It is mainly associated with TNFRSF1A mutations affecting cysteine residues [58].

Evaluation and management

As in the other familial auto-inflammatory syndromes, laboratory investigations during inflammatory attacks show a clear acute phase response, and again even in between fever attacks such an inflammatory response may be measured. Autoantibodies are generally not detected in TRAPS. The IgD level may be elevated, but the value is almost always less than 100 IU/mL[59,60]. Most patients exhibit a significantly lower concentration of soluble TNFRSF1A, most prominently in symptom-free intervals, compared with appropriate controls[59], although this does not appear to be a universal rule[54]. Also, since soluble TNFRSF1A is cleared by the kidneys, TRAPS patients with renal insufficiency (e.g. due to renal amyloidosis) may have normal or elevated plasma concentrations of this protein[63].

A set of clinical diagnostic criteria was proposed by Hull et al. [54] as indicators of TRAPS (*table 5*). These are not validated by epidemiological measures, but may be used as a first step in evaluation of patients. TRAPS is ultimately a genetic diagnosis, defined by a missense mutation in the gene for TNFRSF1A. However, it must be borne in mind that clinical penetrance of TRAPS mutations is not 100%, even for cysteine mutations, and asymptomatic carriers are not uncommon.

Since proteinuria is the initial manifestation of renal amyloidosis, it is advisable to screen urine samples from TRAPS patients regularly by dipstick examination, especially affected family members from a TRAPS patient with amyloidosis.

Table 3. Diagnostic indicators of TRAPS [54]

Recurrent episodes of inflammatory symptoms spanning a period of >6 months duration (several symptoms generally will occur simultaneously)
fever
abdominal pain
myalgia (migratory)
rash (erythematous macular rash occurs with myalgia)
conjunctivitis/periorbital edema
chest pain
arthralgia or monoarticular synovitis
Episodes last >5 days on average (although variable)
Responsive to glucocorticosteroids but not colchicine
Affect family members in autosomal dominant pattern (although may not always be present)
Any ethnicity may be affected

Treatment

Both nonsteroidal antiinflammatory drugs (NSAIDs) and glucocorticoids in relatively high doses (more than 20 mg oral prednisone) are able to alleviate the symptoms of fever and inflammation in most TRAPS patients, although they do not alter the frequency of attacks. They can be used beneficially at times of attack, and glucocorticoids can usually be tapered in the course of one or two weeks, as tolerated. There is no response to colchicine or immunosuppresive drugs such as azathioprine, cyclosporin, thalidomide or cyclophosphamide [54].

With regard to the pathogenesis of TRAPS, intravenous infusion of a synthetic TNFRSF1A fusion protein was tried in one patient by Drewe et al. [54], but this seemed to provoke a severe attack. Use of etanercept, a fusion product of TNFRSF1B (the receptor that is not defective!), has been more successful [54,58,64]. A pilot study with twice weekly administration of etanercept, in a dose

of 25 mg for adults or 0,4 mg/kg for children, in 9 TRAPS patients with various mutations revealed an overall 66% reponse rate as determined by decreased number of attacks over a 6 months period [54]. A similar regimen of etanercept reversed the nephrotic syndrome in a patient with amyloidosis [65]. We have shown that use of etanercept 25 mg at the time of an attack may abort the symptoms and induce a long-lasting remission [63]. Drewe et al. have described one patient whose symptoms were resistant to administration of etanercept, who responded favourably to use of oral sirolimus (4-6 mg) [54]. Infliximab, a monoclonal antibody against TNF, has been shown to be less effective than etanercept in TRAPS.

Muckle-Wells Syndrome (MWS)/ Familial Cold Autoinflammatory Syndrome (FCAS)/ CINCA or NOMID

These three syndromes have all been traced back to mutations in one common gene, but their phenotypes differ, at least to some degree. After the recognition of the genetic defect it became clear that there is considerable overlap between these three disorders. Most notably families have been described with features of both Muckle-Wells syndrome and FCAS [66]. It has been suggested that FCAS, MWS, and CINCA/NOMID appear to represent a spectrum of disease with FCAS the mildest and NOMID/CINCA the most severe form. Given their common genotype, they will be discussed under one heading here.

Epidemiology

All three are rare, autosomal dominantly inherited syndromes. Since its original description in 1940, FCAS has been described in some 20 published reports. Most articles describe large families from Europe and Northern America with extensive pedigrees but sporadic cases have been described. There appears to be a founder effect in American families of Northern European extraction [67]. MWS has been described for the first time in 1962 and has since described in large families although it is seen in isolated cases and small nuclear families. Most families come from France and the United Kingdom[68]. CINCA/NOMID is rare and to date some 70 cases, and only a few families, have been described. Most patients stem from France and Argentina, but cases are seen in other European countries and the United States [69,70].

Etiology

The first indications that MWS and FCAS are allelic stem from early linkage studies demonstrating that both FCAS and MWS were linked to the same region on the long arm of chromosome 1 (1q44) [68,71]. In 2001 the gene for FCAS and MWS was identified. In a large scale positional cloning effort using 3 families with FCAS and one family with MWS, missense mutations in a new gene were found. This gene, CIAS1 (synonyms are *NALP3* and *PYPAF*), encodes for a protein denoted as cryopyrin, which consists of three recognized protein domains, including an N-terminal pyrin domain, a central nucleotide binding site (NBS) domain, and a leucine-rich repeat domain [72]. The protein is predominantly expressed in granulocytes and monocytes. It has been shown that overexpressed crypoyrin interacts with apoptosis-associated-specklike protein and activated NF-κB and caspase1, suggesting a proinflammatory effect. Later studies demonstrated that CIAS1 mutations were also associated with CINCA/NOMID[70,73]. All
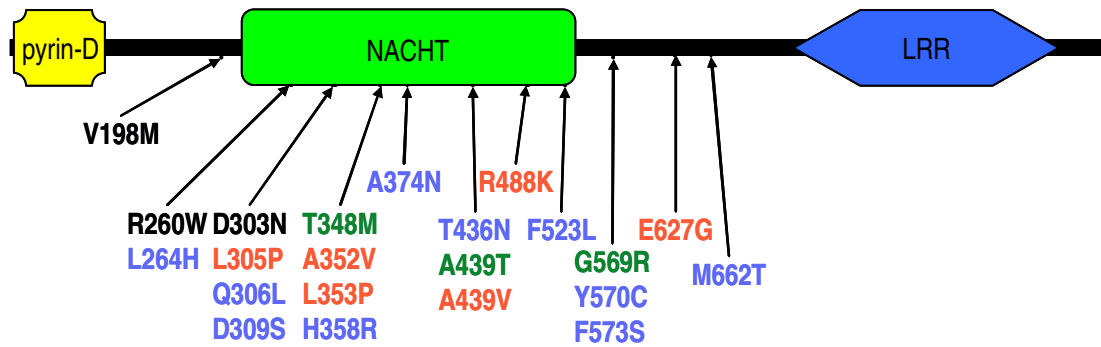
Figure 10. Cryopyrin protein, containing an N-terminal pyrin domain (yellow), a nucleotide binding site (NBS or NACHT, green), and a leucine-rich repeat domain (blue). Indicated are the missense mutations identified in patients with FCAS (red), MWS (green) or CINCA (blue), and mutations which are found in common in two or all of these clinical syndromes.

mutations ascertained so far can be found in exon 3 of the CIAS1 gene which encodes the NBS domain of crypopyrin, lending support to the suggestion that this domain is crucial in cryopyrin function. So far 21 missense mutations have been identified in these 3 disorders [8]. Some mutations occur in MWS as well as in FCAS (R260W) or in both MWS and NOMID/CINCA (D303N) (*figure 10*) [70].

Clinical features

Familial cold autoinflammatory syndrome (FCAS; MIM 120100) is characterized by episodes of rash, fever and arthralgia after generalized exposure to cold (*figure 1*). The disease occurs in large families as an autosomal dominant inherited disorder with an almost complete penetrance [67]. The rash usually starts on the exposed extremities, and in most episodes extends to the remainder of the body. It consists of erythematous macules and plaques (*figure 11, 12*), urticarial lesions and sometimes petechiae [74] and can cause a burning or "itchy" sensation. In some cases, localized edematous swelling of extremities is reported. The arthralgias, present in 93% of cases, most often affect the hands, knees and ankles, but can also involve feet, wrists and elbows [75]. Frank arthritis is not seen. The majority of patients (84%) also report conjunctivitis during a fever episode. Other symptoms which have been reported are myalgia, profuse sweating, drowsiness, headache, extreme thirst and nausea.

Figure 11. Fine confluent erythematous macules in FCAS on the upper leg. Picture courtesy of Dr. Johnstone, Medical College of Georgia, Augusta, Georgia, USA.

Figure 12. Detail of upper leg with fine confluent erythematous macules in FCAS. Picture courtesy of Dr. Johnstone, Medical College of Georgia, Augusta, Georgia, USA

Figure 13. Urticarial skin rash in a MWS patient. Picture courtesy of Dr. D.L. Kastner, National Institute of Health, Bethesda, USA

Figure 14. Urticarial skin rash on the arm of a MWS patient. Picture courtesy of Dr. D.L. Kastner, National Institute of Health, Bethesda, USA

Figure 15. Severe deformative arthropathy of the knees in a patient with CINCA/NOMID. Picture courtesy of Dr. A.M. Prieur, Hopital Necker-Enfants Malades, Paris, France

Figure 16. Radiograph of the knee in CINCA/NOMID showing greatly enlarged epiphyses and patella with punctate increased density. Picture courtesy of Dr. A.M. Prieur, Hopital Necker-Enfants Malades, Paris, France
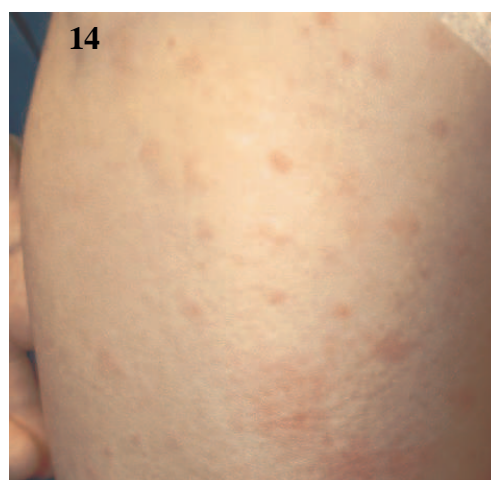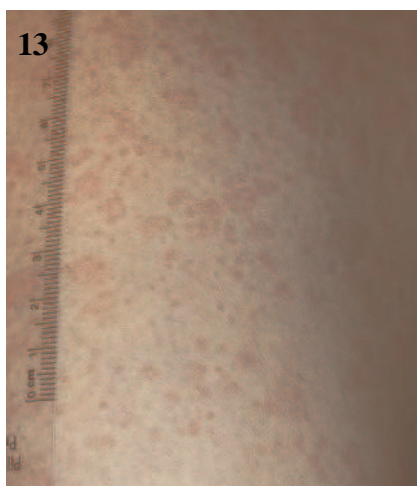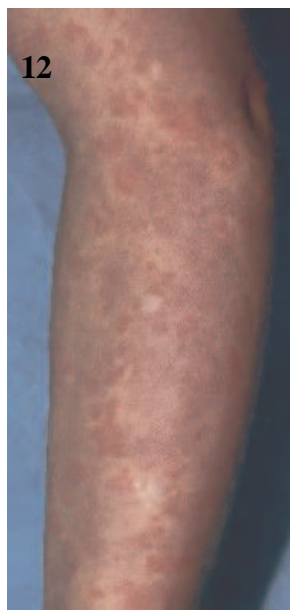
A typical feature of FCAS is the requirement of cold exposure to elicit the symptoms; the delay between cold and onset of symptoms reported in a study by Hoffman et al [75] varied from 10 min to 8 hours. The subsequent fever attack can be variable in length, also depending on the degree of cold exposure; generally it will last a few hours to a maximum of three days. These episodes will start at an early age, 95% of patients having had their first fever episode in the first year of life; 60% even within the first days. The symptoms tend to become less severe with advancing age [74]. Type AA amyloidosis complicated by renal insufficiency has been described in 3 FCAS families [75].

Muckle-Wells syndrome (MWS; MIM 191900) is a rare autosomal dominant inflammatory disorder with incomplete penetrance. Patients suffer from recurrent episodes with fever, abdominal pain, myalgia, urticarial rash (*figure 13, 14*) and conjunctivitis frequently accompanied by arthralgias and/or arthritis with limb pain. Attacks start in adolescence and can be provoked by hunger, tiredness, and sometimes by exposure to cold [76]. The inflammatory episodes generally last 24 to 48 hours (*figure 1*) and start with ill-defined malaise and transient chills and rigour followed by aching and lancinating pains in distal limbs and larger joints. Arthralgia is a common feature of the attacks, but synovitis of the large joints is less common [77]. The rash consists of usually aching and sometimes pruritic erythematous papules of 1-7 cm in diameter. In a few cases genital and buccal aphthous ulcers have been seen [78]. Ocular symptoms may include uveitis and conjunctivitis. Symptoms typically start in adolescence, although debut at earlier age can be seen. Late onset development of perceptive deafness is common in MWS. Bone involvement such as clubbing of nails and pes cavus can be seen as well. Most often, patients have a positive family history for the disease indicative for autosomal dominant inheritance, but isolated cases have been reported. The most feared complication of the inflammatory attacks is type AA amyloidosis which mostly affects the kidneys first and will lead to proteinuria and subsequent rapid progression to renal failure.

Chronic infantile neurological cutaneous and articular syndrome (CINCA; MIM 607115) is a rare congenital disorder defined by the presence of a triad of (a) neonatal onset of skin lesions, (b) chronic aseptic meningitis and (c) recurrent fever along with joint symptoms [79]. CINCA is also known in the Anglo-Saxon literature as the "neonatal onset multisystemic inflammatory disease (NOMID) " and appears to be an autosomal dominant inherited disorder [70]. The key clinical feature of CINCA is a skin rash accompanied by peculiar joint manifestations and central nervous system involvement.
The symptoms in CINCA/NOMID present right after birth or in the first monts of life most notably with a generalised skin rash. The disease follows an unpredictable course with persistent non-pruritic and migratory rash with fever, hepatosplenomegaly and lymphadenopathies. Central nervous sytem involvement is not clear from the outset, unless patients present with symptoms such as seizures, spasticity, or transient episodes of hemiplegia. In the majority of patients there are signs of chronic persistent aseptic meningitis [80]. Cerebral fluid analysis may show mild pleiocytosis and there may be an increased intracranial pressure. Brain imaging shows mild ventricular dilatation, prominent sulci and central atrophy, and in longstanding cases calcifications of faulx and dura. In older children, headache is often a prominent feature as a sign of chronic meningitis.

There is mental retardation in almost all cases. Progressive sensoneural impairment leading to high frequency hearing loss can be seen in a minority. Ocular manifestations are prominent with optic disc changes such as optic disc edema, pseudopapilledema and optic atrophy, and anterior segment manifestations such as chronic anterior uveitis [69]. These symptoms may lead to visual impairment. Hoarseness, especially in older children, is typical. Osteoarticular symptoms are a prominent feature of CINCA and these present as bone inflammation giving rise to major arthropathies due to epiphyseal and metaphyseal disorganisation. Growth cartilage alterations such as enlarged epiphyses and patellar overgrowth can be an impressive feature of the disease (*figure 15, 16*). Erosive changes occur, especially in the phalanges of hands and feet. There are typical dysmorphic features such as frontal bossing and a saddle nose. These common physical features are the reason that CINCA/NOMID patients give the impression that (totally) unrelated patients are siblings. The prognosis of these patients is grave as 20% die in childhood because of infections, vasculitis and amyloidosis [79].

Evaluation and management

Again, most important for diagnosis are patient and family history (*table 1*). Hoffman et al suggested a set of diagnostic criteria for FCAS [75] after studying six large families with this syndrome (*table 6*), but these have not been validated in an independent cohort. Laboratory examination during a fever episode in FCAS, but also MWS and NOMID/CINCA will show an acute phase response with polymorphonuclear leukocytosis and raised sedimentation rate but this will not differentiate between theperiodic fever disorders. Symptoms such as an urticarial rash after cold exposure highly favor a diagnosis of FCAS.. The ice cube test, i.e. holding an ice cube to a patch of skin to provoke urticaria, which is diagnostic in acquired cold urticaria, however is negative in FCAS. Typical facial features as frontal bossing and a long pediatric history including chronic aseptic meningitis will point to NOMID/CINCA There appears to be genetic heterogeneity in NOMID/CINCA since not all patients have CIAS1 mutations.

Table 6. Diagnostic criteria for Familial cold autoinflammatory syndrome (FCAS) [75]

Recurrent intermittent episodes of fever and rash that primarily follow generalized cold exposures.
Autosomal dominant pattern of disease inheritance.
Age of onset <6 months of age.
Duration of most attacks <24 hours.
Presence of conjunctivitis associated with attacks.
Absence of deafness, periorbital edema, lymphadenopathy, and serositis.

Treatment

No specific treatment can be offered for patients suffering from FCAS, MWS or CINCA/NOMID. In FCAS, some patients benefit from high-dose corticosteroids, and NSAIDs may be effective in selective cases [75]. Most effective is the avoidance of precipitating factors such as exposure to cold. In MWS colchicine was effective in controlling arthritic events in one patient [81] but failed in other patients [76,82]. Oral corticosteroids may provide relief for relief of skin and joint symptoms but the role for azathioprine and ciclosporin is less clear [76,83]. CINCA/NOMID is a chronic remitting disease and while corticosteroids may have a beneficial effect on fever, pain and general wellbeing they will not induce a

remission. NSAIDs, disease modifying antirheumatic drugs and cytostatics do not affect the disease course.

Other familial auto-inflammatory syndromes

Two recent suggested additions to the group of familial autoinflammatory syndromes are Blau syndrome and PAPA syndrome.

Blau syndrome (MIM 186580) is an autosomal dominant disorder characterized by recurrent granulomatous inflammation with a varying age of onset [84]. Typical granulomatous inflammations are acute anterior uveitis, arthritis and skin rash; other symptoms include camptodactyly, cranial neuropathies, fever, and arteritis [85]. Blau syndrome is caused by mutations in the NOD2/CARD15 gene [85,86]. The NOD2/CARD15 protein is thought to have a function in regulation of apoptosis. Polymorphisms in the same NOD2/CARD15 gene on chromosome 16 are associated with a susceptibility to Crohn's disease [87]; the risk of developing Crohn's disease is increased up to 40-fold in persons homozygous for these polymorphisms. There are certainly some shared features between the two diseases: both are characterized by granulomatous inflammation, and while bowel inflammation is not seen in Blau syndrome, Crohn's disease can present with uveitis, arthritis and skin rash.

PAPA syndrome (MIM 604416) is an acronym for Pyogenic sterile Arthritis, Pyoderma gangrenosum and Acne syndrome, an autosomal dominant disorder first described by Lindor et al. [88]. The episodic inflammation in this syndrome will lead to eventual destruction of joints, muscle and skin. Wise et al. identified mutations in the CD2-binding protein 1 (CD2BP1) gene linked with PAPA syndrome [9]. CD2BP1 has a role in actin reorganization during cytoskeletal-mediated events, and as such is hypothesized to have a functional relationship with pyrin.

Summary and conclusion

The familial auto-inflammatory syndromes are characterised by recurrent episodes of fever and inflammation. This group of disorders needs to be considered in a patient with a history of years of such inflammatory attacks with symptom-free intervals in between (except for CINCA/NOMID, in which some symptoms and morphological features will persist).

The discovery of the causative genes has had an enormous impact in the field of periodic fever . This has been made possible only because of the accurate phenotypical characterisation of patients with periodic fever. Careful analysis and proper clustering of these patients is indispensable in order to allow the elucidation of the genetic background as well as the evaluation of possible treatment options. This is greatly stimulated by development of central periodic fever registries, which afford the opportunity to appreciate of previously unrecognised symptoms, to give insight in the long-term prognosis and to allow better evaluation of drug regimens. Even yet, despite recent research, many periodic fever patients do not fall in one of the above-mentioned disease categories. It is to be expected that in the future other periodic fever syndromes and corresponding genes will be discovered.

Internet resources

HIDS.net - http://hids.net

Online Mendelian Inheritance in Man –
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM

INFEVERS – the repertory of FMF and hereditary inflammatory disorders mutations - http://fmf.igh.cnrs.fr/infevers/

ORPHANET – a database dedicated to information on rare diseases and orphan drugs - http://orphanet.infobiogen.fr/

METAFMF – a meta-analysis for the study of FMF phenotype-genotype correlation - http://fmf.igh.cnrs.fr/metaFMF/

GeneDx – provide mutation testing in rare disorders – http://genedx.com

Molecular analysis of MVK mutations and enzymatic activity in hyper-IgD and periodic fever syndrome

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Abstract

Hyperimmunoglobulinemia D and periodic fever syndrome (HIDS) is an autosomal recessive inflammatory disorder characterized by recurrent episode of fever associated with lymphadenopathy, abdominal distress, joint involvement and skin lesions. We recently demonstrated that mutations in the mevalonate kinase gene (*MVK*) are associated with HIDS. Direct DNA sequencing was done to screen the entire coding region of *MVK* in 25 unrelated patients with HIDS. Mutations were detected in the coding region of the gene including 11 missense mutations, one deletion, the absence of expression of one allele, as well as 3 novel polymorphisms. Seven of these mutations are novel. The large majority of the patients were compound heterozygotes for two mutations. Of these, V377I (G->A) is the most common mutation occurring in 20 unrelated patients and was found to be associated with I268T in 6 patients. Mutations were associated with a decreased of mevalonate kinase (MK) (ATP: mevalonate 5-phosphotransferase, EC 2.7.I.36) enzymatic activity but not as profound as in mevalonic aciduria, a syndrome also caused by a deficient activity of MK. In HIDS the mutations are located all along the protein which is different from mevalonic aciduria where MK mutations are mainly clustered to a same region of the protein. On the basis of this study, we propose that the diagnostic screen of *MVK* in HIDS should be first directed on V377I and I268T mutations. Three patients are also described to illustrate the genotypic and phenotypic overlap with mevalonic aciduria.

2

Introduction

The hyperimmunoglobulinemia D and periodic fever syndrome (HIDS [MIM 260920], http://www.hids.net) is an autosomal recessive disorder with recurrent febrile attacks as its main feature [2,3,46,89]. During these attacks patients also suffer from a variable presence of lymphadenopathy, arthralgias, splenomegaly, skin lesions and headache. Symptoms start in early childhood and may persist during life. So far, 13 HIDS families with two or more affected siblings are known to us. The elevated serum IgD (to values beyond 100 IU/ml) serves as convenient biochemical marker [244,100]. The clinical presentation is rather homogeneous although severity and intensity of the febrile crises may differ among patients. A recent genome wide search linked HIDS to the long arm of chromosome 12 (12q24) [2,245] and the linked interval contains the gene encoding for mevalonate kinase (*MVK*). MK, a homodimeric enzyme present in the peroxisomes of every mamalian cell, follows 3-hydroxy-3-methylglutaryl-CoA reductase in the cholesterol synthesis and converts mevalonate into 5-phosphomevalonate. A near complete deficiency of this enzyme causes mevalonic aciduria [MIM 251170]. This rare autosomal recessive disorder is characterised by developmental delay, failure to thrive, hypotonia, ataxia, myopathy, cataracts. Most notably, many patients suffer from recurrent febrile attacks with vomiting, diarrhoea, leukocytosis, elevated erythrocyte sedimentation rate, arthralgia and morbilliformic rash [44,45,246,247]. These phenotypical similarities with HIDS lead us to consider mevalonate kinase as a candidate gene for HIDS. Effective linkage and the serendipitous finding by others of minor elevated urinary excretions of mevalonic acid in a HIDS patient, led to the identification of *MVK* as the causative HIDS gene [2,3,249]. Preliminary molecular analysis revealed 4 different missense mutations (V377I, I268T, H20P and P167L), the absence of expression of one allele and a 92bp deletion in the *MVK* gene. These mutations result in a functional defect of MK and preliminary studies indicated a residual activity of 5-15%, values higher than those observed in mevalonic aciduria. At present it is unclear whether HIDS and mevalonic aciduria are extremes of a continuous spectrum or that they are truly two different entities. For example, 2 mutations (I268T and H20P) are common in HIDS and mevalonic aciduria patients, but have been reported heteroallelic with different mutations in both [100,245]. Therefore, the different nature of HIDS and mevalonic aciduria may be caused by a differential mutational spectrum, but information to this end is lacking.

Therefore, we have undertaken studies in order to gain more insight in the genotypes in a large cohort of HIDS patients. We expected to shed light on the molecular pathology of HIDS and to aid the development of an effective molecular testing strategy for diagnosis and carrier testing.

Subjects and Methods

Patients

Patients for this study were selected on the basis of previous inclusion in the Nijmegen HIDS registry [46] and the availability of Epstein-Barr immortalized cell lines. The Nijmegen HIDS registry was set up in 1992 and hosts clinical and laboratory data on these patients. Twenty five unrelated patients agreed to participate in the present study and in eight cases, father and mother were included. The numbering of the patients reflects the entry number in the Nijmegen HIDS registry. A thorough clinical examination was performed on all subjects and HIDS was diagnosed in concordance to set criteria. Biochemical proof of affected status was obtained by measurement of the IgD content in serum. The hereditary pattern in all included families was compatible with an autosomal recessive hereditary trait [46]. The study was carried out after informed consent from all individuals and formal approval was obtained by the Medical Ethical Comittee (CWOM) of the University Medical Center St. Radboud, Nijmegen, The Netherlands.

Databases

Online Mendelian Inheritance in Man (OMIM) is http://www.ncbi.nlm.nih.gov (for HIDS [OMIM 260920] and mevalonic aciduria [OMIM 251170]). Blast searches of htgs (high throughput genomic sequences) database of the National Center for Biotechnology Information (NCBI; http://www.ncbi.nlm.nih.gov) were performed using the cDNA sequence of *MVK* (GeneBank M88468).

Screening of MVK mutations

RNA was extracted from Epstein-Barr immortalised cell lines according to established protocols. Total RNA was prepared using the RNA B reagent (Bioprobe) according to the instructions of the manufactor. Four microgrammes of RNA were used to synthetise cDNA with Superscript reverse transcriptase II (Gibco BRL), followed by PCR using Platinium *Taq* DNA polymerase (Gibco BRL) in a GeneAmp PCR system 9600 (Perkin Elmer). We determined the nucleotide sequences of the amplified fragments by standart semi-automated methods on an ABI PRISM 377 (Perkin Elmer). The primers used in this study have already been described [2]. The complete coding region of *MVK* was amplified from cDNA in two overlapping fragments using primers 29/788 and 591/1381. Their sequences were determined using primers 29, 381, 535, 921, 1000 and 1381. When the homozygous status of a mutation in a proband could not be controlled by the analysis of both parents an additional PCR was performed on cDNA using primers 381/1000 followed by sequence analysis to eliminate the presence of a short deletion spanning primers 788 and 591.

MVK enzyme analysis

Mevalonate kinase enzyme activity in extracts of cultured lymphocytes was performed by a radiometric assay. DL-[Mevalonic-2-14C], DBED salt was purchased from NEN Life Science Products, Inc. Boston. Non-isotopic DL-mevalonolactone, from Sigma Chemical (St Louis, Mo.), was converted before use to the potassium salt as described [93]. Preparation of the lymphoblasts lysates was carried out as described by earlier [94]. Mevalonate kinase assay in lymphoblast

lysates, including separation of substrate and products by thin layer chromatography, was performed according to Gibson et al. [93].

Results

Clinical data

The study group consisted of 25 patients: 22 Dutch, 1 British, 1 Czech and 1 Spanish. The mean age was 30.3± 16.4 years and there was an even male-female ratio (13 female and 12 male). The clinical features of the first 50 patients in the Nijmegen HIDS registry have been described in detail elsewhere [46]. Briefly, all patients had recurrent attacks of fever (≥38.5°C) with an acute phase response with elevated erythrocyte sedimentation rate and a brisk leukocytosis. All had a constantly elevated serum IgD (>100 U/mL) measured at 2 occasions with at least one month apart, and one or more of the following symptoms during attacks; lymphadenopathy; abdominal distress (vomiting, diarrhoea, pain); skin manifestations (erythematous macules and papules); arthralgias / arthritis; and splenomegaly. Although affected siblings were available for the study we elected to study unrelated patients in order to obtain a representative survey of mutational spectrum and enzyme activities of mevalonate kinase in HIDS.

Genomic structure of the MVK gene

The genomic organization of the *MVK* gene is shown in figure 1 and was determined to help in the understanding of the deletion found in HIDS patients. We deduced it by comparison between a human genomic clone from the working draft sequencing database (accession number AC007623) and the human cDNA (accession number M88468). The gene contains 10 coding exons and one non coding exon spanning over 21kb. The size of the exons varies from 62 to 837 bp and the size of the introns from 378 to 4470 bp. The first and last base belonging to each exon is indicated in figure 1 and refers to the numbering of the human cDNA. The last exon contains 152 bp of the open reading frame (ORF) and 685 bp of the 3' UTR.
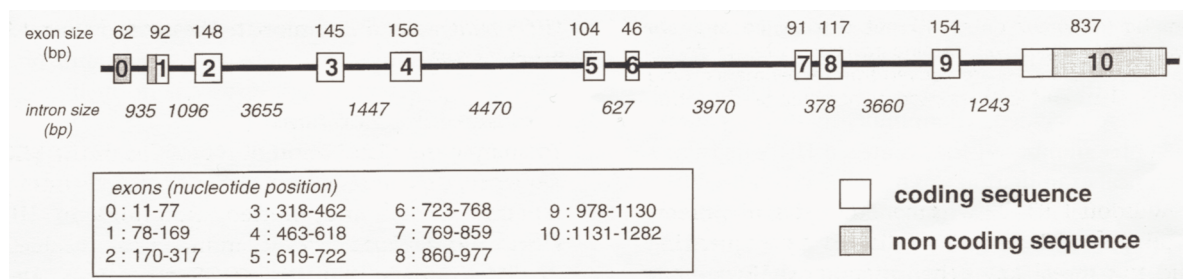

Figure 1. Genomic organization of the MVK gene. This structure has been deduced by comparison between a genomic clone (AC007623) and the human cDNA (M88468). In the large square are indicated the first and last nucleotide position belonging to each exon and refers to the numbering of the human cDNA.

Patient No	Exon	Nucleotide Change	Amino Acid Change[a]	MK Enzyme Activity[b]
1	10	1220G->A	V377I	8.9
	?	?	?	
4	8	894T->C	I268T	5
	10	1220G->A	V377I	
10	9	1016G->A	G309S	3.6
	10	1220G->A	V377I	
11	1	150A->C	H20P	8.5
	10	1220G->A	V377I	
12	2	207T->C	L39P	2.1
	10	1220G->A	V377I	
13	8	894T->C	I268T	7.2
	10	1220G->A	V377I	
15	10	1220G->A	V377I homo	4.5
	?	?	?	
17	10	1220G->A	V377I homo	28
	ND	ND	Abs RNA	
18	8	894T->C	I268T	4
	10	1220G->A	V377I	
21	1	150A->C	H20P	11.4
	10	1220G->A	V377I	
26	10	1220G->A	V377I homo	11.3
	ND	ND	Abs RNA	
31	8	894T->C	I268T	15.3
	10	1220G->A	V377I	
35	10	1220G->A	V377I homo	ND
	ND	ND	Abs RNA	
47	1	149C->A	H20N	3.5
	6	735G->A	R215Q	
52	4	591C->T	P167L	1.8
	5	695G->A	G202R	
55	8	894T->C	I268T	10.7
	10	1220G->A	V377I	
56	8	894T->C	I268T	7.7
	10	1220G->A	V377I	
61	4	540C->T	S150L	17.3
	10	1220G->A	V377I	
66	9	1067G->A	G326R	16.4
	10	1220G->A	V377I	
71	9	1091G->A	A334T	0
	?	?	?	
72	10	1220G->A	V377I homo	8
	?	?	?	
89	10	1220G->A	V377I homo	5.7
	?	?	?	
100	4	465-619del	del exon 4	8.3
	10	1220G->A	V377I	
103	8	894T->C	I268T homo	7.3
	?	?	?	
116	4	591C->T	P167L homo	9.7
	ND	ND	abs RNA	

Table 1. Mutations in the *MVK* cDNA.

del = deletion, homo = homozygote, ND = not determined, abs RNA = absence of RNA expression. a = amino acids are given in single-letter code; b = enzyme activities are derived from experiments using cultured lymphoblasts and are expressed as percentage of control.

Mutation Analysis

Of the 25 unrelated patients investigated for sequence variations in the coding region of the *MVK* gene, we were able to identify a total of 13 mutations and 3 new polymorphisms. Seven mutations are novel and have never been described before. Mutations were detected in all exons except in exon 3 and 7 (table 1). Most of the mutations are missense mutations; nonsense mutations were not found. The position of the affected nucleotide refers to the human *MVK* cDNA (accession number M88468). Where available both parents were studied (patients: 17, 21, 26, 31, 35, 47, 100, 116).

Missense Mutations

We found 7 new mutations: R215Q, L39P, G202R, G326R, G309S, S150L and H20N. Each of them was detected on only one allele in 7 unrelated patients indicating that they are not very frequent in HIDS. Among the previously reported *MVK* mutations we detected the 4 known HIDS missense mutations V377I, I268T, H20P and P167L, and surprisingly one patient carried the A334T allele which previously only had been described in mevalonic aciduria. V377I, which is located at the C-terminal end of the protein, was found in 20 of 25 patients and therefore constitutes the most frequent mutation in HIDS. I268T occurred in 7 unrelated patients and is the second more frequent mutation in HIDS. V377I and I268T were found heteroallelic in 6 patients. Interestingly, one patient carrying I268T was found homozygous for that mutation. The status of the parents could not be determined as they both were dead at the start of the study. Patient 116 was found homozygous for P167L.

Absence of expression of one allele

Analysis of the parents of this last patient (patient 116) showed that the mother was heterozygote for P167L and that the father displayed a normal allele, c suggesting that the second mutation in the proband led to the absence of expression of the second allele. In the group of the 20 patients carrying V377I ([1220G->A]), six were found to be homozygous upon examination on cDNA. For only 3 of them, both parents were available (patients 17, 26 and 35) and tested. In these 3 families, one parent was heterozygous G/A at position 1220 whereas the other parent was found to be homozygous for the normal G at the same position. This reveals that in these three families one parent and the patient also carry an unknown mutation on the second allele that leads to the absence of expression of mRNA. This observation has been reported in our first study [2]. Unfortunately, patients 15, 72, 89 could not been investigated further because samples from their parents and genomic DNA from these 3 patients were not available. Additional RT-PCR using other sets of primers spanning primers 788 and 591 followed by sequencing analysis did not reveal any other alterations. However it cannot be excluded that these patients carry a mutation spanning the 5' or 3' end of the cDNA that would prevent the primer annealing.

Deletions

In patient 100 we found a deletion spanning from nucleotide 465 to 619. Comparison with the deduced genomic organization reveals that this mutation corresponds to the complete deletion of exon 4 suggesting that this patient would

carry a mutation affecting the splicing of the transcript. The same deletion was found in her healthy father, whereas the second mutation of the patient (V377I) was transmitted by her healthy mother. The determination of the genomic structure allowed us to conclude that the 92 bp deletion we reported for family 13 in our previous study [46] corresponds to a complete deletion of exon 1 suggesting that this patient would also carry a mutation affecting the splicing of one transcript.

Polymorphisms

Three novel polymorphisms were identified. A 601C->T transition within exon 4 resulting in a silent change (170D->D) was found in 5% of the individuals analyzed. Another polymorphism, 246G->A with a resulting 52S->N, was detected in 10% of individuals. Although this modification changes the amino acid sequence, we expect it to be silent because it was found in one healthy mother and one independent healthy father (both carrying another heterozygous mutation). In addition, it did not segregate with HIDS as it was not transmitted to their respective affected children. The functional significance of this polymorphism is not known. In addition to these missenses, a 1336insG was found. This polymorphism was located 50 bp after the stop codon and therefore did not affect the coding sequence. It was found in 5% of individuals including a healthy father (carrying another heterozygous mutation) who did not transmit this polymorphism to his affected. None of these rare polymorphisms were specifically associated with a mutated allele and therefore did not give any information about a potential founder effect in the disease.

MK activity

Analysis of the MK enzyme activities in cultured lymphoblasts were greatly affected in HIDS patients. When we excluded patient 71, who had a nearly absent MK activity, the average activity was decreased to 9.2% (SD 6%) of that of healthy controls (table 1). There was a great variation among HIDS patients and enzyme activities varied from 1.8% to even 28%.
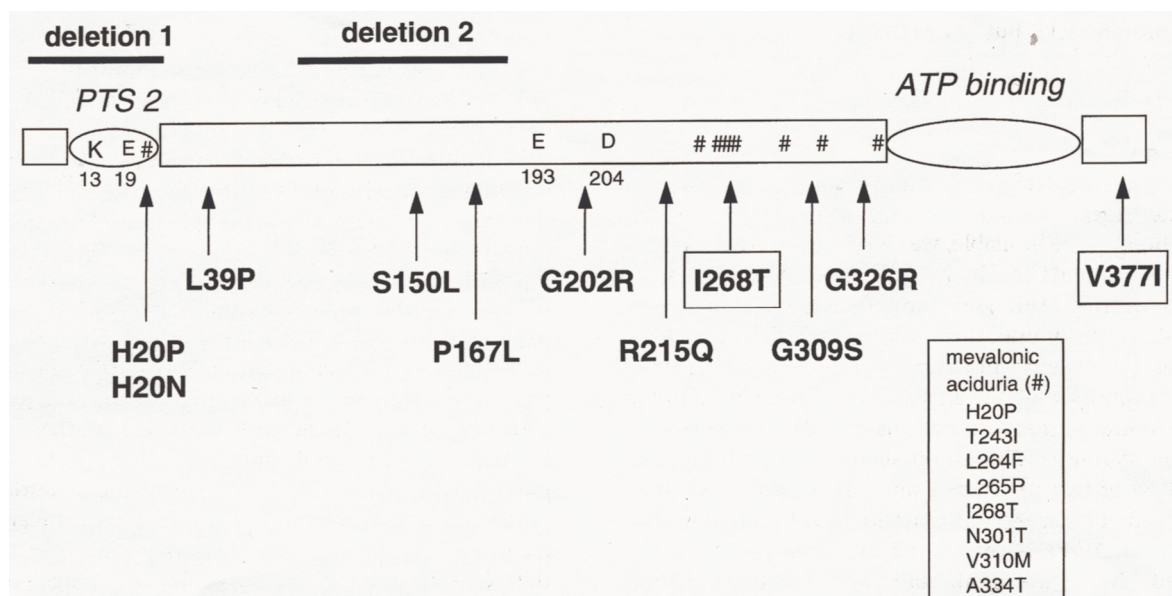
Figure 2. Summary of mutations identified in HIDS and mevalonic aciduria patients [1]. The bar depicts the MK protein. PTS2 homology domain and putative ATP binding domain are indicated. K13, E19, E193 and D204 are known important functional amino acids [12]. Deletion 1 refers to our previous report [1] and deletion 2 refers to this study. HIDS mutations are indicated in bold characters and mevalonic aciduria mutations (#) are summarized in the large square.

Position of the mutations

To analyze the distribution of mutations in the MK protein sequence, we combined our data with results from previous reports [2,3,44,100,245,247] and plotted mutations in HIDS and mevalonic aciduria against amino acids residues (fig. 2). This shows that the HIDS mutations are evenly distributed along the coding region of *MVK*, which contrasts to that of mevalonic aciduria which essentially clusters between amino acids 243 and 334. Except for H20P and I268T, none of the mutations are common between HIDS and mevalonic aciduria. MK contains a peroxisomes targeting sequence 2 (PTS2) domain in the N terminal part and an ATP binding site in the C terminal part. Four amino acids (K13, E19, E193 and D204) have been shown to play a crucial role in the enzymatic activity of the protein [248,249] and some of the HIDS mutations are very close to these important amino acids.

Genotype-phenotype correlation

To evaluate the functional significance of the mutations identified we undertook a careful evaluation of the phenotypes and compared them with the different types of the mutations observed. Patients were evaluated for age of onset of disease, frequency and length of attacks and symptoms accompanying the attacks. Laboratory data with emphasis on immunoglobulins and parameters of the acute phase response were monitored.

We compared the phenotype of those who were compound heterozygote for V377I to the 5 patients who did not carry this mutation. The frequency or severity of febrile attacks did not differ between V377I positive and negative patients, nor was there a difference in symptomatology during attacks.

One patient, a 39-year-old woman (#71) carried an A344T mutation on one allele. This mutation is thought to be associated with mevalonic aciduria [247]. She had typical febrile attacks from birth onwards but also developed a tapetoretinal blindness and optical nerve atrophy at the age of 2. After an episode of measles with encephalitis she developed a clinical picture of severe mental retardation, cerebellar ataxia, athethosis, and microcephalia with epilepsy. She continued to have febrile attacks. Immunoglobulins are elevated: IgD (465 IU/ml) and IgA (4 g/L). The MK enzyme activity was <1% and the excretion of mevalonic acid in urine was strongly elevated 7200 mmol/mol creatinine. On the basis of the massive urinary mevalonic acid excretion and the complete absence of enzymatic activity she must now be classified as having mevalonic aciduria instead of HIDS. It is likely that the severe neurological symptoms fit with mevalonic aciduria and cannot be attributed as a sequel to measles encephalitis.

