The phenotype and genotype of Mevalonate Kinase Deficiency: a series of 114 cases from the Eurofever Registry

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Keywords
Mevalonate Kinase Deficiency
Eurofever Project

Hereditary Autoinflammatory Disease
Hyper IgD syndrome
Mevalonic Aciduria

ABSTRACT

Objectives: Mevalonate kinase deficiency (MKD) is a rare metabolic disease characterized by recurrent inflammatory episodes. This study aims to describe the genetic and phenotypic characteristics and the response to treatment in an international cohort of MKD patients.

Methods: MKD cases were extracted from the Eurofever registry (EAHC Project No. 2007332), an international, multicentre registry that retrospectively collects data on children and adults suffering from periodic fevers. We analysed cases that had been genetically or biochemically validated.

Results: One hundred and fourteen patients were included in this study. The median age of onset was 0.5 years. Patients had on average 12 episodes per year. Most patients had gastrointestinal symptoms (n=112), mucocutaneous involvement (n=99), lymphadenopathy (n=96) or musculoskeletal symptoms (n=89). Neurological complaints included headache (n=43), but also cerebellar syndrome (n=2), mental retardation (n=4) and seizures (n=6). AA-amyloidosis was noted in six patients. Macrophage activation syndrome arose in one patient. Between attacks patients were generally well, but over one third of patients suffered from constitutional symptoms, such as fatigue, between febrile attacks. Patients with combined p.V377I/p.I268T heterozygosity suffered significantly more often from AA-amyloidosis. Patients without p.V377I mutation suffered more often from severe musculoskeletal involvement.
Treatment with NSAIDs can relieve symptoms. Steroids given during attacks, anakinra and etanercept appear to improve symptoms and can induce complete remission in MKD patients.

Conclusion: This study describes the clinical and genetic characteristics of 114 MKD patients, which is the largest cohort so far. The clinical manifestations confirm earlier reports. However, the prevalence of AA-amyloidosis was higher than expected.

INTRODUCTION

Mevalonate kinase deficiency (MKD) is a rare autoinflammatory syndrome characterized by fever and generalized inflammation. The disease encompasses a continuum of two phenotypes, known as the Hyper Immunoglobulinemia D and periodic fever syndrome (HIDS, MIM#260920) and Mevalonic Aciduria (MA, MIM#610377).[1-3] Patients suffering from MKD present with fever, gastrointestinal complaints, lymphadenopathy, arthralgia, myalgia, skin rash and mucosal ulcers. Furthermore, patients suffering from the more severe phenotype Mevalonic Aciduria can also experience dysmorphic features, pre- and postnatal growth retardation and neurological and ocular involvement.[4]

Both phenotypes are caused by mutations in the mevalonate kinase (*MVK)* gene.[5] This gene encodes mevalonate kinase, an enzyme that is part of the mevalonate pathway. This pathway produces cholesterol and unsaturated lipid chains, known as non-sterol isoprenoids.[6] Activity of mevalonate kinase is reduced in MKD patients, varying from 1.8% to 28% in patients with the HIDS phenotype to below 0.5% in patients affected by the MA phenotype, although overlap occurs.[5,7,8] The substrate of this enzyme, mevalonic acid, accumulates and is excreted in the urine. Patients suffering from MKD therefore often excrete elevated amounts of mevalonic acid.[8-12] Due to the lack of clinical criteria, patients can only be diagnosed by the identification of two pathogenic *MVK* mutations or by detection of decreased enzyme activity.[13]

The first MKD patients were described in 1984.[2] Currently, just several hundred patients with this rare disease are known. It has been more frequently reported in patients with a Caucasian ethnicity; a disproportionate number of Dutch HIDS patients have been described. This is probably caused by a founder mutation (p.V377I) in the Dutch population.[14] The current number of MKD patients is certainly an underestimate as many patients will not be diagnosed with MKD.[6] As many physicians are still not familiar with this disease, the diagnostic delay is currently 7.1 years.[15] MKD patients are often suspected of many other diseases (e.g. infections, immunodeficiency or other autoinflammatory syndromes) before being diagnosed correctly.[15]

This study aims to describe the clinical and genetic characteristics and the response to treatment in a large, international cohort, in order to increase knowledge about this rare disease and hence facilitate diagnosis and inform the discussion on treatment and prognosis with affected families.

METHODS

All patients were enrolled in the Eurofever registry (EAHC Project No. 2007332), an international, multicentre registry that retrospectively collects information on patients suffering from periodic fever. Patients were enrolled since November 2009.[16] Epidemiological, demographic and clinical data were collected anonymously by local physicians. Independent ethical committee approval for enrolling patients was granted in accordance with local requirements. Written informed consent was obtained from patients according to local ethical regulations. Two experts on MKD (AS and JF) checked all enrolled patients on genetic, biochemical and clinical characteristics. For this analysis, all cases enrolled until November 2014 were included. Patients harbouring two *MVK* mutations or harbouring one mutation in combination with an abnormal metabolic study result were considered as true MKD patients. These metabolic studies were either measurement of raised urinary mevalonic acid or reduced mevalonate kinase enzyme activity in leukocytes or fibroblasts.

