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Diagnosis and treatment of patients with undefined autoinflammatory diseases

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Summary

Sterile inflammation characterizes a heterogeneous group of primary immunodeficiency disorders named autoinflammatory diseases (AID). Less than 30% of AID patients are molecularly defined. To increase the diagnostic rate and treatment outcome of patients with undefined AID, "omics" technologies, as the next-generation sequencing and mass spectrometry, and clinical data registries analysis are applied.

In the present study, I describe:

- patients with undefined [page 3] and known rare AID (i.e. RAS-associated autoimmune leukoproliferative disease [pages 25] and SAMD9L-associated AID [pages 33]);
- the first 100 genes and related pathways associated with AID [page 42], the actin-related AID [page 71] and the syndrome of undifferentiated recurrent fever [page 89];
- the proteomic signature of hereditary recurrent fevers [page 104].

Furthermore, I analyze the efficacy of interleukin 1 inhibitors in:

- systemic juvenile idiopathic arthritis [page 163];
- cryopyrin associated periodic syndrome [page 198];
- refractory hyperferritinemic syndromes [page 202].

Finally, I present a metadata registry called MERITA [page 210], developed to increase the interoperability and data sharing among clinical registries of the European Reference Network on Rare Immunological Disorders (ERN-RITA).

Next generation sequencing panel in undifferentiated autoinflammatory diseases identify patients with colchicine-responder recurrent fevers

Systemic autoinflammatory diseases (SAID) are a heterogeneous group of innate immune system disorders, characterized by sterile inflammation without evidence of pathogenic autoantibodies or auto-reactive T lymphocytes.[1,2] Originally, these disorders were limited to a handful of rare monogenic diseases (recurrent fevers), with the first autoinflammatory gene, *MEFV*, responsible for familial Mediterranean fever (FMF), discovered in 1997.[3,4] Since then, a substantial progress has been made, with the identification of at least thirty autoinflammatory genes accounting for conditions showing overlapping features.[5-12] Depending on the mode of inheritance and on the either *de novo* or inherited occurrence of the mutation(s), the identification of one or more variants with a known pathogenic impact and high penetrance represents an essential final step for the diagnosis of monogenic SAIDs. However, in a considerable proportion of patients (70-80%) molecular analysis is unable to provide a diagnostic confirmation. It is part of daily practice that a relevant percentage of patients with clinical manifestations clearly consistent with SAID were classified as affected by undifferentiated or undefined SAID (uSAID).[13-16] Little information is available on the clinical presentation, outcome and response to treatment in this heterogeneous group. On the other hand, NGS technologies are revolutionary diagnostic tools for genetic conditions, allowing the simultaneous analysis of different genes associated with a given group of inherited disorders.[17] Massive sequencing, therefore, represents a powerful approach to enable a definitive diagnosis in patients with uSAIDs, as preliminary shown by our group with a 10-genes panel.[18-23]

This study is aimed to test a novel 41-gene NGS diagnostic panel tool and to explore its possible utility in improving the diagnostic yield in a population of uSAID followed in a tertiary center for SAID.

NGS panel design and patients recruitment

An NGS diagnostic panel with forty-one genes related to SAIDs was developed, including genes reported in the Infevers database in 2015, in addition to 15 genes known at that time to be responsible for interferonopathies), and the SERPING1 gene. Ion AmpliSeq™ designer software was used to design the panel, composed of the gene coding portions, plus 20 bp flanking each exon, for a total of 139.35 Kb, covered with 501 amplimers (two primer pools). Libraries preparation was

carried out according to Thermo Fisher's protocols. According to the main clinical manifestations, genes were clustered in seven subpanels: A, recurrent fevers; B, urticarial rash; C, skin bone and articular, involvement; D, intestinal involvement; E, panniculitis, vasculopathy (type I interferonopathies); F, Aicardi-Goutierrez syndromes; G, others (supplementary 1). The inclusion criteria for the study were the following: i) patients with symptoms related to SAID with a pediatric onset; ii) exclusion of other common etiologies, such as neoplasms, infections, autoimmune diseases, and immunodeficiency; iii) negative or not conclusive molecular diagnosis based on Sanger sequencing of suspected genes, iv) for patients with recurrent fevers, exclusion of the PFAPA syndrome by means of the absence or infrequency (less than 30% of the fever episodes) of classical clinical symptoms triad: pharyngitis, aphthous stomatitis, and cervical adenitis.[24] A written consent approved by G. Gaslini Institute Ethical Board was obtained from all the participants. All clinical, laboratory and radiological data were available at the private software of the Pediatric Rheumatology Clinic and the Medical Genetics Unit of our Institute.

Sequencing and variants validation

FastQ data were generated by Ion PGM™ semiconductor and analyzed by the Ion Reporter™5.0. Coverage analysis was performed by the Ion Coverage Analysis plug-in v5.2.1.2. Coding regions that were not included in the experimental design and amplimers covered <10X in the requested genes were analyzed by Sanger sequencing. All missense, frameshift and splice-site variants were confirmed by the Sanger method if reported with a frequency lower than 3% in the general population, according to the ExAC or 1000 Genomes databases.[25, 26]

Genotype-phenotype correlation

For patients without a confirmatory genotype, each variant was considered consistent with the clinical phenotype or not according to the data about associated symptoms in the literature (supplementary 2). Furthermore, each variant was considered possibly pathogenic (PPV) if reported as damaging by 3 or more software tools between the five available on http://varsome.com, or if showed a CLINVAR score higher than 3.[27-33] PPVs were labeled as either likely pathogenic (LP), variants of unknown significant (VUS), or likely benign (LB) according to different criteria (supplementary 3). Family segregation analysis was performed when parental samples were available. Four groups of patients were identified: patients with a confirmatory genotype (group 1), patients with PPV consistent with clinical phenotype (group 2), patients with PPV not consistent with clinical phenotype (group 3), and patients with no new pathogenic, benign variants or no variants (group 4).

Statistical analysis

Categorical data were expressed as number and percentage, and continuous variables as median and range. Comparison of disease characteristics between patients groups were performed by chi-square test or T test as appropriate and where significant interaction were determined a post-hoc analysis was performed to determine if significant differences existed between different populations and adjust for multiple comparisons. A p value <0.05 was considered statistically significant. R statistics version 3.5.0 was used for all statistical analyses.

Demography and clinical features

Fifty patients were enrolled at the median age of 11 years (table 1). All were Caucasian with the median disease duration of 7 years, with at least two years of follow-up in our Centre. All the patients had previously been screened for at least one SAID-related gene, with an average of three genes for each patient (supplementary 4). The majority (36 patients; 72%) presented recurrent fevers but fourteen displayed a prevalent chronic inflammatory disease course (supplementary 5). Two of them (#5 and #22 in table 2) had urticarial rashes as major clinical feature. Both were negative for mutations or mosaicisms of the *NLRP3* or *NLRP12* genes. Six patients (#1, #8, #9, #19, #25 and #48 in table 2) showed a peculiar skin, bone or joints inflammatory involvement, presenting cutaneous abscesses, periostitis or osteitis. Except for two patients, all achieved a complete or partial response with steroids and one required hormonal therapy for steroid-caused puberty delay. Genes previously studied in these patients were *NOD2*, *PSTPIP1* and *ILRN1*. Only one patient (#43 in table 2) presented a prominent intestinal involvement and five (#15, #18, #24, #31, and #50 in table 2) displayed clinical features suggestive for a possible interferonopathy, as panniculitis, discoid lupus-like lesions, polyarthritis or myositis. All these patients were steroid-dependent, but colchicine, methotrexate and rapamycin were not effective in this subgroup. No patient was suspected for disease related to genes included in the F or G subpanels. Three patients (#27, #44, #50 in table 2) displayed at least one episode of macrophage activation syndrome and were also screened for hereditary forms of hemophagocytic lymphohistiocytosis.

NGS results

A mean coverage of 318.7X was achieved in the 50 DNA samples, with only four amplicons represented at <10X (figure 1). A mean of 173.17 variants for each patient was detected, and 100 variants were finally considered (figure 2). All the validated variants were heterozygous (except for four homozygous variants of patient #1, #11, #19, and #38 in table 2) and missense (except for the

c.2807+1G>A substitution of the *IFIH1* gene of patient #5 in table 2). Fifteen variants were detected in our dataset with an allele frequency higher than in public databases (supplementary 6).

Genotype-phenotype correlation

Patients with a confirmatory genotype

Bi-allelic mutations of *MVK* gene with a clear pathogenic relevance were detected in two patients, who received a final diagnosis of MKD. The first patient (#1 in table 2) displayed an atypical presentation with a chronic and persistent inflammatory disease course dominated by a severe bone involvement (supplementary 7). The urinary secretion of mevalonic acid confirmed the diagnosis. Anakinra was started with a prompt amelioration of the inflammatory manifestations. The other patient (#2 in table 2) was previously analyzed for *MVK* exons 2, 8, 9, 10, and 11 with standard Sanger sequencing, allowing the identification of a V377I variant only. At the time of examination the patient was adult and presented a sub-chronic disease course with persistent inflammation, arthralgia, sporadic abdominal pain, and gastrointestinal complains, beside displayed recurrent fevers during childhood. The NGS analysis detected the second *MVK* variant. Canakinumab was started with complete response.

Patients with PPV consistent with clinical phenotype

Two patients (#3 and #4 in table 2) carried novel missense variants of the *PLCG2* gene. Patient #4 presented episodes of urticarial rash and systemic inflammation, while patient #5 had neither systemic inflammation nor immune abnormalities, as expected in PLAID.[34] Functional tests are ongoing and will help to determine the actual pathogenic relevance of the variants found in these patients.

Patients with PPV not consistent with clinical phenotype

Sixteen patients (30%) carried PPV specific for unrelated autosomal dominant (AD) diseases and nine displayed at least one PPV responsible for autosomal recessive (AR) disorders, being therefore asymptomatic carriers. Indeed, eighth patients without skin involvement (#5, #6, #7, #8, #9, #10, #11, #12 in table 2) showed PPV associated with bone disorder, psoriasis or chronic urticarial rash, and two (#13 and #14 in table 2) displayed *NOD2* variants but no inflammatory bowel manifestations. Six patients (#15, #16, #17, #18, #19, #20 in table 2) presented variants associated with type 1 interferonopathies. Four patients (#22, #23, #24, #25 in table 2) presented skin/joint inflammatory involvement and pathogenic variants. In particular, patients #22 and #23 presented *IL36RN* mutations and complete/partial response to anti-IL1 treatment, and it is known that

individuals with *IL36RN* gene mutations up-regulate IL-1 production in response to IL-36 stimulation.[35] Furthermore, the p.Ser113Leu variant of the *IL36RN* gene has been found to be significantly more frequent in patients with inflammatory skin manifestations.[36] Patient #24 displayed the p.Ala177Thr variant of the *RNASE2B* gene, whose carriers have been reported to be asymptomatic, while patient #25 had a positive interferon signature.[37,38] Four patients (#26, #27, #28, #29 in table 2) presented recurrent fevers that characterize the Majeed syndrome, but their *LPIN2* gene variants are considered of unknown significance.

Patients with no new pathogenic variants, benign variants or no variants

The NGS panel was not able to identify any PPV in 21 patients (42%). The heterozygous p.Val726Ala variant of the *MEFV* gene has previously been identified by Sanger analysis in patients #30 and #31 in table 2. This variant is considered pathogenic: the lack of other variants in genes of the NGS panel together with the clinical manifestations associated with fever episodes prompted to classify the patient #30 as heterozygous FMF.[39] The patients had a complete response to colchicine. Conversely, the patient #31 is still considered affected by uSAID because the clinical picture was inconsistent with FMF. The remaining nineteen patients displayed only likely benign variants or did not show any noteworthy variant.

The subset of patients with undifferentiated recurrent fever episodes

This study has provided the opportunity to describe a large subgroup of patients presenting with recurrent fever episodes inconsistent with a classical PFAPA phenotype and negative for genes associated with hereditary recurrent fevers.[40-44] The main clinical features of this subgroup (marked with asterisk in table 2) are described in figure 3. Fever episodes lasted on average of six days ($P<0.0001$), with a median symptoms-free period of three weeks (range 1-6), similar to PFAPA syndrome. Abdominal pain (usually not associated with nausea, vomiting or diarrhea) and limb pain were the most common symptoms during the fever episodes (supplementary 8). The classic PFAPA triad (pharingotonsillitis, apthousis and cervical lymphadenopathy) was less frequently reported ($P<0.0001$). On the other hand, skin rash and arthritis were significantly more frequent ($P<0.0001$). Atypical for PFAPA syndrome, hepato-splenomegaly was reported in 15% of our patients. Rare symptoms in our cohort were periorbital edema (2 patients) and pericarditis (1 patient). Eighteen patients were exclusively treated with steroid on demand with a high response rate (supplementary 8). In 18 patients colchicine treatment was used with an overall complete or partial response in 14 of them (78%). Anakinra was ineffective in 3 out of 4 colchicine-resistant patients, suggesting an IL1β-independent pattern of inflammation in these patients. Only a partial response was achieved

with mycophenolate, mofetil, or etanercept in some cases (supplementary 5). Other disease modifying anti-rheumatic drugs (azathioprine or methotrexate) were also used without response. Tonsillectomy was ineffective in one patient.
We analyzed 6 patients with disease onset after 10 years of age (#8, #22, #23, #31, #44, #48, in table 2) and not highlighted relevant differences of clinical manifestations and treatment response from the entire study cohort. Furthermore, variants are randomly distributed among the different phenotypes according to their main pathogenic pathways (supplementary 9).

In this study, we tested a 41-gene NGS diagnostic panel in a group of fifty consecutive patients referred to our tertiary-care Center and affected by uSAID to explore the pros and cons of such NGS approach in their diagnostic work-up. The 41-gene panel allowed us to achieve a definitive diagnosis in two patients. Interestingly, they presented an atypical form of MKD. This might have been one of the reasons why, despite initial suspects, the solution of these cases came only after NGS based testing.
Indeed, after a long-standing experience and broad literature data, the Sanger sequencing approach has turned out ineffective unless the clinical picture is totally consistent with one of the known monogenic simple SAIDs and a clinical diagnosis can already be made with a high degree of certainty. Therefore, NGS may represent a valuable and effective technique when either the first determination by Sanger sequencing results to be inconclusive or the clinical pictures is not typical for a specific conditions.
However, the final diagnostic yield achieved by present NGS panel in uSAIDs was rather low, with 2 out of 50 patients receiving a genetic confirmatory diagnosis and only other two patients displayed genetic variants possibly causative of their condition. On the other hand, many patients were carriers of possible pathogenic variants in genes that were not consistent with the clinical phenotype, and another relevant group displayed either no pathogenic variants or monoallelic variants for AR diseases. These cases raise the question of the careful interpretation of data coming from the NGS analysis. Indeed, variants should be assessed in the context of a multidisciplinary discussion among geneticists and expert clinicians. To facilitate the NGS data interpretation, and to homogenize diagnosis among different centers, editing of proper guidelines for genotype interpretation and variants reporting would be of great value. A first attempt has recently been carried out by the INSAID project for the four inherited periodic fevers (CAPS, TRAPS, MKD and FMF) [45] and the classifications are available in the Infevers database (http://fmf.igh.cnrs.fr/ISSAID/infevers/).
One possible concern about the systematic use of the NGS in the diagnostic work-up is related to

the costs of this technique. Patient #1 had a very intense history of admissions in different local hospitals during his first 6 years of disease trying to reach the correct diagnosis. A rough calculation of the direct costs imputable to these admissions reveals the presumptive amount of 30.000 Euros. Conversely, the costs of the present study (including materials and man-power for the personnel involved) are calculated to be less than 500 Euros/sample, including the final Sanger Seq validations, for a total of almost 25.000 euros for all the 50 samples. Thus, at least in the present experience, the diagnosis of patient #1 provides alone the justification of the costs of our whole study. Indeed the actual economic impact of the NGS approach was far behind the aims of the present study. In fact, such evaluation would require a specialized methodological approach using specific pharma economic tools.

It is conceivable that the use of NGS panels with a larger number of genes would significantly increase the rate of diagnosis, as recently shown by a similar experience in an equivalent number of vasculitic and SAID patients screened with two panels of 113 and 166 genes, respectively.[19] In these panels, genes related to other immune-mediated diseases were included. The clinical overlap of SAID with the enlarging spectrum of monogenic conditions characterized by an immune dysregulation suggests to adopt either larger panels or, ultimately, the whole exome sequencing (WES) with an intermediate step of *in silico* analysis of all the genes possibly involved, including those of recent identification. This would overcome the present main limitation of NGS panel that does not allow a fast update of the list of genes possibly involved. The progressive reduction of the costs for WES and the improvements in bioinformatics analysis will likely allow the adoption of WES for routinely diagnostic procedures in the next future. This approach would also provide the advantage to identify new candidate genes, involved or not in pathways already known to be associated with SAID, with relevant implications also on the research side.

The low detection rate with the 41-gene panel observed in the present study is also likely due to the high percentage of patients with recurrent fever. It is conceivable that a small percentage of these patients could be secondary to mutations of genes not identified yet. However, it is also possible that most of them might present a multifactorial autoinflammatory condition different from the well-characterized PFAPA syndrome. Indeed, the ambiguity of the available PFAPA criteria may allow to classify these latter patients as affected by PFAPA syndrome, independently of the actual frequency of the cardinal manifestations.[24,41] In this subgroup, the presence of low penetrance variants or polymorphisms does not seem to influence the clinical phenotype. Of note the same variants could be also observed in a number of other multifactorial conditions, such as multiple sclerosis and recurrent pericarditis,[46,47] however their actual impact in conferring susceptibility to mount a pro-inflammatory response in these disorders is still largely debated.

The present study offers an original description of this particular subset of patients. Recurrent abdominal and limb pain and skin rash were the most frequent clinical manifestations. Whenever present, pharyngitis was reported to be sporadic and only seldom associated with a clear exudative tissue reaction. Beside steroid on-demand, that is usually effective, a relevant percentage of these patients display a complete or at least partial (i.e. evident reduction of the frequency and intensity of fever episodes) response to colchicine. This percentage is significantly higher than classical PFAPA patients. In fact, beside the fact that a recent paper has described a good response to colchicine in a PFAPA cohort, patients with one *MEFV* mutation were not excluded in that study and a lower frequency of fever episodes was considered sufficient to evaluate colchicine as effective.[43] Furthermore, at variance with PFAPA syndrome, in the present study tonsillectomy was not used as a possible strategy to reduce fever attacks showing a failure in the single case in which it was performed.

This heterogeneous group of undefined periodic fever does represent an interesting subgroup for future genetic and functional investigations. According to the historical Tel-Hashomer diagnostic criteria, the response to colchicine would allow, independently of the presence of *MEFV* mutations, to classify these patients as clinical FMF.[48] However, this subgroup presented a longer duration of fever episodes (median 6 days) and a high prevalence of skin rash that is not usually observed in FMF. It is in fact possible that a proportion of these patients could be carriers of mutations typical of novel genetic defects, but it is also conceivable that most of them present a multifactorial inflammatory condition, possibly affecting the RhoA-dependent activation of pyrin inflammasome, independently of the presence of mutations of *MEFV* gene. With the aim to characterize this distinct clinical subgroups of patients from the more common PFAPA syndrome we suggest to use the term of systemic undefined recurrent fevers (SURF), previously proposed,[49] and a flow chart for the identification and treatment of these patients is proposed in figure 4.

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Table 1. Overview of our cohort.

Characteristics	**Study cohort (N=50)**
Demography	
Male	27 (54)
Adults	7 (14)
Age at enrolment (median, range; years)	11 (5-38)
Age at onset (median, range; years)	4 (0-21)
Disease duration (median, range; years)	7 (2-23)
Tested genes per patient (median, range)	3 (1-7)
Clinical manifestations	
Periodic fever	36 (72)
Chronic urticaria	2 (4)
Prevalent skin/bone/joints involvement	6 (12)
Prevalent intestinal involvement	1 (2)
Suspected type 1 interferonopathies	5 (10)
Episodes of macrophage activation syndrome	3 (6)
NGS results	
Variants per patient(median, range)	2 (0-6)
Variants in suspected subsets	29 (23)
New diagnosis	2 (4)
Recurrent variants *	38 (30)

The results are shown as number (percentage) if not specified; *supplementary table 5.

Table 2. Genotype-phenotype correlation in 50 patients with uSAID.

N°	**Required subpanel**	**Clinical features and response to treatments**	**Gene**	**Mutation**	**CLINVAR**	**ExAC**	**Eur 1000 Genomes**	**Mutation Taster**	**Fathmm-MKL**	**Meta-SVM**	**PROVEAN**	**SIFT**	**CADD Phred**	**Final results**
GROUP 1 - Patients with a confirmatory genotype														
1	C	Poliarthritis, periostitis, generalized lymphadenopathy, hepatosplenomegaly, dysmorphisms (epicanthus, frontal bossing, saddle nose), small cerebral hemisphere with pachygyria. Complete response to anakinra.	**MVK**	**Gly326 Arg HOMO**	1	-	-	DC	D	D	D	T	4.40	MKD
2	A	Recurrent fever episodes since childhood with generalized lymphadenopathy, abdominal pain and erythematous rash. Complete response to steroids on-demand.	**MVK**	**Val377Ile**	5	1,60E-03	-	DC a	D	D	N	T	20.3	MKD
			MVK	**Ala147 Thr**	-	4.06E-6	-	DC	D	D	D	D	29.1	
GROUP 2 - Patients with possibly pathogenic variant consistent with the clinical phenotype														
3	A	Recurrent fever episodes, every 8-10 days, with erythema nodosum and aphthosis. Lobar granulomatous panniculitis with interstitial and perivascular infiltrate of lymphocytes and histiocytes at skin biopsy. Complete response to steroids. No response to rapamycin,	**PLCG2**	**Asn571 Ser (LB)**	-	6,70E-03	1,39E-02	DC	N	T	N	D	22.9	Suspected APLAID*
			SH3BP2	Arg609 Gly	-	1.966 E-05	-	DC	N	T	N	T	14.22	

		azathioprine, anakinra and thalidomide. Partial response to anti-TNF treatment.												
4	B	Recurrent episodes of urticarial rash, aphthosis, exudative pharyngitis, cervical lymphadenopathy, abdominal pain, and arthromyalgia. Partial response to on-demand steroids. Infiltration of mast cells at skin biopsy. Partial response to anti-histaminic drugs and steroids. A sister with recurrent episodes of urticarial rash after cold exposure and recurrent upper airways infections with sinusitis.	PLCG2	Ala1130Ser	-	8,12E-06	-	DC	D	T	N	D	24.5	Suspected PLAID
			PLCG2	**Pro1127Arg (VUS)**	-	1,63E-05	-	DC	D	D	D	D	29.7	
GROUP 3 - Patients with possibly pathogenic variant not consistent with the clinical phenotype in AD disease														
5	A	Recurrent febrile episodes of 7 days, every month, with sporadic exudative pharyngitis, arthritis, and headache. Complete response to steroids on-demand.	CARD14	Ser200Asn	1	6,38E-03	2,00E-03	P	N	T	N	T	0.004	Undefined*
			TNFRSF11A	**Lys240Glu (VUS)**	0	1,07E-03	1,00E-03	DC	D	T	-	-	28.4	
			IFIH1	Glu627*	-	3,20E-03	8,90E-03	DC	D	-	-	-	26.8	
6	A	Recurrent fever episodes of 7-10 days, with complete response to colchicine. One episode of polyarthritis at the right foot.	**AP1S3**	**Phe4Cys (LB)**	255	7,66E-03	9,90E-03	DC	D	D	D	D	27.2	Undefined*
7	A	Few self-limited fever episodes of 21 days, with severe malaise, erythematous rash, exudative pharyngitis, arthromyalgia and abdominal pain. Recurrent aphthosis. Partial response to maintenance therapy with colchicine. No response to tonsillectomy. Twin sister with chronic entero-colitis and recurrent aphthosis, father with recurrent aphthosis and psoriasis.	IL10RA	Val113Ile (LB)	0	6,75E-03	6,00E-03	P	N	T	N	T	0.006	Undefined*
			NLRP12	**Asp84Asn (VUS)**	-	8,16E-06	-	DC	D	T	D	D	28.1	
			RBCK1	Asn122His (LB)	-	-	-	P	N	T	N	T	10.66	
8	C	Non-suppurate nodular panniculitis with fever and arthralgia. Partial response to cyclosporine and anakinra. Complete response to steroids and anti-TNF.	CARD14	Glu422Lys (LB)	-	2,30E-02	2,88E-02	P	N	T	N	T	8.682	Undefined
			CARD14	**Arg682Trp (VUS)**	1	1,10E-02	1,79E-02	DC	D	T	D	D	35	
			NLRC4	Gly786Arg (VUS)	-	-	-	-	N	T	N	T	23.2	
			NLRP3	Val200Met (LB)	5-3	8,49E-03	1,19E-02	DCa	N	T	N	T	0.002	
			NLRP7	Lys511Arg (LB)	2	1,31E-02	1,09E-02	P	N	T	N	T	0.001	
			NOD2	Arg684Trp (VUS)	-	4,04E-04	2,00E-04	P	D	T	N	T	23.2	
9	C	Chronic bilateral osteomyelitis at the lower limbs with cutaneous abscesses treated with long-term antibiotic treatment with complete response.	**CARD14**	**Arg69Trp (VUS)**	-	1,49E-04	-	DC	D	T	D	D	24.3	Undefined
10	A	Recurrent fever episodes of 5-6 days, every 2-4 weeks, with exudative pharyngitis, aphthosis, hepatomegaly and arthritis. Complete response to steroids on demand.	**CARD14**	**Arg610His (VUS)**	-	1,48E-04	-	DC	N	T	D	D	25.8	Undefined*
			MEFV	Glu148Gln (VUS)	2-3-5	7,08E-02	8,90E-03	Pa	N	T	N	D	23.3	
11	A	Recurrent fever episodes of 10 days, every 2-4 weeks, with abdominal pain, vomit, diarrhea, hepatosplenomegaly, periorbital edema and urticarial rash. Complete response to steroids.	ADAR1	Ser281Arg (LB)	-	-	-	P	N	T	N	T	0.015	Undefined*
			CARD14	Glu422Lys HOMO	-	2,30E-02	2,88E-02	P	N	T	N	T	8.682	

		Partial response to colchicine. Complete response to anakinra as maintenance therapy. Father with granulomatous hepatitis.		(LB)										
			MEFV	Glu148 Gln (VUS)	2-3-5	7,08E-02	8,90E-03	Pa	N	T	N	D	23.3	
			CARD14	**Arg682 Trp (LB)**	1	1,10E-02	1,79E-02	DC	D	T	D	D	35	
12	A	Recurrent fever episodes of 5-6 days, every 10-15 days, with erythematous rash, cervical lymphadenopathy, exudative pharyngitis, arthralgia, abdominal pain and diarrhea. Partial response to colchicine. Mild inflammation at gastroscopy and colonoscopy. Inflammatory bowel disease was ruled out during infancy.	**PLCG2**	**Lys775 Arg (VUS)**	-	1,37E-03	2,00E-03	DC	D	T	N	T	22.5	Undefined*
13	A	Recurrent fever episodes with abdominal pain, headache and arthralgia. Sometimes arthritis. Partial response to colchicine.	ADAR1	Ser281 Arg (LB)	-	-	-	P	N	T	N	T	0.009	Undefined*
			CARD14	Pro506 Leu (LB)	-	1,38E-02	1,39E-02	P	N	T	N	T	5.070	
			NOD2	**Gly908 Arg (VUS)**	25 5-0-3	1,13E-02	9,90E-03	DC	D	T	D	D	31	
14	A	Few fever episodes with severe malaise, exudative pharyngitis, erythematous rash, arthromyalgia, generalized lymphadenopathy and hepatosplenomegaly. Laboratory exams showed hyperferritinemia and hypertransaminasemia. Complete response to high-dose steroids.	**NOD2**	**Pro427 Leu (VUS)**	-	1,10E-04	-	DC	D	D	D	D	25.7	Undefined*
15	E	Lipophagic panniculitis at the lower limbs with fever, arthritis and alopecia. Negative interferon signature. Complete response to steroids and methotrexate.	SH3BP2	Arg534 Trp (VUS)	3	4,62E-03	4,20E-03	P	N	T	N	T	32	Undefined
			NLRP7	Cys399 Tyr (VUS)	0	4,64E-04	-	P	N	T	D	D	22.3	
			SAMHD1	**Met362 Ile (VUS)**	-	-	-	DC	D	D	D	T	23.8	
16	A	Recurrent fever episodes of 4-5 days, every 2-3 weeks, with exudative pharyngitis, aphthosis, generalized lymphadenopathy, abdominal pain and urticarial rash. No response to colchicine and anakinra. Positive family history for rheumatoid arthritis.	**TMEM173**	**Pro317 Leu (VUS)**	-	4.07E-06	-	DC	D	T	N	D	22.4	Undefined*
17	A	Recurrent fever episodes of 5-6 days, every month, with malaise, abdominal pain, exudative pharyngitis, and cervical lymphadenopathy. Complete response to steroids on demand. Brother with similar symptoms.	**IFIH1**	**Lys349 Arg (LB)**	-	3,22E-03	3,20E-03	DC	D	T	N	D	15.54	Undefined*
18	E	Recurrent fever episodes of 3-4 days, every 5-10 days, with maculo-papular rash, headache, abdominal pain and arthralgia. Sometimes aphthosis and diarrhea. Normal colonoscopy. No response to colchicine as maintenance therapy. Positive family history for recurrent fever syndromes, multiple sclerosis and systemic lupus erythematosus.	IFIH1	Thr100 Arg (LB)	-	2,03E-05	-	P	N	T	N	D	14.74	Undefined
			SH3BP2	**Val438 Met (VUS)**	0	2,54E-04	-	DC	D	D	N	D	23.8	
19	C	Discoid skin lesions and polyarthritis. C3 deposit at dermal-epidermal junction. Partial response to steroid.	**SH3BP2**	**Ala212 Val (VUS)**	2-3	5,62E-03	4,0E-03	P,DC	D	T	N	D	22.6	Undefined
			RNASEH2A	Asp205 Glu	2-3	1,09E-02	8,40E-03	DC	D	T	N	T	14.56	

20	A	Recurrent fever episodes of 6 days, every month, with urticarial rash, myalgia and exudative pharyngitis. Low immunoglobulin A and autoimmune neutropenia. Complete response to steroids on demand.	**ADAR1**	**Pro193 Ala HOMO (LP)**	5-3	2,14E-03	4,00E-03	DC	D	D	D	D	-	Undefined*
			NLRP12	Asp979 His (LP)	-	2,03E-05	-	P, DC	D	T	D	T	23.6	
			IL10RB	Glu25Lys (VUS)	0	7,07E-04	-	P	N	T	N	T	21.3	
GROUP 3 - Patients with possibly pathogenic variant not consistent with the clinical phenotype in AR diseases														
21	A	Recurrent fever episodes with abdominal pain, periorbital edema, exudative pharyngitis, cervical lymphadenopathy, headache and arthromyalgia. Sometimes aphthosis. No response to maintenance therapy with colchicine, anakinra, mesalazine or rapamicine.	**SLC29A3**	**Ser203 Pro (LP)**	5	1,25E-05	-	DC	D	D	D	D	25.6	Undefined*
			TNFRSF1A	Arg121 Gln (VUS)	5	1,32E-02	6,00E-03	P	N	D	D	D	15.30	
			IFIH1	Hys460 Arg (LB)	-	9,53E-02	1,19E-02	P a	N	T	N	T	9.6	
22	B	Recurrent/intermittent episodes of diffuse urticarial rash, bilateral conjunctivitis, erythema nodosum and arthralgia/arthritis. Laboratory exams during episodes showed low C4 and monoclonal light chain gammopathy. Complete response to steroids on demand and canakinumab. No response to colchicine.	DNASE1L3	Met1Leu (LP)	5	1,58E-04	1,60E-03	DC	D	-	-	-	22	Undefined
			IL36RN	**Ser113 Leu (LP)**	-	2,90E-03	1,60E-03	DC	D	T	N	D	27.8	
23	A	Recurrent fever episodes of 7-10 days, every 2 weeks, with aphthosis, generalized lymphadenopathy and malaise. Sometimes arthritis. No response to colchicine. Complete response to steroids on demand and anakinra as maintenance therapy.	CARD14	Glu422 Lys (LB)	-	2,30E-02	2,88E-02	P	N	T	N	T	8.682	Undefined*
			DNASE1	Val185Ile (VUS)	-	3,58E-03	4,00E-03	DC	N	T	N	T	0.086	
			IL36RN	**Ser113 Leu (LP)**	5	2,29E-03	1,60E-03	DC	D	-	-	D	27.8	
24	E	Recurrent fever episodes of 15-20 days, with abdominal pain, painful urticarial-like rash or panniculitis, polyarthritis or arthromyalgia at the lower limbs. Complete response to steroids, partial response to colchicine.	PSMB8	Gly8Arg (VUS)	3	1,94E-02	2,88E-02	DC	N	T	N	D	-	Undefined
			NLRC4	Ala929 Ser (LB)	-	7,07E-03	1,29E-02	N	N	T	N	T	0.381	
			RNASEH2B	**Ala177 Thr (VUS)**	5	1,31E-03	-	DC a	D	D	N	T	19.86	
25	C	Recurrent erythema nodosum, sometimes with fever, arthritis, uveitis and generalized lymphadenopathy. Complete response to steroids. Lipoblastoma arborescens of the knee.	TNFRSF1A	Pro75Leu (VUS)	2	5,50E-03	-	P	N	T	D	D	22.2	Undefined
			PSMB8	**Thr74Ser (LP)**	-	4,49E-03	2,00E-03	DC	D	T	D	D	31	
26	A	Recurrent fever with skin rash, arthromyalgia, myositis and hepatosplenomegaly. No response to cyclosporine. Complete response to steroids and anakinra as maintenance therapy. Positive family history for autoimmune trombocitopenia.	CARD14	Pro506 Leu (LB)	-	1,38E-02	1,39E-02	P	N	T	N	T	5.070	Undefined*
			NLRP3	Val200 Met (LB)	5-3	8,49E-03	1,19E-02	DC a	N	T	N	T	0.002	
			LPIN2	**Cys874 Phe (VUS)**	0	1,37E-03	9,20E-03	DC	D	T	D	T	21.9	
27	A	Some episodes of MAS during neonatal period with complete response to steroids. Recurrent infections and self-limited fever episodes of urticarial rash, panniculitis, splenomegaly, generalized lymphadenopathy and malaise. High immunoglobulin A and low platelet count. Mild dismorphisms and growth delay. Consanguineous parents.	IL10RB	Val148 Met (VUS)	-	1,14E-03	-	P	N	T	N	D	15.82	Undefined*
			CECR1	Met309 Ile (VUS)	-	1,74E-03	2,00E-03	P	D	T	N	T	2.643	
			LPIN2	**Glu601 Lys (VUS)**	2	8,88E-03	9,90E-03	DC	D	T	N	T	22	
2	A	Episodes of polyarthritis and	C1NH	Thr48A	-	4,87E-	-	P	N	T	D	T	0.0	Undefined*

8		pericarditis, sometimes with fever and urticarial rash, treated with steroids with complete response. No response to methotrexate. Complete response to colchicine as maintenance therapy.		la (VUS)		04							01	
			LPIN2	**Glu601 Lys (VUS)**	2	8,88E-03	9,90E-03	DC	D	T	N	T	22	
29	A	Recurrent fever episodes of 8-9 days, every 20-25 days, with headache, exudative pharyngitis and aphthosis. Complete response to steroids on demand. Now recurrent episodes of arthritis and livedo reticularis at the lower limbs. Partial response to colchicine as maintenance therapy.	NLRC4	Gly786 Arg (VUS)	-	-	-	-	N	T	N	T	23.2	Undefined*
			LPIN2	**Glu601 Lys (VUS)**	2	8,88E-03	9,90E-03	DC	D	T	N	T	22	
			CARD14	Glu422 Lys (LB)	-	2,30E-02	2,88E-02	P	N	T	N	T	8.682	
GROUP 4 - Patients with not new pathogenic variants or benign variants or no variants														
30	A	Recurrent fever episodes of 3-4 day, every week. Complete response to colchicine as maintenance therapy.	CARD14	Glu422 Lys	-	2,30E-02	2,88E-02	P	N	T	N	T	8.682	Heterozygous FMF
			MEFV	**Val726 Ala^**	5	2,17E-03	1,00E-03	DC a	N	T	N	T	0.001	
			PLCG2	His641 Gly	-	-	-	-	-	-	-	-	28.5	
			RNASEH2A	Asp205 Glu	3	1,09E-02	1,09E-02	DC	D	T	N	T	14.56	
31	E	Episodes of recurrent fever with polyarthritis and erythematous rash. Complete response to steroids.	**MEFV**	**Val726 Ala^**	5	2,17E-03	1,00E-03	DC a	N	T	N	T	0.001	Undefined
			NOD2	Ile836Thr	-	4,06E-05	-	P	N	T	N	T	1,230	
32	A	Recurrent autoimmune hemolytic anemia during early childhood treated with steroids. Recurrent febrile episodes, lasting 15-20 days, with abdominal pain, scrotal pain, diarrhea, aphthosis, exudative pharyngitis and arthralgia. Pericarditis during one episode. Complete response to steroids on demand and colchicine as maintenance therapy.	CARD14	Glu422 Lys	-	2,30E-02	2,88E-02	P	N	T	N	T	8.682	Undefined*
33	A	Recurrent fever episodes of 1-2 days, every month, with abdominal pain, nausea, vomiting, headache, aphthosis and arthralgia at the lower limbs. Complete response to colchicine as maintenance therapy.	CECR1	Met267 Ile	-	1,74E-03	2,00E-03	P	D	T	N	T	2.643	Undefined*
34	A	Recurrent fever episodes, lasting 3-4 days, every 2 weeks, with abdominal pain and malaise. Complete response to on-demand steroids. Partial response to colchicine as maintenance therapy.	LPIN2	Ser579Pro	0	2,49E-03	8,00E-04	P	N	T	N	T	16.44	Undefined*
			MEFV	Pro369 Ser	255	1,42E-02	4,00E-03	P	N	T	D	D	15.64	
			MEFV	Arg408 Gly	255	-	4,00E-03	P	N	T	N	T	7,871	
			SH3BP2	Arg534 Trp	3	4,62E-03	4,20E-03	P	N	T	N	T	32	
35	A	Low fever episodes with headache and arthromyalgia lasting 24-48 hours every 1-2 months. Gilbert syndrome. Partial response to on-demand NSAIDs.	PSMB8	Gly8Arg	3	1,94E-02	2,88E-02	DC	N	T	N	D	-	Undefined*
			PSTPIP1	Arg405 Cys	0-3	5,44E-04	1,00E-03	DC	N	T	N	T	23.8	
			CARD14	Glu422 Lys	-	2,30E-02	2,88E-02	P	N	T	N	T	8.682	
			NLRP7	Met192 Leu	-	8,24E-06	8,00E-04	P	N	T	D	D	4.578	
36	A	Recurrent fever episodes of 3 days, every 3 weeks, with aphthosis, maculo-papular rash and arthralgia. Partial response to colchicine.	CARD14	Glu422 Lys	-	2,30E-02	2,88E-02	P	N	T	N	T	8.682	Undefined*
			NOD2	Ala725 Gly	2	3,13E-03	-	P	D	T	N	T	3.629	
37	A	Recurrent fever episodes, every 15-20 days, with exudative pharyngitis, cervical lymphadenopathy, abdominal pain and arthralgia. Partial	TREX1	Glu321 Gly	0-3	1,69E-03	-	P	N	T	N	T	0.046	Undefined*
			IFIH1	His460 Arg	-	9,53E-02	1,19E-02	P a	N	T	N	T	9.6	
			PSTPIP1	Gly258	2-3	-	4,00E	P	D	T	N	T	3.6	

		response to colchicine.		Arg			-03						4	
38	A	Recurrent fever episodes of 7-10 days with oral aphthosis. No response to colchicine or anakinra as maintenance therapy. Partial response to mofetil mycophenolate.	CARD14	Pro506 Leu	-	1,38E-02	1,39E-02	P	N	T	N	T	5.070	Undefined*
			MEFV	Ala744 Ser	4-5	1,84E-03	5,00E-03	P, DC	N	T	N	T	0.003	
			NLRP7	Lys511 Arg HOMO	2	1,31E-02	1,09E-02	P	N	T	N	T	0.001	
			PSTPIP1	Ser323 Leu	-	1,94E-05	-	P	N	T	N	T	17.62	
39	A	Recurrent fever episodes of 3-4 days, every 1-2 months, with severe malaise, abdominal pain, cervical lymphadenopathy and arthromyalgia. Sometimes aphthosis and genital ulcers. No response to steroids on demand. Partial response to colchicine.	DNASE1	Glu35Asp	-	4,06E-06	6,00E-03	P, DC	N	T	N	T	8.484	Undefined*
40	A	Recurrent fever with abdominal pain, nausea and vomiting. Complete response to steroids and anakinra. Partial response to indometacine.	IL10RA	Val233 Met	-	1,78E-03	2,00E-03	P a	N	T	N	D	19.48	Undefined*
			IL10RB	Val148 Met	-	1,14E-03	-	P a	N	T	N	D	15.82	
41	A	Recurrent fever episodes of 1-2 days, every 2-4 weeks, with urticarial rash and arthritis. Partial response to on-demand steroids. Positive family history for Hashimoto's thyroiditis.	SH3BP2	Arg534 Trp	-	4,62E-03	4,20E-03	P	N	T	N	T	32	Undefined*
42	A	Recurrent fever episodes of 3-5 days, with abdominal pain, aphthosis and arthralgia. Partial response to ibuprofen. Hyperintense bone lesions at total-body magnetic resonance.	None											Undefined*
43	D	Chronic liver disease with neutrophilic infiltration and Budd-Chiari syndrome, ulcerative cutaneous lesions and pyodermagangrenosum. History of recurrent pneumonia and myeloid dysplasia.	None											Undefined
44	A	Recurrent fever episodes with diffuse erythematous rash, generalized lymphadenopathy and arthritis. Episodes of MAS. Pulmonary alveolar proteinosis. Partial response to steroids and canakinumab as maintenance therapy.	None											Undefined*
45	A	Recurrent fever episodes with cervical lymphadenopathy, abdominal pain and headache. Eosinophilia. Personal history of dental abscesses. Partial response to maintenance therapy with colchicine. Positive family history for recurrent fevers (father and cousin).	None											Undefined*
46	A	Recurrent fever episodes of 3 days, every 3 weeks, with abdominal pain and erythematous rash. Complete response to colchicine.	None											Undefined*
47	A	Recurrent fever episodes of 3-4 days, every 2 weeks, with abdominal pain, exudative pharyngitis, generalized lymphadenopathy and myalgia. Complete response to colchicine.	None											Undefined*
48	C	Chronic non-bacterial osteomyelitis-like bone lesions at clavicle, humerus and femur with severe acne at trunk and	None											Undefined

		face.		
49	A	Recurrent fever episodes of 4 days, every 20-30 days, with abdominal pain, headache and arthromyalgia. Complete response to steroids on demand.	None	Undefined *
50	E	Recurrent fever episodes of 10 days. An episode of acute demyelinating encephalopathy and MAS with complete response to steroids. Partial response to rapamycin as maintenance therapy.	None	Undefined

For each patient, the most relevant variant is marked in bold. LP =likely pathogenic; VUS = variant of unknown significance; LB = likely benign; CLINVAR code: 0 = uncertain significance; 1= not provided; 2= benign; 3= likely benign; 4= likely pathogenic; 5= pathogenic; 255= other; P = polymorphism; N = neutral; T= tolerated; DC= disease causing; D = damaging; a = automatic; ^ = variants already known; *=patients with undifferentiated recurrent fever episodes.

