

The phenotype and genotype of mevalonate kinase deficiency: a series of 114 cases from the Eurofever Registry

Running head: Phenotype and genotype of MKD

Nienke M. ter Haar^{1*} MD, Jerold Jeyaratnam^{2*} BSc, Helen J. Lachmann³ MD PhD Anna Simon⁴ MD PhD Paul A. Brogan⁵ MD PhD, Matteo Doglio⁶ MD PhD, Marco Cattalini⁷ MD, Jordi Anton⁸ MD PhD, Consuelo Modesto⁹ MD Pierre Quartier¹⁰ MD, Esther Hoppenreijns¹¹ MD, Silvana Martino¹² MD PhD, Antonella Insalaco¹³ MD, Luca Cantarini¹⁴ MD PhD, Loredana Lepore¹⁵ MD, Maria Alessio¹⁶ MD PhD, Inmaculada Calvo Penades¹⁷ MD PhD, Christina Boros¹⁸ MD PhD, Rita Consolini¹⁹ MD PhD, Donato Rigante²⁰ MD, Ricardo Russo²¹ MD, Jana Pachlopnik Schmid²² MD PhD, Thirusha Lane³ RN MSc, Alberto Martini^{6,23} MD PhD, Nicolino Ruperto⁶ MD MPH, Joost Frenkel^{2*} MD PhD, Marco Gattorno^{6*} MD PhD; for the Paediatric Rheumatology International Trials Organisation (PRINTO) and Eurofever Project.

¹ University Medical Center Utrecht, Laboratory for Translational Immunology, Utrecht, The Netherlands

² University Medical Center Utrecht, Department of Pediatrics, Utrecht, The Netherlands

³ University College Medical School, National Amyloidosis Center, Royal Free Campus, London, United Kingdom

⁴ Radboud University Medical Center, Nijmegen Center for Immunodeficiency and Autoinflammation (NCIA), Department of Internal Medicine, Nijmegen, Netherlands

⁵ University College London Institute of Child Health, Infection, Inflammation, and Rheumatology Section; and Great Ormond Street Hospital NHS Foundation Trust, London, United Kingdom

⁶ Istituto Giannina Gaslini, Pediatria II, Reumatologia, Genoa, Italy

⁷ University of Brescia and Spedali Civili di Brescia, Pediatric Clinic, Brescia, Italy

⁸ Hospital Sant Joan de Déu, University of Barcelona, Pediatric Rheumatology Unit, Esplugues (Barcelona), Spain

⁹ Hospital Valle de Hebron, Reumatologia, Reumatologia Pediátrica, Barcelona, Spain

¹⁰ Université Paris-Descartes, Hôpital Necker-Enfants Malades. Centre de référence national pour les Arthrites Juveniles, Unité d'Immunologie, Hématologie et Rhumatologie Pédiatrique, APHP, IHU Imagine, Paris, France

¹¹ Radboud University Medical Center, Nijmegen Center for Immunodeficiency and Autoinflammation (NCIA), Department of Pediatric Rheumatology, Nijmegen, Netherlands

¹² Clinica Pediatrica Università di Torino, Dipartimento di Scienze Pediatriche e dell'Adolescenza, Day-Hospital Immuno-reumatologia, Torino, Italy

¹³ IRCCS Ospedale Pediatrico Bambino Gesù, Division of Rheumatology, Department of Pediatric Medicine, Rome, Italy

¹⁴ University of Siena, Policlinico le Scotte, Rheumatology Unit, Siena, Italy

¹⁵ Università degli Studi di Trieste, IRCCS Burlo Garofolo, Scienze della Riproduzione e dello Sviluppo, Trieste, Italy

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as an 'Accepted Article', doi: 10.1002/art.39763

© 2016 American College of Rheumatology

Received: Oct 25, 2015; Revised: Mar 08, 2016; Accepted: May 17, 2016

This article is protected by copyright. All rights reserved.

¹⁶Università di Napoli Federico II, Dipartimento di Pediatria, Napoli, Italy

¹⁷University Hospital La Fe, Department of Pediatric Rheumatology, Valencia, Spain

¹⁸Women's and Children's Hospital, University of Adelaide, Discipline of Pediatrics, North Adelaide, SA, Australia

¹⁹Ospedale Santa Chiara, Università di Pisa, Pediatria, Pisa, Italy

²⁰Università Cattolica Sacro Cuore, Institute of Pediatrics, Roma, Italy.

²¹Hospital de Pediatria Juan P. Garrahan, Servicio de Inmunología/Reumatología, Buenos Aires, Argentina.

²²University Childrens Hospital Zurich, Division of Immunology, Zurich, Switzerland

²³Università degli Studi di Genova, Genoa, Italy

* N.M. ter Haar and J. Jeyaratnam contributed equally to this study, J. Frenkel and M. Gattorno contributed equally as senior authors.

Correspondence to: Joost Frenkel MD PhD, Department of General Pediatrics, University Medical Center Utrecht, Room KE 04 133 1, PO-Box 85090, 3508 AB Utrecht, The Netherlands. Phone number: 0031-88-7554194. Fax number: 0031-88-7555349. E-mail: j.frenkel@umcutrecht.nl

Financial support

The Eurofever registry was sponsored by the Autoinflammatory Diseases' Working Group of the Paediatric Rheumatology European Society (PRES) and supported by the Executive Agency For Health and Consumers (EAHC, Project No 2007332) and by Coordination Theme 1 (Health) of the European Community's FP7 (Eurotraps, grant agreement number HEALTH-F2-2008-200923). Novartis and SOBI have granted unrestricted educational grants.