Another patient (#15) had a similar phenotype as patient 71 with mental retardation, tapetoretinal blindness, cataracts, and ataxia. In contrast he has the V377I mutation, the MK activity is 4.5% of controls and during remission there is no excretion of mevalonic acid. This labels him as HIDS. Patient 47, a 20-year-old man who is a compound heterozygote for H20N and R215Q has very severe phenotype. He has repetitive febrile attacks with deforming arthritis. Patient 52 who was compound heterozygote for P167L and G202R died of staphylococcal pneumonia at the age of 30 but she did not have any attacks in the 18 months preceeding her death. Patient 103, a 40-year-old male is homozygote for I268T. Neurological examination is completely normal and he has no dysmorphic features. He has a very mild disease with only 3-4 febrile attacks per year. The

sequence variation has been reported before in mevalonic aciduria [100]. The last patient (#116) is a 10-year-old girl who is a compound heterozygote for the P167L mutation and has the absence of expression of the second allele. She has regular febrile attacks but growth and development is unremarkable.

Discussion

Our study presents detailed clinical and mutation analysis data on the largest cohort of patients with HIDS. Although affected siblings were available, we elected to study a total of 25 unrelated patients to obtain a representative survey of mutations in the *MVK* gene and enzyme activity. We identified 13 different mutations, 7 of which were novel, comprising 11 missense mutations, one deletion and the absence of expression of one allele. Three novel polymorphisms were found. Missense mutations of *MVK* were detected at the heterozygous stage on both alleles of 14 patients and on one allele of 2 patients (#1 and #71). In these two last patients the first heterozygous mutation was localized in the 3' part of the cDNA and the second mutation could not be determined. The disease manifestation in these two patients may be explained by a short deletion overlapping the forward primer of the 5' part of the cDNA or by a mutation in intronic sequences as these were not addressed in this study. We consider patient 1 as typical HIDS because she possesses the most frequent *MVK* mutation (V377I) and displays the classical features of HIDS, being the first HIDS patient diagnosed [1]. This others studies allow us to draw a more complete picture of the biochemical and mutational differences between HIDS and mevalonic aciduria. In our HIDS cohort the MK enzyme activities vary widely and, as we previously observed [2], the lower values may overlap with those observed in mevalonic aciduria [45].

However, in mevalonic aciduria, there are very high concentrations of mevalonic acid present constitutively in all bodyfluids, most notably in urine. This is clearly different from HIDS, where only a minor elevated excretion of urinary mevalonic acid can be observed with attacks. In our cohort we detected a patient with grossly elevated urinary mevalonic acid excretion. This patient (#71), a 39-year-old woman presented with febrile attacks typical for HIDS but was heterozygous for A334T, an allele that already had been reported in patients with mevalonic aciduria. On basis of the increased excretion of mevalonic acid in urine we label her now with mevalonic aciduria. Another patient (#15) with similar phenotypical features carries the V377I mutation and lacks mevalonic aciduria. Both patients illustrate the phenotypic overlap between mevalonic aciduria and HIDS. Patient #71 probably represents a mild phenotype of mevalonic aciduria and most notably, she has an unusual long survival. The course of this syndrome is almost invariably fatal and although some patients may reach late adolescence, survival beyond 35 years has not been described before [45]. Patient 15 is a prototype of a severe classical HIDS patient.

Interestingly, mutations H20P and I268T which are shared by both HIDS and mevalonic aciduria diseases were detected in our sample. In most HIDS patients we found it in association with V377I which suggests that this mutation is responsible for the HIDS phenotype. This corroborates with data generated by expression of *MVK* cDNA's in *E. coli* lysates which only showed a decrease of MK enzyme activity to approximately one-third of controls [3]. In previously reported mevalonic aciduria cases, H20P and I268T were found associated with A334T and N301T respectively [100,245] suggesting that these two last mutations

would determine the severity of the phenotype. However, our finding of a HIDS patient who is homozygous for I268T is puzzling given the fact that homozygosity for this mutation was previously found in a patient with very severe mevalonic aciduria. The patient had a nearly absent MK activity and died at the age of 4.5 months [100]. It is intriguing that our HIDS patient lacks the phenotypical characteristics of mevalonic aciduria such as psychomotor retardation, hypotonia, myopathy and ataxia. He has a residual enzyme activity of 7.2%, far higher than that in mevalonic aciduria. These findings provide the first evidence that the two diseases can share the same *MVK* mutations. This suggests that not only these specific mutations but also other, as yet undetermined, factors are important for the resultant phenotype.

Our study also clearly demonstrates that the V377I mutation, as a consequence of a G to A transition, is the most frequent HIDS mutation as it was detected in 20 patients. We also provide evidence that this mutation is not systematically associated with the HIDS phenotype since 4 patients did not possess the mutant V377I allele. The second most frequent mutation in HIDS is I268T and was found in 7 patients. In 6 of them, patients were compound heterozygote with V377I. On basis of these findings we recommend that a diagnostic screen of the *MVK* gene for HIDS mutations should first be directed on V377I and I268T because they both constitute the most common mutations in HIDS. Since V377I abolishes a *BsmAI* site [2] the detection of V377I with restriction enzymes can be done rapidly and this probably represents a very useful tool for molecular diagnosis of HIDS.

Seven patients were found to display a homozygous mutation using cDNA. For 4 of them, analysis of the both parents allowed us to demonstrate that they carry a second unknown mutation that led to the absence of expression of the second allele. We previously reported such observation [2] and the detection of 4 additional patients here suggests this is a relatively frequent event in HIDS. The finding of one common mutation (V377I) suggests however that our families are distantly related and suggests that it could be a founder mutation. Indeed most patients described in this report stem from the Netherlands that is in concordance with the founder hypothesis. Unfortunately the rare polymorphisms found in the cDNA could not address this question and therefore this issue must be directed by analyzing haplotypes generated by microsatellite markers or by single nucleotide polymorphisms within the gene.

Acknowledgements

Molecular analysis of the mevalonate kinase gene in a cohort of patients with the hyper-IgD and periodic fever syndrome:

Its application as a diagnostic tool

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Abstract

Background: hyper-immunoglobulinemia D and periodic fever syndrome (HIDS) is characterized by recurrent attacks of fever, abdominal distress and arthralgia, and is caused by mevalonate kinase mutations. *Objective*: to ascertain the role of mevalonate kinase and usefulness of molecular diagnosis in HIDS. Design: cross-sectional study. *Setting*: Nijmegen HIDS registry. *Patients*: 54 patients from 41 families, who met the clinical criteria for HIDS. *Measurements*: clinical symptoms and signs, concentrations of immunoglobulins, and leukocytes, erythrocyte sedimentation rate, mutation analysis and mevalonate kinase enzyme activity assay. *Results:* There were two groups of patients: 41 patients with mevalonate kinase mutations ("classic type"), and 13 patients without mutations ("variant type"). Classic type HIDS patients had lower mevalonate kinase enzyme activities, higher IgD, and more additional symptoms with attacks. The IgD concentration did not correlate with disease severity, mevalonate kinase enzyme activity or genotype. This study suggests genetic heterogeneity in patients with a clinical diagnosis of HIDS.

Introduction

Periodic fever encompasses a group of disorders characterized by limited periods of fever that recur for years in otherwise healthy subjects. It represents a high level of discouragement and frustration for physicians and patients alike, because in many instances elaborate clinical investigations fail to substantiate the diagnosis [5,6]. Over the last few years several distinct subtypes of periodic fever have emerged [7,15,89-91], one of which is the hyper-IgD and periodic fever syndrome (HIDS) [1]. Patients with HIDS suffer from recurrent episodes of high fever accompanied by lymphadenopathy, abdominal distress and arthralgia [46] (see also website hids.net), which last several days and recur every few weeks. Most patients are from West-European stock, but patients have been identified in the United States [92]. HIDS patients have constantly elevated serum immunoglobulin D (IgD). The diagnosis is made on clinical grounds and elevated serum IgD concentrations but requires a high index of suspicion.

Two studies employing different genetic methods established that mutations in mevalonate kinase cause HIDS [2,3]. Mevalonate kinase is an enzyme which follows 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, early in the cholesterol synthesis. One study searched the genome using affected HIDS families and established linkage with the locus for mevalonate kinase [2]. Another study detected minor elevated urinary excretions of mevalonic acid and an impaired mevalonate kinase activity in a HIDS patient [3]. Both studies independently discovered disease-associated mutations in the mevalonate kinase gene in affected patients.

To ascertain the role of mevalonate kinase in HIDS, we searched for mutations within the gene and examined the enzyme activity of mevalonate kinase in 54 patients who all shared the typical clinical and laboratory features of HIDS.

Subjects and methods

Patients

Patients were selected on the basis of previous inclusion in the Nijmegen HIDS registry, an international database which at present holds data on 176 patients [46], and the fact that DNA and lymphoblast cell lines were available to us. All patients met the following criteria: repeated episodes of fever (>38.5°C) with biochemical evidence of an acute phase response, including elevated erythrocyte sedimentation rate and leukocytosis; a constantly elevated serum IgD (>100 IU/ml) measured at 2 separate occasions; and one or more of the following symptoms during attacks: lymphadenopathy, abdominal distress (vomiting, diarrhea, pain), skin lesions (erythematous macules and papules), arthralgia and/or splenomegaly.

We included 54 patients from 41 families and 12 unaffected parents of patients. Nationalities of patients included 42 Dutch, 4 French, 2 British, 2 Czech, 2 Spanish, 1 Belgian and 1 Turkish. Patients and family members were subjected to a thorough clinical examination during and in between attacks. Blood samples were collected during and in between fever episodes; laboratory results are from samples taken during remissions unless otherwise indicated. The study was carried out after informed consent from all individuals and formal approval was obtained by the Medical Ethical Committee of the University Medical Center St. Radboud, Nijmegen, The Netherlands.

Screening of mutations in the mevalonate kinase gene

RNA was extracted from Epstein-Barr immortalized lymphoblast cell lines and cDNA was produced using standard techniques. The complete coding region of the mevalonate kinase gene was amplified by polymerase chain reactions and mutation detection was done by fluorescent sequencing as described earlier [2,42].

Mevalonate kinase enzyme analysis

Mevalonate kinase enzyme activity was determined in 36 of the 54 HIDS patients, and in 12 parents of classic HIDS patients who are (obligate) heterozygous for the mevalonate kinase mutation. We employed a radiometric assay using extracts of cultured lymphocytes from Epstein-Barr immortalized cell lines as described elsewhere [93,94].

Statistical analysis

Where appropriate, the Chi square test, unpaired nonparametric Mann-Whitney test or nonparametric Spearman correlation coefficients were used for statistical analysis. Significance level, the probability of a type I error, was set at 0.05.

Results

Mutation analysis

Mutation analysis revealed mutations in the gene for mevalonate kinase in 41 of the 54 tested patients, described in more detail elsewhere [2,42]. We detected a missense mutation that is a one-base pair exchange in the gene resulting in an amino-acid change in the protein, in 64 of 82 examined alleles. A deletion of a small number of base pairs was detected in 5 alleles, while RNA was absent from 7 alleles. Of 6 alleles the pertinent mutation could not be determined. Thirty-seven of the 41 patients were compound heterozygous, that is, they possess a different mutation on each allele of the gene.

In 13 of the 54 patients no mutation of the mevalonate kinase gene could be detected even though they did exhibit the clinical phenotype of HIDS and completely fulfilled the clinical criteria. In order to detect whether mutations in the mevalonate kinase gene result in a different clinical picture, we decided to separate our cohort in 2 groups. We designated HIDS patients who met the clinical criteria for HIDS and carried mutations in the mevalonate kinase gene as "classic type HIDS", and the mutation negative patients, who did meet the clinical criteria for HIDS, as "variant type HIDS".
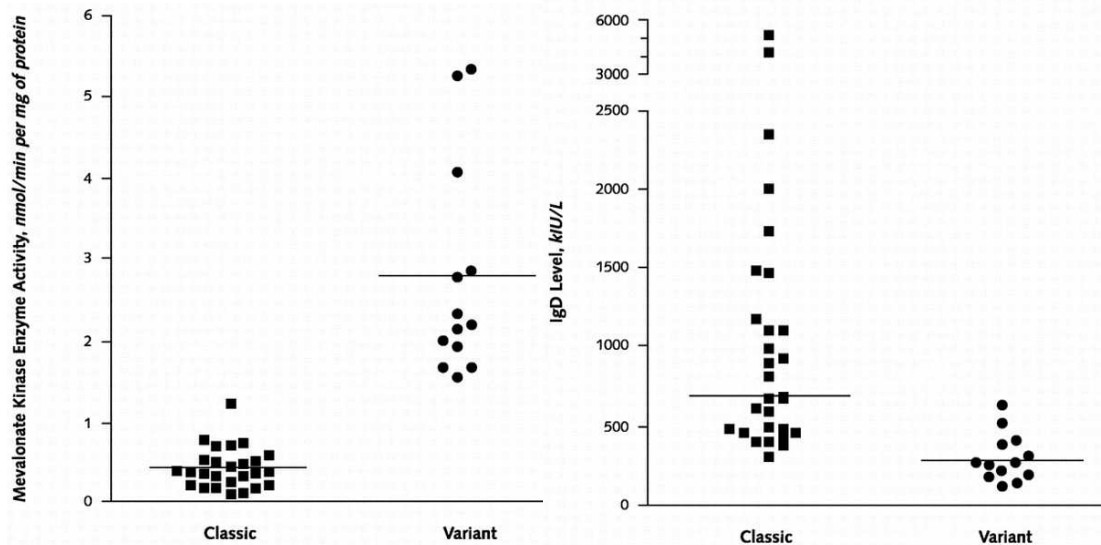
Figure. Mevalonate kinase enzyme activity and IgD levels in patients with classic-type or variant-type hyper-IgD and periodic fever syndrome (HIDS). *Left.* Mevalonate kinase enzyme activity, as measured in lymphoblasts, in patients with classic-type (*squares*) and variant-type (*circles*) HIDS. The horizontal bars represent the mean. *Right.* Serum level of IgD in patients with classic-type versus variant-type HIDS. Horizontal bars represent the median of the groups. Normal range in healthy persons for serum IgD level is <100 kIU/L.

Mevalonate kinase enzyme activity

The left figure shows the mevalonate kinase enzyme activity in individual HIDS patients. In classic HIDS patients, the enzyme activity was greatly depressed to a mean of 0.42 nmol/min/mg protein (SD 0.25), compared to 2.8 nmol/min/mg protein (SD 1.3) in the variant HIDS patients (p=<0.0001). In both groups there was no significant correlation between enzyme activity and clinical disease severity as measured in number of febrile days per year (classic: r_s=0.06, p=0.77, variant: r_s=-0.15, p=0.63) or between enzyme activity and IgD levels (classic: r_s=0.02, p=0.90, variant: r_s=-0.11, p=0.73). Parents of classic HIDS patients, who are heterozygous for the mevalonate kinase mutation, have low enzyme activity (mean 1.7 nmol/min/mg protein, SD 0.77) when compared to variant type HIDS patients (p=0.008), although still significantly higher than their affected offspring (p<0.0001).

Clinical phenotype and laboratory values

The table depicts the pertinent clinical variables. Age of onset of febrile attacks is higher in variant type HIDS patients. There is a tendency for longer attacks (mean number of days 6.9±5.7 vs. 4.7±1.7) and longer attack-free interval (mean number of weeks 9.3±9.2 vs. 5.6±3.8) in the variant type HIDS patients compared to classic HIDS patients, although this does not reach statistical significance (p=0.57 and p=0.74 respectively). Of 13 variant type HIDS patients, one patient had a brother and mother with similar symptoms and one patient had an affected brother and daughter. As shown in table 2, a number of accompanying symptoms in HIDS is more likely to occur in classic type HIDS.

The intensity of the acute phase response during attacks was greater in classic type HIDS patients, as evidenced by a significantly higher erythrocyte sedimentation rate (Table). When compared with variant type patients we observed higher IgA and IgG_3 concentrations, but lower IgG_4 values, in classic HIDS patients. We

detected high serum concentrations of IgD in both groups of patients but the concentrations in classic type HIDS patients greatly exceeded those in variant patients (figure). However, in 2 affected siblings of separate classic HIDS patients we detected low values of IgD, although both patients carried the same mutations, had typical HIDS attacks, and had depressed activity of the mevalonate kinase enzyme similar to their affected sister and brother. Further, concentration of IgD did not correlate with mevalonate kinase enzyme activity (see above), nor was there a significant correlation between IgD concentrations and the number of febrile days per year (classic: r_s=0.05, p=0.76; variant: r_s=0.46, p=0.11).

Table. Characteristics of patients with classical type versus variant type HIDS*

Variable	Patients with classical type HIDS (n=41)	Patients with variant type HIDS (n=13)	*P* value
Clinical feature			
Mean age (yrs)	31.2	30.3	-
Men/Women (n/n)	24/17	8/5	-
Mean duration of attack (shortest-longest, dys)	4.0-5.4	6-7.8	-
Mean attack-free interval (shortest-longest, wks)	4.8-6.5	8.7-10.5	-
Median age at onset (range, in months)	3 (0-120)	24 (0-636)	0.02†
Mean laboratory value ± SD			
Maximum IgD (IU/mL)	1168.8 ± 1090.3	311.8 ±143.9	<0.001†
IgA (g/L)	5.37 ± 2.75	2.66 ± 0.82	0.001†
IgG (g/L)	12.7 ± 3.6	13.9 ± 5.1	
IgG$_1$ (g/L)	8.6 ± 2.4	7.9 ± 3.2	
IgG$_2$ (g/L)	2.0 ± 0.9	2.6 ± 1.5	
IgG$_3$ (g/L)	1.2 ± 0.7	0.5 ± 0.1	<0.001†
IgG$_4$ (g/L)	0.2 ± 0.2	0.5 ± 0.4	0.009†
IgM (g/L)	1.4 ± 0.9	1.5 ± 0.8	
Erythrocyte sedimentation rate (mm/h)‡	92.2 ± 29.3	63.5 ± 35.3	0.02†
Leukocyte count (10^9/L)‡	17.8 ± 7.2	14.4 ± 6.0	
Symptom, n (%)			
Attack after immunization‖	7 (100)	2 (33)	<0.001§
Vomiting	36 (88)	4 (31)	<0.001§
Diarrhoea	28 (68)	4 (31)	<0.001§
Lymphadenopathy	40 (98)	7 (54)	<0.001§
Splenomegaly	20 (49)	2 (15)	<0.001§
Skin lesions	35 (85)	8 (62)	<0.001§
Arthritis	29 (71)	6 (46)	<0.001§
Serositis	5 (12)	0 (0)	<0.001§
Abdominal pain	34 (83)	8 (62)	<0.001§
Arthralgia	36 (88)	9 (69)	0.011§
Aphtous ulcers‖	4 (67)	2 (50)	0.015§
Cold chills	31 (76)	10 (77)	-
Headache	26 (63)	8 (62)	-

*HIDS = hyper-IgD and periodic fever syndrome.

† Calculated by using the Mann-Whitney U test.

‡ Measured during a fever episode.

§ Calculated by using the chi-square test.

‖ Not unequivocally known in all patients; information on attacks after immunizations was obtained from 7 and 6 patients with for classical and variant type HIDS respectively, and on aphtous ulcers from 6 and 4 patients.

Discussion

Recent localization and positional cloning efforts identified mevalonate kinase as the causative gene in HIDS [2,3]. This enabled us to develop and evaluate a molecular genetic test of the mevalonate kinase gene as a diagnostic tool. We could detect disease-associated mutations in 76% of patients in a large cohort of 54 patients. Most patients were compound heterozygotes, that is, they carried a different mutation on each allele of the mevalonate kinase gene.

The mutations have clear physiological consequences as evidenced by the considerably decreased activity of the enzyme mevalonate kinase in cultured lymphoblasts.

We were able to delineate a substantial subgroup of patients (24% of our cohort) who had the typical clinical presentation of HIDS but lacked mutations in the mevalonate kinase gene. This suggests that there is genetic heterogeneity among patients with clinical symptoms indicative of HIDS. Although we did not rule out the possibility of mutations in the promotor or intronic sequences or the occurrence of very large deletions, this is unlikely because these patients display a normal level of mevalonate kinase enzyme activity.

In order to establish if, apart from mutations in the mevalonate kinase gene, there are other distinctive features, we examined the two subgroups. HIDS patients who showed mutations in the gene for mevalonate kinase were denoted as "classic type HIDS", while patients who did not carry any mutation in this gene were designated as "variant type HIDS". Subtle differences in symptoms, signs and laboratory values could be detected (table). In general, classic type HIDS patients are younger at their first attack, tend to have shorter, but more frequent febrile attacks, and have more additional symptoms during attacks. Although variant type patients have high IgD and IgA concentrations, values are lower than those seen in classic type patients. It is intriguing that most of the variant type HIDS patients have a negative family history, which suggests that this type is sporadic. The early onset of symptoms however suggests a genetic predisposition.

It can be hypothesized that, given the phenotypical similarities, both variant and classic HIDS patients share a common inflammatory pathway distal of mevalonate kinase which results in the febrile attacks. Earlier studies indicated the activation of cytokines during fever attacks in HIDS [50,95] which suggests the involvement of macrophage activation. At present it is unclear how a single enzyme defect in the isoprenoid pathway gives rise to an inflammatory disorder such as HIDS. Some insight might be gained from inhibitors of HMG CoA-reductase, the enzyme upstream of mevalonate kinase. These drugs do not only lower cholesterol but also have anti-inflammatory properties [96]. The anti-inflammatory effects of HMG CoA-reductase inhibitors seem contradictory to the pro-inflammatory phenotype of HIDS, which is caused by an enzyme defect one step downstream from HMG CoA-reductase.

This study also raises questions on the significance of IgD in the pathophysiology of HIDS. The height of serum IgD concentrations could not be related to clinical disease severity. Although IgD levels in variant patients were lower than in classic patients, both groups had considerably elevated IgD. This suggests that high IgD values do not result directly from mutated mevalonate kinase. From the study of Houten et al. it appears that patients with periodic fever who do not have raised IgD concentrations may also have mutations in mevalonate kinase [3]. We have demonstrated before that symptoms in HIDS may antedate the rise of serum IgD in

childhood [46,48]. These findings might suggest that IgD acts as a secondary marker which can be found in most, but not all, HIDS patients. Still, we think it is valuable to measure IgD in patients with periodic fever. If the patient has an early onset of symptoms (before the end of the first year of life), has a sibling who is affected with periodic fever and possesses an elevated IgD, the results of the current study indicate that it is likely that the patient suffers from classic type HIDS.

We propose to diagnose HIDS using the clinical criteria as set before [46]. Using a genetic diagnostic tool, this group of HIDS patients can then be divided into a group of patients who have mutations in the gene for mevalonate kinase ("classic type"), and a group who lack mutations ("variant type"). The classic type HIDS patients are a relatively homogeneous group with a predictable inheritance pattern and clinical presentation, which facilitates the counseling of the individual patient.

Acknowledgments

3

Mevalonate kinase deficiency: evidence for a phenotypic continuum

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Abstract

Both mevalonic aciduria, characterized by psychomotor retardation, cerebellar ataxia, recurrent fever attacks and death in early childhood, and hyper-IgD syndrome, with recurrent fever attacks without neurological symptoms, are caused by a functional deficiency of mevalonate kinase. In a systematic review of known mevalonate kinase deficient patients we identified 5 adults with phenotypical overlap between these two syndromes, which argues for a continuous spectrum of disease. Mevalonate kinase deficiency should be considered in adult patients with fitting neurological symptoms, with or without periodic fever attacks.

Introduction

Mevalonate kinase deficiency, caused by mutations in the mevalonate kinase gene, is associated with two disparate clinical syndromes. The hyper-IgD and periodic fever syndrome (HIDS, MIM 260920) is an autoinflammatory disease characterized by lifelong recurrent episodes of fever, abdominal distress, lymphadenopathy and skin rash [2-4], which usually start in the first year of life. There are no apparent neurologic or morphological abnormalities, and in between the febrile attacks patients are remarkably free of symptoms. Mevalonic aciduria (MVA, MIM 251170) is typically a disease of infantile onset, characterized by psychomotor retardation, ataxia, failure to thrive, cataracts and dysmorphic features [45,97]. Patients also suffer from periodic fever attacks, similar to but more severe than in HIDS and, in general, they die in early childhood.

Mevalonate kinase, which phosphorylates mevalonic acid, is a central enzyme in the isoprenoid pathway; end-products include cholesterol, dolichol and ubiquinone. This pathway is also responsible for isoprenylation, a post-translational modification of proteins causing them to become membrane-bound. How a defect in this metabolic pathway leads to either of the clinical phenotypes is obscure. (Random) clinical observation of these patients suggests that the clinical presentation of mevalonate kinase deficiency represents a phenotypic continuum from mevalonic aciduria to HIDS, instead of two separate phenotypical entities. To test this observation, we searched our clinics and database to identify (adult) mevalonate kinase deficient patients with neurological signs and symptoms.

Methods

Patients

The Nijmegen International HIDS registry [46] consists of 81 patients with metabolically and/or genetically confirmed mevalonate kinase deficiency. On screening this registry, four patients with neurological signs were identified. One additional patient with mevalonic aciduria and mevalonate kinase deficiency was identified through the neurology clinics. Some of the patients have been described earlier [42,46,98].For comparison we also summarize the clinical data from 32 classical HIDS patients without neurological signs and symptoms in the HIDS registry of whom all genetic and metabolic data were available. Patients 1,2, and 4 were examined by us. Patients 3 and 5 had been examined by others.

Analysis of mevalonate kinase gene mutations, enzyme activity and urinary mevalonic acid

Genomic DNA was extracted from whole blood or Epstein-Barr immortalized lymphoblasts from the patients, and the mevalonate kinase gene was amplified and sequenced according to established protocols [2]. For analysis of mevalonate kinase enzyme activity, we employed a radiometric assay using extracts of cultured lymphoblasts [42]. Proton NMR spectroscopy (500 MHz) was used to quantify mevalonic acid in urine at pH 2.5, from samples outside fever attacks. Under these conditions mevalonic acid gives a singlet resonance at 1.33 ppm, while in some patients also the lactone of mevalonic acid may be observed as a 1.37 ppm resonance.

	#1	#2	#3	#4	#5	hyper-IgD syndrome[c] n=32	mevalonic aciduria[d] n=11
Current age (years)	45	44	30[a]	54	41	31 (11-58)	4 (0,5-12)
Gender (M / F)	M	M	F	M	F	16 / 16	5 / 6
Clinical characteristics							
Periodic fever attacks	+	+	+	-	+	+	+
Mental retardation	+	+	+	++	++	-	+/++
Language disorder	+	+	?	+	?	-	?
Cerebellar ataxia	+	+	-	+	+	-	+/- (5/10)
Ocular symptoms [e]	+	+	-	+	+	-	+/- (6/11)
Epilepsy	-	-	+	-	+	-	-
Age of onset (months)	0	0	6	12	6	3 (0-96)	ND
IgD (IU/mL)[f]	680	1170	304	20	465	880 (376-5300)	ND
Mevalonate kinase mutations	V377I/ W62X	V377I/ W62X	P167L/ G202R	A334T/ H20P	A334T/ W62X	12 different mutations	ND
Mevalonate kinase enzyme activity (%)[g]	ND	4.5	1.8	1.1	< 0.5	10.5 (2-32)	1.5 (0-4)
Mevalonic acid in urine (mmol/mol creatinine)[h]	4	15.4	raised[b]	2730	7200	ND	10850 (1700-56000)

Table 1. Comparison of patient characteristics. Patient numbering refers to their case number in the International Nijmegen HIDS registry. (a) age at death. (b) no quantitative data available. (c) data from all patients in the Nijmegen International HIDS registry who fulfilled the following criteria: no neurological symptoms, known mutations in the mevalonate kinase gene and known deficient mevalonate kinase enzyme activity. Results presented as median (range). (d) data from 11 mevalonic aciduria patients described in Hoffmann et al [45]; 4 of them had died before age 4. Results presented as median (range). (e) ocular symptoms include cataracts and progressive tapetoretinal degeneration. (f) serum concentration of immunoglobulin D (IgD), normal <100 IU/mL. (g) mevalonate kinase enzyme activity, measured in EBV-transformed lymphoblasts, expressed as percentage of controls. (h) mevalonic acid concentration in urine, normally undetectable. ND = not determined. + present, - absent, ? unknown.

Results

A summary of clinical symptoms and test results is presented in table 1, detailed case descriptions are available in the online supplemental data (see appendix). All patients were of Dutch descent, and except for patient #1 and #2, who were brothers, the family history of the patients was negative. In all five patients, the first symptoms appeared within the first year of life. Four of the five patients had recurrent fever episodes with characteristic accompanying symptoms such as abdominal distress, lymphadenopathy, erythematous skin rash, aphthous ulcera and arthralgia. Patient #4 never experienced such fever episodes. This patient had a normal IgD concentration. Three patients had mild to moderate mental retardation and two had severe mental retardation; all patients except for patient #3 were institutionalized. The three patients examined by us exhibited a similar language disorder: grammatically simple language with a strong predominance of

nouns and adjectives, echolalia and palilalia and phonemic paraphasias, and omission of the first consonant in words. Four of five patients suffered from cerebellar ataxia, more pronounced in gait than in the arms. One patient had left temporal epilepsy with complex partial seizures, one other patient had generalized tonic-clonic seizures. The ocular symptoms in four of the five patients included progressive blindness due to tapetoretinal degeneration and cataracts. Patient #1 and #2 both suffered from hearing loss, presumably due to frequent ear infections and mastoiditis. Generalized muscle hypotonia was observed in patient #4, with normal nerve conduction studies. Figure 1 shows severe cerebellar atrophy in patient #4. Patient #1 was reported to have suffered a left hemispheric ischemic stroke of unknown etiology at age 40, but had recovered with only a very mild residual clumsiness. Patient #3 died at age 30 after a brief intercurrent illness, diagnosed as staphylococcal pneumonia; autopsy was not performed. The other 4 patients are still alive.

4
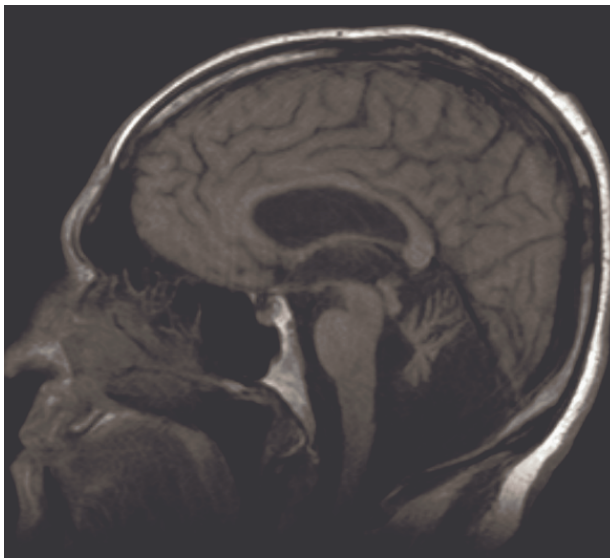
Figure 1. T1 weighted sagittal midline MRI image, to demonstrate severe cerebellar atrophy in patient #4.

Discussion

The patients presented in this paper, who all have mevalonate kinase deficiency, illustrate a phenotypic continuum between HIDS and MVA. Four out of five suffer from characteristic episodes of fever and inflammation, though intriguingly, one patient did not. All have mental retardation with additional neurological signs in a varying degree of severity, such as cerebellar ataxia, epilepsy, and a language disorder that may or may not be specific for the disease. We were unable to determine how this language disorder compares to language deficits in patients with mental retardation of other causes. All patients have survived to adulthood; one patient died at age 30, due to intercurrent pneumonia.

This is in sharp contrast to what has been described in the literature on classical mevalonic aciduria. One study describes the phenotype of 11 mevalonic aciduria patients [45]. The most severely affected had profound developmental delay, dysmorphic features, cataracts, hepatosplenomegaly, lymphadenopathy, and anemia and died in infancy. Less severely affected patients had psychomotor retardation, myopathy, and ataxia. All patients had recurrent crises with fever and lymphadenopathy. The authors did not find a relation between the neurological phenotype and the remaining enzymatic activity [45]. Another report of 4 young patients illustrated that both MVA and HIDS are caused by mevalonate kinase

Figure 2. Proposed genotype-phenotype spectrum for mevalonate kinase deficiency.

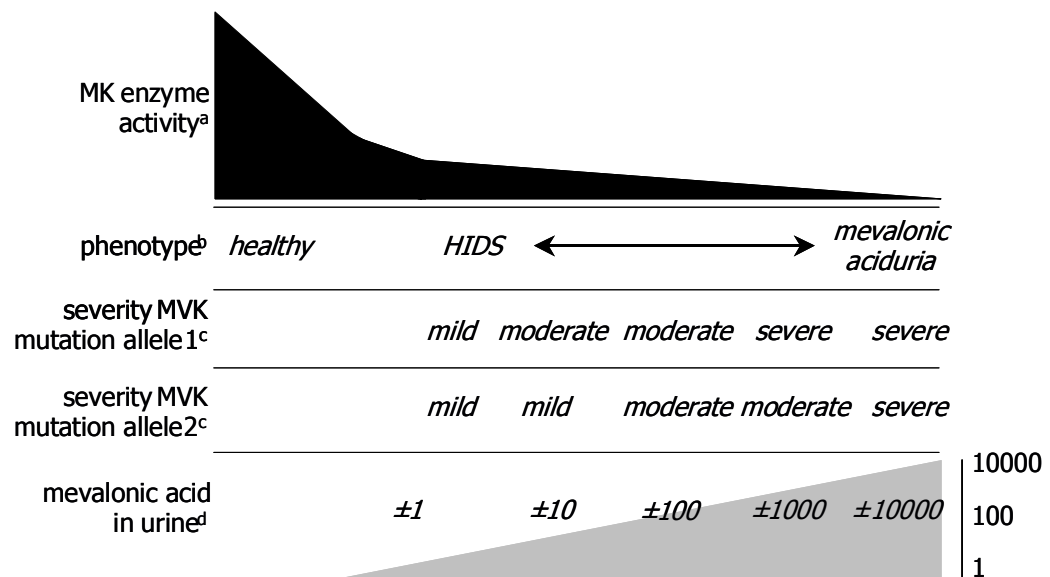(a) Mevalonate kinase enzyme activity, ranging from 100% (left) to <1% (right). (b) Phenotype associated with decreasing enzyme activity, double-headed arrow depicts continuous phenotypic spectrum between two extremes (HIDS and mevalonic aciduria). (c) Genotype consists of a combination of mild (e.g. V377I), moderate (e.g. H20P, I268T) or severe (e.g. A334T) mutations in mevalonate kinase gene. (d) concentration of mevalonic acid detected in urine samples, average estimates, expressed in mmol/mol creatinine.

deficiency [99]. Unfortunately, no genotypes were offered by either of these reports. Patients surviving to their 6th decade, such as our patient #4, have never been described. The patients described here seem to bridge the gap between the classical MVA and HIDS phenotypes. Mevalonate kinase deficiency should be considered in adult patients with fitting neurological symptoms, with or without periodic fever attacks.

In figure 2, a phenotypic spectrum of MVA/HIDS is proposed. One explanation for the varying degree in severity of phenotype might be the specific genotype involved. Generally, patients are compound heterozygous for two different mutations in the mevalonate kinase gene. These mutations may have a different effect on the function of the enzyme, some leading to a minor loss of efficiency (e.g. V377I), others causing near complete loss of function (e.g. A334T). The resulting sum of the residual enzyme activities and efficiency may account for the phenotypical variability (Fig. 2).

But genotype analysis alone is unable to explain the remarkable variability in phenotype. In a study of 3 MVA patients [100] one patient was included with a mild phenotype, still alive at 20 years of age with a genotype identical to our patient #4. In contrast, one other patient in that report, who was homozygous for the I268T mutation, was severely affected and died in the first year of life. We have previously described two adult brothers with this genotype who have HIDS without any neurological symptoms [42]. Therefore, genetic trans-acting or environmental factors have to be considered to explain the phenotypic variability.

Measurement of urinary mevalonic acid by mass spectrometry or proton NMR spectroscopy is the most straightforward step to screen for mevalonate kinase deficiency (Fig. 3). Serum IgD is not always elevated in mevalonate kinase deficiency, and as illustrated by case #4 normal values do not exclude the diagnosis [3].

Clinical phenotype

From <2 yrs of age, 2 or more of the following[a]:
• recurrent inflammatory episodes
• (progresive) cerebellar ataxia
• mental retardation
• tapetoretinal degeneration, cataracts

Urinary mevalonic acid — no → No mevalonate kinase deficiency

Elevated?

yes

Mevalonate kinase deficiency

Confirmation:
►1. mutation analysis mevalonate kinase gene
2. enzyme activity fibroblasts/lymphoblasts

Figure 3. Screening for mevalonate kinase deficiency. (a) Screening would be recommended in cases with 2 or more of these symptoms, starting from an early age; also cases of recurrent inflammatory episodes without other symptoms. It can most directly be done by measuring urinary mevalonic acid concentration. When this is elevated, further diagnostic procedures can be performed for confirmation: (1) mutation analysis mevalonate kinase gene: relatively easy and available, (2) enzyme activity measurement: most direct confirmation but only available in specialist research setting. Data on sensitivity or specificity of these tests are lacking.

Acknowledgements

Appendix Case Descriptions

Patient 1 and 2

Patients 1 and 2 are two brothers with a very similar clinical phenotype. Both were afflicted since their first month of life with recurrent fever episodes accompanied by abdominal distress and an erythematous skin rash, which last a few days and occur every 3 to 5 weeks. They were institutionalized at age 8 and 9 respectively because of mental retardation, and over the years very slowly deteriorated. In their early twenties, tapetoretinal degeneration with cataract was noted in both brothers, and also impaired hearing presumably due to frequent ear infections and mastoiditis. They had eight siblings, who could not be examined because all contact had been severed at an early age. Both lived in an institution where they were able to provide adequate self-care.

The eldest brother (#1) suffered a left hemispheric ischemic stroke of unknown etiology at age 40. When examined at age 43, his major symptoms were gait problems requiring ambulatory aid, as well as minor articulation and swallowing difficulties. At that time the neurological examination showed a small and very lean man with a weight of 45 kg; a mid-thoracic kyphosis; mild to moderate mental retardation; a language disorder that consisted of telegraphic speech with intermittent echolalia and palilalia and phonemic paraphasias; slight dysarthria; gait ataxia with minimal arm ataxia; and brisk reflexes. In addition, he suffered from a unilateral right arm upper motor neuron paresis.

Examination of his brother (#2) at age 42 revealed a mildly to moderately retarded man, weight 44.5 kg, who produced grammatically simple language, with a predominance of nouns and adjectives, echolalia and palilalia, and infrequent omission of the first letter of words; a slurred speech; saccadic intrusions in vertical smooth pursuit eye movements; mild arm ataxia, but more prominent gait ataxia; and brisk tendon reflexes with extensor toe responses but absent Achilles tendon reflexes.

Patient 3

Patient 3 was a Dutch woman referred to us at age 24 because of recurring episodes of fever and a high serum concentration of IgD. The fever attacks had started when she was 12 months old, and were characterized by abdominal distress and arthralgia. Accompanying signs were lymphadenopathy, erythematous skin rash, oral and genital aphthous ulcers en sometimes frank arthritis. Such an episode would last 5 to 7 days, and recur every 3 to 6 weeks. From 6 months-old onwards she had left temporal epilepsy with complex partial seizures. There was a behavioral disorder with mild mental retardation. The family history was negative. Examination in a fever-free interval showed a young woman of 174 cm length with no clear dysmorphic features. Neurologic examination was otherwise unremarkable, without signs of cerebellar ataxia. At age 30 she died after a brief intercurrent illness, diagnosed as staphylococcal pneumonia. Autopsy was not performed.

Patient 4

Patient 4 was institutionalized at a very young age because of mental retardation. Since his late thirties, his vision deteriorated because of cataract and he became wheelchair bound. Episodes of periodic fever were never documented in the institution in all the years he lived there. He was first examined by us at age 52. This revealed corneal opacities OS, bilateral keratoconus and psoriasis-like skin lesion of the abdomen, elbows and knees. Neurological abnormalities included: severe mental retardation with adequate cooperation; a peculiar language disorder in which he used almost exclusively nouns and adjectives with hardly a verb, and with omissions of the first consonant in words; severely decreased visual acuity with intact light / dark discrimination; slurred speech; generalized muscle hypotonia; mild arm ataxia but severe gait ataxia; brisk tendon reflexes with flexor toe responses; and lower leg atrophy with bilateral pes planus valgus. Nerve conduction studies were normal. An MRI scan revealed severe cerebellar atrophy (figure 1). His serum IgD was not elevated (table 1)

Patient 5

Patient 5, a 41-year old woman with negative family history, started from an early age to suffer from periodic fever attacks with all the characteristic accompanying symptoms. These episodes lasted 4 to 5 days and occurred approximately every 6 weeks in childhood. Development was slightly retarded, and although she walked at the age of 12 months it was noticed that she fell very often. At the age of 4 years a crisis occurred which was tentatively diagnosed as measles encephalitis. Subsequently, she developed severe psychomotor retardation requiring institutionalization, epilepsy with generalized tonic-clonic seizures, cerebellar ataxia, and progressive blindness due to tapetoretinal degeneration and atrophy of the optic nerve.

