CLINICAL SCIENCE

Clinical characteristics and genetic analyses of 187 patients with undefined autoinflammatory diseases

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ABSTRACT

Objectives To describe the clinical characteristics, treatment response and genetic findings in a large cohort of patients with undefined systemic autoinflammatory diseases (SAIDs).

Methods Clinical and genetic data from patients with undefined SAIDs were extracted from the Eurofever registry, an international web-based registry that retrospectively collects clinical information on patients with autoinflammatory diseases.

Results This study included 187 patients. Seven patients had a chronic disease course, 180 patients had a recurrent disease course. The median age at disease onset was 4.3 years. Patients had a median of 12 episodes per year, with a median duration of 4 days. Most commonly reported symptoms were arthralgia (n=113), myalgia (n=86), abdominal pain (n=89), fatigue (n=111), malaise (n=104) and mucocutaneous manifestations (n=128). In 24 patients, relatives were affected as well. In 15 patients, genetic variants were found in autoinflammatory genes. Patients with genetic variants more often had affected relatives compared with patients without genetic variants (p=0.005). Most patients responded well to non-steroidal anti-inflammatory drugs (NSAIDs), corticosteroids, colchicine and anakinra. Complete remission was rarely achieved with NSAIDs alone. Notable patterns were found in patients with distinctive symptoms. Patients with pericarditis (n=11) were older at disease onset (33.8 years) and had fewer episodes per year (3.0/year) compared with other patients. Patients with an intellectual impairment (n=8) were younger at disease onset (2.2 years) and often had relatives affected (28.6%).

Conclusion This study describes the clinical characteristics of a large cohort of patients with undefined SAIDs. Among these, patients with pericarditis and intellectual impairment appear to comprise distinct subsets.

Key messages

What is already known about this subject?

► Individuals with undefined autoinflammatory diseases represent the majority of patients approaching the services devoted to the diagnosis and management of autoinflammatory diseases.

What does this study add?

► This study provides a detailed description of the clinical characteristics of a large cohort of patients with undefined autoinflammatory diseases, along with known genetic data and response to treatment.

How might this impact on clinical practice or future developments?

► The detailed description of patients with specific symptoms can be used to identify similar patients in other centres and will aid future research regarding the identification of new autoinflammatory diseases.

INTRODUCTION

One of the most frequently observed groups in autoinflammatory disease clinics are patients with undefined systemic autoinflammatory diseases (SAIDs).[1 2] Providing targeted treatment for these patients is difficult, since a definite diagnosis is lacking. Little is known about the clinical and genetic characteristics of these patients, which impedes the identification of novel clusters within this group. This study provides insight into the clinical picture of a large group of patients with undefined SAIDs.

SAIDs are disorders characterised by periodic or persistent activation of the innate immune system in the absence of infection or autoimmunity. In monogenic SAIDs, this is caused by mutations in a single gene.[3] The best-characterised monogenic SAIDs are familial Mediterranean fever (FMF), cryopyrin-associated periodic syndromes (CAPS), tumour necrosis factor-receptor-associated periodic syndrome (TRAPS) and mevalonate kinase deficiency (MKD).[4 5] Other SAIDs, such as periodic fever, aphthous stomatitis, pharyngitis, adenitis (PFAPA) syndrome or Behçet's disease, are

multifactorial; multiple genes may be involved, but there is no single genetic cause. Clinical diagnostic criteria are available for these diseases.[6 7] However, approximately half of the patients with recurrent inflammation do not fit the clinical picture of any well-defined SAID or do not have pathogenic mutations causing a known hereditary SAID.[1 2] This group is said to have 'undefined SAIDs'.

Characteristics of patients with undefined SAIDs have not been extensively described in the current literature. This might be due to the rarity of SAIDs, hampering sufficient patient numbers for research. To overcome this problem, an international network for the study of SAIDs was established, the Eurofever Project.[8 9] Besides the well-defined SAIDs, the Eurofever Project also collects clinical information on patients with undefined SAIDs, providing a sufficient cohort for our study.

This paper describes the clinical characteristics of a large cohort of patients with undefined SAIDs, along with known genetic data and response to treatment.

METHODS

Eurofever registry

Data of patients with undefined SAIDs were collected from the Eurofever registry (Executive Agency for Health and Consumers project no. 2007332), which has been collecting retrospective patient data since November 2009.[9] Data entered before 2 November 2016 were extracted. To enrol patients as undefined SAID in the registry, other confounding conditions (well-defined SAIDs including PFAPA syndrome, infectious, autoimmune, neoplastic) should have been reasonably excluded.

Ethical committee approval and informed consent was obtained in all participating centres. Detailed epidemiological, demographic and clinical data were collected anonymously.