Statistics
All analyses were performed using Statistical Package for the Social Sciences (SPSS) 21. Categorical variables were described as frequencies and percentages. Median and interquartile ranges (IQR) were used to describe numerical variables. To determine a genotype-phenotype relation, differences in clinical features between groups with specific genotypes were analysed using Fisher's exact test. A p-value <0.05 was considered to be statistically significant.

RESULTS

Demographic data

In November 2014, 161 patients had been enrolled by their local physicians in the Eurofever registry with a diagnosis of MKD. Nineteen of these patients were excluded because genetic testing had not been performed, no *MVK* mutations were found, or clinical data were incomplete. Another 28 patients with one mutation were excluded as MKD could not be confirmed by demonstration of decreased mevalonate kinase activity or elevated urinary mevalonic acid excretion (figure 1).

A total of 114 patients (53 male, 61 female) were entered by 31 centres from twelve countries. The majority of these patients was born in Italy (n=31) and Netherlands (n = 28) (figure 2A). The median age of onset was 6 months (IQR 9 weeks to 19 months). The median age at diagnosis was 6.5 years (IQR 3.5-14.7) (figure 2B). Thus, the median diagnostic delay was six years (IQR 1.9-14.2 years) (figure 2C). The median follow-up period of this study was 11.5 years.

Figure 1. Flowchart of included patients

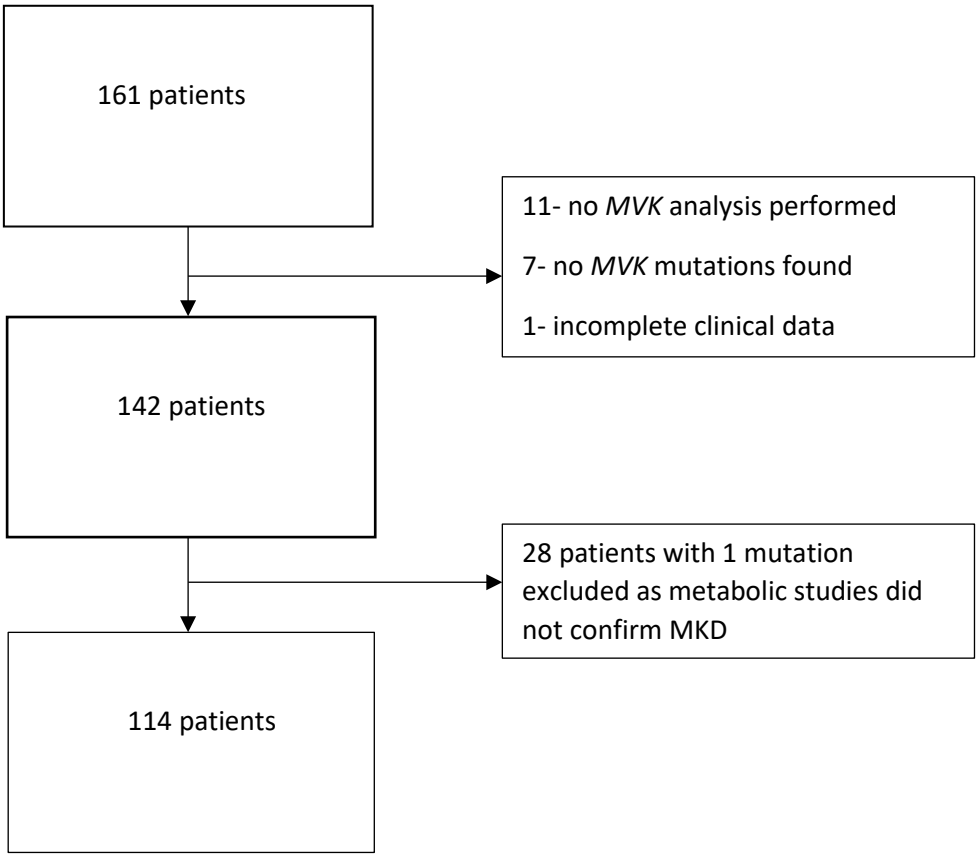
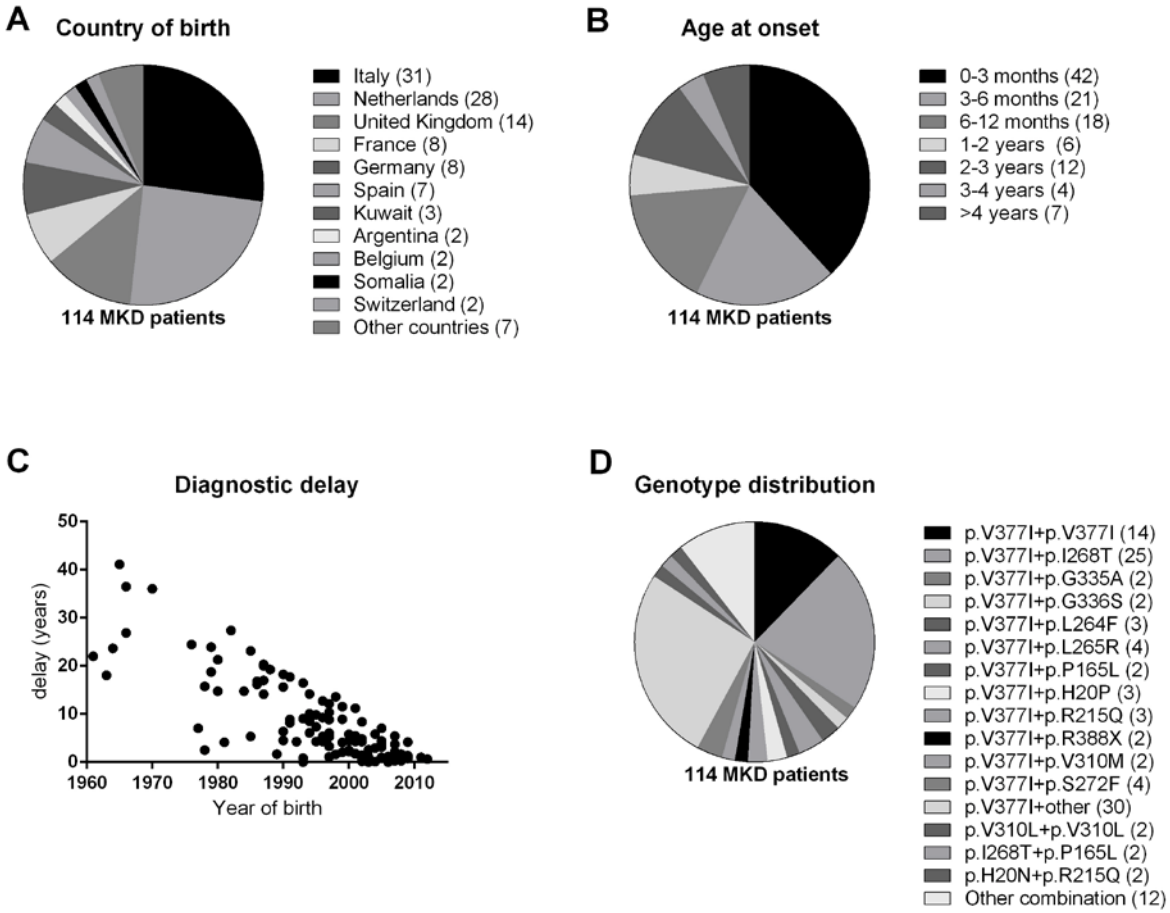