Effect of inflammatory attacks in the classical type hyper-IgD syndrome on immunoglobin D, cholesterol, and parameters of the acute phase response

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Abstract

Background: Classical type hyper-immunoglobulin D (IgD) syndrome (HIDS) is an hereditary auto-inflammatory disorder, characterised by recurrent episodes of fever, lymphadenopathy, abdominal distress and a high serum concentration of IgD. It is caused by mevalonate kinase deficiency.

Objective: to further characterise the acute phase response during fever attacks in HIDS in order to improve diagnosis.

Subjects: 22 mevalonate kinase deficient HIDS patients.

Methods: Blood samples were drawn during and in between febrile attacks, and concentrations of C-reactive protein (CRP), ferritin, procalcitonin, pentraxin 3, IgD and cholesterol in several lipoprotein fractions were determined.

Results: The marked acute phase response at the time of a fever attack in classical type HIDS is reflected by a rise in CRP accompanied by a moderate but statistically significant rise in procalcitonin and pentraxin 3. In only 2 of 22 patients, procalcitonin concentration rose above 2 ng/mL during fever attack, compatible with the non-infectious nature of these attacks. Ferritin does not reach the high concentrations found in Adult-onset Still's disease. Despite the defect in mevalonate kinase, a component of cholesterol metabolism, serum cholesterol did not change during attacks. IgD concentration is elevated regardless of disease activity, though there is appreciable variation during life. Its role in HIDS remains unclear.

Conclusion: The combination of high CRP concentration plus procalcitonin concentration <2 ng/mL in a symptomatic HIDS patient may indicate a febrile attack without (bacterial) infection; this observation warrants further investigation for its usefulness as a marker in clinical practice.

The hyper-immunoglobulinaemia D and periodic fever syndrome (HIDS) is a rare disorder which causes life-long recurrent episodes of fever and inflammation [4]. Clinically, these episodes may be accompanied by abdominal distress, arthralgia, headache, lymphadenopathy, skin rash, and aphthous ulcers [46]. The inflammatory attack lasts about 4 to 6 days followed by a spontaneous remission, only to recur after a symptom-free period of 4 to 6 weeks. Attacks can be triggered by minor trauma, vaccinations, and physical or emotional stress, but often come unexpectedly. Laboratory examination at the time of attacks reveals a vigorous acute phase response with leukocytosis, raised sedimentation rate, and high serum concentrations of C-reactive protein and pro-inflammatory cytokines such as IFNγ, TNFα, and IL-6 [50,95]. Almost all patients have an elevated polyclonal immunoglobulin D (IgD) and IgA concentration. Previously, we have distinguished classical type HIDS and variant type HIDS [40]. While in variant type HIDS the nature of the underlying defect is unclear, classical type HIDS is caused by mutations in the gene encoding for mevalonate kinase, an enzyme that is part of the isoprenoid pathway. The genetic mutations cause a diminished enzyme activity of mevalonate kinase, and during a fever attack its substrate, mevalonic acid, will accumulate in body fluids and be excreted in the urine. End-products of this pathway include cholesterol, dolichol and ubiquinone, and the isoprenoid pathway is also essential for the isoprenylation of proteins, a modification important for membrane bound proteins. The link between the defective isoprenoid metabolism and the inflammatory phenotype of HIDS remains to be resolved.

The main aim of this study was to further define the acute phase response in the inflammatory attacks in classical HIDS patients, actuated by two considerations. In the first place, we sought to discriminate between (serious bacterial) infection and non-infectious inflammation of a HIDS attack. Such a differentiation is needed in clinical practice as the inflammatory episodes in HIDS, with high fever and often severe abdominal distress, can pose a serious diagnostic dilemma and frequently lead to unnecessary use of antibiotics or even avoidable surgical procedures. This prompted us to consider two recently characterised members of the family of acute phase proteins as a biological marker: procalcitonin (PCT) and pentraxin 3 (PTX3) [101-104]. Secondly, we wanted to test serum concentrations of IgD, ferritin and cholesterol in HIDS patients with and without fever, which might be of help in differential diagnosis.

Patients and methods

Patients

We included in this study twenty-two Dutch patients with classical type HIDS, 12 male and 10 female. All patients suffered from typical recurrent fever episodes, shared elevated serum concentrations of IgD and carried mevalonate kinase gene mutations. The main patient characteristics are listed in table 1. Sampling of plasma and serum was performed at two time points, during fever attack and during remission. Our definition of a fever attack for the present study consisted of 1) raised body temperature ($\geq$38 °C), 2) typical symptoms and signs of HIDS as mentioned earlier and 3) no clinical indications for the presence of infection. Remission was defined as the absence of symptoms for at least 1 month. No medication was allowed during the study period. Close follow-up of the patients did exclude bacterial and/or viral infections during the course of the investigation. All patients recovered from the fever episode spontaneously without the use of

antibiotics or antiviral medication. The ethical committee of our institution approved the study protocol, and all patients gave informed consent. In order to delineate the course of IgD during life, we took historical IgD values from patient records from 17 classical type HIDS patients, which were available to us through the Nijmegen HIDS registry [46].

Pt no.	Sex	Age (yrs)	Onset (mnth)	MVK genotype	Fever	Lymph-adenopathy	Abdominal Pain	Arthralgia	Skin lesions
1	F	17	8	V377I/I268T	+	+	+	-	-
2	M	18	4	P167L/I268T	+	+	+	+	+
3	M	22	0	V377I/I268T	+	+	+	+	+
4	M	23	3	V377I/H20P	+	+	+	+	+
5	F	26	2	V377I/H20P	+	+	+	-	+
6	M	30	3	V377I/H20P	+	+	+	+	+
7	M	31	18	P167L/I268T	+	+	+	+	+
8	F	34	0	V377I/unknown*	+	+	+	+	-
9	F	34	12	P167L/G202R	+	+	+	+	+
10	M	37	96	V377I/unknown*	+	+	+	+	+
11	F	37	0	V377I/I268T	+	+	+	+	+
12	F	38	3	V377I/V377I	+	+	+	+	+
13	M	40	24	V377I/deletion	+	+	+	+	+
14	M	41	48	V377I/unknown*	+	+	-	+	+
15	F	42	12	V377I/deletion	+	+	+	+	+
16	M	45	0	V377I/W62X	+	+	+	+	+
17	M	46	0	V377I/W62X	+	+	+	+	+
18	F	52	?	V377I/G309S	+	+	-	-	+
19	F	54	0	V377I/deletion	+	+	-	+	+
20	M	54	3	V377I/I268T	+	+	+	+	+
21	F	54	12	V377I/I268T	+	+	-	-	-
22	M	58	48	V377I/H20P	+	+	-	+	+

Table 1. Patient characteristics.
*In three patients where only one mutation in the *MVK* gene could be identified, the diagnosis classical type HIDS was confirmed by detection of increased urinary mevalonic acid excretion during inflammatory attack (patient 8) or decreased mevalonate kinase enzyme activity (patients 10 and 14).

Serum and plasma determinations

Determinations of each protein was performed in one assay for all samples. CRP concentrations were measured in duplicate by a polyclonal antibody enzyme linked immunosorbent assay (ELISA) as previously reported (normal reference <2,7 mg/L) [105]. Concentration of procalcitonin was determined by chemiluminescent solid phase double antibody immunoluminometric assay (LUMItest; BRAHMS Diagnostica GmbH, Berlin, Germany). Two specific monoclonal antibodies form a sandwich by binding the two major polypeptide chains of procalcitonin (catacalcin and calcitonin); the second antibody carries a luminescent tracer. The light emission triggered by oxidation in the presence of hydrogen peroxide can be measured by luminometer (detection limit 0,05 ng/mL, normal reference <0,5 ng/mL, reference limit used in case of bacterial infection <2 ng/mL) [106,107].

Plasma concentration of pentraxin 3 was measured by ELISA, based on the PTX3-specific monoclonal antibody MNB4 and biotinylated rabbit PTX3-specific polyclonal IgG (normal reference <2 ng/mL) [108,109].

The procedure for the ELISA for measurement of IgD has been published earlier, using rabbit anti-human IgD (Dako, Copenhagen, Denmark) as the primary

antibody [110]. The lower limit of detection of this ELISA was 1 IU/mL (1.4 mg/L), normal reference <100 IU/mL.

Concentrations of cholesterol were measured on the Hitachi 747 analyzer with enzymatic, commercially available reagents (Boehringer–Mannheim, Germany) (normal reference 4,7-6,5 mmol/L). HDL-cholesterol was measured in the supernatant after precipitation with PEG 6000 on the Hitachi 747 analyzer using commercially available reagents [111], LDL-cholesterol was calculated by the Friedewald formula (normal reference limit <4,7 mmol/L) [112].

Ferritin was measured on the Immulite 1 (DPC, Los Angeles, USA) using beads coated with monoclonal anti-ferritin and an alkaline phosphatase polyclonal conjugate (normal reference limit premenopausal women 80 μg/L, men and postmenopausal women 280 μg/L).

Statistical analysis

Statistical analysis was performed using GraphPad Prism version 4 for Windows, GraphPad Software, San Diego California USA, www.graphpad.com. The paired nonparametric Wilcoxon signed rank test was used for statistical comparison of concentrations during remission and during fever attack. Correlation coefficients were calculated with the Spearman correlation test. Data are given as median (range) unless otherwise stated.
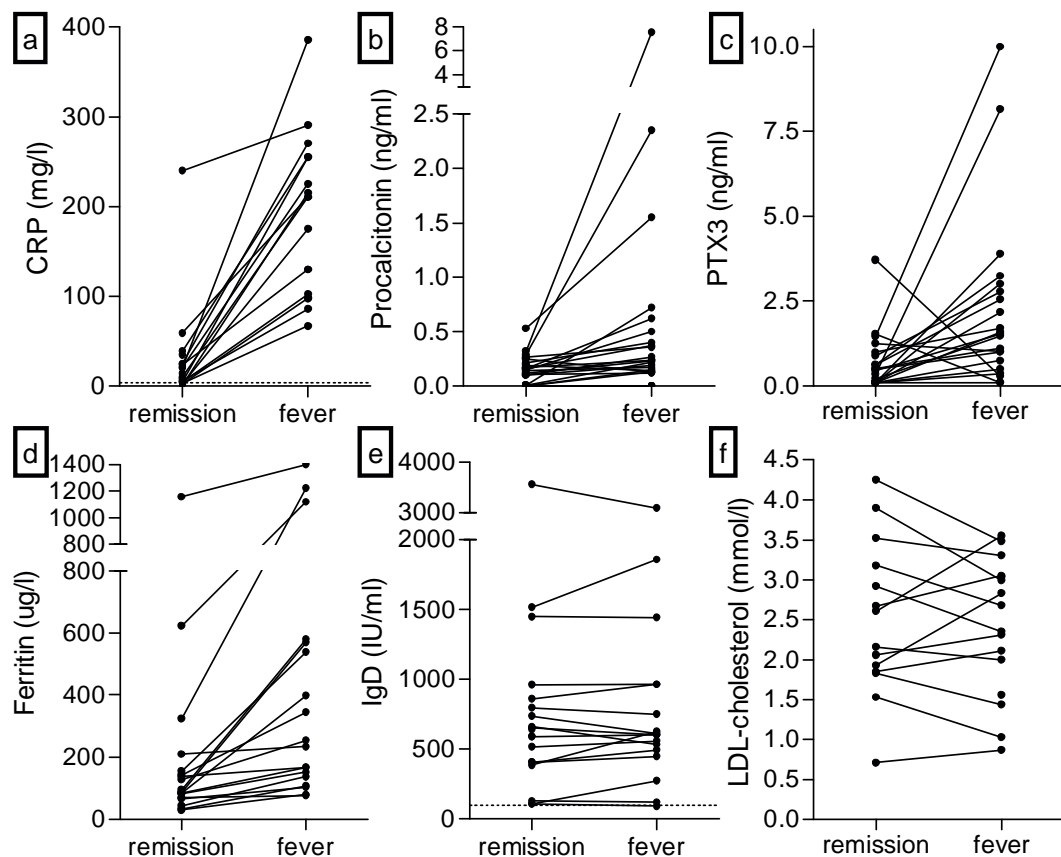
5

Figure 1. The acute phase response in the fever attack of HIDS. (a) serum concentration of CRP; (b) procalcitonin; (c) pentraxin-3; (d) ferritin; (e) immunoglobulin D; and (f) LDL-cholesterol.

Results

During a fever attack, serum concentrations of CRP are invariably and significantly elevated, to a median of 210 mg/L (range 67-385 mg/L; p<0.0001, Figure 1a). During a period of remission when the patient is without any symptoms, CRP concentrations are significantly lower, although often (83% of cases) still elevated above normal (12 mg/L (range 1.3-240 mg/L)).

At the time of fever episodes most patients showed a limited albeit statistically significant rise in serum procalcitonin concentration (Figure 1b; median 0.15 vs. 0.24 µg/L, p=0.0005). A clinically significant increase above the reference limit of 2 ng/mL used for bacterial infection, was seen in only two patients. Procalcitonin concentrations during fever attacks were correlated with CRP concentrations (Figure 2a, Spearman r=0.71, p=0.0013). PTX3 concentration (Figure 1c) increased during fever in the majority of patients (median 0.45 vs. 1.53 ng/mL, p=0.002), with a concentration greater than 2 ng/mL in 8 of 21 patients. However, a correlation between CRP and PTX3 was absent, also in the samples taking during fever attack (Figure 2b).

Serum ferritin concentration increased in 61% of the patients during a fever episode, up to a maximum of 1398 µg/L (p=0.0002, Figure 1d). There was no indication for the presence of hemochromatosis in any of the patients, as ferritin values dropped to below 300 µg/L between attacks and the iron saturation as determined with transferrin saturation or unsaturated iron-binding capacity was below 45% (data not shown).
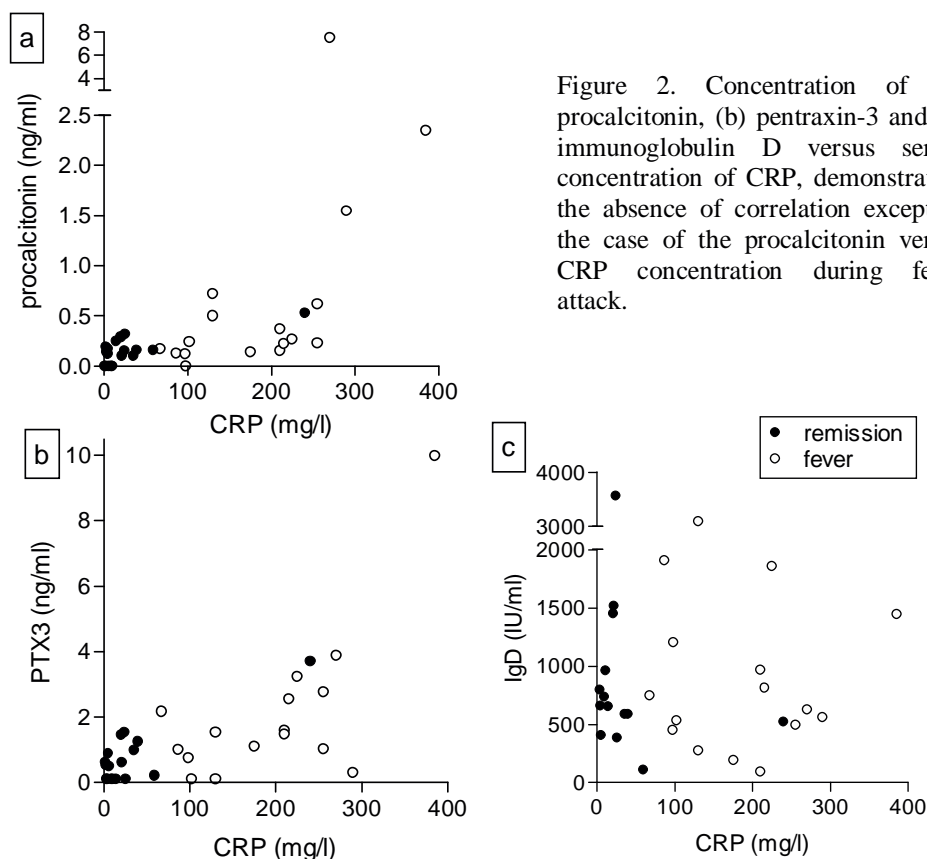

Figure 2. Concentration of (a) procalcitonin, (b) pentraxin-3 and (c) immunoglobulin D versus serum concentration of CRP, demonstrating the absence of correlation except in the case of the procalcitonin versus CRP concentration during fever attack.

Immunoglobulin D (IgD) serum concentrations were elevated, in most patients to at least 5 times the upper level of normal. No difference was found in samples taken during remission or during a fever attack (median 639 IU/mL (range 108-3560 IU/mL) vs. 603 IU/mL (range 92-3090 IU/mL), Figure 1e). There was no correlation between IgD and CRP values, either during attacks, or during remission (Figure 2c). Intraindividual IgD concentrations determined over a period of years show great variation (Figure 3). All HIDS-patients had normal to low-normal serum cholesterol concentrations during remission; this was true for total (mean 3.7 mmol/L $\pm$ SD 1.1), LDL- (mean 2.5 mmol/L $\pm$ SD 0.9) and HDL-cholesterol (mean 0.75 mmol/L $\pm$ SD 0.3). No changes to any of these concentrations occurred during a fever attack (Figure 1f).
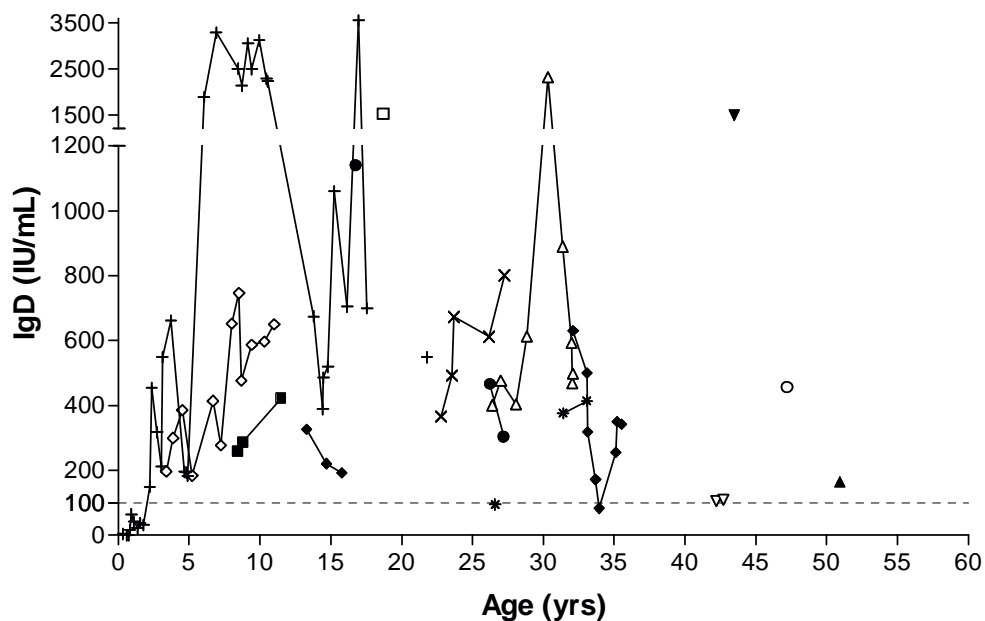

Figure 3.
Serum IgD concentration in 17 HIDS patients. Each symbol represents a separate patient, some of whom have been followed for several years. There is wide intra- and interindividual variation.

Discussion

These results demonstrate the intense acute phase response during the fever episode of classical type HIDS, and provide information on pathophysiological mechanisms. The acute phase response in HIDS is best defined by the sharp rise in serum concentrations of CRP (Figure 1a). This response is similar to that described in patients with familial Mediterranean fever (FMF), although generally the acute phase response is more intense and more prolonged than in FMF [113-116].

Our main objective was to find a biological marker that discriminates between the non-infectious inflammation of a fever attack of HIDS and (bacterial) infection. To this end, we examined the acute phase proteins procalcitonin and PTX3. Both proteins showed a statistically significant rise during fever attacks, although only procalcitonin concentration during fever showed a significant correlation with CRP concentration. Specifically, procalcitonin values rose above the threshold of 2 ng/mL, used as reference limit for bacterial infection, in only in 2 of 22 patients. This is similar to the findings in systemic autoimmune disease [102]. This suggests that the combination of a high serum CRP and procalcitonin concentration below 2 ng/mL may be used to distinguish an inflammatory attack of HIDS from an intercurrent bacterial infection. The sensitivity and specificity of this combination

of markers needs to be determined in future studies. The specific cytokine profile in HIDS might account for the limited increase in PTX3 concentration as this acute phase protein is induced by interleukin-1 (and not by interleukin-6), while in HIDS interleukin-6 is far more upregulated than interleukin-1 [50,103].

The cause of elevated IgD concentrations in the majority of HIDS patients, which prompted the name of this syndrome [1] is still an enigma. In children, the fever attacks may precede the rise in serum IgD for several years [117]. Also, some HIDS patients, despite having a severe phenotype, never develop high IgD[118], while a modest increase in IgD may be found in other hereditary autoinflammatory syndromes. On the other hand, isolated IgD has been shown to elicit an inflammatory response in isolated mononuclear cells [110]. If the IgD elevation is a consequence of the inflammatory phenotype it might be anticipated that its concentration varies with the attacks. In our study, serum IgD concentrations were found to be continuously high, irrespective of inflammatory state and/or CRP concentration. In addition, we found that serum IgD concentrations vary greatly during life (Figure 3), without correlation with clinical symptoms or frequency of attacks. These are further arguments to label the high IgD as an epiphenomenon rather than central to the pathogenesis. These data also indicate that the timing of IgD sampling for diagnostic purposes is irrelevant.

In our patients, we found relatively low serum LDL- and HDL-cholesterol concentrations during remission, but no further decrease with inflammatory attacks. This contrasts with the expected decrease of HDL-cholesterol during inflammation as has been described for numerous inflammatory disorders [119-121], and this might be related to the enzyme defect of mevalonate kinase in HIDS.

Despite an elevated ferritin concentration during fever attack in 61% of HIDS patients, none of the patients showed the extremely high ferritin concentrations (>3000 ng/mL) found in Adult-onset Still's disease (AOSD [122-124]), an inflammatory disorder with unknown aetiology, characterized by spiking fever, arthritis, evanescent rash and onset in adulthood [125].

In conclusion, the fever attack in HIDS is characterized by a strong rise in CRP concentration in all patients. Other acute phase proteins, such as pentraxin-3 and procalcitonin, showed an increase in some but not all patients. Specifically, this study suggests that in classical type HIDS a combination of high CRP concentration with procalcitonin below 2 ng/mL can be used as an indication that a febrile attack is not due to intercurrent bacterial infection, which can reduce unnecessary use of antibiotic therapy. The same may be true for PTX3 when its limit is set higher, although this remains more speculative since less is known about the right cut-off point. The concentration of IgD, which is high in HIDS, is not influenced by the inflammatory episodes.

Acknowledgements

5

A founder effect in the hyper-immunoglobulinemia D and periodic fever syndrome

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Introduction

Hyper-immunoglobulinaemia D and periodic fever syndrome (HIDS [Mendelian Inheritance in Men, 260920]) is an autosomal recessively inherited disorder characterized by recurrent episodes of fever, abdominal distress, arthralgia and aphtous ulcers [4]. The constantly elevated immunoglobulin D (IgD) serves as an apt marker of the disease, and all patients exhibit a marked acute phase response during the fever attacks. It was first described as a separate syndrome in the Netherlands [1], but the disease was later identified in patients from other European countries such as France [126-129], United Kingdom [130], Germany [131,132], Italy [133,134], Turkey [135] and the Czech republic [136] but also in the United States [92] and in Japan [137].

Many HIDS patients are found to have mutations in the gene for mevalonate kinase [2,3,40], which cause a decreased activity of this enzyme. Mevalonate kinase is an essential enzyme in the isoprenoid pathway which eventually results in the production of cholesterol, dolichol, and ubiquinone and in protein isoprenylation [41]. How the deficient activity of this enzyme causes the clinical phenotype is unknown. So far, 18 different mutations have been described, distributed throughout almost all coding regions of the gene [41,42]. One mutation, which leads to a replacement of valine by isoleucine at codon 377 of the protein (V377I), is particularly common, being present in 36 of 45 (80%) unrelated HIDS patients [42,138]. Most HIDS patients are compound heterozygotes, i.e. they have a combination of two different mutations in the mevalonate kinase gene. Usually, patients have a combination of a V377I with an I268T mutation (isoleucine → tyrosine at position 286). The clustering of cases in The Netherlands and in Western Europe, most notably those with a V377I mutation, is remarkable (Figure 1).

Figure 1.

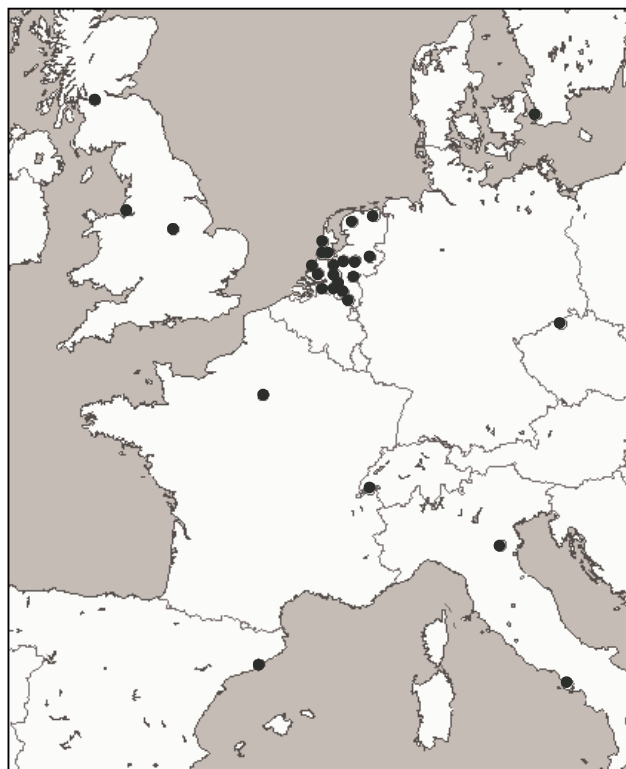Geographical distribution of 32 patients or families with at least one allele with a basepair change 1129G>A in the mevalonate kinase gene, leading to replacement of valine at codon 377 by isoleucine (V377I). Cases, indicated by black dot, are clustered especially in the Netherlands. Not shown: 1 patient in Turkey, 2 patients in the USA. (Figure created with "Online map creation", http://www.aquarius.geomar.de/omc).

In an attempt to gain insight in the ancestral origin and to explain the geographical distribution of HIDS we chose to perform an extensive haplotype study using five closely linked markers surrounding the mevalonate kinase gene in a group of 14 HIDS families.

Subjects and methods

Patients

Patients for this study were selected on the basis of previous inclusion in the Nijmegen International HIDS registry. This registry was set up in 1992 and now hosts clinical and laboratory data on 188 HIDS patients (http://hids.net). Briefly, all patients had recurrent attacks of fever ($\geq$38.5°C) with an acute phase response and a constantly elevated serum IgD (>100 U/mL) measured at 2 occasions with at least one month apart, and one or more of the following symptoms during attacks: lymphadenopathy; abdominal distress; skin manifestations; arthralgia / arthritis; and splenomegaly.

We identified 16 families with one or more affected siblings with HIDS and known mevalonate kinase genotype. Fourteen families were informative enough to allow haplotype analysis (Figure 2); they originate from the Netherlands (7 families), France (2 families), United Kingdom (2 families), Spain (1 family), Czech republic (1 family) and Italy (1 family). We also included 4 patients (Dutch n=3, United States of America n=1) who were homozygous for the V377I mutation. The clinical details and results from genotype analysis of these patients and families have been described elsewhere [42,46,139]. Mevalonate kinase genotype analysis of the 14 families yielded 11 V377I alleles, 7 I268T alleles, and 10 alleles with other mevalonate kinase mutations (P167L, H20P, H20N, R215Q, two deletions and four unknown).
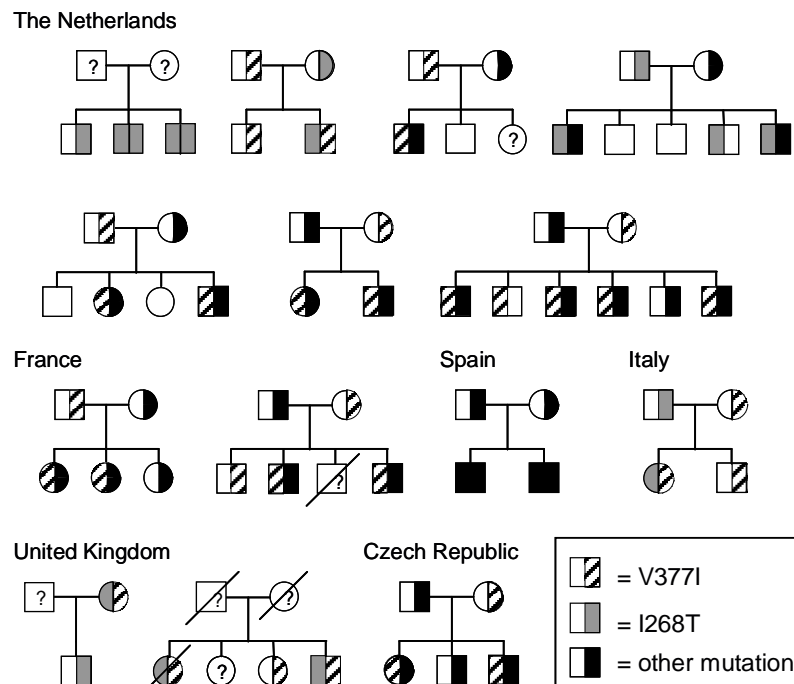

Figure 2. Pedigrees of 14 HIDS families with one or more affected siblings included in this study. The occurrence of the V377I mutation, I268T mutation or an allele with a different mutation in the mevalonate kinase gene is indicated. A question mark denotes a family member not available for genetic analysis.

The control alleles were gathered from unaffected members within the same families, to ensure that they would originate from the same population. The study was carried out after informed consent from all individuals and formal approval was obtained by the Medical Ethical Committee of the University Medical Center Nijmegen, The Netherlands.

Marker

Genomic DNA was extracted from whole blood or Epstein Barr Virus transformed lymphoblastoid cell lines according to standard procedures. The following five markers were used for the haplotype analysis: D12S1605, D12S1339, D12S1645, D12S234 and D12S1583 (GenBank). Primer sequences were obtained from the Genome Database. The markers were amplified using a standard PCR method. The marker allele sizes were analyzed with an ABI PRISM^{TM} 310 Genetic Analyzer (PE Applied Biosystems). Using BLAST search tools, the exact position of the mevalonate kinase gene and markers D12S1339, D12S1645 and D12S234 could be determined within two overlapping DNA sequences deposited in the Genome Database (AC007623 and AC007570). We determined that the HIDS gene is located between markers D12S1645 and D12S234.

Linkage disequilibrium analysis

Linkage disequilibrium indicates association between a genetic marker and a disease associated mutation, and is a measure for co-segregation of a specific haplotype with a mutation in a population. Statistical analysis comparing the frequency of the associated numbered marker allele on chromosomes carrying the V377I mutation with the frequency on normal chromosomes was based on a chi-square test for a 2x2 table. Linkage disequilibrium was assessed by the formula $\delta = (P_D - P_N)/(1-P_N)$, where P_D is the frequency of the associated marker allele on disease chromosomes and P_N the frequency of the same marker allele on normal chromosomes [140].

Results

Base-pair sizes of the different existing alleles of the 5 markers examined and their frequency in the control alleles are shown in table 1. Figure 3 displays the 11 definite V377I haplotypes from the 14 families and it is shown that the haplotype 3-1-4-8 is conserved among persons carrying the V377I mutation. This haplotype of at least the two flanking markers was also found on all of the V377I alleles of the 4 patients homozygous for this mutation, except in one allele of one Dutch patient.Statistical analysis shows a significant linkage disequilibrium for this haplotype, as demonstrated in table 2, and the 3-1-4-8 haplotype was not seen at all on the V377I-negative control alleles, indicating that it is highly mutation-associated.

Seven alleles with the I268T mutation in the mevalonate kinase gene could be collected from the 14 families. Allele 6 of marker D12S234 was only found in association with the I268T mutation and not in 25 control alleles nor in any of the alleles with other mutations in the mevalonate kinase gene. This again suggests a founder effect but the small numbers did not allow statistical analysis.

Origin	D12S1605	D12S1339	D12S1645	MVK gene	D12S234	D12S1583
The Netherlands	3	3	1	V377I	4	8
France	3	3	1	V377I	4	8
The Netherlands	1	3	1	V377I	4	8
The Netherlands	4	3	1	V377I	4	8
The Netherlands	3	3	1	V377I	4	5
The Netherlands	3	3	1	V377I	4	2
United Kingdom	3	3	1	V377I	4	10
Czech Republic	5		1	V377I	4	8
United Kingdom		2	1	V377I	4	8
Italy	3		1	V377I	4	6
France	5	2	1	V377I	5	8

Figure 3. The haplotypes of 11 alleles from the HIDS families carrying the V377I mutation, analyzed for the indicated markers. Numbers indicate different marker alleles, see table 1 for base-pair sizes. The common mutation-related haplotype (3-1-4-8) is marked by the shaded area. Missing numbers stand for missing data.

Discussion

The high prevalence of the V377I mutation among HIDS patients from different families inferred a founder effect with a common ancestor, and the geographical clustering of HIDS seemed to support this assumption. On the other hand, the large number of HIDS patients of Dutch origin might just reflect a reporting bias due to a locally heightened awareness of the disorder, also because measurement of IgD is included as a laboratory test in the diagnostic work-up of patients with periodic fever in The Netherlands [6].

In the present study, we constructed haplotypes surrounding the mevalonate kinase gene in HIDS patients from 7 different countries and our results show that the majority of V377I alleles studiedshare a single, common ancestral haplotype. This indicates that most carriers of the V377I mutation share the same ancestor. As most V377I mutation-positive HIDS patients originate from the Netherlands, it could be speculated that a founder lived there and that the mutation spread out from there, although formal proof is lacking. How frequent is the V377I allele in the general population? For the Dutch population of today, it can be estimated that there are 30 families with the V377I allele. If we assume Hardy-Weinberg equilibrium, the frequency of the V377I allele in the Dutch population would be 0.3%, or about 1:350.

Familial Mediterranean Fever ([Mendelian Inheritance in Men 249100]) is another autosomal recessive periodic fever syndrome which bears phenotypic similarities to HIDS and is caused by mutations in the pyrin gene [4]. Here, a founder effect has been established as well [15]. In contrast to HIDS, the carrier frequency of mutations in the pyrin gene is much higher and a carrier frequency of up to 1:7 has been observed in selected populations [141]. It has been suggested that the high frequency of pyrin mutations might be explained by a survival advantage of heterozygotes (carriers of one pyrin mutation) [15]. In sera of heterozygous carriers of a pyrin mutation, elevated concentrations of acute phase proteins have been detected. This finding has given rise to speculations that carriers have a

primed inflammatory response, which is beneficial to fight infections, and consequently compatible with a better survival [13,114]. In addition to this putative survival advantage, which would lead to natural selection of this mutation, the spread of the mutation might be explained by random genetic drift and migration.

It is remarkable that 17 of 39 (44%) genotypes in unrelated HIDS patients are compound heterozygote for the V377I and I268T mutations, which suggests a similar origin both with respect to age and region of origin.

In conclusion, this paper establishes that the most common mutation in HIDS, the V377I mutation, originates from a common ancestral haplotype in most cases. Current data suggest that a founder of this haplotype and the V377I mutation stems from the Netherlands, and that the mutation was spread from there to the rest of Europe and to the United States of America. This study underscores the importance of establishing the ethnical background of patients in the differential diagnosis of periodic fever syndromes.

Acknowledgements

6

Pro-inflammatory effect of excess mevalonic acid in the hyper-IgD and periodic fever syndrome through interleukin-1

Anna Simon, Jos W.M. van der Meer, Joost P.H. Drenth

Abstract

Growing evidence points to a link between isoprenoid metabolism and inflammation, although details are still lacking. Apart from the pleiotropic anti-inflammatory effects of HMG CoA reductase inhibitors, this link is exemplified by the inflammatory phenotype of the hyper-IgD and periodic fever syndrome (HIDS). This hereditary auto-inflammatory syndrome is caused by deficient enzyme activity of mevalonate kinase, one of the central enzymes of the isoprenoid pathway. Our aim was to explore the effect of excess mevalonic acid, the substrate for mevalonate kinase, on the inflammatory response of peripheral blood mononuclear cells (PBMCs) from healthy controls and six mevalonate kinase deficient HIDS patients. PBMCs from healthy controls preincubated for 4 hours with high dose mevalonic acid secreted twice as much interleukin (IL)-6 as cells stimulated with LPS alone, while the pro-inflammatory cytokines tumor necrosis factor (TNF)-α and IL-β were increased as well. This increased cytokine secretion could be completely suppressed by co-incubation with IL-1 receptor antagonist (IL-1ra). Mevalonate kinase-deficient PBMCs secrete more IL-6 and IL-1β than control PBMCs, but this difference also disappears upon addition of IL-1ra. Preincubation with high dose MVA decreases the secretion of IL-1ra by PBMCs from controls as well as MK-deficient patients.

In conclusion, co-incubation of excess MVA with an inflammatory stimulus has a pro-inflammatory effect, especially on IL-6 production, mediated by the IL-1 system. This may be central to the pathogenesis of HIDS.

Introduction

The isoprenoid metabolism is a metabolic pathway with pleiotropic functions varying from isoprenylation of proteins to production of cholesterol, coenzyme Q, and dolichol [142]. A growing body of evidence points to a previously unanticipated role of the isoprenoid metabolism in inflammation. In the first place, inhibitors of 3',5' hydroxymethylglutaryl coenzyme A (HMG CoA) reductase, the major rate-limiting step early in this metabolic pathway, have anti-inflammatory properties [143]. These properties include decreased expression of adhesion molecules (e.g. intercellular adhesion molecule-1 (ICAM-1) [144]), chemoattractant proteins (e.g. monocyte chemotactic protein-1 (MCP-1) [145]), pro-inflammatory transcription factors such as nuclear factor-κB (NF-κB) and pro-inflammatory enzymes such as cyclooxygenase-2 (COX-2) [146]. In an animal model of a central nervous system autoimmune disease, the HMG CoA reductase inhibitor atorvastatin induced secretion of the inhibitory cytokines interleukin (IL)-4, IL-5, IL-10, and transforming growth factor TGF-beta, while at the same time suppressing secretion of pro-inflammatory cytokines such as IL-2, IL-12, interferon (IFN)-γ and tumor necrosis factor (TNF)-α [147].

Secondly, this link with inflammation is also apparent from the observation that a rare hereditary auto-inflammatory disorder – the hyper-IgD and periodic fever syndrome (HIDS) – is caused by a genetic defect of one of the enzymes of isoprenoid metabolism, i.e., mevalonate kinase [2,3]. Mevalonate kinase deficiency in HIDS results in a phenotype of recurrent episodes of (systemic) inflammation and fever. During inflammatory episodes of HIDS, mevalonic acid, the substrate for mevalonate kinase, accumulates in body tissues and is excreted in urine. HIDS attacks are also accompanied by an impressive release of inflammatory cytokines such as IL-1, IL-6, TNFα and IFNγ [50,95]. In a recent clinical trial in 6 HIDS patients with mevalonate kinase deficiency, we found that simvastatin ameliorates the inflammatory symptoms in HIDS and decreases urinary mevalonic acid excretion [148]. A single study explored whether the increased interleukin-1β (IL-1β) secretion in HIDS was due to an excess mevalonic acid or due to a lack metabolites of the isoprenoid pathway and it concluded that in their model the latter mechanism appeared to be operative [149]. However, further details on this link between isoprenoid metabolism and the inflammatory system are lacking. In this study, we explored the effect of excess amounts of mevalonic acid on the inflammatory response of peripheral blood mononuclear cells (PBMCs) from healthy controls and from mevalonate kinase (MK) deficient patients.

Material & Methods

Patients & controls

Blood donors were 22 healthy controls ranging in age from 26 to 50 years old (12 women and 10 men), and 6 mevalonate kinase deficient HIDS patients, aged between 33 and 43 years old (2 women and 4 men), with a residual mevalonate kinase enzyme activity ranging from 6 to 16%. All patients were symptom-free when venous blood was drawn; none of them used any medication at the time. All participants gave informed consent and the guidelines of the local ethics committee were followed in the conduct of these experiments.

Reagents

Mevalonic acid (MVA) lactone, 3,3-dimethylglutaric acid (DMGA), and *E. coli* 055:B5 TCA and phenol-extracted LPS were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Human recombinant IL-1ra was a gift of Amgen (Boulder, Colorado, USA); IL1α was a gift of Hoffman-La Roche (Nutley, New Jersey, USA). Mevalonic acid was prepared by dissolving the mevalonolactone in a NaOH solution, for 20 minutes at 50˚C; this solution was neutralized with HCl and RPMI buffer.