Conflict of interest

The authors declare that they have no other competing interest.

Acknowledgements

The authors would like to thank Miss Eugenia Mosci, Irene Gregorini and Elisa Patrone for their precious secretarial support.

Word count: 4272

Abstract: 249

ABSTRACT

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

Methods: All MKD cases were extracted from the Eurofever registry (EAHC Project No. 2007332), an international, multicenter registry that retrospectively collects data on children and adults suffering from autoinflammatory diseases.

Results: One hundred and fourteen MKD 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=102) or musculoskeletal symptoms (n=89). Neurological complaints included headache (n=43), but also cerebellar syndrome (n=2) and mental retardation (n=4). AA-amyloidosis was noted in five patients, almost twice as many as expected from previous cohorts. Macrophage activation syndrome occurred in one patient. Between attacks patients were generally well, but 10-20% patients suffered from constitutional symptoms, such as fatigue, between febrile attacks. Patients with p.V377I/p.I268T compound heterozygosity suffered significantly more often from AA-amyloidosis. Patients without a p.V377I mutation suffered more often from severe musculoskeletal involvement.

Treatment with NSAIDs could relieve symptoms. Steroids given during attacks, anakinra and etanercept appeared to improve symptoms and could induce complete remission in MKD patients.

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

Keywords

Mevalonate Kinase Deficiency
Eurofever Project
Hereditary Autoinflammatory Disease
Hyper IgD syndrome
Mevalonic Aciduria

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) (1-4). 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 exhibit dysmorphic features, pre- and postnatal growth retardation and neurological and ocular involvement (5).

Both phenotypes are caused by mutations in the mevalonate kinase (*MVK*) gene (6,7). 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 (8). 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 (6,9,10). 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 (10-14). 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 (15).

The first MKD patients were described in 1984 (2). Currently, several hundred patients with this rare disease have been described. MKD has been more frequently reported in patients with Caucasian ethnicity; a disproportionate number of Dutch HIDS patients have been described, probably caused by a founder mutation (p.V377I) in this population (16). The current number of MKD patients is certainly an underestimate as many patients remain undiagnosed (8). As many physicians are still not familiar with this disease, the diagnostic delay is 7.1 years (17). MKD patients are often suspected of having other diseases, such as infections, immunodeficiency or other autoinflammatory syndromes before being diagnosed correctly (17).

This study aimed 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.

PATIENTS AND METHODS

All patients were enrolled in the Eurofever registry (EAHC Project No. 2007332), an international, multicenter registry which retrospectively collects information on patients suffering from autoinflammatory diseases. Patients were enrolled since November 2009 (18). 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 the enrolled patient data on genetic, biochemical and clinical characteristics. For this analysis, all cases enrolled until November 2014 were included. Patients harboring two *MVK* mutations or harboring one mutation in combination with an abnormal metabolic study result were considered as true MKD patients. The metabolic studies utilized were either raised urinary mevalonic acid or reduced mevalonate kinase enzyme activity in leukocytes or fibroblasts.

Response to different treatments (either on demand or continuous) was assessed as follows: complete response (complete control of the clinical manifestations and normalization of laboratory parameters), partial response (persistence of some clinical manifestations and/or perturbation of laboratory examinations) or failure (absence of any substantial impact on disease activity or worsening).

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 numeric variables. To determine a genotype-phenotype relationship, differences in clinical features between groups with specific genotypes were analyzed using Fisher's exact test. A p-value <0.05 was considered to be statistically significant.

RESULTS

Demographic data

Up to 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 only 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 centers from twelve countries. The majority of these patients was born in Italy (n=31) and the Netherlands (n = 28) (figure 2A). The median age of onset was 6 months (IQR 9 weeks to 19 months) (figure 2B). The median age at diagnosis was 6.5 years (IQR 3.5-14.7 years). Thus, the median diagnostic delay was six years (IQR 1.9-14.2 years) (figure 2C). The median follow-up period since the age at onset was 11.5 years.

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 harbored at least one p.V377I mutation (84%), fourteen of which were homozygous (12%). The second

most frequent mutation was p.I268T, occurring in 29 patients (25%). None of them were p.I268T homozygous (figure 2D).

Two mutations were not present in the Infevers database (19). A p.C152Y mutation was found in one patient. 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.

Clinical characteristics

Ninety-nine of 114 patients suffered from recurrent disease episodes, i.e. they recovered completely between attacks. Six patients suffered from continuous disease without clear-cut exacerbations, whereas nine patients had a continuous course with exacerbations. In these patients the intervals between attacks were characterized by some measure of ongoing inflammation. The median flare duration was four days and the median flare frequency was 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).

MKD symptoms and sequelae

General features

All clinical features are summarized in table 1. Detailed descriptions of patients with severe manifestations can be found in table 2. Seventy-nine patients had constitutional symptoms, such as malaise (n=70), fatigue (n=69) and weight loss (n=16). In 23% of these patients, malaise was reported independent of fever, while fatigue occurred independent of fever in 35% of them. Constitutional symptoms independent of fever were no predictor of a more severe course such as amyloidosis. 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=39).