Figure 2. Characteristics of 114 MKD patients



A. Country of birth of 114 MKD patients. The numbers in brackets indicate the number of patients. Other countries included Albania, Australia, Cyprus, Czech Republic, Morocco, Russia and Turkey. B. Age at onset of 114 MKD patients. The numbers in brackets indicate the number of patients. C. Diagnostic delay according to the year of birth. D. The genotype of all patients. The numbers in brackets indicate the number of patients.

Genetic characteristics

Complete gene screening was performed in 47 (41%) patients, whereas in 46 (40%) patients only the most relevant exons were sequenced. In four (4%) patients only the most relevant point mutations were screened. Ninety-six patients harboured at least one p.V377I mutation (84%), fourteen of them had a homozygous p.V377I mutation (12%). The second most frequent mutation was p.I268T, occurring in 29 patients (25%). None of them were homozygous (figure 2D).

Three mutations are not present in the Infevers database.[17] A rare mutation was found in one patient: p.C152Y. This patient also had a p.V377I mutation and had a mild clinical pattern, as no musculoskeletal and neurological manifestations were reported. As this mutation was not known to be pathogenic, enzyme activity in both fibroblasts and leukocytes was performed, showing decreased activity in both assays.

Further, a 447^448insGCCTAC mutation, which is not known to be pathogenic, was found in one patient who also had a p.V377I mutation. This patient was not severely affected, suffering mostly from gastrointestinal symptoms, myalgia and lymphadenopathy. Metabolic studies were not performed.

One patient with a mild phenotype had a TGA-CGA mutation. This patient also had a p.R388X mutation.

Clinical characteristics

Ninety-nine of 114 patients suffered from recurrent disease episodes, while six patients suffered from a chronic disease and nine patients had a chronic course with exacerbations. Most patients had an episode duration of five days and suffered from twelve episodes per year (figure 3A, 3B). Febrile episodes were provoked by specific triggers in 51 patients, mainly by vaccination (n=38), stress (n=26) and infection (n=18) (figure 3C).

Most frequent MKD symptoms

All clinical features are summarized in table 1. Seventy-nine patients had constitutional symptoms, such as malaise (n=70), weight loss (n=67), fatigue (n=69) and mood disorders (n=23). In 23% of the patients, malaise was seen independent of fever, while fatigue occurred independent of fever in 35% of them. Mood disorders were mentioned independent of fever in 25% of patients.

One-hundred and twelve patients had gastrointestinal complaints. Most of them experienced abdominal pain (n=98), diarrhoea (n=93) and vomiting (n=76). In four patients the recurrent sterile inflammation had led to abdominal adhesions.