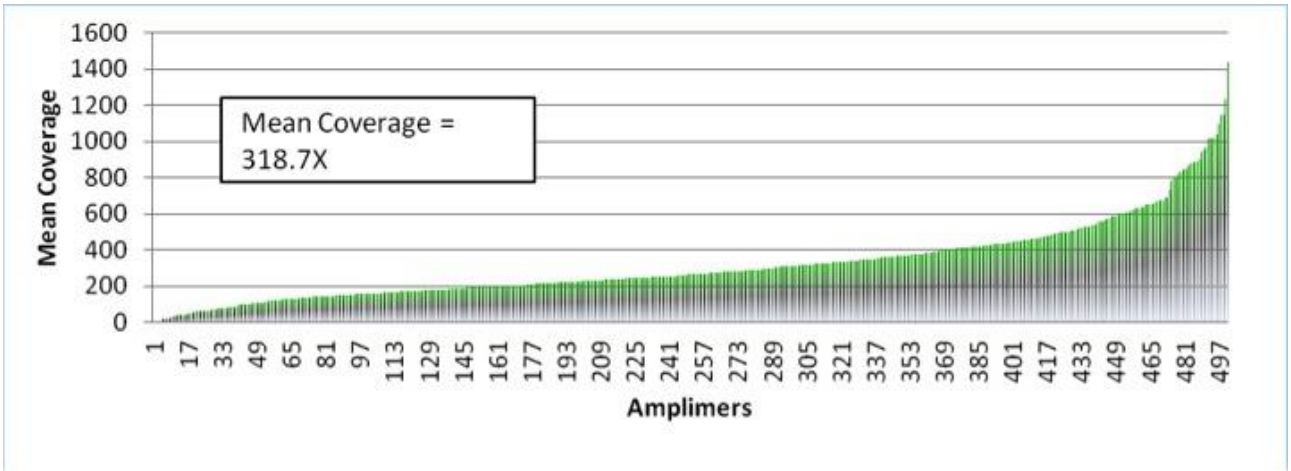

Figure 1. Coverage analysis of all the amplimer reads obtained from all 50 samples. The 501 amplimers used for the enrichment of our gene target are ordered according to the mean coverage, from the least to the most represented in the reads pool, as calculated among the 50 samples tested. The mean coverage is reported in the box.

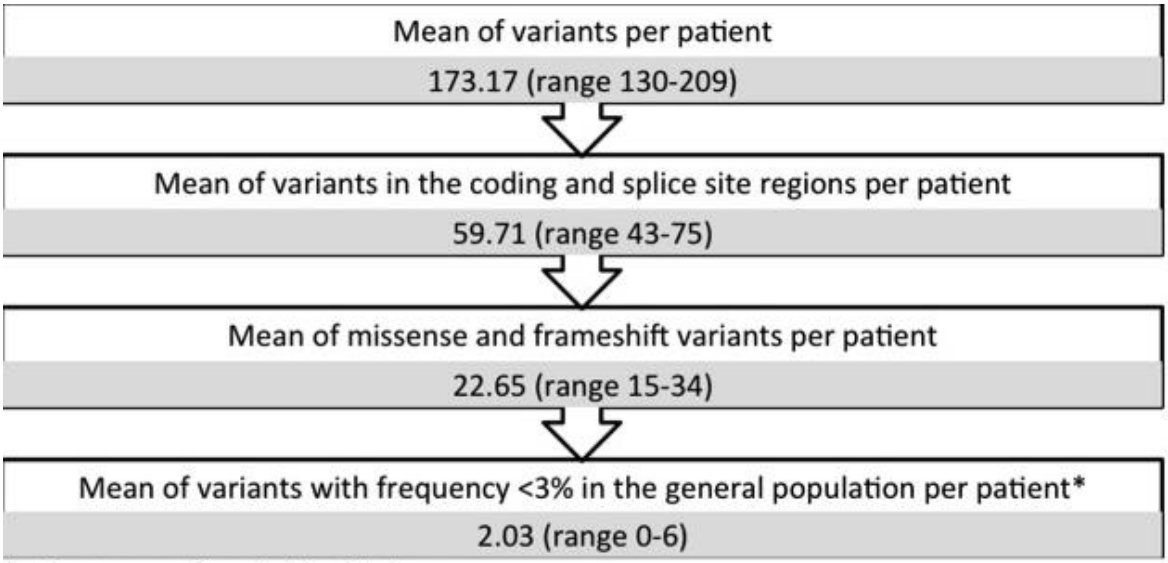

Figure 2. NGS variants validation workflow. The bioinformatics pipeline adopted to filter out variants unlikely to be involved in the uSAID disorders taken into consideration in this study is shown. In particular, criteria, such as position of the variants within either the coding portion or in splice sites, missense, nonsense, and missense nature of the coding change, and the frequency of the variants in the general population, have been applied to reduce the number of candidate variants from an average of 173 to 2 per patient.

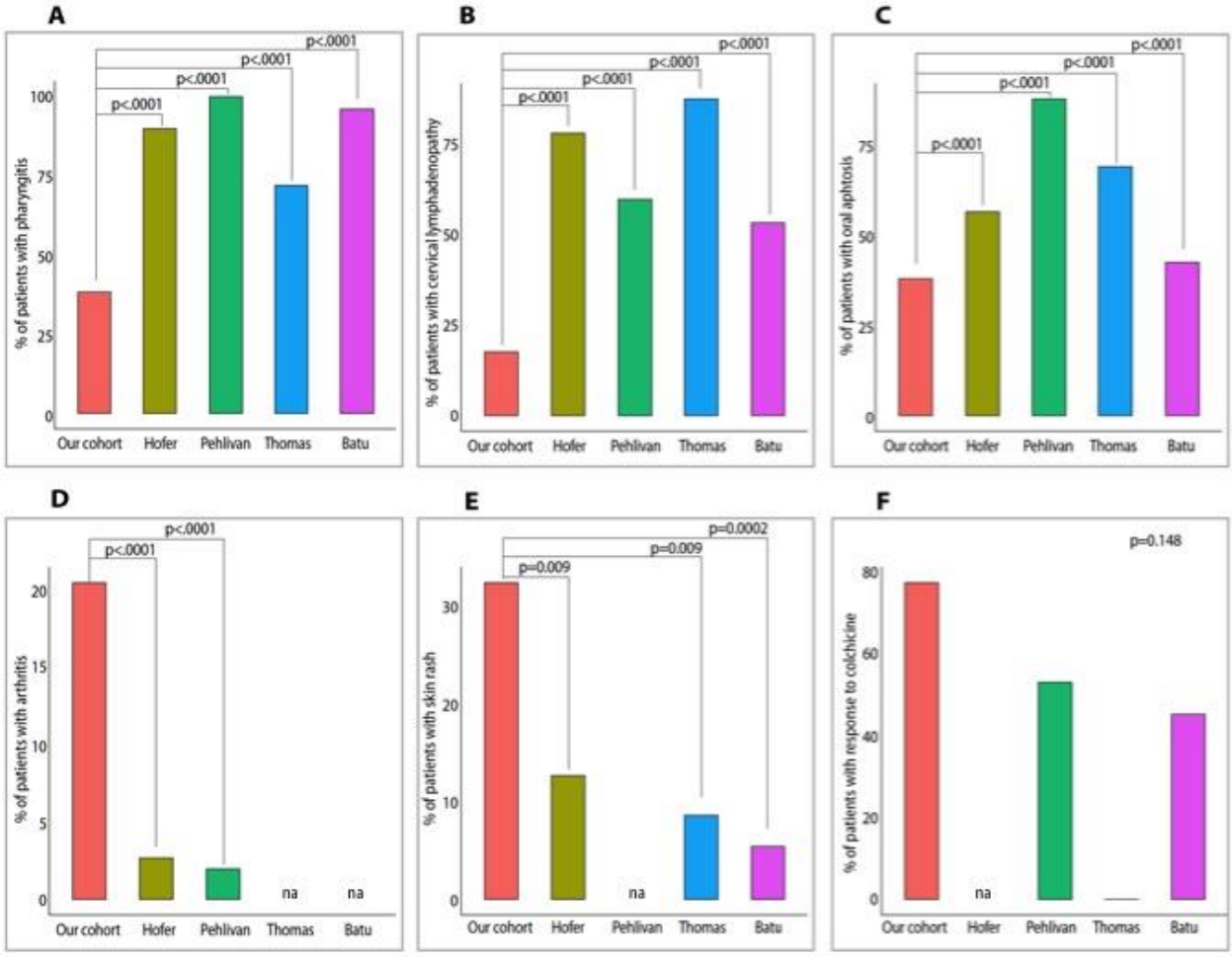

Figure 3. Clinical features of patients with systemic undifferentiated recurrent fevers (SURF) compared to different cohorts of PFAPA available in the literature. Na=not available. Values are number of patient (%). P values were assessed using Chi square test or T-test as appropriate and where significant interactions were determined post-hoc test for multiple comparison were performed. All the post-hoc analyses highlighted significant differences ($p<0.05$) between our cohort and the other PFAPA populations reported by Hofer et al,[41] Pehlivan et al,[44] Thomas et al,[40] Batu et al.[42]

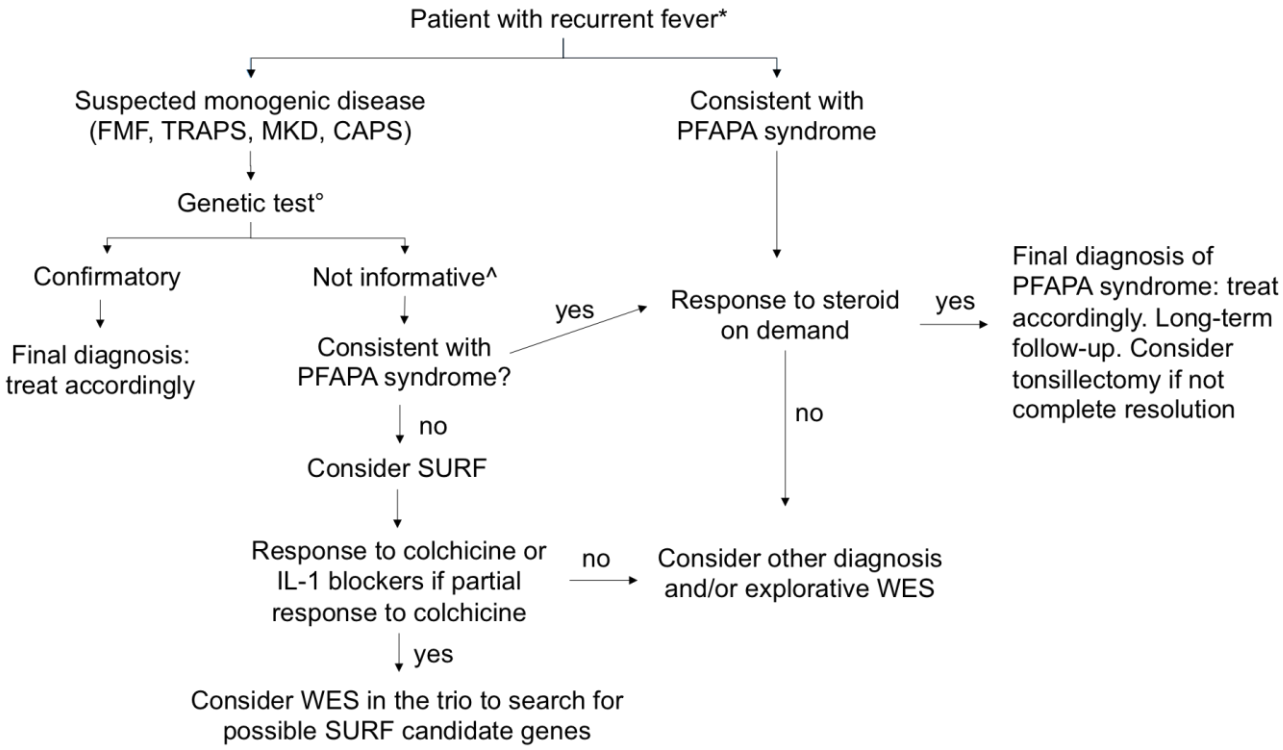

Figure 4. Diagnostic and therapeutic flow-chart for patients with suspected autoinflammatory recurrent fever. * after careful exclusion of other possible causes (infections, neoplasms, etc..); ° with target Sanger sequencing or NGS diagnostic panels; ^ consider carefully variants of unknown origin and low-penetrance variants, consider the possibility of somatic mosaicisms.

Type I interferon activation in RAS-associated autoimmune leukoproliferative disease (RALD)

RAS-associated autoimmune leukoproliferative disease (RALD) is a rare immune dysregulation syndrome characterized by lymphadenopathy, splenomegaly, autoimmunity and monocytosis, secondary to heterozygous somatic gain-of-function mutation of either *NRAS* or *KRAS* gene in a subset of hematopoietic cells.[1,2] RALD patients are at high risk of malignancies and an early diagnosis is essential for the adequate cancer screening.[3,4]

In recent years, a subset of pediatric rheumatic disorders with atypical presentation characterized by early onset, severity of symptoms and resistance to standard treatments, has been associated with mutations of genes primarily affecting the immune system. Among these disorders, RALD can present with multi-organ autoimmunity mimicking systemic lupus erythematosus (SLE).[5,6] Lymphadenopathy and hypergammaglobulinemia are common features of both disorders, complicating the differential diagnosis. The pathological mechanism of these manifestations is not fully determined.

In the present report, we describe a RALD patient with recurrent infections and decreasing serum levels of immunoglobulin (Ig), developing a complex multi-organ autoimmune disease with hepatopulmonary syndrome that required liver transplant. Defects of T and B lymphocytes function and positive type I interferon signature (ISG) were demonstrated.

The patient

The 27-years-old patient was born from non-consanguineous parents and followed from 13 to 22 years of age at our Center. Immunologic and genetic analyses were conducted in the context of a research project approved by the regional IRB (approval BIOL 06/05/2004).

FACS analyses, in vitro immunosuppression assay and ISG analysis

Human peripheral blood mononuclear cells were purified by standard density gradient technique and stained with antibodies against CD4, CD25 and CD127 when the patient was 16 years of age. Intracellular detection of human FOXP3 was performed after incubation for 30 min at RT (Biolegend), according to manufacturer's protocol. All samples were acquired using BD FACS Canto and analyzed using FlowJo software (TreeStar Inc).

After enrichment of CD4+ cells (Miltenyi), CD4+ CD25high CD127− human regulatory T cells and CD4+ CD25− responder T cells from the patient and healthy control were isolated by FACS sorting. Purity was ≥90%. Autologous and allogeneic suppression was performed plating 50,000

responder cells and 25,000 regulatory T cells (ratio 1:0.5). Cells were co-stimulated with an equal number of allogeneic accessory cells, irradiated at 30Gy and 1µg/mL of soluble anti-CD3 (Janssen-Cilag). After 96 hours of co-culture, suppression assays were pulsed for 16 hours with 1 µCi 3 Hthymidine/well (Amersham Biosciences). Cells were harvested and counted in a scintillation counter. [7] Peripheral blood ISG was assessed 4 times during the patient follow-up as previously described by our group.[8]

Genetic analysis

Whole exome sequencing (WES) analysis was performed on the family trio. Target DNA was captured by Nextera Flex Enrichment kit in combination with the xGen Exome Research Panel v1. Libraries quality was checked with 2100 Bioanalyzer instrument. Sequencing was performed on Illumina NovaSeq 6000 System and quality check was achieved by FastQC tool. Obtained reads were aligned against Hg19 Human Genome by BWA-MEM. Initial mappings were processed using the GATK framework version 4.0, according to the GATK best practices recommendations.

Variants were classified as known or novel based on dbSNP146 and annotated using KGGSeq, then filtered based on the allele frequencies in the healthy population and the impact on encoded proteins. In particular, only *de-novo* missense variants with allele frequencies below 1% were considered. The DNA from other tissues of the patient, namely oral brush and urinary sediment, was extracted using the Qiagen DNeasy Blood & Tissue kit. The variant selected after the bioinformatics pipeline was validated by Sanger sequencing in the DNA of available family members (proband, father, mother and healthy brother).

Patient clinical and laboratory characteristics

The patient presented with persistent generalized lymphadenopathy, hepatosplenomegaly and recurrent otitis media at the age of 5 months. Laboratory tests showed anemia and hypergammaglobulinemia (Fig. 1A). Lymph node biopsy showed reactive follicular hyperplasia and chronic EBV infection was suspected. Oral aciclovir for 5 days and monthly intravenous pulses of interferon for 6 months were administered with partial benefit.

At 3 years of age, the patient developed nephrotic syndrome with peripheral edema, abdominal and pericardial effusions, and polyarthritis. Laboratory tests showed elevation of acute phase reactants with persistent anemia and hypergammaglobulinemia (Fig. 1A). Despite the absence of autoantibodies and the normality of complement fractions, the diagnosis of SLE was suggested. Intravenous and subsequently oral steroids were partially effective to control the inflammatory manifestations. However, steroid-sparing drugs, such as hydroxychloroquine and mofetil

mycophenolate, did not allow complete tapering of steroids. Furthermore, the patient displayed three episodes of varicella Zoster virus pneumonia and, at 8 years of age, nephrotic range proteinuria. Kidney biopsy was performed, and histological analysis revealed minimal change disease, suggesting stage 1 lupus nephritis.

Since pubertal age, growth and pubertal delay was evident, and replacement therapy with growth hormone and testosterone was started. Malar rash and multiple areas of alopecia and cutaneous photosensitivity developed. Thorax CT scan revealed lung fibrosis and esophagogastroduodenoscopy showed diffuse atrophy of the intestinal villi. The patient started gluten free diet, oral L-thyroxine for autoimmune hypothyroidism and intravenous Ig replacement therapy with monthly infusions due to the low serum concentration of Ig (Fig. 1A).

At 18 years of age, regenerative nodular hyperplasia (RNH) of the liver was detected by abdominal CT scan (Fig. 1B), later confirmed by histologic analysis of a needle biopsy specimen (Fig. 1C and 1D). During patient follow-up, non-cirrhotic portal hypertension with esophageal varices and hepatopulmonary syndrome developed, requiring liver transplant.

Immune workup

In the context of a study on patients affected by common variable immunodeficiency (CVID), an intrinsic B cell defect in class switch recombination with high frequency of naïve B cell, low expression of CD73 and defect of memory IgG and IgA differentiation were noted (reported in Schena F. et al., Supplemental Figure 8).[9]

The concentration of CD4/CD8 double negative T cells and Fas-mediated apoptotic test were normal. *In vitro* culture experiments revealed increased CD4+ T cell proliferation activity in response to anti-CD3+ stimulus (Fig. 1E) and defective immunosuppressive activity of regulatory T cells, identified as CD25+ CD4+ FoxP3+ T cells, compared to healthy control. Regulatory T cell percentages in peripheral blood were within the normal range (Fig. 1F). Peripheral blood ISG was tested several times during follow up and resulted always positive (Fig. 1G).

Genetic analysis

Considering the early onset phenotype and the atypical symptoms presented by the patient, a genetic disease was suspected. A 41-genes next generation sequencing panel including genes associated with type I interferonopathies, monogenic SLE and other common autoinflammatory diseases, resulted negative for pathogenic variants,[10] thus WES analysis was performed on the family trio. The average read-depth was 85.1X, 74.3X and 67.8X for the father, mother and the patient, respectively. In addition, a mean of 96% and 82.5% of the three exomes was covered at

≥10X and ≥30X read-depth, respectively. Upon variant calling, 253 ultra-rare variants (frequency <10E-04 in public databases) were selected and, among those apparently *de novo*, 12 missense variants were further considered, according to the phenotype. One of these, the c.38G>A of the *NRAS* gene was finally regarded as the causative mutation and thus validated by Sanger sequencing (Fig.2A). The variant was detected only in the patient, according to a *de-novo* inheritance, with allele frequency of 20:8 (ref:alt) for a total of 28 allele coverage at that position. The mutation causes the amino acid substitution p.Gly13Asp, characterized by DANN score >0.99 and classified as pathogenic through InterVar. The wild type genotype was confirmed in the DNA of peripheral blood mononuclear cells of the parents and healthy brother (Fig.2B-D). While germline *NRAS* mutations are associated with the Noonan syndrome type 6 (https://omim.org/entry/164790), RALD is caused by somatic mutations (https://omim.org/entry/614470). For this reason, since the unbalanced allelic coverage of the WES data and despite the fact that looking at the outcome from the Sanger sequencing of the DNA extracted from whole blood (the two allele peaks were perfectly overlapping; Fig.2A), we sought to prove the possible somatic nature of the p.Gly13Asp mutation by direct Sanger sequencing of the DNA extracted from additional tissues of the patient, in particular from oral brush and urinary sediment. Such DNA sequencing, showing only small "T" peaks in both these latter gDNA sources, appeared to confirm the somatic nature of the identified *NRAS* mutation (Fig.2E-F). Unfortunately, we cannot exclude a contamination by leukocytes of the buccal swab and urine samples and, on the other hand, no dermal fibroblast cell line was available to check presence of the "T" allele in a different, uncontaminated gDNA.

In the present study, we report a RALD patient with inflammatory CVID-like manifestations and severe liver complication, showing defective T and B cells and positive ISG.

RALD is usually characterized by generalized lymphadenopathy, hypergammaglobulinemia and monocytosis. During childhood, these features should prompt extensive diagnostic workup to exclude chronic infections, malignancies and immune disorders, such as autoimmune lymphoproliferative syndrome. In recent years, the increased availability of WES technology has improved the discovery of genetic defects associated with immune dysregulation, ameliorating the understanding of pathogenic mechanisms underlying these manifestations.

The present case was referred at our Center at the age of 13 years for suspected SLE evolved into a complex syndrome suggestive of inflammatory CVID.[11] In fact, numerous autoimmune manifestations progressively developed during the patient's follow-up in association with recurrent infections and decreasing hypogammaglobulinemia. Surprisingly, the hypergammaglobulinemia usually reported in RALD was present only during the first years of life of the patient.

The analysis of Ig subclasses revealed class switch defect with consequent high level of IgM caused by intrinsic B cell defect. This peculiar characteristic with the evidence of sustained activation of the type I interferon pathway can be related to the progressive development of a complex multi-organ autoimmunity without evidence of autoantibodies.

Furthermore, the increased proliferation activity of CD4+ T cells and the decreased function of regulatory T cells shown by *in vitro* experiments may be associated with the lymphoproliferative manifestations of the patient. Thus, despite our observations will require further investigations to be confirmed, our case highlights the need of excluding *NRAS* gene mutations in patients with inflammatory CVID, especially in case of associated generalized lymphadenopathy or positive ISG.

The evidence that epithelial cells of the patient carry a very low proportion of the mutant allele compared to circulating lymphocytes, or even no mutant allele at all, confirms the known clonal selection of the *NRAS* mutated cells. This clonal advantage further highlights the need for a rigorous cancer screening during the long-term follow-up of patients with RALD. An early onset presentation as the one described in our patient, related initially only to a EBV infection, has already been reported in other RALD patient and might suggest a pre-natal mutational event.[2]

The severe liver complication presented by our patient markedly affected the disease course. Abdominal CT scan and histological analysis revealed RNH, which is usually associated with a variety of systemic disorders, including SLE and CVID.[12,13] RNH is a rare and frequently asymptomatic condition secondary to obliterative vasculopathy, causing intrahepatic portal hypertension in the absence of cirrhosis. Patients with monogenic disorders associated with persistent activation of the type I interferon pathway are usually affected by a multi-organ vasculopathy involving mainly lungs and skin, however lymph nodes and liver can also be affected.[14] Thus, type I interferon activation may concur to the inflammatory manifestations of our patient leading to the hepatopulmonary syndrome that required liver transplant. It would be of interest to assess the potential role of early treatment with type I interferon antagonists in RALD, as well as in patients with inflammatory CVID and positive ISG. Finally, the origin of type I interferon pathway activation in this case of RALD remains currently unknown and might be linked to the increased amount of DNA errors secondary to the high turnover of hyperproliferative lymphocytes caused by the gain-of-function *NRAS* mutation.[15] Blocking this pathway may favor onset of the well-known malignant complications of RALD.

In conclusion, our report highlights the pleiotropic manifestations of RALD, ranging from recurrent infections, multi-organ autoimmunity and the type I interferon-mediated inflammatory symptoms. RNH can complicate the course of RALD. *NRAS* gene mutations should be screened in patients with inflammatory CVID with generalized lymphadenopathy or positive ISG.

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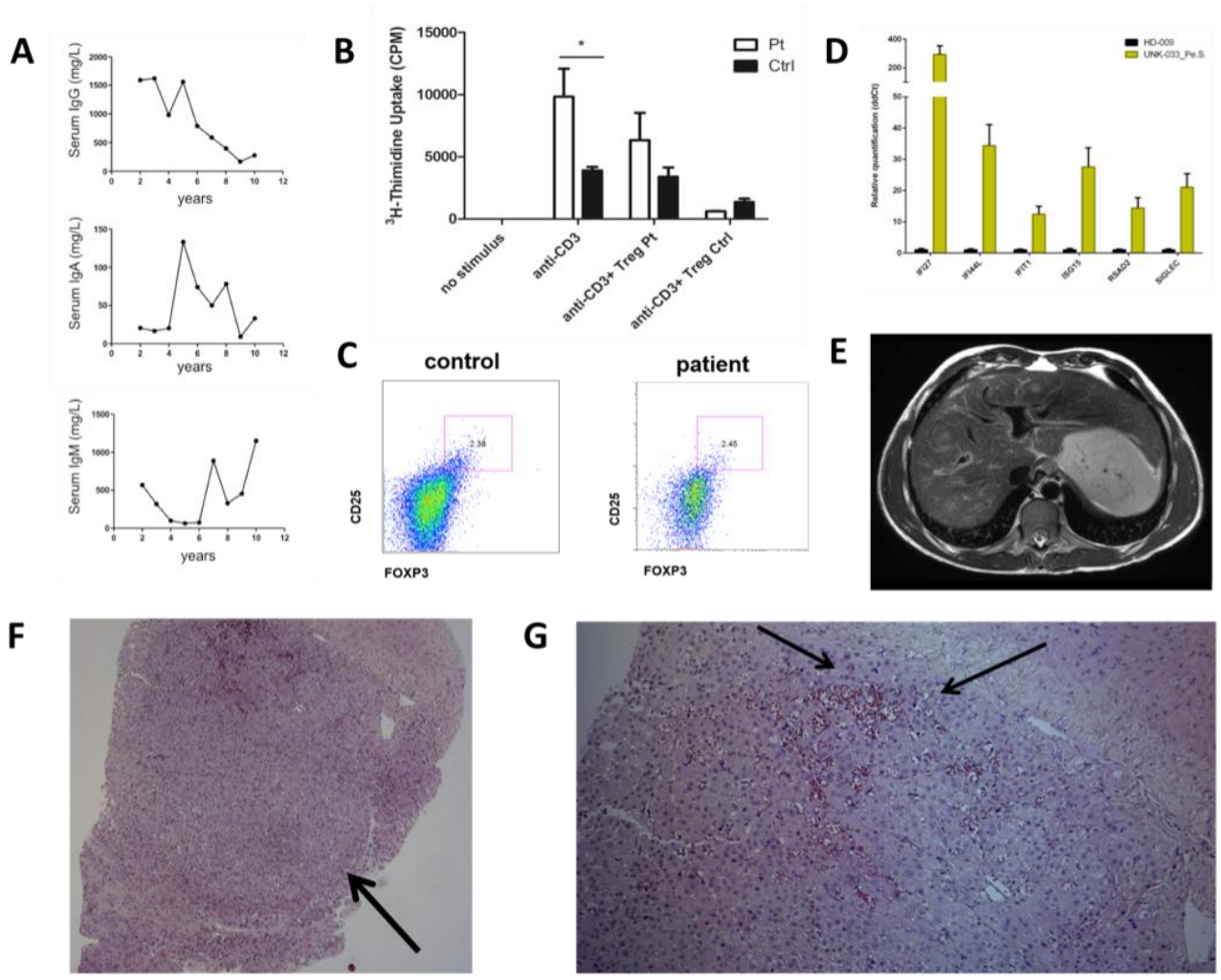

Figure 1. Clinical, histological and immunological characteristics of the patient.

(A) Serum Ig concentration during the follow-up. (B) Abdominal T2-weighted TC scan image showing a regenerative nodular hyperplasia of the liver with portal hypertension. (C) Altered hepatic parenchymal architecture with micronodular pattern (arrow) and dilatation of the vascular network on the needle liver biopsy, H&E, 5X. (D) Blood-filled dilated hepatic sinusoids on the needle liver biopsy, H&E, 10X. (E) High proliferative CD4+ T cells and hypoactive CD25+ CD4+ regulatory T cells of the patient compared with healthy cells (shown results are representatives of three independent experiments; *=$p<0.05$) (F) Normal regulatory T cells percentage in the peripheral blood of the patient compared with a healthy donor. (G) Positive type I interferon signature of the patient compared to a healthy donor.

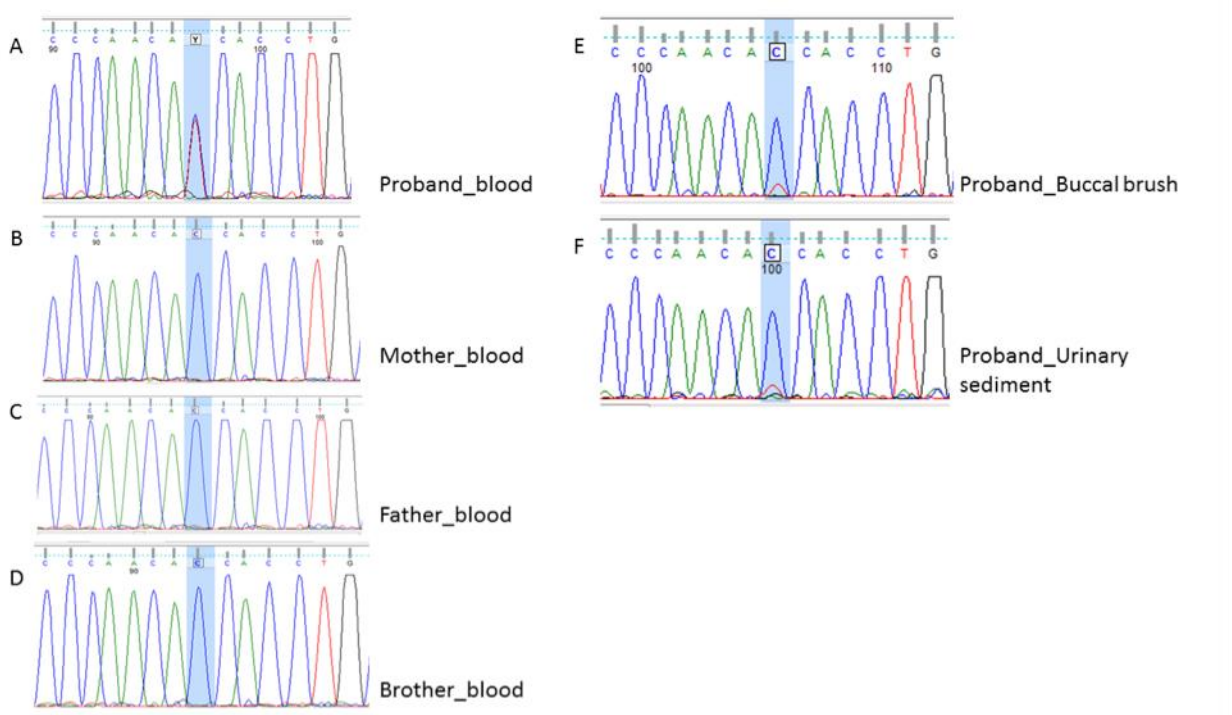

Figure 2. Electropherogram of the *NRAS* gene.

(A-D) Sanger sequencing results of DNA extracted from the peripheral blood mononuclear cells of the family trio and the healthy brother. (E-F) Sanger sequencing results of DNA extracted from oral bush and urinary sediment of the patient. The DNA segment image shows the mutation site c.38G>A.

Progression of non-hematologic manifestations in SAMD9L-associated autoinflammatory disease (SAAD) after hematopoietic stem cell transplantation

Interferonopathies are a growing group of rare monogenic autoinflammatory diseases characterized by overexpression of type I interferon stimulated genes (ISG).[1] Sterile alpha motif domain-containing protein 9 (SAMD9) and SAMD9-like (SAMD9L) are well-known ISG and cell proliferation antagonists whose mutations have been associated to various disorders (Table 1). Recently, frameshift variants of SAMD9L have been shown causing an inflammatory phenotype known as SAMD9L-associated autoinflammatory disease (SAAD).[2,3]

Here, we describe a 3-year-old girl born small for the gestational age from unrelated healthy parents, after a pregnancy characterized by oligohydramnios. At birth, she displayed a diffuse maculopapular erythema, evolving in a fine skin desquamation, associated with fever (Figure 1A). Clinical examination revealed microcephaly, axial hypotonia, hepatosplenomegaly and nail dystrophy. Brain magnetic resonance imaging (MRI) was normal, except for a tiny T2-hypointense spot in the left globus pallidus consistent with a calcific lesion (Figure 1B). Laboratory tests showed elevation of acute phase reactants, leukocytosis with lymphopenia. Congenital infectious diseases were excluded and lymphocytes' subpopulations showed a low number of B and NK cells (Table S1 and Supporting Information). Immunoglobulin levels, NK cell activity and T cell proliferation and clonality were normal. Skin biopsy was consistent with leukocytoclastic vasculitis. Bone marrow aspirate was negative for atypical cells, karyotype was normal, and the type I interferon signature was negative (Figure 1C and Supporting Information). A 41-genes next generation sequencing panel of the more common autoinflammatory diseases was negative.[4]

Increasing doses of steroids were able to achieve only a partial control of the inflammatory manifestations (Figure 1A). Recombinant interleukin-1 receptor antagonist (anakinra) was started with a partial effect despite high dosages (up to 6 mg/kg daily); also sirolimus was attempted without any substantial improvement. At 3 months of age, parenteral nutrition was started due to recurrent episodes of diarrhea, malabsorption and failure to thrive.

In the following months, the patient displayed *Staphylococcus aureus* central catheter infection and *Pneumocystis carinii* pneumonia with severe respiratory distress despite antibiotic prophylaxis with

trimethoprim sulfamethoxazole. Follow-up brain imaging showed bilateral T2 hyperintensities (Figure S1) and several symmetrical calcifications (Figure 1B).

The patient was referred to the hematopoietic stem cell transplantation (HSCT) Unit and received alpha/beta/CD19-depleted haploidentical HSCT at the age of 7 months (Supporting Information). Three months later, multiple absence-like epileptic events occurred. Brain MRI revealed new focal and contrast enhancing lesions (Figure 1D). She was treated with levetiracetam at increasing dosages and valproic acid. After 2 weeks, brain MRI revealed spontaneous resolution of the contrast enhancing lesions. After 18 months from haplo-HSCT, she developed hypoxia, cough and respiratory distress without infectious etiology. Lung CT scan was suggestive for obliterative bronchiolitis (Figure 1E). Imatinib was started with clinical benefit and discontinued 1 year later.

At last follow-up, at the age of 3 years and 7 months, she is affected by severe growth retardation (height -3,1 SD; weight and cranial circumference <-2 SD), facial dysmorphisms (Figure 1F) and neurodevelopmental delay, failing to meet language milestones and walking with passive support on a broad base. Only nutritional support therapy continues and montelukast and azithromycin for anti-inflammatory purposes on the lung (Supporting Information). Family support is required. Brain imaging shows progressive leukoencephalopathy, brain atrophy and increased calcifications (Figure 1B, Figure S1). Whole exome sequencing analysis of the patient revealed a *de novo* c.2658-2659del, p.F886Lfs*11 mutation of the *SAMD9L* gene, confirmed by Sanger sequencing and known to be associated with SAAD.[3,5]

The present case highlights the benefit of early HSCT in SAAD, as reported in the literature (Table S2).[3,5-7] The absence of acute graft versus host disease and the low acute toxicity displayed by our patient after alpha/beta/CD19-depleted haplo-HSCT may suggest this approach as a valid therapeutic strategy. HSCT cured the immunological status of the patient and reduced the risk of severe infection but it did not impact on extra-hematological manifestations of SAAD as neurological and pulmonary symptoms.

Two myelodysplastic syndrome (e.i. the myelodysplasia and leukemia syndrome with monosomy of chromosome 7 (MLSM7), MIM 252270, and the myelodysplasia, infection, restriction to growth, adrenal hypoplasia, genital phenotypes and enteropathy syndrome (MIRAGE syndrome), MIM 617053) have been associated with SAMD9/9L mutations (Table 1). Thus, a myelodysplastic risk should also be considered in SAAD and supports the indication of HSCT.

Brain calcifications are characteristic of SAAD patients, and were also present in our patient. This complication might be due to an altered calcium deposition secondary to an increased type I interferon response, as reported in type I interpheronopathies.[3] However, as in the present case, type I interferon signature is usually negative in SAAD but it raises rapidly over normal values during viral infections only.[3] Thus, recurrent viral infections may worsen the progression of brain calcifications, as suggested for patients with normophosphatemic familial tumoral calcinosis (NFTC), MIM 610455, carrying germline loss-of-function SAMD9 mutation (Table 1).[8] Whether the type I interferon blockade with JAK inhibitors might have some benefit to control SAAD manifestations is still to be assessed.

One of the most relevant extra-hematological features characterizing our patient after HSCT is the presence of a progressive leukoencephalopathy with a severe developmental delay.

Recently, the autopsy of a child carrying the same mutation of our patient showed patchy loss of Purkinje cells in the cerebellum without signs of demyelination.[5] On the other hand, progressive leukoencephalopathy and brain atrophy have been described in patients with ataxia pancytopenia syndrome (ATXPC), MIM 159550, secondary to germline gain-of-function SAMD9L missense mutations (Table 1).[9]

The rapid improvement of the brain contrast enhancing lesions developed three months after HSCT by our patient have never been reported in the literature and may suggest an immune reconstitution inflammatory syndrome-like phenomenon,[10] whose correlation with the SAMD9L mutation requires further studies.

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Table 1. SAMD9/9L associated diseases.

Gene	Loss of function	Gain of function
SAMD9L	MLSM7 [somatic]	ATXPS [germline, missense]; SAAD [germline, frameshift]
SAMD9	MLSM7 [somatic]; NFTC [germline]	MIRAGE [germline]

[Apex] = type of mutation. MLSM7, myelodysplasia and leukemia syndrome with monosomy of chromosome 7; NFTC, normophosphatemic familial tumoral calcinosis; ATXPS, ataxia pancytopenia syndrome; SAAD, SAMD9L-associated autoinflammatory disease; MIRAGE, myelodysplasia, infection, restriction of growth, adrenal hypoplasia, genital phenotypes and enteropathy syndrome.

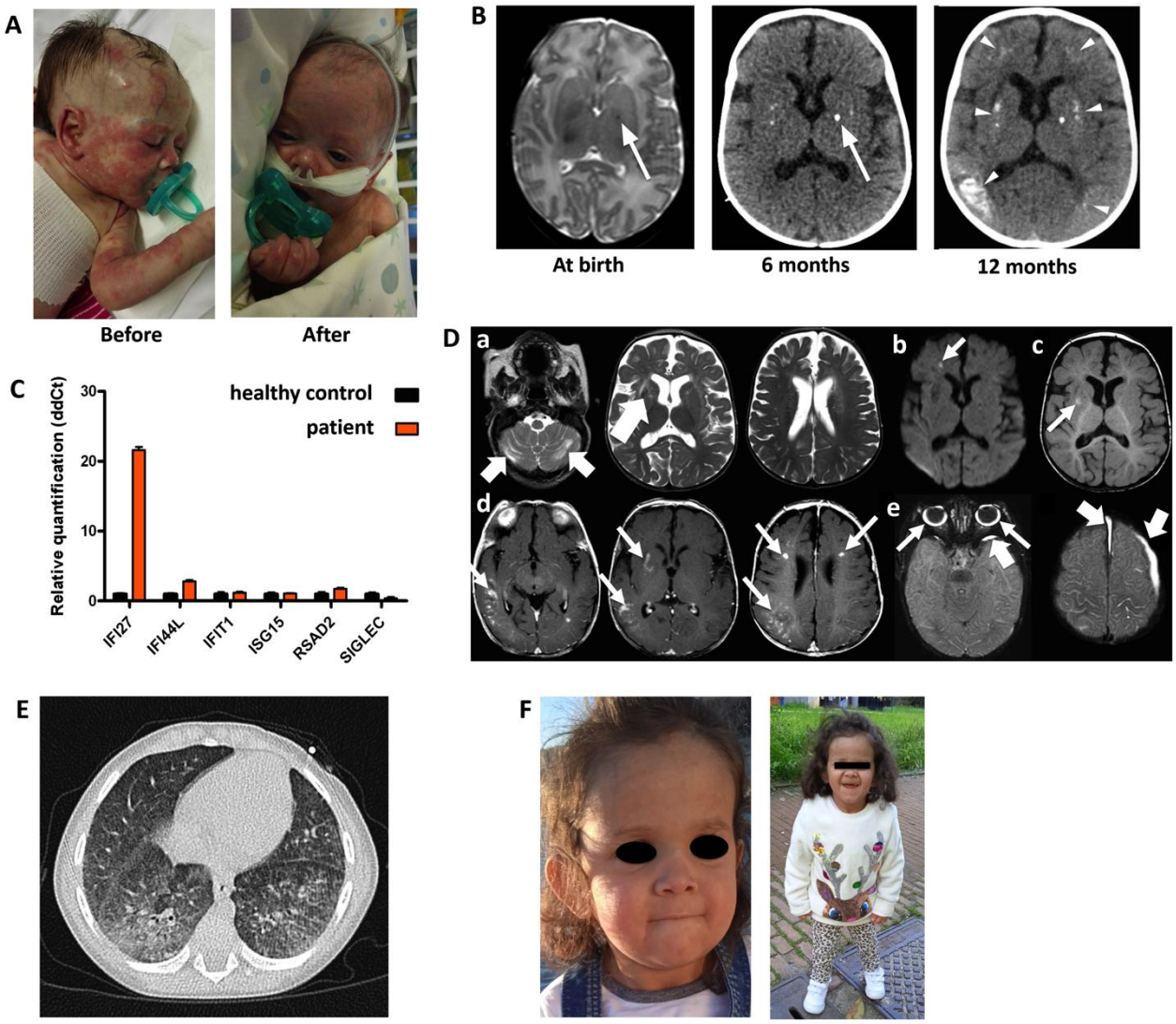

Figure 1. Clinical and imaging features of the patient.
A) Skin manifestations at one month of life before and after steroids. B) At birth, brain MRI axial T2-weighted image showing a left focal hypointensity in the left globus pallidus consistent with a small calcification; head CT performed at 6 months of age before the haplo-HSCT showing multiple spot-like calcifications (arrowhead); head CT performed at 3 years and 7 months of age, revealing new calcifications (arrowheads). C) Negative type I interferon signature of the patient compared to healthy control (Supporting Information). D) Brain MRI performed at 10 months of age, 3 months after the haplo-HSCT: a) axial T2-weighted images revealing worsening of the cerebellar and right putaminal lesions, and faint diffuse T2 hyperintensity of the frontal periventricular white matter; b) Axial diffusion-weighted image revealing a focal area of restricted diffusion in the right periventricular white matter; c) Axial T1-weighted image demonstrating a focal hyperintensity within the putaminal lesion, suggestive of microhemorrage; d) axial post-contrast T1-weighted and e) fat-saturated FLAIR images showing multiple nodular/punctate contrast enhancing lesions in the right basal ganglia and bilateral frontal white matter, associated with linear perivascular contrast enhancement in the right temporo-parietal subcortical white matter, diffuse leptomeningeal enhancement, pronounced bilateral choroidal and anterior eye segments contrast enhancement, and bilateral contrast enhancing subdural fluid collections. D) Lung CT showing interstitial lung disease developed 18 months after haplo-HSCT. K) Facial features at the last follow-up.