Inclusion and exclusion criteria

Patients of whom clinical information was available were included in this study. The exclusion process consisted of two steps. First, patients were excluded from analysis if there was no evidence of increased acute phase reactants during episodes, if there was no fever reported or if they carried pathogenic mutations classifying them as having a well-defined SAID.

Second, the Federici criteria were retrospectively applied on the cohort. The Federici criteria are clinical criteria for well-defined monogenetic SAIDs.[10] Additional data were collected from patients with a clinical picture consistent with a defined SAID, but without genetic analysis performed on the associated gene.[10] Centres were asked if additional genetic analysis was performed since registration. Subsequently, patients were excluded from analysis if further genetic analysis revealed a defined SAID, if they received a different diagnosis explaining their symptoms or if no additional genetic analysis was performed.

Clinical and genetic information

The clinical characteristics included the disease pattern, disease manifestations and response to treatment. Clinical manifestations were reported by the entering physician as being present never, sometimes/often or always during episodes. Treatment response was graded as complete (absence of clinical manifestations with normalisation of inflammatory markers), partial (general amelioration of the clinical picture but not complete normalisation of the clinical manifestations and/or systemic inflammation) or failure (lack of response). Information on molecular genetic analyses regarding the main SAIDs was collected. Genetic variants were classified as being pathogenic, likely pathogenic, of uncertain significance, likely benign or benign.[11 12] Only pathogenic or likely pathogenic variants and variants of uncertain significance were noted in this study and were regarded as genetic variants in further analyses.[13]

Statistical analysis

Categorical variables were described as frequencies and percentages. Numeric variables were reported as the median and IQR. To compare dichotomous variables with interval or ordinal variables, the Mann-Whitney U test was performed. Correlations between two dichotomous variables, or dichotomous variables and nominal variables were assessed using the χ^2 or Fisher's exact test. The Spearman's rank correlation was performed to assess differences between two interval variables or between interval and ordinal variables.

The threshold for statistical significance was $p<0.05$. Statistical analysis was performed with the IBM Statistical Package for the Social Sciences (SPSS) V.24.

RESULTS

Patient inclusion and clinical classification criteria

In total, 337 patients were included from the Eurofever registry. Patients came from 30 different centres. Clinical information was available for 235 patients. See figure 1 for a detailed flowchart of included and excluded patients. Patients were excluded when inflammatory markers were not elevated during fever episodes (n=26) or no fever was reported (n=3). Additionally, one patient was excluded because he had a pathogenic mutation in the *MEFV* gene and had a positive clinical classification score for FMF, classifying him as FMF patient.[10]

When applying the Federici criteria, a majority of the patients (n=1364,9%) did not classify for any of the major periodic fever syndromes.[10] Twenty-nine patients, coming from 10 centres, had a clinical picture consistent with a monogenetic SAID according to the Federici criteria, without genetic analysis having been performed on the associated gene.[10] For these patients, a specific query was raised to the enrolling centres. Eleven patients had additional genetic analysis performed on the associated genes and turned out to be negative or not confirmatory. The other 18 patients were excluded from analysis (figure 1). Four of them received an alternative diagnosis. In one patient, who clinically classified as MKD, additional genetic tests revealed pathogenic mutations in the *MVK* gene. The other three patients were diagnosed with diseases other than their clinical classification: systemic juvenile idiopathic arthritis (n=2) and ARPC1B-combined immunodeficiency (n=1).

In total, 187 patients were included in this study. Fifty-four patients classified as clinically compatible with one or two hereditary periodic fever syndromes. In four of them, genetic variants were found in the associated genes (figure 2). These variants were not confirmatory of a defined SAID.

Baseline characteristics

Patients came from 17 different countries (online supplementary table 1). Most patients came from Italy (n=103) and the Netherlands (n=21). Almost half of the patients were female (49%). The median age at disease onset was 4.3 years (IQR 1.3–12.9) (figure 3A). Thirty-five patients had a disease onset in adulthood.

Episode characteristics

Seven patients had a chronic disease course, 17 patients had a chronic disease course with recurrent acute exacerbations and 163 patients had a disease course with recurrent episodes.