PBMC isolation and experimental procedures

Blood for the isolation of PBMCs was drawn in 10 ml EDTA anti-coagulated tubes (Vacutainer System; Becton Dickinson, Rutherford, NJ). PBMCs were isolated by density gradient centrifugation over Ficoll-Hypaque (Pharmacia Biotech AB, Uppsala, Sweden). The cells from the interphase were aspirated, washed three times in sterile PBS, and resuspended in culture medium RPMI 1640 (Dutch modification; Flow Labs, Irvine, UK) supplemented with L-glutamine (2 mmol), pyruvate (1 mmol), and gentamicin (50 mg/ml).

Cells were incubated at 5×10^5 cells/well in 96-wells polystyrene plates (Greiner, Alphen a/d Rijn, The Netherlands), at 37°C and 5% CO_2, with or without MVA (1 or 10 mM) and IL-1ra (10 µg/mL) for 4 hours. After this preincubation, LPS (1 ng/mL) or IL-1α (10 ng/mL) were added and supernatants were collected after 5 h or 24 h of incubation, and stored at -80°C until assay. Viability of cells was checked at end of incubation by trypan-blue staining.

Cytokine measurements

Concentration of IL-6 was determined by a commercially available ELISA kit (Pelikine compact; Sanquin, Amsterdam, The Netherlands). For TNF-concentration, a specific ELISA was used with two avian (duck, chicken) antibodies in the pre-analyte and two mammalian (rabbit, goat) antibodies in the post-analyte stage [150]. IL-1ß was determined by radioimmunoassay as described by us previously [151]. Concentration of IL-1ra was determined with an ELISA Development Reagens kit (R&D Systems, Inc.; Minneapolis, MN, USA).

Statistical analysis

Statistical analysis was performed using an unpaired or paired (where appropriate) two-sided *t*-test; $P < 0.05$ was considered significant. The software package used for statistical analysis and graphs was GraphPad Prism, version 4.00 (GraphPad Software, Inc.).

Results

Excess mevalonic acid

The addition of MVA in a concentration of 10 mM to PBMCs of healthy controls has no effect on cytokine production or viability (data not shown). However, when cells are preincubated with 10 mM MVA for 4 hours, and subsequently exposed to a low dose of LPS (1 ng/mL) for 6 hours, twice as much IL-6 is produced as when cells are exposed to LPS alone, without MVA (figure 1a). The same twofold increase in IL-6 response is seen when cells preincubated with 10 mM MVA are

exposed to a 1000-fold concentration of LPS (1 µg/mL; data not shown). Preincubation with MVA is necessary: when MVA and LPS are added at the same time, the pro-inflammatory effect of MVA disappears (data not shown). Preincubation with saponificated DMGA, a fatty acid with similar chemical characteristics as MVA, does not have any effect on the LPS response either (data not shown). These last two findings support the theory that MVA has a specific effect, and does not work through direct destabilization of LPS micelles.
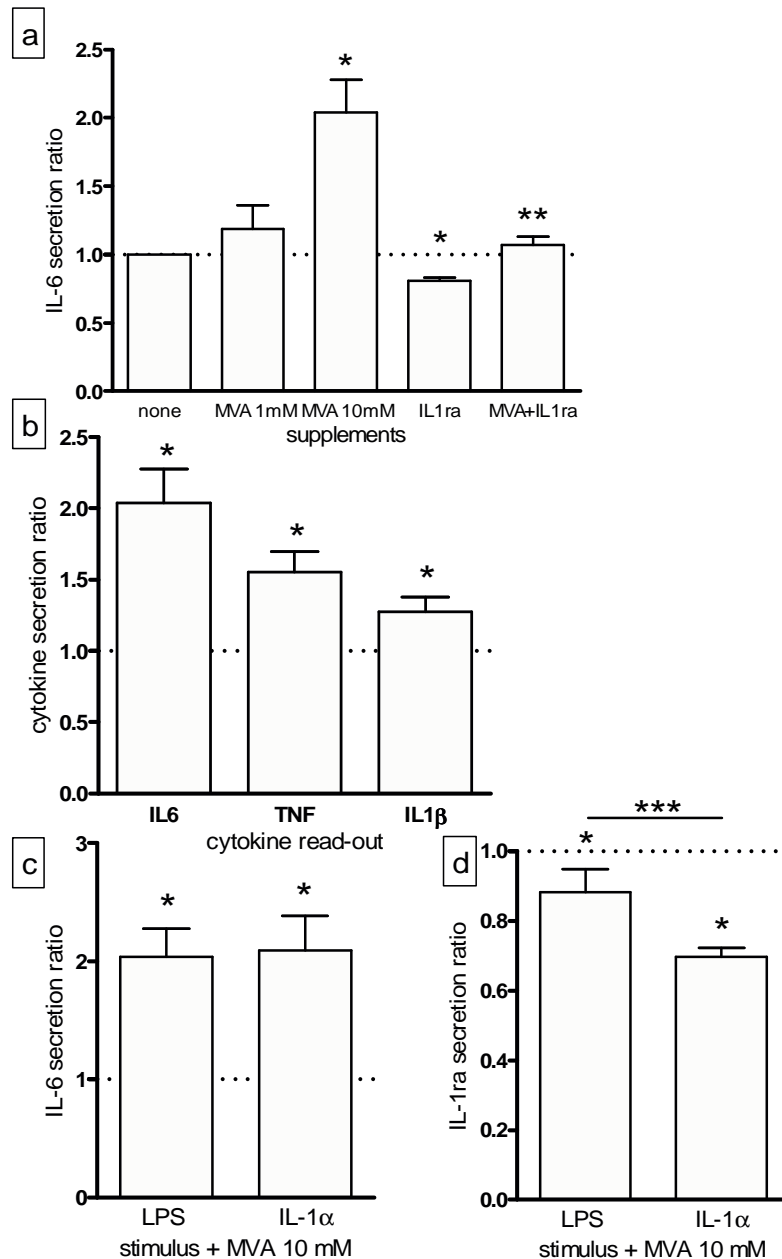
Figure 1 (a) Ratio of IL-6 secretion on stimulation with low dose LPS (1 ng/mL) after preincubation with mevalonic acid (MVA), interleukin-1-receptor antagonist (IL-1ra) or both, relative to IL-6 secretion on LPS stimulation alone. IL-1ra completely blocks the pro-inflammatory effect of preincubation with MVA. (b) Increased secretion of IL-6, TNF and IL-1β on stimulation with low dose LPS after pre-incubation with MVA. The effect is most pronounced for IL-6. (c) Stimulating effect of pre-incubation with MVA on IL-6 secretion is similar for stimulation with either low dose LPS or IL-1α. (d) Pre-incubation with MVA decreases IL-1ra secretion by LPS stimulation, but even more on stimulation with IL-1α. * = p<0.05 for effect of pre-incubation; ** = p<0.05 when compared to pre-incubation with MVA alone; ***=p<0.05 when comparing stimulus LPS with IL-1α.

Role of interleukin-1

The effect of excess MVA on cells exposed to LPS can be completely inhibited by addition of IL-1 receptor antagonist (IL-1ra) (figure 1a). This suggests a role of IL-1 in the pro-inflammatory effect of excess MVA. On the other hand, our data suggest also that the effect is not completely mediated by IL-1β as the response of IL-1β after incubation with LPS is much more subtle than that seen for IL-6 or TNF-α (figure 1b). We next investigated whether increased IL-1 receptor expression might be involved, by stimulating with IL-1α instead of LPS. This shows a similar pro-inflammatory effect of MVA preincubation (figure 1c). At the same time, secretion of IL-1ra is lower after preincubation with MVA, most notably when stimulated with IL-1α (figure 1d).

Mevalonate kinase deficient PBMCs and excess MVA

PBMCs from MK-deficient HIDS patients were used to compare the response to that of PBMCs from healthy controls. Incubation with only 10 mM MVA (without LPS) resulted in increased secretion of IL-6 in the MK-deficient PBMCs, while this was clearly not the case in PBMCs from healthy controls (figure 2); a similar pattern was seen for IL-1β secretion.

The following experiments with low dose LPS were performed with an incubation time of 24 hours, which resulted in an even more pronounced effect of preincubation with MVA in healthy controls than with the incubation time of 6 hours, as measured in terms of secretion of IL-6 (figure 3a) or IL-1β (figure 3b). However, the effect is still greater on IL-6 (5 times increased secretion after preincubation with MVA) than on IL-1β (3.2 times increased secretion).

MK-deficient PBMCs produce slightly more IL-6 after stimulation with low dose LPS (figure 3a). Preincubation with MVA also increases IL-6 secretion in MK-deficient PBMCs, but the effect is smaller and IL-6 secretion by PBMCs on LPS after preincubation with MVA is similar to that of healthy controls (figure 3a).
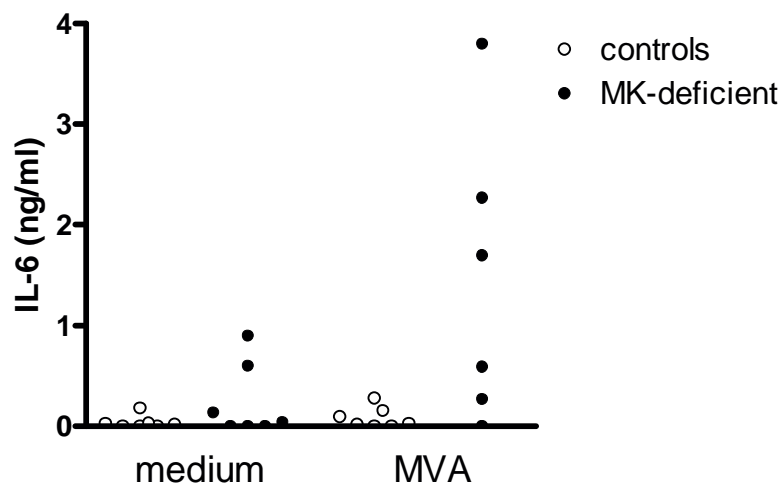
Figure 2. Incubation of PBMCs from healthy controls with 10 mM MVA alone does not lead to IL-6 secretion, while MK-deficient PBMCs from HIDS patients do secrete more IL-6 on incubation with MVA compared to medium alone.

Mevalonate kinase deficient PBMCs and interleukin-1

In accordance with the experiments with healthy PBMCs, we observed that the increased IL-6 secretion of MK-deficient PBMCs on low dose LPS could be inhibited by addition of IL-1ra (figure 3a). The effect of MVA can also be completely reversed by co-incubation with IL-1ra. IL-1β secretion on stimulation with low dose LPS with or without MVA mirrors the response of IL-6 (figure 3b). MK-deficient PBMCs secrete more IL-1ra when exposed to LPS than control PBMCs; in both MK-deficient and control PBMCs IL-1ra secretion is diminished after preincubation with MVA (figure 3c). Stimulation with IL-1α results in a different picture: MK-deficient PBMCs secrete less IL-6 on stimulation with IL-1α when compared with control PBMCs, with or without preincubation with MVA (figure 3d).
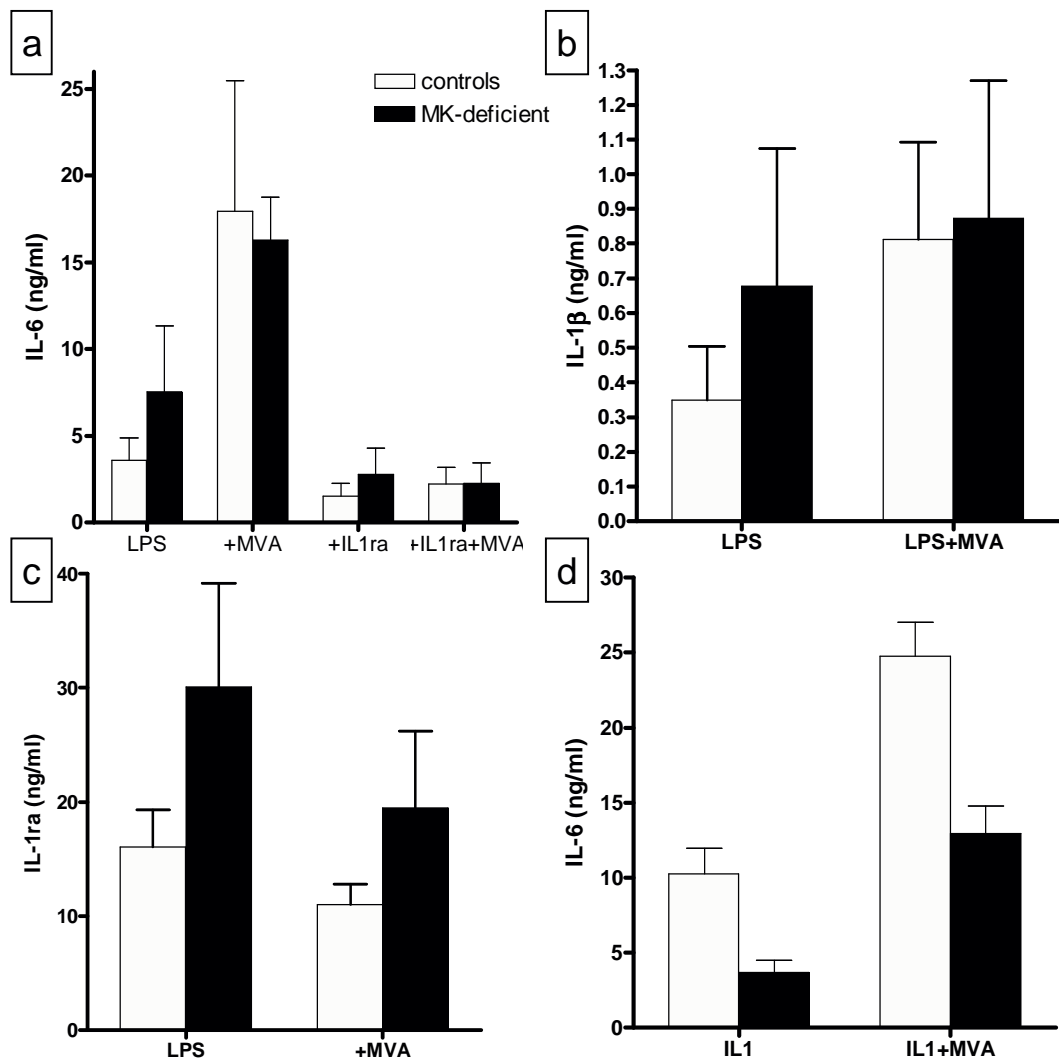
Figure 3. MK-deficient PBMCs (n=6) secrete more IL-6 (a) and IL-1β (b) than control PBMCs after stimulation with LPS. Upon pre-incubation with MVA, IL-6 secretion in both groups is increased to the same concentration. Co-incubation with IL-1ra inhibits the extra IL-6 secretion seen in MK-deficient PBMCs on LPS stimulation as well as inhibiting the pro-inflammatory effect of pre-incubation with MVA. (c) MK-deficient PBMCs also secreted more IL-1ra on LPS stimulation, but pre-incubation with MVA decreases IL-1ra secretion in both. (d) Upon stimulation with IL-1α, MK-deficient PBMCs secrete less IL-6 than controls, with or without pre-incubation with MVA.

Discussion

This study shows that an excess amount of mevalonic acid has a pro-inflammatory effect, most pronounced with regard to IL-6 secretion. The effect is apparently mediated through IL-1, as the production of IL-6 can be completely blocked by addition of IL-1ra. The effect we observed is dose-dependent, and is only seen after preincubation with MVA before adding an inflammatory stimulus (LPS or IL-1α alike). This suggests that MVA primes the cells to become more sensitive to a subsequent pro-inflammatory stimulus. The decreased secretion of IL-1ra after MVA preincubation may contribute to this effect.

PBMCs (lymphocytes and monocytes) from HIDS patients are deficient in mevalonate kinase enzyme activity due to the underlying germ-line genetic defect. To compensate for this, the activity of HMG-CoA reductase, the enzyme preceding MK in the isoprenoid pathway, is upregulated [43]. Together this will lead to elevated intracellular MVA concentrations, and this is thought to subsequently restore the flux through the mevalonate pathway and help to maintain near normal levels of isoprenylated end-products in these patients [43]. This effect is mirrored by mevalonic acid excretion in urine, which increases to detectable levels during attacks, but decreases to much lower values during remission. On the other hand, this compensatory increase of MVA concentration may at the same time, in an autocrine fashion, prime cells to respond excessively to inflammatory stimuli (figure 3a, 3b). This might then result in the fever attack which is central to the HIDS phenotype.

Our results also show that MK-deficient cells secrete more IL-1ra than controls. This is in line with earlier experiments using a whole blood culturing system [50] and this autocrine inhibitory loop may explain why MK-deficient cells respond less to an IL-1α stimulus, probably through a compensatory effect (figure 3d). Preincubation with MVA causes a decrease of secretion of the anti-inflammatory cytokine IL-1ra in both MK-deficient cells and controls, which may contribute to the pro-inflammatory effect of MVA.

Treatment of HIDS patients with simvastatin, which reduces urinary MVA excretion, leads to improvement of symptoms with fewer days of illness and inflammation [148]. This also fits with the growing body of evidence for anti-inflammatory and immuno-modulatory effects of statins [143]. Frenkel et al. in their experiments with mononuclear cells from controls and HIDS patients did not detect a pro-inflammatory effect of preincubation with MVA [149]. This may have been due either to the relatively low concentration of MVA used (1 mM, compared to 10mM in our study), or to the inflammatory stimulus used: anti-CD2-CD28 antibody, a specific T-cell stimulus, which was previously used in one of the few models demonstrating a pro-inflammatory effect of statins [152].

The central role of IL-1 in HIDS as demonstrated in these experiments emphasizes the link with other hereditary autoinflammatory syndromes. Defective regulation of IL-1 by proteins from the pyrin family has been shown to be one of the central pathogenic mechanisms in familial Mediterranean fever and the cryopyrin-associated periodic syndromes [153-156]. These results also suggest that treatment of HIDS patients with a recombinant IL-1ra (Anakinra) may decrease the inflammatory symptoms. In fact, our first results with this drug underscore this (Bodar et al, unpublished observation).

In conclusion, co-incubation of excess MVA with an inflammatory stimulus has a pro-inflammatory effect, especially on IL-6 secretion, probably mediated by the IL-1 system. This may be central to the pathogenesis of HIDS.

Acknowledgments

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Limited efficacy of Thalidomide in the treatment of febrile attacks of the Hyper-IgD and periodic fever syndrome

A Randomized, Double-Blind, Placebo-Controlled Trial

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Abstract

Hyper-IgD and periodic fever syndrome (HIDS) is an autosomal recessive disorder featured by recurrent febrile attacks. Previous unpublished experience (J. van der Meer and R. Powell) suggested that thalidomide may prevent febrile attacks. Six HIDS patients (5 male and 1 female) who had at least one febrile attack every 6 weeks, entered a randomized, double-blind, placebo-controlled crossover trial to explore the efficacy of a daily 200-mg thalidomide dose in the treatment of recurrent febrile attacks of HIDS. The patients received either thalidomide, 200-mg daily, or placebo for 16 weeks, followed by a 4-week washout period and another 16-week treatment (crossover) with either thalidomide or placebo. Patients completed a weekly diary card noting attacks and side effects. During the study, C-reactive protein (CRP), serum amyloid A (SAA), interleukin (IL)-6, tumor necrosis factor (TNF)-α, IL-1 receptor antagonist, soluble TNF receptor p55 and p75, and lipopolysaccharide-stimulated IL-1β and TNF-α production were measured at six different points, whereas urine neopterin levels were measured weekly. During the active treatment with thalidomide, there were 10 attacks compared with 13 attacks with placebo. Thalidomide resulted in a nonsignificant decrease of CRP and SAA, but the concentrations of other inflammatory mediators, including urine neopterin, remained unchanged. One patient developed sensory polyneuropathy, but this resolved when thalidomide administration was stopped. The effect of thalidomide in HIDS is limited to a decrease in acute phase protein synthesis without an effect on the attack rate.

Introduction

In 1984, van der Meer and colleagues described six patients with periodic fever and a constantly elevated serum polyclonal IgD and called the syndrome hyperimmunoglobulinemia D and periodic fever syndrome (HIDS) [1]. HIDS is an autosomal recessive disorder, and to date more than 150 familial and isolated cases are known [46]. It should be distinguished from other periodic fever syndromes, such as the autosomal recessive familial Mediterranean fever and autosomal dominant tumor necrosis factor receptor-associated periodic fever syndrome (http://hids.net; [157]). HIDS patients have a long history of recurrent attacks of fever, frequently preceded by chills and accompanied by headache, bilateral cervical lymphadenopathy, and occasionally by oral and vaginal aphthous ulcers, abdominal pain, and diarrhea. Laboratory analyses invariably reveal an acute phase response during attacks and a constantly elevated serum level of polyclonal IgD [46]. Symptoms commence at an early age and persist throughout life, and the febrile attacks occur every 4 to 8 weeks, lasting 3 to 7 days. Patients are asymptomatic between attacks, although the acute phase response may persist. Symptomatic episodes are associated with increased concentrations of inflammatory cytokines, such as TNF-α, interleukin (IL)-6, and interferon (IFN)-γ, and of the anti-inflammatory compounds IL-1ra and soluble TNF receptor p55 and p75 [50]. HIDS is caused by missense mutations in the mevalonate kinase gene, which leads to reduced enzyme activity of mevalonate kinase and results in small amounts of mevalonic acid in the urine [2,3,42]. Despite the progress in understanding the pathogenesis of the disorder, treatment remains largely supportive. Although initial reports suggested benefits from colchicine, subsequent experience with the drug failed to substantiate this [1,133]. Similarly, isolated case reports have mentioned success with various dosages of steroids, immunoglobulin infusions, and cyclosporine, but application in a larger group did not confirm this.

Thalidomide, infamous for its severe teratogenicity, is able to inhibit TNF-α production by human mononuclear cells and to normalize elevated plasma TNF-α concentrations [158,159]. In addition, thalidomide inhibits IFN-γ synthesis and constrains leukocyte chemotaxis [160]. The drug has been shown to have a consistent benefit in a wide variety of inflammatory disorders, such as erythema nodosum leprosum [161,162] and Behçet's syndrome [163]. A large randomized trial showed that thalidomide is very effective for treating oral and genital ulcers and follicular lesions of Behçet syndrome [164]. Preliminary data obtained in two HIDS patients showed that thalidomide resulted in a dramatic relief of symptoms (J. van der Meer and R. Powell, unpublished observations). Therefore, we initiated a 36-week randomized, double-blind, placebo-controlled trial to assess the effect of a daily 200-mg thalidomide dose on the frequency, intensity, and duration of attacks in HIDS patients.

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Materials and Methods

Patients

Patients were selected from the Nijmegen HIDS registry. This database carries the pertinent clinical and laboratory data of patients with HIDS. Because all patients were required to visit the University Medical Center St. Radboud regularly throughout this trial, we recruited only Dutch patients. The patients were informed about the trial by a nation-wide patient meeting in Nijmegen, August 1999. Only male HIDS patients and women who had had a surgical sterilization procedure were invited to take part in the study. Additionally, female patients were required to either use condoms or refrain from sexual intercourse during the study. Only patients over 18 years old and having frequent febrile attacks (more than one attack every 6 weeks) were eligible for the study. All patients who enrolled in this study were screened for mutations in the mevalonate kinase gene. The numbering of the patients in this study refers to the original patient number in the Nijmegen HIDS registry [46]. Given the average attack frequency in our patients (at least one every 6 weeks), we defined effective thalidomide treatment as treatment that was able to decrease the number of attacks by 50%. Our study was designed to have an 80% power of detection and a decrease of 45% in the frequency of attacks.

Trial Design

The trial was a 36-week randomized, placebo-controlled crossover trial evaluating the efficacy of thalidomide taken at a dosage of 200 mg daily in the treatment of HIDS patients suffering from recurrent febrile attacks. The study consisted of two periods of 16-week treatments with thalidomide or placebo separated by a 4-week washout. The trial was conducted at the outpatient clinic of the Division of General Internal Medicine of the University Medical Center St. Radboud in Nijmegen, The Netherlands. Permission of the local Medical Ethical Committee had been obtained. A clinical pharmacist who was not directly involved in the trial prepared a simple, computer-generated, random-number list. The code was kept at the Department of Clinical Pharmacy and was opened only after all data had been entered into a computer for analysis. Before study entry, the prospective candidates were informed about the design, purpose, and duration of the study and received oral and written information concerning adverse effects of thalidomide. Patients gave written informed consent before enrolling in the trial. Subjects were screened at the beginning of the trial, and baseline demographic and relevant clinical information was obtained. They all had normal results on clinical neurological evaluations. Physical examinations and laboratory measurements were performed at baseline and at weeks 8, 16, 20, 28, 36.

Drugs

At baseline and at the 20-week visit, the patients received two bottles containing either 100-mg thalidomide tablets (Grünenthal GmbH, Aachen, Germany) or placebo tablets identical in appearance to the thalidomide tablets. Patients were instructed to take two tablets each evening. At week 16 and week 36, unused tablets were collected and counted to determine compliance. Patients were allowed to use antipyretic drugs, such as acetaminophen or nonsteroidal anti-inflammatory drugs, throughout the study period. One patient (patient 4) used a fixed low-dose steroid (prednisone 10 mg/day) throughout the study period.

Outcome Measures

Attacks

The primary outcome measure was defined as the number of attacks. To register the number and severity of attack, all patients were asked to complete a weekly diary card to register the presence or absence of symptoms and the severity using a visual analog scale. The following characteristics were listed: lymphadenopathy, abdominal pain, nausea, diarrhea, vomiting, arthralgia, skin lesions, aphthous ulcers, and headache. The patients could indicate the severity of the symptoms on a scale ranging from 0 to 7. Attacks were defined as fever ($>38.5°C$) together with one or a combination of the following symptoms: abdominal distress (pain, vomiting, diarrhea), joint involvement (arthralgia, arthritis), skin lesions, and/or lymphadenopathy. Each week this symptom card was mailed to the principal investigator by the patient.

Acute Phase Response and Cytokine Measurements

Serum IgD was measured at the onset of the trial using an enzyme-linked immunosorbent assay (ELISA), as described elsewhere [110]. Blood samples were drawn six times throughout the whole study period: at baseline and at weeks 8, 16, 20, 28, and 36, and the serum/plasma concentrations of the following inflammatory mediators were measured. C-reactive protein (CRP) and serum amyloid A (SAA) were measured using sensitive ELISAs [105]. Measurements of serum TNF-, IL-1ra, and IL-1β were performed using fluid phase radioimmunoassays, whereas IL-6 was measured with an ELISA [151]. The concentrations of sTNFr p55 and sTNFr p75 were measured in serum using an ELISA developed by Hoffman-La Roche (Basle, Switzerland) [50]. Cytokine production was measured using a whole blood culture system developed in our laboratory's assay [50]. Briefly, two 2-ml tubes containing 24 µl of EDTA-K3 (10,000 kallikrein-inactivating units per milliliter; Bayer, Leverkusen, Germany) were drawn. One tube was incubated immediately, and the other tube was incubated after addition of 25 µl of lipopolysaccharide (Escherichia coli serotype 055:B5; Sigma, St. Louis, MO; final concentration 10 µg/ml of blood). After 24 h of incubation at 37°C, both tubes were centrifuged at 2250g for 10 min and then at 15,000g for 5 min to obtain platelet-poor plasma. Aliquots were stored at 70°C until assay.

Neopterin Measurement

Previous data indicated that the excretion of neopterin in urine conveniently correlated with the febrile attacks. We therefore asked the patients to collect weekly urine samples, which were stored at 20°C until assay. Neopterin was determined by reversed-phase high-performance liquid chromatography [165]. Briefly, urine samples were centrifuged to remove debris, diluted in a 1 to 10 ratio with water containing dimethylpterine as an internal standard, and injected directly onto a Techsphere 5 ODS column (HPLC Technology, Ltd., Welwyn Garden City, Hertfordshire, UK). A binary gradient elution was used with an initial mobile phase of 2% methanol in 15 mM phosphate buffer, pH 6.4, increasing to 25% methanol after 12 min, and creatinine was detected separately using a kinetic alkaline picrate (Jaffe) method. The ratio of neopterin to creatinine was calculated to compensate for variations of urine density. The median urine neopterin value for a group of 65 healthy controls was 149 µmol/mol creatinine (range 62-273).

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Characterization of HIDS by Mutation Analysis

RNA was extracted from Epstein-Barr immortalized cell lines, and cDNA was produced using standard techniques. The complete coding region of the mevalonate kinase gene was amplified by polymerase chain reactions, and we determined the nucleotide sequences of the amplified fragments by standard semiautomated methods on an ABI PRISM 377 (PerkinElmer Life Science, Boston, MA) [2].

Monitoring of Side Effects

Because thalidomide can cause peripheral neuropathy, a careful neurological examination was performed at each visit. Patients who developed any sign of neuropathy were excluded from continuation of the trial. The patients mailed a weekly diary card, which contained a list of potential side effects. In total, they could choose from a list of 16 precoded side effects.

Statistical Analysis

Continuous variables were compared by using the Wilcoxon rank-sum test. Probability (P) values were calculated on the basis of two-tailed t tests. A P value of less than 0.05 was considered to be the lowest level of significancy.

Results

Patients

Six patients (5 male and 1 female) enrolled in the study between August and September 1999. The demographic data are depicted in Table 1. The mean age at the start of the study was 32.5 (standard deviation 13.7) years and all patients had had their first attacks before the end of their first year of life. Patients reported an attack frequency once every 2 to 9 weeks. The length of the febrile episodes varied between 2 to 7 days in these HIDS patients. All patients had elevated serum IgD concentrations, which ranged from 154 to 4224 IU/ml.

Mutation Analysis

Four patients carried the V377I missense mutation (valine replacing isoleucine at codon 377 of the mevalonate kinase protein). This mutation is considered to be the prototype mutation in HIDS. One patient was homozygous for this mutation, and three were compound heterozygote, most commonly in combination with I268T (isoleucine replacing a threonine at codon 268). In patient 26, we were unable to detect a mutation on one of the chromosomes.

No. in HIDS registry	Sex	Age (years)	Age at onset (months)	Average length of attack (days)	Average attack/ weeks	Maximal IgD (IU/ml)	Mutations in MVK gene
4	M	51	0	3-4	1/4	991	V377I/I268T
26	M	20	3	2-3	1/3-4	4224	V377I/?
27	M	28	18	2-3	1/2	1731	P167L/I268T
31	M	19	0	7	1/4	656	V377I/I268T
89	F	35	3	4	1/2-3	376	V377I/V377I
102	M	48	12	4-5	1/6-9	154	I268T/I268T

Table 1. Clinical characteristics of the patients. The average attack frequency was reported by the patient at time of the diagnosis.

Treatment Outcomes

All patients were able to complete the trial. However, one patient (patient 102) receiving thalidomide discontinued treatment after 9 weeks because he developed numbness and paraesthesias of the extremities. Four days after discontinuation of the trial medication, the signs of sensorial neuropathy had disappeared, and a physical examination performed at the end of the trial 6 weeks later showed normal discriminative and vibration sensations and physiological muscle stretch reflexes. The data from this patient were analyzed on an intention-to-treat analysis. Compliance rates for thalidomide or placebo use, as calculated from the returned pill counts, were similar in both groups (95% versus 96%). Three patients used antibiotics at some point during the trial because of suspected upper airway infections. During thalidomide treatment, patients 31 and 89 were treated with a combination of amoxicillin/clavulanic acid, and patient 34 was treated with erythromycin.

In the complete study period, including the washout period, the six patients registered a total of 30 attacks (one attack every 7.2 weeks). During active treatment with thalidomide, there were 10 attacks compared with 13 attacks with placebo (Table 2). The number of symptomatic days was similar with thalidomide (65 days) or placebo (87 days). The length of attacks was similar during both treatment blocks (thalidomide 6.3 days versus placebo 6.2 days). With thalidomide, the number of symptoms was 2.9 per attack compared with 3.5 with placebo. The severity of the separate symptoms during the febrile episodes, as indicated on a scale from 0 to 7, was 3.8 (standard deviation 2.2) with thalidomide and 4.2 (standard deviation 2.8) with placebo.

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Table 2. The distribution of the number of attacks in the six individual HIDS patients during the crossover trial among placebo and thalidomide (200 mg/day).

No. in HIDS Registry	Placebo	Washout	Thalidomide
4	1	0	0
26	3	3	2
27	3	2	1
31	1	0	3
89	4	1	3
102	1	1	1
Total	13	7	10

Circulating Mediators

Thalidomide treatment resulted in a moderate decrease of CRP and SAA when compared with placebo. At start of the active treatment, mean CRP concentration was 40.1 mg/l, and this decreased to 12.2 mg/l at the end of the trial. A similar effect was seen on SAA concentrations, which decreased from 30.8 mg/l to 3.9 mg/l (Fig. 1, upper panel). These differences were not significant. Thalidomide treatment did not affect plasma IL-1RA or IL-6 concentrations. With placebo, both these parameters rose during the last 6 weeks of the trial (Fig. 1, lower panel). Likewise, active treatment did not influence the sTNFr concentrations in plasma when compared with placebo.

Ex Vivo Production

Figure 2 shows the LPS-stimulated production of the various cytokines tested. The production of IL-1β in patients with HIDS during thalidomide treatment was similar to that of placebo. Given the specific TNF-α inhibiting property of thalidomide, we anticipated a decrease of the TNF-α production. However, we failed to note an effect of the drug on the ex vivo LPS-stimulated production of TNF-α.

Neopterin

Patients collected 92 weekly urine samples in the placebo period and 88 urine samples with thalidomide treatment. Figure 3 shows box and whisker plots of the values throughout the trial. With placebo the median value was 270.5 µmol/mol creatinine, which did not differ from those obtained during thalidomide (281 µmol/mol creatinine) or during the washout period (264.5 µmol/mol creatinine). In all 204 samples submitted, 51% had values above the upper limit of those found in healthy individuals (273 µmol/mol creatinine). Febrile attacks clearly influenced neopterin levels, and higher values were seen during periods with disease activity. During the 30 attacks, the average neopterin value was 657 µmol/mol creatinine, clearly higher than with remission (mean values 329 µmol/mol creatinine). There was no significant difference between thalidomide and placebo with respect to the neopterin excretion during attacks (data not shown). Most notably, we detected very high values (>1000 µmol/mol creatinine) in 5 of 167 samples of patients who reported to have a remission. One patient had a value of 2598 µmol/mol creatinine but denied any clinical disease activity before or at the point of sampling.

Side Effects

Three patients did not note any side effects. The other three patients recorded a total of 165 symptoms, as indicated by their diaries. During thalidomide, 115 symptoms were recorded compared with 50 during placebo. As depicted in Table 3, patients most commonly complained about a dry mouth and fatigue. As previously mentioned, one patient reported symptoms of polyneuropathy necessitating termination of thalidomide treatment. No patient developed neuropathy after the trial (Table 3).

CRP

SAA

IL-1RA

IL-6
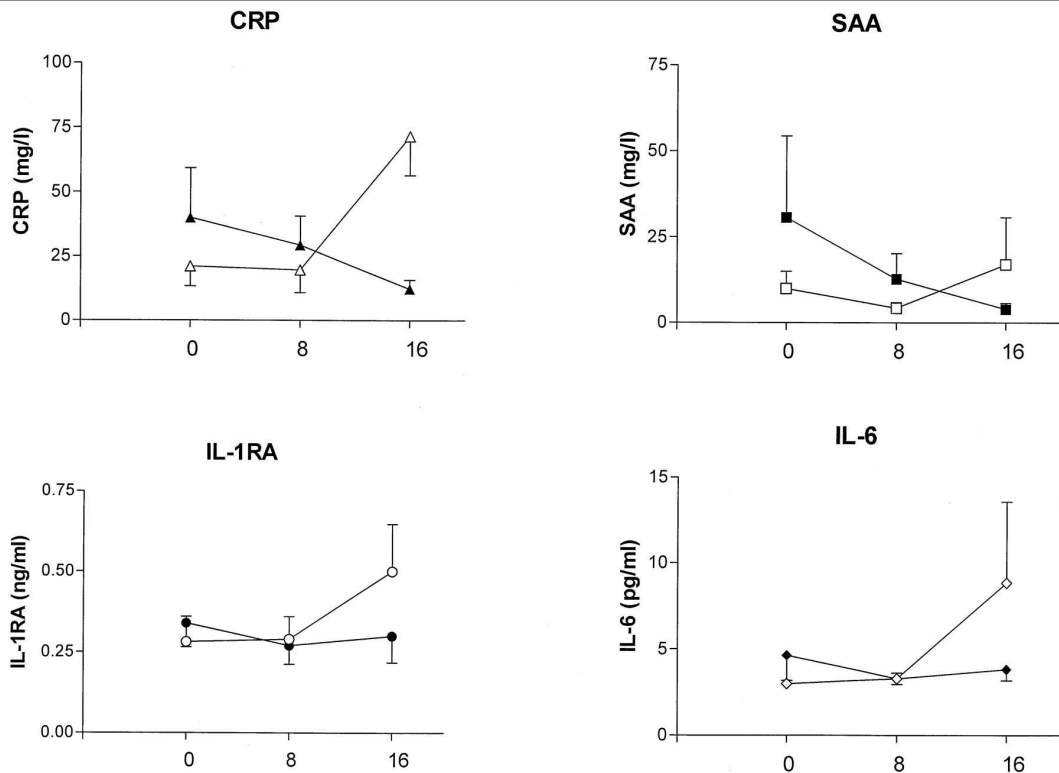
Figure 1. The effect of treatment with thalidomide (closed shapes) or placebo (open shapes) on CRP (left upper panel), SAA (right upper panel), IL-1RA (lower left panel), IL-6 (right lower panel). Data are given as mean ± S.E.M. The values at the *x*-axis refer to time of sampling during the trial and indicate baseline (0), 8 weeks (8), and 16 weeks (16). Note that there is a trend of lower CRP, SAA, IL-1RA, and IL-6 values after 16 weeks of thalidomide treatment as compared with the same time point during placebo.

TNFα production
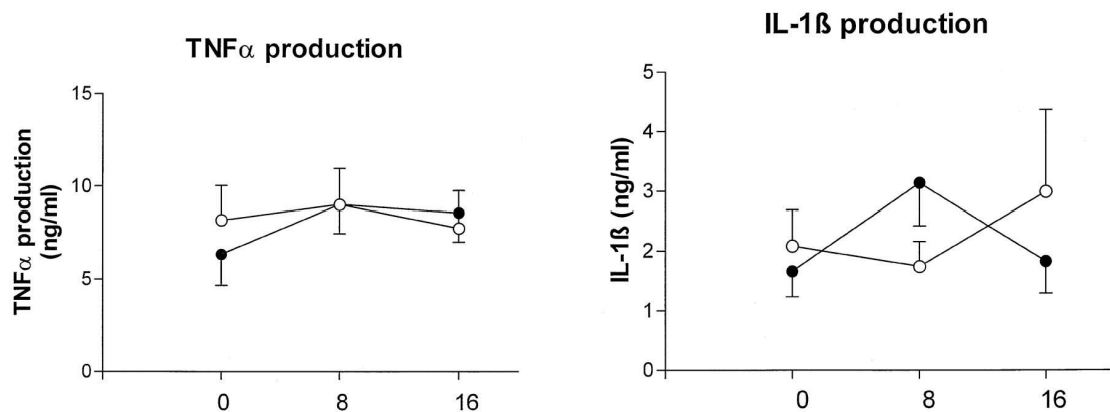
IL-1ß production

Figure 2. The effect of thalidomide treatment (closed bullets) or placebo (open bullets) on the *E. coli* lipopolysaccharide stimulated ex vivo production of TNF-α (left pannel) and IL-1β (right panel) as assessed in a whole-blood culturing system. The values at the *x*-axis refer to time of sampling during the trial and indicate baseline (0), 8 weeks (8), and 16 weeks (16). Note the absence of any effect of thalidomide treatment on ex vivo TNF-α production.

Figure 3. Box and Whisker plots of urine neopterin excretion. Samples were collected weekly, and data reflect the results from treatment with placebo (n = 92 weeks), washout (n = 24 weeks), and thalidomide (n = 88 weeks).

Thalidomide did not influence the neopterin excretion in HIDS patients.

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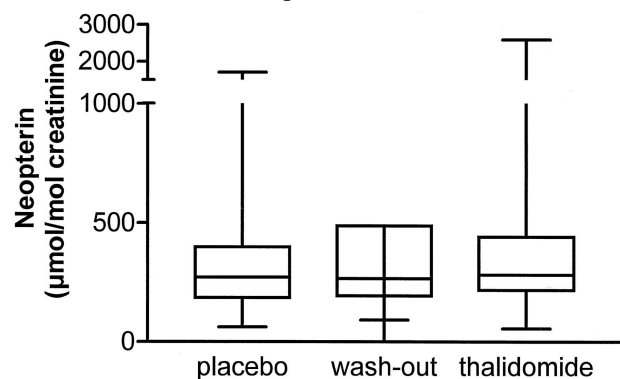
Event	Placebo		Thalidomide	
	No. of weeks	No. of patients	No. of weeks	No. of patients
Fatigue	14	1	28	3
Numbness	3	1	16	2
Paresthesias	5	2	15	2
Dry mouth	9	1	28	3
Drowsiness		0	10	2
Sleepiness		0	5	3

Table 3. Comparative incidence of adverse events among patients treated with thalidomide and placebo during the 36 weeks of the randomised trial. Values are given as number of weeks as indicated on diary card returned by the patients and by the number of patients who experienced the adverse at any time during the trial.