Ninety-nine patients suffered from mucocutaneous symptoms, such as aphthous stomatitis (n=67) and pharyngitis (n=31). Fifteen percent of patients had aphthous stomatitis irrespective of fever. Maculopapular rash (n=43) and urticarial rash (n=16) were seen only during febrile episodes in 92% and 62%, respectively.

Most patients (n=102) had lymphadenopathy, which was usually, but not exclusively cervical (n=96) and tender (n=59). Generalized lymphadenopathy occurred in a sizeable minority (n=30).

Musculoskeletal symptoms were noted in 89 patients. Most had myalgia (n=64) and arthralgia (n=80), specifically during fever episodes (85% and 82%). Arthritis was less common (n=31).

Severe manifestations

Patients with severe manifestations are mentioned in table 2 and some of them are discussed in greater detail below.

Eleven patients had severe neurological manifestations, namely seizures (n=6), cerebellar syndrome (n=2) and mental retardation (n=4). One patient with a heterozygous p.A334T and c.421dupG (A141fs) mutation, who suffered from a recurrent disease, experienced both a cerebellar syndrome and mild mental retardation. This patient also had retinitis pigmentosa and has been described before as a case report.[18] Another patient who suffered from a cerebellar syndrome also had a recurrent disease. He was p.V377I homozygous and was diagnosed with type 1 Arnold Chiari cerebellar syndrome by an incidental finding upon a brain MRI performed at the age of two years; hence the relationship between this cerebellar syndrome and MKD is not clear. Despite this malformation, he showed normal psychomotor development. He had a twin who died in utero. One patient with mental retardation was heterozygous for p.I268T and p.P165L and had a colitis as part of the MKD. The two other patients with mental retardation were both mildly affected concerning the other features.

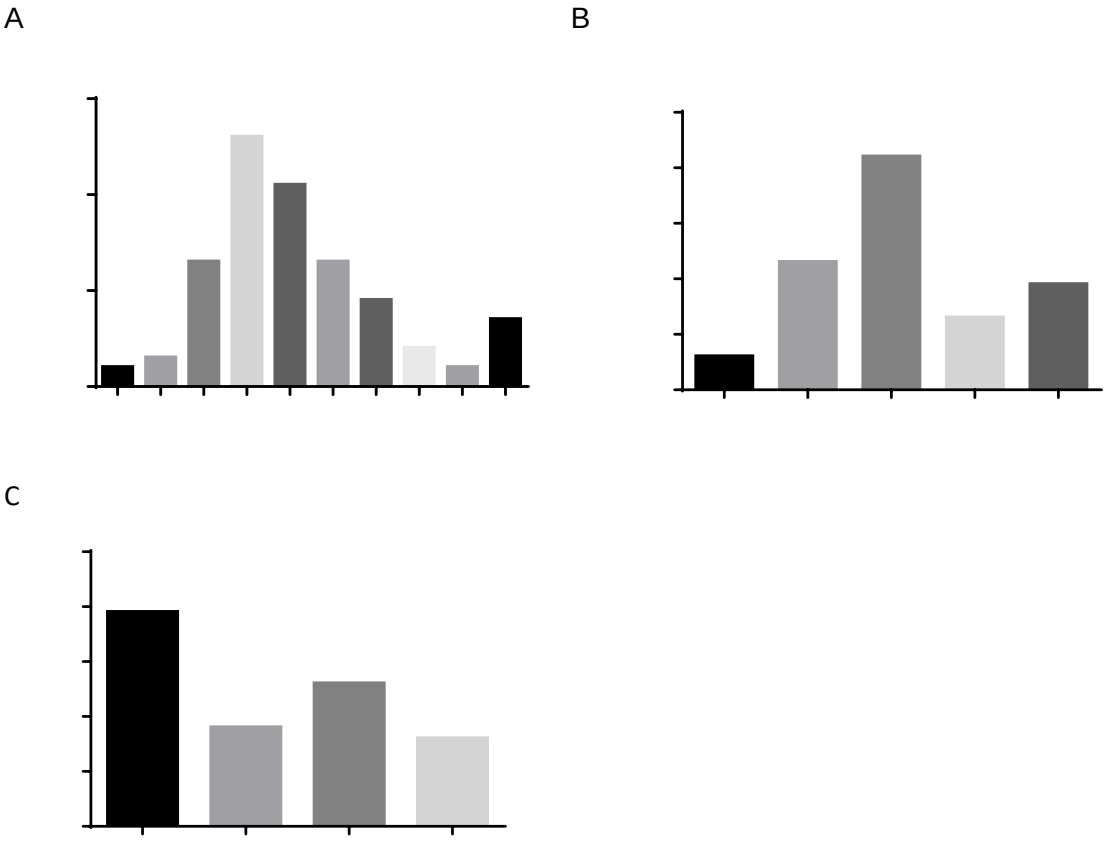
Five patients suffered from severe musculoskeletal involvement: flexion contractures (n=5), bone erosion (n=2), osteolytic lesions (n=2) and bone deformity (n=1). One patient with a

homozygous p.H20Q mutation suffered from severe polyarthtritis with involvement of all joints. The inflammation led to contractures and bone deformity. Two brothers, who had a heterozygous p.H20N and p.R215Q mutation, had flexion contractures, bone erosion and osteolytic lesions. The older brother had involvement of two joints. The younger brother, who suffered from a continuous disease based on clinical features and chronic elevation of inflammatory parameters, had involvement of thirteen joints. The first contractures occurred at the age of two years.