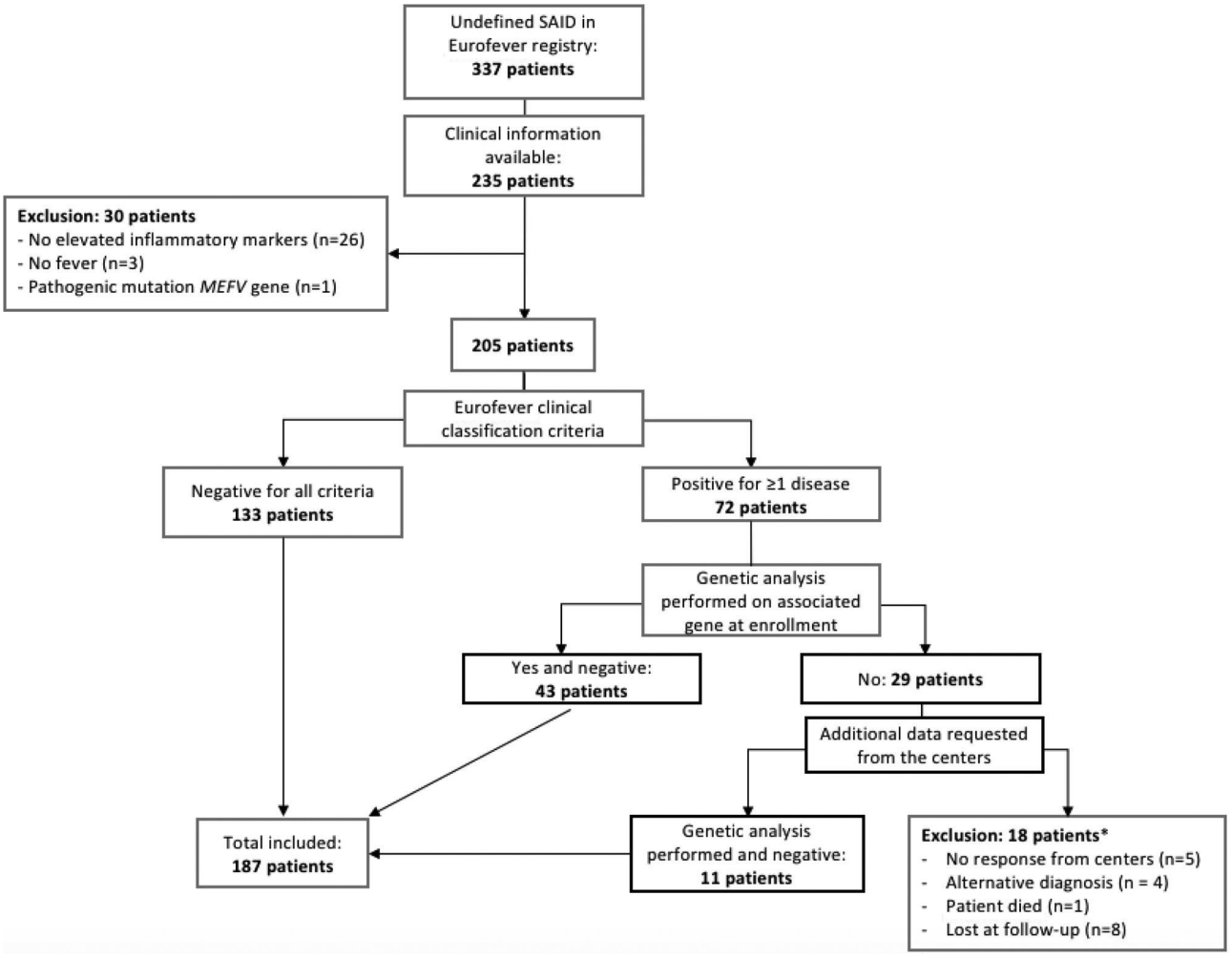

Figure 1 Flowchart of included patients. Eurofever clinical classification criteria: Federici criteria for monogenetic SAID.[10] *In five patients, no response was received from the centres, four patients received an alternative diagnosis (MKD, systemic juvenile idiopathic arthritis, ARPC1B-combined immunodeficiency), one patient died before additional genetic testing was performed and eight patients were lost at follow-up before additional genetic testing was performed. MKD, mevalonate kinase deficiency; SAID, systemic autoinflammatory disease.

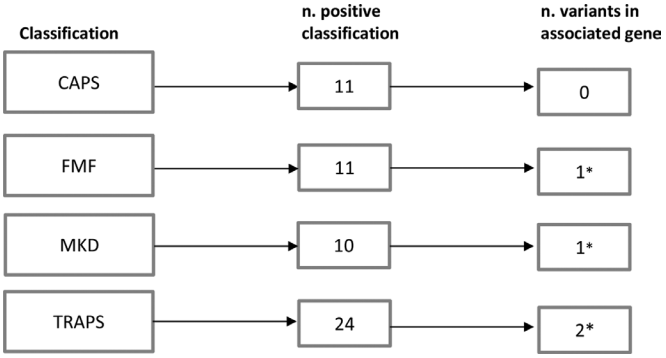

Figure 2 Clinical classification criteria and genetic variants. This figure shows the classification of the 54 patients who fulfilled the clinical criteria for a hereditary periodic fever syndrome.[10] Two of them scored positive for two syndromes: TRAPS and MKD, TRAPS and CAPS. The third column displays the number of patients in whom variants were found in the associated gene. * Variants found in the associated genes: FMF p.A744S; MKD p.T356M; TRAPS p.R92Q; n.=number of patients. CAPS, cryopyrin-associated periodic syndromes; MKD, mevalonate kinase deficiency; TRAPS, tumour necrosis factor-receptor-associated periodic syndrome.