Mucocutaneous involvement

Ninety-nine patients suffered from mucocutaneous symptoms, such as aphthous stomatitis (n=67) and pharyngitis (n=31). Fifteen percent of patients had aphthous stomatitis independent of fever. Maculopapular rash (n=43) and urticarial-like rash (n=16) were seen between febrile episodes in 8% and 38%, respectively.

Musculoskeletal involvement

Musculoskeletal symptoms were noted in 89 patients. Most had arthralgia (n=80) and myalgia (n=64), specifically during fever episodes (85% and 82%). Arthritis was less common (n=31) and resolved when the fever episode subsided in 91%. In total eight patients suffered from severe musculoskeletal involvement: flexion contractures (n=5), persistent arthritis (n=2), bone erosion (n=2), osteolytic lesions (n=2), osteitis (n=2), and bone deformity (n=1). These patients are described in more detail in the following paragraphs and table 2.

One patient (pt 1) with persistent polyarthritis developed contractures and bone deformity. This patient also had severe gastrointestinal complaints, such as gastrointestinal bleeding and gastrointestinal and (peri)anal ulcers. The other patient with persistent arthritis did not have other severe manifestations.

Two brothers, who had a compound heterozygous p.H20N and p.R215Q mutation, had flexion contractures, bone erosion and osteolytic lesions. The older brother (pt 2) had involvement of two joints and also had severe gastrointestinal involvement including aseptic peritonitis, gut perforation, intestinal occlusion and abdominal adhesions. Besides he suffered from scleritis. The younger brother (pt 3), who suffered from a continuous disease based on clinical features and chronic elevation of inflammatory parameters, had involvement of thirteen joints. Joint contractures appeared at the age of two years.

Two patients suffered from osteitis. One of them (pt 4) had a continuous disease pattern and suffered from bacterial osteomyelitis. This patient also experienced aseptic peritonitis, intestinal occlusion and abdominal adhesions. The other patient (pt 5) suffered from osteitis without other severe manifestations. The osteitis was located in the right calcaneus. The blood culture was negative. One patient only had flexion contractures reported.

Gastrointestinal involvement

One-hundred and twelve patients had gastrointestinal (GI) complaints. Most of them experienced abdominal pain (n=98), diarrhoea (n=93) and vomiting (n=76). In more than 80% of patients, these symptoms were only seen during fever episodes.

Eighteen patients had severe gastrointestinal symptoms: aseptic peritonitis (n=7), GI-bleeding (n=7), (peri)anal ulcers (n=5), intestinal occlusion (n=3), gut perforation (n=2) and gastrointestinal ulcers (n=2).

Abdominal adhesions were reported in four patients, although the modality of identification of this complication (imaging or after surgery) was not specified. Two sisters (pt 6 and 7) both had gastrointestinal bleeding. The elder sister also had uveitis and scleritis, while the younger sister had flexion contractures.

Patient 8 suffered from aseptic peritonitis and (peri)anal ulcers. Another patient (pt 9) suffered from aseptic peritonitis and abdominal adhesions. One patient (pt 10), who had a continuous disease course, suffered from aseptic peritonitis, GI bleeding, gut

perforations and abdominal adhesions. This patient also experienced aseptic meningitis. She died due to acute respiratory distress syndrome before the age of one year. Ten additional patients had one severe GI manifestations namely GI bleeding (n=3), (peri)anal ulcers (n=3), aseptic peritonitis (n=2), intestinal occlusion (n=1) or GI ulcers (n=1), without other severe organ involvement.

Neurological involvement

Headache was the most common neurological complaint (n=43) and was reported independent of fever in 33% of the patients. Mood disorders were reported in 23 patients. Moreover, 25% of these patients reported mood disorders between febrile episodes, thus reflecting the psychological impact of the disease, irrespectively from its recurrent or continuous course. Six patients had severe neurological manifestations, namely mental retardation (n=4), cerebellar syndrome (n=2) and aseptic meningitis (n=1). One patient (pt 11), who had a recurrent disease course, experienced both a cerebellar syndrome and mild mental retardation. This patient also had retinitis pigmentosa and has been described before as a case report (20). Another patient (pt 12) who had cerebellar disease, also suffered from a recurrent disease course. He was diagnosed with type 1 Arnold Chiari cerebellar syndrome as an incidental finding upon brain magnetic resonance imaging (MRI) performed at the age of two years. Despite this malformation, he showed normal psychomotor development. He had a twin who died in utero. One patient (pt 13) with mental retardation was heterozygous for p.I268T and p.P165L and had colitis as part of the MKD. The two other patients (pt 14 and 15) with mental retardation had no other severe manifestations.

Macrophage activation syndrome

Macrophage activation syndrome (MAS) arose in one patient (pt 16), who had a heterozygous p.V377I and del Exon 8 mutation. This patient has been described before (21). At the time, the patient only used flurbiprofen at full dosage and the disease was apparently well-controlled. The MAS presented initially as a typical MKD episode with high fever, arthralgia, myalgia and oral aphthae, which led to hospitalization. Two days after hospitalization, the patient was treated with several antibiotics due to presumed sepsis. The clinical picture worsened with initial heart failure and lethargy. Intensive care support with continuous ventilation was required. A bone marrow aspiration revealed activated macrophages and numerous hemophagocytic cells. The patient was treated with high dose methylprednisolone (30mg/kg/day) for four days. Hereafter, maintenance steroid therapy (1mg/kg/day) and ciclosporin (4mg/kg/day) were given.