Details of immunologic and genetic analysis

Immunologic and genetic analyses were conducted in the contest of a research project approved by the regional IRB (approval BIOL 06/05/2004). In particular, the interferon signature was assessed analyzing the expression of six interferon-related genes as previously reported with minor modifications.[1] The whole exome sequencing analysis was performed on the family trio. Target DNA was captured by Nextera Flex Enrichment kit in combination with the xGen Exome Research Panel v1. Libraries quality was checked with 2100 Bioanalyzer instrument. Sequencing was performed on Illumina NovaSeq 6000 System and a quality check was achieved by FastQC tool. Obtained reeds were aligned against Hg19 Human Genome by BWA-MEM. Initial mappings were processed using the GATK framework version 4.0, according to the GATK best practices recommendations. Variants were classified as known or novel based on dbSNP146 and annotated using KGGSeq, then filtered based on the allele frequencies in the healthy population and the impact on encoded proteins. Only *de-novo* missense variants with allele frequencies below 1% were considered. The pathogenic variant was selected after the bioinformatic pipeline and validated by Sanger sequencing.

Details of the HSCT

The mother was selected as donor. The conditioning regimen included treosulfan (10 gr/m2 days -6,-5,-4), fludarabine (30 mg/m2 days -6,-5,-4,-3), anti-thymocyte globulin (4 mg/kg days -4,-3,-2) and rituximab (200 mg/m2 day -1). Prophylaxis with trimethoprim sulfamethoxazole (5 mg/kg in 2 doses), liposomal amphotericin B (2,5 mg/kg/dose twice a week) and antiviral prophylaxis with acyclovir (30 mg/kg/die in 3 doses) were administered. The patient received manipulated graft containing $12{,}36 \times 10^9$/kg mononuclear cells, specifically 35.69×10^6/kg CD34+ with alpha/beta $0{,}205 \times 10^5$/Kg. The source of stem cells was peripheral blood mobilized with granulocyte colony-stimulating factor from a haplo-parent. All grafts were manipulated with selective TCR □lpha/□eta+/CD19+ depletion using an immunomagnetic method, according to the manufacturer's recommendations (Miltenyi Biotec, Bergisch Gladbach, Germany). When more than one haplo-parent was suitable for peripheral blood stem cell donation, donor selection was performed according to current guidelines[2] and excluding parent for whom the patient had donor-specific antibodies, privileging donors characterized by NK alloreactivity, and/or a B/X KIR genotype.

Engraftment was reached 14 and 10 days after haplo-HSCT for platelets and neutrophils, respectively, and full and stable donor chimerism was demonstrated in peripheral blood cells. No major infections or toxicity occurred in the peri-transplantation period.

Details of the follow-up

The patient stopped immunosuppressant treatments and antibiotic prophylaxis at 29 months after the HSCT and removed the central catheter shortly after. Due to the satisfactory immunological reconstitution, the vaccination calendar was started without relevant problems and with a satisfactory antibody response. Since then, she required three hospitalizations for a viral febrile infection, a *Pseudomonas oryzihabitans* febrile infection and an episode of upper urinary tract infection by *Escherichia Coli*. From the respiratory point of view, she did not present any relevant further complication. The last chest CT was stable.

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Table S1. Immunological laboratory tests before and after HSCT.

Laboratory tests, unit	Before HSCT	After HSCT	Normal range
Erythrocyte sedimentation rare, mm/h	74	<10	<10
C reactive protein, mg/dl	14.9	<0.46	<0.46
Immunoglobulin A	<4	197	0 - 83
Immunoglobulin G	207	1280	230 - 1400
Immunoglobulin M	<4	108	0 - 145
Lymphocytes, cells/mm^2	3130	2410	3700 - 9600
CD3+, cells/mm^2 (%)	2998 (96.7)	1518 (63)	3300 - 6500
CD3+ CD4+, cells/mm^2 (%)	2504 (80)	561 (23.3)	1500 - 5000
CD3+ CD8+, cells/mm^2 (%)	513 (16.4)	554 (23)	500 - 1600
CD3+ HLA DR+, cells/mm^2 (%)	247 (7.9)	330 (13.7)	70 - 500
CD19+, cells/mm^2 (%)	19 (0.6)	446 (18.5)	600 - 3000
CD16+ CD56+ CD3-, cells/mm^2 (%)	88 (2.8)	434 (18)	100 - 1300

Table S2. Literature review of patients with SAAD compared to our case.[3,5,6]

Characteristics	Our case	Literature (N=9)
Gender	Female	Female:Male:not known=2:2:5
Neonatal onset	+	3/9 (not known in 6/8 patients)
Fever	+	9/9
Rash erythematous	+	3/9
Panniculitis	-	6/9
Lip erosion	-	3/9
Nail dystrophy	+	0/9
Epiglottitis	-	2/9
Hepatosplenomegaly	+	8/9*
Chronic diarrhea	+	0/9
Facial dysmorphisms	+	0/9
Viral infections	-	RSV (3/9), rhinovirus (3/9), parainfluenza (2/9), coronavirus (1/9), CMV (1/9), adenovirus (1/9), enterovirus (1/9)
Bacterial infections	Staphylococcus aureus, pneumocystis carinii	0/9**
Lung disease	Obliterative bronchiolitis after HSCT	Interstitial lung disease (4/9), lung opacities in the upper lobes (1/9)
MRI brain change	Calcification, white matter disease, atrophy	Brain calcification (5/9), white matter disease (2/9), atrophy (1/9)
Skin biopsy	Leukocytoclast vasculitis	Neutrophilic infiltration (8/9), leukocytoclast vasculitis (1/9)
Serum inflammatory markers	+	3/9 (not known in 6/9 patients)
B cell lymphopenia	+	9/9
Low immunoglobulin concentrations	-	3/9

*one patient only with splenomegaly; ** Staphylococcus aureus was found post-mortem in one patient.

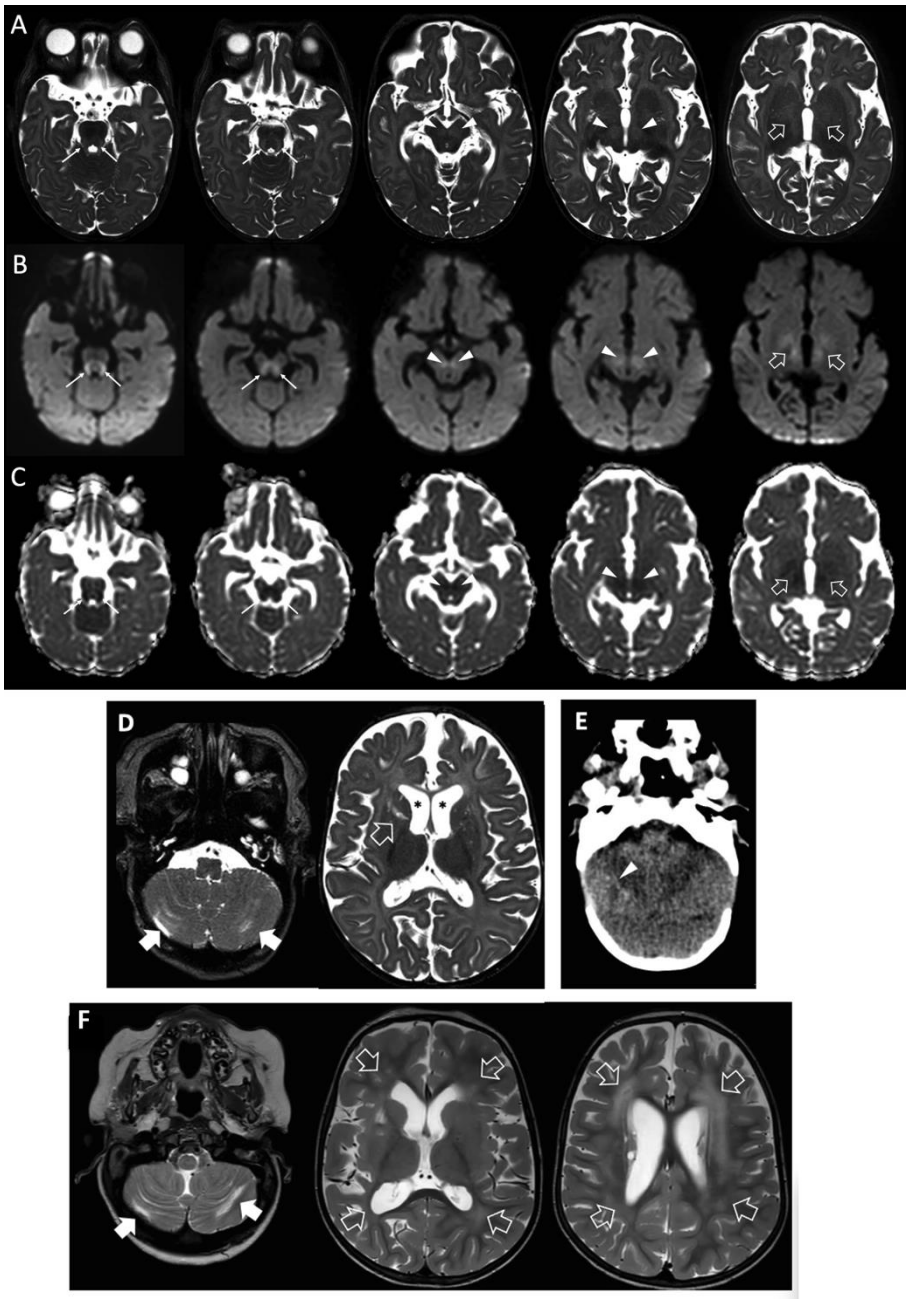

Figure S1. Imaging follow-up of the brain.
A) Axial T2-weighted, B) diffusion weighted (DWI), and C) apparent diffusion coefficient (ADC) map images demonstrating bilateral T2 hyperintensities with restricted diffusion and low ADC values of the superior cerebellar peduncles (thin arrows), red nuclei (arrowheads), and mesial thalami and subthalamic nuclei (empty arrows). No contrast enhancement is present. D) Brain MRI and E) head CT at 6 months of age before the haplo-HSCT; axial T2-weighted images demonstrating reduction of the white matter volume with enlargement of the lateral ventricles (asterisks) and subarachnoid spaces, associated with focal T2 hyperintensities at the level of the right putamen (empty arrow) and bilateral cortico-subcortical cerebellar regions (thick arrows). L) Brain MRI performed at last follow-up, at 3 years and 7 months of age, revealing progression of the leukoencephalopathy (empty arrows) and brain atrophy with reduction of the white matter volume, stable cerebellar lesions (thick arrows).

The expanding pathways of autoinflammation: a lesson from the first 100 genes related to autoinflammatory manifestations

Autoinflammatory diseases (AIDs) are a heterogeneous group of innate immune system disorders, characterized by sterile inflammation without evidence of pathogenic autoantibodies or auto-reactive T lymphocytes.[1,2] Originally, AIDs were characterized by recurrent fever episodes with urticarial rash and serositis, with possible severe long term complications as mental impairment, infertility and AA amyloidosis.[3,4] After the discovery of the first disease-causing gene, namely *MEFV* for Familial Mediterranean Fever (FMF),[5,6] an expanding spectrum of monogenic disorders have been associated with errors of the intra-cellular pathways primarily involving the innate immunity.[7,8] All these disorders are mediated by the overproduction of various inflammatory cytokines, such as interleukin (IL)-1, IL-18, and type I interferons (IFN). According to the mainly intracellular pathways involved in the pathogenesis of these rare conditions, treatments with specific inhibitors of the cytokines involved or their downstream mediators have proven to be very effective in most patients.[9]

Due to the clinical overlap of symptoms among different monogenic AIDs, the diagnostic approach is usually rather complex. Recently developed technologies for the molecular analysis, as well as the next generation sequencing, allow the simultaneous analysis of various genes associated with a given group of inherited disorders. Next generation sequencing panels are increasingly used in patient with undefined AID with variable success.[10–14] Furthermore, geneticists try to design appropriate workflow for gene variants interpretation.[15]

Interesting, inherited errors in the regulatory system of the immune response can result not only in AIDs, but also in primary immunodeficiencies (PIDs). These latter conditions are characterized by autoimmune manifestations caused by defective tolerance to self-antigens inducing organ dysfunctions. However, a growing number of PID can also present with a clear inflammatory phenotype overlapping the clinical manifestations observed in AIDs. These group of diseases are named immune dysregulation.[16,17] Thus, the clinical presentation may not favor a correct molecular diagnosis in patients affected by immune disorders.

The knowledge of the underlying dysfunctional pathways may aid clinicians detecting the pivotal cytokine causing inflammatory symptoms, thus providing the best target for treatments even in patients without diagnosis.

Here, we review the genes associated to diseases possibly presenting with a clear inflammatory phenotype, and we propose a pathways-oriented classification of monogenic conditions associated to an inflammatory phenotype.

Autoinflammatory pathways

Discovery of AID-causing genes reveals key roles of proteins involved in the common inflammatory response. The number of genes related to AID (Table 1) and inflammatory manifestations associated with disorders alternatively classified (Table 2) are constantly increasing and consent to highlight at least six main autoinflammatory pathways.

Inflammasomopathies

Enlarging number of AID are caused by constitutive activation of inflammasomes. Inflammasomes are multiprotein intracellular complexes that promotes the maturation and secretion of pro-inflammatory cytokines, as IL-1β and IL-18, in response to pathogenic microorganisms and sterile stressors.[18,19] Three of the four best-known recurrent fever syndromes follow this pathway: FMF, mevalonate kinase deficiency (MKD) and cryopirinopathies, also named cryopin-associated periodic syndromes (CAPS).

NLRP3 inflammasome

CAPS are linked to gain of function (GOF) mutations of the nucleotide-binding oligomerization domain-containing protein (NOD)-like receptor family pyrin domain containing 3 (NLRP3) gene causing a constitutive activation of the NLRP3 inflammasome (Table 1). Three clinical phenotypes with increasing severity have been identified: familial cold autoinflammatory syndrome type 1 (FCAS1), Muckle-Wells syndrome (MWS) and Chronic infantile neurological, cutaneous and Articular syndrome (CINCA). Patients with CAPS suffered of systemic inflammation, fever and urticarial rash. Patients with CINCA also present chronic aseptic meningitis with consequent brain atrophy, mental retardation and sensor neural deafness, in addition to osteoarticular manifestations.[20–24]

The discovery of the underlying pathogenic mechanisms led to the use of IL-1 inhibitors for the treatment of these patients.[25,26] Although the treatment response is often optimal, some patients respond only partially, requiring relevant dose escalation. Moreover, some manifestations (for example, the bone abnormalities) are refractory to IL-1 inhibition.[27]

In recent years, it has been shown that the secretion of IL-1 and IL-18 mediated by inflammasomes needs the cleavage of gasdermine D induced by caspase-1 and translocation of the N-terminal fragment towards the plasma membrane.[28,29] At this level, the gasdermine D forms pores by binding to membrane phospholipids through which cytokine secretion occurs. A continuous activation of gasdermine D and the excessive formation of pores at the level of the plasma membrane determines

the inflammatory form of programmed cell death, called pyroptosis, which is characterized by cellular edema, loss of the plasma membrane integrity with consequent release of inflammatory cytokines in the extracellular environment. Human CAPS murine models demonstrated a close correlation between NLRP3 inflammasome activation, gasdermine D maturation and subsequent pyroptosis, confirming that gasdermine D is an important effector of this pathway. Recently, a novel knock-in murine model of CAPS demonstrated the development of AA amyloidosis and partial response to proton pump inhibitors.[30] Thanks to the demonstration of the NLRP3 involvement in atherosclerosis and other common adult diseases, [31,32] studies about molecules able to regulate the NLRP3-dependent inflammation are greatly increasing during the recent years[33–40] and intramolecular/intracellular regulators have also been discovered.[41,42] Of particular interest, lipin-2 protein acts as a magnesium-dependent phosphatidate phosphatase enzyme which catalyzes the conversion of phosphatidic acid to diacylglycerol during triglyceride, phosphatidylcholine and phosphatidylethanolamine biosynthesis in the reticulum endoplasmic membrane. Lipin-2 restricts overphosphorylation of protein kinases with extracellular regulation (ERK), p38, and c-Jun NH2-terminal Kinase (JNK), thus inhibiting the activation of the NLRP3 inflammasome.[43] Mutations of the *LPIN2* gene coding for lipin-2 protein are related to the Majeed syndrome (MJDS), a rare AID characterized by recurrent fever, congenital dyserythropoietic anemia and sterile multifocal osteomyelitis, whose patients respond to IL-1 inhibition (Table 1).[44]

Pyrin inflammasome

FMF and the pyrin-associated autoinflammation with neutrophilic dermatosis (PAAND) are monogenic AIDs both related to variants in different domains of the *MEFV* gene and associated with an excessive activation of the pyrin inflammasome (Table 1).[45,46] Pyrin consists of four domains with different functions.[47] The N-terminal domain is necessary for the formation of the pyrin inflammasome. Other domains seem to have a self-inhibitory role. In particular, mutations in the C-terminal domain have been associated with FMF. Other FMF-related mutations affect a region of the protein close to the N-terminal domain. In the stationary conditions, this protein region is maintained phosphorylated by kinases dependent on molecules that are sensitive to any cytoskeletal alterations. In case of aggression by pathogens not previously encountered by the immune system, the perturbation induced in the organization of the cytoskeleton is able to activate the pyrin inflammasome causing the secretion of IL-1β and guaranteeing an adequate inflammatory response.[47,48] Colchicine is able to stabilize the cytoskeleton of the cell, limiting the pro-inflammatory effect of a pyrin that is more prone to activation due to specific *MEFV* mutations.[49,50]

On the other side, constitutive activation of the pyrin is able to affect cytoskeleton-dependent properties of neutrophils. In particular, neutrophilic dermatosis, peripheral neutropenia, and sterile abscesses have been reported in response to mild stimuli in patients with PAAND, the pyogenic sterile arthritis, pyoderma gangrenosum, acne (PAPA) syndrome, and the PSTPIP1-associated myeloid-related proteinemia inflammatory syndrome (PAMI), these two latter conditions both related to *PSTPIP1* gene mutations (Table 1).[51,52]

Hidrosadenitis supporativa (HS), also known as acne inversa, is a long-term skin disease characterized by recurrent inflammatory sterile abscesses. HS manifestations may occur in patients with PAPA syndrome[53] and genes related to familial forms of HS are newly recognized AID-related genes (Table 2).[54] In this group of disorders, treatment with IL-1 inhibitor may be effective, despite anti-tumor necrosis factor (TNF) or IL-6 inhibitors are the first line biological therapy for HS.[55–57]

Homozygous or compound heterozygous mutations in the *MVK* gene resulting in a loss of function (LOF) of the mevalonate kinase enzyme greater than 1% cause MKD. Patients with MKD usually present with recurrent febrile episodes associated with cervical lymphadenopathy, abdominal pain with vomiting and diarrhea, arthralgia and cutaneous manifestations; atypical manifestations may occurs.[58,59] Less than 0.5% of the enzymatic activity determines the more severe disease manifestation, also call mevalonic aciduria, characterized by the early development of systemic inflammation with facial dysmorphisms, growth delay, convulsions and progressive cerebellar ataxia. Patients with an intermediate phenotype can present at birth with persistent inflammation. The pathophysiology of inflammation in MKD is related to the loss of isoprenoid lipids synthesis and, in particular, with the geranylgeranyl diphosphate deficiency. The geranylgeranyl diphosphate is necessary for the prenylation of small GTPases, as well as RhoA, whose inactivation causes constitutive activation the pyrin inflammasome, IL-1 release and inflammation.

On this basis, the main therapeutic attempts for patients with MKD were focused on the IL-1 inhibition, despite the response is partial in 70% of patients. More recently Canakinumab, an anti-IL1β monoclonal antibody, has shown superior efficacy, with a complete response in more than 50% of patients.[26] These patients may require higher dose and frequency of drug administration compared to patients with other periodic fevers.[60] The involvement of other pro-inflammatory cytokines, including IL-6 and TNF is increasingly emerging in the pathogenesis of MKD. This evidence may explain the limited efficacy of IL-1 blockade and supports alternative pharmacological treatments. Tocilizumab is a humanized monoclonal antibody against the IL-6 receptor that binds to the soluble and membrane receptor of this cytokine, inhibiting IL-6 mediated signaling. Limited experience on the use of tocilizumab in MKD has been reported in the

literature.[61] However, blockade of IL-6 should represent the third-line treatment before considering hematopoietic stem cell transplantation.[62]

Other inflammasomes

Patients with autoinflammation and infantile enterocolitis (AIFEC) carry a heterozygous GOF variant of the *NLRC4* gene and may present with fatal episodes of macrophage activation syndrome (MAS). An exaggerated IL-18 production is the *primu movens* alternative to IL-1, whose inhibition is not effective in these patients. Murine models lacking IL-18 inhibition or with constitutively active IL-18 highlighted the role of toll-like receptor 9 and intestinal mucosa in hyperferritinemic syndromes.[63,64] Interestingly, according to these evidences, a patient with AIFEC resistant to treatment with humanized anti-IFN gamma was treated with transplant of maternal stool with benefit.[65,66] In healthy individuals, the IFN regulatory factor 8 is a critical regulator of NLRC4 inflammasome during the host defense against Gram-negative bacteria.[67] The nucleotide-binding domain and leucine-rich repeat receptor family apoptosis inhibitory proteins are the cytosolic receptors that recognize conserved bacterial proteins, as flagellin. IFN regulatory factor 8 is required for the transcription of genes encoding this group of cytosolic receptors that activate the NLRC4 inflammasome, leading to cell death and IL-1/IL-18 secretion.

Patients with C/EBPe-associated autoinflammation and immune impairment of neutrophils (CAIN) present recurrent fevers lasting 4-5 days, every 2-4 weeks, characterized by abdominal pain and oral aphthosis.[68] Sterile abscesses of the tongue, paronychia, and hemorrhagic diathesis not related to platelets abnormalities are associated. Laboratory tests show high acute phase reactants and defects in segmentation of neutrophils. The genetic cause is a homozygous variant of the *CEBPE* gene, coding for the transcription factor CRP1 or C/EBPe. The reduced ability of binding transcriptional repressors results in over-expression of many NLRP3-related inflammatory genes and hypersecretion of IL-1 and IL-18 by neutrophils. Patients do not suffer of serious long-term complications, presenting an average life span of more than 70 years. However, the reduced quality of life can suggest using IL-1 or IL-18 inhibitors.

On the other side, the evidences of NLRP12 and NLRP7-related AID are scanty and many different inflammatory manifestations have been associated with *NLRP12* and *NLRP7* gene variants.[69–72] The high prevalence of variants of unknown significance in the general population may suggests to be very careful in considering a pathogenic role of the NLRP12 or NLRP7 variants (Table 2).

Rare variants of the *NLRP1* gene cause LOF of the auto-inhibitory N-terminal domain of the NLRP1-inflammasome that acts in keratinocytes preventing the development of the NLRP1-associated autoinflammatoty disease with arthritis and dyskeratosis (NAIAD).[73,74] The anthrax

lethal factor metalloprotease and small-molecule DPP8/9 inhibitors are both able to cleavage the N-terminal domain of the NLRP1, activating the NLRP1-dependent inflammatory response.[75–77] NLRP1 inflammasome seems to have an antibacterial role in mucosal innate immunity, preventing the development of inflammatory bowel disease (IBD).[78,79]

Patients with mutations in the *F12* or *ADGRE2* gene also present autoinflammatory urticarial manifestations (Table 2).

Immuno-proteinopathies

Proteinopathies are a group of diseases where proteins are abnormally associating and aggregating, due to pathological conformational changes. Intracellular deposition of misfolded proteins causes endoplasmic reticulum stress and pro-inflammatory reaction called the unfolded proteins response (UPR). Here, we propose to collect AIDs causing UPR activation under the term of immune-proteinopathies to distinguish them from the neurologic diseases related to misfolded proteins accumulation.

The TNF receptor-associated periodic syndrome (TRAPS) is one of the best-known monogenic hereditary recurrent fever syndromes, resulting from an autosomal dominant variant of the *TNFRSF1A* gene.[80] TRAPS patients usually present recurrent episodes of fever, abdominal, chest or limb pain, rash, ocular symptoms, and up to 10% develop systemic AA amyloidosis.[81] On demand anti-inflammatory drugs in case of flares and regular blood and urine tests to check high acute phase reactants and proteinuria during the symptoms free intervals are required. NSAIDS or steroids are effective in the short term, but colchicine, DMARDs and biologics may be needed as maintenance therapy.[82] Initially, it was hypothesized that TNF receptor mutations are able to increase the binding affinity or induce a state of constitutive activation even in the absence of TNF, but both hypotheses were incorrect.[83] Under physiological conditions, the extra-cytosolic portions of TNF receptor undergo a cleaving mediated by the transmembrane glycoprotein called ADAM17 or TNF-converting enzyme 23. Following the shedding, a soluble pool of TNF binding proteins prevents the TNF contact with the receptors on the cell membrane, blocking the transmission of the pro-inflammatory signal. The presence of a receptor-shedding defect in TRAPS patients has been demonstrated in 1999.[80] On this basis, a therapeutic role for the TNF receptor fusion protein was hypothesized but later not confirmed.[84] Subsequent studies have shown that the pathogenic mechanisms related to TRAPS are much more complex. Mouse model showed that TRAPS mutations cause a defect in the transport of TNF receptor towards the cell membrane, leading to its accumulation within the endoplasmic reticulum and consequent formation of aggregates able to induce UPR independently of the extracellular TNF binding. A protein sensor was identified in the

endoplasmic reticulum of hepatocytes, called cAMP-responsive-element-binding protein H that is capable of directly increasing the expression of acute phase proteins, including the serum amyloid A. The accumulation of the TNF receptor in the cytoplasm and Golgi has been also associated with nuclear factor kappa-B (NFkB) hyper activity. Furthermore, the exaggerated inflammatory response observable in the TRAPS patient also appears to be supported by the activation of the p38 and JNK proteins. Together with ERK, p38 and JNK constitute the family of the mitogen-activated protein kinases that are crucial in the recruitment and activation of leukocytes at the inflammatory site. The intra-cytosolic accumulation of the unfolded protein is able to inhibit the phosphatases that normally block mitogen-activated protein kinases activity.[85–87] This data has been demonstrated by the fact that homozygous knock-in mice for TNF receptor mutations are protected from any inflammatory insult. It is therefore likely that the induction of the inflammatory response in TRAPS requires the simultaneous presence of the mutated receptor and the wild type receptor on the membrane, as indeed happens in TRAPS subjects who have 50% of normal membrane receptors.

The UPR is also believe to be the relevant in the sideroblastic anemia with B-cell immunodeficiency, periodic fever and developmental delay (SIFD), as well as in infantile cortical hyperostosis (ICH), also known as Caffey disease (Table 2).

Patients with SIFD present with persistent systemic inflammation since first weeks of life, associated with mucocutaneous and musculoskeletal manifestations, as septal panniculitis and mononuclear myofasciitis.[88] The need for blood transfusions increase during flares.[89,90] SIFD is caused by homozygous or compound heterozygous LOF mutation in the *TRNT1* gene, coding for the tRNA nucleotidyltransferase cytidine-cytidine-adenosine-adding 1 that is essential for maturation of tRNAs, which accumulation causes higher spontaneous production of reactive oxygen species and NLRP3-dependent inflammatory cytokines in cultured patients cells secondary to augmented endoplasmic reticulum stress response. Activation of UPR causes the abnormal B cell maturation leading to Ig deficiency.[91] Furthermore, mutant cells showed deficient clearance of the ubiquitin-scaffold protein p62, which is a substrate for the ubiquitine-proteasome system (UPS), which primary defects are associated with other AIDs, as described later in details. Anti-TNF treatments are more effective then IL-1 inhibitors in these patients.

Caffey disease is a familial disorder manifesting in the late fetal period or infancy with excessive periosteal bone formation.[92] Signs and symptoms regress spontaneously within months. Caffey diseases is caused by the c.3040C>T mutation of the *COL1A1* gene. The consequent p.Arg836Cys substitution leads to increased disulfide crosslinking within or between collagen fibrils, causing the UPR activation. The *COL1A1* mutations in the intron 5 and 6 have been associated with Ehlers-Danlos syndrome, and exon mutations predominately cause osteogenesis imperfecta.[93]

Immuno-actinopathies

Abnormal regulation of the cytoskeleton assembly determines complex clinical pictures that associate inflammatory manifestations and immunodeficiency. Actin is a major component of cytoskeletron architecture, and then we propose immuno-actinopathies as name to refer to this group of conditions in which there is a clear overlap between pure autoinflammation and immune-dysregulation.

Wiskott Aldricht syndrome (WAS) is an X-linked immunodeficiency characterized by the clinical triad of microthrombocytopenia, eczema, and recurrent infections (Table 2). The syndrome has a wide clinical spectrum ranging from autoimmunity to atopy and autoinflammation.[94] Autoinflammatory manifestations typically present early in life, are often refractory to therapy, and are associated with a worse clinical prognosis and an increased risk of developing a malignancy.[95] Deficiency of WAS protein causes defects in chemotaxis and podosomes formation, leading to abnormal autophagosome and inflammasome function and thus favoring the onset of autoinflammatory manifestations.[96]

The autoinflammatory periodic fever, immunodeficiency, and thrombocytopenia (PFIT) syndrome is caused by mutation in the actin-regulatory *WDR1* gene, coding for the WD-repeat protein 1 (Table 1).[97] The protein plays a critical role in dynamic reorganization of the cytoskeleton.[98] Patients present periodic fevers lasting 3–7 days, every 6–12 weeks, with severe acute phase reactants, hyperferritinaemia, and oral mucosal inflammation, causing an acquired microstomia. Severe infections, recurrent perianal ulceration, and pyoderma gangrenosum were also observed. Platelets abnormalities characterize also the platelet abnormalities with eosinophilia and immune-mediated inflammatory disease (PLTEID) caused by bi-allelic mutations of the *ARPC1B* gene.[99–103] The disease is characterized by systemic inflammation with lympho-proliferation and immunodeficiency resembling the Wiskott-Aldrich syndrome, with early onset vasculitis, severe infections, eczema. Laboratory tests show microthrombocytopenia, eosinophilia, T lymphopenia with reduced T naïve cells and high serum levels of immunoglobulin (Ig)E and IgA. Functional studies can detect the defective migration and proliferation of T lymphocytes. A functional fMLP/phalloidin test can differentiate symptomatic patients from asymptomatic mutation carriers.[104] Patients with PLTEID present low production of ARPC1B protein, determining a defect of the protein complex adepts to regulate the elongation of actin filaments in the blood cells.

A new monogenic AID characterized by IL-18 hypersecretion related to cytoskeletal abnormalities has diagnosed in four patients presented with Neonatal onset cytopenia with autoinflammation, rash, and hemophagocytosis (NOCARH).[105] Patients present with fever, hepatosplenomegaly and

urticarial rash. Growth retardation and facial dimorphisms similar to those are present in patients with cryopirinopathy or Noonan syndrome were noted.[106] Laboratory tests showed increased acute phase reactants, high serum concentration of IL-18 and transfusion-dependent cytopenia. Whole exome sequencing highlights three stop-codon variants of the *CDC42* gene, coding for the Cell division control protein 42 homolog (CDC42) protein. The variants involve the C-terminal domain that normally allows CDC42 binding to lysosomal membrane containing phosphatidyl-inositol-4,5-bisphosphate, necessary for actin assembly. Patients respond to IL-1 inhibitor with complete control of inflammatory symptoms and growth re-catch. However, severe infections occurred during the follow-up, in association with persistence of increased IL-18 concentration.

An increased IL-18 concentration also characterizes familial forms of hemophagocytic lymphoistiocytosis (HLH) and other diseases with immunodysregulation (Table 2).

Interleukin receptors signaling defects

IL-1R and IL-36R signaling defects

Generalized pustular psoriasis (GPP) is an extremely rare type of psoriasis, presenting with a whole body-covered pus-filled blisters that can be associated with systemic inflammation.[107] Three AIDs are characterized by GPP caused by inflammation of the resident cells of the skin, namely keratinocytes: the IL-1 receptor antagonist deficiency (DIRA), the IL-36 receptor antagonist deficiency (DITRA), and the caspase recruitment domain (CARD) 14-mediated psoriasis (CAMPS). A patient with coexisting mutations of the *CARD14* and *IL36RN* genes have been also described.[108] Furthermore, the AP1 deficiency, secondary to specific polymorphisms of the *AP1S3* gene, seems to modify symptoms severity in patients with DITRA.[109–111] Anti IL-1 treatment is the first-line treatment in patients with DIRA, that usually presents more significant signs of systemic inflammation then patients with DITRA or CAMPS, as persistent fever and osteitis.[112–114] Anti IL-1 treatment is less effective in patients with DITRA who dramatically respond to Ustekinumab. The availability of recombinant IL-36 receptor antagonist will represents the treatment of choice in this condition.[115–117]

IL10R signaling defects

Patients with rare forms of very early-onset (VEO)-IBD may carry mutations of genes coding for IL10 or IL-10 receptor subunit A and B. Furthermore, two siblings presenting with early-onset

ANA-positive multidrug-resistant oligoarthritis carried LOF variants of the *NFIL3* gene that has been related to the IL-10 signaling defects in mice and IBD susceptibility in humans.

Relopathies

Hyper activation of the NFkB/Rel, leading to inflammatory manifestations, is the final pathway involved in many AIDs, as well as the CARD14 and CARD15/NOD2 signaling defects, and the newly discovered group of disorders with abnormal ubiquitination.[118]

CARD14 and CARD15/NOD2 signaling defects

CARD14 is a proinflammatory signaling molecule whose expression is predominantly restricted to epidermal keratinocytes.[119,120] The N-terminal domain acts as NFkB activator thought blocking the activity of the inhibitor of kappa B kinase complex. The C-terminal domain seems to have an auto-regulatory activity. Dominant GOF mutations in the *CARD14* gene are associated with psoriasis and LOF mutations are related to severe variant of atopic dermatitis.[121,122] Patients with CAMPS may present with a wide range of psoriasis-like manifestations, from palmoplantar pustulosis to papulosquamous eruptions, requiring high doses of Ustekinumab.[123–127]

Patients carrying a variant of the CARD15/*NOD2* gene can be affected by Blau's syndrome, early onset sarcoidosis, or be more prone to develop Crohn's disease. The number of NOD2 variants considered likely benign or of unknown significance according the classification of the International study group for systemic autoinflammatory diseases, freely available at the Infevers web-site (https://infevers.umai-montpellier.fr/web/), are numerous and therefore caution should be used in confirming clinical diagnosis especially in case of the presence of intronic variants.[128–130]

The *NOD2* gene coding for a cytosolic pattern recognition receptor and is composed of three domains.

Variants in the C-terminal leucine-rich-repeat domain, which is essential for the muramyl-dipeptide sensing ability of NOD2, are associated with susceptibility to Crohn's disease in relation to their ability to disrupt intestinal mucosal homeostasis.[131–133]

Intriguingly, stimulation of innate immunity by administration of NOD2 agonists represents a new way to prevent and treat infectious diseases and gastrointestinal cancer.[134,135] On the other hand, NOD2 variants in the central nucleotide binding and oligomerization domain of the protein, which acts as auto-inhibitor domain, cause Blau's syndrome. The classic triad of clinical manifestations are boggy arthritis, panuveitis, and granulomatous dermatitis, despite atypical sites of granulomas

have been reported.[136–138] The severity of articular involvement, even if isolated, or multi-drug resistant uveitis may require anti IL-6 treatment.[139–148]

Patients with rare forms of very early-onset (VEO)-IBD may carry mutations of genes whose encoded protein affects the NOD2 signaling and, in particular, those with TRIM22 and XIAP deficiency (Table 2).

Ubiquitination defects

The ubiquitin-proteasome system (USP) allows clearance of abnormal intracellular proteins. Proteasome is a intracellular multi-protein complex able to degrade molecules bended to ubiquitin chains thanks to the action of the Linear ubiquitin chain assembly complex (LUBAC).[149,150] Negative regulation of the NFκB pathway, which is essential to stop the inflammatory response, depends on the level of ubiquitination of proteins associated with TNF receptor 1 signaling. LUBAC consists of at least three proteins: ring finger protein 31 (RNF31/HOIP), RanBP-type and C3HC4-type zinc finger containing 1 (RBCK1/HOIL1) and SHANK-associated RH domain interacting protein (SHARPIN/SIPL1). The clinical manifestations presented by patients with the *HOIL1* gene variants are amylopectinosis, chronic inflammation and severe bacterial infections.[118] HOIP deficiency has been described in a patient with recurrent fevers, chronic steatorrhea and severe bacterial infections.[151] A second HOIP patient showed early-onset seronegative polyarthritis that was resistant to steroids, methotrexate and TNF inhibitors.[152] Generalized epilepsy and eczematous dermatitis were also associated. The skin biopsy showed an extensive CD4+ cells infiltration. Laboratory tests showed high acute phase reactants, low number of memory B cells with hypogammaglobulinemia, and no evidence of immune response to vaccination against Pneumococcus. SHARPIN deficiency was recently described by Oda et al. and characterized by colitis, polyarthritis, mumps and hepato-splenomegaly with partial response to anti IL-6 therapy.[153]

On the other hand, A20 and OTU deubiquitinase with linear linkage specificity (OTULIN) removes linear polyubiquitin from proteins that have been modified by LUBAC.[154] Patients with OTULIN-related autoinflammatory syndrome (ORAS) showed relapsing episodes of nodular panniculitis with neutrophil infiltrate and fevers. Reduced growth parameters and painful swollen joints, as well as elevated immunoglobulin levels and serum autoantibodies were reported. Patients with A20 haploinsufficiency (HA20) manifest a Behçet's disease-like syndrome with oral and genital ulcers and, rarely, uveitis. Transmission is dominant, symptoms occurs at early age, and severe inflammatory manifestations, as well as inflammatory bowel disease, arthritis, and thyroiditis, can be associated.[155–158] Treatment with TNF and IL-1 inhibitors is partially effective.[159] However,

other factors seems to be involved in the inflammatory pathways of relopathies, and combined biologics therapy may be required.[160–162]

The interest of neurologists in LUBAC complex is increasing due to the evidence of an impact into the valosin containing protein-related diseases.[163]

Interferonopathies

Interferonopathies are a group of monogenic AIDs characterized by severe inflammatory manifestations caused by high secretion of IFNα/β. The type I IFN-dependent inflammatory response is a highly conserved innate immune pathway against viral infections. To the date, several monogenic diseases have been included in this group (Table 2). High production of type I IFN-related mRNAs, called IFN signature, is commonly used biomarker in these patients.[164–166] The discovery of the underlying molecular mechanism leading to the IFNα/β secretion led to the development of target drugs called JAK/STAT inhibitors.[167,168]

Proteasome-associated autoinflammatory syndromes

The UPS is also involved in a group of interferonopathies called proteasome-associated autoinflammatory syndromes (PRAAS).

The Chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperature (CANDLE) is the PRAAS paradigm causing by LOF mutations in the *PSMB4, PSMB8, PSMB9, PSMA3* genes that encodes the proteasome subunits.[169] Clinically, patients with CANDLE present with fever, neutrophilic panniculitis that leads to lipodystrophy, joint contractures, myopathy, and aseptic lymphocytic meningitis. During the first decade of life, almost half of the patients develop systemic arterial hypertension, metabolic syndrome, autoimmunity and hepatic steatosis.[170,171] Intellectual disability, generalized lipodystrophy and joint contractures have been reported as long-term outcomes in these patients. Similar clinical picture has been described in patients carrying mutations of proteins that regulate the catalytic activity of proteasome. In particular, an Algerian patient with early onset recurrent fevers, polymorphic rash and hepatosplenomegaly, carrying homozygous LOF mutations of the *PSMB10* gene, has been described as the last proteasome subunit-related PRAAS.[153]

Proteasome maturation protein (POMP) is a chaperone for proteasome assembly and is critical for the incorporation of catalytic subunits into the proteasome. Heterozygous frame shift variants of the *POMP* gene result in a truncated protein that perturbs proteasome assembly by a dominant-negative mechanism. POMP-related autoinflammation and immune dysregulation disease (PRAID) has been

discovered in two unrelated individuals with early-onset combined immunodeficiency, neutrophilic dermatosis, and autoimmunity.[172]

Heterozygous LOF mutations of the *PSMG2* gene, coding for the PAC2 protein, whose mutations result in a lower proteolytic activity of the UPS, has been found by Jesus et al. in patient with early-onset recurrent fevers, panniculitis, edema of the lower extremities.[173] Hepatosplenomegaly, generalized lymph adenomegaly, transfusion-dependent hemolytic anemia, and myositis were associated.

Homozygous LOF mutations of the *USP43* gene, coding for a protein capable in deubiquitinating proteasome subunits, cause early onset recurrent fevers, lipodystrophy and polyarthritis in a Tartar patient.[153] The lack of such intracellular regulation also causes a reduced proteolytic activity of the proteasome.

Other autoinflammatory interferonopathies

The STING associated vasculopathy with onset in infancy (SAVI) is caused by GOF mutations in the STING viral sensor.[174,175] Patients show interstitial lung disease associated with peripheral ulcerative vasculitis of extremities, rarely leading to amputation. Interstitial lung disease also characterize patients affected by COPA syndrome that usually present early-onset symmetric polyarthritis at the disease onset.[176,177]

Patients with PRAAS, SAVI and COPA syndrome poorly respond to DMARDS or biological drugs that inhibit primary inflammatory cytokines (IL-1, TNF and IL-6). Baricitinib, leading to the block of STAT phosphorylation, has been proven some efficacy, despite viral respiratory infections and poliomavirus reactivation may developed, rarely leading to acute renal failure.[178,179] New target therapies are under studying.[180]

Others

The adenosine deaminase (ADA) 2 deficiency (DADA2) is a complex systemic AID in which vasculopathy that histologically resembles panarteritis nodosa, dysregulated immune function, and/or cytopenias predominate.[181,182] ADA2 acts as a growth-factor for monocytes and endothelial cells, altering the balance between pro and anti-inflammatory macrophages and disrupting the integrity of vasa wall.[183] Mutations of the *CECR1* gene affect the sites of N-linked glycosylation of the ADA2, altering the enzyme trafficking and secretion.[184]An abundance of circulating low-density granulocytes that are prone to spontaneous neutrophil extracellular traps formation seems also to play a role in DADA2 manifestations.[185]

A loss of the auto-inhibitor domain functionality characterizes the disorders related to PLCG2 gene. Patients with the auto-inflammation and phospholipase Cg2 (PLCg2)-associated antibody deficiency and immune dysregulation (APLAID) syndrome, carrying GOF heterozygous mutation that causes a constitutive activation of the PLCG2 protein. The syndrome is characterized by early-onset blistering skin lesions, posterior uveitis, IBD, and cutis laxa, and has been recently described in a second family.[186,187] On the contrary, patients carrying small intragenic deletions of the auto-inhibitor SH domains, show episodes of cold-induced urticarial rash due to PLCG2 activation in case of a low temperature exposition. Higher calcium efflux in B cells in response to lower temperature exposition is a useful functional tests in patients with PLCg2-associated antibody deficiency and immune dysregulation (PLAID) syndrome, also known as FCAS type 3.[188] Polymorphisms of the protein regulatory domain, causing mild enzymatic hyperactivity, seem protective against the development of beta-amyloid related neurological diseases.[189–192] Furthermore, a mild hypogammaglobulinemia with consequent recurrent sinopulmonary infections have been reported in patients with PLCG2 variants and are related to abnormalities in B cells by Bruton's tyrosine kinase receptor activation. In fact, variants of the PLCG2 gene have been reported in tumor cells of patients with chronic lymphocytic leukemia resistant to Bruton's tyrosine kinase inhibitors.[193,194]

The LACC1 gene codes for the laccase domain-containing 1 (LACC1) oxidoreductase, that associates with fatty acid synthase on peroxisomes promoting fatty-acid oxidation, and NOD2-signaling complex, inducing mitochondrial production of the reactive oxygen species, cytokine secretion and bacterial clearance by macrophages.[195,196] Variants of this gene has been associated to Crohn's disease, leprosy, and an early-onset multi-drugs-resistant systemic and polyarticular form of juvenile monogenic arthritis.[197] LACC1-deficient mouse produces high level of TNF and IL-17, thus suggesting a possible new therapeutic approach for these patients.[198,199]

RANKL signalling defects

Frameshift GOF mutations of the *TNFRSF11A* gene have been associated to autosomal dominant recurrent fever syndrome by Jeru et al. However, *TNFRSF11A* autosomal recessive LOF mutations are known to cause the osteoclast-poor osteopetrosis with hypogammaglobulinemia,[200] and heterozygous duplications have been associated with the familial expansile osteolysis or early-onset Paget disease of bone.[201,202] The reason why the *TNFRSF11A* GOF mutations do not affect bones remains unclear. The gene codes for the type I transmembrane Receptor Activator of NFkB (RANK), an osteoclast differentiation factor that mediates essential signals for osteoclastogenesis.