Patients with recurrent episodes had a median of 12.0 episodes per year (IQR 5.0–14.5), with a median duration of 4.0 days (IQR 3.0–7.0) (figure 3B and C). An irregular disease pattern was more frequently seen than a regular disease pattern (55.6% vs 38.5%). A minority of patients (13.4%) reported specific triggers for disease episodes, including emotional stress and infection (figure 3D). Clinical manifestations for patients with chronic and recurrent disease course are summarised in table 1. Most commonly reported symptoms were arthralgia, myalgia, abdominal pain, fatigue, malaise and mucocutaneous manifestations.

Treatment response

Non-steroidal anti-inflammatory drugs (NSAIDs) and steroids were frequently used during attacks. NSAIDs were beneficial in 80/105 patients, but were rarely completely effective. Steroids were beneficial in 85/104 patients (41 complete, 44 partial response). With colchicine therapy, 7/49 patients had a complete response and 22 had a partial response. Thirty patients used colchicine as maintenance therapy and 8 patients used colchicine on demand (11 treatment schedule unknown). Thirteen patients got treated with anakinra, five had a complete response and three had a partial response. Four patients used anakinra as maintenance therapy and four patients used anakinra on demand (five treatment schedule unknown). Methotrexate was

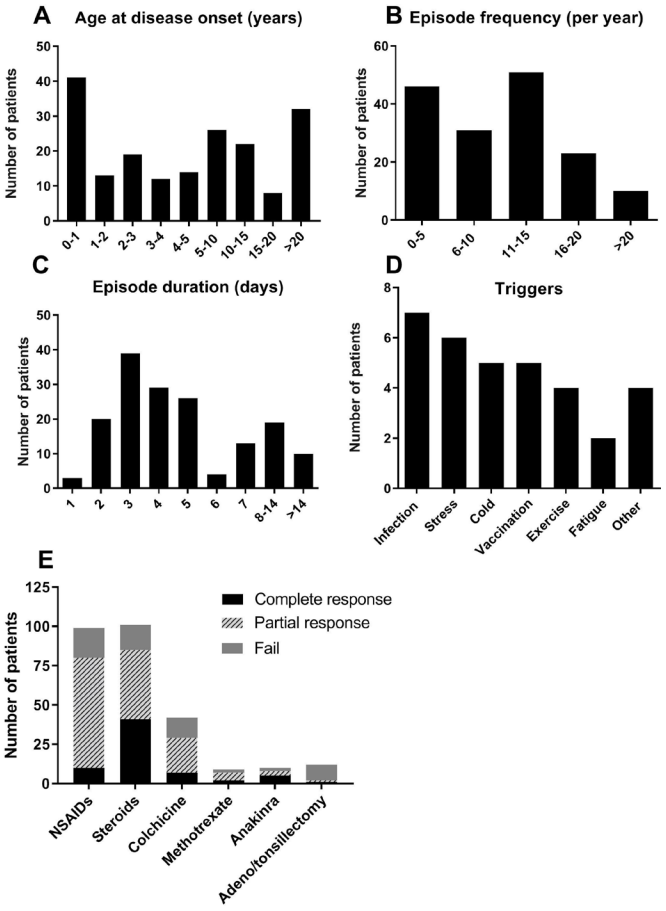

Figure 3 Disease characteristics, medication response. Other triggers were teething (1), surgery (1), constipation (1), heat (1).

given in 10 patients, with a complete response in 2 and a partial response in 5 patients. Adenoidectomy and/or tonsillectomy had limited effect; 8/9 adenotonsillectomies, 1/1 tonsillectomies and 1/2 adenoidectomies were ineffective. Figure 3E summarises the responses to treatment.

Comparing treatment response to other clinical information, we found that patients with a good response to colchicine had a shorter episode duration compared with poor responders (p=0.030). In addition, a regular pattern of febrile episodes was more often described in patients with a good response to steroids or colchicine (p=0.050 and p=0.002, respectively). Patients with a good response to anakinra had a lower episode frequency (p=0.037), were older at disease onset (p=0.018) and more often had an irregular disease pattern (p=0.018) compared with patients with a moderate or bad response to anakinra.