Macrophage activation syndrome arose in one patient, who had a heterozygous p.V377I and IVS7-1G>C mutation. Six patients suffered from AA-amyloidosis. One patient had a p.V377I mutation in combination with a p.S272F mutation. The other five patients were compound heterozygous for the p.V377I mutation and the p.I268T mutation. One of these five had a continuous disease course and deceased around the age of nineteen years. The other five patients with amyloidosis suffered from a recurrent disease.

Three patients with reported continuous disease had no other severe manifestations. One patient, who was heterozygous for p.T237S and p.I268T, had died due to acute respiratory distress syndrome before the age of one year.

Figure 3. Features of inflammatory attacks in 108 MKD patients.



A. Episode duration in days. B. Frequency of the number of episodes per year. C. Triggers in 51 MKD patients. Other triggers include cold (n=4); exercise (n=5); trauma (n=2); food (n=1); menstruation (n=4); fatigue (n=6); travel (n=1).

Table 1. Clinical characteristics of 114 MKD patients

	n		% during [+] every episode	% during[++] fever only
Disease pattern				
Recurrent	99	(87%)		
Chronic with exacerbations	9	(8%)		
Chronic	6	(5%)		
Constitutional symptoms*	79/111	(71%)		
Malaise	70/108	(65%)	53%	77%
Fatigue	69/109	(63%)	52%	65%
Mood disorders	23/95	(24%)	43%	75%
Weight loss	16/102	(16%)		
Mucocutaneous involvement	99/113	(87%)		
Aphthous stomatitis	67/111	(60%)	37%	85%
Maculopapular rash	43/111	(39%)	21%	92%
Urticarial rash	16/109	(15%)	38%	62%
Exudative pharyngitis	31/109	(28%)	16%	90%
Musculoskeletal involvement	89/113	(79%)		
Arthralgia	80/113	(71%)	34%	85%
Myalgia	64/112	(57%)	34%	82%
Arthritis	31/109	(28%)	24%	87%
Severe musculoskeletal involvement**	5/110	(5%)		
Gastrointestinal symptoms	112/114	(98%)		
Abdominal pain	98/111	(88%)	44%	84%
Diarrhoea	93/111	(84%)	38%	89%
Vomiting	76/110	(69%)	29%	82%
Ocular involvement	17/113	(15%)		
Conjunctivitis	11/113	(10%)	9%	64%
Uveitis	2/113	(2%)		
Impaired vision	2/113	(2%)		
Cataract	3/113	(3%)		
Neurological involvement	46/114	(41%)		
Headache	43/114	(38%)	40%	67%
Severe neurological involvement***	11/114	(10%)		
Lymphoid organs	102/113	(90%)		
Generalized enlargement	39/103	(38%)	36%	78%
Cervical lymphadenopathy	96/113	(85%)	64%	85%
Painful lymph nodes	59/99	(60%)	42%	88%

The denominator varies as missing values are disregarded in this table. The percentage is taken from the number of known values.
*Constitutional symptoms were defined as fever, malaise, fatigue, mood disorders or weight loss.
**Severe musculoskeletal involvement was defined as flexion contractures, bone deformity, bone erosion or osteolytic lesion.
***Severe neurological involvement was defined as seizures, cerebellar syndrome or mental retardation.
[+]During every episode means that the symptom was present during every febrile episode.
[++]During fever only means that the symptom was only present during febrile episodes and not in between.

Table 2. Severely affected patients

Patient	Mutation 1	Mutation 2	age at onset (years)	episode duration (days)	disease course	Amyloidosis	Seizures	Cerebellar syndrome	Mental retardation	Flexion contractures	Osteolytic lesion	Bone erosion	Bone deformity	MAS	Treatment with biologicals
1	p.V377I	p.I268T	0.12	x	continuous	x									
2	p.V377I	p.I268T	4	not known	recurrent	x systemic									
3	p.V377I	p.I268T	4	10	recurrent	x systemic									partial response on anakinra maintenance
4	p.V377I	p.S272F	3	13	recurrent	x									partial response on anakinra maintenance
5	p.V377I	p.I268T	1	7	recurrent	x systemic									partial response on etancercept
6	p.V377I	p.I268T	2.06	6	recurrent	x									partial response on anakinra used during attacks, partial response on etanercept
7	p.H20Q	p.H20Q	0.16	4	recurrent					x			x		Partial response on etanercept
8	p.H20N	p.R215Q	0.08	5	recurrent					x	x	x			complete response on anakinra maintenance, partial etanercept
9	p.H20N	p.R215Q	0		continuous					x	x	x			partial response anakinra, fail on etanercept
10	p.V377I	p.L265R	0.26	6	continuous and recurrent					x					
11	p.V310L	p.V310L	3	x	continuous					x					partial response on anakinra maintenance
12	p.V377I	p.V377I	0.04	3	recurrent		x								
13	p.V377I	p.I268T	0.22	5	recurrent		x								complete response on anakinra used during attacks and as maintenance therapy
14	p.V377I	p.V377I	0.14	5	recurrent		x								
15	p.V377I	p.S272F	0.5	9	continuous and recurrent		x								complete response on anakinra maintenance, fail on etanercept, complete response on canakinumab
16	p.V377I	c.790delC	0.03	5	recurrent		x								
17	p.V377I	p.I268T	0.32	>20	recurrent		x								
18	p.A334T	p.A141fs	0.01	4	recurrent			x	x						fail on anakinra maintenance
19	p.V377I	p.V377I	0.5	4	recurrent			x							
20	p.V377I	c.606insG	0.34	3	recurrent				x						
21	p.V377I	p.I268T	0.62	5	recurrent				x						