Discussion

In this article, we describe the results from the first randomized placebo-controlled therapeutic trial in HIDS. Given the propensity of the HIDS symptoms to wax and wane over time, we developed a thorough randomized trial to assess the therapeutic efficacy of thalidomide. The assessment period of 36 weeks was long enough because all patients had repetitive attacks at either phase of the trial. Intervention with thalidomide did result in a minor, nonsignificant decrease of febrile attacks in HIDS patients, and four of six patients had fewer attacks with thalidomide. However, this effect is clinically insignificant. One reason for disappointing results might be that the dosage of thalidomide was too low. On the other hand, if increasing the dosage would lead to a greater benefit, this would probably come at the cost of a higher incidence of unwanted side effects. In light of the results of this randomized trial and given the small balance between efficacy and side effects, thalidomide is not the drug of choice in the treatment of HIDS attacks. However, we need to consider other (biochemical) effects of treatment. Biochemically, treatment with thalidomide was associated with a small decrease of serum CRP, SAA, and IL-1ra. Decrease of acute phase proteins such as SAA may be a desired goal in the treatment of a periodic fever syndrome. SAA is thought to be the precursor of AA-type amyloidosis, a much feared complication of periodic fever syndromes. For example, in familial Mediterranean fever colchicine treatment, results not only in a decrease of the frequency of attacks but also a decrease in the incidence of amyloidosis [22]. In our patients, high CRP and SAA values were detected throughout the study period. In all samples collected during the complete study period, the average CRP value was 32.7 mg/l (S.D., 44.3), and that of SAA was 15.9 mg/l (S.D., 57.7). However, despite important elevated SAA levels, amyloidosis has not yet been observed as a complication of HIDS, and a clear pathogenetic interpretation is lacking here. We noted important elevations of urine neopterin during the whole trial regardless of treatment. Neopterin is considered a nonspecific marker of T-cell activation, and it is released from macrophages and monocytes following interferon- stimulation. Elevated urine neopterin levels have been found in a variety of disorders, such as systemic lupus

erythematosus [166], familial Mediterranean fever [167], and tuberculosis [168]. Previously, we showed that urine neopterin levels accurately reflect disease activity, and this study confirmed this notion [95]. Our patients had active disease as assessed by the neopterin excretion because 51% of the samples had values above the upper limit of normal [165]. Again, thalidomide did not influence urine neopterin levels, which parallels the observed lack of clinical efficacy. The intensity of the attacks, as reflected by the height of the excretion of neopterin, was not affected by thalidomide when compared with placebo.

We did not find an effect of thalidomide treatment on either plasma TNF-α concentration or on the LPS-stimulated ex vivo production of TNF-α. This was surprising considering the in vitro ability of thalidomide to decrease the production of TNF-α in human monocytes by inhibition of the transcription of TNF-α mRNA. It is possible, however, that the dosage given to our patients is too low to reproduce the aforementioned (in vitro) effects. On the other hand, our results correspond with other clinical trials, which also showed that thalidomide is not an effective systemic TNF-α inhibitor [169]. We could speculate that this lack of TNF-α inhibition explains the limited efficacy of the drug, as observed in our study. On the other hand, thalidomide was able to heal aphthous ulcerations in the esophagus of patients infected with human immunodeficiency virus despite absence of an effect on plasma TNF-α level [170]. This suggests that the inhibitory effect of thalidomide on TNF-α is apparent only on tissue level. Extrapolated to HIDS, this might indicate that TNF-α is not as important in the pathogenesis of HIDS as suggested earlier, and that other factors may be operative.

In our trial, thalidomide treatment was associated with certain toxicity. We gave thalidomide at bedtime to minimize somnolence and dizziness. Although this might increase the acceptability of the drug, we saw that fatigue, drowsiness, and sleepiness were more frequent with thalidomide as opposed to placebo. Likewise, numbness and paraesthesias were more frequently observed with thalidomide. One patient developed polyneuropathy after 9 weeks of treatment, but this was completely reversible. The occurrence of neurotoxicity appears to be, in part, disease specific, and the severity of neurotoxicity has not been consistently correlated with the total dose of the drug. In general, the neurotoxicity is reversible if the symptoms are recognized early; however, prolonged and irreversible polyneuropathy can occur [171]. The two HIDS patients who had a good clinical response to thalidomide both developed polyneuropathy, and this was irreversible in one patient (J. van der Meer and R. Powell, unpublished observations). The rather limited efficacy of thalidomide in decreasing the number of febrile attacks and the potential risk of (ir)reversible neurotoxicity precludes its prolonged use in HIDS. The use of thalidomide is further limited to adult males or sterilized females. This is a problem because, given the autosomal recessive inheritance pattern of HIDS, half of the patients are female. Most female patients are in their reproductive years; the average age of the patients carrying HIDS mutations in the Nijmegen HIDS registry is 30.2 ± 17.7 years.

Given the rather limited effect of thalidomide, we think that a search for new potential therapeutic avenues is warranted. Given the molecular defect of HIDS, it is tempting to speculate that inhibition of 3-hydroxy-3-methylglutaryl coenzyme A reductase, the enzyme that precedes mevalonate kinase in the cholesterol synthesis, might be fruitful. Currently, we are performing a drug trial aimed to study the effect of high-dose simvastatin in the treatment of HIDS attacks.

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Acknowledgments

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Chapter 9

Simvastatin treatment for inflammatory attacks of the hyper-IgD and periodic fever syndrome

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Abstract

Hyper-IgD and periodic fever syndrome, a hereditary autoinflammatory syndrome, is characterized by lifelong recurrent episodes of fever and inflammation. No effective treatment is known. It is caused by a defect of mevalonate kinase, an enzyme following HMG-CoA-reductase in the isoprenoid pathway. We wanted to test the hypothesis that inhibition of HMG-CoA reductase would ameliorate the inflammatory attacks. Six patients with hyper-IgD syndrome and proven mevalonate kinase deficiency were followed for two treatment periods with either simvastatin 80 mg/day or placebo for 24 weeks, separated by a 4-week washout period in a double-blind fashion. Simvastatin resulted in a drop in urinary mevalonic acid concentration in all patients and decreased the number of febrile days in five out of six patients. No side effects were observed. These data offer preliminary evidence for the hypothesis that simvastatin may improve inflammatory attacks in the hyper-IgD syndrome. This highlights the anti-inflammatory properties of HMG-CoA reductase inhibition.

Introduction

The hyper-IgD and periodic fever syndrome (HIDS; Mendelian Inheritance in Men (MIM) #260920) is a rare hereditary autoinflammatory syndrome caused by mutations in the mevalonate kinase gene [2,3]. Patients with HIDS suffer from periodic fever episodes that usually start from the first year of life and are characterized by high fever, lymphadenopathy, abdominal distress, myalgias and skin lesions [4]. These inflammatory episodes generally last four to six days, and recur every four to six weeks.

Mevalonate kinase is one of the central enzymes in isoprenoid metabolism, which has numerous end products such as cholesterol, ubiquinone, and dolichol and is also essential for isoprenylation of proteins (figure 1). Mevalonic acid, or mevalonate, is the product of the enzyme 3'-hydroxy-3'-methylglutaryl-coenzyme A (HMG-CoA) reductase [142]. The mutations in the mevalonate kinase gene lead to a deficient mevalonate kinase enzyme activity and accumulation of its substrate mevalonic acid, most notably during fever attacks. However, it is not exactly clear how this results in the inflammatory phenotype of HIDS.
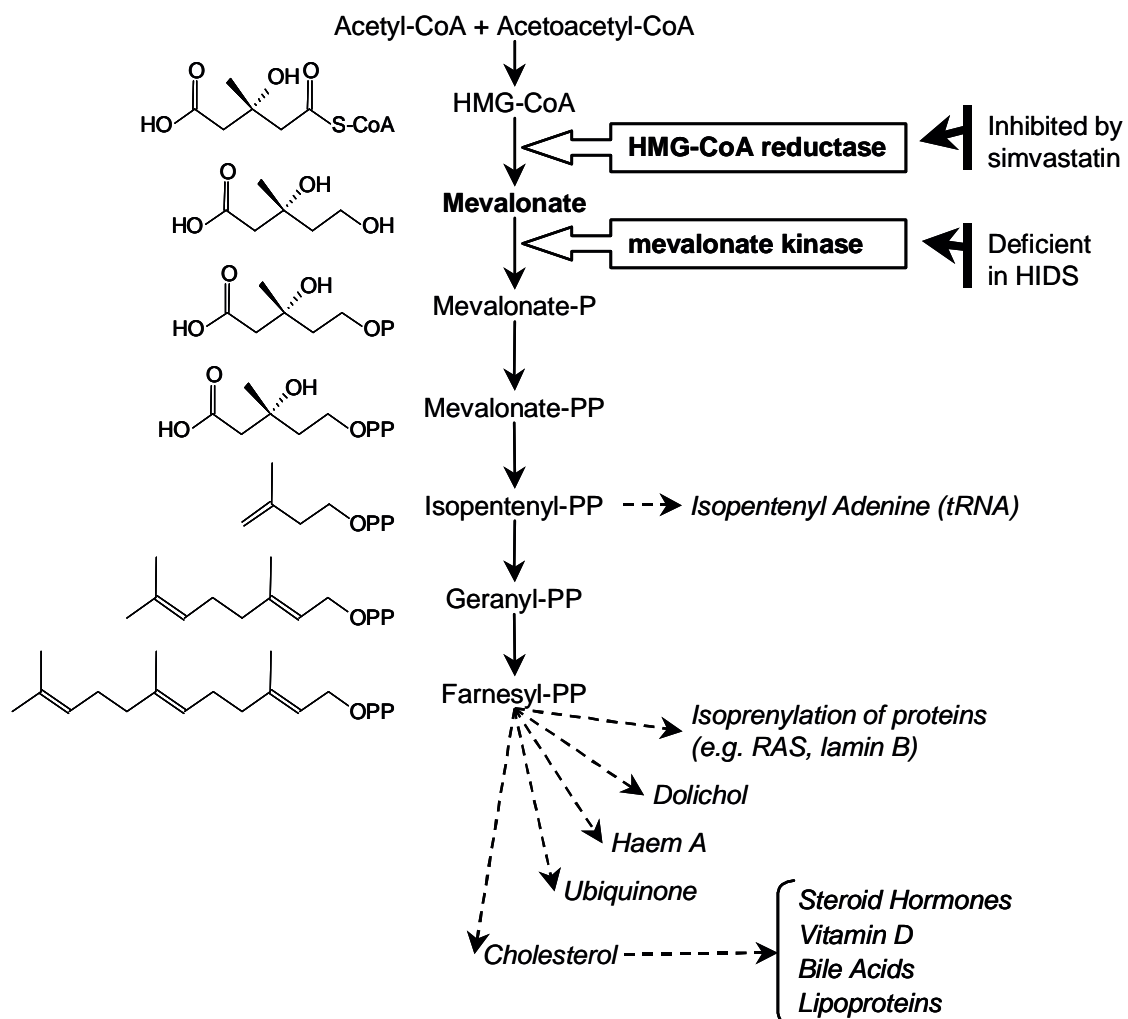

Figure 1. Schematic representation of the isoprenoid metabolism with its end-products. Indicated is the metabolic defect in HIDS and the point of inhibition by simvastatin.

Treatment options available for HIDS do not reflect our current understanding of pathophysiology of the disease. So far, therapeutic experiments have sought to curtail the inflammatory response in HIDS and have met with mixed results. For example, thalidomide (200 mg) given as an inhibitor of TNF response, failed to elicit a beneficial effect in a controlled clinical trial [52]. Uncontrolled evidence suggests that a small proportion of patients benefit from prednisolone, and recently open-labelled use of etanercept reduced the frequency and severity of symptoms in two HIDS patients [172]. Although etanercept has shown a beneficial effect, potential side effects such as the increased risk on infections and severe neurological damage [173,174] may preclude its prolonged use. Moreover, it is not known whether the beneficial initial response is followed by sustained improvement over a longer period of time. However, in our opinion non-controlled clinical observations are not the most optimal setting in HIDS, because of the highly variable nature of frequency and severity of the fever attacks. This calls for a more rigorous approach.

The discovery of mevalonate kinase as the gene implicated in HIDS led us to hypothesize that HIDS patients might benefit from the use of a HMG-CoA reductase inhibitor such as simvastatin. In theory, this would restore the balance in the isoprenoid metabolism and reduce the mevalonic acid overload. Despite these theoretical considerations, preliminary data from a single study in 2 children with classical mevalonic aciduria, the severe form of mevalonate kinase deficiency, are not encouraging [45]. Short term treatment with low dose lovastatin (initially 5 mg, later 10 mg) precipitated a severe crisis in both, prompting discontinuation of the statin. Therefore, we first explored whether statins could be tolerated by HIDS patients (who have a mild mevalonate kinase deficiency). In a 4-week pilot study with high dose atorvastatin (80 mg/day) in three HIDS patients we observed no precipitation of inflammatory attacks, and more importantly, no side effects. These results encouraged us to investigate our hypothesis in a randomized double-blind crossover study of six HIDS patients.

Patients and methods

We selected patients from the Nijmegen HIDS registry [46] when they fulfilled the entry criteria for the study, i.e. 1) mevalonate kinase gene mutations on both alleles, 2) age over 16 years and 3) frequent attacks (more than 1 per 6 weeks). The study was approved by the local Medical Ethical Committee and all participants gave written informed consent.

The follow-up period of 52 weeks consisted of two treatment periods of 24 weeks with a 4-week washout period in between. Patients were randomized to receive simvastatin 80 mg/day in the first or second treatment period, with placebo tablets in the other period. A clinical pharmacist not directly involved in the study prepared a simple, computer-generated, random-number list for randomization. The code was held at the Department of Clinical Pharmacy, UMC St. Radboud, Nijmegen, and was only opened after all data had been entered into a computer for analysis. Concomitant medications were allowed throughout the study and recorded.

The primary outcome measure was defined as the number of febrile days. Secondary outcome parameters were number of fever attacks, urinary concentration of mevalonic acid and serum lipid concentrations. Attacks were defined as fever (>38.5°C) together with one or more of the following symptoms:

abdominal distress (pain, vomiting, or diarrhoea), joint involvement (arthralgia, arthritis), skin lesions, lymphadenopathy. Patients used weekly diary cards to register number, duration and severity of attacks on a visual analogue scale and to record possible side-effects of study medication. At each clinic visit (week 0, 12, 24, 28, 40 and 52) blood was drawn to determine concentration of plasma Alanine Transferase (ALT) and Creatine Kinase (CK) and serum lipids using an automated enzymatic assay. Patients collected weekly urine samples on which mevalonic acid concentration was measured by isotope-dilution gas-chromatography/mass spectrometry as described before [175] albeit slightly modified.

GraphPad Prism (version 4.00 for Windows, GraphPad Software, San Diego, California, USA) was used for statistical analysis of data. Where appropriate, we used the two-tailed paired t-test or two-tailed Mann-Whitney test. Data are given as mean $\pm$ standard deviation, unless stated otherwise. A *p* value <0.05 was considered as the threshold for statistical significance.

Table 1 – patient characteristics

Patient no.	Mutations in mevalonate kinase	Mevalonate kinase enzyme activity (%)	Maximal serum IgD (U/mL)
1	V377I/H20P	8.5	450
2	V377I/I268T	7.2	900
3	V377I/H20P	11.3	4224
4	V377I/del92bp	10.5	809
5	V377I/V377I	5.7	376
6	V377I/H20P	ND	1140

ND = not done

Results

Patients

At the start of the study, the Nijmegen HIDS registry contained data on 58 HIDS patients with proven mevalonate kinase deficiency. Patients were excluded because of age (<16 yrs) (n=25), infrequent attacks (n=8), refusal to participate (n=12) or miscellaneous reasons (n=7, this includes lost to clinical follow-up, residence abroad or homelessness at start of study). Four patients (two men and two women) were enrolled in December 2000, two other male patients started in March and September 2001, with an average age of 34 years (range 17-56 years). Patient characteristics are summarized in table 1. Three patients were randomly assigned to start with placebo in the first six-month period, to go on with simvastatin in the last six months; the other three patients were treated in the reverse order. All six patients completed the follow-up.

Medication used concomitantly with the study medication consisted primarily of acetaminophen, in equal amounts in the two periods. Patient no. 1 was on a continuous dose of colchicine 1.5 mg throughout the year of follow-up. Patient no. 6 had been on oral prednisolone for the year previous to the study because of severe joint involvement; within 6 weeks of the start of the simvastatin treatment period (in her case the first period) prednisolone had been tapered and stopped without complications.

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Table 2. Overview of results

Pt no.	Start[a]	Order[b]	Subjective preference	Febrile attacks, length and severity score[c] First period	Wash-out	Second period
1	Dec	S-P	Equal	4 d; vas 2 4 d; vas 5 3 d; vas 1	5 d; vas 3 4 d; vas 3	3 d; vas 2 3 d; vas 1 4 d; vas 4 3 d; vas 2
2	Sept	S-P	Placebo	4 d; vas 7 5 d; vas 9 4 d; vas 6 5 d; vas 4		3 d; vas 3 6 d; vas 7
3	Dec	P-S	Simvastatin	7 d; vas 8 8 d; vas 8 9 d; vas 9 8 d; vas 10	7 d; vas 8	7 d; vas 5 14 d; vas 9
4	Mar	P-S	Simvastatin	12 d; vas 6 6 d; vas 4 6 d; vas 7		1 d; vas 8 7 d; vas 9
5	Dec	P-S	Simvastatin	8 d; vas 4 7 d; vas 9 7 d; vas 3 8 d; vas 9 4 d; vas 1		9 d; vas 7 6 d; vas 8
6	Dec	S-P	Simvastatin	4 d; vas 1 4 d; vas 3 5 d; vas 1 4 d; vas 1 10 d; vas 1		9 d; vas 2 3 d; vas 1 6 d; vas 1 10 d; vas 1 6 d; vas 3

[a]Month of start in study protocol (Dec = December, Sept = September, Mar = March).
[b]Order of treatment: S-P = simvastatin-placebo, P-S = placebo-simvastatin
[c]Individual fever episodes per patient; indicated are duration in days (d) and severity as measured by the patient on a visual analogue scale (vas) from 0-10 points, with 10 as most severe.

Clinical outcome

In the complete study period of 52 weeks, the patients registered a total of 44 fever attacks (on average, one every 7 weeks per patient) and 262 days of illness (table 2). During the placebo period, a total of 146 febrile days were reported versus 100 days in the simvastatin treatment period – this amounts to an average of 24.3 ± 11.0 *vs.* 16.7 ± 6.9 days per patient per 6 months (decrease of 7.6 days or 31%; $p=0.12$, 95% confidence interval –3 to 18.36, figure 2). The effect seemed to be more pronounced in the patients who used simvastatin in the second period than in those who started with simvastatin. This might indicate a carry-over effect, but the small number of patients precludes a definitive conclusion.

The number of fever attacks was low (total 23 vs. 18 for placebo vs. simvastatin; table 2). For the whole group, a mean number of 3.8 ±1.2 was observed in the placebo period, versus 3.0 ±1.3 in the simvastatin period ($p=0.29$). No consistent effect of drug treatment was observed on either severity or duration of attacks (table 2). Four patients had a subjective preference for simvastatin, one patient considered the periods equal, and one patient preferred the placebo period although classifying the past year as a "light one" (both of these in the simvastatin-placebo order group, table 2).
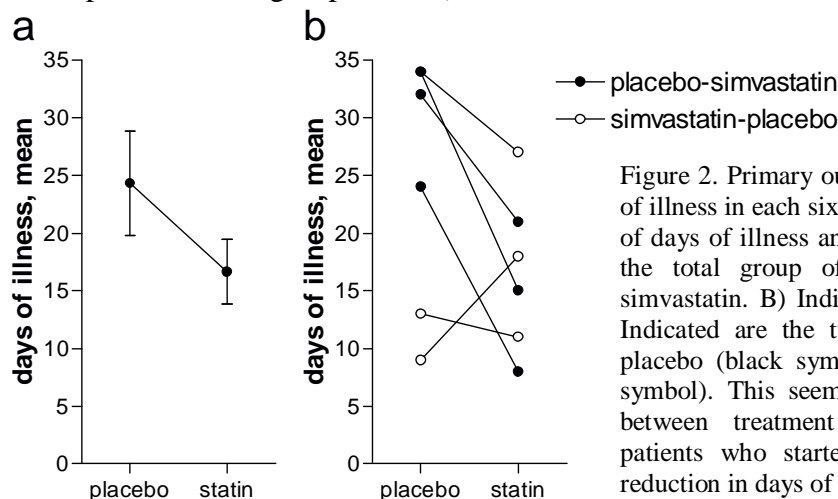
Figure 2. Primary outcome measure: number of days of illness in each six months period. A) Mean number of days of illness and standard error of the mean for the total group of patients during placebo and simvastatin. B) Individual results for the 6 patients. Indicated are the two groups, starting either with placebo (black symbol) or with simvastatin (white symbol). This seems to demonstrate an interaction between treatment order and treatment effect: patients who started with placebo have a larger reduction in days of illness during simvastatin.

Lipid spectrum

At baseline, the HIDS patients had normal to low-normal serum concentrations of LDL-cholesterol (mean 2.6 mmol/l ±1.0). As expected, simvastatin significantly reduced serum total cholesterol (mean 4.8 ±1.2 vs. 3.0 ±0.5 mmol/l at end of treatment, 38% reduction, p=0.016) and LDL-cholesterol (mean 3.3 ±0.9 *vs.*1.5 ±0.7 mmol/l at end of treatment, 55% reduction, p=0.0052; figure 3), with no difference between the two treatment-order groups (data not shown).
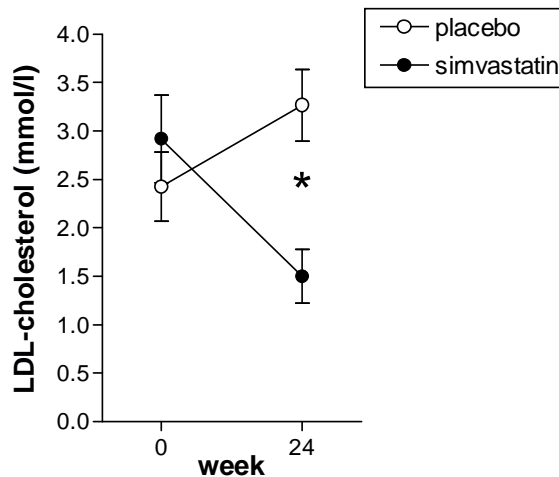
Figure 3. Serum LDL-cholesterol concentration, mean of 6 patients at beginning and end of treatment period (* p<0.0052).

Urinary mevalonic acid (MVA)

The six patients collected 133 urine samples in the placebo period and 135 samples in the simvastatin period (total 20 missing samples). The MVA concentration mirrored the clinical symptoms, with high peaks up to 14.6 mg/g creatinine during a fever attack (normal values 0.4 ± 0.18 mg/g creatinine), although discrepancies between MVA concentration and clinical symptoms can be observed (figure 4). The mevalonic acid concentration in the placebo period was significantly higher than in the simvastatin treatment period, whether looking at median absolute concentrations (4.9 *vs.* 1.9 mg/g creatinine, p<0.001, 61% reduction, figure 5), or at median area-under-the-curve (115.5 *vs.* 59.9 mg/g creatinine*wk, p=0.004). This pattern was consistent for all six patients.

Side effects

Side-effects were not reported by five out of six patients. Patient no. 1, who remained on a steady dose of 1.5 mg colchicine throughout the study period, reported symptoms of flatulence during the whole year, specifically nightmares with simvastatin and increased tiredness and myalgias whilst on placebo. Neither treatment affected CK or ALT concentrations.
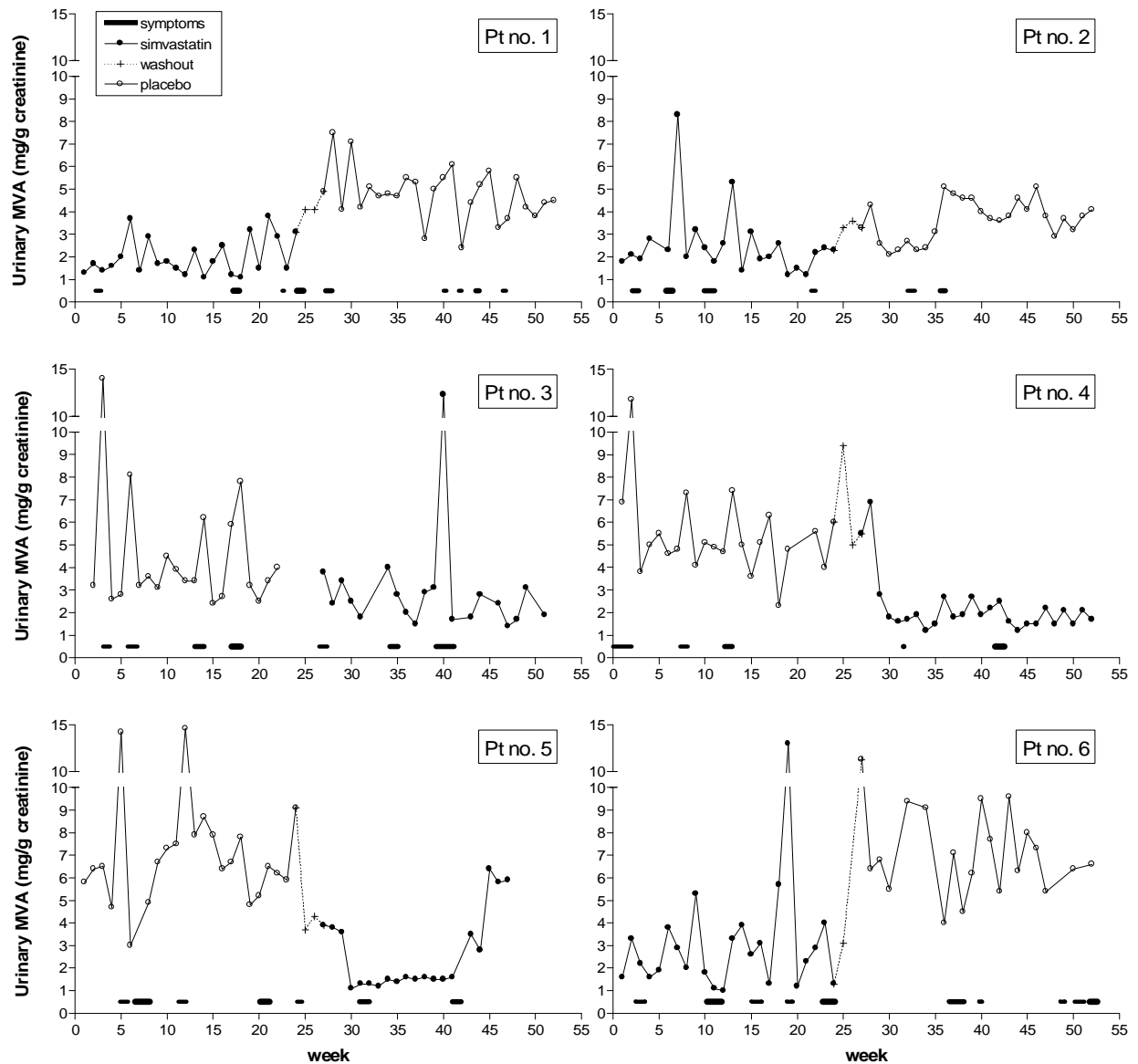
Figure 4. Urinary mevalonic acid (MVA) concentration in weekly collected urine samples in the six individual patients, in the placebo period (white symbols), 4-week washout period (crossed symbol and dotted line) and simvastatin period (black symbols). Also indicated are the fever episodes of each patient (black horizontal bars). Patient no. 3 omitted to collect urine samples during the washout period, patient no. 5 admitted to non-compliance at the end of the study period.
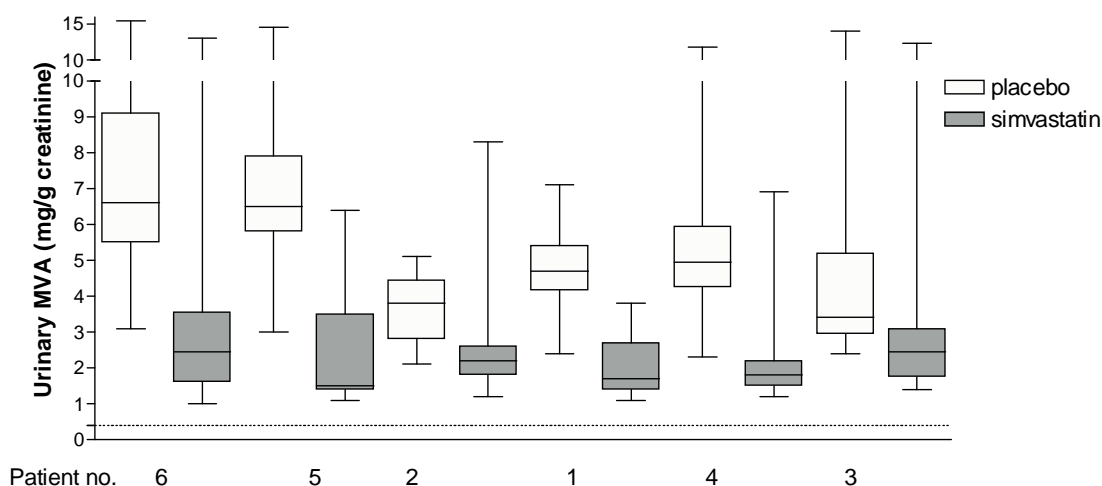
Figure 5. Box-and-whisker plots of urinary mevalonic acid (MVA) concentration in the six individual patients in the placebo period *vs.* the simvastatin period, arranged from lowest (patient no. 5) to highest (patient no. 3) known mevalonate kinase enzyme activity (mevalonate kinase activity in patient 6 not determined). The dotted line represents the normal concentration detected in healthy controls (0.4 ±0.18 mg/g creatinine).

Discussion

This study is the first to investigate a pharmacological intervention specifically designed to target the metabolic defect in HIDS. The cohort of patients included in this study is small, which is largely related of the rarity of the disorder (6 patients represent almost 10 percent of the worldwide known patient population at the start of study). We observed a positive clinical effect in five of the six patients, with a decrease in the number of febrile days, although the difference did not reach statistical significance.

HIDS has been linked to mutations in the mevalonate kinase gene and decreased mevalonate kinase enzyme activity since 1999 [2,3], but despite intensive research efforts the actual pathogenesis has yet to be determined. It has been suggested that the primary defect lies in the decreased flux through the isoprenoid metabolism leading to shortage of end-products, which is compensated by increased mevalonic acid [43,149]. Alternatively, high mevalonic acid concentrations may in itself be toxic and result in the inflammatory attacks. Mayatepek et al. reported a close correlation between the concentration of the proinflammatory mediator leukotriene E4 with the urinary level of mevalonic acid [176]. Although the suggested beneficial effect observed in the current study would not refute the former hypothesis, the results are more in line with the latter hypothesis, even more so since clinical improvement is mirrored by lower mevalonic acid excretion. However, it is possible that inhibition of HMG-CoA reductase results in a new equilibrium in the isoprenoid metabolism through feedback mechanisms or other selection methods. This could favour the production of certain end-products over others, thereby correcting a putative shortage. Such an effect is reminiscent of the situation in Smith-Lemli-Opitz syndrome, a rare hereditary disorder characterized by pathologically low concentrations of cholesterol. It is caused by a deficiency of 7-dehydrocholesterol reductase, the last step in the Kandutsch-Russell cholesterol biosynthetic pathway. Inhibition of HMG-CoA reductase by statins in this syndrome results in a paradoxical increase of cholesterol [177], most likely through augmentation of residual enzyme activity.

The possible benefit of simvastatin appears to go beyond the period the drug was actually used. In particular, we observed a possible carryover effect in clinical outcome parameters in patients first treated with simvastatin and then by placebo. How does this relate to the biological action of simvastatin? The high dose of simvastatin resulted in the expected decrease of cholesterol (figure 3) from the normal serum cholesterol values at the start of this study. Interestingly, the possible carryover effect was not mirrored by the cholesterol concentration, asat the end of the wash-out period cholesterol concentrations had normalized while the clinical effect still persisted..

The mechanism for the observed carryover effect is not clear. It is possible that prolonged treatment with simvastatin results in a depletion of accumulated mevalonic acid restoring the balance within the isoprenoid pathway. However, this hypothesis does not seem to be corroborated by the rapid increase of mevalonic acid excretion observed in our patients after discontinuation of simvastatin. Alternatively, the simvastatin treatment has restored depleted concentrations of one or more isoprenoid end-products, such as isoprenylated proteins, which may persist for a prolonged period.

We did not observe side effects in our patients, which differs appreciably with results obtained in children with severe mevalonate kinase deficiency where statins allegedly induced attacks [45]. Several factors might be responsible for this discrepancy. In the first place, we observed that although simvastatin had a beneficial effect, it did not result in a complete absence of inflammatory crises. Possibly the attacks seen by Hoffmann et al. were part of the 'normal' phenotype of the disease and not caused by the statin. A longer treatment of the 2 children in Hoffmann's study might have resolved this issue. Secondly, both the children described by Hoffmann used supplements that exert an influence on the isoprenoid metabolism, including cholesterol, ubiquinone and vitamin E. The resulting effect of this combination treatment on the isoprenoid metabolism is hard to predict, but may be disadvantageous. A third possible explanation is the higher residual mevalonate kinase enzyme activity in HIDS compared to classical mevalonic aciduria (5.7-11.3% *vs.* 0-1.5%) [45]. Therefore, the results of this study can not be extrapolated to patients with classical mevalonic aciduria.

In conclusion, we describe preliminary evidence for a possibly effective treatment for the hyper-IgD and periodic fever syndrome (HIDS) in a randomized double-blind follow-up study of six patients. Although our trial is small, randomised trials are clearly to be preferred over observational studies and, in our opinion, controlled studies provide the only way that any unbiased measurements of effectiveness can be made. In any case, we hope to set the stage for a larger trial and additional clinical experience with HMG-CoA reductase inhibitors in HIDS in more patients over a longer period of time is desirable to prove its standing in clinical practice. This study again highlights the relation between inflammation and the isoprenoid metabolism, and offers additional evidence for the anti-inflammatory activity of HMG-CoA reductase inhibitors [143,178].

Acknowledgements

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The value of genetic screening in the diagnosis of hereditary autoinflammatory syndromes compared to clinical examination plus targeted genetic analysis

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*see appendix

Abstract

Hereditary autoinflammatory syndromes are characterized by recurrent episodes of fever and inflammation. Six subtypes have been described, caused by mutations in 4 different genes. Apart from a common phenotype of lifelong recurrent inflammatory attacks, all of them have distinct features and specific therapeutic options, which emphasizes the need for a specific diagnosis in each case. The aim of this study was to examine whether genetic screening would allow classification of previously unclassified patients. We also examined whether individual patients suffering from an autoinflammatory syndrome carry additional mutations in one of the other autoinflammatory genes. We included 60 patients with an unclassified autoinflammatory syndrome, 87 patients diagnosed with either hyper-IgD syndrome (HIDS), familial Mediterranean fever (FMF) or TNF-receptor associated periodic syndrome (TRAPS), and 50 healthy controls. DNA samples were screened for the most prevalent mutations in the *MEFV*, *TNFRSF1A, MVK* and *CIAS1* genes. This yields only one possible diagnosis of FMF in the 60 previously unclassified patients. Two low-penetrance mutations were found in equal numbers in the groups of patients and controls. Thus, screening of known genes involved in these disorders does not yield additional relevant information. Differential diagnosis of hereditary autoinflammatory syndromes can be made by thorough clinical examination followed by targeted genetic analysis of one or two most likely syndromes. High prevalence, low penetrant mutations from autoinflammatory genes do not occur more frequently in hereditary autoinflammatory patients compared to the general population.

Periodic fever syndromes are characterized by recurrent episodes of fever and inflammation [4]. Currently, they are designated by the term (hereditary) autoinflammatory syndromes, because fever may be absent, while periodic inflammation (triggered by an often unknown stimulus and without signs of autoimmunity) is a key feature. Six distinct hereditary autoinflammatory syndromes have been genetically characterized (table 1). Apart from lifelong recurrent inflammatory attacks, these syndromes have distinctive features, such as age of onset, duration of attacks, accompanying symptoms, prognosis and ethnic origin. The differential diagnosis remains a challenge. A correct diagnosis enables specific therapeutic interventions, and options have expanded considerably [154,179,180].

The advent of genetic testing for the autoinflammatory syndromes has had a number of consequences: (i) the clinical phenotypes and ethnic distribution of each of these syndromes turned out to be much more variable than anticipated; (ii) quite some patients presenting with a clear phenotype cannot be classified by genetic testing; (iii) patients with a combination of mutations from two autoinflammatory genes have been reported [181,182], which raises the question whether this might be more common.

For this study, we asked whether more rigorous and non-restricted genetic screening would allow classification of patients with undiagnosed periodic fever. Further, we aimed to examine whether the combination of gene mutations of various autoinflammatory syndromes in a single patient occurs more often than expected.

Table 1. Hereditary autoinflammatory syndromes.

Syndrome	Inheritance pattern	Gene	Protein	Most frequent mutations
Familial Mediterranean Fever (FMF)	Autosomal recessive	MEFV	Pyrin (marenostrin)	M694V, V726A, M694I, M680I, E148Q[†]
Hyper-IgD and periodic fever syndrome (HIDS)	Autosomal recessive	MVK	Mevalonate kinase	V377I
TNF-receptor associated periodic syndrome (TRAPS)	Autosomal dominant	TNFRSF1A	TNF-receptor type I (p55)	R92Q[†], P46L[†]; various mutations in exon 2-4
Cryopyrin-associated periodic syndrome (CAPS)*	Autosomal dominant	CIAS1	Cryopyrin	Various mutations exon 3

*This includes Muckle-Wells syndrome (MWS), Familial Cold Autoinflammatory Syndrome (FCAS) and Chronic Infantile Neurological Cutaneous and Articular syndrome (CINCA; also known as Neonatal Onset Multisystemic Inflammatory Disease (NOMID)).

[†]These mutations are alternatively described as low-penetrance mutations, disease-modifying factors or even polymorphisms instead of disease-causing mutations.

Not included in this table or study: Blau syndrome, caused by mutations in the NOD2/CARD15 gene, and Pyogenic Sterile Arthritis, Pyoderma gangrenosum and Acne (PAPA) syndrome, caused by mutations in CD2BP1-gene.

Patients and Methods

Patients

For this study we included all patients with suspected autoinflammatory syndrome referred to our clinic (as a tertiary referral center in the Netherlands and through the International HIDS registry (hids.net)) between January 1992 and December

2003. Control DNA samples from 50 healthy Dutch were donated by the Department of DNA Diagnostics, UMC Nijmegen. We discerned two groups of patients: those without a classifying diagnosis (unclassified) and those with a confirmed diagnosis (diagnosed).

Unclassified patients

Clinical workup in these patients had failed to establish a specific diagnosis. Inclusion criteria were: [1] recurring episodes with fever and documented acute phase response for more than two years; [2] no genetic diagnosis, despite one or two specific genetic tests of most likely syndrome; [3] DNA sample or nucleated cells available. We were able to collect 60 patients. Clinical information was collected with a standardized questionnaire (24 patients) or by our clinical observation (36 patients). A positive family history was present in 9 patients. Fifty-five patients were of North-West European Caucasian origin, including 36 Dutch patients; others came from Italy [2], Greece [1], Slovakia [1] and Japan [1]. Twenty-nine of these patients were designated as variant type HIDS [40], defined as a clinical phenotype compatible with HIDS, elevated serum immunoglobulin D (IgD) and no mevalonate kinase gene mutations.

Diagnosed patients

This group encompassed 87 patients, whom we previously diagnosed using clinical workup (history taking, physical examination) and specific genetic analysis. It includes 64 classical type HIDS patients from 49 families in the International HIDS registry, 15 Dutch patients with TRAPS from 7 families and 8 unrelated patients with FMF.

Table 2.