Table 1 Clinical manifestations during episodes

	Chronic disease course (7)	Recurrent disease course patients (180)*	
	n (%)†	n (%)† Always	Sometimes/often
Mucocutaneous	***5 (71%)***	***123 (69%)***	
Aphthous stomatitis	1 (14%)	10 (6%)	43 (24%)
Erythematous pharyngitis	1 (14%)	5 (3%)	42 (23%)
Exudative pharyngitis	0	6 (3%)	27 (15%)
Maculopapular rash	4 (57%)	10 (6%)	25 (14%)
Gastrointestinal	***2 (29%)***	***104 (58%)***	
Abdominal pain	2 (29%)	23 (13%)	64 (36%)
Vomiting	0	7 (4%)	37 (21%)
Diarrhoea	1 (14%)	2 (1%)	28 (16%)
Musculoskeletal	***7 (100%)***	***118 (66%)***	
Arthralgia	6 (86%)	27 (15%)	80 (44%)
Myalgia	6 (86%)	17 (9%)	63 (35%)
Oligoarthritis	1 (14%)	2 12%)	10 (6%)
Ocular	***1 (14%)***	***29 (16%)***	
Conjunctivitis	1 (14%)	0	17 (9%)
Periorbital oedema	0	1 (1%)	8 (4%)
Lymphoid	***5 (71%)***	***89 (49%)***	
Enlarged cervical lnn	1 (14%)	20 (11%)	56 (33%)
Hepatomegaly	1 (14%)	8 (4%)	13 (7%)
Splenomegaly	1 (14%)	6 (3%)	14 (8%)
Cardiorespiratory	***2 (29%)***	***29 (16%)***	
Chest pain	1 (14%)	2 (1%)	19 (11%)
Pericarditis	1 (14%)	1 (1%)	9 (5%)
Neurological	***2 (29%)***	***74 (41%)***	
Headache	2 (29%)	22 (12%)	45 (25%)
Morning headache	0	3 (2%)	19 (11%)
Genito-urinary	***0***	***13 (7%)***	
Urethritis/cystitis	0	0	6 (3%)
Gonadal pain	0	1 (1%)	2 (1%)
Constitutional	***7 (100%)***	***179 (99%)***	
Fatigue	5 (71%)	33 (18%)	73 (41%)
Malaise	5 (71%)	34 (19%)	65 (36%)

Clinical manifestations of all patients, separated for patients with a chronic disease course and recurrent disease course. In grey: number of patients that reported at least one symptom of that organ system. In white: most commonly reported symptoms of that organ system. For patients with a recurrent disease course the separate symptoms are split into always (left column) or sometimes/often present during episodes (right column).
*Patients with recurrent disease course and chronic disease course with recurrent acute exacerbations.
†Percentage of total with chronic or recurrent disease course (7 or 180 patients).
lnn, lymph nodes; n, number of patients.

Family history

Twenty-four patients had affected relatives. In 12 patients, first degree relatives were affected. Three patients had multiple relatives affected. Within our cohort, two patients were related to each other: two sisters from Italy with a disease onset at 1.0 year and 12.0–13.0 attacks/year with a duration of 3.0 days. Common features of these sisters were a recurrent disease course, exudative pharyngitis, bilateral enlarged cervical lymph nodes, fatigue and malaise.

Patients with relatives affected were significantly younger at disease onset (2.0 vs 5.8 years, p=0.007) and more often had a regular disease pattern (63.6% vs 31.5%, p=0.007) compared with patients without relatives affected. Furthermore, genetic variants were more often found in patients with relatives affected (27.3% vs 5.4%, p=0.005).

Genetic characteristics

Analysis of one or more SAID-related genes was performed in 159 patients (85.0%), either by complete gene screening, screening of most relevant exons or screening of most relevant point mutations. In total, 15 patients carried likely pathogenic variants or variants of uncertain significance. Two patients had a genetic variant in the *NLRP3* gene, seven in the *MEFV* gene, two in the *MVK* gene, four in the *TNFRSF1A* gene and last, one patient had a variant in the *NOD2* gene (table 2). No variants were reported in the *PSTPIP1*, *NLRP12*, *ADA2* or *IL1RN* gene.

Table 2 Genetic characteristics

		Molecular analyses						
	n.tested	Complete gene screening	Most relevant exons	Most relevant point mutations	Unknown	n.variants found	Variants	Genetic class*
MEFV	113	33	68	4	8	7	p.A744S p.E148Q p.K25R p.R761H p.S339F	3 3 4 4 3
NLRP3	39	17	15		7	2	p.R488K p.V198M	3 3
TNFRS1A	119	28	84		7	4	p.R92Q	3
MVK	79	40	28	1	10	2	p.T356M	3
NOD2	9	7	2			1	p.R702W/SNP8	3
NLRP12	6	3	1		2	0		
PSTPIP1	3	1	2			0		
ADA2	1	1				0		
IL1RN	1	1				0		

*Genetic classification: 3=uncertain significance, 4=likely pathogenic.
class, classification; n, number of patients.

One patient had variants found in two genes, the p.R92Q variant in the *TNFRSF1A* gene and the p.V198M variant in the *NLRP3* gene.

Distinctive manifestations

More distinctive manifestations were reported in 46 patients (table 3); most frequently reported were seizures (n=10), pericarditis (n=11), intellectual impairment (n=8) and bone alteration/deformity (n=5). A detailed description for these more severely affected patients can be found in online supplementary table 2.