The ligand of RANK (RANKL) activates brain regions involved in thermoregulation, inducing fever.[203]

On the other hand, variants of the Filamin-binding LIM protein 1, coded by the FBLIM1 gene, have been associated with a hereditary form of chronic recurrent multifocal osteomyelitis (CRMO). The protein localizes at cell junctions and may link cell adhesion structures to the actin cytoskeleton. Authors demonstrated an increased production of RANKL in these patients.

Cherubism is a rare autosomal dominant disorder of the jaws caused by mutation of the *SH3BP2* gene.[204,205] Patients present with a childhood onset bilateral and symmetric proliferative fibro-osseous lesions limited to the mandible and maxilla. SH3BP2 is an adaptor protein involved in lymphocyte activation, osteoclast differentiation and bone remodeling. In these patients, hyperactive macrophages secrete a high level of TNFα that drives systemic inflammation, stimulates secretion of RANKL by stromal cells, and ultimately results in bone loss.

Conclusions

Throughout history, the doctors classified diseases according main clinical manifestation, the most involved organ, or the name of physician who firstly described each condition. In light of the little advantage of this method, during the last century, thanks to the development of new gene sequencing technologies, reclassification of hereditary human diseases has been proposed, according to the locus of causative mutations. However, a symptom may be secondary to different gene variants and many variants of a gene cause numerous symptoms, thus the gene-targeted classification of monogenic disorders does not seem to provide a useful taxonomy for physicians. On the contrary, molecular pathways cannot only be useful in classifying monogenic disease, but also highlight roles of proteins that can represent future therapeutic targets to better control the deregulated machinery. In fact, thanks to advances in understanding of cell biology, many drugs selectively inhibiting a target protein can treat efficaciously specific symptoms and limit adverse effects. The availability of these molecules makes early diagnosis even more important in order to avoid the development of complications and long-term outcomes.

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Table 1. Genotypes considered as consistently associated with AIDs, according pathways description in the text.

Gene	Protein	Mechanism	Effect	Disease	Inheritance	Main symptoms
NLRP3	NLRP3	GOF	Constitutive activation of NLRP3 inflammasome	CAPS (FCAS1, MWD, CINCA)	AD	Urticaria, recurrent fever, CNS inflammation, bone overgrowth
LPIN2	LPIN2	LOF	Increased potassium efflux through P2X7R resulting in activation of TLR4 and NLRP3 inflammasome	MJDS	AR	CRMO, congenital dyserythropoietic anemia, recurrent fever, Sweet syndrome
MEFV	Pyrin	LOF	Activation of pyrin inflammasome	FMF, PAAND	AR or AD	Recurrent fever, serositis, rash, AA amyloidosis
PSTPIP1	PSTPIP1	GOF	Dysregulation of cytoskeleton resulting in activation of pyrin inflammasome	PAPA, PAMI	AD	Sterile abscesses, pioderma gangrenosum arthritis
MVK	MVK	LOF	Deficit in geranygeranylation of RhoA resulting in activation of pyrin inflammasome	MKD	AR	Recurrent fever, lymphadenopathy, vomiting, rash
NLRC4	NAIP-NLRC4	GOF	Constitutive activation of NLRC4 inflammasome	AIFEC, FCAS4	AD	Recurrent MAS, enterocolitis, urticaria, recurrent fever, CNS inflammation
NLRP1	NLRP1	GOF	Constitutive activation of NLRP1 inflammasome	NAIAD	AR or AD	Hyperkeratotic ulcers, vitiligo, arthritis
CEBPE	C/EBPe	GOF	Cytoskeleton abnormalities	CAIN	AD	Recurrent fevers, sterile abscesses, pyoderma gangrenosum
TNFRSF1A	TNFR1A	Misfolding	Activation of unfolded-protein response	TRAPS	AD	Recurrent fever, serositis, rash, AA amyloidosis
TRNT1	TRNT1	LOF	ER stress due to mature tRNA deficiency resulting in activation of unfolded-protein response	SIFD	AR	Immunodeficiency, recurrent fevers, panniculitis
COL1A1	COL1A1	Misfolding	Activation of unfolded-protein response	ICH	AD	Infantile cortical hyperostosis
WDR1	WDR1	LOF	Dysregulation of cytoskeletron resultin in activation of pyrin inflammasome	PFIT	AR	Thrombocytopenia, immunodeficiency, fever
ARPC1B	ARPC1B	LOF	Dysregulation of cytoskeletron resulting in ARP2/3-dependent F-actin polymerization deficiency	PLTEID	AR	Thrombocytopenia, immunodeficiency, vasculitis, fever
CDC42	CDC42	GOF	Dysregulation of cytoskeletron	NOCARH	AD	Fever, cytopenia, hemophagocitosis, rash, lymphedema, macrothrombocytopenia
CARD14	CARD14	GOF	Constitutive activation of NFkB in keratinocyte	CAMPS	AD	Pustolar psoriasis, Pytiriasis rubra pylaris
IL36RN	IL36RA	LOF	Deficit of IL-36 receptor agonist	DITRA	AR	Pustolar psoriasis
IL1RN	IL1RA	LOF	Deficit of IL-1 receptor agonist	DIRA	AR	Pustolosis, CRMO
NOD2	CARD15	GOF	Constitutive activation of NOD2 signalling	Blau syndrome	AD	Arthritis, uveitis, rash
PLCG2	PLCg2	GOF	Incresed production of inositol trisphosphate, dyacylglycerol and calcium	APLAID, PLAID, FCAS3	AD	Colitis, ocular inflammation, cold-induced urticaria, infections
LACC1	LACC1	LOF	Deficit in endogenous fatty acid oxidation, peroxisomal dysfunction and decreased ROS production	LACC1 deficiency	AR	Arthritis, colitis, oral ulcers

RBCK1/RNF 31/*SHARPIN*	HOIL1/HOIP /SHARPIN	LOF	Deficit in linear ubiquitination	LUBAC deficiency	AR	Fever, lymphangiectasia
OTULIN	OTULIN	LOF	Deficit in hydrolisis of linear Met1-linked ubiquitin chains	ORAS	AR	Lypodistrophy, arthralgia, GI inflammation
TNFAIP3	A20	LOF	Deficit in hydrolisis of Lys63-linked ubiquitin chains	HA20	AD	Arthritis, GI ulcers, ocular inflammation
PSMG2/A3/B 4/8/9/10	PSMβ5i	LOF	Deficit in proteasome assembly	CANDLE	AR or AD	Lypodistrophy, fever, panniculitis, CNS calcification
POMP	POMP	LOF	Deficit in proteasome assembly	PRAID	AD	Neutrofilic dermatitis, autoimmunity, immunodeficiency
USP43	USP43	LOF	Deficit in proteasome assembly	USP43-AID	AR	Recurrent fevers, erythema nodosum, lipodystrophy and polyarthritis
TMEM173	STING	GOF	Constitutional activation of DNA sensor	SAVI	AD	Polyarthritis, interstitial lung disease, vasculitis
COPA	COPA	LOF	Deficit in retrograde Golgi-to-ER transport resulting in ER stress and Th17 upregulation	COPA	AD	Polyarthritis, interstitial lung disease, vasculitis
CECR1	ADA2	LOF	Deficit in growth factor ADA2 activity and stimulation of inflammatory macrophage response	DADA2	AR	Panarteritis nodosa, CNS stroke, livedo reticularis, immunodeficiency

Table 2. Genotypes associated with autoinflammatory manifestations.

Gene	**Protein**	**Mechanism**	**Effect**	**Disease**	**Inheritance**	**Main symptoms**
PSEN1	Presenilin1	LOF	Catalytic component of gamma-secretase	HS	AD	Sterile abscesses
PSENEN	Presenilin2	LOF	Component of gamma-secretase	HS	AD	Sterile abscesses
NCSTN	Nicastrin	LOF	Component of gamma-secretase	HS	AD	Sterile abscesses
NLRP7	NLRP7	GOF	Activation of NLRP7 inflammasome	VEO-IBD	AD	Ulcerative colitis, hydatiform mole
NLRP12	NLRP12	GOF	Deficit in inhibition of ASC filaments nucleation resulting in accelerated activation of inflammasome	FCAS2	AD	Urticaria, recurrent fever
F12	FXII	GOF	Increased FXII amyloidotic activity	F12-associated FCAS	AD	Cold-induced urticaria
ADGRE2	ADGRE2	GOF	Deficit in inhibition of mast cells degranulation	Vibratory Urticaria	AD	Vibratory-induced orticaria
WAS1	WAS	LOF	Cytoskeleton abnormalities due to CDC42 signaling	WAS	XLR	Macrotrombocytopenia, eczema, recurrent infections
FHL1/PRF1/ UNC13D/ST X11/STXBP2	FHL1/Perfori n/Munc13-4/synthaxin11 /synthaxin-binding-protein2	LOF	Defective cytotoxicity	Familal HLH	AR	Recurrent MAS with fever, cytopenias, hepatosplenomegaly
HAVCR2	HAVCR2	LOF	Defective cytotoxicity	SPTCL	AR	HLH, panniculitis
RAG1/2	RAG1/2	LOF	Defective V(D)J recombination	CCHIDG	AD	HLH, granulomas, eosinophilia
STAT1	STAT1	GOF	Increase transcription of acute phase response genes	CVID	AD	Mucocutaneous candidiasis, recurrent infections, autoimmunity

STAT3	STAT3	GOF	Increase transcription of acute phase response genes	CVID, ADMIO1	AD	Autoimmunity, lymphoproliferation, recurrent infections, short stature
CTLA4	CTLA4	LOF	T cells costimulation defects	CVID, CHAI	AD	Hypogammaglobulinemia, cytopenia, lymphoproliferation, autoimmunity
LRBA	LRBA	LOF	Ligand-receptor complexes endocytosis dysregulation	CVID	AR	Hypogammaglobulinemia, interstitial lung disease, enteropathy, recurrent infections
TNFRSF11A	TNFR11A	GOF	Increased RANK signaling	TRAPS-like disease	AD	Recurrent fever, amelogenesis imperfecta
FBLIM1	FBLP1	LOF	Deficit of IL-10/ STAT3/NR4A2-dependent inhibition of ERK1/2 phosphorylation resulting in increased RANKL production	Monogenic CRMO	AR	CRMO
SH3BP2	SH3BP2	GOF	Increased response to RANKL stimulation in myeloid cells and osteoclast	Cherubism	AD	Cyst-like overgrowth of the lower head bones
GALNT3	GALNT3	LOF	Deficit of O-glycosylation of FGF23 resulting in accumulation of fragmented FGF23 in osteocytes	HFTC1	AR	Cortical hyperostosis, CRMO, periarticular calcinosis, hyperphosphatemia
TGFB1	TGFb	GOF	Decreased osteoclastic resorption and increased osteoblastic bone formation	Camurati-Engelman disease	AD	Symmetrical long bone hyperostosis, cytopenias, recurrent fevers
ALPL	TNSALP	LOF	Deficit of tissue non specific alkaline phosphatase resulting in hyperprostaglandinism and demineralization	Hypophosphatasia	AR or AD	CRMO, fractures, premature loss of teeth
SLC29A3	ENT3	LOF	Defect of macrophage colony-stimulator factor clearance resulting in proliferation	PHID	AR	Recurrent fever, hyperpigmentation with hypertrichosis, dysmorphic features, hepatosplenomegaly, sensorineural deafness
FATP1	FATP1	LOF	Defect of long chain fatty acid transportation in classically activated macrophages resulting in increased pro-inflammatory glucose metabolism	MRS	AD	Recurrent facial nerve paralisis, emifacial swelling, fissured tongue
AP1S3	AP1	LOF	Deficit of TLR3-dependent IFN1β production resulting in hypersecretion of IL-1β	AP1 deficiency	AD	Pustolar psoriasis
IL10/IL10RA /B	IL-10/IL10R	LOF	Deficit of IL-10 inhibition and STAT3 phosphorilation	VEO-IBD	AR	Colitis, failure to thrive
TRIM22	TRIM22	LOF	Deficit in K63-linked polyubiquitination of NOD2	VEO-IBD	AR	Colitis, severe perianal disease, infections
XIAP	XIAP	LOF	Impaired NOD2 signaling and lymphocytes apoptosis	VEO-IBD	XL	Colitis, failure to thrive, lymphoproliferation
NFIL3	NFIL3	LOF	Excessive IL-1β and TNFα production in macrophages	NFIL3 deficiency	AR	Oligoarthritis, autoimmune thyroiditis, positive HLA-B27, ANA, and pANCA
LYN	LYN	GOF	Costitutive activation of tyrosin kinase	LAID	AD	Vanishing bile duct disease, vasculitis, cytopenia
SKIV2L	SKIV2L	LOF	Loss of cytosolic RNA exosome function in RNA turnover	THES	AR	Severe diarrhea, immunodeficiency, trichorrhexis nodosa, facial

						dysmorphism
PDGFRB	PDGFRB	GOF	Activation of STAT3	Penttinen syndrome	AD	Lypoatrophy, scar-like skin lesions, hair abnormalities, hypereosinophilia
JAK1	JAK1	GOF	Activation of STAT1/3	Hypereosinophilic syndrome	AD	Severe dermatitis, hepatosplenomegaly, hypereosinophilia
TREX1	DNAse3	LOF	Deficit of exonuclease resulting in accumulation of nucleic acid	AGS1, RVCL	AR or AD	CNS calcification, SLE, chilblains
RNASEH2A/ B/C	RNH2A/B/C	LOF	Deficit of RNAse resulting in accumulation of nucleic acid	AGS4/3/2	AR	CNS calcification
SAMHD1	SAMH	LOF	Deficit of nuclease resulting in accumulation of nucleic acid	AGS5	AR	CNS calcification, chilblain, oral ulcers, deforming arthropaty, cerebral vasculopathy with erly onset stroke
ADAR1	DSRAD	LOF	Deficit of RNA deaminase resulting in accumulation of nucleic acid	AGS6, DSH	AR/AD	CNS calcification, dyscromatosis symmetrica hereditaria
IFIH1	MDA5	GOF	Activation of RNA sensor	AGS7, SMS	AD	Aortic calcification, glaucoma, psoriasis, skeletal abnormalities with dental dysplasia
DDX58	RIG1	GOF	Activation of dsRNA sensor	SMS2	AD	Aortic calcification, glaucoma, psoriasis, skeletal abnormalities with acro-osteolysis
C1qA/C1qB/ C1qC/C1r/C 1s/C2/C3/C4 a	C1q/C1r/C1s/ C2/C3/C4a	LOF	Deficit of immune complex clearance resulting in activation of autoreactive B cells	Monogenic SLE	AR	SLE-like
DNASE1/DN ASE1L3/DN ASE2	DNAse1/DN Ase1L3/DNA se2	LOF	Deficit of deoxyribonuclease resulting in accumulation of nucleic acid	Monogenic SLE	AD or AR	SLE-like
ACP5	TRAP	LOF	Accumulation of phosphorilated osteopotin in peripheral dendritic cells	SPENCDI	AR	Scleroderma, bone dysplasia, spasticity
ISG15	ISG15	LOF	Reduced intracellular IFNα/β-inducer USP18 accumulation	MSMD	AR	CNS calcification, mycobacterial infections
USP18	USP18	LOF	Reduced intracellular IFNα/β-inducer USP18 accumulation	PTORCH2	AR	CNS calcification, liver dysfunction, cytopenia
POLA1	POLA1	LOF	Deficit of DNA polimerase alpha resulting in deficit of cytosolic RNA:DNA hybrids and costitutive activation of IFN regulatory factor and NFkB	XLPDR	XLR	Recurrent pneumonia, diffuse reticulate hyperpigmentation, hypohydrosis, colitis, uretral stenosis, corneal inflammation, failure to thrive
PNPT1	PNP	LOF	Mitochondrial double-stranded RNA accumulation resulting in interferon upregulation	Mitochondrial disease	AR	Infantile encephalopathy, movement disorder
NGLY1	N-glicase1	LOF	Accumutaltion of misfolded glycoprotein resulting in interferon upregulation	Mitochondrial disease	AR	Infantile encephalopathy, movement disorder, hypo/alocrimia, thyroid and adrenal insufficiency

Actin remodeling defects leading to autoinflammation and immune dysregulation

Actin is a family of globular proteins that form microfilaments of cell cytoskeleton. In the past, the most important function of actin was related to the binding of myosin, collaborating to the muscle contraction with troponin. These properties can easily be tested adding pure myosin to water and actin, causing an increase in viscosity and birefringence of the liquid due to the formation of the actomyosin complex.(1) Thus, the term of actinopathies was originally considered for a well-defined group of monogenic muscle diseases secondary to the actomyosin complex dysfunction.(2) During the recent years, a growing number of disorders of the immune system have been linked to actin cytoskeleton abnormalities (numbers are related to the Table and Figure).(3) Furthermore, evidences that actin cytoskeletal deregulation in immune cells causes inflammatory manifestations are increasing.(4) In this review we illustrate the inflammatory and immunological disorders associated with different pathways of actin-binding molecules.

Elongation defects

Actin is the most abundant protein in the majority of eukaryotic cells, contributing to acquire and maintain cell structure and functions. Vertebrates express three actin isoforms, including the α-isoform of skeletal, cardiac, and smooth muscles cells, and the β- and γ-isoforms.(5) The conformation of actin monomer, called globular (G)-actin, is the same among different isoforms. G-actin assembles into polarized filaments, called filamentous (F)-actin, that form cortical actin network (CAcN) and cell protusions.(6) Monomer binding proteins, such as the Profilin-1, control polymerization. Individual filaments lifetime can be as short as ten seconds or lasting for days, depending on the extracellular stimulus duration and intracellular conditions.(7) Inhibiting the actin polymerization through activity of the capping proteins, or stimulating actin disassembly through the Cofilin/actin depolarizing factor (ADF) influences the intracellular concentration of G-actin, usually relatively equal throughout the cell cytoplasm.

Profilin-1 is ubiquitously expressed in human cells.(8) Its main function is to chaperone G-actin to the positive-charged barbed end of F-actin in response to an increased concentration of the phosphatidylinositol (4,5)-bi-phosphate (PIP2). Mutation of the *PFN1* gene coding for the Profilin-1 causes the familial form of amyotrophic lateral sclerosis(9) and deletions have been recently related to an early-onset form of Paget's disease (n°1).(10) This condition is characterized by anomalies of the appendicular bone, favoring malign tumors. Pre-osteoblasts lacking Profilin-1 lose their differentiation and adhesion capability and fail to mineralize efficiently the appendicular bone,

acquiring invasive properties. Depletion of the Profilin-1 in breast tumor cells causes defects in formation of filopodia, limiting cell motility and favoring proliferation through upregulation of the transcriptional factor SMAD3.(11) On the other hand, deficiency of Profilin-1 acts against invasion of cytotoxic T lymphocytes in tumors and haploinsufficiency of Profilin-1 seems protective against subcutaneous inflammation induced by high fat diet.(12) Furthermore, activation of the Profilin-1 pathway has been related to the inflammatory vascular damage in patients with diabetic retinopathy.(13,14)

Heterozygous gain-of-function (GoF) variant of the *ACTB* gene, coding for the β-isoform of actin, has been reported in a female with recurrent infections due to neutrophil chemotaxis and oxidative burst defect (n°2).(15) Intellectual disabilities and short stature was also present. No other patients have been reported to the present date. However, Authors demonstrated that mutant β-isoform of actin bound the Profilin-1 less efficiently than normal, despite normal actin polymerization capability. Loss-of-function (LoF) variants of the *ACTB* gene, as well as of the *ACTG1* gene, coding for the γ-isoform of actin, have been related to the highly variable spectrum of the Baraitser-Winter syndrome, a rare condition without relevant immunological manifestations.(16)

Cofilin/ADF activation is dependent by phospholipase Cγ (PLCγ) in tumors and Rac2 signaling in neutrophils.(17) Reduction of Cofilin/ADF expression in leukocytes is associated with abnormal chemotaxis.(18) In neurons, Cofilin/ADF controls axon elongation and regeneration(19) and serum levels are significantly higher in patient with Alzheimer's disease.(20) Cofilin/ADF is also upregulated in patients with Friedreich's ataxia, whose mutations correlate with an altered immune-related genes transcription.(21,22) However, contrary to anecdotal reports, the clinical trial of oral steroids in patients with Friedreich's ataxia fails its primary end-point.(23,24)

Proteins containing a short structural motif of approximately 40 amino acids, often terminating in a tryptophan-aspartic acid (WD) dipeptide, called WD40 repeat, can accelerate the Cofilin/ADF activity. The best-known example is the WD40 repeat protein 1 (WDR1), also known as Actin interacting protein 1 (AIP1). Homozygous LoF mutations of the *WDR1* gene cause a monogenic autoinflammatory disease characterized by periodic fever, immunodeficiency, and thrombocytopenia (PFIT; n°3).(25,26) Patients display recurrent fever attacks lasting 3–7 days, every 6–12 weeks, with high acute phase reactants and hyperferritinaemia. Recurrent mucosal inflammation, causing a peculiar acquired microstomia, may resemble the Behcet's disease's attacks during childhood.(27) Lymphocytes of patients with PFIT show adhesion and motility defects.(28) Coronin-1A is another WD40 repeat-containing protein whose LoF mutants have been related to a severe combined immunodeficiency characterized increased susceptibility to viral and

mycobacterial infections (n°4).(29–32) Patients usually present with mucocutaneous manifestations, sinopulmonary diseases and neurocognitive disorders without inflammatory manifestations.

On the other hand, the capping proteins are heterodimers composed by two unrelated subunits with highly conserved amino acid sequences. The RGD, leucine-rich repeat, tropomodulin and proline-rich containing protein (RLTPR), also called CARMIL2, is a cytosolic protein that acts as scaffold between the nuclear factor kappa-light-chain-enhancer of activated B cells (NFkB) and CD28.(33,34) Autosomal recessive (AR) LoF mutations of the *RLTPR* gene cause a primary immunodeficiency (PID) characterized by allergy, increased incidence of bacterial and fungal infections, and virus-related tumors (n°5).(35) The abnormal cytoskeleton of T-cell in patients with CARMIL2 deficiency causes defects of activation and is related to an abnormal activity of the capping proteins.(36)

Activation defects

Over 40 years ago, studies on the ligand-induced movement of immunoglobulin on the surface of lymphocytes called attention to a special relationship between CAcN and antigen-presenting cells.(37) A specialized cell–cell junction, the immune synapse,(38) is required for the activation of lymphocytes and begin with the formation of thousands of transient, low affinity interactions between antigens and integrins, such as the lymphocyte function-associated antigen 1 (LFA-1).(39) These interactions require a minimum distance of 40 nm, while the major histocompatibility complexes require 15 nm. The consequent antigen-induced CAcN rearrangements leads to morphological changes that are crucial for adhesion, migration, endocytosis, division, gene expression, and calcium flux, as well as for the releasing of cytokines and cytotoxic granules in lymphocytes, neutrophils and monocytes.(40)

In particular, on resting leucocytes, LFA-1 is maintained in a low activity state by an inhibitory interaction with the CAcN.(41,42) Therefore, activation of leucocytes requires the release of CAcN-integrin interactions, so that LFA-1 can diffuse in the cell membrane and start binding activities.(39) The essential role of CAcN in phagocyte function can be highlighted during chronic infections.(43) In fact, microbes are able to lose their integrin ligands in order to escape the immune response.(44) The abnormal rolling of leukocytes seems the main affected mechanism in patients with PID caused by LFA-1 defects (n°6-9).(45) The deficiency of the β2 integrin subunit of the LFA-1 causes the leukocyte adhesion deficiency (LAD) type I, and the defective activation of LFA-1 subunits has been related to the LAD type III, both nowadays effectively treated with the hematopoietic stem cells transplantation.(46,47) On the other side, the administration of oral fucose did not seem effective to control the LAD type II clinical manifestations.(48)(49)

Finally, a monocyte-selective adhesion defect has been recently noted in patients with cystic fibrosis (CF) and called LAD type IV.(50–52) *CFTR* heterozygous LoF variants cause hyper activation of the small G-proteins Rho family that controls integrins activation.(53) Interestingly, these small G-proteins are also well-known inhibitor of the pyrin inflammasome.(54) Furthermore, CFTR interacts with Ezrin protein via its C-terminal domain. Ezrin is the most prominent members of the Ezrin-Radixin-Moesin (ERM) domain-containing protein family that links CAcN to the cell membrane, regulating tension during motility and endocytosis.(55,56) In hematopoietic cells, Ezrin and Moesin are highly expressed, whereas Radixin is mostly absent. Hemizygous LoF mutations of the *MSN* gene coding for Moesin is associated to a PID called X-linked MSN-associated immunodeficiency (X-MAID; n°10).(57) Patient T cells displayed impaired proliferative responses after activation by certain mitogens, and a variable defects in cell migration and adhesion, whereas the formation of immunologic synapses is normal. Thus, CAcN dysfunctions impair epithelial tight junction formation as well as lymphocytes adhesion capability in X-MAID patients.

Protrusions defects

The collapse of CAcN to the side of cells occupied by microtubule organizing centers creates an opening for new actin polymerization to form membrane protrusions at the leading edge. This process is controlled by the small G-proteins Rho family, including the Cell division control protein 42 homolog (Cdc42) and Rac2.(58)

Small G-proteins are a superfamily of ubiquitously expressed cytosolic hydrolase enzymes that can independently bind and hydrolyze guanosine triphosphate (GTP) to guanosine diphosphate (GDP), becoming inactive.(59) The best-known subfamily members are the Ras GTPases that are divided into five main families: Ras, Rho, Ran, Rab and Arf. The Ras family is generally responsible for cell proliferation, Rho for cell morphology, Ran for nuclear transport and Rab and Arf for vesicle transport. The Ras guanyl nucleotide-releasing protein 1 (RASGRP1) is a diacylglycerol-regulated nucleotide exchange factor specifically activating Ras and regulating T and B cells development, homeostasis and differentiation. Rasgrp1 deregulation in mice results in a systemic lupus erythematosus-like disorder (60) and RASGRP1 deficiency in humans causes a PID characterized by impaired cytoskeletal dynamics (n°11).(61) Patients with RASGRP1 deficiency suffer from recurrent bacterial and viral infections especially affecting the lung with a severe failure to thrive and can develop EBV-related lymphomas.

The localization of small G-proteins on the cell membrane is due to their prenylation, a post-translational modification characterized by the addition of twenty-carbon lipophilic isoprene units to the cysteine residues at the C-terminus.(62) Furthermore, most of the Rho family

members contain a cluster of positively charged residues (i.e. polybasic domain), directly preceding their geranylgeranyl moiety that serves to fine-tune their localization among different cell membrane sites. Overall, the prenylation of small G-proteins is involved in the regulation of cytokines production (63) and can be regulated by statins in monocytes and macrophages.(64)

On 2D surfaces, activated Cdc42 and Rac2 generate filopodia and lamellipodia, respectively. The formation of these membrane protrusions consents leucocytes to reach the damaged tissue passing through an intact vessel wall, a process called diapedesis. The local concentration of the complement system C3 fraction also contributes to this process.(65) However, in 3D environment, the blebbing motility seems the more common migratory strategy of blood cells.(66,67) Stop-codon variants of the *CDC42* gene has been recently associated with a novel autoinflammatory disease characterized by neonatal-onset of cytopenia, rash, and hemophagocytosis (NOCARH), successfully treated with interleukin-1β inhibition (n°12).(68) Furthermore, heterozygous *CDC42* missense variants have been related to the Takenouchi-Kosaki syndrome (TKS).(69–71) TKS patients do not usually display autoinflammatory manifestations but hematologic and/or lymphatic defects, including macrothrombocytopenia, lymphedema, intestinal lymphangiectasia and recurrent infections. Platelets and B cell dysfunctions have been recently clarified,(72–74) whereas autoinflammatory symptoms are still unclearly related to the mutant protein. A recent extensive genotype-phenotype correlation study allows to classify three groups of the *CDC42* variants regarding involved protein domain.(75) Based on these evidences, the NOCARH-associated variants occur at the C-terminus that usually allows PIP2 interaction, whereas variants associated with TKS resembling Noonan syndrome occurs at the N-terminus. Thus, different roles of the Cdc42 protein may be subverted in these conditions with different clinical manifestations.

The Rho guanosine triphosphatases Rac2 is expressed only in hematopoietic cells. Patients with Rac2 dysfunction secondary to dominant negative or homozygous LoF mutations present early-onset recurrent abscesses, neutrophilia, and defective wound healing, whereas monoallelic germline GoF mutations of the same *RAC2* gene cause a severe combined immunodeficiency (n°13).(76–79) Interestingly, Rac2 activation in neutrophils is primarily mediated by the dedicator of cytokinesis (DOCK) 2, an atypical guanine nucleotide exchange factor (GEF) that rapidly translocate to the plasma membrane in a phosphatidylinositol 3,4,5-trisphosphate (PIP3)-dependent manner upon stimulation, resulting in increased local CAcN polymerization.(80,81) DOCK2 is mainly expressed in peripheral blood leukocytes and DOCK2 deficiency causes an early-onset PID characterized by a T-cell defective chemotactic responses with bacterial and viral infections (n°14).(82)

On the other side, DOCK8 is a Cdc42-specific GEF that regulates interstitial migration of dendritic cells and DOCK8 deficiency causes the AR Hyper-IgE syndrome (HIES), a combined

immunodeficiency characterized by recurrent viral infections, early-onset malignancy and atopic dermatitis (n°15). Patients with HIES display severe viral skin infections, such as chronic anogenital ulcers, multiple acral warts, and disfiguring molluscum contagiosum.(83–85) Selective loss of group 3 innate lymphoid cell has been described in these patients.(86)

Branching defects

Cdc42 and Rac2 transmit many signals through the GTP-dependent binding of effector proteins containing the Cdc42/Rac interactive binding (CRIB) motif, such as the Wiskott–Aldrich syndrome (WAS) protein (WASP).(87) WASP is restricted to hematopoietic cells, while neuronal WASP (N-WASP), closely related in amino acids sequence, is more widely expressed.(88) Other members of this proteins family include the Scar/WAVE proteins. N-WASP has been implicated in filopodia formation downstream of Cdc42, and the Scar/WAVE proteins family has been shown to contribute to the formation of lamellipodia downstream of Rac2. Recently, an immune dysregulation disorders characterized by deficit of the hematopoietic-specific WAVE complex regulator HEM1, coded by the *NCKAP1L* gene, has been characterized (n°16).(89) Patients with HEM1 deficiency suffer from recurrent infections, asthma and lymphoproliferation.

N-WASP deficiency increases the production of inflammatory cytokine.(90) Human LoF mutations of *WAS* gene cause severe defects in hematopoietic cell functions, leading to the well-known triad of microthrombocytopenia, immunodeficiency and eczema (n°17).(91) The cytoskeletal defects of megakaryocytes are responsible for the low number of platelets in patients with WAS and others CRIB-related disorders.(92) WASP deficiency promotes T-cell cytoskeletal tension decay and phosphorylation of a serine/threonine protein kinase 4 (STK4) that usually increase T-cell migration, therefore promoting immune synapse breaking and secondary B cells dysfunction.(93,94) WASP-deficient lymphocytes fails to differentiate into memory cells(95) and are more prone to develop DNA damages due to the loss of the Golgi-dispersal response, a recently described mechanism of cell survival after ionized radiation exsposure.(96) The STK4 deficiency causes a PID characterized by B and T cell lymphopenia, neutropenia, and cardiac malformations (n°18).(97) STK4 phosphorylates the Forkhead box O1 transcription factor, increasing NFkB-mediated production of interleukin 12 in dendritic cells and limiting the oxidative stress susceptibility.(98) No platelets anomalies have been described in patients with STK4 deficiency. Equally, deficiency of the WASP interacting protein family member 1 (WIPF1) causes a WAS-like syndrome with normal platelet volume (n°19). WIPF1 is able to stabilize WASP, preventing its degradation in lymphocytes.(99)

WASP controls the rate of actin branching by activating the actin related protein (ARP) 2/3 complex constituted by seven subunits. Two of them, the ARP2 and 3, closely resemble the structure of the G-actin, allowing the formation of a thermodynamically stable dimer that serves as a nucleation site for the new actin filaments at 70° angle from the main filament. Homozygous LoF variants of the *ARPC1B* gene, coding for the p41 regulatory subunits of the ARP2/3 complex, cause the platelet abnormalities with eosinophilia and immune-mediated inflammatory disease (PLTEID; n°20).(100–104) Patients with PLTEID usually present systemic inflammation with lymphoproliferation and immunodeficiency resembling WAS, with early onset vasculitis, severe infections, and eczema. A functional test has been recently described to detect asymptomatic carriers.(105)

Additional WASP activators include the proline–serine–threonine phosphatase-interacting protein 1 (PSTPIP1), PIP2, and the c-Src protein-tyrosine kinases family. Heterozygous GoF mutation of the *PSTPIP1* gene cause the pyogenic sterile arthritis, pyoderma gangrenosum, and acne (PAPA) syndrome and the PSTPIP1-associated myeloid-related proteinemia inflammatory (PAMI) syndrome (n°21).(106,107) PAMI syndrome is caused by variants that substantially alter electrostatic properties of the PSTPIP1 critical region for auto-inhibiting dimerization, resulting in a GoF mutant protein that constitutively activates the underlying Pyrin inflammasome.(108) Pyrin is the pivotal protein of the related inflammasome, a member of cytosolic multiprotein oligomers family responsible for the activation of inflammatory responses in human cells. The Pyrin-associated autoinflammation with neutrophilic dermatosis (PAAND) and familial Mediterranean fever (FMF) are well-known monogenic autoinflammatory diseases both related to GoF variants at different locus sites of the *MEFV* gene and associated with an excessive activation of the Pyrin inflammasome (n°22). Recently, the mevalonate kinase deficiency (MKD) caused by homozygous or compound heterozygous LoF mutations in the *MVK* gene has been related to the constitutive activation of Pyrin (n°23).(109)

Production defects

Megakaryoblastic leukemia 1 (MKL1) is a member of the myocardin-related transcription factors and usually held in an inactive state in the cytoplasm in a reversible complex with G-actin.(110) Stimulation of the small Rho GTPases promotes incorporation of G-actin into F-actin, allowing MLK1 to enter into the nucleus, stimulating transcription of actin and other cytoskeletal proteins genes. Homozygous LoF mutation in the *MKL1* gene result in a PID characterized by susceptibility to severe bacterial infection and recurrent skin abscesses (n°24).(111) MKL1 deficiency causes reduced phagocytosis and almost complete abrogation of neutrophils spreading properties.(112)

MLK1 participates in differentiation of megakaryocytes and mild thrombocytopenia has been noted in patients with MKL1 deficiency.(113)

Finally, LoF variants of the gene coding for the transcription factor CCAAT enhancer binding protein epsilon (C/EBPε) cause a PID called AR neutrophil-specific granule deficiency-1 (SGD),(114) whereas heterozygous GoF variants have been recently related to an autoinflammatory disease called the C/EBPε-associated autoinflammation and immune impairment of neutrophils (CAIN; n°25). Patients with CAIN display recurrent fevers characterized by abdominal pain, lasting 4-5 days, and skin inflammatory manifestations, such as sterile abscesses, pyoderma gangrenosum and oral ulcerations. The mutant C/EBPε causes deregulated transcription of interleukins and interferon response genes in neutrophils.(115)

The field of autoinflammation is moving from a gene-centric view of innate immune-mediated diseases towards a systems-based concept, which describes how various convergent molecular pathways, including actin cytoskeleton, contribute to the autoinflammatory process(116) and to a number of conditions characterized by the coexistence of inflammation, autoimmunity and defective immune response. Indeed, the complex regulation of the actin remodeling represents an example of autoinflammatory diseases merging with immunodeficiencies. Cytoskeleton-targeted therapies, such as colchicine, may play new roles in these disorders. The study of the molecular and modular diversity of these immune responses to the changing conditions has only recently become possible through the development of the new ‘omics’-based screening technologies.(117) The adoption of ‘omics’ and systems-based concepts will have implications for the discovery of novel diseases and for the possible development of targeted diagnostic tests and treatment options.

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Table. Monogenic immune system diseases characterized by actin remodeling defects.