Restriction enzyme analysis			
Gene	**Mutation**	**Enzyme**	**Primers**
TNFRSF1A	R92Q	Nci-I	5'-GCTGCTCCAAATGCCGAAAG-3' 5'-GGTTTTCACTCCAATAATGCCG-3'
	P46L	Nci-I	5'-CTTGTGTTCTCACCCGCAG-3' 5'-CTTTCGGCATTTGGAGCAGC-3'
MVK	V377I	BsmA-I	5'-TGTAAAACGACGGCCAGTGTCTCGAAGTGGAGGCCACGAAGCAG-3' 5'-CCAGCACAGAGTCGAACTGCAG-3'
MEFV	M694V	Hph-I	5'-GAATGGCTACTGGGTGGAGAT-3' 5'-GGCTGTCACATTGTAAAAGGAG-3'
	V726A	Alu-I	5'-GAATGGCTACTGGGTGGAGAT-3' 5'-GGCTGTCACATTGTAAAAGGAG-3'
	M694I	BspH-I	5'-GGCTGTCACATTGTAAAAGGAG-3' 5'-GCTACTGGGTGGTGATAATCAT-3'
	M680I	Hinf-I	5'-GAGGTTGGAGACAAGACAGCATGG-3' 5'-TCGGGGGAACGCTGGACGCCTGGTACTCATTTTCCT-3'
	E148Q	Bst-NI	5'-GCCTGAAGACTCCAGACCACCCCG-3' 5'-AGGCCCTCCGAGGCCTTCTCTCTG-3'
DNA sequencing			
TNFRSF1A	Exon 2,3,4		5'-GATCTGCTACCCCTAAGC-3' 5'-CCCACACACCACTCAAGACC-3' 5'-GTGTTCTCACCCGCAGCCTA-3' 5'-GGAAGTGCCACCGCATGGG-3'
CIAS1	Exon 3		5'-GCATCTCAGGTGGATGTGTG-3' 5'-CTGCCAGTCAGTGCAGAGCGG-3' 5'-GACCTGATCATGAGCTGCTGC-3' 5'-TGGTTCCAGATTCCATCTGC-3' 5'-CATGTGGAGATCCTGGGTTT-3' 5'-CCAGAAGGACTGTCACGTCTCG-3' 5'-TCTACAGCTTCATCCACATG-3' 5'-GGGCTGTGGCAACAGTATTTGG-3'

DNA analysis

DNA was isolated from blood lymphocytes by standard procedures. Polymerase chain reaction (PCR) amplification of specific segments of the *MVK*, *MEFV*, and *TNFRSF1A* gene was performed for restriction fragment length polymorphism (RFLP) analysis (table 2). We amplified exon 2, 3 and 4 of *TNFRSF1A* gene and exon 3 of *CIAS1* gene for DNA sequencing (table 2). Primers were either designed by us or adapted from available literature with minor modifications [12,183-185]. For the RFLP analysis, PCR products were digested by restriction enzymes (table 2) at appropriate temperatures and incubation times. Digested samples were run on a 2-3% agarose gel (Biozym) and stained with ethidium bromide. Using purified PCR products, sequencing was performed according to standard procedures on both DNA strands using the same forward and reverse primers as those in the PCR step.

Results

Table 3 lists the results of the elaborate genetic screening.

Gene	Mutation	Autoinflammatory syndrome nos (of which variant type HIDS) n=60 (29)	Healthy controls n=50	Classical type HIDS n=64	TRAPS n=15	FMF n=8
MVK	V377I	0 (0)	0	NA (52)[†]	0	0
MEFV	M694V	1 (1)	0	0	0	NA
	V726A	0 (0)	0	1	0	NA
	M694I	0 (0)	0	0	0	NA
	M680I	0 (0)	0	0	0	NA
	E148Q	1 (1)	2	2	1	NA
TNFRSF1A	P46L	0 (0)	0	0	NA	0
	R92Q	1 (0)	1	1	NA	0
	Sequencing exon 2,3,4	0 (0)*	ND	ND	NA	ND
CIAS1	Sequencing exon 3	0 (0)	ND	ND	ND	ND

Table 3. Autoinflammatory syndrome nos = patient with autoinflammatory syndrome not otherwise specified; NA= not applicable; ND = not determined. *except for the R92Q mutation as detected by restriction enzyme analysis. [†]the V377I mutation was previously found in 52 of the 64 classical type HIDS patients.

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Unclassified patients

In 60 unclassified patients, we identified 3 patients who carried one mutation for one of the genes mentioned in table 1. No patients were found with a V377I mutation in the *MVK* gene, and neither did DNA sequencing of exon 3 of the *CIAS1* gene and exon 2, 3 and 4 of the *TNFRSF1A* gene yield any additional mutations.

A 13-year-old Slovakian girl appeared to carry one M694V *MEFV* gene mutation. This patient had her first fever episode at age 5; these episodes lasted ~3 days and were accompanied by abdominal pain and lymphadenopathy. In the last few years, she has been free of symptoms. Maximal IgD concentration: 134 U/mL (normal reference value <100 U/mL). Her family has no Jewish or Mediterranean origin, and colchicine had not been tried. As the phenotype resembled HIDS, she had

been analyzed for HIDS, but comprehensive sequencing of MVK was negative. She did not carry any of the other FMF mutations tested.

Secondly, we detected a R92Q *TNFRSF1A* gene mutation in a 10-year-old Swedish boy, presenting with fever episodes, lasting about 7 days, since the age of 4 months. He also had headache, diarrhea, abdominal pain and lymphadenopathy. Laboratory examination shows high concentration of C-reactive protein during fever, and a continuously high IgD (680 U/mL). His older sister and his father have similar symptoms and laboratory values, but did not carry this mutation. Elaborate sequencing of the *TNFRSF1A* gene yields no other abnormalities. Lastly, we detected a E148Q mutation in *MEFV* gene in a 12-year-old Japanese boy, whose case history was published earlier [186]. This boy has recurrent episodes of fever, cervical lymphadenopathy, headache and occasional abdominal pain, with a continuously raised serum IgD (maximal 371 U/mL). Comprehensive DNA analysis of the *MVK* gene failed to detect mutations.

Diagnosed patients

In the group in whom we had previously made a clinical and genetic diagnosis of HIDS, TRAPS, or FMF, we detected 5 patients with mutations in other autoinflammatory genes.

An Armenian refugee girl, aged 7, living in the Netherlands, had been diagnosed as classical HIDS because of monthly episodes of fever, arthralgia, abdominal pain, oral ulcers and erythematous rash, which last 4-6 days and had started in her first year of life. She did not respond to colchicine. Her IgD serum concentration was 700 U/mL and *MVK* gene analysis demonstrated homozygosity for the V377I mutation, confirmed by sequencing of her parents. Now, she was found to be a carrier of a V726A mutation in *MEFV* gene. No other *MEFV* mutations were found.

An E148Q mutation in the *MEFV* gene was detected in DNA samples from two brothers of Dutch Caucasian origin, with classical HIDS (*MVK* genotype P167L/I268T). A man of Dutch origin, previously diagnosed with TRAPS (D93E mutation in *TNFRSF1A* gene) was identified as carrier of E148Q mutation in the *MEFV* gene. None of the latter three are of Mediterranean or Jewish descent. A Dutch woman with classical HIDS (*MVK* genotype V377I/I268T) was found to be a carrier of a R92Q mutation in the *TNFRSF1A* gene.

Control DNA samples

We detected one R92Q *TNFRSF1A* gene mutation and two E148Q *MEFV* gene mutations in 50 control DNA samples.

Discussion

Systematic genetic screening of patients with unclassified periodic fever, who had previously undergone one or two specific genetic tests, yields no mutations in 57/60 patients. Our screen netted only one possible diagnosis of FMF. Before this study, we had considered HIDS in this patient, but in hindsight FMF was more likely given her clinical phenotype.

Another patient carried an E148Q mutation in the *MEFV* gene, and one a R92Q mutation in the *TNFRSF1A* gene. The latter are regarded as low-penetrance mutations, disease-modifying factors or even polymorphisms instead of disease-causing mutations, because of the high prevalence in control populations

(frequency 1.2-6.4%) [58,187,188]. We confirm this in our study, as 4% and 2 % respectively of our relatively small group of 50 healthy Dutch controls carries these mutations. It is therefore questionable whether these patients should be diagnosed with either FMF or TRAPS.

These results demonstrate that clinical examination combined with directed genetic testing for one or two of the most likely syndromes forms the basis for diagnosis of the autoinflammatory syndromes included in this study. While it is true that clinical phenotype of the autoinflammatory syndromes may overlap and that therefore clinical diagnostic criteria, such as those of Tel-Hashomer for FMF [29], are not useful for differential diagnosis within this group of disorders, in the large majority of patients clinical examination will point towards one or two specific syndromes. DNA analysis can be focused on syndromes with highest clinical suspicion. As we have shown, screening of all known genes involved in these disorders is unlikely to yield additional relevant information.

This notion is confirmed by the clinical workup of the 87 previously diagnosed patients. We made a positive diagnosis in 84/87 using a single genetic test, while in only 3 cases an additional genetic test was necessary to establish a positive diagnosis.

This study also reemphasizes that a substantial proportion of patients with a definite autoinflammatory syndrome does not fall into any known genetic category. Most are sporadic cases, although there are patients with affected family members. The cause of disease in these patients remains elusive.

The second part of this study focused on the prevalence of combinations of mutations in more than one autoinflammatory gene. Although we found the low penetrance gene mutations R92Q in *TNFRSF1A* and E148Q in *MEFV* in patients with a different autoinflammatory disorder, their prevalence was not different from that in controls. Thus, the combination of disease-causing mutation(s) with a mutation in another autoinflammatory gene in a single patient only reflects the high prevalence of these latter low-penetrance mutations (or polymorphisms) in the general population [181,182]. The same holds true for the V726A *MEFV* mutation found in the Armenian girl with HIDS: carrier frequency in healthy Armenians for exon 10 mutations in this gene is 16%, of which about one fifth is the V726A mutation [14].

In conclusion, for diagnosis of an autoinflammatory syndrome we recommend a thorough clinical examination, including detailed medical history, use of medication, family history, ethnic origin and physical examination during an attack. An acute phase response (raised C-reactive protein or leukocytosis) should be present during the inflammatory episode. With current clinical knowledge of autoinflammatory syndromes, this information will yield a working diagnosis of one or two possible syndromes [4]. Subsequently, confirmation of this working diagnosis can be sought by directed genetic tests. Screening for mutations in all possible genes is not recommended, because this is not likely to yield extra information. Development of a set of validated discerning clinical criteria for differential diagnosis of the hereditary autoinflammatory syndromes is more likely to be helpful.

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Acknowledgements

Appendix

Contributing member of the International HIDS Study Group: Prof. J.W.J. Bijlsma, Department of Rheumatology, University Medical Center Utrecht, the Netherlands; Dr. C.A.M. de Swart, Department of Internal Medicine, Spaarne Hospital, Heemstede, the Netherlands; Dr. P.T.A. Schellekens, Department of Internal Medicine, Academic Medical Center, Amsterdam, the Netherlands; Dr. R.J. Powell, Clinical Immunology Unit, Queen's Medical Centre, Nottingham, United Kingdom; Dr. M. van Deuren, Department of Internal Medicine, University Medical Center St. Radboud, Nijmegen, the Netherlands; Dr. C.M.R. Weemaes, Department of Pediatrics, University Medical Center St. Radboud, Nijmegen, the Netherlands; Dr. C. Chapelon, Department of Internal Medicine, Hopital Pitie-Salpetriere, Paris, France; Dr. T. Espanol, Department of Immunology, Hospital General, Ciutat Saitaria i Universtiaria, Barcelona, Spain; Dr. H.R. van den Brink, Department of Rheumatology, Medical Center Alkmaar, the Netherlands; Prof. E. Elias, The Liver & Hepatobiliary Unit, Queen Elizabeth Hospital, Birmingham, United Kingdom; Dr. J. Louis, Department of Pediatrics, Clinique Notre Dame, Charleroi, Belgium; Dr. C.E.M. de Maat, Department of Internal Medicine, St. Antonius Hospital, Nieuwegein, the Netherlands; Prof. T.W. Kuijpers, Department of Pediatrics, Academic Medical Center, Amsterdam, the Netherlands; Dr. C.G. Arvidsson, Department of Pediatrics, Central Hospital Vastmanland, Vasteras, Sweden; Prof. U. Saatci, Department of Pediatrics, Hacettepe Children's Hospital, Ankara, Turkey; Dr. R. Tamminga, Department of Pediatrics, Academic Hospital Groningen, the Netherlands; Dr. D. Jilek, Department of Immunology, Regional Hygiene Institute, Usti nad Labem, Czech republic; Prof. C.D.A. Stehouwer, Department of Internal Medicine, Free University Medical Center, Amsterdam, the Netherlands; Prof. L. Businco, Department of Pediatrics, University La Sapienza, Rome, Italy; Dr. E.V. Granowitz, Infectious Disease Division, Tufts School of Medicine, Springfield, MA, USA; Dr. J.J. David, Comprehensive Care for Infants, Lincoln Pediatric Group, Lincoln, USA; Dr. K. Riesbeck, Department of Medical Microbiology, Malmö University Hospital, Malmö, Sweden; Prof. R.M. Scolozzi, Department of Internal Medicine, University of Ferrara, Italy; Dr. P. Betts, Southampton General Hospital, United Kingdom; Dr. P. Pohunek, Division of Pediatric Pulmonology, University Hospital Motol, Prague, Czech Republic; Dr. E. Drewe, Clinical Immunology Unit, Queen's Medical Centre, Nottingham, United Kingdom; Dr. A.M. Farrell, Department of Histocompatibility & Immunogenetics, Glasgow Royal Infirmay, Glasgow, United Kingdom; Dr. A. Ahlin, Sach's Children's Hospital, Stockholm, Sweden; Prof. J.J. Rasker, Department of Rheumatology, Medisch Spectrum Twente, Enschede, the Netherlands; Prof. J. Palmblad, Department of Medicine, Karolinska Institutet at Huddinge Unversity Hospital, Stockholm, Sweden; Dr. C.J. Darroch, Department of Immunology, Royal Liverpool University Hospital, Liverpool, United Kingdom; Prof. H.P.H. Kremer, Department of Neurology, University Medical Center St. Radboud, Nijmegen, the Netherlands; Dr. T. Fiselier, Department of Pediatrics, University Medical Center St. Radboud, Nijmegen, the Netherlands; Dr. K. Takada, National Institute of Health, Bethesda, MD, USA; Dr. H.J. Hasper, Department of Internal Medicine, Deventer Hospital, Deventer, the Netherlands; Prof. D.C. Knockaert, Department of General Internal Medicine, University Hospital Gasthuisberg, Leuven, Belgium; Dr. T. Tribolet de Abreu, Department of Internal Medicine, Hospital do Espírito Santo-Évora, Évora, Portugal; Prof. M. Korppi, Department of Pediatrics, Kuopio University Hospital, Kuopio, Finland.
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Genetic analysis: valuable key to diagnosis and treatment in periodic fever

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Abstract

Two Dutch patients suffering from recurrent fever attacks who went undiagnosed for more than 40 years are described. Their diagnosis of periodic fever was made when molecular analysis revealed novel mutations in the tumor-necrosis factor (TNF)-receptor (*TNFRSF1A*) gene, establishing the diagnosis of TNF-receptor associated periodic syndrome (TRAPS). TRAPS is an autosomal dominant disorder, characterized by recurring episodes of fever, arthralgia and skin lesions. It is caused by mutations in the 55kDa *TNFRSF1A* gene. This finding has facilitated the treatment for TRAPS as blocking of TNF signaling seems to alleviate the symptoms. The use of a short course of recombinant p75-TNFR:Fc fusion protein (etanercept) induced prolonged remission in one patient described here.

Introduction

Prolonged or recurrent fevers are one of the greatest challenges to the diagnostic skills of the clinician. In a prospective study of 167 immunocompetent patients with fever of unknown origin, no diagnosis could be made in 50 patients [6]. In those with recurrent fever, the chance of reaching a diagnosis was even lower [6]. Until recently, the different forms of periodic fever were diagnosed using clinical criteria because knowledge on the pathogenesis was absent. Consequently, treatment was limited to supportive measures. The recent discovery of the genetic background of a number of familiar periodic fever syndromes [2,3,7,15,90] has greatly simplified diagnostic procedures in patients with recurrent febrile attacks [89].

Tumor necrosis factor (TNF)-receptor associated periodic syndrome (TRAPS, OMIM [142680]) is an autosomal dominant disorder characterized by recurrent febrile attacks accompanied by abdominal distress, arthralgia or arthritis, myalgia, migrating erythematous skin lesions and conjunctivitis. Mutations of the 55kD TNF-receptor gene (*TNFRSF1A*) on chromosome 12 have been established as the cause of this syndrome and due to this finding effective therapy could be developed. In the cases presented here we demonstrate the role of genetics in diagnosis and treatment of TRAPS. In both cases, the positive family history was an important clue leading to genetic screening and diagnosis.
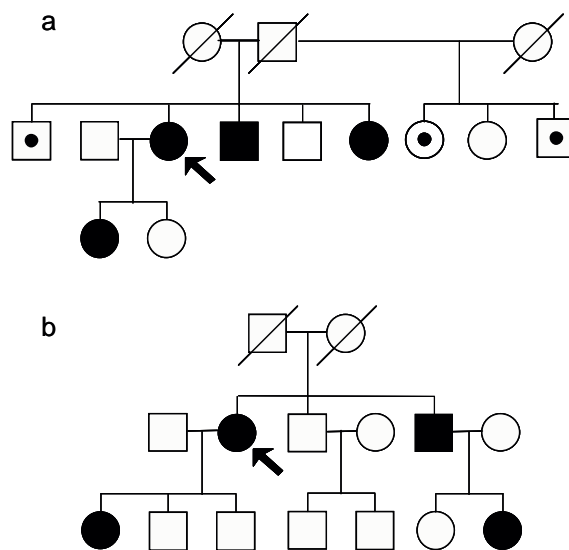
Figure 1. Family trees of case 1 (a) and 2 (b); cases indicated by arrow. Line through a symbol = deceased. ■= affected, □ = unaffected, ▣ = carrier of mutation without clinical symptoms.

Report of cases

Case 1

A 62-year old woman of Dutch origin contacted us after reading a local newspaper article on periodic fever. She recognized the patient history described in that article as similar to her own. Six years previously, she had been examined at our center for fever of unknown origin, participating in a prospective multicenter study [6], but no diagnosis could be made at that time. For over 40 years, she has suffered twice a year from fever attacks characterized by a spiking fever up to 40°C, accompanied by general malaise, chills, fatigue, myalgia and abdominal pain. The

attacks resolved spontaneously only after 4 to 6 weeks, but could be aborted earlier when non-steroid anti-inflammatory drugs were started early. The further medical history revealed two uncomplicated pregnancies, an appendectomy and two gynecological operations including hysterectomy because of the fever. A brother, sister and a daughter had similar attacks (figure 1a). With the exception of fever, physical examination during an attack was normal. Laboratory examination showed a raised erythrocyte sedimentation rate (93 mm/hr), leukocytosis ($22.7*10^9$/l) and a normal serum IgD concentration (41 U/ml). Numerous cultures of blood, urine, feces, sputum and bone marrow had remained negative. Additional diagnostic interventions, including computer tomographic (CT) scanning of abdomen, an [111]Indium-IgG scan for detection of focal inflammation and endoscopic cholangiography revealed no abnormalities. We considered the diagnosis TRAPS because of the low frequency and long duration of the febrile attacks and the autosomal dominant inheritance pattern in the family. DNA analysis revealed a novel missense mutation in the *TNFRSF1A* gene, which leads to a replacement of tyrosine at position 38 of the *TNFRSF1A* protein for a cysteine (Y38C). This mutation was subsequently detected in three other family members. One of her brothers and a half-brother and half-sister, aged between 39 and 63 years, were without symptoms despite having the disease associated genotype.

Case 2

A 62-year old woman of Dutch origin was referred because of recurrent episodes of fever from the age of six. Attacks were characterized by fever (> 40°C), accompanied by conjunctivitis, erythematous skin lesions, myalgia /arthralgia especially of hands and legs, and abdominal pain. In general, these episodes lasted 2 to 3 weeks, and recurred several times a year. Treatment with prednisone (up to 20 mg/day) during an attack only slightly ameliorated the symptoms. The medical history further revealed appendectomy, hysterectomy and diagnostic laparotomy, all because of unexplained fever and abdominal pain. Her father, brother, daughter and niece had similar, albeit milder, attacks with fever. In the 2 years before admission, the severity, frequency, and duration of the fever attacks increased, despite increasing steroid dosages. When prednisone was tapered in order to exclude an underlying infection, the febrile attacks did not abate but persisted. At admission she appeared ill, febrile (40°C), had severe abdominal distress, skin lesions and arthralgia. There was an important acute phase response as mirrored by the elevated erythrocyte sedimentation rate (100 mm/hr), C-reactive protein (219 mg/L) and leukocyte count ($34.2*10^9$/L). Elaborate investigations, including CT scanning of thorax and abdomen, an [111]Indium-IgG scan and microscopic examination and cultures of bone marrow failed to yield a diagnosis. Although IgD was elevated (269 IU/mL; normal <100 IU/mL), the clinical picture was not considered compatible with another periodic fever syndrome: hyper-IgD and periodic fever syndrome [46]. Because of the long duration of the attacks and the apparent autosomal dominant inheritance pattern of the disease in the family, TRAPS was suspected. Sequencing of the *TNFRSF1A* gene of the patient revealed a novel missense mutation resulting in a cysteine to phenylalanine substitution at codon 29 of the protein (C29F).

Given the central role of TNF signaling in the pathogenesis of TRAPS, and the persistence and severity of the attack in patient 2, we explored the efficacy of treatment with recombinant p75TNFR:Fc fusion protein (Etanercept, Enbrel®) [189]. On three successive days, a subcutaneous injection of Etanercept (25 mg)

was given. As shown in Figure 2, within two days the body temperature dramatically declined and the symptoms of myalgia, arthralgia and malaise rapidly improved, while the inflammatory parameters returned to baseline. Two weeks after treatment, the patient was discharged. She remained in clinical remission for six months without any therapy. After that time she returned with fever, myalgia, abdominal pain and skin lesions. Laboratory examination again showed increased inflammatory parameters. Because of the relative mildness of the attack, and the limited availability of the drug in our country, she was treated with a single dose of Etanercept (25 mg s.c.). This again resulted in a disappearance of fever and symptoms, and decline in inflammatory parameters. However, this time the remission lasted only two weeks. Subsequently, she received four successive injections of Etanercept of the same dose (25 mg s.c.) which now induced a long-lasting remission.
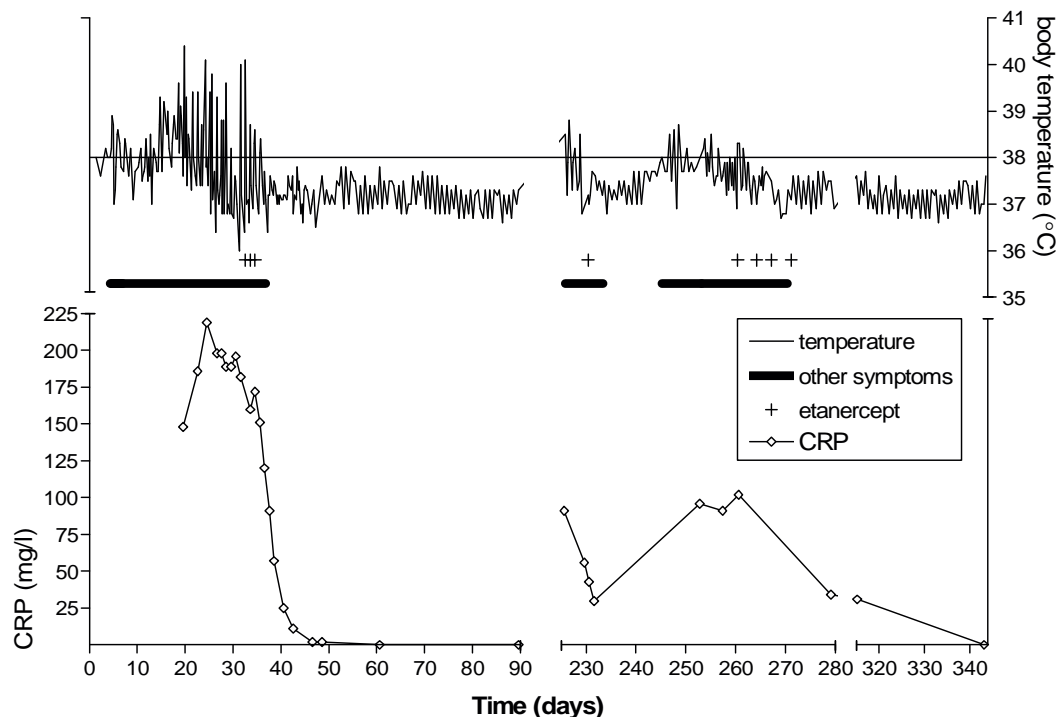
Figure 2. Effect of treatment with etanercept in case 2. The upper panel depicts body temperature, the thick straight lines indicate the time when the patient had accompanying symptoms as described in the text. Time of subcutaneous injection with 25 mg etanercept is indicated by a cross. In the lower panel, the dotted line shows the serum concentration of C-reactive protein (CRP) in mg/L during this period.

Discussion

Long before the elucidation of the molecular pathophysiology of fever a number of families with autosomal dominant inherited periodic fever were reported in the literature [53,56,59,190-192]. Although there were some similarities in presentation different names were coined, including Familial Hibernian Fever, Familial Periodic Fever and Autosomal Dominant Recurrent Fever. A genome-wide linkage in an Australian family with familial periodic fever indicated linkage with chromosome 12 and this finding was confirmed in an Irish/Scottish family with familial Hibernian fever suggesting that these families were suffering from the same disease [55,193]. Subsequent investigations demonstrated that affected

patients from these families all had mutations in gene encoding for the 55 kD TNF receptor protein (*TNFRSF1A*). In order to group these families it was proposed to refer to this syndrome as TRAPS [7].

Up to now, 14 different missense mutations in the *TNFRSF1A* gene, located on chromosome 12, have been described in different families with TRAPS. The novel missense mutations reported here (C29F and Y38C) affect cysteine residues within the extracellular domain of TNFRSF1A involved in disulphide bonding, similar to most of the previously described mutations [7,63,189]. Most TRAPS mutations have a penetrance of around 75%, as is seen in case 1. Genetic analysis of the *TNFRSF1A* gene should be performed in a patient with recurrent episodes of fever of unknown origin that last at least one week and a positive family history in an autosomal dominant inheritance pattern.

TNF is an important pro-inflammatory cytokine, and cellular signaling is mediated by the TNFRSF1A receptor. Upon activation the TNFRSF1A is clipped off from the cell surface and released into the circulation. Consequently, TNF signaling is interrupted and soluble TNFRSF1A can bind to and inactivate circulating TNF, thereby exhibiting anti-inflammatory effects. It is postulated that TRAPS mutations impair TNFRSF1A shedding and reduce the pool of the circulating protein. Indeed, when measured during remissions serum TNFRSF1A concentrations are low relative to normal controls [7]. It is suggested that the impaired shedding results in continuous pro-inflammatory TNF signaling. Treatment of patients with TNF inhibitors, therefore, provides a rational therapeutic approach.

Etanercept is a recombinant fusion protein that consists of the other soluble TNF-receptor (p75) linked to the Fc portion of human IgG$_1$ and it has profound anti-inflammatory actions by blocking TNF. Etanercept is used as a disease modifying agent in rheumatoid arthritis, where the drug is given twice weekly. Using this scheme, continuous administration of etanercept was reported to be beneficial in improving clinical and laboratory parameters in a small number of TRAPS patients [189]. As demonstrated in our patient it does not seem necessary to subject TRAPS patients to twice weekly etanercept injections. The repeated response strongly suggests that a short course is sufficient to induce prolonged remission of TRAPS attacks. This should be investigated in a formal clinical trial.

In the case of TRAPS, the spin off of genetic research has clearly improved the quality of life of patients by giving the opportunity for a clear diagnosis avoiding various unnecessary medical interventions and offering the possibility of genetic counseling. Elucidation of the molecular background of this syndrome has furthermore afforded the development of novel therapeutic strategies.

Acknowledgements

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Familial periodic fever and amyloidosis due to a new mutation in the *TNFRSF1A* gene

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Introduction

Establishing a diagnosis in patients with recurrent episodes of fever is often very difficult [5,6]. The episodes of fever and signs of inflammation may lead to a considerable morbidity and to notable frustration in patients and doctors as extensive diagnostic attempts often fail to reveal the cause. However, after ruling out infectious causes for the febrile attacks one should consider syndromal causes of periodic fever. Currently, at least three separate periodic fever syndromes have been recognized: hyper-IgD and periodic fever syndrome (HIDS, MIM260920), the tumor necrosis factor (TNF)-receptor associated periodic syndrome (TRAPS, MIM142680), and the best known and most prevalent syndrome familial Mediterranean fever (FMF, MIM249100). The identification of causative genes for each of them [2,3,7,15,90] has greatly advanced diagnostic possibilities and has implications for treatment as well.

We wish to present the following observation as an example of a typical familiar case of periodic fever and amyloidosis. With the use of molecular tools we were able to make a definitive diagnosis of TRAPS.

Case report

A 61-year old woman of Dutch-Indonesian ancestry was referred because of recurrent episodes of high fever and proteinuria. She had her first attack of fever as a 9-year-old child in Indonesia. At that time no elaborate diagnostic procedures were performed and the recurrent fever was attributed to attacks of malaria. She moved to the Netherlands at the age of 21 years and shortly thereafter she was admitted to a hospital because of a 3-week long attack of fever which subsided spontaneously. No pertinent diagnosis could be made. From that time onwards the fever episodes occurred approximately twice a year and generally lasted up to 3 weeks.
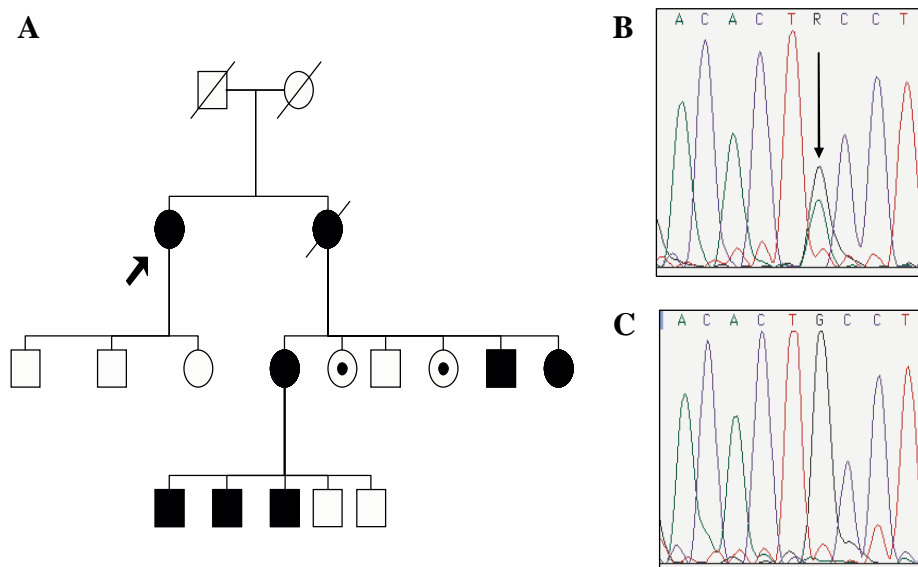
Figure 1. a. Family tree, index patient indicated by arrow. Line through a symbol = deceased. filled symbol = affected, open symbol = unaffected, dotted symbol = carrier of mutation without clinical symptoms. b. DNA sequence electrospherogram of C70Y mutation (TGC → TAC, indicated by arrow) in the gene for the type 1 TNF-receptor which is found in the affected members of this family. c. DNA sequence of healthy individual with normal pattern (TGC) at position 70.

Table 4. Clinical features and laboratory results of affected family members

Individual	Age (yrs)		Attacks		Symptoms and signs						Laboratory results			
	Current	Onset	Length (dys)	Frequency	Myalgia	Abdominal pain	Pleural pain	Rash	Red eyes	Inguinal hernia	CRP (mg/l)	SAA (mg/l)	sTNFR p55 (ng/ml)	sTNFR p75 (ng/ml)
II:1*	61	9	21	†	Yes	No	No	No	No	NA	2.7	0.9	10.5	22.0
III:4	45	8	14	1x/yr	Yes	Yes	Yes	Yes	Yes	NA	15	2.5	0.81	3.23
IV:1	20	5	14	1x/yr	Yes	Yes	No	Yes	Yes	No	8	3.2	0.66	3.28
IV:2	18	0	14	1x/yr	Yes	Yes	Yes	Yes	Yes	Yes	28	5.9	1.00	3.80
IV:3	15	2	21	1-2x/yr	No	Yes	Yes	Yes	Yes	No	105	11	0.69	3.15

* proband and main subject of this clinical observation. † varying frequency during life, now no attacks anymore, see text for details. Individual = generation and number from family tree (fig. 1). CRP= C-Reactive Protein (normal <2.7 mg/l) measured in serum; SAA = serum amyloid A protein (normal <4.2mg/l) measured in serum; sTNFR = soluble TNF-receptor concentrations in plasma, p55 = type 1 (normal <1,50 ng/ml), p75 = type 2 (normal <2,51 ng/ml). All laboratory measurements were done from samples taken in the attack-free interval.

Usually, a short course of steroids was administered because the impression existed that this ameliorated the attacks. Apart from fever, generalized myalgia and fatigue during the attacks she had no specific symptoms. During each of her three (uncomplicated) pregnancies she was free from symptoms, but after each delivery she experienced a severe febrile bout. Attacks could further be provoked by emotional and physical stress. She gave a positive family history. Her sister, who died at age 47 due to breast carcinoma, had experienced similar febrile attacks during life. Three of her sister's six children and three of her sister's five grandsons were also affected (fig. 1).

Symptoms and pertinent laboratory results of our patient and four affected relatives are summarized in Table 1. There are not enough data available to determine definitely which of the parents of our patient transmitted the disease to their offspring. It is also unclear whether the disease originated from the Dutch or Indonesian side of the family. Our patient noticed an increase in the frequency of the fever attacks after reaching menopause to about 4 to 5 times each year.

In the following years she developed renal failure, despite colchicine treatment. Both rectal and renal biopsies showed evidence of type AA amyloidosis. Since 1997 she has been dependent on intermittent hemodialysis. Most remarkably, the decrease of renal function coincided with a cessation of fever episodes, and since 1996 she is free from attacks (fig. 2). The autosomal dominant inheritance, the long duration of attacks, and the (partial) response to steroids reminded us of TRAPS. After sequencing the *TNFRSF1A* gene, which codes for the p55 TNF-receptor, the incriminated molecule in TRAPS [183], we discovered a novel missense mutation in our patient. This replaces cystine by tyrosine at position 70 of the amino acid sequence of the protein (C70Y, fig. 1). The C70Y mutation has been found in 10 of 17 tested family members, two of whom remain asymptomatic up to this day, which suggests a penetrance of 80%. With the exception of our patient (II:1), the affected family members had low concentrations of soluble TNFRSF1A in their blood (table 1) as measured by ELISA (La Roche) [50].

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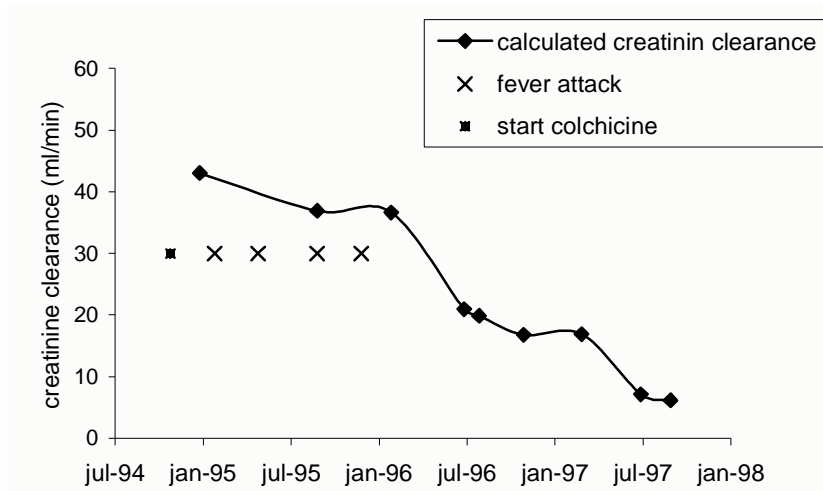
Figure 2. Calculated creatinine clearance as a measure of renal function in relation to occurrence and cessation of fever attacks. The last fever attack occurred November 1995.

Discussion

The acronym TRAPS was recently suggested by McDermott et al. [7] to be used for a group of autosomal dominantly inherited periodic fever syndromes previously known by several names (e.g. Familial Hibernian Fever, Familial Periodic Fever). Patients experience recurrent attacks of fever accompanied by abdominal pain, myalgia, skin lesions and conjunctivitis. Nephropathic reactive amyloidosis of the AA type has been reported in four patients up till now [59,194]. The attacks occur in varying frequency and generally last two to three weeks. Laboratory examination reveals acute phase response. The autosomal dominant inheritance pattern is one of the main characteristics which distinguishes TRAPS from the two other main periodic fever syndromes, familial Mediterranean fever and Hyper-immunoglobulinemia D and periodic fever syndrome, both being autosomal recessive.

The gene responsible for TRAPS is located on the short arm of chromosome 12 [55,193] and encodes for the type I receptor of TNF (TNFRSF1A) [7]. The TNFRSF1A protein is a cell membrane coupled receptor expressed on a large variety of cells. TNF has many effects, including induction of cytokine secretion, increased expression of adhesion molecules on leukocytes and endothelial cells, activation of leukocytes, fever and cachexia. Activation of the receptor also leads to a specific negative feedback mechanism called shedding: the receptor is cleaved by a protease, by which the extracellular part of the receptor is released in a soluble form into the extracellular space, where it can act as a competitive inhibitor of TNFRSF1A. Mutations in this gene lead to a structural change in the extracellular part of the receptor and it has been suggested that this impairs shedding of the receptor [7]. Following this hypothesis, the auto-inflammatory phenotype of TRAPS results from an impaired downregulation of membrane-bound TNFRSF1A, and decreased formation of soluble TNFRSF1A [7]. As a consequence, a trivial trigger leads to an uncontrolled inflammatory response.

Until recently, no adequate treatment for TRAPS was available and attacks were treated symptomatically by oral steroids and colchicine with varying results. After the discovery of the molecular defect, the administration of recombinant soluble TNF receptors became a logical therapeutic step. Indeed, in selected patients it

seems beneficial with a decrease in frequency of attacks [189]. We did not treat our patient with recombinant soluble TNF receptors because she did not have any further attacks or evidence of acute phase response. As soluble TNF receptors are cleared from the body by the kidneys, we expected high concentrations in our patient because of coexisting renal failure. This was confirmed: plasma concentrations of soluble TNF receptor were clearly elevated (table 1). As shown in Fig. 2 the cessation of fever episodes in our patient coincided with the deterioration of her renal function. A relation between these two observations could be explained by the rise in concentration of soluble TNFRSF1A, which could protect her from further attacks of fever.

Acknowledgements

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Beneficial response to interleukin-1-receptor antagonist in a patient with severe, treatment-resistant TNF-receptor associated periodic syndrome (TRAPS)

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To the editor:

Interleukin-1 (IL-1) is one of the central pro-inflammatory cytokines, and *in vitro* studies have implicated IL-1 as important pathogenic factor in several autoinflammatory syndromes [70,149,156]. Hawkins et al. reported good results with the recombinant IL-1 receptor antagonist (IL-1ra) anakinra [195] in two patients with Muckle-Wells syndrome, one of the autoinflammatory syndromes [196]. This prompted us to try anakinra in a patient with severe, treatment-resistant tumor necrosis factor (TNF)-receptor associated periodic syndrome (TRAPS; Mendelian Inheritance in Men Database no. 142680).

The patient is a 19-year old Dutch woman, diagnosed with TRAPS, confirmed by a C43Y-mutation in the TNF-receptor type 1 (TNFRSF1A) gene. From the age of 1, she suffers from episodes of fever, general malaise, abdominal pain, myalgia and painful erythematous skin lesions, accompanied by vigorous acute phase response. Numerous therapies were tried, including non-steroidal anti-inflammatory drugs (NSAIDs), methotrexate, and cyclosporin, with disappointing results; the only reasonably effective drug was prednisone, at least 30 mg daily. After the diagnosis TRAPS was made she started with the TNF-inhibitor etanercept [179]. This improved symptoms temporarily, but the acute phase response persisted and prednisone could not be tapered. A brief trial of sirolimus, an inhibitor of T-cell activation [197], had to be stopped because of severe allergic reaction. The last year, inflammatory symptoms had become unremitting and occurred daily. Between July 2002 and November 2003 (total: 22 measurements), median CRP concentration was 117.5 mg/l (interquartile range 70.5-157.5) and leukocytes 20.9×10^9/l (interquartile range 14.95-23.6). The patient consented to use of anakinra, given subcutaneously at a dose of 100 mg daily. This resulted in remarkable improvement of symptoms and an unprecedented decrease of CRP-concentration and leukocyte count within days (figure 1). Prednisone could rapidly be tapered to 10 mg daily. After 1 month the patient was symptom-free and feeling well for the first time in many years. She did suffer pain and redness at the anakinra injection site (figure 2).