AA-amyloidosis

AA-amyloidosis was diagnosed in five patients. The median diagnostic delay to the diagnosis of MKD in these five patients was 23 years (range 15 - 41 years). The median disease duration before the diagnosis of amyloidosis was 23 years (range 8 - 37 years).

One of these five patients (pt 17) had a p.V377I mutation in combination with a p.L234P mutation. He presented with end stage renal failure at the age of 39 years and also had involvement of the liver and spleen. A renal transplantation was performed two years after the diagnosis of amyloidosis with good graft function.

The other four patients (pt 18-21) were compound heterozygous for the p.V377I mutation and the p.I268T mutation. In one of these four patients (pt 18), AA-amyloidosis presented with end stage renal failure at the age of 27 years and was diagnosed by biopsy, showing involvement of the kidneys and spleen. She suffered from persistent inflammation with continuously elevated inflammatory parameters. She died at the age of 45 due to complications after eighteen years of dialysis.

Patient 19 presented with end stage renal failure at the age of 19 years. Serum Amyloid P (SAP) component scintigraphy showed involvement of kidneys, spleen and liver. He underwent a live-related renal transplant twice. The first kidney was lost due to recurrent amyloid deposition. The second transplantation led to good graft function. After the first renal transplantation the disease was not well controlled with anakinra and subsequently etanercept. During a period of five years the patient did not use any biological. Four months before the second transplantation treatment with tocilizumab was started with complete response. In his brother (pt 20) amyloidosis was diagnosed at the age of 37 years after presentation with end stage renal failure. SAP scintigraphy showed involvement of the kidneys, spleen and liver. He underwent a live-related renal transplant a year after diagnosis with good result.

The last patient (pt 21) was diagnosed with amyloidosis at the age of eight years. Treatment with colchicine was started due to suspected FMF. At the age of twelve years she underwent kidney transplantation. When she was eighteen years old, she was diagnosed as having MKD. She died at the age of nineteen years.

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. IgM was within normal range in 81 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. This patient has been described before in a diagnostic study (22). The second patient was compound heterozygous for p.R388X and stop397R. In this patient mevalonic acid was measured as part of the whole organic acid screening. It is unknown whether this patient was well or febrile during urine sampling. 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 whilst febrile.

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 exhibited normal enzyme activity in both fibroblasts and leukocytes.

Associations between the genotype and phenotype

To analyze genotype-phenotype associations, we divided all patients into four groups: homozygous p.V377I; compound heterozygous p.V377I and p.I268T; patients with one p.V377I mutation and a second mutation other than p.V377I or p.I268T; and patients without a p.V377I mutation. The frequency of MKD features was compared between the groups. Four out of 25 patients with p.V377I/p.I268T combined heterozygosity experienced amyloidosis as compared to one out of 89 patients with other genotypes ($p=0.049$). Further, patients with mutations other than p.V377I mutation suffered more often from a continuous course, musculoskeletal, severe musculoskeletal involvement and severe gastrointestinal involvement (table 3).

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 reported 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 in eight patients, with three of them having a complete response and the other five a partial response. Nineteen patients used anakinra 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 pattern. One of them was severely affected and suffered from cerebellar syndrome, mental retardation and retinitis pigmentosa. The two other patients were mildly affected and did not have severe manifestations. In at least seven patients with an initial failure or partial response on anakinra the dose of anakinra was raised. This did not lead to complete remission. Four of these seven patients have switched to another therapy, three of them are still using anakinra. In another six patients with an initial partial response, the dose was not raised. Though, three of them are still using anakinra.

Five patients were treated with canakinumab; four of whom achieved complete remission, while one had a partial response. The patient with a partial response was a p.V377I/p.G338D compound heterozygote and had failed on NSAIDs, steroids, anakinra, etanercept and adalimumab, before the initiation of canakinumab. Two patients with a complete response had a partial response on anakinra before treatment with canakinumab.

Etanercept was prescribed to 27 patients: it had a beneficial effect in sixteen patients, of whom two had a complete response. Eleven 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 (87) of these patients has not been described in previous cohorts (10,23). 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 fever, abdominal pain, diarrhoea, vomiting, lymphadenopathy, arthralgia, myalgia and aphthous stomatitis. Five patients suffered from AA-amyloidosis, a frequency almost twice of that reported in previous cohorts (23). This might be due to reporting bias, since these patients are more likely to be enrolled in the registry. Still, this number might be an under-representation due to the limited follow-up: the median age of enrolled patients was only 12 years, while amyloidosis occurred after 18 years in 4/5 patients. Only one patient in our cohort experienced a macrophage activation syndrome, which is less than described in the study of Bader-Meunier *et al* (10).

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 and has been found in increased numbers in rheumatic diseases (24,25). In our cohort patients with constitutional symptoms between attacks did not have a more severe course such as amyloidosis.