N	Location	Gene	Protein	Mechanism	Effect	Diseases	MIM	Inheritance	Main symptoms	Main laboratory characteristics
1	17p13.2	PFN1	Profilin 1	LOF	Failure to differentiate pre-osteoblast	Early-onset Paget's disease	None	AR	Polyostotic Paget's disease, osteosarcome	None
2	7p22.1	ACTB	Beta-actin	GOF	Failure to polarize cytoskeletron in response to fMLP	ACTB-related immunodeficiency	102630	DN	Reurrent infections, trombocytopenia, intelletual impairment and short stature	Poor neutrophil chemotaxis and oxidative burst
3	4p16.1	WDR1	WDR1	LOF	Defect of cofilin activation	PFIT	None	AR	Recurrent fevers and infections genital and oral ulcers, pyoderma gangrenosum, microstomia	Trombocytopenia, neutrophil and lymphoid dysfunction, hyperferritinaemia
4	16p11.2	CORO1A	Coronin1A	LOF	Defect of WDR1 activation	Coronin1A deficiency	615401	AR	Mycobacterial and viral infections, neurological disorders	Naive T-cells lymphopenia
5	16q22.1	RLTPR	Carmil2	LOF	Defective regulation of capping protein and CD28-mediated costimulation in T-cell	CARMIL2 deficiency	618131	AR	Bacterial and fungal infections, atopy, disseminated EBV-positive smooth muscle tumors	T-cells functional defect
6	21q22.3	ITGB2	ITGAL/M/X	LOF	Deficit of the beta-2 integrin subunit of the LFA-1 causing delayed motility of neutrophils	LAD type I	116920	AR	Recurrent bacterial infections, delayed separation of the umbilical cord and delayed wound healing	Severe granulocytosis
7	11p11.2	SLC35C1	GDP-L-fucose transporter	LOF	Deficit of CD15 causing delayed motility of neutrophils	LAD type II/CDG2C	266265	AR	LAD1-like immune deficiency, psychomotor retardation, mild dysmorphism	Severe granulocytosis , Bombay blood type
8	11q13.1	FERMT3	Kindlin-3	LOF	Deficit in inside-out signaling that enable high-avidity binding of integrin to ligands on leucocytes and platelets	LAD type III/I variant	612840	AR	LAD1-like immune deficiency, Glanzmann thrombasthenia-like bleeding problems, osteopetrosis	Severe granulocytosis
9	7q31.2	CFTR	CFTR	LOF	Defect of monocyte adhesion	LAD type IV/Cystic fibrosis	219700	AR	Recurrent lung infections, pancreatic insufficiency, male infertility	Hypergammaglobulinemia
10	Xq11	MSN	Moesin	LOF	Impaired T cells proliferation, migration and adhesion	X-MAID	300988	XLR	Recurrent bacterial and varicella zoster virus infections, eczema and other skin manifestations (recurrent molluscum, thrombotic thrombocytopenic purpura), acute stroke	Leukopenia with defective T-cell proliferation and fluctuating neutropenia, hypogammaglobulinemia, ADAMTS13+ thrombocytopenia
11	15q14	RASGRP1	RasGRP1	LOF	Defect in Ras activation in T-cells and B-cells	RASGRP1 deficiency	618534	AR	Bacterial and viral infections, autoimmunity	T-cells and B-cells functional defect
12	1p36.12	CDC42	CDC42	GOF	Dysregulation of cytoskeletron	NOCARH/TKS	616737	AD	Fever, rash, lymphedema	Cytopenia, hemophagocitosis, macrothrombocytopenia

N	Location	Gene	Protein	Mechanism	Effect	Diseases	MIM	Inheritance	Main symptoms	Main laboratory characteristics
13	22q13.1	RAC2	RAC2	LOF/GOF	Defect in fMLF-induced actin remodeling; increased neutrophil superoxide production	RAC2 dysfuntion	608203	AR/AD/DN	Recurrent sterile abscesses (frequently perirectal)	Leukocytosis with neutrophilia, low-normal T and B cells number, hypogammaglobulinemia
14	5q35.1	DOCK2	DOCK2	LOF	Deficit of RAC2 activation	DOCK2 deficiency	616433	AR	Early-onset invasive bacterial and viral infections, autoimmunity	Lymphopenia and defective lumphocytes migration
15	9p24.3	DOCK8	DOCK8	LOF	Deficit of CDC42 activation	HIES	243700	AR	Recurrent viral infections, early-onset malignancy and atopic dermatitis	Lymphopenia, hypergammaglobulinemia, mild-to-moderate eosinophilia
16	12q13.13	NCKAP1L	HEM1	LOF	Deficit of WAVE regulatory complex	HEM1 deficiency	None	AR	Recurrent infections, asthma, hepatosplenomegaly and lymphadenopathy	Increased T and memory T cells, neutrophils migration defects, decreased NK cytotoxicity
17	Xp11.23	WAS	WASP	LOF/GOF	Deficit of ARP2/3 complex activation causing lack of actin braching	WAS/X-linked thrombocytopenia/X-linked neutropenia	301000	XLR	Recurrent infections, eczema	Trombocytopenia, defective T cell and NK cell functions, increased number of NK cells/Neutropenia
18	20q13.12	STK4	STK4	LOF	Deficit of L-plastin phopshorilation causing abnormal T-cell migration	STK4 deficiency	614868	AR	Recurrent bacterial and viral infections with warts and abscesses, autoimmunity, cardiac malformations	CD4+ and naive CD8+ T-cell and B-cell lymphopenia, neutropenia
19	2q31.1	WIPF1	WIPF1	LOF	Deficit of ARP2/3 complex activation causing lack of actin braching	WAS type 2	614933	AR	WAS-like immune deficiency	Trombocytopenia, defective T-cell and NK-cell functions, increased number of NK cells
20	7q22.1	ARPC1B	ARPC1B	LOF	Deficit of ARP2/3-dependent F-actin polymerization	PLTEID	617718	AR	Recurrent viral infections, vasculitis, periodic fevers	Thrombocytopenia, hypogammaglobulinemia with high IgE, reduced CD8+ T cell count
21	15q24.3	PSTPIP1	PSTPIP1	GOF	Dysregulation of cytoskeleton resulting in activation of pyrin inflammasome	PAPA, PAMI	604416	AD	Sterile abscesses, pioderma gangrenosum, arthritis	High acute phase reactants
22	16p13.3	MEFV	Pyrin	GOF	Dysregulation of cytoskeleton resulting in activation of pyrin inflammasome	FMF/PAAND	134610	AR/AD	Recurrent fevers with abdominal pain and arthralgia	High acute phase reactants/Neutropenia
23	12q24.11	MKD	Mevalonate kinase	LOF	Dysregulation of cytoskeleton resulting in activation of pyrin inflammasome	MKD	260920	AR	Reurrent fevers, lymphadenopathy, arthralgia, skin rash	High concentration of mevalonate acid in urine during fever attacks
24	22q13.1	MLK1	MLK1	LOF	Deficit of actin production	MLK1 deficiency	None	AR	Severe bacterial infections, skin abscesses	Mild trombocytopenia, selective defect of T-cell proliferation to anti-CD3 antibody, neutrophil migration defect
25	14q11.2	CEBPE	C/EBPe	LOF/GOF	Defecit in regulation of actin-related genes transcription	SGD/CAIN	245480	AD	Recurrent fevers, sterile abscesses, pyoderma gangrenosum	Atypical Pelger-Huët anomaly with neutrophil hyposegmentation, and impaired chemotaxis

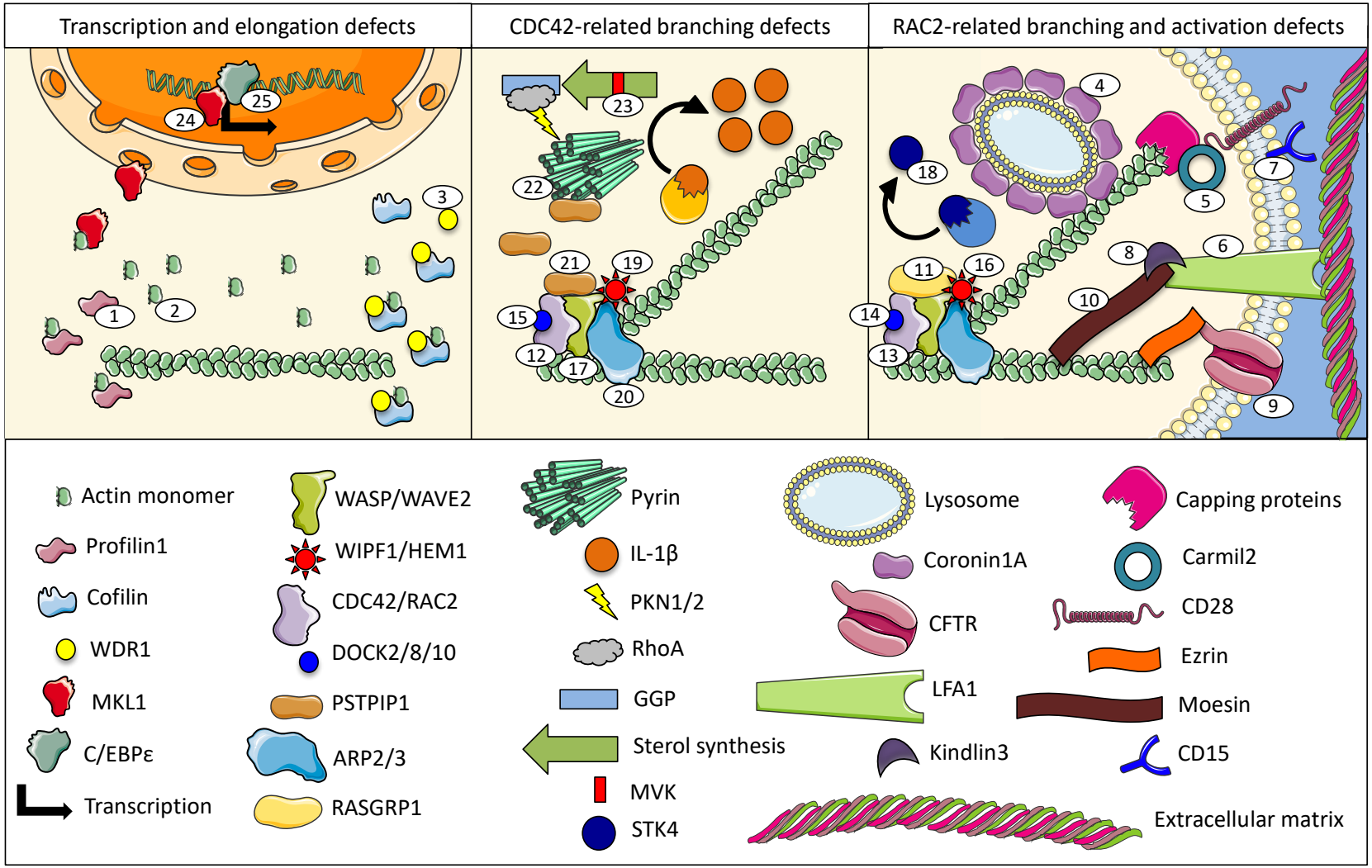

Figure. Proteins and pathways involved in monogenic immune system diseases characterized by actin remodelling defects (numbers in Text and Table).

Systemic undefined recurrent fevers (SURF): an emerging group of autoinflammatory recurrent fevers

Systemic undefined recurrent fevers (SURF) is a heterogeneous group of autoinflammatory diseases (AID) characterized by self-limiting episodes of systemic inflammation without a confirmed molecular diagnosis. Firstly defined by Broderick et al.,[1] SURF is increasingly diagnosed in patients with a suspected hereditary recurrent fever (HRF) after exclusion of all the known AID-related mutations by the next generation sequencing (NGS) analysis.[2] Recent evidences suggest a multi-organ presentation and at least a partial response to the long-term treatment with colchicine as the best clinical criteria to distinguish SURF from the more common periodic fever, aphthous stomatitis, pharyngitis and adenopathy (PFAPA) syndrome.[3] Omics-based technologies provide a wide-range opportunity to analyze the functional characteristics of immune cells in SURF patients, highlighting the pathological relevance of the new mutations and supporting the development of new diagnostic tests. On the other hand, the colchicine response suggests a pivotal role of cell cytoskeleton and related proteins in the genesis of the inflammatory attacks. In fact, cytoskeleton dysregulation causes abnormal motility and differentiation of immune cells that are suggested as triggers of inflammation in other waxing and waning immune diseases, such as rheumatoid arthritis.[4] In this review, we will focus on the clinical manifestations and treatment response of this newly defined group of AID called SURF, giving an overview of the currently used diagnostic and therapeutic approaches, with the aim to harmonize future studies in the field.

Studies selection and main characteristics

Eighteen studies regarding the performance of NGS analysis in patients suspected of AID have been found in the PubMed database according with the systematic literature review strategy of the Figure 1, and are reported in the Table 1. The number of these studies is increased overtime (Figure 2). Recurrent fever is the only symptom included in the enrollment criteria and required by 6/18 (33%) studies, despite a selection bias should be taken into account. A total of 2179 patients suspected of AID have been genotyped by NGS since 2014. Studies enrolling a large amount of patients usually did not perform an analysis of many genes and viceversa (Figure 3). However, the number of analyzed genes in the NGS panels used in the available studies only referred to AID did not exceed 55. The major enrolled ethnic groups of patients are Caucasian, Middle Eastern and Asian. The exclusion criteria of a previous diagnosis of PFAPA or clinical familial Mediterranean

fever (FMF) is referred to the modified Marshall's criteria and the Tel-Hashomer's criteria, respectively.

Genotype-phenotype assessment

In the analyzed studies of the Table 1, the pathogenicity assessment of each discovered variant was obtained by using the minor allele frequency (MAF) in healthy population, predictive software, classification tools and Sanger sequencing confirmation analysis in 12/18 (67%), 11/18 (61%), 14 (78%) and 10/18 (56%) studies, respectively. Some studies considered also the pattern of inheritance and available family data. For assessing the MAF, the 1000 Genome Project (http://www.1000genomes.org), the Exome Variant Server (http://esv.gs.washington.edu/ESV/), the Exome Aggregation Consortium database (http://exac.broadinstitute.org/) and the Genome Aggregation database (https://gnomad.broadinstitute.org/) are used. The Sorting Intolerant From Tolerant (SIFT; https://sift.bii.a-star.edu.sg/) is the most frequently used predictive *in silico* software (Figure 4), followed by the Polymorphism Phenotyping version 2 (PP2; http://genetics.bwh.harvard.edu/pph2/index.shtml) and Mutation Taster (MT; http://www.mutationtaster.org/). Since its first description in 2014, the Combined Annotation Dependent Depletion software (CADD; https://cadd.gs.washington.edu/) is also routinely implemented. The most used variant classification tools are the ClinVar and the AID-focused website Infevers (https://infevers.umai-montpellier.fr/web/index.php) that reports the International Study Group for Systemic Autoinflammatory Diseases (INSAID) variant classification (Figure 5).

Variants characteristics

In total, more than 1100 variants were reported, ranging from 0.2 to 6.5 per patients. The median rate of detection of a pathogenic or likely pathogenic variant in an undefined AID patient was 20%, ranging from 0% to 89%. Thus, the number of undefined AID patients persists quite high even if the NGS or the whole exome sequencing (WES) approach has been used (73% in Wang et al.). No studies using a whole genome sequencing approach in undefined AID patients have been published to the date.

Clinical manifestations

Considering as affected by SURF who was suspected of AID for undefined recurrent fevers and did not reach a molecular diagnosis even after the NGS analysis, we found a detailed clinical description of 486 SURF patients in 5/18 (28%) studies of the Table 1 and in other 4 studies found in the PubMed database according with the systematic literature review strategy of the Figure 1.

Clinical features of these patients are reported in the Table 2.

The larger cohorts of patients come from the international Eurofever registry and Japan. Another highly enrolled ethnic group is the Middle East. The median ages at the symptoms onset and patient enrollment are 13 and 25 years, respectively. The median diagnosis delay is 35 months (range 13-78) in the 4 cohorts enrolling only children (Chandrakasan et al., De Pauli et al., Garg et al. and Demir et al). Males are 42% of the total. A positive family history has been reported in 0% to 32% of patients in the different cohorts.

The median duration of inflammatory attacks is 4 ± 1 days with a monthly frequency (11 ± 2 attacks/years). The most frequently reported symptoms during fever attacks are fatigue and malaise (>70% of the patients; Figure 6). Arthralgia, abdominal pain, myalgia and eye manifestations are reported in >40% of the patients. Lymphadenopathy, rash/erythema and oral ulcers are less frequently reported (20-40% of the patients). Headache, pharyngitis, arthritis, nausea/vomiting, diarrhea and hepato/splenomegaly are reported in 10-20% of the patients, and chest pain and pericarditis by less than 10%. Sinusitis, urethritis/cystitis, genital ulcers, gonadal pain, neck stiffness, morning headache, febrile seizure, pleuritis, proteinuria, amyloidosis and sensorineural hearing loss are reported by only single studies.

Treatment response

Treatments are differently considered as effectives in different studies. However, a general consideration of an evident amelioration of the clinical manifestations of the patients after the treatment onset has been considered as a sign of efficacy. Only some studies reported a difference between a partial and complete response, and not all authors carefully described the differences between these types of treatment response. Taking into account these general considerations, the efficacy rate of treatments used in SURF patients is shown in the Figure 7. The most frequently administered therapy are on demand steroids (308 patients) with at least a partial efficacy described in >50% of patients, followed by colchicine (190 patients) and NSAIDs (127 patients) with a similar efficacy rate (56% and 65%, respectively). Anakinra is the most effective and frequently used biologic therapy, administered to 46 patients with an efficacy rate of 74%. DMARDs are less frequently used and less effective: 32 patients with an efficacy rate of 48%. Adenoidectomy and tonsillectomy have been performed in only 24 patients with a very low efficacy rate (9%).

In the present study, we systematically reviewed the original English articles enrolling groups of suspected AID patients who were extensively genotyped by NGS technology in order to define the clinical manifestations and treatment response of patients with recurrence of undefined

inflammatory attacks, not fulfilling any PFAPA criteria[5,6] and identified under of the new term of SURF.

Inflammation is the most evident sign of immune system activation against pathogens and damaging signaling in living organisms. Despite it is usually an index of a well being, in case of occurring secondary to an inborn error of immunity, the so-called *horror autoinflammaticus* may onset.[7] At first, the most characteristic manifestation of this pathological process was the recurrence of self-resolving fever attacks. However, a deeper analysis often revealed a subclinical inflammation in affected patients causing long term or life-threatening complications with an evident shortening of the life expectancy and quality. An early diagnosis and targeted treatment may prevent these severe outcomes.

Despite the recurrence was present even in the definition of the first AID group of HRF, the periodicity dilemma persists nowadays, suggesting the presence of an unbalanced regulation of the inflammatory response to common hits, inducing a negative feedback against the primary cause of the immune system hyperactivation. This virtuous cycle prevents an early *exitus* of people with minor defects of the innate immune system that can cause milder AID phenotypes and allowing these mutations to be inherited across future generations. The molecular definition of numerous monogenic AID during the last 20 years dramatically increased our knowledge of the pathways and proteins involved in the innate immune system.[8] However, the large amount of patients displaying undefined recurrent fevers even after the NGS approach, suggests the needing of further discoveries in the field.

In this review, we define a subset of undefined AID patients with recurrent inflammatory attacks and systemic manifestations. Fever is the physiological reaction to an increased concentration of inflammatory cytokines in the blood during an inflammatory response. In case of a single organ or tissue is primarily inflamed, fever does not always suggest an inborn error of immunity. Despite also interesting, as in case of somatic mosaicisms, a defined localization of the primary hit usually suggests that a local treatment can be effective to switch off the clinical manifestations. On the other hand, a primary systemic inflammation often requires systemic drugs, such as targeted agents against a specific cytokine or other therapies able to prevent the unbalanced inflammatory response.

Among these drugs, colchicine is an old cytoskeleton stabilizer with a renowned efficacy in some AID. The concept that a cytoskeleton deregulation can cause an immune disease is not new, but not fully understood.[9] The clinical definition of SURF may be useful to investigate further the molecular basis of this regulatory effect on the inflammatory response. Furthermore, future studies may delineate novel treatments able to control clinical manifestations of SURF.

In conclusion, we reviewed the literature data regarding an emerging group of AID called SURF, suggesting a set of the clinical parameters that could help to distinguish SURF from PFAPA and HRF (Table 3). A proper statistical analysis comparing this group of patients with those with PFAPA and other HRF will allow a proper proposal of the possible evidence-based classification criteria for SURF, with the final aim of favoring the harmonization of future studies in the fascinating field of AID without a molecular diagnosis.

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Table 1. Studies about the NGS analysis in patients suspected of AID.

N°	Study	Date	Enrollment criteria	Pts	Ethnicity	Genes	MAF	Predictive *in silico* tools	Variant classification tools	Sanger confirmation	Variants	Variants for pts, median (range)	Pts with clearly pathogenic variants	Pts with likely pathogenic variants	Pts with VUS	Pts with likely benign or benign variants	Pts without variants
1	Chandrakasan et al.[10]	2014	Periodic fever	66*	Caucasian (14), African (7), others (5)°	7	ND	ND	Infevers	Yes	44	0.8 (0-4)*	25 (42)	0 (0)	6 (10)	0 (0)	28 (48)
2	De Pieri et al.[11]	2015	Periodic fever with negative or indefinite genetic analysis; PFAPA syndrome with very early onset and/or poor response to steroids or tonsillectomy	42	Caucasian	5	Any	SIFT, PP2, MT, MutationAssesor, HSF, NNSplice	EMGQN	Yes	38	0.9 (0-4)	0 (0)	0 (0)	24 (57)	5 (12)	13 (31)
3	Rusmini et al.[2]	2016	Systemic AID with at least one mutation in one AID-related gene by Sanger sequencing	50**	Caucasian	10	< 5%	SIFT, PP2	ND	Yes	254	5 (ND)	23 (68)	7 (21)	4 (12)	0 (0)	0 (0)
4	Nakayama et al.[12]	2017	Clinical diagnosis of AID	108	Asian	12	< 1%	ND	ND	Yes	27	0.25 (ND)	ND	ND	ND	ND	ND
5	Omoyinmi et al.[13]	2017	Undiagnosed inflammatory diseases with clinician suspicion of a genetic cause and negative conventional genetic tests	50	Mixed	166	< 1% ^	SIFT, PP2, MT	ACGS	Only VUS	325	6.5 (1-16)	6 (12)	11 (22)	31 (62)	0 (0)	2 (4)
6	Kostik et al.[14]	2018	Clinical suspicious of primary immunodeficiency with periodic fever	65	ND	302	< 3%	SIFT, PP2, MT, CADD	ClinVar	ND	ND	ND	ND	ND	ND	ND	ND
7	Karacan et al.[15]	2019	Symptoms suggestive of a systemic AID; exclusion of typical FMF	196	Middle Eastern	15	< 1%	ND	ClinVar, Infevers, HGMD	ND	ND	ND	14 (10)	27 (14)	97 (50) §	97 (50) §	58 (30)
8	Ozyilmaz et al.[16]	2019	Periodic fever	64	Middle Eastern	3	Any	ND	ClinVar	ND	13	0.2 (0-1)	4 (6)	0 (0)	3 (5)	6 (9)	51 (80)
9	Hua et al.[17]	2019	Chinese adults suspected of systemic AID	92	Asian	5	ND	ND	EMGQN, Infevers	ND	49	0.5 (0-4)	5 (5)	0 (0)	33 (36)	0 (0)	54 (59)
10	Boursier et al.[18]	2019	Suspected monogenic AID (except FMF, DADA2 and MKD after March 2018)	631	ND	55	ND	SIFT, PP2, MT, MES, HSF, NNSplice, SSF,	Infevers	ND	176	0.3 (ND)	44 (7)	50 (8)	63 (10)	0 (0)	474 (75)

11	Papa et al.[3]	2020	Pediatric onset systemic AID; exclusion of PFAPA syndrome and others etiologies; negative or not conclusive Sanger sequencing of suspected genes	50	Caucasian	41	< 3%	SIFT, MT, FATHMM, MetaSVM, PROVEAN, CADD	ClinVar	Yes	100	2 (0-6)	3 (8)	3 (8)	25 (50)	10 (20)	9 (18)
12	Suspitsin et al.[19]	2020	Periodic fever	56	ND	354	ND	ND	ClinVar	Yes	ND	ND	9 (16) §	9 (16) §	7 (13)	40 (71) §	40 (71) §
13	Sözeri et al.[20]	2020	Symptoms suggestive of a systemic AID; exclusion of FMF, PFAPA syndrome and other common etiologies; positive Eurofever score for MKD, TRAPS and CAPS	71	Caucasian, Middle Eastern	16	< 1%	SIFT, PP2, MT, GERP	EMGQN, ClinVar, HGMD, Eurofever criteria	ND	74	1 (0-3)	35 (49)	0 (0)	36 (51) §	36 (51) §	36 (51) §
14	Hidaka et al.[21]	2020	Unexplained fever	176	Asian	11	< 1%	ND	ND	ND	ND	ND	29 (17)	0 (0)	53 (30)	0 (0)	94 (53)
15	Kosukcu et al.[22]	2020	Recurrent fever and high C-reactive protein along with clinical features of inflammation with a possible AID; infections excluded; negative analysis of 14 AID-related genes	11	Middle Eastern	WES	< 1%	SIFT, PP2, MT, CADD, REVEL, VEST4	ND	ND	ND	ND	4 (36) §	4 (36) §	7 (64)	0 (0)	0 (0)
16	Wang et al.[23]	2020	Pediatric patients suspected of monogenic AID	288	Asian	3/347/WES	< 1%	SIFT, PP2, MT, CADD, UMD-Predictor	ClinVar, Infevers, HGMD	Yes	ND	ND	79 (27)	ND	ND	ND	ND
17	Demir et al.[24]	2020	Symptoms suggestive of a systemic AID; exclusion of FMF, PFAPA syndrome, Blau syndrome, infantile sarcoidosis and other common etiologies; positive Eurofever score for MKD, TRAPS and CAPS	64	Caucasian, Middle Eastern	16	< 1%	SIFT, PP2, MT, GERP	ClinVar, HGMD	Yes	ND	ND	15 (23)	21 (33) §	21 (33) §	28 (44) §	28 (44) §
18	Rama et al.[25]	2021	Symptoms of AID (>3 attacks, elevated CRP, age of onset <30 years); exclusion of Armenian, Turkish, Sephardic and Arabic when mentioned and other causes of inflammation	99	ND	55	< 1%	SIFT, PP2, MT,, MES, HSF, NNSplice, GVGD, Grantham score	Infevers	Yes	ND	ND	10 (10) §	10 (10) §	20 (20)	69 (70) §	69 (70) §

*7 patients were not analyzed; °Hispanic, Vietnamese, Asian-Indian, Puerto Rican-Filipino-Mixed European; **16 patients were not classified; ^ except for the PRF1 p.A91V, TNFRSF1A p.R92Q, and NLRP3 p.V198M variants; § classification was not specified.

Results are shown as numbers (%) unless stated otherwise. ND, not declared.

Table 2. Characteristics of SURF patients published in the English literature.

Study	Chandrakasan et al.[10]	Harrison et al.[26]	De Pauli et al.[27]	Ozyilmaz et al.[16]	Ter Haar et al.[28]	Garg et al.[29]	Papa et al.[3]	Hidaka et al.[21]	Demir et al.[24]
Year	2014	2016	2018	2019	2019	2019	2020	2020	2020
Patients	25	11	23	9	180	22	34	133	49
Ethnicity (patients)	Caucasian (14), African (7), others (5)°	Caucasian (10), Jewish (1)	Caucasian (20), Middle Eastern (2), others (1)	Middle Eastern	Mixed	Caucasian (11), Asian (5), Jewish (1), African (1), others (4)	Caucasian	Asian	Caucasian, Middle Eastern
Age at enrollment, median (range), years	2.5 (0-9)	ND	4.3 (2-9)	18 (1-47)	ND	ND	ND	39.9 (22-57)	5.9 (3-9)
Age at onset, median (range), years	1.4 (0-5)	35 (24-76)	0 (0-2)	ND	4.3 (1-12)**	0.61 (0-13.5)	ND	33.4 (13-53)	3 (1-6)
Adults onset	0 (0)	11 (100)	0 (0)	0 (0)	65 (35)**	0 (0)	ND	ND	ND
Gender, M:F	16:9	5:6	5:18	5:4	51:49**	8:14	ND	66:67	34:15
Positive family history	0 (0)	0 (0)	ND	1 (11)	24 (13)**	7 (32)	ND	ND	12 (24)
Attacks/year, median (range)	8 (4-12)	ND	ND	ND	12 (5-14.5)	ND	12 (7-24)	ND^	10 (6-12)
Attacks duration, median (range), days	4 (3-5)	ND	ND	ND	4 (3-7)	ND	5.9 (4.5-7.3)	ND^	3 (2-4)
Fever	25 (100)	11 (100)	ND	6 (67)	180 (100)	13 (59)	34 (100)	133 (100)	49/49 (100)
Abdominal pain	1 (4)	2 (18)***	12 (52)	8 (89)	87 (48)	4 (18)	17 (50)	ND	31 (63)
Nausea/Vomiting	ND	2 (18)***	ND	ND	44 (24)	5 (23)	3 (9)	ND	8 (16)
Diarrhea	2 (8)	2 (18)***	ND	ND	30 (17)	3 (14)	3 (9)	40 (30)	5 (10)
Rash/Erythema	3 (12)	9 (82)	ND	ND	35 (20)	12 (55)	11 (32)	10 (8)	22 (45)
Genital ulcers	ND	1 (9)	ND	ND	ND	ND	ND	ND	ND
Oral ulcers	1 (4)	3 (27)	12 (52)	ND	53 (29)	ND	13 (38)	ND	14 (29)
Pharyngitis/Tonsillitis	1 (4)	ND	13 (57)	ND	47 (18)	ND	13 (38)	ND	5 (10)
Eye manifestations	ND	ND	ND	ND	ND	14 (64)	ND	ND	11 (22)
Arthritis	2 (8)	5 (46)	ND	1 (11)	12 (7)	12 (55)	7 (21)	ND	4 (8)
Arthralgia	ND	8 (72)	ND	ND	107 (59)	10 (46)	12 (35)	57 (43)	27 (55)
Myalgia	ND	8 (72)	15 (65)	ND	80 (44)	13 (59)	9 (27)	25 (19)	23 (47)
Headache	1 (4)	5 (46)	ND	1 (11)	67 (37)	1 (5)	7 (20)	ND	10 (20)
Morning headache	ND	ND	ND	ND	22 (12)	ND	ND	ND	ND
Fatigue	ND	11 (100)***	ND	ND	106 (59)	ND	ND	ND	ND
Malaise	ND	11 (100)***	ND	ND	99 (55)	ND	ND	ND	ND

Study	Chandrakasan et al.[10]	Harrison et al.[26]	De Pauli et al.[27]	Ozyilmaz et al.[16]	Ter Haar et al.[28]	Garg et al.[29]	Papa et al.[3]	Hidaka et al.[21]	Demir et al.[24]
Lymphadenopathy	1 (4)	4 (36)	ND	ND	76 (42)	12 (55)	6 (18)	ND	ND
Splenomegaly	ND	ND	ND	ND	20 (11)	ND	5 (15)***	ND	1 (2)
Hepatomegaly	ND	ND	ND	ND	21 (12)	ND	5 (15)***	ND	ND
Chest pain	ND	1 (9)	0 (0)	0 (0)	21 (12)	5 (23)	ND	17 (13)	4 (8)
Pericarditis	ND	2 (18)	ND	ND	10 (6)	ND	ND	ND	1 (2)
Urethritis/cystitis	ND	ND	ND	ND	6 (3)	ND	ND	ND	ND
Gonadal pain	ND	ND	ND	ND	3 (2)	ND	ND	ND	ND
Neck stiffness	1 (4)	ND	ND	ND	ND	ND	ND	ND	ND
Sinusitis	ND	6 (55)	ND	ND	ND	ND	ND	ND	ND
Febrile seizure	ND	ND	ND	ND	ND	ND	ND	ND	4 (8)
Pleuritis	ND	ND	ND	ND	ND	ND	ND	ND	1 (2)
Proteinuria	ND	ND	ND	ND	ND	ND	ND	ND	1 (2)
Amyloidosis	ND	ND	ND	ND	ND	ND	ND	ND	1 (2)
Sensorineural hearing loss	ND	ND	ND	0 (0)	ND	ND	ND	ND	0 (0)
Treatment response	ND	11 (100)	ND	ND	ND	22 (100)	18 (53)	ND	ND
NSAIDs	ND	ND	ND	ND	80/105 (76)	3/22 (14)	ND	ND	ND
Steroids	ND	6/10 (60)	16/21 (76)	ND	85/104 (82)	11/22 (50)	17/18 (94)	29/133 (22)	ND
Colchicine	15/25 (60)	0/3 (0)	6/13 (46)	ND	29/49 (59)	ND	14/18 (78)	44/133 (33)	31/49 (63)
DMARDs	ND	0/10 (0)	ND	ND	7/10 (70)	13/22 (59)	ND	ND	ND
Anakinra	ND	10/11 (90)	ND	ND	8/13 (62)	16/22 (73)	ND	ND	ND
Tonsillectomy/Adenoi dectomy	ND	ND	0/12 (0)	ND	2/12 (17)	ND	ND	ND	ND

° Hispanic, Vietnamese, Asian-Indian, Puerto Rican-Filipino-Mixed European; **including 7 patients with a chronic disease course; ^57.1% >1 episodes/months and 54.9% <=3 days; ***not specify.

Results are shown as numbers (%) unless stated otherwise. ND, not declared.

Table 3. Proposed indications for the clinical suspicion of SURF.

Required indications
Recurrent Fever [1]
Negative criteria for PFAPA [2]
Negative genotype for HRF [3]
Additional supporting indications
Monthly attacks
Attacks duration of 3-5 days
Fatigue/malaise
Arthralgia/myalgia
Abdominal pain
Eye manifestations [4]
Colchicine/anakinra response [5]

1) At least 6 similar episodes of fever of unknown origin in 6-12 months.
2) According to the modified Marshall's and/or Eurofever criteria.
3) NGS and/or Sanger sequencing of at least the most commonly associated genes.
4) Periorbital edema and/or corneal erythema.
5) Amelioration of symptoms and/or acute phase reactants.

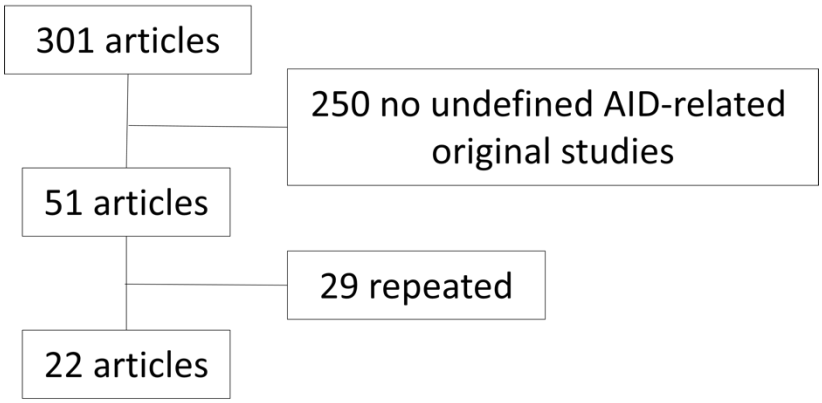

Figure 1. Original English studies found in the PubMed database (https://pubmed.ncbi.nlm.nih.gov/ accessed on 02.02.2020) with the queries: "periodic/recurrent fever/s" AND "NGS/Sanger"; "undefined/undifferentiated" AND "autoinflammatory"; "NGS/Sanger" AND "autoinflammatory".

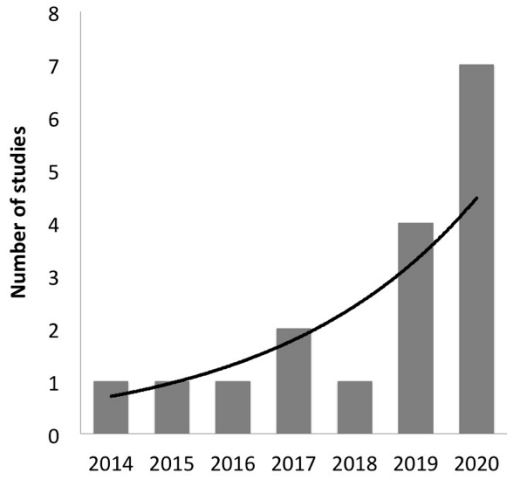

Figure 2. Trend line of studies in Table 1.

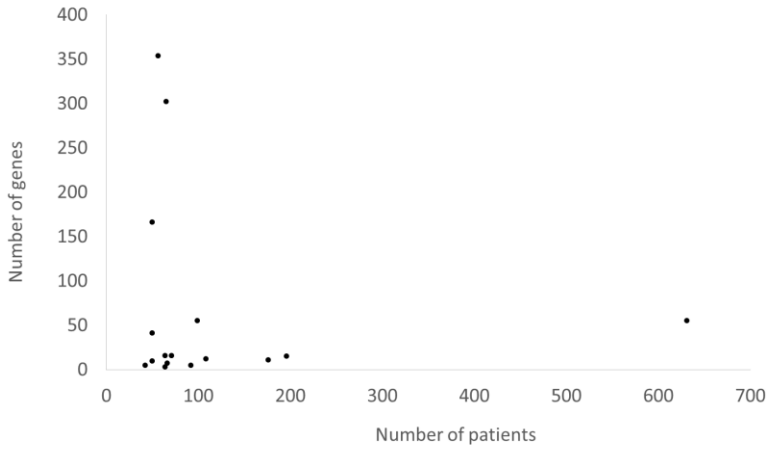

Figure 3. Correlation between the numbers of enrolled patients and analyzed genes of studies in Table 1 except the two using WES.

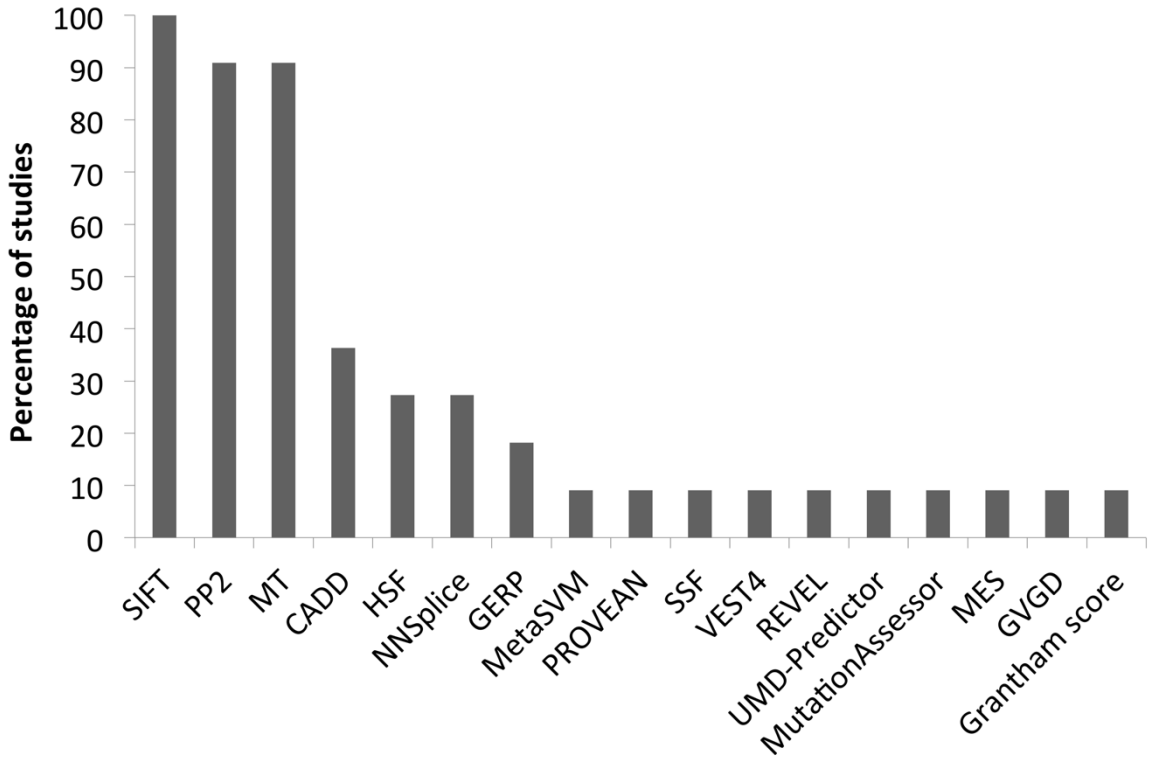

Figure 4. Predictive software of studies in Table 1.

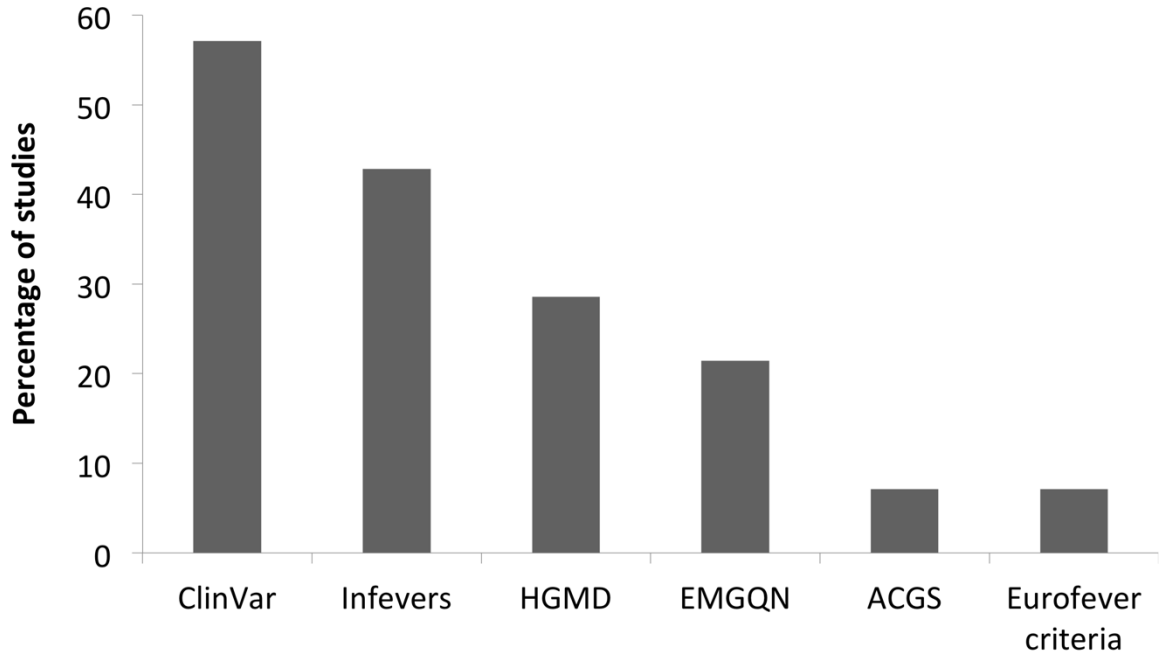

Figure 5. Classification tools of studies in Table 1.

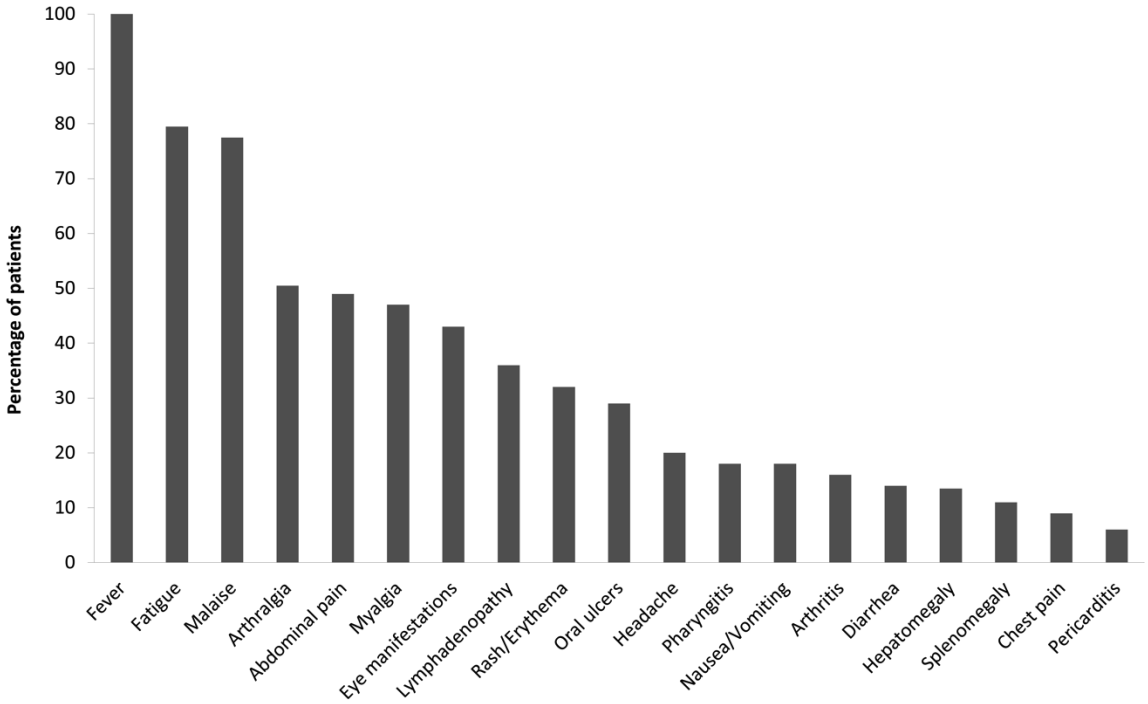

Figure 6. Clinical manifestations of SURF patients reported by at least two studies of Table 2.

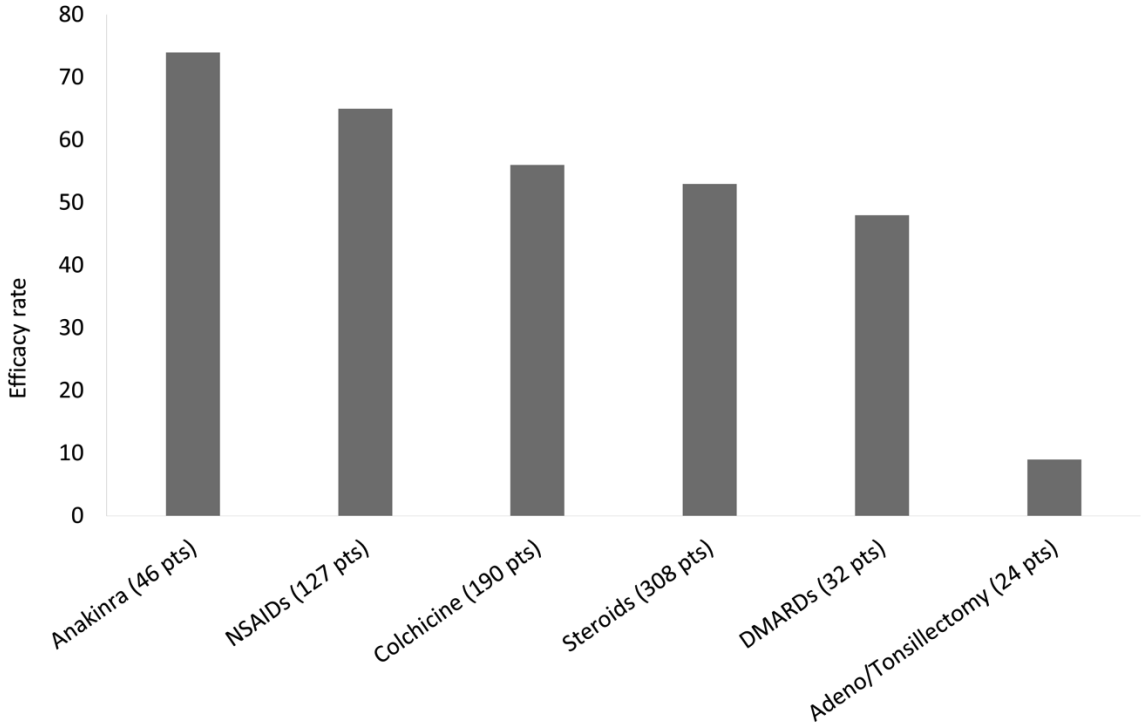

Figure 7. Treatment efficacy in SURF patients.