Two groups of patients stood out. First, patients with pericarditis (n=11) were older at disease onset (33.8 vs 4.0 years, p<0.001) and had a lower episode frequency (3.0 vs 12.0/year, p=0.001), which was more often reported as irregular (9/11 vs 95/167, p=0.011). Patients with pericarditis often reported arthralgia (5/11), myalgia (5/11) and abdominal pain (3/11). In 3/9 tested patients, genetic variants were found. The *TNFRSF1A* gene was screened in eight patients, in two patients, the p.R92Q variant was found. The *MEFV* gene was tested in seven patients; in one patient, the p.A744S variant was found, and he did not present a clinical phenotype consistent with FMF.

Second, patients with an intellectual impairment (n=8) were younger at disease onset (2.2 vs 4.7 years, p=0.034). Their median episode duration was 4.5 (3.0–6.3) days, they had a median of 12.0 (5.5–15.8) episodes per year and in 28.6% relatives were affected, and this did not differ from other patients. The following symptoms were more frequently reported in patients with an intellectual impairment: abdominal pain (100% vs 46.9%, p=0.043), arthralgia (100% vs 59.5%, p=0.045), headache (87.5% vs 34.1%, p<0.001), seizures (28.6% vs 4.6%, p=0.050) and generalised lymph node enlargement (57.2% vs 10.1%, p<0.001). Seven of these patients had genetic analyses performed (*TNFRSF1A,* n=5; *MEFV*, n=5; *NLRP3,* n=2; *MVK,* n=5; *PSTPIP,* n=1; *ADA2,* n=1), all without genetic variants found.

Table 3 Distinctive manifestations in 46 patients

	n.		n.
Musculoskeletal	***7***	***Gastrointestinal***	***6***
Bone alteration/deformity	5	Aseptic peritonitis	3
Flexion contractures	3	Gastrointestinal ulcers	2
Osteitis	2	Gastrointestinal bleeding	1
Osteolytic lesions	2	Intestinal occlusion	1
Muscular atrophy	3	Peritoneal adhesions	1
Hyperostotic lesions	1	Gut perforation	1
Neurological	***21***	***Cardiorespiratory***	***15***
Seizures	10	Pericarditis	11
Intellectual impairment	8	Venous thrombosis	1
Aseptic meningitis	2	Arterial thrombosis	1
Cranial neuropathy	3	Pulmonary fibrosis	3
Peripheral neuropathy	1	(mild; severe)	(2; 1)
Hydrocephalus	2		
Cerebellar syndrome	1		
Mucocutaneous	***4***	***Ocular***	***1***
Genital ulcers	2	Retinal vasculitis	1
Pyoderma gangrenosum	1	***Other***	***1***
Necrotic lesions extremities	1	Macrophage activation syndrome	1

n, number of patients.

DISCUSSION

We described a large well-defined cohort of patients with undefined SAIDs, enabling us to provide a broad description of the clinical characteristics, the genetic characteristics and the treatment response.

An advantage of our study is the standardised and elaborate list of symptoms, which yields a comprehensive clinical picture of the patients. In addition to fever, most commonly reported symptoms were: arthralgia, myalgia, abdominal pain, mucocutaneous manifestations, fatigue and malaise. Arthralgia, myalgia and mucocutaneous manifestations were frequently reported in other, smaller cohorts of patients with undefined periodic inflammation as well.[14–18] Fatigue and malaise were reported by more than half of our patients, but were only mentioned in one other study.[18] As fatigue and malaise are generally often encountered by patients with rheumatic diseases, an under-reporting of these symptoms in other cohorts seems to be the most likely explanation of this discrepancy.[18 19] Most of our patients had a disease onset before the age of 5 years. However, even though a relevant number of patients with an adult-onset have been included, a selection bias could have decreased the average age

of onset, due to an over-representation of paediatric centres in the Eurofever project. In other studies, the age of disease onset varied from 4 to 43 years.[14–18]

As in most of the defined SAIDs, the majority of patients in our cohort had a favourable response to NSAIDs, steroids, colchicine and anakinra, but patients rarely achieved complete response with NSAIDs alone.[20–23] Contrary to the good effect observed in PFAPA syndrome, tonsillectomy and/or adenoidectomy were rarely effective in patients with undefined SAIDs.[21 22] Nonetheless, we cannot exclude a reporting bias, as physicians tend to enrol patients with a long-standing or difficult-to-treat disease course and thus leave out patients with a complete response to NSAIDs, tonsillectomy and/or adenoidectomy.