TRAPS is an hereditary autoinflammatory syndrome, characterized by recurrent episodes of fever and inflammation, caused by TNFRSF1A gene mutations. Its clinical features can vary greatly between patients, from a recurrent mild localized myalgia to the incapacitating severe symptoms as described in our patient [54]. In mild cases, the occasional use of NSAIDs is usually sufficient for symptom-management. After discovery of the genetic etiology, anti-TNF treatment in the form of etanercept has been introduced and found to be successful in many cases [54,64,179]. However, not all patients benefit and initial response may wear off with prolonged therapy, necessitating alternative therapies. In one severe case unresponsive to etanercept, Drewe et al. [197] describe a beneficial response to sirolimus. This could not be used in our patient because of an allergic reaction.
Anakinra is a recombinant form of IL-1ra, an endogenous inhibitor of IL-1 signaling, used in the treatment of rheumatoid arthritis [195]. It is usually well tolerated; the most common side effect is an injection-site reaction with pain, erythema and local inflammation [198]. This beneficial response to anakinra argues for a central role of IL-1 in the pathogenesis of TRAPS [70,149,156,196]. It is generally assumed that in TRAPS the inflammatory effects of TNFα and

lymphotoxin (LT) are not countered adequately, because of a lack of soluble TNF-receptor, the major inhibitor of TNF. In that respect it is hard to understand that the TNF-receptor construct, etanercept, had hardly any therapeutic effect in our patient. Perhaps even more difficult to explain is the effectiveness of IL-1ra, since IL-1β and IL-1α are responsible for only a small part of the biological effects of TNFα and LT. Further research is required here, while also long-term efficacy needs yet to be determined.
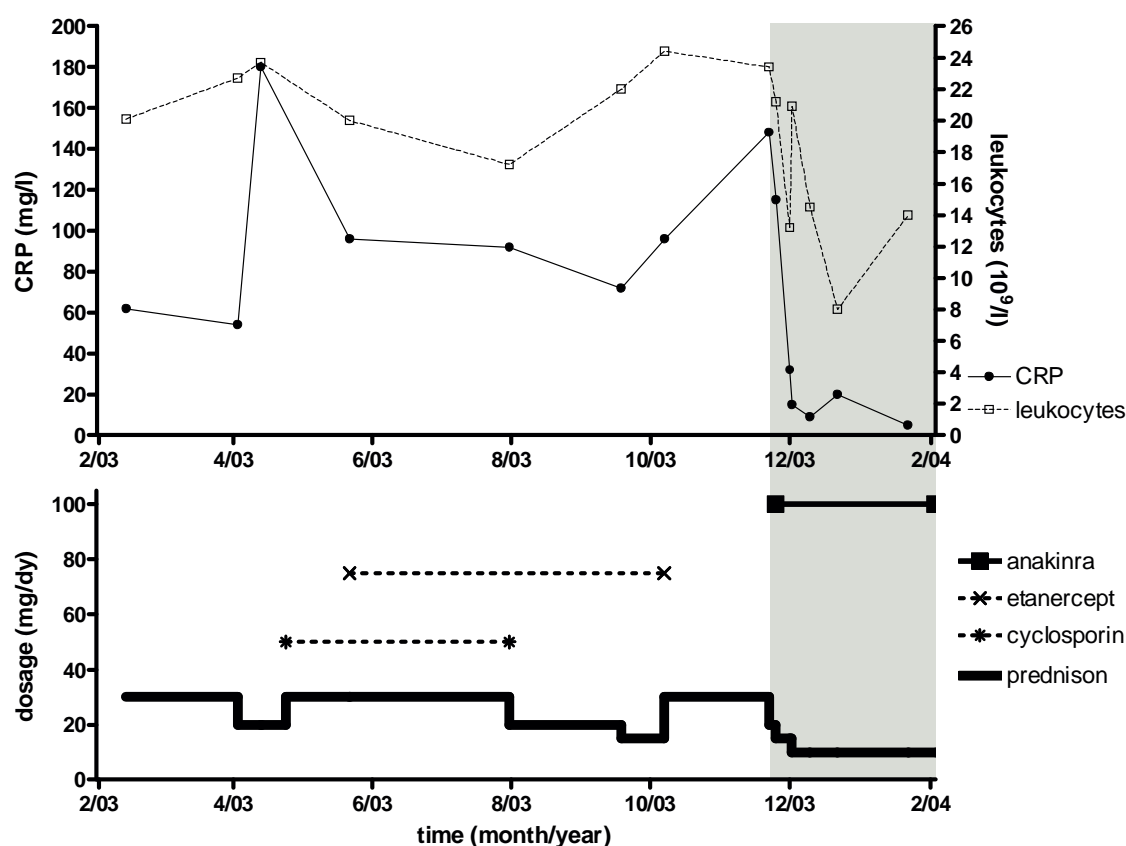
Figure 1. Concentration of C-reactive protein (CRP) and leukocytes in a patient with TRAPS, from February 2003 to January 2004. Treatment used is shown in the bottom half of the figure, in dosage of mg per day (except for etanercept, which is dosage per week). Shortly after start of anakinra (gray area of graphs), the concentration of CRP and leukocytes dropped to the normal range.

Figure 2. Erythematous and painful injection site reaction associated with subcutaneous use of anakinra on the upper leg of the patient. Such reactions clear up after a few days, and are less severe when anakinra is used in the abdominal region.

Familial Mediterranean fever – A not so unusual cause of abdominal pain

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Abstract

Familial Mediterranean fever is a hereditary syndrome characterised by recurrent episodes of fever and serositis, resulting in pain in the abdomen, chest, joints and muscles. It is primarily diagnosed in people of Jewish, Arabic, Turkish or Armenian ancestry and is caused by mutations in the gene encoding for pyrin. Abdominal FMF attacks resemble the clinical presentation of 'acute abdomen', with severe abdominal pain and rigidity, but in FMF symptoms always resolve spontaneously. It is important to distinguish these regular pain episodes from small bowel obstruction due to adhesions to prevent life-threatening bowel strangulation. In most cases, colchicine will prevent new painful attacks. This seminar also discusses other causes of abdominal pain in FMF patients.

Case report

A 24-year old man of Turkish origin, living in the Netherlands, was referred to our University hospital because of unexplained abdominal pain. Since the age of 19, he suffers from episodes of severe abdominal pain, occurring about once every month. He abstains from eating or drinking during attacks because this increases the pain. Accompanying symptoms are often (though not always) high fever, mild diarrhoea, and sometimes chest pain. The pain resolves spontaneously within 2 or 3 days. Family history is negative, but his parents are cousins. On presentation to the emergency ward of a district hospital, which occurred regularly, physical examination was usually compatible with 'acute abdomen'. Laboratory evaluation during such an episode showed evidence of inflammation with increased serum concentration of C-reactive protein, but otherwise no abnormalities. Twice, laparotomy was performed because of suspected appendicitis or gastric perforation, which yielded a normal appendix, while biopsies showed microscopic evidence of haemorrhagic peritonitis. The patient met the clinical criteria for FMF and was started on colchicine 1 mg daily, with favourable effect. There was no evidence of amyloidosis. DNA analysis revealed that he was homozygous for a Met694Val mutation in the *MEFV* gene.

Introduction on Familial Mediterranean fever (FMF)

This is a typical story for a patient with Familial Mediterranean Fever (FMF). FMF is a hereditary autoinflammatory disorder characterised by lifelong recurrent episodes of fever and serositis, resulting in pain in the abdomen, chest, joints and muscles (table 1) [4,36]. In the majority of patients, these inflammatory attacks start before the age of 20 [24]. The onset of a typical FMF attack is acute, and the symptoms persist for only a short time (6 to 96 hours) before they resolve spontaneously. It is an autosomal recessive disease, which is most prevalent in people from the Mediterranean basin, including Sephardic Jews, Arabs, Turks and Armenians. Prevalence is estimated to be as high as 1 in 700 in Israel and 1 in 1400 in Turkey. There is often a delay of 5 to 10 years from the onset of symptoms before the correct diagnosis is made; major factors contributing to this delay were found to be patient´s neglect of symptoms and physician´s unawareness of FMF [199]. Even in a region where FMF can be encountered frequently, at least 11 FMF patients could be detected in a group of 59 children who had been given the diagnosis functional abdominal pain *per exclusionem* [200]. The major long-term complication, which occurs in up to 40% of untreated patients with FMF, is AA type amyloidosis resulting in renal insufficiency.

Clinical feature	Frequency n=1558
Fever	100%
Peritonitis	94%
Arthritis	54%
Pleuritis	39%
Rash	30%

Table 1. Frequency of clinical features of FMF among 1558 patients, which include Jews (n=515), Turks (n=601), Arabs (n=227) and Armenians (n=215) (adapted from Ben Chetrit et al. [36])

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FMF is caused by mutations in the *MEFV* gene, which encodes for the protein pyrin (or marenostrin) [15,90]. About 40 different mutations have been described until now, although just four or five mutations account for the majority of FMF cases. The function of pyrin was unknown at the time of discovery, but it has since been identified as a cytoplasmic protein and member of a family of proteins that all contain a domain first described in pyrin, the so-called pyrin domain. These proteins are involved in regulation of inflammation, apoptosis and/or cytokine secretion. Pyrin is thought to indirectly regulate caspase-1 function, and therefore influence interleukin-1β processing and apoptosis. Thus, FMF is caused by a disordered regulation of inflammation, resulting in excessive inflammatory attacks after 'trivial' stimuli.

Abdominal attacks in FMF

Clinical presentation

Abdominal pain is the most frequent symptom encountered in FMF; 95% of patients report this as the main symptom during at least some of their fever episodes, while 50% cite such an 'abdominal attack' as the first symptom of their disease [201]. Presentation of a typical abdominal attack will resemble that of 'acute abdomen'. Onset is sudden and acute, leading to rapid development of symptoms within 1-2 hours. The abdominal pain is usually diffuse throughout the entire abdomen, although in some cases it may be localized; it may be very severe in intensity. Patients prefer to lie still, as pain is aggravated on movement. This may be accompanied by any bowel activity, ranging from constipation (most often) to diarrhoea. Findings on physical examination will be compatible with 'acute abdomen' as well: distension of the abdomen, rigidity, direct and rebound tenderness, and reduced peristaltic sounds [201]. The intensity of symptoms and signs of an inflammatory attack in FMF will decrease spontaneously after 12-24 hours, and usually, the attack resolves over the following 48 hours [201]. Thus, after about three days the patient will be symptom-free again. The duration of remission is variable between patients and within patients and may last for weeks or months, or even longer [202]. So-called incomplete abdominal attacks may occur. These differ from 'typical' abdominal attacks in one or two features, which may include absence of fever, minimal change in acute phase parameters, absence of 'true' peritonitis and/or localisation of the abdominal pain to a single specific abdominal area. It may be difficult to differentiate an 'incomplete' abdominal attack from other causes of abdominal pain, mainly because of its atypical presentation [24,29].

Table 2. CT findings in 14 patients with FMF during acute abdominal attack (adapted from Zissin et al. [203]).

No. of patients	CT findings
12	Engorged mesenteric vessels and/or thickened mesenteric folds
6	Mesenteric and/or retroperitoneal lymphadenopathy
6	Ascites
4	Ascites with focal peritonitis
3	Splenomegaly
2	Dilated small bowel loops
1	Mural thickening of the ascending colon

Laboratory and radiological evaluation

Laboratory examination during an FMF attack will reveal a vigorous acute phase response, including leukocytosis with a left shift, increased erythrocyte sedimentation rate and increased concentrations of C-reactive protein, fibrinogen and serum amyloid A protein [113]. Plain abdominal radiography may show dilatation of small bowel with air-fluid levels; in a study by Aharoni et al. this was seen in 11 of 34 plain abdominal films made in symptomatic FMF patients [204]. On computed tomography (CT) scanning, the most common finding during an abdominal attack in FMF is non-specific mesenteric pathology, which may include engorged mesenteric vessels, thickened mesenteric folds and mesenteric and/or retroperitoneal lymphadenopathy [203]. Minimal ascites, with or without focal peritonitis, splenomegaly and dilated small bowel loops may also be present on CT scanning (table 2). No role for MRI in FMF has been defined yet.

On laparoscopy or laparotomy during an acute attack, it is possible to observe signs of peritonitis, including oedematous and hyperaemic peritoneal folds or greater omentum. Microscopic examination of omentum biopsies will reveal a sterile non-specific inflammation, which may be purulent or haemorrhagic and accompanied by signs of chronic recurrent peritonitis, such as peritoneal inclusion cysts [201,205]. A sterile peritoneal exudate containing fibrin and polymorphonuclear cells is usually present in the peritoneal cavity [23]. Fibrous adhesions may ensue (see below).

Diagnosis of FMF

FMF is a clinical diagnosis, and for diagnostic purposes, a set of clinical criteria was designed by Livneh et al. [29] (table 3). However, this was validated in Israel in a population with a very high prevalence of FMF, and it is unknown whether they work as well in other populations [4]. The criteria include a beneficial response to colchicine. Additionally, DNA testing of the *MEFV* gene may be used as a confirmatory test.

Major criteria:	Typical attacks with peritonitis (generalized)
	Typical attacks with pleuritis (unilateral) or pericarditis
	Typical attacks with monarthritis (hip, knee, ankle)
	Typical attacks with fever alone
	Incomplete abdominal attack
Minor criteria:	Incomplete attacks involving chest pain
	Incomplete attacks involving monarthritis
	Exertional leg pain
	Favorable response to colchicine

Table 3. Diagnostic criteria for diagnosis of FMF (Tel-Hashomer) (adapted from Livneh et al. [29]). Requirements for diagnosis of FMF are ≥1 major criteria or ≥2 minor criteria. Typical attacks are defined as recurrent (≥3 of the same type), febrile (≥38°C) and short (between 12 hours and 3 days). Incomplete attacks are defined as painful and recurrent attacks not fulfilling all the criteria for a typical attack.

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Differentiating abdominal attack from other pathology in a known FMF patient

The inflammatory peritoneal exudate and recurrent peritonitis in FMF may lead to intraperitoneal adhesions. Adhesive small bowel obstruction is not uncommon in FMF patients, especially after previous (often unnecessary) abdominal surgical procedures. In a study of 355 children with FMF with a mean follow-up of 8 years, Ciftci et al. identified 11 patients with adhesive small bowel obstruction, where adhesiolysis or resection of strangulated ileum or jejunum was necessary [206].

It can be very difficult to differentiate between small bowel obstruction (a potentially life-threatening complication) [201] and a severely painful, but otherwise harmless abdominal attack in FMF, which will resolve spontaneously. In the first case, surgical intervention must not be delayed, while in the second case this course of action might lead to further complications. The same is true for the differential diagnosis of acute appendicitis. In women, it can be difficult to differentiate an FMF attack restricted to the pelvic region from pelvic inflammatory disease [207]. Such pelvic attacks are often precipitated by menstruation or pelvic instrumentation [201].

The clinical course of the abdominal pain will often enable differentiation. In a typical FMF attack, signs and symptoms will peak within a few hours, remain stable for 12-24 hours and then gradually subside within an additional 6-12 hours, to resolve completely within 24-72 hours [201]. In the case of small bowel obstruction leading to strangulation, or appendicitis, there will be a continuous progressive deterioration of the clinical picture. Most FMF patients are intimately familiar with the 'normal' pattern of symptoms during a typical abdominal attack, and close observation is warranted when the patient reports abdominal complaints which are experienced differently [201]. In a small retrospective study of abdominal CT scanning in 17 known FMF patients with abdominal symptoms, Zissin et al. were able to correctly identify two patients with appendicitis and one patient with complicated small bowel obstruction, while in the remaining 14 patients CT findings were compatible with mild peritonitis and acute surgical intervention could be correctly avoided (table 2) [203]. Thus, the use of abdominal CT may be a promising tool for differential diagnosis in complicated cases.

Laleman et al. report the use of fluoro-deoxy-glucose positron-emission tomography (FDG-PET) in a patient with suspected FMF and abdominal pain [208]. FDG-PET demonstrated diffuse peritoneal uptake, fitting with diffuse peritoneal inflammation, but without local obstruction, appendicitis or other bowel diseases, such as Crohn's disease. The authors suggest that the use of FDG-PET during an FMF attack may be helpful for establishing the differential diagnosis, but its use is precluded by limited availability. Further research into its clinical applicability is warranted.

Treatment and management

Colchicine prophylaxis

First-line treatment of FMF is colchicine. Its clinical efficacy as prophylaxis in FMF was established thirty years ago in three small randomized controlled double-blind trials [33,34,209]. All three had a crossover design and recorded number of fever attacks as primary outcome measure. Zemer et al. [33] included 22 patients (18 of whom were men) of Sephardic Jewish origin and 13 patients were included in the final analysis. The treatment consisted of twice daily 0.5 mg colchicine or placebo for two months each, in random order. The patients had a total number of

67.5 attacks during two months of placebo versus 17.5 during colchicine (atypical attacks were counted as half; p<0.01). Eleven of 13 patients had fewer attacks on colchicine [33]. Dinarello et al. [34] used an alternative crossover design with alternating 28-day cycles of colchicine and placebo in random order in each of their 11 adult patients. Initial colchicine dose was three times daily 0.6 mg, but this dose would be reduced when gastrointestinal side-effects occurred, which in effect happened in all patients. In the final analysis, the most used dosage of colchicine was twice daily 0.6 mg. The study was terminated after 11 months when an interim analysis showed good results. The number of attacks for the total group of 11 patients was 38 in the placebo versus 7 in the colchicine courses (p<0.001). Four of 6 patients who completed the trial benefited significantly from colchicine (defined in this study as a difference of at least 5 between total number of attacks on placebo versus colchicine) [34]. Goldstein and Schwabe [209] performed a study with two 90-day periods of colchicine 0.6 mg thrice daily versus placebo. Ten of fifteen patients completed this study; 7 of these were of Armenian origin, and 8 were male. On placebo therapy the patients reported a total of 59 attacks versus 5 attacks in the colchicine period. In 7 of the 10 patients, no attacks occurred while on colchicine. No mention is made of any side-effects or dose reduction of colchicine, despite the relatively high treatment dose of 1.8 mg daily [209].

Numerous clinical observational studies have corroborated the results of the initial trials. The accumulated experience tells us that colchicine provides significant improvement in up to 90% of patients, while in 60% of patients it will prevent attacks altogether [22]. Optimal dosage should be determined in each individual patient, to gain a maximal effect with minimal side-effects (see below). General recommendation is to start colchicine in a dose of 1 mg daily (either 0.5 mg twice daily or 1 mg once a day [24]), and increase this slowly until remission is achieved, to a maximum of 2.5 mg/day.

Colchicine, fertility and children

Colchicine may affect male fertility by inducing oligospermia or azoospermia, but this is relatively rare [210]. Male FMF patients wanting to create progeniture should not be deterred from continuous use of colchicine. Colchicine does not seem to affect female fertility, frequency of miscarriage or teratogenicity [35,211], despite contrary results from in vitro studies. In fact, female fertility and outcome of pregnancy have improved in FMF patients using colchicine, due to a decreased incidence of peritoneal adhesions and of acute attacks which cause miscarriage and/or early delivery [210]. In Israel, pregnant female FMF patients on colchicine are offered amniocentesis with karyotyping [210]. However, there is no robust evidence that suggests that colchicine use throughout pregnancy carries a substantial teratogenic or mutagenic risk when used at recommended doses.Colchicine is detected in breast milk of women using the drug, but the dose ingested by the infant is less than one tenth of the therapeutic dose (per kg) in adults [212], and clinical follow-up of children whose mother continued on colchicine while breast-feeding is favourable [210]. Thus, breast feeding is not contraindicated during colchicine use. In children, long-term colchicine use has been shown to be safe and without a negative effect on growth; rather, the cessation of FMF attacks and return to health will improve growth and development [210].

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Alternatives to daily oral colchicine

Although oral colchicine remains the mainstay of treatment, alternatives to daily colchicine to suppress the periodic inflammatory attacks in colchicine-resistant patients are actively sought. Before the discovery of the benefit of colchicine, numerous other therapies were reported to be effective (e.g. estrogen therapy [213] or low-fat diet [214]) but none of these have withstood the test of time or thorough controlled investigation.

It would be desirable to have a drug available that is able to abort inflammatory attacks at the very onset of symptoms. Wright et al. [215] report this effect of intermittent use of colchine – starting at the earliest suspicion of an attack with a course of six tablets of 0.6 mg on the first day of (preliminary) symptoms, and two tablets on each of the two following days, distributed evenly over time. In a double-blind controlled study, they compared this with placebo in a total of 9 patients, and found that 21 of 28 attacks were aborted by colchicine (an aborted attack was defined by symptoms lasting less than 8 hours and absence of fever), while only 3 of 31 attacks were aborted when treated with placebo [215]. The only side-effect reported was diarrhoea.

Tunca et al. [216] published a pilot study on the use of interferon (IFN) alpha as an adjunct to daily colchicine at the onset of a fever attack, after they observed a cessation of attacks in a colchicine-resistant FMF patients during 6 months of IFN alpha treatment for his chronic hepatitis B [217]. In an open-labelled, uncontrolled study, 7 FMF patients on daily colchicine who still experienced fever attacks used IFN alpha (3 million IU s.c.) at the earliest onset of a total of 18 attacks, which led to rapid resolution of symptoms, in a median of 3 hours time [216]. A formal double-blind trial by the same investigators failed to confirm the initial positive results [218]. Calguneri et al. [219] reported use of IFN alpha for 6 months in 7 colchicine-resistant patients, in a dose of 4.5 million IU s.c. three times a week. None of the patients experienced any attack during this period, in contrast to the mean number of 26 attacks per year before start of IFN alpha.

Lidar et al. [220] have tried the addition of weekly intravenous colchicine at a dose of 1 mg to oral colchicine in an open-labelled study of 3 months in 13 patients with frequent FMF attacks despite high dose of oral colchicine. They found a significant reduction in number (mean 4.2 to 1.9 attacks per person) and severity of attacks, particularly abdominal and pleural attacks, without side effects.

However, daily use of colchicine is still recommended in all FMF patients because this is the only treatment that has been proved beneficial against development of amyloidosis, as follows below.

Colchicine and amyloidosis

Compliance to colchicine treatment should be strongly emphasized, even in the face of apparent unresponsiveness, as it has been shown to prevent the occurrence of the major long-term complication of FMF: amyloidosis. This was nicely demonstrated by Zemer et al. [221] in a follow-up study of 960 FMF patients during 4 to 11 years after they were recommended to start colchicine (1 to 2 mg daily). All patients were free of proteinuria at the onset of the follow-up. Persistent proteinuria, taken as a sign of amyloidosis, developed in only 4 of 906 patients who adhered to the prophylactic schedule while it occurred in 16 of 54 who admitted non-compliance [221]. This latter percentage exactly corresponded to the incidence of amyloidosis in a historic control group without treatment. Colchicine also seemed to stop further deterioration of renal function in 73 of another group of 86 FMF patients with proteinuria at the onset of the study. In 24 FMF patients

who had already developed nephritic syndrome or uremia, colchicine did not cause improvement, suggesting that amyloidosis had developed to far to be resolved in these patients [221]. This same group demonstrated in a subsequent study of 68 FMF patients with amyloidosis in a follow-up of 5 or more years that the therapeutic dosage of colchicine for amyloidosis in FMF is >1.5 mg/day, and that this is only effective in this first stages of amyloidosis before overt deterioration of renal function [222].

Since the introduction of colchicine, the incidence of amyloidosis in FMF has dropped dramatically. Amyloidosis was previously observed in 30-50% of FMF patients and in areas with a high prevalence of FMF, where colchicine is not routinely available, such as Armenia, amyloidosis is still common.

Management of abdominal attack

During an abdominal attack, conservative management is the mainstay. Colchicine is ineffective for the full-blown acute FMF attack. Diclofenac (75 mg administered intramuscularly) may be used for pain relief [24]. Other agents (e.g. reserpine, steroids) have only limited efficacy. Many patients become familiar with their disorder and deal with the inflammatory attacks at home, without consulting a doctor, once they know the diagnosis and its consequences.

When a FMF patient presents with an atypical course of an abdominal attack, close observation is warranted to exclude complications, such as adhesive small bowel obstruction and strangulation (see above). Elective laparoscopic appendectomy has been suggested in FMF patients to prevent unnecessary emergency surgery [223], but this has not been recommended by others, because any surgical procedure may provoke an attack and add to the formation of adhesions [24].

Other causes of abdominal pain in FMF

Colchicine toxicity

Crampy abdominal pain, diarrhoea and nausea due to hyperperistalsis are frequent side effects of colchicine [224,225]; these adverse effects are most pronounced with maximum therapeutic doses. Gastrointestinal intolerance is used in clinical practice as a parameter of dose titration and may serve as a warning to protect patients from toxic doses.

Colchicine intoxication is a rare, but serious and potentially lethal complication. In general, the severity of the reaction correlates with the dose administered and a high case-fatality rate is associated with an oral dose above 40 mg in adults, but there is considerable individual variability and lethal toxicity has been described with doses as low as 7 mg orally [224]. Risk factors for colchicine intoxication [224] are intravenous use, use of loading doses, use in elderly patients, prior maintenance colchicine use, excretory organ failure (renal or hepatic dysfunction) and drug interactions (especially cimetidine and other cytochrome P-450 inhibitors and cyclosporine [201]).

Clinical presentation of intoxication starts with a gastrointestinal phase characterized by diffuse abdominal pain, nausea, vomiting and severe (haemorrhagic) diarrhoea, which may develop into paralytic ileus. This is accompanied by volume depletion and hypotension [224,225]. From 24 to 72 hours after ingestion, multiorgan failure may develop, including bone marrow depression, renal failure, adult respiratory distress syndrome, heart failure and disseminated intravascular coagulation [224]. If the patient survives this second

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phase, recovery will set in thereafter, typically accompanied by alopecia. The hair loss is usually reversible, but permanent alopecia has been described [224].

Management of colchicine intoxication is mainly supportive and symptomatic. Because of the rapid distribution of colchicine and its high binding affinity, haemodialysis and plasma exchange are ineffective [224]. There is no specific antidote available; immunotherapy with anticolchicine antibodies is still in development.

Amyloidosis of the gastrointestinal tract

As mentioned above, type AA systemic amyloidosis is a serious complication of FMF. This type of amyloid fibrils may be deposited in many organs, including the kidneys, adrenals, intestines, spleen, liver, stomach, thyroid gland, heart and lungs [201]. Clinically, the kidneys are the most significant organs involved, as amyloidosis will eventually impair renal function and lead to kidney failure.

Microscopic evidence for amyloid depositions in the gastrointestinal tract can be found early in the course of FMF. Nevertheless, amyloidosis of the intestines will only result in clinical symptoms in a minority of patients and often only after many years of asymptomatic amyloid deposition, when the entire wall of the small intestine is affected [226]. These symptoms may include constipation or intractable diarrhoea and severe malabsorption, especially in the case of bacterial overgrowth and bile acid deconjugation due to decreased mobility of the small intestine [201]. However, it may be difficult to differentiate amyloidosis-induced diarrhoea from colchicine-induced diarrhoea [201].

Another possible manifestation of amyloidosis in the intestinal tract is ischemic enterocolitis [227,228], which may result in fibrosis, mucosal ulceration or colonic obstruction or perforation. Clinical, radiological and histopathological features of amyloid-associated ischemic enterocolitis may mimic those of Crohn's disease, except for the absence of transmural lymphoid aggregates and granulomas [228,229]. Spontaneous rupture of an amyloid spleen, resulting in acute abdomen, as is sometimes described in AL type amyloidosis [230], is rarely seen in AA type amyloidosis [231], and has never been described in FMF.

In recent years, the incidence of clinical manifestations of extra-renal amyloidosis is increasing and associated with longer survival in patients with renal amyloidosis due to hemodialysis and renal transplantation [201], although the overall incidence of amyloidosis has declined due to the use of colchicine. It is not yet known whether colchicine also has a beneficial effect on gastrointestinal amyloidosis, as is seen in renal amyloidosis. Often, the dose of colchicine optimal for anti-amyloidogenic effect (2 mg per day) cannot be tolerated by patients with gastrointestinal amyloidosis, which aggravates the diarrhoea.

Diagnosis of amyloidosis requires tissue biopsy, preferentially of the involved organ (usually rectal biopsy or renal biopsy); an alternative to obtain tissue is fine-needle aspiration of abdominal fat pad, although one small study suggests that this might not be very sensitive in FMF [232] The tissue sample is then stained with Congo Red to detect amyloid deposits. Further classification of the type of amyloid can be done by immunohistochemistry with specific antibodies [233].

Disorders associated with FMF and accompanied by abdominal pain

Several inflammatory diseases have been found in association with FMF, some of which may be accompanied by abdominal pain. Such associations may complicate clinical interpretation of the symptoms and may thwarth diagnosis.

An increased prevalence of inflammatory bowel disease in FMF was reported in two studies. Cattan et al. [234] described 3 patients with concomitant FMF and either Crohn's disease or ulcerative colitis in a group of 300 FMF patients from 173 families of non-Ashkenazi Jewish descent; eight other persons in these families studied had inflammatory bowel disease without FMF. Crohn's disease was diagnosed in 7 of nearly 5000 FMF-patients from the FMF-registry in Tel Hashomer, Israel [235]. While Cattan et al. [234] found that the patients with inflammatory bowel disease in the FMF families were more severely affected, Fidder et al. [235] report similar severity on comparison with a Crohn's disease control group, although the prevalence of amyloidosis was increased. This association of Crohn's disease with FMF is interesting in the light of the recent findings of *NOD2* gene mutations as a susceptibility factor in Crohn's disease. The NOD2 (or CARD15) protein belongs to a family of proteins involved in inflammation and apoptosis and is (indirectly) related to the pyrin domain family. The defects underlying Crohn's disease and FMF are thus thought to be located in closely related areas of inflammation and might have a modifying effect on each other. It remains to be seen whether FMF patients with concomitant inflammatory bowel disease do have specific NOD2 gene mutations. However, questions might be raised about the diagnosis of inflammatory bowel disease in some FMF patients, in the light of the similarity between ischemic enterocolitis due to amyloidosis and Crohn's disease [228] and based on the fact that not all FMF-cases were confirmed by demonstration of transmural granulomas and lymphoid aggregates in biopsies [234,235], while proteinuria due to amyloidosis was more prevalent in this group.

A number of types of vasculitis, which may be accompanied by abdominal symptoms, have been associated with FMF as well [201,236], although it could be questioned in some cases, whether vasculitis is a manifestation of FMF, rather than an associated disorder.

Behçet's disease (found in 16 of 4000 FMF patients [237]) is an episodic inflammatory disorder of unknown pathogenesis, with a broad clinical picture that may include oral and genital aphthous ulcers, pustulosis, erythema nodosum, arthritis, central nervous system involvement and pathergy (hyperreactive skin lesion evoked by a needle prick) [238]. Abdominal pain and diarrhea are common as well; this type of abdominal pain is usually more prolonged than the short abdominal attacks seen in FMF. A second type of vasculitis, reported more frequently in FMF patients, is Henoch-Schönlein´s purpura (4-7% of FMF patients [23,239,239]) often in a severe form; gastrointestinal symptoms in Henoch-Schönlein´s purpura may include colicky abdominal pain, nausea, vomiting, diarrhoea or constipation and frequently stools with blood or mucus are observed [201]. Thirdly, the systemic necrotizing vasculitis, polyarteritis nodosa (PAN), is found in 1% of FMF patients [239,240] and may lead to abdominal symptoms ranging from vague non-specific pain to bowel infarction and perforation due to ischemia [201].

Three case reports have been published on patients who developed peritoneal mesothelioma after years of abdominal FMF attacks [241-243]. The development of malignancy was suggested to be linked to the chronic recurrent peritonitis, but questioned by others [201].

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Abdominal pain in other autoinflammatory syndromes

FMF is part of an expanding group of hereditary disorders known as familial autoinflammatory syndromes, which are commonly characterized by recurrent episodes of fever and inflammation [4]. Abdominal pain and other signs of abdominal distress are also frequently seen in the TNF-receptor associated periodic syndrome (TRAPS) [54] and the hyper-IgD and periodic fever syndrome (HIDS) [46].

In HIDS, the clinical presentation of abdominal pain is usually less dramatic, and often the pain is more crampy than fitting with full-blown serositis. Other frequent symptoms and signs are (cervical) lymphadenopathy, headache, myalgia, arthralgia and a erythematous skin rash [46]. The fever episodes generally last somewhat longer than in FMF, usually 4 to 6 days, and they start within the first year of life [4]. Characteristically, a continuously high concentration of immunoglobulin D (IgD) and IgA is detected in serum. Inheritance pattern of HIDS is autosomal recessive. HIDS is caused by mutations in the gene encoding for mevalonate kinase, an enzyme in the isoprenoid pathway [2,3].

TRAPS is usually distinguished by inflammatory episodes of longer duration; attacks of 14 to 21 days are often seen, although episodes of a few days may occur [58,60]. Apart from abdominal distress, the high spiking fever during such episodes may be accompanied by myalgia, arthralgia, migratory erythematous skin lesions and ocular involvement, including conjunctivitis and periorbital edema [4]. The genetic mutations in TRAPS are found in the gene encoding the type 1 TNF-receptor [7].; it is autosomal dominantly inherited

Unnecessary laparotomies, intraperitoneal adhesions and small bowel obstruction are complications of both HIDS and TRAPS, as seen in FMF. Abdominal pain is more rarely reported in the clinical spectrum of cryopyrin-associated periodic syndromes, which are characterized by joint or skin manifestations, among other features [66,70,73].

Summary

Familial Mediterranean fever is a hereditary syndrome characterized by recurrent episodes of fever and serositis, resulting in pain in the abdomen, chest, joints and muscles. It is primarily found in people of Jewish, Arabic, Turkish and Armenian ancestry. Causative mutations are located in the *MEFV* gene, which encodes for the protein pyrin.

Abdominal FMF attacks present clinically as an 'acute abdomen' with severe abdominal pain and rigidity, which resolve spontaneously within 3-4 days. It is important to distinguish these attacks from small bowel obstruction due to adhesions, a serious complication of FMF. In the case of a FMF attack, surgery will be of no help and will only increase the risk of adhesions, while in adhesive small bowel obstruction surgical intervention should not be delayed in order to prevent life-threatening bowel strangulation. Distinction can be made on close observation of the clinical course, while abdominal imaging by CT may be helpful. FMF is effectively treated by use of oral colchicine in more than 90% of patients. However, abdominal pain and diarrhoea are frequently observed side-effects of colchicine use, and may also represent the first phase of colchicine intoxication. Abdominal pain in FMF patients may also be caused by one of the associated types of vasculitis, such as Behçet's disease or Henoch Schönlein´s purpura.

Recurrent episodes of fever and abdominal pain are also seen in other autoinflammatory syndromes, especially TNF-receptor associated periodic syndrome and hyper-IgD syndrome.

Practice points

- Abdominal attacks in FMF are characterized by signs of 'acute abdomen', but will resolve spontaneously within 3-4 days.
- FMF is a hereditary syndrome characterized by lifelong recurrent episodes of fever and serositis (mainly peritonitis).
- FMF is especially prevalent in people from the Mediterranean, including Sephardic Jews, Arabs, Turks, and Armenians.
- Diagnosis of FMF is based on a set of clinical criteria, and may be confirmed by DNA analysis of the *MEFV* gene.
- Treatment of FMF consists of colchicine.
- The recurrent peritonitis may lead to peritoneal adhesions and small bowel obstruction
- It is important to differentiate between small bowel obstruction and the abdominal FMF attack.
- The first phase of potentially life-threatening colchicine intoxication is characterized by diffuse abdominal pain, nausea, vomiting and (haemorrhagic) diarrhoea.

Research agenda

- The exact function of pyrin, the mutated protein in FMF, needs to be determined.
- Detailed controlled studies are necessary to define the best method of pain relief in FMF.
- The precise mechanism of action of colchicine needs to be determined.
- Further development of a diagnostic tool to distinguish an abdominal FMF attack from other causes of 'acute abdomen' is warranted.
- Other therapies to prevent and combat inflammatory attacks and to prevent amyloidosis should be sought for.

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General summary

The subject of this thesis is a group of rare hereditary disorders, collectively called auto-inflammatory syndromes, with a specific focus on the hyper-IgD and periodic fever syndrome (HIDS). The autoinflammatory disorders are characterized by lifelong recurrent episodes with fever, usually accompanied by other inflammatory symptoms such as abdominal pain, diarrhoea, rash or arthralgia. The inflammatory episodes are separated by symptom-free intervals.

In **chapter 1** we describe the clinical characteristics of these syndromes in detail. Diagnosis of an autoinflammatory syndrome needs to be considered in a patient with a history of years of inflammatory attacks with symptom-free intervals in between. The inheritance pattern along with specific hallmarks will usually point to the right diagnosis.

The discovery of the causative genes has had an enormous impact in the field of periodic fevers. Earlier investigations had shown that mutations in mevalonate kinase (*MVK*) gene cause HIDS. **Chapter 2** reports our investigations into the genotype in a series of HIDS patients and offers a much broader view as we detected 11 different missense mutations, one deletion and the absence of expression of one allele. The large majority of the patients were compound heterozygotes for two mutations. Of these, V377I is the most common mutation, followed by I268T. On the basis of this study, we propose that the diagnostic screen of the *MVK* gene in HIDS should be first directed on V377I and I268T mutations.

In the study described in **chapter 3**, we wanted to ascertain the role of *MVK* mutations and usefulness of molecular diagnosis in HIDS in a cross-sectional study of 54 patients. Mutation analysis revealed mutations in the *MVK* gene in 41 patients (subsequently designated as "classic type HIDS"), while in 13 of the 54 patients no mutation could be detected even though they did exhibit the clinical phenotype of HIDS and completely fulfilled the clinical criteria ("variant type HIDS"). Classic type HIDS patients had lower mevalonate kinase enzyme activities, higher IgD concentrations, and more additional symptoms with attacks. We propose to diagnose HIDS using clinical criteria; these patients can then be divided into classic type and variant type HIDS patients by genetic analysis. The classic type HIDS patients are a relatively homogeneous group with a predictable inheritance pattern and clinical presentation, which facilitates the counselling of the individual patient. The group of variant type HIDS patients is more heterogeneous and may represent several different as-yet-undefined disorders.

Both mevalonic aciduria, characterized by psychomotor retardation, cerebellar ataxia, recurrent fever attacks and death in early childhood, and HIDS, with recurrent fever attacks without neurological symptoms, are caused by a functional deficiency of mevalonate kinase (MK). In a systematic review of known MK deficient patients (**chapter 4**), we identified 5 adults with phenotypical overlap between these two syndromes, which argues for a continuous spectrum of disease. One explanation for the varying degree in severity might be the genotype involved, but this cannot be the whole answer. Genetic trans-acting or environmental factors will have to be considered to explain the phenotypic variability.

The aim of the study in **chapter 5** was to further define the acute phase response in the inflammatory attacks in classical HIDS patients, primarily to try to discriminate between (serious bacterial) infection and non-infectious inflammation of a HIDS attack. One of the proteins of interest was procalcitonin, which reaches high serum concentrations during infection. In only 2 of 22 HIDS patients, procalcitonin concentration rose above 2 ng/mL during fever attack, compatible with the non-infectious nature of these attacks. Thus the combination of high CRP concentration plus procalcitonin concentration <2 ng/mL in a symptomatic HIDS patient may indicate a febrile attack without (bacterial) infection; although this observation warrants further investigation for its usefulness as a marker in clinical practice.

In **chapter 6**, we asked ourselves the question why classic type HIDS families, especially those carrying a V377I mutation in the *MVK* gene, are mainly from the Netherlands. Through an extensive haplotype study, we established that this V377I mutation, originates from a common ancestral haplotype. Data suggest that a founder of this haplotype came from the Netherlands, and that the mutation was spread from there to the rest of the world.

Chapter 7 examines the effect of an excessive amount of mevalonic acid, the substrate for mevalonate kinase, on isolated peripheral blood mononuclear cells. This study shows that an excess amount of mevalonic acid exhibits a pro-inflammatory effect, most pronounced in terms of IL-6 secretion. The effect is primarily mediated via IL-1 as it can be completely blocked by addition of IL-1ra. This may be central to the pathogenesis of HIDS.

The next two chapters describe two clinical trials in HIDS.
In **chapter 8**, six classic type HIDS patients who had at least one febrile attack every 6 weeks, entered a randomized, double-blind, placebo-controlled crossover trial to explore the efficacy of a daily 200-mg thalidomide dose in the treatment of recurrent febrile attacks. The effect of thalidomide, a drug known to inhibit production of several cytokines, in HIDS was limited to a slight decrease in acute phase protein concentration without an effect on the attack rate. Next, we explore the effect of simvastatin, an inhibitor of HMG-CoA reductase, one enzyme step prior to MK in **chapter 9**. Six classic type HIDS patients were enrolled a double-blind clinical trial consisting of two treatment periods with either simvastatin 80 mg daily or placebo for 24 weeks, separated by a 4-week washout period. Simvastatin resulted in a drop in urinary mevalonic acid concentration in all patients and decreased the number of febrile days in five out of six patients. No side effects were observed. These data suggest that simvastatin improves inflammatory attacks in the hyper-IgD syndrome. This also highlights the anti-inflammatory properties of HMG-CoA reductase inhibition.

Apart from a common phenotype of lifelong recurrent inflammatory attacks, all of the hereditary autoinflammatory syndromes have distinct features and specific therapeutic options, increasing the need for a specific diagnosis in each case. In **chapter 10**, we examined whether genetic screening would allow classification of previously unclassified patients. This yields only one possible diagnosis of FMF in the 60 previously unclassified patients. Thus, screening of known genes involved in these disorders does not result in significant additional information. Differential

diagnosis of hereditary autoinflammatory syndromes is possible with thorough clinical examination followed by targeted genetic analysis of one or two most likely syndromes.

In the next three chapters, we take a closer look at TRAPS.
In **chapter 11**, we describe two Dutch TRAPS patients suffering from recurrent fever attacks who went undiagnosed for more than 40 years. The use of a short course of etanercept, a recombinant p75-TNFR:Fc fusion protein, at the time of attack consistently induced prolonged remission in one of these patients. This suggests that it is not necessary to subject TRAPS patients to continuous twice weekly etanercept injections, but that a short course at time of attack is sufficient.
Another TRAPS patient is described in **chapter 12.** This patient developed the most dreaded complication of TRAPS, systemic amyloidosis, leading to renal failure. She experienced a cessation of inflammatory attacks, which coincided with the deterioration of her renal function. We demonstrated elevated plasma concentrations of soluble TNF receptor, which is cleared from the body by the kidneys. It is our impression that this increased soluble TNF receptor concentration protects her from further attacks of fever.
Chapter 13 reports the beneficial response of a young patient with a severe, treatment-resistant form of TRAPS to the recombinant IL-1ra protein Anakinra. It is generally assumed that in TRAPS the inflammatory effects of TNFα and lymphotoxin (LT) are not countered adequately, because of a lack of soluble TNF-receptor, the major inhibitor of TNF. In that light it is difficult to explain the effectiveness of IL-1ra, since IL-1 is responsible for only a small part of the biological effects of TNFα and LT. Further research is required to elucidate the enigmatic role of IL-1 even in TRAPS.