According to the enrolling centers, up to 25% of the patients displayed some mood disorders. In the original survey the definition of mood disorders was left to the discretion of the enrolling physicians. However, this relatively high frequency possibly reflects the psychological impact of the disease, as already reported by van der Hilst *et al* (23).

Although uncommon, some patients in our series did have severe musculoskeletal manifestations, such as persistent arthritis. In these severe cases, the differentiation between MKD and other pediatric rheumatic diseases such as systemic juvenile idiopathic arthritis (sJIA) can be hard. However, the disease course with frequent, short fever attacks alternating with prolonged episodes of spontaneous remission renders diagnoses like sJIA highly unlikely.

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 (15). 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 (10,22). The failure to detect mevalonic acid in some may be due to methodological limitations, as isotope dilution had not been employed in all of these patients. Unexpectedly in one patient, measurement of mevalonate kinase enzyme activity was reportedly 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 (8). However, apparently even enzyme activity assays may yield false negative results.

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 (23,26). Seventy-five percent of the patients reported at least some benefit when using NSAIDs, but efficacy was rarely complete. Corticosteroids are more effective to terminate inflammatory attacks, but long-term side effects are a major drawback. Interleukin (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 (26). 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 (26). Some patients did not benefit from the use of biologicals. From the available data it cannot be excluded that these patients received an insufficient dose. However, from seven known patients, dose increase of anakinra did not result in complete control of the symptoms.

The retrospective design of the Eurofever registry comes with a number of limitations. Due to this design, there were a number of variables reported as “not known” by the centers. This was mainly due to the fact that some variables requested by the Eurofever registry could not be retrieved from the clinical chart, because they were not investigated at the moment of the patients’ evaluation. This issue might have induced a bias, with a possible under-representation of some symptoms. 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 (pediatric) immunologists and (pediatric) rheumatologists. As Mevalonic Aciduria patients experience predominantly neurological symptoms, these patients are more likely to be seen by a (pediatric) 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 under-represented as they are more likely to die at a young age (27). Still, our cohort included some patients who can be classified as MA. Also, many patients were enrolled by European centers as the Eurofever registry is predominantly known in Europe. Finally, the involvement of more pediatric than adult specialists may have introduced an age bias. MKD does not seem to carry a high mortality rate, yet the median age of enrolment in our cohort was 12 years. A long follow-up is needed to acquire information about long term complications. A limitation to the interpretation of therapeutic interventions is the absence of clear criteria for complete and partial response. The response was left to the interpretation of the physician. Bias is inevitable due to the lack of control groups and randomization. Furthermore, as it is unknown whether drugs were used simultaneously or sequentially, 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 have described the clinical and genetic features in the largest, international cohort of MKD patients. Most MKD patients suffered from fever, accompanied by gastrointestinal symptoms, lymphadenopathy, arthralgia and aphthous stomatitis. AA-amyloidosis (4%) and macrophage activation syndrome (0.9%) were rare, but severe complications of MKD. Patients could benefit from treatment with NSAIDs, steroids and biologics, mainly IL-1 blockers and etanercept. Statins and colchicine were 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, van Nieuwkoop JA, Meyer CJ, Lobatto S, 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, le Coultre R. Mevalonic aciduria: an inborn error of cholesterol biosynthesis? *Clin Chim Acta* 1985 Oct 31;152(1-2):219-222.
4. Drenth JP, Haagsma CJ, van der Meer JW. Hyperimmunoglobulinemia D and periodic fever syndrome. The clinical spectrum in a series of 50 patients. International Hyper-IgD Study Group. *Medicine (Baltimore)* 1994;73(3):133-44.
5. Simon A, Kremer HP, Wevers RA, Scheffer H, De Jong JG, Van Der Meer JW, et al. Mevalonate kinase deficiency: Evidence for a phenotypic continuum. *Neurology* 2004 Mar 23;62(6):994-997.
6. Houten SM, Kuis W, Duran M, de Koning TJ, van Royen-Kerkhof A, Romeijn GJ, et al. Mutations in MVK, encoding mevalonate kinase, cause hyperimmunoglobulinaemia D and periodic fever syndrome. *Nat Genet* 1999 Jun;22(2):175-177.
7. Drenth JP, Cuisset L, Grateau G, Vasseur C, van de Velde-Visser, S D, de Jong JG, et al. Mutations in the gene encoding mevalonate kinase cause hyper-IgD and periodic fever syndrome. International Hyper-IgD Study Group. *Nat Genet* 1999;22(2):178-81.
8. van der Burgh R, Ter Haar NM, Boes ML, Frenkel J. Mevalonate kinase deficiency, a metabolic autoinflammatory disease. *Clin Immunol* 2013 Jun;147(3):197-206.
9. Cuisset L, Drenth JP, Simon A, Vincent MF, van der Velde Visser S, van der Meer JW, 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.
- 10 Bader-Meunier B, Florkin B, Sibilia J, Acquaviva C, Hachulla E, Grateau G, et al. Mevalonate kinase deficiency: a survey of 50 patients. *Pediatrics* 2011 Jul;128(1):e152-9.
11. Prietsch V, Mayatepek E, Krastel H, Haas D, Zundel D, Waterham HR, et al. Mevalonate kinase deficiency: enlarging the clinical and biochemical spectrum. *Pediatrics* 2003 Feb;111(2):258-261.
12. Hoffmann GF, Charpentier C, Mayatepek E, Mancini J, Leichsenring M, Gibson KM, et al. Clinical and biochemical phenotype in 11 patients with mevalonic aciduria. *Pediatrics* 1993 May;91(5):915-921.