Looking at patients with distinctive manifestations, we found that patients with pericarditis, in line with published data concerning idiopathic recurrent pericarditis, had a disease onset in adulthood and a low episode frequency.[24] However, patients with pericarditis in our cohort seem to form a specific cluster, since they also often suffered from musculoskeletal symptoms and abdominal pain, usually not reported in typical recurrent pericarditis.[25] This could mean that these patients either display an extension of the spectrum of idiopathic recurrent pericarditis or they form a distinct entity. Second, patients with an intellectual impairment often had relatives affected and were young at disease onset. Possibly these patients form a distinct entity on their own as well. Online supplementary table 2 can be used to identify similar patients in other centres with distinctive symptoms.

A limitation of our study is its retrospective design. As mentioned previously, we cannot exclude a bias in the selection of patients entered in the registry, favouring patients with more severe disease. An additional selection bias was introduced by the design of this analysis, excluding patients with normal acute phase proteins. Furthermore, for some patients, parts of the clinical variables were missing as they were not retraceable from their clinical charts. More importantly, the lack of prospective follow-up data hampers conclusions regarding outcome and long-term therapy response in these patients. The treatment response was also difficult to interpret due to the possibility that the natural disease course or simultaneous use of other drugs influenced the response to therapy.[21] To overcome these limitations in future research, a follow-up registry has recently been implemented by the Eurofever working group.[26]

We have found a correlation between the presence of genetic variants and a positive family history of (undefined) SAIDs. Whether this represents a causal relation is uncertain. One might reason that these genetic variants, although not by themselves pathogenic, could contribute to autoinflammation in combination with environmental triggers or other (epi)genetic factors. However, there may be mere confounding by indication as patients with a positive family history might have been more likely to undergo genetic testing. Furthermore, the method of genetic screening varied among patients and this registry was not designed for in-depth analyses of family history nor disease aetiology. Laboratory experiments and population-based genetic studies are necessary to define a causal relation between genetic variants and autoinflammation.

One of the main limitations of this study is the lack of homogeneous and complete genetic analysis in the entire population of patients included in our study. Hence, genetic diagnoses might have been missed, because the relevant genes or the relevant regions of the affected gene were not tested. In particular, genes that had not been identified as cause of autoinflammatory disease when the registry started, like *TNFAIP3* causing A20 haploinsufficiency, may not have been tested in patients with the relevant phenotype.[27] Similarly, somatic mosaicism for autosomal dominant mutations would not have been detected. Genetic screening was often limited, due to the large geographical distribution of the enrolling centres and limited availability of molecular screening in some centres. Notably, most patients were enrolled from 2009 onwards, far before next generation sequencing of gene panels was routinely used. We tried to minimise the chance of erroneously including patients with a well-defined SAID, by excluding those with a clinical picture indicative of such a disease, but in whom the relevant genetic analysis had not been performed.

We want to stress the importance of thorough diagnostics. Many patients in our cohort classified positive with the Federici clinical score.[10] This confirms the difficulty in differentiating between undefined and defined SAIDs on clinical grounds only and suggests a need for new classification criteria for monogenic autoinflammatory diseases, which should combine both genetic and clinical variables. The age of sequential single gene analysis is over. Patients with undefined SAIDs deserve next generation sequencing with gene panels and, if negative, to proceed to whole exome sequencing, where and when available and affordable.

In conclusion, we provide a detailed description of the clinical characteristics of a large well-defined international cohort of patients with undefined SAIDs. This protean group of patients represents one of the most frequent subsets observed in the daily practice of autoinflammatory disease clinics.[1 2] Despite the large variability of this heterogeneous group of patients, the availability of a relevant number of affected individuals allowed to identify some interesting clues. A relevant proportion of the patients had other affected family members. When available, a whole exome sequencing approach would be appropriate in such families in order to identify possible new genes. Moreover, some distinctive manifestations (like pericarditis or intellectual impairment) could allow the identification of novel SAID clinical clusters, possibly related to specific genes.

In this study, we described the characteristics of patients with undefined SAIDs as a single group. However, different underlying causes for autoinflammation are undoubtedly present in this cohort. Future research, combining extensive genetic data with functional and phenotypic data, is likely to provide insight into genotype-phenotype relation, leading to the eventual identification of new SAIDs within this group.[28]