Proteomic signatures of hereditary recurrent fevers

Hereditary recurrent fevers (HRF) are a group of monogenic autoinflammatory diseases characterized by auto-resolving attacks of sterile inflammation caused by mutations of the *MEFV*, *MVK*, and *TNFRSF1A* gene. (1-3) The familial Mediterranean fever (FMF) is the HRF prototype caused by autosomal gain-of-function mutations of the *MEFV* gene, coding for pyrin. (4, 5) Mutated pyrin is able to process interleukin (IL)-1β by easily nucleating in a pyrin inflammasome. Furthermore, pyrin can be activated by the cortical actin cytoskeleton remodeling induced by bacterial toxins via RhoA, sensing pathogens without recognition of non-self antigens. On the other hand, patients with the mevalonate kinase deficiency (MKD) carry bi-allelic loss-of-function mutations of the *MVK* gene, causing a reduced sterol synthesis and prenylation of small G proteins such as RhoA, favoring activation of the pyrin inflammasome. (6-8) Patients with the TNF receptor-associated periodic syndrome (TRAPS) carry an autosomal dominant mutation of the *TNFRSF1A* gene, resulting in a heap of misfolded TNF receptor 1 in the endoplasmic reticulum, causing oxidative stress, defective autophagy, and high production of pro-inflammatory cytokines (9, 10).
Clinical manifestations of HRF are similar and unspecific (fever, abdominal pain, and skin rash), causing a sensitive diagnostic and treatment delay. Furthermore, a group of patients with systemic undefined recurrent fevers (SURF) do not reach the molecular diagnosis even after an extensive genetic screening with the modern next-generation sequencing (NGS) technologies (11). Moreover, there are no specific serum markers for HRF.
Recently, large-scale proteomics based on high-resolution mass spectrometry (MS) has offered a systems-wide hypothesis-free method to analyze intracellular pathways in order to identify new disease biomarkers. (12-14) Here, we analyzed the proteomics data from untreated and lipopolysaccharide (LPS)-treated monocytes of patients with FMF, TRAPS and MKD, describing dysregulated intracellular pathways with possible future diagnostic and therapeutic implications.

Monocytes isolation and activation
After approval of the study by the Ethics Committee of the Gaslini institute, 5 blood samples of 5 different patients affected by each HRF (Table 1) and 15 healthy donors (HD) were obtained during the routine follow-up. Clinicians evaluated the disease activity considering the presence of symptoms related to each HRF. Laboratory tests were considered abnormal if C-reactive protein (CRP) was > 0.46 mg/dl and serum amyloid A (SAA) was > 10 mg/l. Peripheral blood mononuclear cells (PBMCs) were isolated by gradient (Ficoll Hystpaque) and then purified with CD14 microbeads positive selection (Miltenyi Biotec). Then, monocytes were cultured for 4h in RPMI

1640 with 5% fetal calf serum and in the presence or absence of the LPS 100 ng/ml. The treatment with LPS minimizes the impact of the disease activity and/or ongoing therapy on the monocytes responsiveness. (15, 16)

Samples preparation and MS setup

Monocytes were analyzed by high-resolution liquid chromatography and tandem MS. The cellular pellets were re-suspended in 25 µl of lysis buffer (6M GdmCl, 10mM TCEP, 40mM CAA, 100mMTris pH8.5). The samples were reduced, alkylated and lastly digested in a single step, and then loaded into the Stage Tip. (17) Peptides were analyzed by nano-UHPLC-MS/MS using an Ultimate 3000 RSLC with EASY spray column (75 µm x 500 mm, 2 µm particle size, Thermo Scientific) and with a 180-minute non-linear gradient of 5-45 % solution B (80% CAN and 20% H2O, 5% DMSO, 0.1% FA) at a flow rate of 250 nl/min. Eluting peptides were analyzed using an Orbitrap Fusion Tribrid mass spectrometer (Thermo Scientific Instruments, Bremen, Germany). Orbitrap detection was used for both MS1 and MS2 measurements at resolving powers of 120 K and 30 K (at m/z 200), respectively. Data-dependent MS/MS analysis was performed in top speed mode with a 3 seconds cycle time, during which precursors detected within the range of 375−1500 m/z were selected for activation in order of abundance. Quadrupole isolation with a 1.4 m/z isolation window was used and dynamic exclusion was enabled for 45 s. Automatic gain control targets were 2.5 × 10^5 for MS1 and 5 × 10^4 for MS2, with 50 and 45 ms maximum injection times, respectively. The signal intensity threshold for MS2 was 1 × 10^4. HCD was performed using 30% normalized collision energy. One Microscan was used for both MS1 and MS2 events.

Statistical analysis and Network analysis

MaxQuant software was used to process the MS raw data, setting a false discovery rate (FDR) of 0.01 for the identification of proteins, peptides and peptide-spectrum matches. Moreover, a minimum length of six amino acids was required for peptide identification. MaxQuant-incorporated Andromeda engine was used to search MS/MS spectra against the Uniprot human database. For protein digestion, allowing for N-terminal to proline cleavage, trypsin was chosen. Cysteine carbamidomethylation was selected as fixed modification, whereas N-terminal protein acetylation, oxidation (M) and deamidation (N, Q) have been selected as variable modifications. A tolerance of 7 ppm was set for the mass deviation of the precursor ions, while the maximum mass deviation for MS2 events was 0.5 Da. Algorithm MaxLFQ (12) was chosen for protein quantification, with the activated option ‘match between runs’ to reduce the number of missing proteins. All bioinformatics analyses were done with the Perseus software of the MaxQuant computational platform (13).

Protein groups were filtered up to require 70% valid values in at least one experimental group. The label-free intensities were expressed as base log2, and empty values were imputed with random numbers from a normal distribution for each column, to best simulate the low abundance values close to the noise level. For each group, a t-test with permutation-based FDR of 0.05 and a s0 of 0.1 was used. The Venn diagram of identified proteins was calculated using an online tool (14). A Student's t-test analysis (FDR < 0.05 and S0 > 0.1) was performed to characterize differences and reciprocal relationships between the two groups of each HRF. Principal Component Analysis (PCA) was performed to visualize similarities or differences between samples. The significant proteins were plotted using a Volcano plot. We performed a Cytoscape analysis using the ClueGo app to identify molecular interaction networks and related biological functions of significantly modulated proteins through the t-test. In order to gain biological information from statistically modulated proteins, we highlighted which pathways were altered in the two groups of each disease. Proteomic data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository with the dataset identifier PXD0000000. Using the significantly modulated proteins, the STRING database protein network analysis was performed and then integrated with a DISEASES database query in order to highlight proteins and intracellular pathways already related to each HRF.

Proteins quantification and identification

We identified about 5000 proteins for each HRF (Figure 1A, 1B, 1C). PCA shows discrimination between samples of patients with different HRF and HD (Figure 1D). In particular, 115 and 99 proteins were found significantly modulated in untreated and LPS-treated monocytes of FMF patients (Figure 2A/B and Supplementary Table 1 and 2). In TRAPS and MKD patients, significantly modulated proteins were 16/90 (Figure 2C/D, and Supplementary Table 3 and 4) and 36/415 (Figure 2E/F, and Supplementary Table 5 and 6), respectively. Thus, the rate of LPS-treated over untreated proteins was higher in MKD patients (1.(16), 5.625 and 11.52(7) for FMF, TRAPS and MKD monocytes, respectively). However, LPS treatment maximizes differences between up-regulated proteins in monocytes of HRF patients and HD, independently from the disease activity and/or ongoing treatments (Figure 3). Thus, data from untreated monocytes were not analyzed in details (Supplementary Figure 1).

Interaction Networks

Significantly modulated proteins and pathways of each HRF were analyzed (Figure 4).

FMF pathways

In LPS-treated FMF monocytes (Figure 4A), significantly up-regulated proteins were IL1β, TNFα, interferon-induced protein with tetratricopeptide repeats 1 (IFIT1), IFIT2, and IFIT3, ubiquitin-like protein (ISG15), 60S ribosomal protein L35a (RPL35A) and histone H2B type 1-K (HIST1H2BK), while significantly down-regulated proteins compared to HD were the signal transducer and activator of transcription 3 (STAT3), cyclin-dependent kinase 9 (CDK9), 60S ribosomal protein L36 (RPL36), histone acetyltransferase (HAT), death-domain associated protein 6 (DAXX), retinoblastoma-associated protein (RB1) and carbon anhydrase 2 (CA2).

Looking at the interaction networks (Supplementary Figure 2), major activated pathways were the advanced glycation end-products and its receptors (AGE-RAGE) pathway and the Ras-related C3 botulinum toxin substrate 1 (RAC1) pathway. The signaling related to IL-2/3/4/5/6/7/9/11, type I interferon, adipocytokine, leptin, prolactin, alfa 6 beta 4 intergrin, thymic stromal lymphopoietin (TSLP), platelet-derived growth factor (PDGF), met, oncostatin M, erythropoietin (EPO) receptor and kit receptor were up-regulated. Furthermore, regulation of the microtubule cytoskeleton, heat shock proteins and Fas ligand pathway was altered. Other significantly altered pathways are related to a specific disease, such as diabetes, insulin resistance, leukemia and the type 1 papillary renal cell carcinoma.

TRAPS pathways

In LPS-treated TRAPS monocytes (Figure 4B), significantly up-regulated proteins were the disintegrin and metalloproteinase domain-containing protein 17 (ADAM17), transthyretin (TTR), adapter molecule CRK, 14-3-3 protein theta (YWHAQ), Ras-related protein Rap-1A (RAP1A), signal recognition particle 9 kDa protein (SRP9) and eukaryotic translation initiation factor 3 subunit M (EIF3M), while significantly down-regulated proteins compared to HD were again STAT3, proto-oncogene VAV1, alpha-centractin (ACTR1A), nucleoporin NUP43, acyl-protein thioesterase 2 (LYPLA2) and ribosomal proteins S15, 35A and P2 (RPLS15/35A/P2). Major activated pathways were related to the RNA splicing and transport, inflammatory anti-viral response, chemokine signaling, and leucocytes activation and degranulation (Supplementary Figure 3).

MKD pathways

In LPS-treated MKD monocytes (Figure 4C), significantly up-regulated proteins were the tyrosine-protein phosphatase non-receptor type 18 (PTPN18), protein transport protein Sec61 subunit beta (SEC61B), ubiquitin-like modifier NEDD8, RANBP2-type and C3HC4-type zinc finger containing protein 1 (RBCK1), zinc and ring finger 2 (ZNRF2), F-Box protein 6 (FBXO6), syntaxin-7 (STX7),

chloride nucleotide-sensitive channel 1A (CLNS1A), dynactin subunit 6 (DCTN6), chromobox 5 (CBX5) and protein homolog LSM12 , while significantly down-regulated proteins compared to HD were the nuclear factor kappa-B p105 subunit (NFKB1), toll-like receptor 2 and 8 (TLR2/8), VAV1, tripartite motif containing 4 (TRIM4), lectin and mannose binding protein 2 (LMAN2), Ras-related protein RAP2B, carnitine palmitoyltransferase 2 (CPT2), enoyl-CoA delta isomerase 1 (ECI1), vacuolar protein sorting-associated proteins 16, 29 and 33A (VPS16/29/33A), exportin 1 (XPO1), tubulin beta 1 class VI (TUBB1), ribophorin II (RPN2), mitochondrial citrate and phosphate carriers (SLC25A1/3), ribosomal protein S6 kinase A3 (RPS6KA3), voltage-dependent anion-selective channel 1 (VDAC1), histone cluster 2 H3 family member (HIST2H3A), GPI transamidase component PIG-S, threonyl-tRNA synthetase (TARS), AP-1 complex subunit gamma-like 2 (AP1G2), mitofusina-2 (MFN2), mitochondrial carrier 2 (MTCH2), copine 2 (CPNE2), CA2, myosin-Va (MYO5A), DNA topoisomerase II Beta (TOP2B), NADH:ubiquinone oxidoreductase subunit A9 (NDUFA9), isocitrate dehydrogenase subunit gamma (IDH3G), ethylmalonyl-CoA decarboxylase 1 (ECHDC1), mitochondrially encoded cytochrome C oxidase II (MT-CO2), and aldehyde dehydrogenase 3 family member A2 (ALDH3A2).

Major activated pathways were related to the intermediate cell metabolism of glucose and other carbohydrates, glycoprotein VI-mediated activation cascade, recycling pathways of L1 and activation of the immune system. In particular, neutrophils degranulation, nucleotide-binding oligomerization domain (NOD) signaling, NOD-like receptor family pyrin domain containing 3 (NLRP3) inflammasome, and ILs signaling were modulated (Supplementary Figure 4).

In this study, we report the proteomics data analysis of untreated and LPS-treated monocytes of 15 patients with HRF compared to HD. Despite the identification of the gene mutations causing FMF, TRAPS and MKD shred new lights on their pathogenesis, intracellular consequences are far to be fully understood. Here, we discuss modulated proteins and pathways from a clinical view.

In LPS-treated FMF monocytes, high expression of IL1β, TNFα and proteins related to the type 1 interferon pathway, such as IFIT1/2/3 and ISG15, recalls treatment targets of FMF. (18-20) The higher concentration of the IL-6-induced STAT3/CDK9 complex in LPS-treated HD monocytes suggests a lower activation of the IL-6-mediated inflammatory response in FMF monocytes, (21) thus acting against the use of flavonoids as CDK9 inhibitors in FMF patients. (22) However, an increased oxidative stress in FMF patients seems confirmed by the high concentration of the macrophage migration inhibitory factor (MIF) in untreated FMF monocytes (Supplementary Table 7). (23)

Other finely modulated proteins in FMF monocytes are CA2, cell cycle regulators RB1 and DAXX, and the nuclear RPL35A, RPL36, HIST1H2BK and HAT. Up-regulated CA2 recalls efficacy of NSAIDs during FMF attacks. (24) On the other hand, RB1 is the only protein dramatically reduced after LPS treatment (Supplementary Table 7). Noteworthy, RB1 stabilizes microtubules-associated α-tubulin protein, suggesting that its loss may contribute to the cytoskeleton instability of FMF potentially controlled by colchicine. (25)

The analysis of interaction networks highlights the abnormal regulation of pathways related to the chronic inflammation, microtubule cytoskeleton and cell apoptosis. The activation of the AGE-RAGE pathway triggers generation of free radicals and the expression of pro-inflammatory gene mediators such as the SA100A12, an extracellular ligand of RAGE also found up-regulated in untreated FMF monocytes (Supplementary Table 7), causing a dangerous feed-forward loop capable of shifting acute to chronic inflammation. (26-28) This effect has been involved in many metabolic diseases, such as insulin resistance and diabetes, and contributes to modulate the signaling of adipocytokine, leptin, and prolactin, as an endocrine disruptor. (29, 30) A fascinating hypothesis poses the Mediterranean diet at defense against these secondary effects of the *MEFV* gene mutations. (31) Several proteins and pathways related to cell proliferation and apoptosis can contribute to the significantly lower incidence of cancers in FMF patients. (32)

In LPS-treated TRAPS monocytes, up-regulation of the shedding protein ADAM17 suggests an ineffective attempt to correct the well-known TNF receptor 1 shedding defect. (33) Moreover, the up-regulated amyloidogenic TTR recalls the highest prevalence of systemic amyloidosis in TRAPS patients. (34) Differently from FMF data, pyrin inflammasome is not up-regulated but even inhibited by the up-regulated 14-3-3 protein theta, a well-known pyrin inhibitor.

Other modulated proteins in LPS-treated TRAPS monocytes are related to the translational machinery (SRP9, EIF3M and RPS15), vesicles transport (ACTR1A and NUP43) and mitogen-activated protein kinase pathway (LYPLA2, CRK, VAV1 and RAP1A). These evidences support the hypothesis that a defect in the translation and/or transportation of the mutated TNF receptor 1 causes accumulation of the misfolded protein in the cytosol and the Golgi, causing an unfolded-protein response. (35-38) Interestingly, ,OPTN seems partially reduced after LPS treatment, as well as the anti-inflammatory tyrosine kinase LYN, maybe increasing the stress of the endoplasmic reticulum and autophagy during TRAPS attacks (Supplementary Table 8), confirming our previous evidences. (39, 40) The modulated RNA pathways may justify the lower concentration of the microRNAs observed in TRAPS fibroblasts (41).

MKD is a metabolic autoinflammatory disease and showed the higher complexity of proteins regulation. Essentially, the significantly modulated pathways are involved in the intermediate cell

metabolism, ubiquitination, intracellular trafficking, and inflammation. In particular, NEDD8, ZNRF2, FBXO6, LSM12 and RBCK1, also known as HOIL-1, mediate ubiquitination, and SEC61B, STX7, DCTN6, AP1G2, TUBB1, MYO5A, LMAN2, VPS16/29/33A and CPNE2 are liked to the intracellular trafficking regulation, both emerging pathways in autoinflammation. (42, 43) On the other hand, up-regulated PTPN18 can bind PSTPIP1, even modestly un-regulated in untreated and LPS-treated MKD monocytes (Supplementary Table 9), whose mutations have been related to the well-known IL-1-mediated autoinflammatory pyogenic sterile arthritis, pyoderma gangrenosum, and acne (PAPA) syndrome. Finally, the up-regulated CBX5, also known as haptoglobin 1 alpha, have been associated with several inflammatory disorders due to its ability to modulate the helper T-cell type 1 and 2 balance and antioxidant properties. (44)

Down-regulated proteins in the LPS-treated MKD monocytes are mainly related to the NFkB-mediated inflammatory response (NFKB1, TLR2/8, RAP2B and TRIM4), as well as DNA transcription and RNA nuclear export (CLNS1A, TOP2B, TARS and XPO1), maybe explain the reduced microRNA-mediated silencing complex activity of the mevalonate pathway. (45) These evidences recall the increased susceptibility to infections of MKD patients. Furthermore, several down-regulated proteins participate to the fatty acid oxidation and glycolipid biosynthesis (PIG-S, ALDH3A2, RPN2, CPT2, ECI1) supporting a prenylation defects of small GTPases in MKD. (46, 47) Finally, the modulated MT-CO2, MFN2, MTCH2, IDH3G, NDUFA9, VDAC1, ECHDC1 and SLC25A1/3 proteins confirmed previous evidences of an instable mitochondrial membrane and altered autophagy regulation in MKD monocytes. (48)

In conclusions, single amino acid substitution in a key immune related protein can alters a large amount of proteins and pathways, showing the relevance of systems-base biology. Proteomics data may support the design of new diagnostic tools to integrate NGS technologies for HRF patients. Furthermore, new drug targets may be discovery. On the other hand, the integration of proteomic and genomic data of HRF patients may help to define the molecular diagnosis of patients with SURF.

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Table 1. Characteristics of patients.

No	Diagnosis	Mutation	Disease activity	CPR (mg/dL)	SAA (mg/L)	Treatment
1	FMF	M694V/M694V	Inactivity	1,82	145	Colchicine
2	FMF	M694V/V726A	Inactivity	1,16	26	Colchicine
3	FMF	M694V/V726A	Inactivity	<0,46	15,3	None
4	FMF	M680I/V726A	Inactivity	<0,46	14,2	Colchicine
5	FMF	M694V/M694V	Inactivity	<0,46	4,2	Colchicine
6	TRAPS	C55Y	Inactivity	<0,46	2,07	Canakinumab
7	TRAPS	C88Y	Inactivity	<0,46	1,8	Canakinumab
8	TRAPS	C52Y	Inactivity	<0,46	5,39	Canakinumab
9	TRAPS	T50M	Inactivity	<0,46	9,94	Canakinumab
10	TRAPS	C55Y	Inactivity	<0,46	3,86	Canakinumab
11	MKD	L265R/V377I	Inactivity	<0,46	4,1	Canakinumab
12	MKD	C605insG/V377I	Activity	1,21	135	Canakinumab
13	MKD	C785_790delC/V377I	Inactivity	<0,46	7,2	Canakinumab
14	MKD	V310M/V377I	Activity	3,08	130	None
15	MKD	V377I omo	Inactivity	<0,46	16,8	None

FMF, familial Mediterranean fever; TRAPS, TNF receptor-associated periodic syndrome; MKD, mevalonate kinase deficiency; CPR, C-reactive protein; SAA, serum amyloid A.

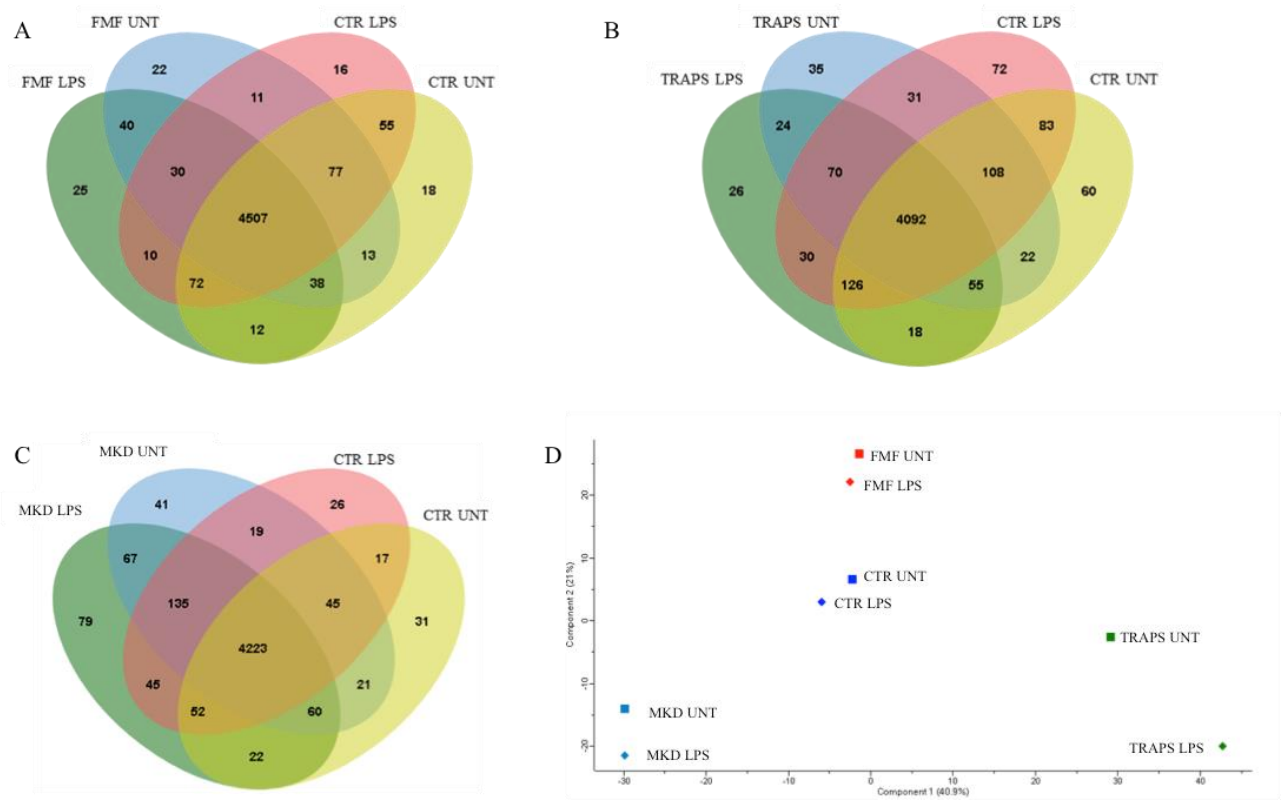


Figure 1. Venn diagram shows the number of proteins in untreated and LPS-treated monocytes of patients with FMF (A), TRAPS (B), and MKD (C) compared to HD. Principal component analysis (D) shows discrimination between proteins of untreated (squares) and LPS-treated (circles) monocytes of patients with FMF (red), TRAPS (green), MKD (light blue) and HD (blue).

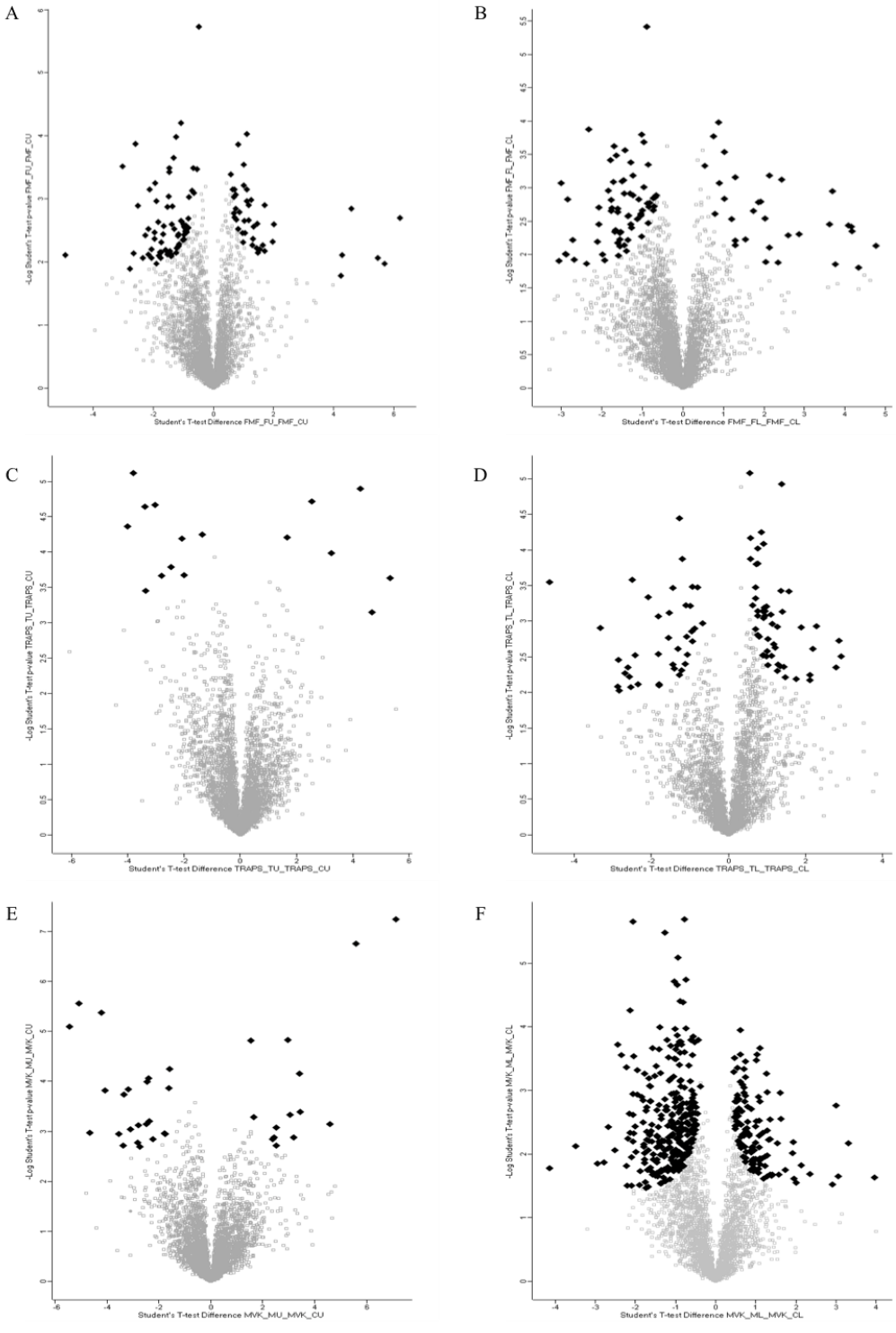


Figure 2. Volcano plots shows proteins of untreated and LPS-treated monocytes of patients with FMF (A and B), TRAPS (C and D) and MKD (E and F). Black dots represent proteins with large magnitude fold-changes (x-axis) and high statistical significance (y-axis; p = −log10).

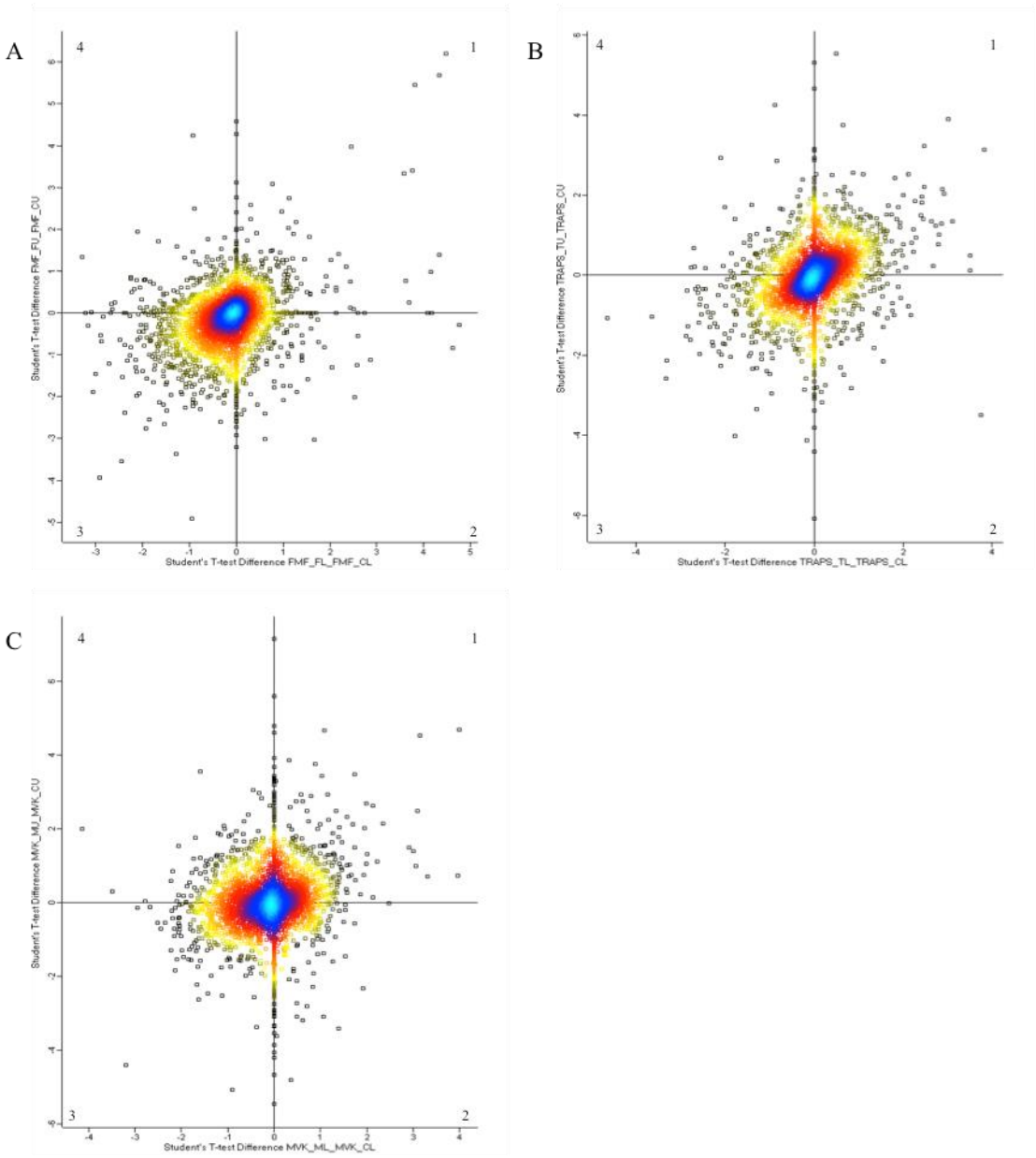


Figure 3. Volcano plots shows up-regulated proteins of FMF (A), TRAPS (B), and MKD (C) monocytes (upper right) compared to HD (lower left), independently from the treatment. For each HRF, the figure also shows up-regulated proteins of LPS-treated monocytes with down-regulated proteins of untreated monocytes (lower right) and up-regulated proteins of untreated monocytes with down-regulated proteins of LPS-treated monocytes (upper left).

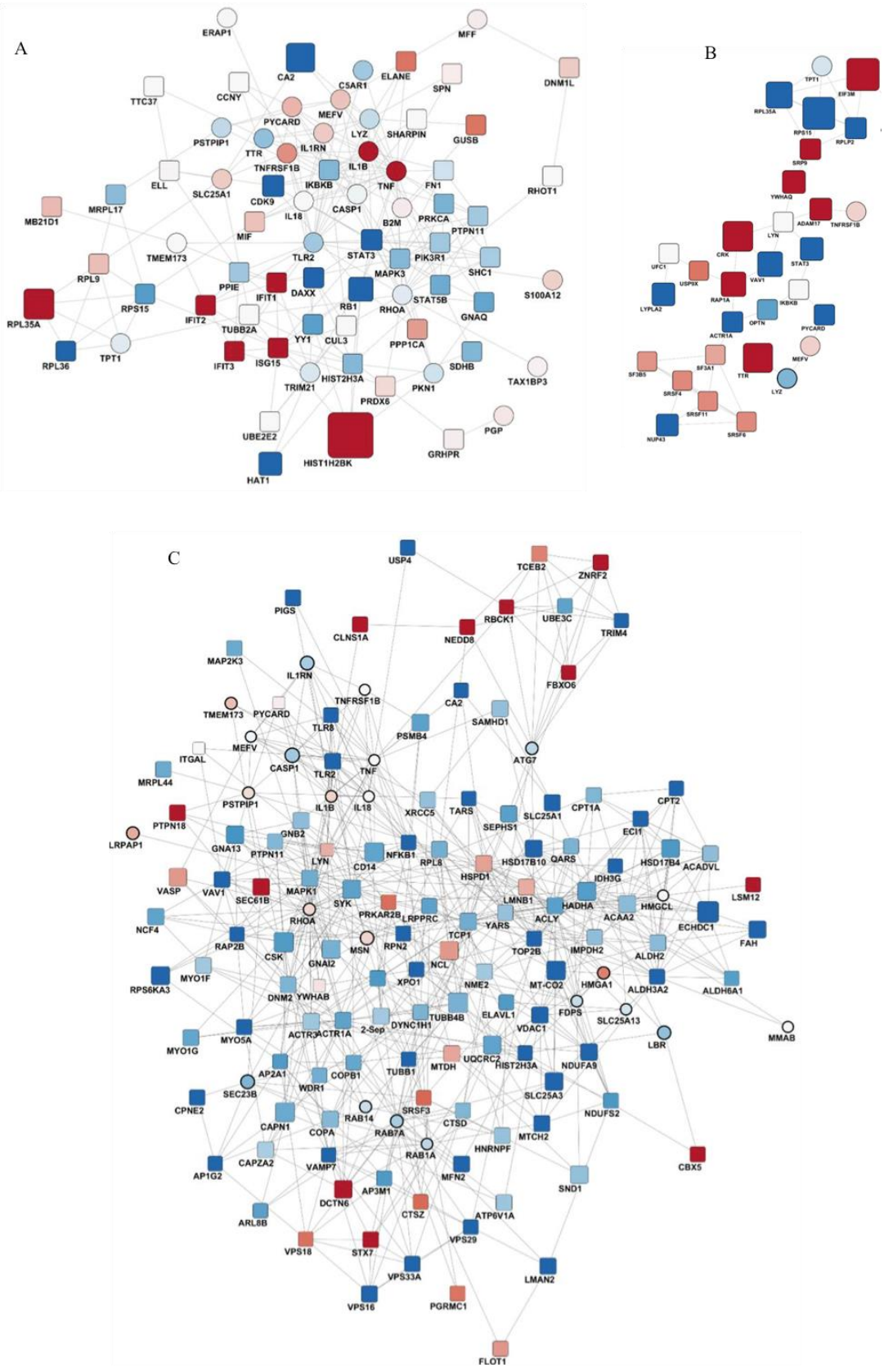
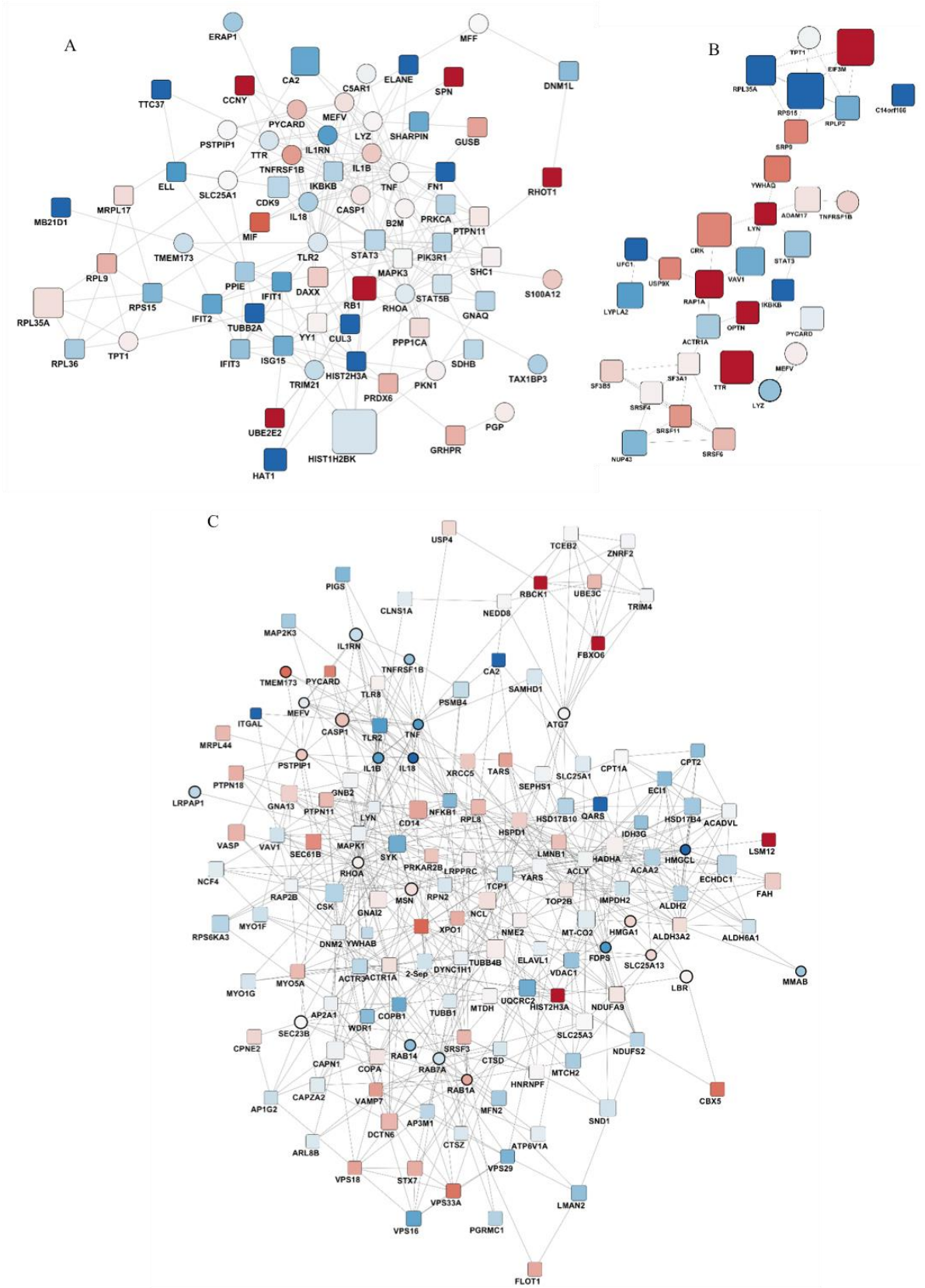
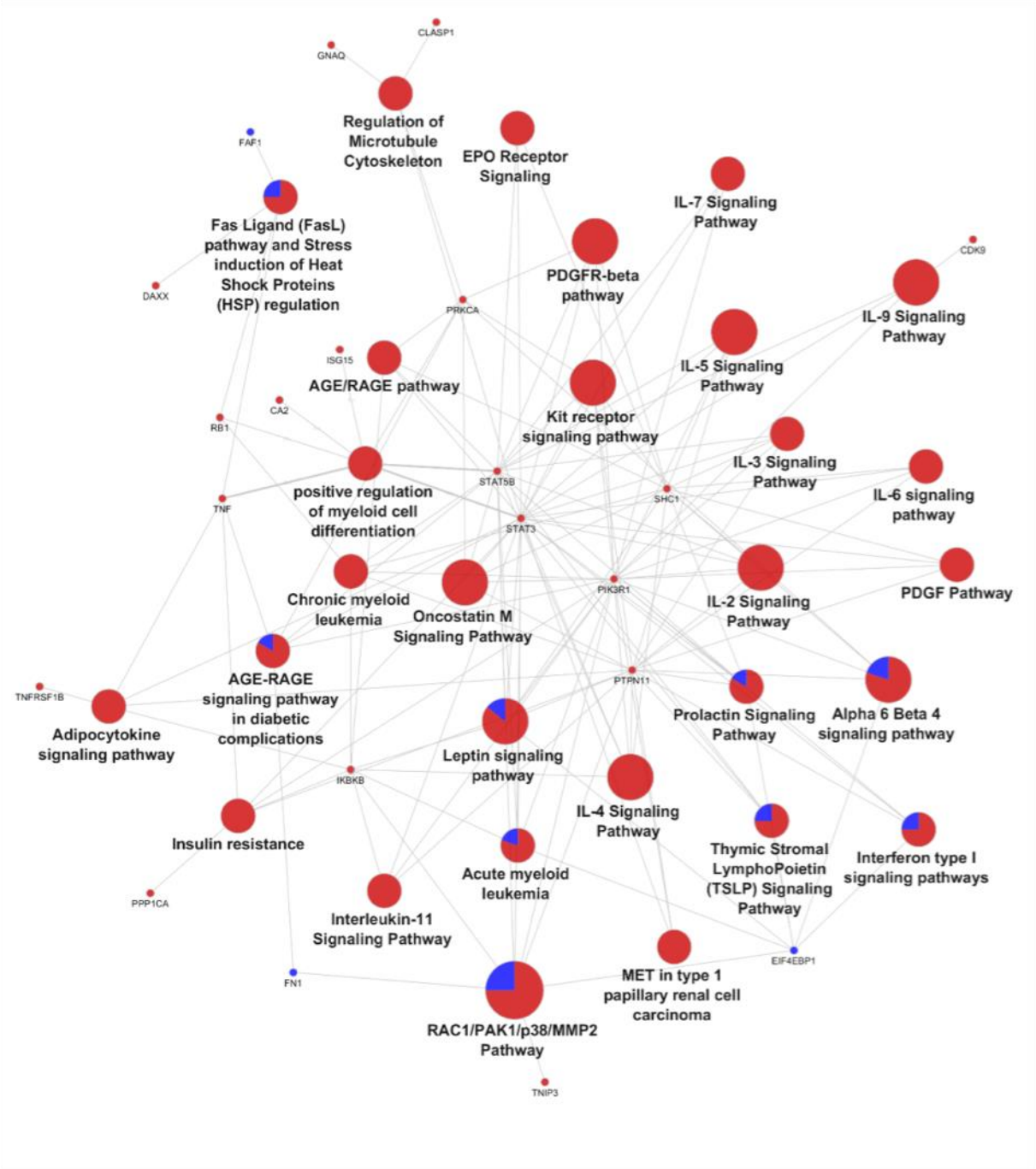


Figure 4. Interaction network of already related (circle) and new (square) significantly up-regulated proteins in LPS-treated monocytes (red) of FMF (A), TRAPS (B), and MKD (C) patients compared with HD (blue). The size of each node expresses the percentage of protein concentration.