22	p.P165L	p.I268T	0.33	no specific pattern	recurrent				x						Partial response on anakinra used during attacks
23	p.V377I	IVS7-1G>C	2	5	recurrent									x	partial response anakinra used during attacks and as maintenance therapy
24	p.P165L	p.I268T	0	x	continuous										partial response on anakinra maintenance, fail on etanercept
25	p.V310L	p.V310L	3.33	x	continuous										
26	p.T237S	p.I268T	0.01	x	continuous										

Laboratory findings

Abnormal immunoglobulin (Ig) D levels were found in 55 of 76 tested patients. Other immunoglobulin levels were also measured: IgA was elevated in 48 of 90 patients, while IgG was elevated in fourteen of 91 tested patients. Immunoglobulin M was within normal range in the majority of the 91 tested patients.

Measurement of urinary mevalonic acid was performed in 40 patients; 37 of them showed elevated excretion. Thus, three patients excreted normal amounts of mevalonic acid. All three of them suffered from typical MKD symptoms. The first patient was homozygous for p.V377I and had a confirmed impairment of mevalonate kinase enzyme activity. Urine was collected during a febrile episode. The second patient was compound heterozygous for p.R388X and TGA-CGA stop codon. In this patient mevalonic acid was measured as part of the whole organic acid screening. The last patient was compound heterozygous for p.V377I and p.V310M and had a mild clinical pattern. In this patient urine was not collected during fever.

Enzymatic studies in both leukocytes and fibroblasts were performed in 19 patients. Reduced mevalonate kinase enzyme activity in fibroblasts was found in seven of eight tested patients, while fifteen of sixteen tested patients showed reduced enzyme activity in leukocytes. Unexpectedly, one patient with a homozygous p.V377I mutation, typical MKD symptoms and elevated urinary mevalonic acid excretion reported normal enzyme activity in both fibroblasts and leukocytes.

Associations between the genotype and phenotype

To analyse genotype-phenotype associations, we divided all patients into four groups: homozygous p.V377I, combined heterozygous p.V377I and p.I268T, patients with one p.V77I mutation and another second mutation than p.V377I or p.I268T and patients without a p.V377I mutation. The frequency of MKD features were compared between the groups. Five out of 25 patients with p.V377I/p.I268T combined heterozygosity experienced amyloidosis as compared to 1 out of 89 patients with other genotypes (p=0.01). Further, patients without a p.V377I mutation suffered more often from a chronic course, musculoskeletal and severe musculoskeletal involvement (table 3).

Table 3. Associations between the genotype and clinical characteristics

Genotype/ phenotype	p.V377I+ p.V377I (n=14)	p.V377I+ p.I268T (n=25)	p.V377I+ other (n=57)	patients without p.V377I (n=18)	p-value
Chronic course	0%	4%	0%	28%	0.000
Family history	45%	38%	29%	53%	ns
Constitutional symptoms	85%	68%	85%	89%	ns
Mucocutaneous involvement	100%	76%	91%	78%	ns
Gastrointestinal involvement	100%	96%	100%	94%	ns
Lymphoid involvement	93%	92%	89%	89%	ns

Ocular involvement	21%	8%	11%	35%	ns
Neurological involvement	64%	48%	30%	47%	ns
Severe neurological involvement	21%	12%	5%	11%	ns
Musculoskeletal involvement	86%	64%	77%	100%	0.025
Severe musculoskeletal Involvement	0%	0%	2%	22%	0.006
Amyloidosis	0%	20%	2%	0%	0.01

Patients were compared with patients from the other groups regarding the clinical variables, using Fisher exact test. A p-value <0.05 was considered to be significant.

Treatment

NSAIDS

Non-steroidal anti-inflammatory drugs (NSAIDs) were used in 66 patients, usually to treat the symptoms of attacks, and were beneficial in 48 of them. Seven of these patients reported a complete response to NSAIDs. Five of them used NSAIDs during attacks only and not as maintenance therapy. Two of them used NSAIDs as monotherapy, the other five in combination with corticosteroids. The response to steroids was reported as complete in four of them and as partial in one.