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REFERENCES

1 Hernández-Rodríguez J, Ruíz-Ortiz E, Tomé A, *et al*. Clinical and genetic characterization of the autoinflammatory diseases diagnosed in an adult reference center. *Autoimmun Rev* 2016;15:9–15.
2 Rigante D, Vitale A, Lucherini OM, *et al*. The hereditary autoinflammatory disorders uncovered. *Autoimmun Rev* 2014;13:892–900.
3 Kanazawa N. Rare hereditary autoinflammatory disorders: towards an understanding of critical in vivo inflammatory pathways. *J Dermatol Sci* 2012;66:183–9.
4 Rigante D, Frediani B. Cantarini L. A comprehensive overview of the hereditary periodic fever syndromes. *Clin Rev Allergy Immunol* 2016:1–8.
5 Ozen S, Bilginer Y. A clinical guide to autoinflammatory diseases: familial Mediterranean fever and next-of-kin. *Nat Rev Rheumatol* 2014;10:135–47.
6 Thomas KT, Feder HM, Lawton AR, *et al*. Periodic fever syndrome in children. *J Pediatr* 1999;135:15–21.
7 Koné-Paut I, Shahram F, Darce-Bello M, *et al*. Consensus classification criteria for paediatric Behçet's disease from a prospective observational cohort: PEDBD. *Ann Rheum Dis* 2016;75:958–64.
8 Ozen S, Frenkel J, Ruperto N, *et al*. The Eurofever project: towards better care for autoinflammatory diseases. *Eur J Pediatr* 2011;170:445–52.
9 Toplak N, Frenkel J, Ozen S, *et al*. An international registry on autoinflammatory diseases: the Eurofever experience. *Ann Rheum Dis* 2012;71:1177–82.
10 Federici S, Sormani MP, Ozen S, *et al*. Evidence-based provisional clinical classification criteria for autoinflammatory periodic fevers. *Ann Rheum Dis* 2015;74:799–805.
11 Richards S, Aziz N, Bale S, *et al*. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of medical genetics and genomics and the Association for molecular pathology. *Genet Med* 2015;17:405–23.
12 Van Gijn ME, Ceccherini I, Shinar Y, *et al*. New workflow for classification of genetic variants' pathogenicity applied to hereditary recurrent fevers by the International Study Group for Systemic Autoinflammatory Diseases (INSAID). *J Med Genet* 2018:jmedgenet-2017-105216.
13 Shinar Y, Obici L, Aksentijevich I, *et al*. Guidelines for the genetic diagnosis of hereditary recurrent fevers. *Ann Rheum Dis* 2012;71:1599–605.
14 Simon A, van der Meer JWM, Veselý R, *et al*. Approach to genetic analysis in the diagnosis of hereditary autoinflammatory syndromes. *Rheumatology* 2006;45:269–73.
15 Cantarini L, Rigante D, Merlini G, *et al*. The expanding spectrum of low-penetrance TNFRSF1A gene variants in adults presenting with recurrent inflammatory attacks: clinical manifestations and long-term follow-up. *Semin Arthritis Rheum* 2014;43:818–23.
16 Harrison SR, McGonagle D, Nizam S, *et al*. Anakinra as a diagnostic challenge and treatment option for systemic autoinflammatory disorders of undefined etiology. *JCI Insight* 2016;1:1–15.
17 Yang JA, Choi JY, Kang EH, *et al*. Clinical and genetic features of Korean patients with recurrent fever and multi-system inflammation without infectious or autoimmune evidence. *J Korean Med Sci* 2016;31:196–201.
18 Levy R, Gérard L, Kuemmerle-Deschner J, *et al*. Phenotypic and genotypic characteristics of cryopyrin-associated periodic syndrome: a series of 136 patients from the Eurofever registry. *Ann Rheum Dis* 2015;74:2043–9.
19 Ter Haar NM, Jeyaratnam J, Lachmann HJ, *et al*. The phenotype and genotype of mevalonate kinase deficiency: a series of 114 cases from the Eurofever registry. *Arthritis Rheumatol* 2016;68:2795–805.
20 ter Haar NM, Oswald M, Jeyaratnam J, *et al*. Recommendations for the management of autoinflammatory diseases. *Ann Rheum Dis* 2015;74:1636–44.
21 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;72:678–85.
22 Vanoni F, Theodoropoulou K, Hofer M. PFAPA syndrome: a review on treatment and outcome. *Pediatr Rheumatol* 2016;14:1–5.
23 Demirkaya E, Erer B, Ozen S, *et al*. Efficacy and safety of treatments in familial Mediterranean fever: a systematic review. *Rheumatol Int* 2016;36:325–31.
24 Lazaros G, Imazio M, Brucato A, *et al*. Anakinra: an emerging option for refractory idiopathic recurrent pericarditis: a systematic review of published evidence. *J Cardiovasc Med* 2016;17:256–62.
25 Soler-Soler J, Sagristà-Sauleda J, Permanyer-Miralda G. Relapsing pericarditis. *Heart* 2004;90:1364–8.
26 Girschick H, Finetti M, Orlando F, *et al*. The multifaceted presentation of chronic recurrent multifocal osteomyelitis: a series of 486 cases from the Eurofever international registry. *Rheumatology* 2018;57.
27 Zhou Q, Wang H, Schwartz DM, *et al*. Loss-of-function mutations in TNFAIP3 leading to A20 haploinsufficiency cause an early-onset autoinflammatory disease. *Nat Genet* 2016;48:67–73.
28 Rusmini M, Federici S, Caroli F, *et al*. Next-generation sequencing and its initial applications for molecular diagnosis of systemic auto-inflammatory diseases. *Ann Rheum Dis* 2016;75:1550–7.