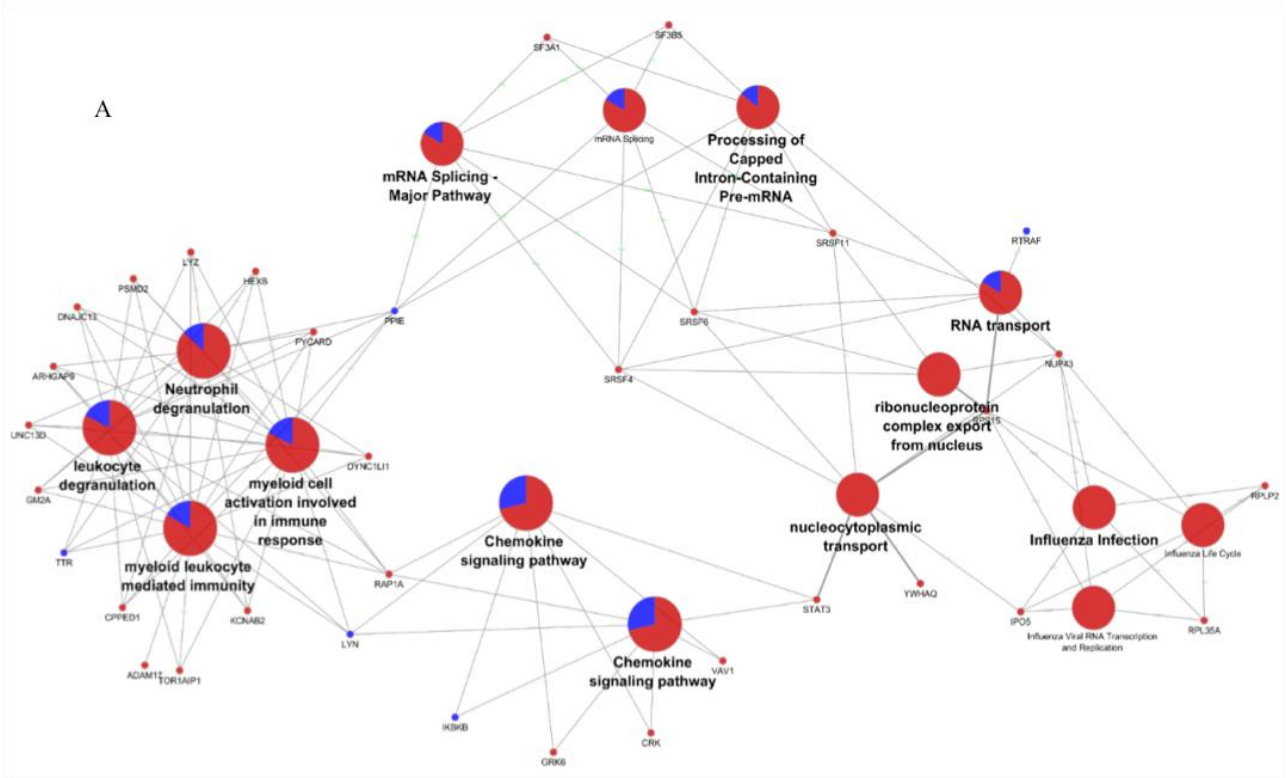


Supplementary Figure 1. Interaction network of already related (cicle) and new (square) significantly up-regulated proteins in untreated monocytes (red) of FMF (A), TRAPS (B), and MKD (C) patients compared with HD (blue). The size of each node expresses the percentage of protein concentration.

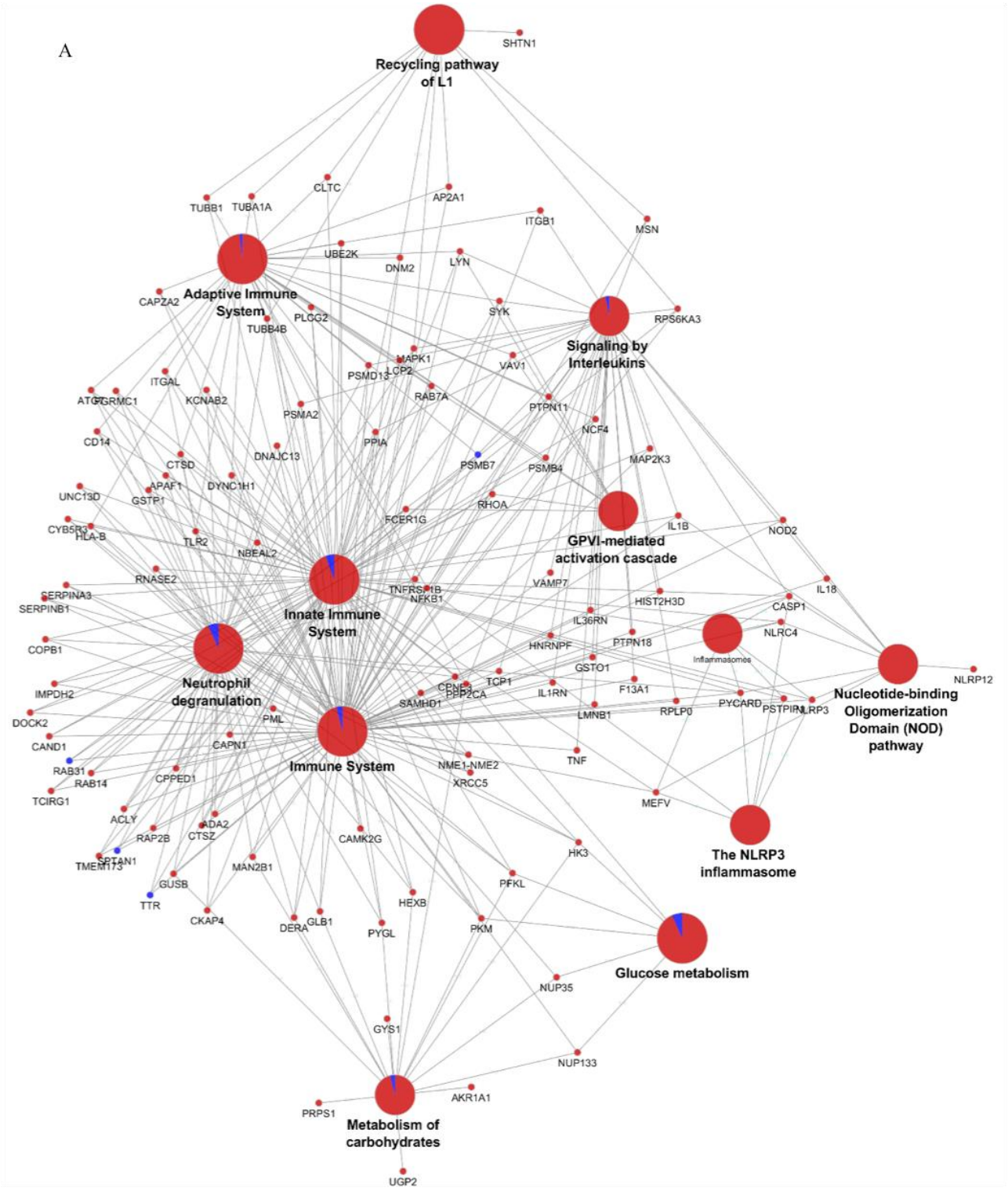
A



Supplementary Figure 2. Interaction network of untreated (blue) and LPS-treated (red) FMF monocytes. The size of each node expresses the degree of network activation.



Supplementary Figure 3. Interaction network of untreated (blue) and LPS-treated (red) TRAPS monocytes. The size of each node expresses the degree of network activation.



Supplementary Figure 4. Interaction network of untreated (blue) and LPS-treated (red) MKD monocytes. The size of each node expresses the degree of network activation.

Supplementary Table 1. Significantly modulated proteins of untreated FMF monocytes shown in Figure 2A.			
Protein names	**Gene names**	**Student's T-test p-value (-log)**	**Student's T-test Difference**
Translation initiation factor eIF-2B subunit delta	EIF2B4	2,6491	-1,08
BCL2/adenovirus E1B 19 kDa protein-interacting protein 3-like	BNIP3L	2,64253	-1,04628
Proteasomal ubiquitin receptor ADRM1	ADRM1	3,42823	-1,45877
Thromboxane-A synthase	TBXAS1	2,14246	-2,65853
Ubiquitin-protein ligase E3A	UBE3A	2,34873	-1,01414
Baculoviral IAP repeat-containing protein 1	NAIP	2,35951	-0,962915
Exosome complex component CSL4	EXOSC1	3,25102	-1,94119
von Willebrand factor A domain-containing protein 9	VWA9	3,21249	1,0017
Myeloid-associated differentiation marker	MYADM	2,46876	-0,901073
COP9 signalosome complex subunit 1	GPS1	2,57068	-1,6512
Actin beta	ACTB	1,78203	4,23435
Lactotransferrin	LTF	1,97549	5,68489
Serine/threonine-protein kinase MARK2	MARK2	2,1941	-1,57728
3-phosphoinositide-dependent protein kinase 1	PDPK1	2,29002	-1,1278
DNA (cytosine-5)-methyltransferase 3A	DNMT3A	2,88017	-1,36095
Translocation protein SEC62	SEC62	2,69098	-0,83733
Protein transport protein Sec24C	SEC24C	3,09638	-0,6466
Sjoegren syndrome/scleroderma autoantigen 1	SSSCA1	2,66599	1,16753
Cathelicidin antimicrobial peptide	CAMP	2,11231	4,27064
28S ribosomal protein S7, mitochondrial	MRPS7	2,15092	-1,50467
ORM1-like protein 3	ORMDL3	2,12002	-1,40518
Actinin alpha 4	ACTN4	2,06711	-2,05933
Transmembrane protein 205	TMEM205	2,42134	-2,26277
Dynamin-1-like protein	DNM1L	2,31057	-1,73744

Supplementary Table 1. Significantly modulated proteins of untreated FMF monocytes shown in Figure 2A.			
Protein names	**Gene names**	**Student's T-test p-value (-log)**	**Student's T-test Difference**
Histone acetyltransferase type B catalytic subunit	HAT1	1,88924	-2,76738
2-deoxynucleoside 5-phosphate N-hydrolase 1	DNPH1	2,31399	0,988897
Syntaxin-6	STX6	2,06827	-2,33611
Uncharacterized protein KIAA0513	KIAA0513	2,1456	-1,23255
Galactosylgalactosylxylosylprotein 3-beta-glucuronosyltransferase 3	B3GAT3	2,22009	-1,21531
Thioredoxin domain-containing protein 12	TXNDC12	2,84474	0,747147
Fibronectin	FN1	3,87831	-2,59544
Non-histone chromosomal protein HMG-14	HMGN1	2,56373	-0,967116
Prosaposin	PSAP	2,79447	0,875078
Annexin A3	ANXA3	2,0623	5,45663
Macrophage migration inhibitory factor	MIF	2,60382	1,46934
Leukosialin	SPN	2,59416	2,01043
High mobility group protein HMG-I/HMG-Y	HMGA1	2,41716	-1,1831
Diacylglycerol kinase alpha	DGKA	2,87246	-1,53102
Peroxiredoxin-6	PRDX6	2,77405	0,686326
60S ribosomal protein L9	RPL9	3,02732	0,67707
Prohibitin	PHB	3,38393	0,576388
Replication factor C subunit 5	RFC5	2,15914	-1,77835
Cysteine--tRNA ligase, cytoplasmic	CARS	3,65283	-1,31223
RNA-binding protein 25	RBM25	3,0307	-0,811191
Ras-related protein Rab-9A	RAB9A	3,98751	-1,23249
RNA polymerase II elongation factor ELL	ELL	2,09464	-1,41141
Methionine--tRNA ligase, cytoplasmic	MARS	2,12011	-1,51632

Supplementary Table 1. Significantly modulated proteins of untreated FMF monocytes shown in Figure 2A.			
Protein names	**Gene names**	**Student's T-test p-value (-log)**	**Student's T-test Difference**
ATP synthase subunit e, mitochondrial	ATP5I	2,46174	1,055
40S ribosomal protein S15	RPS15	2,60154	-0,989971
Histone H3.2	HIST2H3A	2,11215	-4,91382
Mitochondrial 2-oxoglutarate/malate carrier protein	SLC25A11	2,25039	-1,14861
Sialic acid-binding Ig-like lectin 14	SIGLEC14	2,19903	-2,05611
Heat shock protein 75 kDa, mitochondrial	TRAP1	2,53508	-0,936696
TAR DNA-binding protein 43	TARDBP	2,54596	-0,825465
Eukaryotic translation initiation factor 4E-binding protein 1	EIF4EBP1	3,06487	0,743391
Inositol-tetrakisphosphate 1-kinase	ITPK1	2,65068	1,03663
Cullin-3	CUL3	2,89467	-2,51483
Tubulin beta-2A chain	TUBB2A	2,10634	-1,80102
Nuclear transcription factor Y subunit gamma	NFYC	2,71195	0,712255
V-type proton ATPase subunit F	ATP6V1F	2,17341	1,71373
Putative coiled-coil-helix-coiled-coil-helix domain-containing protein CHCHD2P9, mitochondrial	CHCHD2P9	2,63145	-1,8274
Ubiquitin-associated protein 2	UBAP2	2,17427	-1,44638
Rootletin	CROCC	2,96526	1,10605
Vacuolar protein sorting-associated protein 53 homolog	VPS53	2,51984	-2,16291
Rap1 GTPase-activating protein 2	RAP1GAP2	2,36566	1,31747
Tetratricopeptide repeat protein 37	TTC37	2,12155	-2,16444
Vacuolar protein sorting-associated protein 13C	VPS13C	3,49152	-0,673022
UPF0317 protein C14orf159, mitochondrial	C14orf159	1,97671	-1,88973
Coiled-coil domain-containing protein 91	CCDC91	3,14619	-2,12756
Nitrilase homolog 1	NIT1	2,48105	-0,895555

Supplementary Table 1. Significantly modulated proteins of untreated FMF monocytes shown in Figure 2A.			
Protein names	**Gene names**	**Student's T-test p-value (-log)**	**Student's T-test Difference**
Copine-8	CPNE8	2,15429	-1,72138
Cytokine receptor-like factor 3	CRLF3	2,42804	-0,981761
LysM and putative peptidoglycan-binding domain-containing protein 2	LYSMD2	2,56517	1,33936
Mitochondrial Rho GTPase 1	RHOT1	2,26671	1,64559
Misshapen-like kinase 1	MINK1	2,36364	-1,95205
Oxidation resistance protein 1	OXR1	2,4458	-1,16105
Eukaryotic translation initiation factor 4E type 3	EIF4E3	2,06687	-1,74517
Cyclic GMP-AMP synthase	MB21D1	3,51982	-3,01569
GTPase IMAP family member 8	GIMAP8	5,72817	-0,482056
Cyclin-Y	CCNY	2,32371	1,97999
ATP-binding cassette sub-family F member 1	ABCF1	2,48835	-0,929731
Retinol dehydrogenase 11	RDH11	2,10449	-1,42718
Nuclear pore membrane glycoprotein 210	NUP210	3,86408	0,818427
AP-3 complex subunit sigma-1	AP3S1	2,27	1,30312
Protein YIPF5	YIPF5	4,20425	-1,07156
Coiled-coil domain-containing protein 47	CCDC47	2,65329	-1,0721
RalBP1-associated Eps domain-containing protein 1	REPS1	3,54157	1,00333
Copine-2	CPNE2	2,45587	0,974257
ERO1-like protein alpha	ERO1L	3,15015	1,10495
Zinc finger RNA-binding protein	ZFR	3,03535	-1,48686
Ubiquitin-conjugating enzyme E2 E2	UBE2E2	2,84301	4,58013
Calcium and integrin-binding protein 1	CIB1	2,43848	-1,66321
N-terminal Xaa-Pro-Lys N-methyltransferase 1	NTMT1	2,52139	0,823927

Supplementary Table 1. Significantly modulated proteins of untreated FMF monocytes shown in Figure 2A.			
Protein names	**Gene names**	**Student's T-test p-value (-log)**	**Student's T-test Difference**
RNA-binding protein 4	RBM4	3,14836	0,634053
Sharpin	SHARPIN	2,26113	-1,16509
Phosducin-like protein 3	PDCL3	2,90454	1,1818
Translation initiation factor IF-3, mitochondrial	MTIF3	2,2022	1,54116
MIP18 family protein FAM96A	FAM96A	2,97977	1,25787
Calcyclin-binding protein	CACYBP	3,47277	-0,546935
Integrator complex subunit 7	INTS7	2,47123	-1,96085
Aurora kinase A-interacting protein	AURKAIP1	2,54637	-0,819901
Cell growth-regulating nucleolar protein	LYAR	2,96601	-1,88782
NADH dehydrogenase [ubiquinone] 1 alpha subcomplex assembly factor 4	NDUFAF4	3,49299	-1,45721
Core histone macro-H2A.2	H2AFY2	2,90307	1,70402
Glyoxylate reductase/hydroxypyruvate reductase	GRHPR	3,14723	0,703817
Protein NipSnap homolog 3A	NIPSNAP3A	2,5962	-1,38459
U6 snRNA-associated Sm-like protein LSm7	LSM7	2,14617	1,48891
Glucocorticoid modulatory element-binding protein 2	GMEB2	2,49792	-0,921182
DNA helicase INO80	INO80	2,58241	-0,919009
FAS-associated factor 1	FAF1	2,66964	0,754553
Peptidyl-prolyl cis-trans isomerase E	PPIE	3,13143	-0,697405
Sialic acid-binding Ig-like lectin 9	SIGLEC9	2,52277	-1,3022
WD repeat-containing protein 7	WDR7	4,03392	1,10474
Myeloblastin	PRTN3	2,21776	1,43862

Supplementary Table 2. Significantly modulated proteins of LPS-treated FMF monocytes shown in Figure 2B.			
Protein names	**Gene names**	**Student's T-test p-value (-log)**	**Student's T-test Difference**
Apolipoprotein C-II	APOC2	2,65529	0,0144
AP-2 complex subunit mu	AP2M1	2,70471	0,0113846
Ig kappa chain C region	IGKV3-11	2,96108	0,01525
Transmembrane emp24 domain-containing protein 7	TMED7-TICAM2	3,15646	0,011619
Killer cell lectin-like receptor subfamily F member 1	KLRF1	2,42047	0,0124286
Thromboxane-A synthase	TBXAS1	2,34402	0,0320714
Phosphatidylinositol 3-kinase	PIK3C3	2,45059	0,0132381
Basic leucine zipper and W2 domain-containing protein 1	BZW1	3,07285	0,014
Myeloid-associated differentiation marker	MYADM	2,3412	0,0256296
COP9 signalosome complex subunit 1	GPS1	2,65123	0,0148235
G protein subunit beta 2	GNB2	2,54111	0,0146316
Protein FAM195B	FAM195B	3,41629	0,0112
Lactotransferrin	LTF	1,8043	0,046381
Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-3	GNB3	2,79654	0,0110909
2,5-phosphodiesterase 12	PDE12	2,60798	0,0304918
Aminoacyl-tRNA synthetases	WARS	3,12091	0,014
Spermatogenesis-defective protein 39 homolog	VIPAS39	3,48995	0,0186667
Translocating chain-associated membrane protein 1	TRAM1	2,66561	0,0471064
REST corepressor 1	RCOR1	2,00124	0,0365294
Cytochrome B5 type B	CYB5B	2,12077	0,0455584
Myelin basic protein	MBP	1,91818	0,0479167
ATP-dependent RNA helicase DDX39A	DDX39A	2,85283	0,0458353
Insulin-like growth factor 2 mRNA-binding protein 3	IGF2BP3	2,01345	0,0350448

Supplementary Table 2. Significantly modulated proteins of LPS-treated FMF monocytes shown in Figure 2B.			
Protein names	**Gene names**	**Student's T-test p-value (-log)**	**Student's T-test Difference**
Inhibitor of nuclear factor kappa-B kinase subunit beta	IKBKB	3,013	0,016
Cytochrome b-c1 complex subunit 8	UQCRQ	2,71608	0,0392877
Histone H2B type 1-K	HIST1H2BK	2,13071	0,0161702
Mitochondrial import receptor subunit TOM70	TOMM70A	2,84649	0,0461579
NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 3	NDUFA3	2,43581	0,010963
L-lactate dehydrogenase A chain	LDHA	3,77584	0,015027
Carbonic anhydrase 2	CA2	2,22256	0,0201569
Tumor necrosis factor	TNF	2,10769	0,0355758
Ubiquitin-like protein ISG15	ISG15	2,30345	0,0140465
Retinoblastoma-associated protein	RB1	2,19513	0,0315088
Epoxide hydrolase 1	EPHX1	2,82814	0,0165217
Beta-glucuronidase	GUSB	2,20811	0,044439
Neutrophil elastase	ELANE	2,1428	0,0480455
Interferon-induced protein with tetratricopeptide repeats 1	IFIT1	1,88922	0,0483232
Protein kinase C alpha type	PRKCA	2,27297	0,0471579
60S ribosomal protein L35a	RPL35A	2,95199	0,016
Tumor necrosis factor receptor superfamily member 1B	TNFRSF1B	3,53611	0,0122
Succinate dehydrogenase [ubiquinone] iron-sulfur subunit, mitochondrial	SDHB	3,68361	0,0128421
Oxysterol-binding protein 1	OSBP	2,47088	0,0470769
Immunoglobulin alpha Fc receptor	FCAR	1,86006	0,0476129
Transcriptional repressor protein YY1	YY1	2,45033	0,032069
Phosphatidylinositol 3-kinase regulatory subunit alpha	PIK3R1	2,72729	0,0475056
SHC-transforming protein 1	SHC1	2,88778	0,0485977

Supplementary Table 2. Significantly modulated proteins of LPS-treated FMF monocytes shown in Figure 2B.			
Protein names	**Gene names**	**Student's T-test p-value (-log)**	**Student's T-test Difference**
Enoyl-CoA hydratase, mitochondrial	ECHS1	3,98197	0,0162667
ATP synthase subunit gamma, mitochondrial	ATP5C1	2,45458	0,012
Signal transducer and activator of transcription 3	STAT3	2,69252	0,014
Guanine nucleotide-binding protein G(q) subunit alpha	GNAQ	3,18343	0,0106087
Cyclin-dependent kinase 9	CDK9	3,08943	0,0187692
Signal transducer and activator of transcription 5B	STAT5B	2,53191	0,03
Pterin-4-alpha-carbinolamine dehydratase	PCBD1	2,61119	0,048186
Serine/threonine-protein phosphatase PP1-alpha catalytic subunit	PPP1CA	3,06993	0,0158333
U6 snRNA-associated Sm-like protein LSm6	LSM6	1,88535	0,0469778
40S ribosomal protein S15	RPS15	2,58079	0,02056
SUMO-conjugating enzyme UBC9	Ube2i	3,88061	0
Signal peptidase complex catalytic subunit SEC11A	SEC11A	2,88991	0,0142564
Neutrophil gelatinase-associated lipocalin	LCN2	1,85332	0,0461266
Tyrosine-protein phosphatase non-receptor type 11	PTPN11	2,86577	0,0451084
Quinone oxidoreductase	CRYZ	2,82319	0,0221818
Mitochondrial-processing peptidase subunit alpha	PMPCA	2,40649	0,0318095
TAR DNA-binding protein 43	TARDBP	3,80335	0,0203333
Cytoskeleton-associated protein 5	CKAP5	2,77314	0,0384789
Putative heat shock protein HSP 90-alpha A4	HSP90AA4P	3,18752	0,0124444
BRO1 domain-containing protein BROX	BROX	2,05345	0,0474227
Microtubule-associated protein 1S	MAP1S	2,9133	0,0152727
Type-1 angiotensin II receptor-associated protein	AGTRAP	1,98753	0,0482857
UPF0317 protein C14orf159, mitochondrial	C14orf159	1,91081	0,0467733

Supplementary Table 2. Significantly modulated proteins of LPS-treated FMF monocytes shown in Figure 2B.			
Protein names	**Gene names**	**Student's T-test p-value (-log)**	**Student's T-test Difference**
CLIP-associating protein 1	CLASP1	2,35413	0,01575
Putative phospholipase B-like 2	PLBD2	2,57368	0,0209796
Tudor domain-containing protein 7	TDRD7	1,9284	0,0449744
Splicing factor U2AF 26 kDa subunit	U2AF1L4	2,29238	0,0156444
Nuclear pore complex protein Nup133	NUP133	2,82081	0,0123333
Ubiquitin carboxyl-terminal hydrolase 7	USP7	3,38129	0,0174286
MOB kinase activator 3A	MOB3A	2,52959	0,0326545
TNFAIP3-interacting protein 3	TNIP3	2,22684	0,0372174
Retinoid-binding protein 7	RBP7	2,00695	0,04555
Sorting nexin-18	SNX18	3,35005	0,013561
Calcineurin B homologous protein 1	CHP1	3,56584	0,0112
Gamma-soluble NSF attachment protein	NAPG	2,17734	0,0379444
Death-inducer obliterator 1	DIDO1	2,5816	0,0321875
Protein PBDC1	PBDC1	2,66488	0,031
UBX domain-containing protein 6	UBXN6	2,36709	0,0235472
Rab3 GTPase-activating protein non-catalytic subunit	RAB3GAP2	2,63818	0,0139
Rab GTPase-binding effector protein 2	RABEP2	2,22598	0,0366857
Complement component C1q receptor	CD93	2,6965	0,0144516
39S ribosomal protein L17, mitochondrial	MRPL17	5,41162	0
Structural maintenance of chromosomes protein 4	SMC4	2,22263	0,0465946
CKLF-like MARVEL transmembrane domain-containing protein 6	CMTM6	3,1136	0,0135556
ER membrane protein complex subunit 3	EMC3	2,91134	0,0116
Protein RCC2	RCC2	3,3307	0,0465652

Supplementary Table 2. Significantly modulated proteins of LPS-treated FMF monocytes shown in Figure 2B.			
Protein names	**Gene names**	**Student's T-test p-value (-log)**	**Student's T-test Difference**
COMM domain-containing protein 3	COMMD3	2,83432	0,0197692
Death domain-associated protein 6	DAXX	2,32283	0,0315254
Ragulator complex protein LAMTOR2	LAMTOR2	2,78285	0,01184
WW domain-binding protein 11	WBP11	2,1449	0,0449877
ARF GTPase-activating protein GIT1	GIT1	2,76892	0,0316923
60S ribosomal protein L36	RPL36	3,10071	0,0143529
Mitochondrial carrier homolog 2	MTCH2	3,62523	0

Supplementary Table 3. Significantly modulated proteins of untreated TRAPS monocytes shown in Figure 2C.			
Protein names	**Gene names**	**Student's T-test p-value (-log)**	**Student's T-test Difference**
U4/U6.U5 tri-snRNP-associated protein 2	USP39	4,64476	-3,37603
Beta-hexosaminidase	HEXA	4,36552	-4,00728
Inhibitor of nuclear factor kappa-B kinase subunit beta	IKBKB	3,78714	-2,44653
Glutaredoxin-3	GLRX3	4,24581	-1,35854
Transthyretin	TTR	4,71492	2,5282
Tyrosine-protein kinase Lyn	LYN	3,1506	4,66983
Ubiquitin-conjugating enzyme E2 K	UBE2K	4,66944	-3,01738
Eukaryotic translation initiation factor 3 subunit M	EIF3M	3,98755	3,2244
Parkinson disease 7 domain-containing protein 1	PDDC1	4,19438	-2,06145
Optineurin	OPTN	4,89887	4,25239
Type I inositol 3,4-bisphosphate 4-phosphatase	INPP4A	3,67887	-2,00061
39S ribosomal protein L39, mitochondrial	MRPL39	4,20496	1,66681
Peptidyl-prolyl cis-trans isomerase E	PPIE	3,66788	-2,80125
UPF0568 protein C14orf166	C14orf166	5,11778	-3,80364
Ubiquitin-fold modifier-conjugating enzyme 1	UFC1	3,4539	-3,37135

Supplementary Table 4. Significantly modulated proteins of LPS-treated TRAPS monocytes shown in Figure 2D.			
Protein names	**Gene names**	**Student's T-test p-value (-log)**	**Student's T-test Difference**
Acyl-coenzyme A thioesterase 1	ACOT1	2,91761	1,8944
Killer cell lectin-like receptor subfamily F member 1	KLRF1	2,35408	2,78495
Zinc finger Ran-binding domain-containing protein 2	ZRANB2	2,92689	2,29108
Ubiquitin-like modifier-activating enzyme 6	UBA6	2,34883	-2,60249
Phosphoglycolate phosphatase	PGP	3,87107	-1,19608
GTP-binding protein Rheb	RHEB	3,06775	-1,81305
Protein PRRC2C	PRRC2C	2,8109	0,757051
ATP synthase F1 subunit beta	ATP5B	3,34007	-2,09055
Serine/threonine-protein phosphatase	PPP4C	2,37124	1,35351
Serine/arginine-rich splicing factor 2	SRSF2	5,07821	0,553397
Sulfatase-modifying factor 2	SUMF2	2,50839	0,993273
Lon protease homolog, mitochondrial	LONP1	2,22189	-2,56644
Transcription initiation factor TFIID subunit 4	TAF4	2,52059	0,902915
Importin-5	IPO5	3,58239	-2,49514
Long-chain-fatty-acid--CoA ligase 4	ACSL4	2,33436	-1,4109
Syntaxin-10	STX10	2,39669	-1,44128
DnaJ homolog subfamily C member 13	DNAJC13	2,1122	-2,3428
Acyl-protein thioesterase 2	LYPLA2	2,1156	-1,79507
Apoptosis-inducing factor 1, mitochondrial	AIFM1	2,31319	-1,21197
Transthyretin	TTR	2,17034	2,11562
Superoxide dismutase [Mn], mitochondrial	SOD2	3,47564	-0,795899
60S acidic ribosomal protein P2	RPLP2	2,24316	-1,28098
Uroporphyrinogen decarboxylase	UROD	3,79867	0,718335
Beta-hexosaminidase subunit beta	HEXB	2,85845	-0,943622

Supplementary Table 4. Significantly modulated proteins of LPS-treated TRAPS monocytes shown in Figure 2D.			
Protein names	**Gene names**	**Student's T-test p-value (-log)**	**Student's T-test Difference**
Histone H2A.V	H2AFV	3,2165	-0,997572
Prolyl 4-hydroxylase subunit alpha-1	P4HA1	3,20409	0,991196
Proto-oncogene vav	VAV1	2,52521	-2,42122
Ganglioside GM2 activator	GM2A	2,90487	-3,32058
60S ribosomal protein L35a	RPL35A	2,02909	-2,83904
Oxysterol-binding protein 1	OSBP	2,92113	1,26053
DNA replication licensing factor MCM3	MCM3	2,76898	-1,55605
14-3-3 protein theta	YWHAQ	4,92329	1,3736
Proteasome subunit beta type	PSM8	3,48298	-0,942297
Signal transducer and activator of transcription 3	STAT3	2,54145	-1,81738
G protein-coupled receptor kinase 6	GRK6	3,42739	1,3553
Adapter molecule crk	CRK	2,61655	2,1844
Dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit STT3A	STT3A	2,26965	-2,68238
Signal recognition particle 9 kDa protein	SRP9	2,51648	1,1249
Rap1 GTPase-GDP dissociation stimulator 1	RAP1GDS1	3,31793	0,695963
Arginine--tRNA ligase, cytoplasmic	RARS	4,44209	-1,27305
Adenosine kinase	ADK	2,21462	1,47635
6.8 kDa mitochondrial proteolipid	MP68	2,5083	2,93227
Alpha-centractin	ACTR1A	2,40034	-1,12017
Lysozyme C	LYZ	2,97255	-0,677104
Ras-related protein Rap-1A	RAP1A	3,41596	1,57156
40S ribosomal protein S15	RPS15	3,55048	-4,64983
Disintegrin and metalloproteinase domain-containing protein 17	ADAM17	2,39435	1,27999
Tumor necrosis factor alpha-induced protein 2	TNFAIP2	2,08198	-2,86997

Supplementary Table 4. Significantly modulated proteins of LPS-treated TRAPS monocytes shown in Figure 2D.			
Protein names	**Gene names**	**Student's T-test p-value (-log)**	**Student's T-test Difference**
Serine/arginine-rich splicing factor 11	SRSF11	3,07982	0,768003
Serine/arginine-rich splicing factor 4	SRSF4	3,80635	0,766953
Syntaxin-5	STX5	3,04753	0,905947
26S proteasome non-ATPase regulatory subunit 2	PSMD2	4,24552	0,847585
Serine/arginine-rich splicing factor 6	SRSF6	4,02532	0,769634
Voltage-gated potassium channel subunit beta-2	KCNAB2	3,46974	-1,43573
Cold-inducible RNA-binding protein	CIRBP	2,74965	1,03243
Splicing factor 3A subunit 1	SF3A1	3,87414	0,572037
NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 5	NDUFA5	2,78074	-1,08153
Putative oxidoreductase GLYR1	GLYR1	3,1385	0,765809
Torsin-1A-interacting protein 1	TOR1AIP1	3,22079	0,621812
Ubiquitin-associated protein 2	UBAP2	3,14002	0,946587
Aftiphilin	AFTPH	2,29981	1,27546
Protein unc-13 homolog D	UNC13D	2,71693	-0,935266
COMM domain-containing protein 1	COMMD1	2,60889	-1,30781
Nucleoporin Nup43	NUP43	3,12088	-1,54425
TBC1 domain family member 15	TBC1D15	2,67981	1,16709
Serine/threonine-protein phosphatase 4 regulatory subunit 1	PPP4R1	3,14244	0,889703
TBC1 domain family member 5	TBC1D5	2,07304	-2,53172
La-related protein 4B	LARP4B	2,1023	-1,80506
Probable ubiquitin carboxyl-terminal hydrolase FAF-X	USP9X	4,08315	0,908494
Protein YIPF5	YIPF5	3,19344	0,935504
Mediator of RNA polymerase II transcription subunit 8	MED8	3,09076	1,09395
MMS19 nucleotide excision repair protein homolog	MMS19	2,19116	1,76638

Supplementary Table 4. Significantly modulated proteins of LPS-treated TRAPS monocytes shown in Figure 2D.			
Protein names	**Gene names**	**Student's T-test p-value (-log)**	**Student's T-test Difference**
Protein NipSnap homolog 1	NIPSNAP1	2,62543	1,20919
Serine/threonine-protein phosphatase CPPED1	CPPED1	2,96519	1,12858
Rho GTPase-activating protein 9	ARHGAP9	4,16595	0,576696
RNMT-activating mini protein	FAM103A1	3,08062	0,949156
N-terminal Xaa-Pro-Lys N-methyltransferase 1	NTMT1	2,88891	0,713439
Splicing factor 3B subunit 5	SF3B5	3,47688	0,699482
Chitinase domain-containing protein 1	CHID1	2,57685	1,00737
Protein phosphatase 1 regulatory subunit 12C	PPP1R12C	2,7828	0,802731
GTP-binding protein SAR1a	SAR1A	2,35959	1,43886
Mitochondrial import receptor subunit TOM22 homolog	TOMM22	2,38449	1,0312
Myoferlin	MYOF	2,45846	-2,84924
Apoptosis-associated speck-like protein containing a CARD	PYCARD	2,52971	-1,07121
Zinc finger protein 330	ZNF330	2,52607	0,902349
Hypoxia up-regulated protein 1	HYOU1	3,22441	-1,1079
Transforming acidic coiled-coil-containing protein 3	TACC3	3,13558	1,40283
Cytoplasmic dynein 1 light intermediate chain 1	DYNC1LI1	2,89763	-0,883852

Supplementary Table 5. Significantly modulated proteins of untreated MKD monocytes shown in Figure 2E.			
Protein names	**Gene names**	**Student's T-test p-value (-log)**	**Student's T-test Difference**
A-kinase anchor protein 9	AKAP9	5,09991	-5,45275
COMM domain-containing protein 4	COMMD4	3,28256	1,655
von Willebrand factor A domain-containing protein 8	VWA8	3,1464	4,5966
Heat shock protein family A member 8	HSPA8	3,11882	-2,77979
HEAT repeat-containing protein 5A	HEATR5A	5,3754	-4,19927
Transmembrane and coiled-coil domain-containing protein 1	TMCO1	2,71126	2,52264
Ras-related protein Rab-4B	RAB4B	3,39498	3,42834
Fructose-1,6-bisphosphatase isozyme 2	FBP2	3,98947	-2,4602
Transthyretin	TTR	2,97015	-4,66502
Anion exchange protein 2	SLC4A2	3,86877	-1,61614
Spermidine synthase	SRM	2,71081	-3,37023
High mobility group nucleosome-binding domain-containing protein 5	HMGN5	3,20722	-2,36694
Aminoacylase-1	ACY1	4,24728	-1,58658
T-lymphoma invasion and metastasis-inducing protein 1	TIAM1	4,06037	-2,40614
Myotubularin-related protein 3	MTMR3	2,69437	-2,73138
Ras-related protein Rab-31	RAB31	4,81537	1,53276
Spectrin alpha chain, non-erythrocytic 1	SPTAN1	2,76857	-2,80785
Protein RRP5 homolog	PDCD11	4,82902	2,97891
Rootletin	CROCC	5,55965	-5,07579
Ninein	NIN	7,24404	7,13879
Tetratricopeptide repeat protein 9C	TTC9C	3,83862	-3,18524
Mitochondrial import inner membrane translocase subunit TIM14	DNAJC19	3,04649	-3,09963
Proteasome subunit beta type-7	PSMB7	3,33627	3,04137

Supplementary Table 5. Significantly modulated proteins of untreated MKD monocytes shown in Figure 2E.			
Protein names	**Gene names**	**Student's T-test p-value (-log)**	**Student's T-test Difference**
CD180 antigen	CD180	3,74148	-3,34947
M-phase phosphoprotein 8	MPHOSPH8	2,88289	2,43691
Ubiquitin-like protein 5	UBL5	4,15996	3,42452
Mitochondrial fission factor	MFF	2,83858	2,37847
Kinesin-like protein KIF13A	KIF13A	2,95261	-3,54405
Olfactory receptor 10A2	OR10A2	2,84901	-2,22888
Conserved oligomeric Golgi complex subunit 4	COG4	2,87489	3,17967
Phosphopantothenate--cysteine ligase	PPCS	3,81922	-4,05984
Histidine triad nucleotide-binding protein 3	HINT3	3,14403	-2,48642
3-oxoacyl-[acyl-carrier-protein] synthase, mitochondrial	OXSM	2,95555	-1,79409
Protocadherin beta-15	PCDHB15	6,76271	5,59694
General transcription factor 3C polypeptide 5	GTF3C5	2,95287	-1,74294

Supplementary Table 6. Significantly modulated proteins of LPS-treated MKD monocytes shown in Figure 2F.			
Protein names	**Gene names**	**Student's T-test p-value (-log)**	**Student's T-test Difference**
40S ribosomal protein S9	RPS9	3,6641	-1,56307
Proteasome subunit alpha type	PSMA2	1,98229	-0,758658
Zinc finger protein 787	ZNF787	2,89637	1,0644
40S ribosomal protein S24	RPS24	2,43325	-1,99903
Clathrin heavy chain	CLTC	3,06215	-0,871793
GMP reductase	GMPR2	2,85874	-0,977707
NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 10, mitochondrial	NDUFA10	2,2165	-1,33103
ATPase ASNA1	ASNA1	1,95426	-0,795187
Selenoprotein H	C11orf31	3,29106	0,445317
Proteasome activator complex subunit 2	PSME2	1,73527	0,931124
Calcium/calmodulin-dependent protein kinase type II subunit gamma	CAMK2G	2,31833	-1,06501
Isovaleryl-CoA dehydrogenase, mitochondrial	IVD	1,72859	-1,19169
Integrin-linked protein kinase	ILK	2,62889	-0,740769
Sulfotransferase	SULT1A4	2,26674	-1,4465
UPF0693 protein C10orf32	C10orf32-ASMT	1,98054	1,27572
Serine/arginine-rich splicing factor 7	SRSF7	2,86164	0,583411
Dolichyl-diphosphooligosaccharide--protein glycosyltransferase 48 kDa subunit	DDOST	2,2896	-1,07467
DNA-directed RNA polymerase	POLR2A	3,52349	-1,16738
Thromboxane-A synthase	TBXAS1	3,55787	-2,35376
Fatty acid synthase	FASN	1,9973	-1,56089
Shootin-1	KIAA1598	2,02041	0,883891
Aconitate hydratase, mitochondrial	ACO2	2,64385	-0,568282
Small ubiquitin-related modifier 3	SUMO3	1,61329	1,95791
Pituitary tumor-transforming gene 1 protein-interacting protein	PTTG1IP	2,17257	1,53228

Supplementary Table 6. Significantly modulated proteins of LPS-treated MKD monocytes shown in Figure 2F.			
Protein names	**Gene names**	**Student's T-test p-value (-log)**	**Student's T-test Difference**
Unconventional myosin-Ig	MYO1G	2,56182	-0,850527
Polyadenylate-binding protein	PABPC4	3,38119	-1,486
Branched-chain-amino-acid aminotransferase	BCAT2	1,91725	-1,76094
Septin-11	11-set	1,72841	-1,04883
Annexin	ANXA6	2,49616	-0,943773
Protein PRRC2C	PRRC2C	2,91316	0,74127
Casein kinase II subunit alpha	CSNK2A1	2,93641	-0,547729
Complement receptor type 1	CR1	2,32872	-2,04296
Transducin beta-like protein 2	TBL2	2,31121	-0,550451
Transformation/transcription domain-associated protein	TRRAP	2,01142	1,9173
Protein transport protein Sec23A	SEC23A	2,95576	-1,05487
2,5-phosphodiesterase 12	PDE12	2,046	-0,666538
CAD protein	CAD	2,90084	-1,85107
Phosphatidylinositol 4,5-bisphosphate 3-kinase catalytic subunit delta isoform	PIK3CD	2,62517	-1,09182
Eukaryotic translation initiation factor 2A	EIF2A	2,45773	0,625928
Translocation protein SEC62	SEC62	2,18751	1,92992
Aminoacyl-tRNA synthetases	WARS	2,76214	3,00623
Sp110 nuclear body protein	SP110	1,51842	2,91582
AMP deaminase 2	AMPD2	2,13904	0,600659
Methyltransferase-like protein 7A	METTL7A	2,63543	-2,1536
RNA-binding protein with serine-rich domain 1	RNPS1	2,85446	0,561779
Beta-hexosaminidase	HEXA	1,95113	-2,08827
Proteasome-associated protein ECM29 homolog	KIAA0368	1,70311	-1,46049
REST corepressor 1	RCOR1	2,03264	0,763764

Supplementary Table 6. Significantly modulated proteins of LPS-treated MKD monocytes shown in Figure 2F.			
Protein names	**Gene names**	**Student's T-test p-value (-log)**	**Student's T-test Difference**
Ubiquitin thioesterase OTUB1	OTUB1	2,1114	-0,781033
Tubulin-specific chaperone D	TBCD	1,91019	-1,46345
Protein SSXT	SS18	1,92048	0,988246
Histone H3	H3F3B	2,12753	-3,47977
Lon protease homolog, mitochondrial	LONP1	1,77697	-1,1821
Transmembrane protein 205	TMEM205	1,7569	-1,23679
Mitochondrial import inner membrane translocase subunit TIM44	TIMM44	2,9368	-1,94859
Unconventional myosin-If	MYO1F	2,44848	-0,495675
Membrane-associated progesterone receptor component 1	PGRMC1	2,05226	0,858472
Dynactin subunit 6	DCTN6	3,56454	1,02879
High mobility group nucleosome-binding domain-containing protein 4	HMGN4	1,5482	1,99868
Lysosomal alpha-mannosidase	MAN2B1	3,21644	-1,05135
Fructose-1,6-bisphosphatase isozyme 2	FBP2	2,12745	-1,42789
Apoptotic protease-activating factor 1	APAF1	3,31953	-1,94591
Exportin-1	XPO1	1,95858	-1,28982
FYN-binding protein	FYB	1,93861	0,74564
Actin-related protein 2/3 complex subunit 3	ARPC3	1,78974	-0,900927
Serine palmitoyltransferase 1	SPTLC1	1,81264	-0,802845
Syntaxin-7	STX7	2,52298	1,08607
U4/U6 small nuclear ribonucleoprotein Prp3	PRPF3	2,22094	1,32621
Sjoegren syndrome nuclear autoantigen 1	SSNA1	1,78858	0,937857
A-kinase anchor protein 8	AKAP8	1,7539	1,7313
Isocitrate dehydrogenase [NAD] subunit beta, mitochondrial	IDH3B	3,15707	-1,1633
Intron-binding protein aquarius	AQR	1,87165	-2,78409