13. Poll-The BT, Frenkel J, Houten SM, Kuis W, Duran M, de Koning TJ, et al. Mevalonic aciduria in 12 unrelated patients with hyperimmunoglobulinaemia D and periodic fever syndrome. *J Inher Metab Dis* 2000 Jun;23(4):363-366.
14. 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.
15. Ammouri W, Cuisset L, Rouaghe S, Rolland MO, Delpech M, Grateau G, 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.
16. Houten SM, van Woerden CS, Wijburg FA, Wanders RJ, Waterham HR. 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.
17. Berody S, Galeotti C, Kone-Paut I, Piram M. A retrospective survey of patients's journey before the diagnosis of mevalonate kinase deficiency. *Joint Bone Spine* 2015 Feb 9.
18. Toplak N, Frenkel J, Ozen S, Lachmann HJ, Woo P, Kone-Paut I, et al. An international registry on autoinflammatory diseases: the Eurofever experience. *Ann Rheum Dis* 2012 Jul;71(7):1177-1182.
19. Infevers, the registry of Hereditary Auto-inflammatory Disorders Mutations URL: <http://fmf.igh.cnrs.fr/infevers>. Accessed on 25-05-2015
20. Balgobind B, Wittebol-Post D, Frenkel J. Retinitis pigmentosa in mevalonate kinase deficiency. *J Inher Metab Dis* 2005;28(6):1143-1145.
21. Rigante D, Capoluongo E, Bertoni B, Ansuini V, Chiaretti A, Piastra M, et al. First report of macrophage activation syndrome in hyperimmunoglobulinemia D with periodic fever syndrome. *Arthritis Rheum* 2007 Feb;56(2):658-61.
22. Jeyaratnam J, ter Haar NM, de Sain-van der Velden MG, Waterham HR, van Gijn ME, Frenkel J. Diagnostic value of urinary mevalonic acid excretion in patients with a clinical suspicion of Mevalonate Kinase Deficiency (MKD). *JIMD Rep.* 2015 Sep 27.
23. van der Hilst JC, Bodar EJ, Barron KS, Frenkel J, Drenth JP, van der Meer JW, 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.
24. Overman C, Kool M, Da Silva JAP, Geenen R. The prevalence of severe fatigue in rheumatic diseases: an international study. *Clin Rheumatol* 2015.

25. Nijhof L, van de Putte, Elise M, Wulffraat N, Nijhof S. Prevalence of severe fatigue among adolescents with pediatric rheumatic diseases. *Arthritis Care Res (Hoboken)* 2015.
26. Ter Haar N, Lachmann H, Ozen S, Woo P, Uziel Y, Modesto C, et al. Treatment of autoinflammatory diseases: results from the Eurofever Registry and a literature review. *Ann Rheum Dis* 2013 May;72(5):678-685.
27. Hoffmann GF, Charpentier C, Mayatepek E, Mancini J, Leichsenring M, Gibson KM, et al. Clinical and biochemical phenotype in 11 patients with mevalonic aciduria. *Pediatrics* 1993 May;91(5):915-921.

Table 1. Clinical characteristics of 114 MKD patients

	n	% during ⁺ every episode	% also present between episodes ⁺⁺
<i>Disease pattern</i>			
Recurrent	99 (87%)		
Continuous with exacerbations	9 (8%)		
Continuous	6 (5%)		
<i>Constitutional symptoms*</i>			
Malaise	70/108 (65%)	53%	23%
Fatigue	69/109 (63%)	52%	35%
Weight loss	16/102 (16%)		
<i>Lymphoid organs</i>			
Generalized enlargement	39/103 (38%)	36%	22%
Cervical lymphadenopathy	96/113 (85%)	64%	15%
Painful lymph nodes	59/99 (60%)	42%	12%
<i>Mucocutaneous involvement</i>			
Aphthous stomatitis	67/111 (60%)	37%	15%
Maculopapular rash	43/111 (39%)	21%	8%
Urticarial rash	16/109 (15%)	38%	38%
Exudative pharyngitis	31/109 (28%)	16%	10%
<i>Musculoskeletal involvement</i>			
Arthralgia	80/113 (71%)	34%	15%
Myalgia	64/112 (57%)	34%	18%
Arthritis	31/109 (28%)	24%	9%
Severe musculoskeletal involvement**	8/110 (7%)		
<i>Gastrointestinal symptoms</i>			
Abdominal pain	98/111 (88%)	44%	16%
Diarrhoea	93/111 (84%)	38%	11%
Vomiting	76/110 (69%)	29%	18%
Severe gastrointestinal involvement***	18/114 (16%)		
<i>Ocular involvement</i>			
Conjunctivitis	11/113 (10%)	9%	36%

Uveitis	2/113	(2%)		
Impaired vision	2/113	(2%)		
Cataract	3/113	(3%)		
<i>Neurological involvement</i>	46/114	(41%)		
Headache	43/114	(38%)	40%	33%
Mood disorders	23/95	(24%)	43%	25%
Severe neurological involvement****	6/114	(5%)		

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, persistent arthritis, osteitis or osteolytic lesion.