Lastly, we turn to FMF in **chapter 14**. Abdominal FMF attacks present clinically as an 'acute abdomen' with severe abdominal pain and rigidity, which resolve spontaneously within 3-4 days. It is important to distinguish these attacks from small bowel obstruction due to adhesions, a potentially life-threatening complication of FMF. Distinction can be made by close observation of the clinical course, while abdominal imaging by CT may be helpful.

Future perspectives

The results described in chapter 7, on the role of mevalonic acid and IL-1 in HIDS, have left a number of questions unanswered concerning the pathogenesis of this disorder. Current evidence points to toxicity of MVA rather than shortage of an isoprenoid end-product. How does mevalonic acid exert its pro-inflammatory effect – through its influence on the isoprenoid pathway, or through some more direct effect? How can the pathogenesis of HIDS be linked to that of the other hereditary autoinflammatory syndromes – in other words, where does the inflammasome, thought to be central in the pathogenesis of FMF and cryopyrin-associated periodic syndromes, fit in? Another field which is likely to yield interesting results for this group of disorders is (regulation of) apoptosis.
Unravelling of the pathogenesis of HIDS will continue to provide suggestions for possible new treatment options. Simvastatin is successful in reducing the number of days of illness in HIDS, as reported in chapter 9, though it is not a complete cure. It will especially be of interest to find a treatment which can be offered at

time of attack to curtail the inflammatory symptoms, and which could if necessary be used in addition to continuous prophylactic treatment with simvastatin. Taking into account the beneficial report on the TNF-inhibitor etanercept in two young HIDS patients [172], this will be a promising option to investigate. The results of chapter 7 suggest that it will be worthwhile to investigate the effect of IL-1ra (Anakinra) in HIDS.

The discovery of causative genes, treatment options and research into pathogenesis, as also described in this thesis, has been made possible only because of the accurate phenotypical characterisation of patients with periodic fever. Careful analysis and proper clustering of these patients is indispensable in order to allow the elucidation of the genetic background as well as the evaluation of possible treatment options. This is greatly stimulated by development of central periodic fever registries, such as the International HIDS registry in Nijmegen (see website hids.net), which also afford the opportunity to appreciate previously unrecognised symptoms. Even yet, despite recent research, many periodic fever patients do not fall in one of the above-mentioned disease categories. This represents a great challenge. It is to be expected that in the future other periodic fever syndromes and corresponding genes will be discovered.

Up till now, the International HIDS registry mainly contains data gathered at the time patients are included in the database, which allows us to do cross-sectional studies. It is our plan to try and extend this in the near future with follow-up data, to enable us to study natural course and prognosis of HIDS. Such information has not been obtained in any systematic way so far. One of the interesting aspects of the prognosis of HIDS is the virtual absence of systemic amyloidosis as a complication of this chronic recurrent inflammatory disorder; research in this field is likely to yield valuable information on the pathogenesis of systemic amyloidosis in general.

Even after twenty years, the role of IgD in HIDS remains obscure (see also chapter 5), and IgD is still the orphan among the immunoglobulins. Not all patients that have MVK gene mutations and a diminished MK activity will have a high serum IgD, while IgD concentration may be (moderately) high in other disorders. This had led some researchers to suggest that the name HIDS (Hyper-IgD and periodic fever syndrome) is not appropriate anymore to describe this hereditary autoinflammatory disorder, because it puts too much emphasis on IgD. While this may be true, we have to point out that this name is well anchored in the medical literature and enjoys wide recognition. Given the fact that the exact pathogenesis of HIDS is still unknown, we feel that suggestions for a change of name are rather premature at this time.

Nederlandse samenvatting

Het onderwerp van dit proefschrift is een groep zeldzame erfelijke aandoeningen die autoinflammatoire syndromen genoemd worden, met speciale aandacht voor het hyper-IgD en periodieke koorts syndroom (HIDS). De autoinflammatoire syndromen worden gekenmerkt door levenslang terugkerende episoden met koorts, meestal vergezeld van andere symptomen van ontsteking zoals buikpijn, diarrhee, huiduitslag of arthralgie. Deze perioden met ontsteking worden afgewisseld door een symptoom-vrij interval.

In **hoofdstuk 1** beschrijven we de klinische kenmerken van deze syndromen. De diagnose van een autoinflammatoir syndroom dient te worden overwogen bij een patiënt met een voorgeschiedenis van jarenlange ontstekingsaanvallen afgewisseld met perioden zonder symptomen. Het overervingspatroon in combinatie met een aantal specifieke kenmerken geeft meestal voldoende aanwijzingen om tot de juiste diagnose te komen.

De ontdekking van de betrokken genen heeft een enorme invloed gehad op het onderzoek naar periodieke koorts. Eerder onderzoek had uitgewezen dat HIDS wordt veroorzaakt door mutaties in het mevalonaat kinase (*MVK*) gen. **Hoofdstuk 2** vermeldt de resultaten van ons onderzoek naar het genotype in een groep HIDS patiënten. De meerderheid van de patiënten was samengesteld heterozygoot voor twee mutaties. V377I is de meest voorkomende mutatie, gevolgd door I268T. Op basis van dit onderzoek raden wij aan bij een diagnostisch onderzoek van het *MVK* gen in HIDS eerst te kijken naar V377I en I268T mutaties.

Het onderzoek beschreven in **hoofdstuk 3** had als doel de rol vast te stellen van *MVK* mutaties en het nut van DNA-diagnostiek in HIDS. In een groep van 54 patiënten werden mutaties in het *MVK* gen gevonden bij 41 patiënten (hierna aangeduid als "klassieke type HIDS"), terwijl in de 13 andere patiënten geen mutaties gevonden werden hoewel ze voldeden aan de klinische criteria voor HIDS ("variant type HIDS"). Klassieke type HIDS patiënten hadden minder mevalonaat kinase enzymactiviteit, hogere concentraties van IgD, en meer bijkomende symptomen tijdens een koortsaanval. Het is ons voorstel om de diagnose HIDS te stellen op basis van klinische criteria; deze patiënten kunnen dan onderverdeeld worden in klassieke type en variant type HIDS door genetisch onderzoek. De klassieke type HIDS patiënten vormen een relatief homogene groep met een bekend overervingspatroon en klinische presentatie – dit kan helpen in de begeleiding van de individuele patiënt. De groep van variant type HIDS patiënten is veel diverser en het is goed mogelijk dat deze groep bestaat uit patiënten die in feite leiden aan andere tot op heden nog-niet-beschreven autoinflammatoire aandoeningen.

Zowel klassieke mevalonacidurie, gekenmerkt door psychomotore retardatie, cerebellaire ataxie, periodieke koortsaanvallen en overlijden op jonge leeftijd, als HIDS, met periodieke koortsaanvallen zonder neurologische symptomen, wordt veroorzaakt door een functionele deficiëntie van mevalonaat kinase (MK). Na systematissche evaluatie van bekende MK-deficiënte patiënten (**hoofdstuk 4**) konden we 5 volwassen patiënten identificeren met een overlappend fenotype van deze twee syndromen. Dit pleit voor een continu spectrum van ziekte. De variatie in ernst zou deels veroorzaakt kunnen worden door het specifieke onderliggende genotype, maar dit kan niet alles verklaren. Om deze fenotypische variabiliteit

volledig te verklaren zal gekeken moeten worden naar "genetic trans-acting" en/of naar omgevingsfactoren.

Het doel van het onderzoek in **hoofdstuk 5** was het nader beschrijven van de acute fase respons tijdens de ontstekingsaanvallen in klassieke type HIDS patiënten, met name om te proberen het onderscheid te maken tussen (ernstige bacteriële) infectie en de niet-infectieuze ontsteking van een HIDS-aanval. Hierbij hebben we onder andere gekeken naar het eiwit procalcitonine, dat tijdens infectie een hoge serum concentratie bereikt. In slechts 2 van 22 HIDS patiënten kwam de procalcitonine concentratie boven de 2 ng/mL tijdens een koortsaanval, wat past bij het niet-infectieuze karakter van deze aanvallen. De combinatie van een hoge CRP-concentratie met een procalcitonine concentratie <2 ng/mL in een symptomatische HIDS-patiënt zou dus kunnen wijzen op een koortsaanval zonder (bacteriële) infectie. Nader klinisch onderzoek zou moeten uitwijzen of dit bij de begeleiding van individuele patiënten van nut zou kunnen zijn.

In **hoofdstuk 6** vroegen we onszelf af waarom klassieke type HIDS families, met name diegenen met een V377I mutatie in het *MVK* gen, vooral afkomstig zijn uit Nederland. Met behulp van een uitgebreid haplotype onderzoek hebben we kunnen vaststellen dat deze V377I mutatie deel uitmaakt van een gemeenschappelijk voorouderlijk haplotype. Onze data suggereren dat de grondlegger van dit haplotype afkomstig was uit Nederland, en dat de mutatie zich hiervandaan verspreid heeft over de rest van de wereld.

Hoofdstuk 7 onderzoekt het effect van een overmatige hoeveelheid mevalonzuur, het substraat van mevalonaat kinase, op geïsoleerde mononucleaire cellen uit perifeer bloed. Dit onderzoek laat zien dat zo'n overmatige hoeveelheid mevalonzuur een pro-inflammatoir effect heeft, met name in de vorm van IL-6 secretie. Het effect wordt gemedieerd door IL-1, want het kan volledig geblokkeerd worden door toevoeging van de IL-1 antagonist IL-1ra. Dit effect zou een centrale rol kunnen spelen in de pathogenese van HIDS.

De volgende twee hoofdstukken beschrijven twee klinische trials in HIDS.
In **hoofdstuk 8** hebben zes klassiek type HIDS patiënten met tenminste één koortsaanval iedere zes weken deelgenomen aan een gerandomiseerd, dubbelblind, placebo-gecontroleerd cross-over onderzoek om het effect van een dagelijkse dosis van 200 mg thalidomide op de periodieke koortsaanvallen te bestuderen. Het effect van thalidomide, een geneesmiddel waarvan bekend is dat het de productie van verscheidene cytokinen remt, bleef beperkt tot een lichte daling van de concentratie van acute fase eiwitten zonder effect op de duur, ernst of frequentie van de koortsaanvallen.
Aansluitend bestudeerden we het effect van simvastatine, een remmer van HMG-CoA reductase, één enzymstap voor mevalonaat kinase, zoals beschreven in **hoofdstuk 9**. Zes klassiek type HIDS patiënten werden geïncludeerd in een dubbelblind klinisch onderzoek bestaande uit twee behandelperiodes met simvastatine 80 mg per dag of placebo gedurende 24 weken, gescheiden door een uitwasperiode van 4 weken. Simvastatine gaf een daling van urine concentraties mevalonzuur in alle patiënten en verminderde het aantal dagen koorts in vijf van de zes patiënten. Er werden geen bijwerkingen waargenomen. Deze resultaten laten zien dat simvastatine een gunstig effect heeft op de koortsaanvallen in HIDS.

Ze benadrukken daarnaast nog eens de anti-inflammatoire eigenschappen van remming van HMG-CoA reductase.

Ook al hebben alle erfelijke autoinflammatoire syndromen een gemeenschappelijk fenotype van levenslang terugkerende aanvallen van ontsteking, toch hebben ze onderscheidende kenmerken en specifieke therapeutische opties, wat het vinden van de specifieke diagnose in elke patiënt steeds belangrijker maakt. In **hoofdstuk 10** hebben we onderzocht of genetische screening nadere classificatie van voorheen ongeclassificeerde periodieke koorts patiënten mogelijk zou maken. Dit leverde slechts één mogelijke diagnose van FMF op in een groep van 60 voorheen ongeclassificeerde patiënten. Screening van alle bekende genen die betrokken zijn bij deze aandoeningen levert dus geen significante extra informatie op. Differentiële diagnostiek van de erfelijke autoinflammatoire syndromen is mogelijk door grondig klinisch onderzoek gevolgd door gericht genetisch onderzoek van één of twee van de meest waarschijnlijke syndromen.

In de volgende drie hoofdstukken wordt meer aandacht besteed aan TRAPS.
In **hoofdstuk 11** beschrijven we twee Nederlandse TRAPS-patiënten met periodieke koortsaanvallen waarvoor gedurende meer dan 40 jaar geen diagnose gesteld kon worden. Toediening van een korte kuur met etanercept, een recombinant p75-TNFR:Fc fusie-eiwit, op het moment van een aanval resulteerde in een langdurige remissie in één van deze patiënten. Dit wijst erop dat het niet nodig is om TRAPS-patiënten continu te behandelen met etanercept injecties, omdat een korte kuur op het moment van een aanval voldoende is.
Een andere TRAPS-patiënt wordt beschreven in **hoofdstuk 12**. Deze patiënt ontwikkelde de meest gevreesde complicatie van TRAPS, systemische amyloidose, leidend tot nierfalen. Op het moment dat haar nierfunctie ernstig achteruitging stopten haar koortsaanvallen. We vonden een verhoogde plasma concentratie van de oplosbare TNF-receptor, die normaal gesproken door de nieren geklaard wordt. Het is onze indruk dat deze verhoogde oplosbare TNF-receptor concentratie haar beschermt tegen de koortsaanvallen.
Hoofdstuk 13 rapporteert de goede respons op het recombinante IL-1ra eiwit Anakinra in een jonge patiënte met een ernstige vorm van TRAPS, die tot die tijd nauwelijks te behandelen was. De geldende hypothese over de pathogenese van TRAPS luidt dat het inflammatoire effect van TNFα en lymphotoxine (LT) niet voldoende wordt afgeremd door een tekort aan oplosbare TNF-receptor, de voornaamste remmer van TNF. Bezien in dat licht is het moeilijk om de effectiviteit van IL-1ra te verklaren, omdat IL-1 slechts verantwoordelijk is voor een klein deel van de biologische effecten van TNFα en LT. Meer onderzoek is nodig om de enigmatische rol van IL-1, zelfs in TRAPS, op te helderen.

Als laatste besteden we aandacht aan FMF in **hoofdstuk 14.** Abdominale FMF aanvallen presenteren zich klinisch als een 'acute buik' met ernstige buikpijn en "défense musculaire", die binnen 3-4 dagen spontaan geneest. Het is belangrijk om deze aanvallen te kunnen onderscheiden van dunne darmileus ten gevolge van adhesies, een potentieel levensbedreigende complicatie van FMF. Dit onderscheid kan gemaakt worden door nauwgezette observatie van het klinisch beloop, terwijl een CT-scan van het abdomen hierbij behulpzaam kan zijn.

Toekomst-perspectieven

De resultaten beschreven in hoofdstuk 7, over de rol van mevalonzuur en IL-1 in HIDS, laten een aantal vragen over de pathogenese van deze aandoening onbeantwoord. Deze resultaten wijzen op een toxiciteit van mevalonzuur, in plaats van tekort aan een isoprenoid eindproduct. Op welke manier geeft mevalonzuur dit pro-inflammatoire effect – door beïnvloeding van het isoprenoid metabolisme, of op een meer directe manier? Hoe kan de pathogenese van HIDS gerelateerd worden aan dat van de andere erfelijke autoinflammatoire syndromen – anders gezegd, wat is de plaats van het inflammasoom, dat zo centraal lijkt te staan in de pathogenese van FMF en cryopyrine-geassocieerde syndromen? Een ander onderzoeksterrein waarvan interessante resultaten te verwachten zijn voor deze groep aandoeningen is (regulatie van) apoptose.

Het ophelderen van de pathogenese van HIDS zal steeds nieuwe suggesties voor mogelijke behandeling opleveren. Simvastatine vermindert het aantal ziektedagen in HIDS (hoofdstuk 9), hoewel het niet leidt tot complete genezing. Het is met name interessant om een therapie te ontwikkelen die op het moment van een aanval de ontstekingssymptomen kan afremmen, en die zo nodig naast de continue profylactische behandeling met simvastatine gebruikt kan worden. Gelet op de gunstige resultaten van de TNF-remmer etanercept in twee jonge HIDS patiënten [172], lijkt dit een veelbelovende optie voor nader onderzoek. Naar aanleiding van de resultaten beschreven in hoofdstuk 7 is het ook van belang om het effect van IL-1ra (Anakinra) in HIDS te bestuderen.

De ontdekking van de betrokken genen, therapeutische mogelijkheden en onderzoek naar de pathogenese, zoals ook beschreven in dit proefschrift, is slechts mogelijk geworden door de accurate fenotypische beschrijving van patiënten met periodieke koorts. Nauwkeurige analyse en de juiste groepering van deze patiënten is onmisbaar om onderzoek zowel naar de genetische achtergrond als naar behandeling mogelijk te maken. Dit wordt sterk bevorderd door de ontwikkeling van centrale periodieke koorts registers, zoals het Internationale HIDS Register in Nijmegen (zie de website hids.net), waardoor ook eerder niet onderkende symptomen ontdekt kunnen worden. Veel periodieke koorts patiënten vallen nog steeds niet in een van de bekende categorieën. Dit is een grote uitdaging. Het is de verwachting dat in de toekomst meer periodieke koorts syndromen en bijbehorende gen-defecten ontdekt zullen worden.

Tot nu toe bevat het Internationale HIDS register met name gegevens verzameld op het moment dat patiënten geïncludeerd worden in de database. Dit maakt het mogelijk om dwarsdoorsnede onderzoek te doen. We zijn van plan om dit in de nabije toekomst aan te vullen met follow-up data, zodat we meer te weten komen over het natuurlijke beloop en de prognose van HIDS. Zulke informatie is tot op heden nog niet op een systematische manier verzameld. Eén van de interessante aspecten van de prognose van HIDS is de extreme zeldzaamheid van systemische amyloidose als een complicatie van deze chronische ontstekingsziekte; onderzoek op dit gebied zal naar alle waarschijnlijkheid waardevolle informatie opleveren over de pathogenese van systemische amyloidose in het algemeen.

Zelfs 20 jaar na de eerste beschrijving van HIDS is de rol van IgD nog steeds onduidelijk (zie ook hoofdstuk 5), en IgD is nog steeds het verwaarloosde broertje onder de immunoglobulines. Niet alle patiënten met MVK gen mutaties en een verminderde MK activiteit hebben een hoge serum IgD concentratie, terwijl de IgD concentratie ook in andere aandoeningen (licht) verhoogd kan zijn. Daarom

wordt door sommige onderzoekers gesuggereerd dat de naam HIDS (Hyper-IgD en periodieke koorts syndroom) niet langer geschikt is om te gebruiken voor deze erfelijke auto-inflammatoire aandoening, omdat het te veel de nadruk legt op IgD. Echter, HIDS is een gevestigde naam in de medische literatuur en aangezien de exacte pathogenese van HIDS nog steeds onbekend is, zijn wij van mening dat een naamsverandering op dit moment alleen tot meer verwarring zal leiden, hetgeen het ziektebeeld en de patiënten niet ten goede komt.

Samenvatting voor leken

Dit proefschrift gaat over autoinflammatoire ziekten en dan met name over de zeldzame ziekte HIDS. Alle onderzoeken die we de afgelopen jaren in het UMC St. Radboud te Nijmegen hebben verricht komen aan bod.

Autoinflammatoire ziekten zijn zeldzame erfelijke aandoeningen, die gekenmerkt worden door steeds terugkerende perioden met koorts en symptomen van ontsteking. Bij deze ziekten lijkt het alsof er regelmatig vanzelf (="auto") een ontstekingsreactie (="inflammatie") ontstaat die zich richt tegen het eigen lichaam. Er lijkt een defect te zijn waardoor ontstekingsreacties niet goed afgeremd kunnen worden.

Het lichaam heeft een ingewikkeld ontstekings- en afweerapparaat, waarvan we nog lang niet alle details kennen. Het wordt onder andere gebruikt om stoffen die niet in het lichaam thuis horen snel weg te werken. Denk bijvoorbeeld aan een splinter in je vinger: als die blijft zitten ontstaat vaak na korte tijd een ontsteking op die plek om de splinter weg te krijgen. Dat merk je omdat de plek warm en rood wordt, en gaat kloppen. Als het nog erger wordt, kun je er zelfs koorts bij krijgen. Maar als de splinter er eenmaal uitgevallen is, is de ontsteking niet meer nodig en alleen nog maar hinderlijk. Het lichaam heeft daarom ook de mogelijkheid om een ontsteking weer stop te zetten als deze niet meer nodig is. Het lichaam is normaal zo goed ingesteld dat het de meeste dreigende problemen, zoals een wondje of een bacterie, kan oplossen met behulp van een kleine ontsteking, waar je zelf niet eens iets van merkt. Dit gebeurt vrijwel dagelijks. Pas als het probleem te groot is om zomaar op te lossen ga je het merken: je krijgt dan bijvoorbeeld verkoudheidsverschijnselen als je een virus oploopt of diarree na het eten van besmet voedsel.

Schijnbaar wordt een ontstekingsreactie bij iemand met een autoinflammatoire ziekte zoals bijvoorbeeld HIDS niet voldoende afgeremd. Daardoor kan een ontsteking veel makkelijker uit de hand lopen. Pas na een langere periode dooft de ontstekingsreactie uit.

Het hyper-IgD syndroom, ook wel HIDS genoemd, is een ziekte die gepaard gaat met steeds terugkerende perioden met hoge koorts met daarbij andere ontstekingsverschijnselen, zoals opgezette lymfklieren, vermoeidheid, hoofdpijn, spierpijn en buikpijn. Zo'n koortsaanval duurt meestal een paar dagen, om dan weer spontaan te verdwijnen. In het bloed wordt een hoge concentratie van een bepaald eiwit, het IgD, gevonden. (zie voor meer uitleg de website hids.net).

In 1999 is ontdekt wáár precies in de erfelijke informatie de mutatie zit bij patiënten met HIDS. Het blijkt te gaan om het stuk DNA (=gen) dat informatie bevat voor het maken van één bepaald eiwit, dat mevalonaat kinase genoemd wordt. Mevalonaat kinase speelt een centrale rol in een aantal processen in de cel, waarbij onder andere cholesterol gevormd wordt. Bij HIDS werkt dit mevalonaat kinase niet goed. Hoe dit dan leidt tot de koortsaanvallen is nog niet bekend.

Hoofdstuk 1 van dit proefschrift is een overzicht van de kenmerken van de verschillende autoinflammatoire ziekten. Ze geven allemaal koortsaanvallen, maar de bijkomende symptomen zijn vaak net even anders, evenals de duur van de koortsaanval of de manier van overerving. In de afgelopen jaren is van elk van deze ziekten ontdekt waar de genetische fout zit. Van deze autoinflammatoire ziketen is HIDS al genoemd. Twee andere ziekten uit deze groep zijn FMF en TRAPS.

Familiaire Middellandse Zeekoorts (FMF) komt het meeste voor. Zoals de naam al doet vermoeden komt het vooral voor bij mensen die afkomstig zijn uit landen rond de Middellandse Zee, bijvoorbeeld Turkije en Israël. FMF wordt eigenlijk nooit gevonden bij mensen van Nederlandse afkomst, maar wel bij immigranten, waardoor tegenwoordig ook Nederlandse artsen het regelmatig tegenkomen. Bij FMF staat vaak de buikpijn tijdens een koortsaanval op de voorgrond, of gewrichtsklachten. Aanvallen duren vaak 2 of 3 dagen. Voor FMF bestaat een goede behandeling: door dagelijks colchicine te gebruiken blijven de meeste aanvallen weg; sommige patiënten hebben zelfs helemaal geen aanvallen meer.

TRAPS (afkorting van de Engelse naam "TNF-receptor associated periodic syndrome) is een ziekte met koortsaanvallen die meestal langer duren. Bij veel patiënten duurt een aanval 2 tot 3 weken; meestal blijft het dan wel beperkt tot 2 of 3 aanvallen per jaar. TRAPS heeft een andere manier van overerven dan FMF en HIDS. Het komt ook voor in Nederland. (Meer informatie over TRAPS is te vinden op de website http://hids.net/TRAPS/grond.htm).

Deze aandoeningen kunnen sterk op elkaar lijken, maar in het algemeen is het wel mogelijk onderscheid te maken door te letten op de kleine verschillen.

De volgende 8 hoofdstukken gaan specifiek over HIDS.

In **hoofdstuk 2** wordt een studie beschreven over DNA-onderzoek waarmee de diagnose HIDS gesteld kan worden. We onderzochten een stukje DNA van een groep HIDS-patiënten. Al deze patiënten hadden fouten (=mutaties) in het gen voor mevalonaat kinase, maar dit waren niet altijd dezelfde mutaties. In totaal werden 13 verschillende mutaties gevonden. Er bleken echter twee mutaties te zijn die bij bijna iedere HIDS-patiënt voorkomen. Om vast te stellen of er sprake is van HIDS hoeft in het begin alleen gekeken te worden of de patiënt één van deze twee mutaties heeft. Dat maakt het DNA-onderzoek een stuk eenvoudiger.

In de volgende studie wilden we weten of alle patiënten die, gezien hun klachten, HIDS leken te hebben ook daadwerkelijk de passende DNA-mutaties hadden (**hoofdstuk 3**). Dat bleek niet zo te zijn: van 54 patiënten die klachten hadden passend bij HIDS hadden 41 patiënten ook echt de mutaties in het mevalonaat kinase gen. De andere 13 patiënten hadden een normaal mevalonaat kinase gen zonder fouten, en dus een normaal werkend mevalonaat kinase eiwit. Om deze twee groepen te onderscheiden noemden we de eerste groep "klassieke type HIDS" en de laatste groep "variant type HIDS". Toen we daarna opnieuw keken naar de klachten en kenmerken van de twee groepen patiënten bleken er kleine verschillen te zijn. Van de patiënten met "variant type HIDS" weten we nog niet wat de oorzaak van de koortsaanvallen is. Het is goed mogelijk dat deze patiënten zelfs niet allemaal dezelfde ziekte hebben. Het is opvallend dat de patiënten met "variant type HIDS" eigenlijk nooit familieleden hebben met dezelfde klachten. Mogelijk dat dit geen erfelijke ziekte is.

Al jaren voor de ontdekking van de mutaties in het mevalonaat kinase gen in HIDS was er al een ziekte beschreven die veroorzaakt wordt door mutaties in hetzelfde gen. Deze ziekte wordt klassieke mevalonacidurie (letterlijk: uitplassen van mevalonzuur) genoemd. In klassieke mevalonacidurie hebben patiënten ook koortsaanvallen die veel lijken op die van HIDS. Maar ze hebben ook nog veel andere afwijkingen, met name aan de hersenen. De meeste van deze patiënten zijn verstandelijk gehandicapt en ze overlijden op jonge leeftijd. Het verbaasde ons dat

mutaties in hetzelfde gen konden leiden tot twee ziekten die zo veel verschillen van elkaar. We hadden het vermoeden dat het eigenlijk gaat om één ziekte, die zich meer of minder ernstig kan uiten. Dit vermoeden wordt ondersteund door het onderzoek beschreven in **hoofdstuk 4**. We vonden namelijk vijf volwassen patiënten met mutaties in het gen voor mevalonaat kinase, van wie het ziektebeeld niet echt paste bij klassieke mevalonacidurie maar ook niet helemaal bij HIDS. Hoe ernstig ziek iemand wordt hangt waarschijnlijk af van het soort mutatie in het gen, maar ook van andere, nog onbekende factoren.

In de volgende studie hebben we bestudeerd wat er verandert in het bloed van een HIDS patiënt tijdens een koortsaanval (**hoofdstuk 5**). We hebben gekeken naar bepaalde eiwitten die met ontsteking te maken hebben. Hierbij waren we op zoek naar een bepaling in het bloed die zou kunnen helpen om het onderscheid te maken tussen een "gewone" koortsaanval, passend bij HIDS, en een infectie met een bacterie, die ook koorts geeft. Dit zou van belang kunnen zijn bij de behandeling van HIDS: door makkelijker vast te stellen dat het om HIDS gaat, kan onnodig gebruik van antibiotica (wat zou helpen als het om een bacterie zou gaan) voorkomen worden. In dit onderzoek wordt aangetoond dat een combinatie van een hoge concentratie van één eiwit (namelijk CRP) met een niet-verhoogde concentratie van een ander eiwit (namelijk procalcitonine) waarschijnlijk alleen bij een HIDS-aanval gevonden wordt, en niet bij een bacterie. Er is meer onderzoek nodig alvorens deze resultaten in de praktijk gebruikt zullen kunnen worden bij een individuele patiënt.

In ons centrum onderhouden we een register waarin we gegevens van alle HIDS-patiënten ter wereld proberen te verzamelen. Het viel ons op dat veel patiënten met HIDS afkomstig waren uit Nederland, en we vroegen ons af hoe dat kwam. Eén oorzaak is dat er juist in Nederland veel wetenschappelijk onderzoek gedaan wordt naar HIDS, onder andere in Amsterdam (vanuit een biochemisch laboratorium), Utrecht (vanuit de afdeling kindergeneeskunde) en Nijmegen (vanuit de afdeling interne geneeskunde). Hierdoor weten meer artsen in Nederland van het bestaan van deze zeldzame ziekte en zal de diagnose eerder gesteld worden. Er blijkt echter nog een andere oorzaak te zijn: door middel van DNA-onderzoek hebben we aangetoond dat een groot aantal patiënten met HIDS ergens in de geschiedenis, honderden jaren geleden, van een zelfde voorouder afstamt (**hoofdstuk 6**).

Hoe het defect in mevalonaat kinase leidt tot koortsaanvallen is nog steeds niet precies bekend. In het volgende onderzoek in dit proefschrift (**hoofdstuk 7**) hebben we geprobeerd dit te onderzoeken in het laboratorium. Daarbij hebben we witte bloedcellen uit bloed van gezonde vrijwilligers en van verschillende HIDS-patiënten gehaald en in een testbuisje bloot gesteld aan verschillende stoffen die met mevalonaat kinase en ontsteking te maken hebben. Dit heeft een aantal nieuwe aanwijzingen opgeleverd over het ontstaan van de koortsaanvallen.

In de volgende twee hoofdstukken worden twee studies met geneesmiddelen beschreven met als doel een goede behandeling van HIDS te vinden. Aan beide studies werkten 6 patiënten met HIDS mee die zeer vaak last hadden van koortsaanvallen. In beide studies werd het effect van een bepaald geneesmiddel vergeleken met dat van een nep-pil (=placebo), waarbij de onderzoekers en de patiënt niet wisten wanneer ze wat gebruikten.

Eerst werd het effect van het geneesmiddel thalidomide onderzocht (**hoofdstuk 8**). Helaas leidde thalidomide niet tot in aantal minder aanvallen of tot minder ernstige aanvallen; het gaf alleen een lichte daling van de concentratie van ontstekingseiwitten in het bloed. Dit is dus geen goede behandeling voor HIDS.

Daarna onderzochten we het effect van simvastatine (**hoofdstuk 9**). Simvastatine is een geneesmiddel dat meestal gebruikt wordt om het cholesterol te verlagen. Het remt een bepaald eiwit dat veel te maken heeft met mevalonaat kinase, en daarom was het interessant om dit te onderzoeken. We wisten uit ervaring dat het leek te helpen bij één of twee HIDS-patiënten. Dus hebben we een uitgebreider onderzoek gedaan gedurende een jaar, waarbij de deelnemers een half jaar simvastatine gebruikten en een half jaar een placebo, zonder te weten welke ze gebruikten. Gedurende dat jaar hielden zij bij hoe vaak ze een koortsaanval hadden en hoe hevig die aanvallen waren. Het resultaat was goed: vijf van de zes patiënten waren duidelijk minder dagen ziek tijdens het gebruik van simvastatine dan tijdens de nep-pil. Simvastatine heeft weinig bijwerkingen en lijkt dus een veelbelovende behandeling om het aantal dagen ziekte te verminderen. De komende jaren moet in de praktijk duidelijk worden hoe goed het werkt en wat de beste dosering is. Helaas onderdrukte simvastatine niet alle aanvallen. Het kan dus alleen als een onderhoudsbehandeling gebruikt worden om het aantal ziektedagen te verminderen.

Daarnaast zou het mooi zijn als er een geneesmiddel gevonden wordt dat de patiënt kan gebruiken op het moment dat hij of zij een aanval voelt opkomen, om de aanval te stoppen. Op dit moment zijn we bezig met het onderzoeken van een dergelijk geneesmiddel; de resultaten hiervan zijn nog niet bekend. Binnenkort gaat waarschijnlijk een landelijk onderzoek van start waarbij we een ander geneesmiddel willen testen, dat ook gebruikt kan worden op het moment dat de patiënt een koortsaanval voelt aankomen

In **hoofdstuk 10** beschrijven we een studie naar de beste manier om de juiste diagnose te stellen bij patiënten met periodieke koortsaanvallen. Hierbij komt onder andere naar voren dat er nog steeds veel patiënten met koortsaanvallen zijn bij wie we geen naam aan de ziekte kunnen geven. Deze patiënten hebben dus bijvoorbeeld geen HIDS, FMF of TRAPS en dit zou kunnen betekenen dat er nog een aantal ziekten is die we niet kennen. Er is nog veel onderzoek nodig om deze nieuwe ziekten te ontdekken.

De volgende drie onderzoeken gaan over TRAPS. In **hoofdstuk 11, 12 en 13** beschrijven we een aantal patiënten met TRAPS en het verloop van de ziekte bij deze mensen. Ook beschrijven we hier onze ervaringen met de behandeling van TRAPS met behulp van ontstekingsremmers, zoals het zogenaamde etanercept en anakinra.

Het laatste hoofdstuk (**hoofdstuk 14**) gaat over FMF, en met name over de FMF-aanvallen die gepaard gaan met hevige buikpijn. We beschrijven de kenmerken van zulke aanvallen, de beste manier is om onderscheid te maken tussen een FMF-aanval en een gevaarlijke complicatie en de beste behandeling.

Al deze wetenschappelijke onderzoeken hebben informatie opgeleverd over de autoinflammatoire syndromen; de oorzaak, de verschijnselen tijdens de koortsaanvallen en de behandeling. Stap voor stap komen we zo steeds meer te

weten. Maar er is nog veel onderzoek nodig voor we alles begrijpen en voor we weten hoe we patiënten en families met deze aandoeningen het beste kunnen behandelen. Daarom wordt het onderzoek naar HIDS en andere autoinflammatoire ziekten in het Universitair Medisch Centrum St. Radboud in Nijmegen voortgezet. Hierbij wordt zowel samengewerkt met artsen en onderzoekers in Amsterdam en Utrecht, als met onderzoeksinstituten in het buitenland.

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Abbreviations

CIAS1 gene	cold induced autoinflammatory syndrome gene (= NALP3 = PYPAF)
CINCA	chronic infantile neurological cutaneous and articular syndrome (=NOMID)
CRP	C-reactive protein
FCAS	familial cold autoinflammatory syndrome
FMF	familial mediterranean fever
HIDS	hyper-IgD and periodic fever syndrome
HMG-CoA	3'5'-hydroxymethylglutaryl coenzyme A
IgA	immunoglobulin A
IgD	immunoglobulin D
LPS	lipopolysaccharide
MEFV gene	mediterranean fever gene, associated with FMF
MIM	Mendelian inheritance in men database
MK	mevalonate kinase
MVA	mevalonic acid
MVK gene	mevalonate kinase gene
MWS	Muckle-Wells syndrome
NOMID	neonatal onset multisystemic inflammatory disease (=CINCA)
NSAIDs	non-steroid antiinflammatory drugs
PAPA	pyogenic sterile arthritis, pyoderma gangrenosum and acne
PBMCs	peripheral blood mononuclear cells
PCT	procalcitonin
PFAPA	periodic fever, aphtous stomatitis, pharyngitis and adenitis
PTX3	pentraxin-3
SAA	serum amyloid A
TNF	tumour necrosis factor
TNFRSF1A	TNF-receptor type 1 (= p55)
TNFRSF1B	TNF-receptor type 2 (= p75)
TRAPS	TNF-receptor associated periodic syndrome

Dankwoord

Curriculum vitae

Dankwoord

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plotseling uit het onderzoek weggeroepen werden om de Spoedeisende Hulp of een klinische afdeling van het UMC St. Radboud te bemannen, dat schept een band.

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Curriculum vitae

De schrijver van dit proefschrift werd op 21 mei 1974 te Hilvarenbeek geboren. Zij behaalde haar eindexamen gymnasium aan het Jacob Roelantslyceum te Boxtel in 1992. In de zomer van 1992 werd zij uitgezonden als één van de twee Nederlandse deelnemers aan het "dr. Bessie F. Lawrence 24th International Summer Science Institute for pre-university students", aan het Weizmann Institute of Science te Rehovot, Israel. Daarna begon zij haar studie Geneeskunde in Utrecht, die in 1997 werd afgesloten met het doctoraalexamen (cum laude), in 1999 gevolgd door het artsexamen. Tijdens deze studie werkte ze twee jaar als student-assistent Microscopische Anatomie. Een wetenschappelijke stage op de afdeling Endocrinologie van het Academisch Ziekenhuis Utrecht (onder leiding van prof.dr. C. Lips en dr. H. Koppeschaar) werd wegens interesse een aantal maanden uitgebreid en leidde in 1997 tot een bezoek van 3 maanden aan het laboratorium van prof. M. Levine, Johns Hopkins University, Baltimore, Maryland, USA.

In augustus 1999 begon zij met haar opleiding tot internist in het Canisius Wilhelmina Ziekenhuis te Nijmegen (opleider dr. R. de Koning). Na toekenning van een NWO AGIKO subsidie volgde in januari 2000 overplaatsing naar het UMC St. Radboud te Nijmegen (opleider prof.dr. J.W.M. van der Meer) en werd gestart met het onderzoek beschreven in dit proefschrift. In april 2004 zette zij haar opleiding tot internist voort in het Canisius Wilhelmina Ziekenhuis te Nijmegen (opleider dr. A.S. Dofferhoff).

Sebright Bantam and science, or: Why the chickens?

> *Just as man can give beauty, according to his standard of taste, to his male poultry [...], can give to the Sebright bantam a new and elegant plumage, an erect and peculiar carriage – so it appears that female birds [...] have by a long selection of the more attractive males, added to their beauty or other attractive qualities.*
> Charles Darwin, 1882, in "The Descent of Man".

The cover illustration of this thesis is no random choice. I first encountered the Sebright bantam while working on my predoctoral research in the field of pseudohypoparathyroidism. It is a good example of my motto, the observation by William Harvey (page 5) that "nature is nowhere accustomed more openly to display her secret mysteries than in cases where she shows traces of her workings apart from the beaten path". Observation of such traces is central to scientific research – and the Sebright bantam rooster has in this way inspired a whole series of renowned scientists since the beginning of the 19th century.

First, what is so special about the Sebright bantam rooster? You can see it in the illustration: the Sebright bantam rooster in the foreground has the large comb of a rooster, but the short, upright tail feathers, called henny feathers, normally seen on a hen (the other chicken is a Black bantam rooster with regular long and curvy cocky feathers). So the Sebright bantam rooster is a genetic male with female tail feathers. It was discovered by Sir John Sebright, an avid animal breeder, in the beginning of the 19th century.

Sebright's work caught the attention of Charles Darwin, who used the Sebright bantam as one of the examples to support his theory of evolution. In the beginning of the 20th century, Thomas Hunt Morgan, who later won a Nobel prize for his discovery of gene linkage and the chromosomal basis of heredity, chose the Sebright bantam as a model system for his studies on embryonic development and showed that henny feathering was transmitted by an autosomal dominant gene. The brilliant endocrinologist Fuller Albright, who found out about the Sebright bantam through Morgan's work, used it as an example of his postulation that hormones must have receptors in their target tissues. In 1942, he proposed that the female feathering was caused by a resistance to the action of the male sex hormone, testosterone, similar to the defect he had just demonstrated in patients with pseudohypoparathyroidism, whose kidneys and bone were resistant to parathyroid hormone.

Almost forty years later, this theory was proved wrong by Jean Wilson and his colleagues. They demonstrated that the androgen receptors in these chickens are perfectly normal. The problem is located at the level of aromatase, the enzyme that converts testosterone to estradiol. While aromatase is normally only expressed in very specific tissues in chickens, in the Sebright bantam the tissue specificity of aromatase expression is lost, and estrogen is formed in many tissues including skin. The feathers of the Sebright bantam rooster are thus female because of high local estrogen levels. Further examination revealed that this increased expression of aromatase is caused by the presence of a second promotor sequence for the aromatase gene, which is most likely due to an inadvertent retroviral insertion in the chicken's DNA. And as recently as one year ago, the Sebright bantam was referred to again in medical literature, as three human patients were described with a new defect, very similar to that of the Sebright bantam – gain-of-function mutations in the aromatase gene leading to excess estrogen.

Thus, a relatively harmless rare defect in the feathering pattern of a chicken has fuelled scientific minds for the last 200 years, resulting in a series of important discoveries. The study of rare (orphan) diseases, such as hereditary autoinflammatory syndromes, when brought beyond the scope of "stamp collecting", may in a similar way not only benefit the patients suffering from it, but increase our knowledge of life in general.

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