Corticosteroids

Corticosteroids were used by 49 patients to treat fever attacks. Nineteen of them reported complete suppression of inflammatory episodes (16/19 had not used biologics) and 21 patients had some improvement. Five out of seven patients who used maintenance corticosteroids reported some benefit, failure was noted in the other two.

Colchicine and statins

Colchicine was used by 21 patients; thirteen of them did not respond to this treatment and only one patient with p.V377I/p.S135L heterozygosity had a complete response. Mediterranean Fever (MEFV) screening had not been performed in this Caucasian patient from Italy. This patient did not use NSAIDs, steroids or biologics. Statins were used in fifteen patients; in eleven patients this treatment failed, moreover three of them reported worsening of their disease. Four patients noted some improvement of symptoms.

Biologicals

Anakinra was used only during attacks by eight patients, with three of them having a complete response and the other five a partial response. Nineteen patients used anakinra only as maintenance therapy, which led to complete remission in three of them and a partial response in thirteen. In three patients Anakinra was not effective. All three suffered from a recurrent disease. One of them was severely affected and suffered from cerebellar syndrome, mental retardation and retinitis pigmentosa. The two other patients were mildly affected.

Five patients used canakinumab; four went in complete remission, while one had a partial response. The patient with a partial response was p.V377I/p.G338D heterozygous and had

failed on NSAIDs, steroids, anakinra, etanercept and adalimumab, before the initiation of canakinumab. Etanercept had a beneficial effect in sixteen patients, of whom two had a complete response. Ten other patients failed to respond to etanercept.

DISCUSSION

This study describes the phenotypic and genotypic characteristics and the response to treatment in the largest cohort of MKD patients reported so far. Moreover, the vast majority of these patients have not been described in previous cohorts.[19] This large cohort enables us to give a broad description of the clinical features and treatment of this rare disease.

In many respects, our study confirms the clinical characteristics reported by previous studies. Typically, the disease starts within the first year of life. Onset after the age of 4 years makes the diagnosis extremely unlikely. The most common symptoms were abdominal pain, diarrhoea, vomiting, lymphadenopathy, arthralgia, myalgia and aphthous stomatitis. Six patients suffered from AA-amyloidosis, which is almost double compared to previous cohorts. 19] Only one patient in our cohort experienced a macrophage activation syndrome, which is less than described in the study of Bader-Meunier *et al.*[8]

Unexpectedly, many patients had complaints between attacks. This concerned mainly constitutional symptoms such as fatigue, malaise and headache, but also oral aphthous ulcers. It has to be noted that these are also very common symptoms in the general population. Hence, a causal relation between mevalonate kinase deficiency and these symptoms remains uncertain.

This study confirms previous findings that measurement of IgD is not a reliable method to diagnose MKD, as 28% of the tested patients in this cohort did not have elevated IgD levels.[13] Further, measurement of urinary mevalonic acid is a sensitive method for screening on MKD, as 93% of the tested patients excreted elevated amounts of mevalonic acid.[8] Unexpectedly, measurement of mevalonate kinase enzyme activity could be entirely normal in the presence of known pathogenic mutations and elevated urinary excretion of mevalonic acid. Enzymatic studies have been regarded as the diagnostic gold standard for mevalonate kinase deficiency.

The most frequent combination of mutations was p.V377I/I268T heterozygosity occurring in 22% of the patients, followed by p.V377I homozygosity in 12%. Patients with a combined heterozygosity for p.V377I/p.I268T suffered significantly more often from AA-amyloidosis.

As reported previously, treatment with statins and colchicine was not effective in most patients.[19,20] Seventy-five percent of the patients reported at least some improvement of symptoms when using NSAIDs, but a complete effect is rarely seen. Corticosteroids are more effective to terminate inflammatory attacks, but long-term side effects are a major drawback. IL-1 blockade is beneficial in many MKD patients, but apparently not as effective as observed in the IL-1 driven cryopyrin-associated periodic syndromes, at least using the Il-1 receptor antagonist (Anakinra).[20] Although the number of MKD patients reported here is substantially larger than that described in a previous paper on therapy in the Eurofever cohort, the findings on therapy remain essentially unchanged.[20]

The retrospective design of the Eurofever registry comes with a number of limitations. Due to this design, there are quite some missing values, which might have induced a bias. The missing values might cause an underrepresentation as the symptoms might have been present. For that reason, we have chosen to use the number of known values as denominator in the tables. Further, the Eurofever Registry collects data on patients suffering from periodic fever. The participating physicians are (paediatric) immunologists and (paediatric) rheumatologists. As mevalonic aciduria patients experience predominantly

neurological symptoms, these patients are more likely to be seen by a (paediatric) neurologist or a specialist in metabolic diseases. Therefore, some of the more severely affected MKD patients may not have been enrolled. Besides, mevalonic aciduria patients may be underrepresented as they are more likely to die at a young age.[21] Still, our cohort included some patients who can be classified as MA. A limitation to the interpretation of therapeutic interventions is the absence of criteria for complete and partial response. Bias is inevitable due to the lack of control groups and randomization. Furthermore, as it is unknown whether drugs were used simultaneously or solely, it is even more difficult to draw solid conclusions about the efficacy of treatments. However, the Eurofever registry does provide information on current practice.