***Severe gastrointestinal involvement was defined as aseptic peritonitis, gastrointestinal bleeding, intestinal occlusion, gut perforation, abdominal adhesions, gastrointestinal ulcers and (peri)anal ulcers.

****Severe neurological involvement was defined as cerebellar syndrome, mental retardation or aseptic meningitis.

*During every episode means that the symptom was present during every febrile episode.

**Also present between episodes means that the symptom was not only present during fever episodes, but also in between.

Table 2. Severely affected patients

Pt	Severe manifestations	Mut 1	Mut 2	Age at onset (years)	Diagnostic delay (years)	Episode duration (days)	Disease course	Treatment with biologicals
1	MSK, GI	p.H20Q	p.H20Q	0.2	0.2	4	REC	etanercept PR
2	MSK, GI, EYE	p.H20N	p.R215Q	0.1	14.8	5	REC	anakinra maintenance CR, etanercept PR
3	MSK	p.H20N	p.R215Q	0	4.5	x	CNT	anakinra PR, etanercept fail
4	MSK, GI	p.P165L	p.I268T	0	1.5	x	CNT	anakinra maintenance PR, etanercept fail
5	MSK	p.V377I	p.S329R	7.8	0	4	REC	canakinumab CR, etanercept PR
6	GI, EYE	p.V310L	p.V310L	3.3	23.1	x	CNT	
7	MSK, GI	p.V310L	p.V310L	3	14.2	x	CNT	anakinra maintenance PR
8	GI	p.G336S	p.N166I	4.9	36.1	7	REC	

9	GI	p.V377I	p.I268T	0.1	26.9	4	REC	anakinra during attacks PR, etanercept PR
10	GI, NEU	p.T237S	p.I268T	0.01	0.7	x	CNT	
11	NEU, EYE	p.A334T	p.A141fs	0.01	2.1	4	REC	anakinra maintenance fail
12	NEU	p.V377I	p.V377I	0.5	5.5	4	REC	
13	NEU	p.P165L	p.I268T	0.3	5.4	no specific pattern	REC	anakinra during attacks PR
14	NEU	p.V377I	c.606insG	0.3	1.2	3	REC	
15	NEU	p.V377I	p.I268T	0.6	1.1	5	REC	
16	MAS, EYE	p.V377I	del exon 8	2	4.3	5	REC	anakinra PR, canakinumab CR
17	amyloidosis	p.V377I	p.L234P	2.1	41.1	6	REC	anakinra during attacks PR, etanercept PR, tocilizumab PR
18	amyloidosis	p.V377I	p.I268T	4	23.7	not known	REC	
19	amyloidosis	p.V377I	p.I268T	4	14.8	10	REC	anakinra maintenance PR, etanercept fail, tocilizumab CR
20	amyloidosis	p.V377I	p.I268T	3	24.5	13	REC	anakinra maintenance PR
21	amyloidosis	p.V377I	p.I268T	0.1	17.7	x	CNT	

Severe manifestations: GI: gastrointestinal, NEU: neurological, MSK: musculoskeletal, EYE: ocular.
Disease course: CNT: continuous, REC: recurrent. Therapy: CR: complete response, PR: partial response.

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
Continuous 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
Severe gastrointestinal involvement	0%	16%	12%	39%	0.02

Lymphoid involvement	93%	92%	89%	89%	ns
Ocular involvement	21%	8%	11%	35%	ns
Neurological involvement	64%	48%	30%	47%	ns
Severe neurological involvement	14%	4%	2%	20%	ns
Musculoskeletal involvement	86%	64%	77%	100%	0.025
Severe musculoskeletal involvement	0%	0%	4%	50%	0.001
Amyloidosis	0%	16%	2%	0%	0.049

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.

Figure 1. Flowchart of included patients

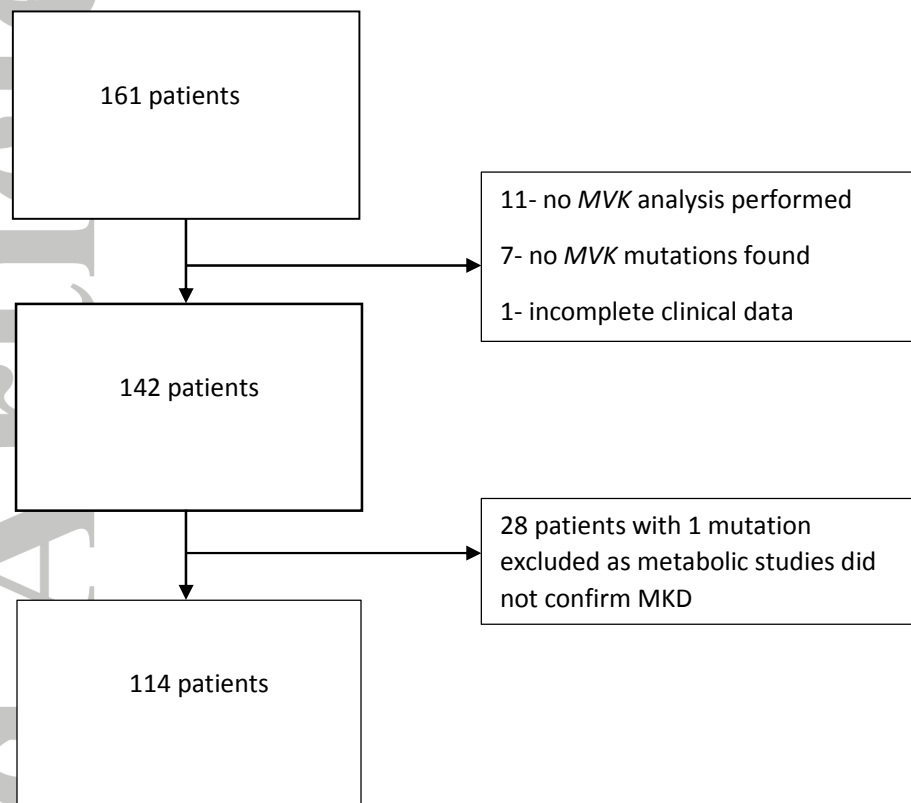
Figure 2. Characteristics of 114 MKD patients

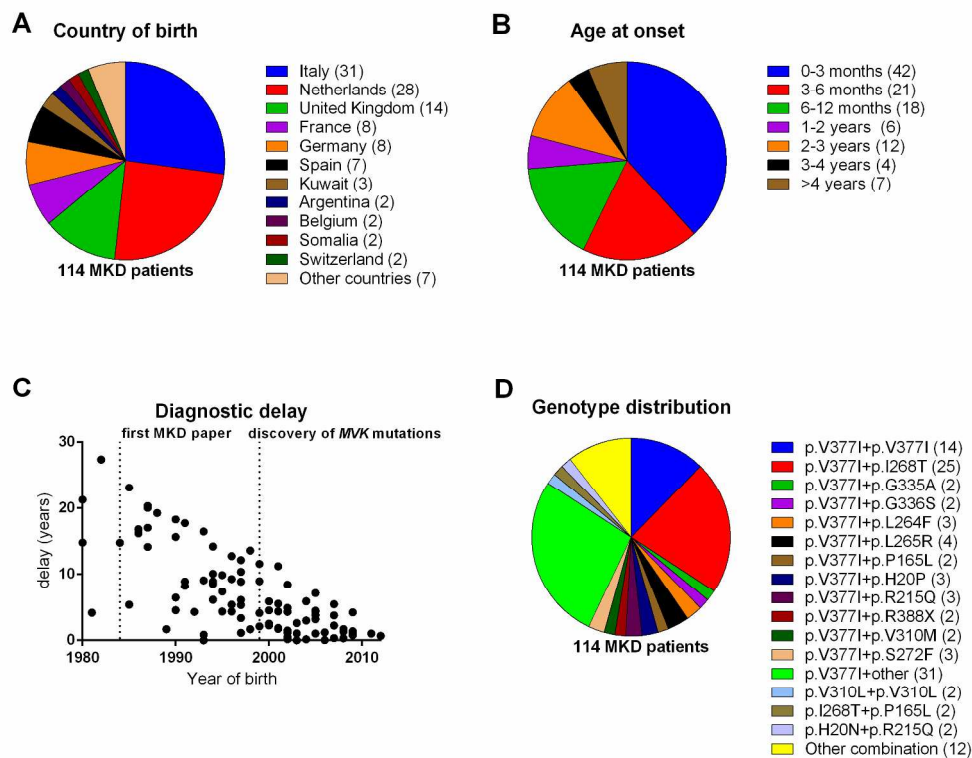
A. Country of birth of 114 MKD patients. The numbers in brackets indicate the number of patients. Other countries included Albania, Australia, Cyprus, Czech Republic, Morocco, Russia and Turkey. B. Age at onset of 114 MKD patients. The numbers in brackets indicate the number of patients. C. Diagnostic delay according to the year of birth from patients born since 1980. D. The genotype of all patients. The numbers in brackets indicate the number of patients.

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. Episode 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).

Figure 1. Flowchart of included patients

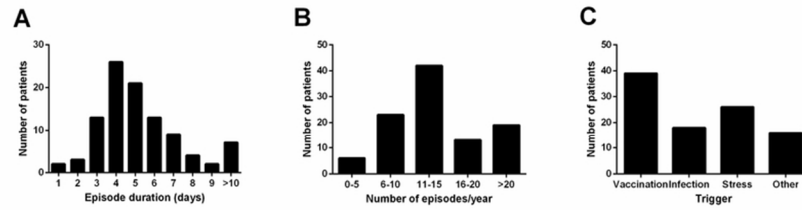




A. Country of birth of 114 MKD patients. The numbers in brackets indicate the number of patients. Other countries included Albania, Australia, Cyprus, Czech Republic, Morocco, Russia and Turkey. B. Age at onset of 114 MKD patients. The numbers in brackets indicate the number of patients. C. Diagnostic delay according to the year of birth from patients born since 1980. D. The genotype of all patients. The numbers in brackets indicate the number of patients.

256x203mm (300 x 300 DPI)

AcceJ



A. Episode duration in days. B. Frequency of the number of episodes per year. C. Episode 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).

82x40mm (300 x 300 DPI)

Accepted