In conclusion, we describe the clinical and genetic features in the largest, international cohort of MKD patients. Most MKD patients suffer from fever, accompanied by gastrointestinal symptoms, lymphadenopathy, arthralgia and aphthous stomatitis. AA-amyloidosis (5%) and macrophage activation syndrome (0.8%) are rare, but severe complications of MKD. Patients can benefit from treatment with NSAIDs, steroids and biologicals, mainly anakinra and etanercept. Statins and colchicine are usually not effective in MKD patients.

References

1. Prieur AM, Griscelli C. Nosologic aspects of systemic forms of very-early-onset juvenile arthritis. Apropos of 17 cases. Sem Hop 1984 Jan 26;60(3):163-167.

2. van der Meer JW, Vossen JM, Radl J, et al. Hyperimmunoglobulinaemia D and periodic fever: a new syndrome. Lancet 1984 May 19;1(8386):1087-1090.

3. Berger R, Smit GP, Schierbeek H, Bijsterveld K, et al. Mevalonic aciduria: an inborn error of cholesterol biosynthesis? Clin Chim Acta 1985 Oct 31;152(1-2):219-222.

4. Simon A, Kremer HP, Wevers RA, et al. Mevalonate kinase deficiency: Evidence for a phenotypic continuum. Neurology 2004 Mar 23;62(6):994-997.

5. Houten SM, Kuis W, Duran M, et al. Mutations in MVK, encoding mevalonate kinase, cause hyperimmunoglobulinaemia D and periodic fever syndrome. Nat Genet 1999 Jun;22(2):175-177.

6. van der Burgh R, Ter Haar NM, Boes ML, et al. Mevalonate kinase deficiency, a metabolic autoinflammatory disease. Clin Immunol 2013 Jun;147(3):197-206.

7. Cuisset L, Drenth JP, Simon A, et al. Molecular analysis of MVK mutations and enzymatic activity in hyper-IgD and periodic fever syndrome. Eur J Hum Genet 2001 Apr;9(4):260-266.

8. Bader-Meunier B, Florkin B, Sibilia J, et al. Mevalonate kinase deficiency: a survey of 50 patients. Pediatrics 2011 Jul;128(1):e152-9.

9. Prietsch V, Mayatepek E, Krastel H, et al. Mevalonate kinase deficiency: enlarging the clinical and biochemical spectrum. Pediatrics 2003 Feb;111(2):258-261.

10. Hoffmann GF, Charpentier C, Mayatepek E, et al. Clinical and biochemical phenotype in 11 patients with mevalonic aciduria. Pediatrics 1993 May;91(5):915-921.

11. Poll-The BT, Frenkel J, Houten SM, et al. Mevalonic aciduria in 12 unrelated patients with hyperimmunoglobulinaemia D and periodic fever syndrome. J Inherit Metab Dis 2000 Jun;23(4):363-366.

12. Prasad C, Salvadori MI, Rupar CA. Severe phenotypic spectrum of mevalonate kinase deficiency with minimal mevalonic aciduria. Mol Genet Metab 2012 Dec;107(4):756-759.

13. Ammouri W, Cuisset L, Rouaghe S, et al. Diagnostic value of serum immunoglobulinaemia D level in patients with a clinical suspicion of hyper IgD syndrome. Rheumatology (Oxford) 2007 Oct;46(10):1597-1600.

14. Houten SM, van Woerden CS, Wijburg FA, et al. Carrier frequency of the V377I (1129G>A) MVK mutation, associated with Hyper-IgD and periodic fever syndrome, in the Netherlands. Eur J Hum Genet 2003 Feb;11(2):196-200.

15. Berody S, Galeotti C, Kone-Paut I, et al. A restrospective survey of patients's journey before the diagnosis of mevalonate kinase deficiency. Joint Bone Spine 2015 Feb 9.

16. Toplak N, Frenkel J, Ozen S, et al. An international registry on autoinflammatory diseases: the Eurofever experience. Ann Rheum Dis 2012 Jul;71(7):1177-1182.

17. Infevers, the registry of Hereditary Auto-inflammatory Disorders Mutations URL: *http://fmf.igh.cnrs.fr/infevers*. Accessed on 25-05-2015

18. Balgobind B, Wittebol-Post D, Frenkel J. Retinitis pigmentosa in mevalonate kinase deficiency. J Inherit Metab Dis 2005;28(6):1143-1145.

19. van der Hilst JC, Bodar EJ, Barron KS, et al. Long-term follow-up, clinical features, and quality of life in a series of 103 patients with hyperimmunoglobulinemia D syndrome. Medicine (Baltimore) 2008 Nov;87(6):301-310.

20. Ter Haar N, Lachmann H, Özen S, et al. Treatment of autoinflammatory diseases: results from the Eurofever Registry and a literature review. Ann Rheum Dis 2013 May;72(5):678-85.

21. Hoffmann GF, Charpentier C, Mayatepek E, et al. Clinical and biochemical phenotype in 11 patients with mevalonic aciduria. Pediatrics 1993 May;91(5):915-921.