Supplementary Table 6. Significantly modulated proteins of LPS-treated MKD monocytes shown in Figure 2F.			
Protein names	**Gene names**	**Student's T-test p-value (-log)**	**Student's T-test Difference**
Procollagen-lysine,2-oxoglutarate 5-dioxygenase 3	PLOD3	1,50536	-2,09434
Toll-like receptor 2	TLR2	2,55182	-1,35848
H/ACA ribonucleoprotein complex subunit 4	DKC1	2,01105	-1,06335
WD repeat-containing protein 1	WDR1	1,97443	-0,770498
Copine-3	CPNE3	1,55489	-1,74597
DnaJ homolog subfamily C member 13	DNAJC13	2,36391	-1,6024
NADH dehydrogenase [ubiquinone] iron-sulfur protein 2, mitochondrial	NDUFS2	2,06543	-0,99331
Core histone macro-H2A.1	H2AFY	3,75721	-0,528851
NADH dehydrogenase [ubiquinone] iron-sulfur protein 3, mitochondrial	NDUFS3	3,06566	-1,62435
Interferon-inducible double-stranded RNA-dependent protein kinase activator A	PRKRA	1,67361	-1,5785
Acyl-protein thioesterase 1	LYPLA1	2,42778	-1,43566
U5 small nuclear ribonucleoprotein 200 kDa helicase	SNRNP200	3,70966	-0,9646
Eukaryotic translation initiation factor 3 subunit J	EIF3J	3,2084	0,74896
AP-1 complex subunit gamma-like 2	AP1G2	1,75285	-1,69973
CAAX prenyl protease 1 homolog	ZMPSTE24	1,85534	-1,77427
Flotillin-1	FLOT1	2,09824	0,637147
Glutaredoxin-3	GLRX3	1,75863	-1,28176
TOX high mobility group box family member 4	TOX4	1,76785	1,06289
Mitofusin-2	MFN2	2,60447	-1,35445
Reticulon-3	RTN3	1,89289	1,07761
Vesicle-associated membrane protein-associated protein B/C	VAPB	1,65561	1,39322
NAD kinase	NADK	1,51436	-1,90481
AP-2 complex subunit alpha-1	AP2A1	1,86978	-0,968019
Peroxisomal membrane protein 11B	PEX11B	2,01916	-1,77389

Supplementary Table 6. Significantly modulated proteins of LPS-treated MKD monocytes shown in Figure 2F.			
Protein names	**Gene names**	**Student's T-test p-value (-log)**	**Student's T-test Difference**
L-lactate dehydrogenase A chain	LDHA	2,39312	-0,764338
NADH-cytochrome b5 reductase 3	CYB5R3	2,49853	-1,81116
Cytochrome c oxidase subunit 2	MT-CO2	4,25766	-2,13599
Coagulation factor XIII A chain	F13A1	2,20862	-1,28347
Aspartate aminotransferase, mitochondrial	GOT2	3,79135	-1,20688
Carbonic anhydrase 2	CA2	1,78639	-1,62715
Alpha-1-antichymotrypsin	SERPINA3	2,21278	-0,619604
Dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit 2	RPN2	2,10215	-1,14633
Guanine nucleotide-binding protein G(i) subunit alpha-2	GNAI2	3,97126	-0,748285
Aldehyde dehydrogenase, mitochondrial	ALDH2	2,62104	-0,633041
60S acidic ribosomal protein P0	RPLP0	2,41834	-1,03796
Integrin beta-1	ITGB1	3,85059	-0,559403
Glycogen phosphorylase, liver form	PYGL	3,48884	-0,993727
Nucleophosmin	NPM1	2,82436	0,529038
Cathepsin D	CTSD	2,07031	-0,680798
Calpain-1 catalytic subunit	CAPN1	4,38378	-0,817368
Tubulin beta chain	TUBB	2,36384	-0,63584
Beta-hexosaminidase subunit beta	HEXB	2,28612	-1,46273
Tyrosine-protein kinase Lyn	LYN	1,78399	-1,02416
Tropomyosin beta chain	TPM2	1,64658	3,05169
Fumarate hydratase, mitochondrial	FH	2,46166	-0,662382
Annexin A6	ANXA6	3,76216	-0,862628
Beta-glucuronidase	GUSB	1,79523	-0,847849
Monocyte differentiation antigen CD14	CD14	4,40179	-0,876353

Supplementary Table 6. Significantly modulated proteins of LPS-treated MKD monocytes shown in Figure 2F.			
Protein names	**Gene names**	**Student's T-test p-value (-log)**	**Student's T-test Difference**
Annexin A5	ANXA5	4,71894	-1,03138
Glutathione S-transferase P	GSTP1	3,9671	-1,00345
Chromosome transmission fidelity protein 8 homolog isoform 2	CHTF8	2,36213	0,763079
Non-secretory ribonuclease	RNASE2	3,36359	-2,21522
Cytochrome c oxidase subunit 5B, mitochondrial	COX5B	1,87498	0,748083
60 kDa heat shock protein, mitochondrial	HSPD1	2,59983	0,559345
Histidine--tRNA ligase, cytoplasmic	HARS	1,85024	-1,05112
Inosine-5-monophosphate dehydrogenase 2	IMPDH2	2,17477	-0,604184
Xaa-Pro dipeptidase	PEPD	1,87983	-0,917921
X-ray repair cross-complementing protein 5	XRCC5	2,34754	-0,580843
Glycogen [starch] synthase, muscle	GYS1	1,88678	-0,991416
Hematopoietic lineage cell-specific protein	HCLS1	2,11451	0,722314
Alcohol dehydrogenase [NADP(+)]	AKR1A1	2,12813	-0,77733
Pyruvate kinase PKM	PKM	2,99865	-0,588267
Proto-oncogene vav	VAV1	2,49495	-1,09344
Carbonyl reductase [NADPH] 1	CBR1	2,88512	-0,574039
Beta-galactosidase	GLB1	2,20238	-0,907407
NADPH--cytochrome P450 reductase	POR	5,65244	-2,06288
1-phosphatidylinositol 4,5-bisphosphate phosphodiesterase gamma-2	PLCG2	2,09533	-0,627927
Fumarylacetoacetase	FAH	3,27055	-1,37436
Calpain-2 catalytic subunit	CAPN2	2,65472	-0,924127
ATP-dependent 6-phosphofructokinase, liver type	PFKL	2,37931	-0,72436
T-complex protein 1 subunit alpha	TCP1	3,04807	-0,777268
Nucleolin	NCL	3,95183	0,622529

Supplementary Table 6. Significantly modulated proteins of LPS-treated MKD monocytes shown in Figure 2F.			
Protein names	**Gene names**	**Student's T-test p-value (-log)**	**Student's T-test Difference**
Hexokinase-1	HK1	2,94551	-1,09912
Casein kinase II subunit alpha	CSNK2A2	2,14554	-0,821901
Nuclear factor NF-kappa-B p105 subunit	NFKB1	1,90645	-1,10802
Transcription factor BTF3	BTF3	2,318	0,827761
Lamin-B1	LMNB1	2,43947	0,495247
Integrin alpha-L	ITGAL	2,48374	-0,843106
Voltage-dependent anion-selective channel protein 1	VDAC1	2,86398	-1,27154
Nucleoside diphosphate kinase B	NME2	2,3839	-0,497314
Cytochrome b-c1 complex subunit 2, mitochondrial	UQCRC2	3,59401	-0,881662
Splicing factor, proline- and glutamine-rich	SFPQ	2,58002	0,475691
NAD-dependent malic enzyme, mitochondrial	ME2	2,23534	-0,596182
Carnitine O-palmitoyltransferase 2, mitochondrial	CPT2	1,79399	-1,19811
ATP synthase F(0) complex subunit B1, mitochondrial	ATP5F1	2,16714	-1,39646
ATP synthase subunit alpha, mitochondrial	ATP5A1	2,64284	-0,513721
Threonine--tRNA ligase, cytoplasmic	TARS	1,75598	-1,26384
Proteasome subunit beta type-4	PSMB4	3,1436	-0,890987
Mitogen-activated protein kinase 1	MAPK1	2,61753	-0,784326
Protein PML	PML	1,8994	0,901271
Elongation factor 1-delta	EEF1D	2,36834	1,27918
Endoplasmic reticulum resident protein 29	ERP29	2,92356	0,541735
High affinity immunoglobulin epsilon receptor subunit gamma	FCER1G	2,38926	0,746802
HLA class I histocompatibility antigen, B-53 alpha chain	HLA-B	2,7472	0,790903
Leukocyte elastase inhibitor	SERPINB1	3,1684	-0,859692
Coronin-1A	CORO1A	2,26719	0,63936

Supplementary Table 6. Significantly modulated proteins of LPS-treated MKD monocytes shown in Figure 2F.			
Protein names	**Gene names**	**Student's T-test p-value (-log)**	**Student's T-test Difference**
cAMP-dependent protein kinase type II-beta regulatory subunit	PRKAR2B	1,83878	0,900121
Cytochrome b-c1 complex subunit 1, mitochondrial	UQCRC1	3,39441	-0,660538
3-hydroxyisobutyrate dehydrogenase, mitochondrial	HIBADH	2,68811	-1,24953
14-3-3 protein beta/alpha	YWHAB	1,67599	1,40275
Cleavage stimulation factor subunit 2	CSTF2	1,74765	0,979084
Oxygen-dependent coproporphyrinogen-III oxidase, mitochondrial	CPOX	2,05769	-2,51356
DNA-directed RNA polymerase II subunit RPB9	POLR2I	2,39122	-0,987918
V-type proton ATPase catalytic subunit A	ATP6V1A	2,47061	-0,527253
T-complex protein 1 subunit zeta	CCT6A	2,77191	-0,578092
Malate dehydrogenase, mitochondrial	MDH2	3,06233	-0,384426
Trifunctional enzyme subunit alpha, mitochondrial	HADHA	3,86287	-0,949674
Tyrosine-protein kinase CSK	CSK	4,6583	-0,96348
Isoleucine--tRNA ligase, cytoplasmic	IARS	2,15091	-1,79023
Enoyl-CoA delta isomerase 1, mitochondrial	ECI1	2,26472	-1,16629
Leucine-rich PPR motif-containing protein, mitochondrial	LRPPRC	2,16936	-0,89247
3-ketoacyl-CoA thiolase, mitochondrial	ACAA2	3,14964	-0,626313
Glycerol-3-phosphate dehydrogenase, mitochondrial	GPD2	2,4452	-1,19893
Tyrosine-protein kinase SYK	SYK	3,76973	-0,901762
Chromobox protein homolog 5	CBX5	2,10899	1,08514
Probable 28S rRNA (cytosine(4447)-C(5))-methyltransferase	NOP2	1,6149	1,19726
Dual specificity mitogen-activated protein kinase kinase 3	MAP2K3	2,15019	-0,813216
Glucosamine-6-phosphate isomerase 1	GNPDA1	2,94327	-2,06763
F-actin-capping protein subunit alpha-2	CAPZA2	2,63881	-0,444223
Eukaryotic translation initiation factor 1A, X-chromosomal	EIF1AX	1,67984	1,34877

Supplementary Table 6. Significantly modulated proteins of LPS-treated MKD monocytes shown in Figure 2F.			
Protein names	**Gene names**	**Student's T-test p-value (-log)**	**Student's T-test Difference**
Glutamine--tRNA ligase	QARS	2,08611	-0,715545
Protein PRRC2A	PRRC2A	2,70659	0,880562
Signal recognition particle 9 kDa protein	SRP9	1,84039	0,937887
Alanine--tRNA ligase, cytoplasmic	AARS	2,04659	-0,991529
Serine--tRNA ligase, cytoplasmic	SARS	3,79332	-0,612337
Very long-chain specific acyl-CoA dehydrogenase, mitochondrial	ACADVL	2,39053	-0,604834
Selenide, water dikinase 1	SEPHS1	2,74704	-0,910801
Carnitine O-palmitoyltransferase 1, liver isoform	CPT1A	2,06977	-0,688274
Vasodilator-stimulated phosphoprotein	VASP	3,56988	0,618461
Dynamin-2	DNM2	2,5002	-0,707044
T-complex protein 1 subunit delta	CCT4	3,40388	-0,896176
Isocitrate dehydrogenase [NAD] subunit gamma, mitochondrial	IDH3G	1,7228	-1,38255
Host cell factor 1	HCFC1	2,33379	1,18256
Fatty aldehyde dehydrogenase	ALDH3A2	2,11063	-1,79095
Peroxisomal multifunctional enzyme type 2	HSD17B4	3,64602	-0,989813
Vesicle-associated membrane protein 7	VAMP7	1,57467	-1,24284
Ribosomal protein S6 kinase alpha-3	RPS6KA3	3,99285	-1,39619
Heterogeneous nuclear ribonucleoprotein M	HNRNPM	3,1392	0,613544
Heterogeneous nuclear ribonucleoprotein F	HNRNPF	2,54012	-0,572794
Hexokinase-3	HK3	2,82374	-0,598927
Tricarboxylate transport protein, mitochondrial	SLC25A1	2,76965	-1,40092
ATP-citrate synthase	ACLY	2,75442	-0,910218
Coatomer subunit beta	COPB1	1,98502	-0,845007
Coatomer subunit alpha	COPA	2,7146	-0,661044

Supplementary Table 6. Significantly modulated proteins of LPS-treated MKD monocytes shown in Figure 2F.			
Protein names	**Gene names**	**Student's T-test p-value (-log)**	**Student's T-test Difference**
Methylosome subunit pICln	CLNS1A	2,28733	1,07814
Tyrosine--tRNA ligase, cytoplasmic	YARS	2,22958	-0,564491
Adenosine kinase	ADK	2,61725	-2,0628
Puromycin-sensitive aminopeptidase	NPEPPS	2,30021	-0,695954
IgG receptor FcRn large subunit p51	FCGRT	1,48641	-1,68566
Methionine--tRNA ligase, cytoplasmic	MARS	1,7956	-1,01287
AP-1 complex subunit sigma-2	AP1S2	3,79375	-0,432019
Protein transport protein Sec61 subunit beta	SEC61B	2,96839	1,28473
Ribose-phosphate pyrophosphokinase 1	PRPS1	1,57901	-1,25981
Ubiquitin-conjugating enzyme E2 K	UBE2K	1,99856	-0,768679
Actin-related protein 3	ACTR3	2,80776	-0,519711
Alpha-centractin	ACTR1A	2,68743	-0,886678
COP9 signalosome complex subunit 2	COPS2	2,74075	-1,22798
Ras-related protein Rap-2b	RAP2B	1,65553	-1,45289
Protein max	MAX	3,66876	1,10512
60S ribosomal protein L27	RPL27	2,11708	-1,35084
40S ribosomal protein S7	RPS7	1,73713	-1,04498
Serine/threonine-protein phosphatase PP1-alpha catalytic subunit	PPP1CA	2,44289	-0,545941
40S ribosomal protein S13	RPS13	1,94349	-1,10148
Histone H4	HIST1H4A	2,47475	-0,484609
60S ribosomal protein L23	RPL23	3,64318	-1,41488
Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-2	GNB2	2,74817	-0,609241
60S ribosomal protein L10a	RPL10A	2,34054	-0,890566
60S ribosomal protein L8	RPL8	2,59349	-0,765245

Supplementary Table 6. Significantly modulated proteins of LPS-treated MKD monocytes shown in Figure 2F.			
Protein names	**Gene names**	**Student's T-test p-value (-log)**	**Student's T-test Difference**
Peptidyl-prolyl cis-trans isomerase A	PPIA	2,46366	0,548413
Guanine nucleotide-binding protein G(s) subunit alpha isoforms short	GNAS	2,68883	-1,20628
Serine/threonine-protein phosphatase 2A 55 kDa regulatory subunit B alpha isoform	PPP2R2A	2,70699	-0,640564
40S ribosomal protein S21	RPS21	2,96434	0,700949
Guanine nucleotide-binding protein subunit beta-2-like 1	GNB2L1	2,4237	-1,0795
SUMO-conjugating enzyme UBC9	Ube2i	1,81859	-1,01179
Serine/threonine-protein phosphatase 2A catalytic subunit alpha isoform	PPP2CA	2,60837	-1,08207
Nuclease-sensitive element-binding protein 1	YBX1	3,09281	0,670355
Signal peptidase complex catalytic subunit SEC11A	SEC11A	2,20306	-1,17365
Tubulin beta-4B chain	TUBB4B	4,74287	-0,732298
Immunoglobulin-binding protein 1	IGBP1	1,80974	1,22574
Glutathione S-transferase omega-1	GSTO1	1,97652	-0,715778
DNA-dependent protein kinase catalytic subunit	PRKDC	3,60594	-0,776316
28S ribosomal protein S36, mitochondrial	MRPS36	3,33779	0,712056
Serine/arginine-rich splicing factor 3	SRSF3	1,921	0,936574
Histone H3.2	HIST2H3A	1,77461	-4,14398
Phosphate carrier protein, mitochondrial	SLC25A3	3,70505	-1,12527
Transcriptional activator protein Pur-alpha	PURA	2,49857	-0,721097
Methylmalonate-semialdehyde dehydrogenase [acylating], mitochondrial	ALDH6A1	1,81749	-0,926159
DNA topoisomerase 2-beta	TOP2B	2,03779	-1,06745
Single-stranded DNA-binding protein, mitochondrial	SSBP1	1,91343	-0,834792
Tyrosine-protein phosphatase non-receptor type 11	PTPN11	2,18192	-0,681671
Cytoskeleton-associated protein 4	CKAP4	2,28852	0,70689
Mitochondrial-processing peptidase subunit alpha	PMPCA	1,98611	-1,26331

Supplementary Table 6. Significantly modulated proteins of LPS-treated MKD monocytes shown in Figure 2F.			
Protein names	**Gene names**	**Student's T-test p-value (-log)**	**Student's T-test Difference**
Vesicular integral-membrane protein VIP36	LMAN2	2,84059	-1,80768
Heat shock protein 75 kDa, mitochondrial	TRAP1	2,20098	-1,40645
Protein flightless-1 homolog	FLII	2,88011	-0,990279
Lymphocyte cytosolic protein 2	LCP2	2,46652	0,61867
Ubiquitin carboxyl-terminal hydrolase 4	USP4	2,06133	-1,2616
Serine/arginine-rich splicing factor 9	SRSF9	2,43055	0,998055
Serine/arginine-rich splicing factor 6	SRSF6	2,05764	0,961327
Voltage-gated potassium channel subunit beta-2	KCNAB2	1,86417	-1,03915
Cytoplasmic dynein 1 intermediate chain 2	DYNC1I2	2,2055	0,565186
V-type proton ATPase 116 kDa subunit a isoform 3	TCIRG1	2,73568	-1,00609
Diacylglycerol kinase zeta	DGKZ	2,18066	-1,16102
Stromal interaction molecule 1	STIM1	2,51708	0,529726
Bleomycin hydrolase	BLMH	2,4213	-2,6743
Ubiquitin-associated protein 2-like	UBAP2L	2,51005	0,794757
Cytoplasmic dynein 1 heavy chain 1	DYNC1H1	2,08893	-0,718312
Guanine nucleotide-binding protein subunit alpha-13	GNA13	3,499	-1,02924
RNA-binding protein 39	RBM39	2,35072	-0,576593
Protein disulfide-isomerase A5	PDIA5	2,29842	-1,65076
Pre-mRNA-splicing regulator WTAP	WTAP	3,47656	1,00766
Septin-2	02-set	2,73964	-0,493138
116 kDa U5 small nuclear ribonucleoprotein component	EFTUD2	2,0319	-0,905758
Neutrophil cytosol factor 4	NCF4	2,8733	-0,884125
Transcription elongation factor B polypeptide 2	TCEB2	2,40538	0,768398
Ubiquitin-protein ligase E3C	UBE3C	1,86271	-0,88677

Supplementary Table 6. Significantly modulated proteins of LPS-treated MKD monocytes shown in Figure 2F.			
Protein names	**Gene names**	**Student's T-test p-value (-log)**	**Student's T-test Difference**
Scaffold attachment factor B1	SAFB	2,39901	0,49421
Splicing factor 1	SF1	2,1738	0,615689
ELAV-like protein 1	ELAVL1	1,98018	-0,963947
NEDD8	NEDD8	2,17462	0,993753
39S ribosomal protein L23, mitochondrial	MRPL23	2,08323	-1,19942
Hsp90 co-chaperone Cdc37	CDC37	2,66815	0,4987
Kynureninase	KYNU	2,52648	-1,09881
NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 9, mitochondrial	NDUFA9	3,07419	-1,64304
UTP--glucose-1-phosphate uridylyltransferase	UGP2	3,22477	-1,51934
Macrophage-expressed gene 1 protein	MPEG1	1,79612	-0,976171
Protein LSM12 homolog	LSM12	1,8273	2,12645
Putative oxidoreductase GLYR1	GLYR1	2,53032	-0,515744
Presequence protease, mitochondrial	PITRM1	2,03165	-1,9872
RRP12-like protein	RRP12	1,46907	-1,74184
Engulfment and cell motility protein 2	ELMO2	2,17375	-0,731397
FAD synthase	FLAD1	1,5441	-1,37372
Microtubule-associated protein 1S	MAP1S	1,50047	-2,20191
Atlastin-3	ATL3	2,73621	-1,72114
Twinfilin-2	TWF2	3,72044	-0,856113
Pre-mRNA-processing-splicing factor 8	PRPF8	2,36247	-0,940774
La-related protein 1	LARP1	2,51979	1,01923
Biogenesis of lysosome-related organelles complex 1 subunit 2	BLOC1S2	1,70029	1,06084
Nesprin-3	SYNE3	2,01681	-0,676152
Neurobeachin-like protein 2	NBEAL2	1,9098	-1,54626

Supplementary Table 6. Significantly modulated proteins of LPS-treated MKD monocytes shown in Figure 2F.			
Protein names	**Gene names**	**Student's T-test p-value (-log)**	**Student's T-test Difference**
Vacuolar protein sorting-associated protein 13C	VPS13C	2,82177	-1,00394
Protein unc-13 homolog D	UNC13D	2,62309	-0,666353
Staphylococcal nuclease domain-containing protein 1	SND1	3,56017	-0,578772
Eukaryotic translation initiation factor 3 subunit M	EIF3M	1,65273	1,27447
Zinc finger CCCH-type antiviral protein 1	ZC3HAV1	2,8254	0,726645
Elongation factor Tu GTP-binding domain-containing protein 1	EFTUD1	2,20326	-1,65264
Myosin-14	MYH14	1,99775	-1,29583
KDEL motif-containing protein 2	KDELC2	1,85801	-0,96589
Protein LYRIC	MTDH	2,63261	0,528735
Cullin-associated NEDD8-dissociated protein 1	CAND1	1,98656	-1,02126
Ankyrin repeat and KH domain-containing protein 1	ANKHD1	3,26598	1,26275
Chromatin complexes subunit BAP18	BAP18	3,35271	0,550715
EH domain-binding protein 1-like protein 1	EHBP1L1	3,50919	0,47355
Cohesin subunit SA-2	STAG2	2,061	-1,67936
ADP-ribosylation factor GTPase-activating protein 1	ARFGAP1	2,31082	0,69476
Serine/threonine-protein phosphatase 6 regulatory ankyrin repeat subunit B	ANKRD44	1,72405	-1,3506
Parkinson disease 7 domain-containing protein 1	PDDC1	1,74804	-1,05617
NHL repeat-containing protein 2	NHLRC2	1,87036	-0,990679
Saccharopine dehydrogenase-like oxidoreductase	SCCPDH	3,26675	-1,76188
Dedicator of cytokinesis protein 8	DOCK8	2,96461	-0,979904
Nucleoporin NUP53	NUP35	2,30424	0,672071
E3 ubiquitin-protein ligase ZNRF2	ZNRF2	2,23478	1,35541
Splicing factor U2AF 26 kDa subunit	U2AF1L4	1,9353	1,16037
Nuclear pore complex protein Nup133	NUP133	1,67874	-1,50911

Supplementary Table 6. Significantly modulated proteins of LPS-treated MKD monocytes shown in Figure 2F.			
Protein names	**Gene names**	**Student's T-test p-value (-log)**	**Student's T-test Difference**
Sec1 family domain-containing protein 1	SCFD1	2,1278	-1,41071
PEST proteolytic signal-containing nuclear protein	PCNP	2,10385	0,909601
GTPase IMAP family member 1	GIMAP1	1,6353	-1,29585
UBX domain-containing protein 4	UBXN4	2,28551	0,822291
Dedicator of cytokinesis protein 2	DOCK2	2,89095	-0,907926
Translational activator GCN1	GCN1L1	5,08812	-0,941544
Pre-mRNA-splicing factor ATP-dependent RNA helicase PRP16	DHX38	2,34844	-1,71038
Ubiquitin carboxyl-terminal hydrolase 7	USP7	2,19789	-2,06639
Transcription elongation factor A protein-like 3	TCEAL3	1,95649	1,21938
Synapse-associated protein 1	SYAP1	1,93329	0,731824
Vacuolar protein sorting-associated protein 33A	VPS33A	2,24503	-2,17975
U8 snoRNA-decapping enzyme	NUDT16	3,72204	-2,43102
Copine-2	CPNE2	2,28787	-1,11777
RNA-binding protein 14	RBM14	2,82584	0,479664
Vacuolar protein sorting-associated protein 35	VPS35	1,98205	-0,870391
GPI transamidase component PIG-S	PIGS	2,46451	-1,59008
Chloride channel CLIC-like protein 1	CLCC1	1,8732	0,925029
3-hydroxyacyl-CoA dehydrogenase type-2	HSD17B10	3,18232	-1,11657
Histone H2B type 1-M	HIST1H2BM	1,6272	3,96336
Tyrosine-protein phosphatase non-receptor type 18	PTPN18	2,54493	1,35423
Protein NipSnap homolog 1	NIPSNAP1	2,32923	-1,65041
Serine/threonine-protein phosphatase CPPED1	CPPED1	2,09883	-0,678389
Partner of Y14 and mago	WIBG	2,88337	0,534585
ADP-dependent glucokinase	ADPGK	3,18701	-1,66906

Supplementary Table 6. Significantly modulated proteins of LPS-treated MKD monocytes shown in Figure 2F.			
Protein names	**Gene names**	**Student's T-test p-value (-log)**	**Student's T-test Difference**
COP9 signalosome complex subunit 4	COPS4	2,42094	-0,875684
Mini-chromosome maintenance complex-binding protein	MCMBP	1,96243	0,679536
Transmembrane protein 43	TMEM43	1,66103	-1,50206
Charged multivesicular body protein 4a	CHMP4A	2,67597	0,6476
RanBP-type and C3HC4-type zinc finger-containing protein 1	RBCK1	1,6776	1,56482
E3 ubiquitin-protein ligase TRIM4	TRIM4	1,64549	-1,45091
Phospholysine phosphohistidine inorganic pyrophosphate phosphatase	LHPP	1,72819	-1,87908
Thioredoxin-related transmembrane protein 4	TMX4	2,10657	-1,46578
Vacuolar protein sorting-associated protein 16 homolog	VPS16	2,68981	-1,46442
Tubulin beta-1 chain	TUBB1	1,84806	-2,94456
39S ribosomal protein L44, mitochondrial	MRPL44	2,38241	-0,816819
Nicotinamide/nicotinic acid mononucleotide adenylyltransferase 1	NMNAT1	2,1957	-1,5928
Calcyclin-binding protein	CACYBP	1,84313	1,10445
Manganese-transporting ATPase 13A1	ATP13A1	2,7988	-0,725937
Charged multivesicular body protein 1a	CHMP1A	2,1657	3,30591
Toll-like receptor 8	TLR8	1,93982	-1,10091
F-box only protein 6	FBXO6	1,68245	2,35402
Epimerase family protein SDR39U1	SDR39U1	1,86874	-1,4544
Protein FAM114A2	FAM114A2	2,28072	-0,888541
Phenylalanine--tRNA ligase beta subunit	FARSB	2,17824	-0,909596
Ethylmalonyl-CoA decarboxylase	ECHDC1	5,48376	-1,26085
Mycophenolic acid acyl-glucuronide esterase, mitochondrial	ABHD10	1,72156	-1,58812
ADP-ribosylation factor-like protein 8B	ARL8B	1,74521	-0,919146
ATP-dependent RNA helicase DDX18	DDX18	1,75179	-1,34623

Supplementary Table 6. Significantly modulated proteins of LPS-treated MKD monocytes shown in Figure 2F.			
Protein names	**Gene names**	**Student's T-test p-value (-log)**	**Student's T-test Difference**
THUMP domain-containing protein 1	THUMPD1	2,116	0,930059
Nuclear distribution protein nudE homolog 1	NDE1	2,31384	-0,925975
UDP-glucose:glycoprotein glucosyltransferase 1	UGGT1	2,71331	-0,868593
Adenosine deaminase CECR1	CECR1	2,8293	-1,63256
E3 ubiquitin-protein ligase RNF181	RNF181	2,96526	1,61198
Vacuolar protein sorting-associated protein 18 homolog	VPS18	1,74559	0,875457
Leucine--tRNA ligase, cytoplasmic	LARS	3,54143	-2,0452
Vacuolar protein sorting-associated protein 29	VPS29	1,98758	-1,37989
Cathepsin Z	CTSZ	1,90528	0,921716
Protein NipSnap homolog 3A	NIPSNAP3A	2,33258	-2,12625
Signal recognition particle subunit SRP68	SRP68	1,75838	-0,944346
Vacuolar protein sorting-associated protein 51 homolog	VPS51	1,81778	-2,21265
Tyrosine-protein kinase BAZ1B	BAZ1B	1,60595	-1,14092
Hematological and neurological expressed 1 protein	HN1	3,45913	0,744209
Apoptosis-associated speck-like protein containing a CARD	PYCARD	1,53768	-1,35594
26S proteasome non-ATPase regulatory subunit 13	PSMD13	2,83225	-1,62535
Zinc finger CCCH domain-containing protein 4	ZC3H4	3,04223	0,711782
Translation machinery-associated protein 7	TMA7	2,82494	1,09774
AP-3 complex subunit mu-1	AP3M1	2,04928	-0,973914
Thyroid hormone receptor-associated protein 3	THRAP3	2,15996	0,623545
Acyl-coenzyme A thioesterase 9, mitochondrial	ACOT9	2,79518	-0,808127
Deoxyribose-phosphate aldolase	DERA	1,83758	-1,00717
Mitochondrial fission 1 protein	FIS1	2,46892	-1,03182
Deoxynucleoside triphosphate triphosphohydrolase SAMHD1	SAMHD1	2,76034	-0,5844

Supplementary Table 6. Significantly modulated proteins of LPS-treated MKD monocytes shown in Figure 2F.			
Protein names	**Gene names**	**Student's T-test p-value (-log)**	**Student's T-test Difference**
Unconventional myosin-Va	MYO5A	2,22747	-1,72203
Leucine-rich repeat flightless-interacting protein 2	LRRFIP2	2,55254	1,61824
Mitochondrial carrier homolog 2	MTCH2	2,68093	-1,74277
Sulfide:quinone oxidoreductase, mitochondrial	SQRDL	5,68902	-0,769156
Apoptotic chromatin condensation inducer in the nucleus	ACIN1	2,72152	0,584889

Supplementary Table 7. Significantly modulated proteins of untreated and LPS-treated FMF monocytes shown in Figure 4A and Supplementary Figure 1A, respectively.

Genes related to FMF	UNT	LPS	Interesting genes	UNT	LPS	Others	UNT	LPS
MEFV			IFIT1			MRPL17		
PYCARD			IFIT2			RPL9		
IL1RN			IFIT3			RPL35A		
IL1B			ISG15			RPL36		
CASP1			MB21D1			RPS15		
IL18			PRDX6			PPIE		
TNF			GNAQ			YY1		
PSTPIP1			SHC1			DAXX		
RHOA			PTPN11			HAT1		
IKBKB			MIF			HIST2H3A		
MAPK3			CDK9			HIST1H2BK		
STAT3			FN1			RB1		
STAT5B			ELANE			SHDB		
PIK3R1			PPP1CA			GUSB		
PKN1			SHARPIN			LYZ		
PRKCA			RHOT1			Ca2		
TLR2			TMEM173			TUBB2A		
S100A12			TAX1BP3			SPN		
C5AR1			CUL3			CCNY		
DNM1L						ERAP1		
TNFRSF1B						TTC37		
TTR						GRHPR		
TPT1						ELL		
TRIM21								
B2M								
SLC25A1								

Supplemenaty Table 8. Significantly modulated proteins of untreated and LPS-treated TRAPS monocytes shown in Figure 4B and Supplementary Figure 1B, respectively.

Genes related to TRAPS	**UNT**	**LPS**	**Interesting genes**	**UNT**	**LPS**	**Others**	**UNT**	**LPS**
MEFV			TTR			SF3A1		
PYCARD			CRK			SF3B5		
TNFRSF1B			YWHAQ			SRSF4		
ADAM17			OPTN			SRSF11		
STAT3			VAV1			SRSF6		
TPT1			RAP1A			NUP43		
LYZ			USP9X			RPS15		
IKBKB			ACTR1A			LYPLA2		
			SRP9			RPL35A		
			EIF3M			RPLP2		
			LYN			UFC1		
						C14orf166		

Supplementary Table 9. Significantly modulated proteins of untreated and LPS-treated MKD monocytes shown in Figure 4C and Supplementary Figure 1C, respectively.

Genes related to MVD	UNT	LPS	Interesting genes	UNT	LPS	Others	UNT	LPS
IL1RN			LYN			RPL8		
IL1B			SYK			CLNS1A		
PSTPIP1			GNA13			MRPL44		
MAPK1			GNA12			HIST2H3A		
MAP2K3			PTPN18			PIGS		
CD14			PTPN11			SEPHS1		
NFKB1			VAV1			QARS		
RHOA			PSMB4			TARS		
LRPAP1			ACTR1A			YARS		
TMEM173			ACTR3A			HSD17B10		
TLR2			VASP			HSPD1		
TLR8			SEC61B			LMNB1		
RAB14			XRCC5			FLOT1		
RAB7			SAMHD1			PGRMC1		
RAB1A			NEDD8			AP1G2		
ATG7			USP4			DCTN6		
Mefv			RBCK1			MFN2		
Pycard			TCEB2			MTCH2		
TNFRSF1B			ZNRF2			HNRNPF		
Casp1			UBE3C			CTSD		
IL18			TRIM4			SRSF3		
TNF			LMAN2			CPNE2		

			RAP2B			MTDH		
			NCF4			CA2		
			SND1			MYO1F		
			FBXO6			MYO1G		
			CPT1A			MYO5A		
			CPT2			LRPPRC		
			ECI1			TCP1		
			CSK			TOP2B		
			DNM2			NCL		
			VPS16			NME2		
			VPS33A			ELAVL1		
			VPS29			UQCRC2		
			VPS18			NDUFA9		
			STX7			NDUFS2		
			ATP6V1A			CBX5		
			CTSZ			SLC25A3		
			XPO1			HMGA1		
			ARL8B			LSM12		
			CAPZA2			MMAB		
			VAMP7			MSN		
			AP3M1			IDH3G		
			COPA			ACADVL		
			COPB1			ECHDC1		
			WDR1			IMPDH2		
			CAPN1			MT-CO2		
			SEC23B			ALDH2		

			AP2A1			ALDH3A2		
			TUBB1			ALDH6A1		
			TUBB4B					
			DYNC1H1					
			RPN2					
			SLC25A1					
			RPS6KA3					
			YWHAB					
			PRKAR2B					
			ACLY					
			HADHA					
			VDAC1					
			LBR					
			FDPS					
			HSD17B4					
			ACAA2					
			FAH					
			ITGAL					

A non-interventional study on the long-term safety of anakinra in patients with systemic juvenile idiopathic arthritis from the Pharmachild registry

Systemic juvenile idiopathic arthritis (SJIA) is characterized by high spiking intermittent fever, chronic arthritis/arthralgias, maculopapular rash, hepatosplenomegaly, lymphadenopathy, serositis, and marked increase in acute-phase reactants.(1-4) SJIA may be associated with complications, either due to chronic arthritis, and/or treatment with glucocorticoids, including joint damage, growth impairment, osteoporosis, or to systemic inflammation, such as the potentially fatal macrophage activation syndrome (MAS) and the rare AA amyloidosis.(5-8) Laboratory and clinical observations suggest an inappropriate activation of the innate immunity, with hypersecretion of proinflammatory cytokines, such as interleukin-1 (IL-1) and 6 (IL-6).(3) Anakinra is a recombinant human IL-1 receptor antagonist that blocks the biological activity of both IL-1α and IL-1β by competitively inhibiting its binding to the IL-1 receptor type 1, thereby controlling active inflammation.(9) Anakinra has been studied for several disease states including the EU approved indications Rheumatoid Arthritis, Still's disease, SJIA and different forms of Cryopyrin-Associated Periodic Syndrome as well as Familial Mediterranean Fever, and its safety profile is now well established.(9, 10)

Beside consolidated data on the efficacy of anakinra in treating glucocorticoid-dependent patients with SJIA(11, 12) and recent data on anakinra as first-line monotherapy in new-onset SJIA patients,(13, 14) little evidence has been collected in the last years on long-term safety in large cohorts of SJIA patients treated with anakinra.(13-18) Events of MAS have been described in patients treated with anakinra for SJIA, however a causal relationship between anakinra and MAS has not been established.(9, 17) On the contrary, anakinra has been reported to be an effective treatment for MAS.(19-21) In 2014, a set of classification criteria for MAS complicating SJIA was developed through a combination of expert consensus and analysis of patient data,(22, 23) then validated by consensus using clinical data.(8) However their performance in patients who develop MAS while on treatment with biologics, including anakinra, is still unclear. Phase III clinical trials of canakinumab (24) and tocilizumab in SJIA (25-27) clearly demonstrated that these treatments do not provide protection against MAS and suggest that IL-1 and IL-6 might not be the only cytokines with a central role in the pathogenesis of this syndrome. As well, scarce literature exists on the role of anakinra in influencing MAS occurrence in SJIA patients, especially in the long-term.(22)

The present study was designed with the objective to evaluate and characterize the long-term safety profile of anakinra when used in standard clinical practice to treat patients with SJIA (NCT03932344), including the occurrence of MAS as the most severe complication of the disease.

Study design

The study was a non-interventional, post-authorization safety study (PASS) and the protocol was developed in accordance with the EMA guidance for the format and content of the protocol of a non-interventional PASS.(28) The study was conducted by the Paediatric Rheumatology International Trials Organisation (PRINTO) using the ENCePP certified pharmacovigilance registry named Pharmachild. In brief, one of the aims of the Pharmachild registry, set up in December 2011, is to evaluate the long-term safety of synthetic conventional and biologic disease modifying anti-rheumatic drugs (sDMARDs and bDMARDs). More details on the Pharmachild registry and data collection and validation are reported elsewhere.(29, 30)

For the present study, data from patients with SJIA, as per the ILAR classification criteria,(31, 32) enrolled in the Pharmachild registry before 30 September 2018 and treated with anakinra at any point in time after SJIA diagnosis were included in the analysis.

All centres obtained ethics committee approval according to national requirements and parents/patients provided consent/assent as appropriate.

Study endpoints

The study endpoints were the occurrence of non-serious adverse events (AEs) of at least moderate severity and serious AEs (SAEs), including MAS as an event of special interest (ESI), and the duration of the anakinra treatment with reasons for discontinuation.

Data collection in Pharmachild

Both retrospective safety and drug exposure data since disease onset collected prior to the enrollment in the registry, and subsequent prospective data were used in this study. All AEs of moderate/severe/very severe intensity and serious AEs are reported in Pharmachild; mild intensity is reported only for those AEs which do not resolve and require a follow-up report. In the present study, mild intensity was also reported for AEs of such intensity determining a reason for discontinuation. AEs are reported in Pharmachild irrespective of a possible suspected causal relationship to anakinra or other therapies and according to the latest release of the Medical Dictionary for Regulatory Activities (MedDRA, version 21.1), which classifies them on a five-level structure depending on the specificity required (from the most specific Lowest Level Term (LLT)

and Preferred Term (PT) to the least, System Organ Class (SOC)). According to this terminology, the PT Haemophagocytic lymphohistiocytosis (HLH) was used in this study as synonymous interchangeably of MAS.

Statistical methods

Data were analyzed overall, including all patients with SJIA and treated at any point in time with anakinra in the registry, and by three defined populations, the long-term treatment set -12, -18, -24, including patients with 12-18-24 months or more of continuous anakinra treatment. Continuous treatment was defined as ongoing treatment when no more than 30 consecutive days of unexposed duration occurred in between treatment periods.

Categorical data were reported in terms of absolute frequencies and percentages. Continuous data were described in terms of mean, standard deviation (SD), median, minimum and maximum, 1st and 3rd quartiles. The IR per 100 patient-years was calculated by taking the number of the specific incident event and dividing by the sum of patient years under risk, i.e., exposed to anakinra, multiplied by 100. A patient could contribute with multiple events of the same AE and to different treatment sets. The IR was derived by a Poisson regression model (with only intercept) and the 95% Confidence Interval (CI) was estimated using the Poisson estimator with a cluster-robust estimate of variance to control for both overdispersion and intra-cluster correlation. AE specific IRs were calculated overall for the complete study period and by 6-month calendar time windows (1-6 months, 7-12 months, 13-18 months, 19-24 months and >24 months). Each period of anakinra treatment exposure was calculated as the period between the start date of anakinra until (and including) the stop date of anakinra plus two days, which accounted for approximately five half-lives of the drug. Where applicable, the stop date was substituted with the end of the time window, date of discontinuation of the anakinra treatment exposure, last visit, or death. AEs occurring outside anakinra treatment exposure, i.e before, and when anakinra treatment was paused or stopped were not counted.

In addition, the IR of MAS was analyzed with respect to 1st occurrence and recurrence, respectively. The rationale for this was to account for a biological distinction in altered risk following a first event. Analysis and presentation were based on available data, i.e., no imputation of missing data was performed. Statistical analyses were performed using SAS software Version 9.3 or later (SAS Institute Inc, Cary, North Carolina, United States).

Analysis and reporting were done independently by PRINTO with final report shared with the marketing authorization holder (MAH) and regulatory authorities.

Population

Out of 944 patients with SJIA included in the Pharmachild registry, 306 (32.4%) had been treated with anakinra (Figure 1) (first documented anakinra treatment in 2004) and were included in the analysis. Among them, 141 (46.1%), 104 (34.0%) and 86 (28.1%) patients were continuously treated with anakinra for at least 12, 18, and 24 months, respectively. (Figure 1 and Table 1) Prospective data were collected for 174 (56.9%) patients. Of the 32 countries participating in the Pharmachild JIA registry, 38 centres from 15 countries (37.5%) reported data on anakinra treatment for SJIA: 97.7% of the patients were from Europe, 2.3% from Asia. Caucasian ethnicity was most prevalent (70.6%). (Table 1) Anakinra had been given as monotherapy or in combination with other glucocorticoids/DMARDs, as per the local standard of care. (Supplementary table S1)

Both genders were equally represented, with a median age at first anakinra treatment of 8.0 years, and a median time between disease diagnosis and anakinra start of 0.3 years. (Table 1)

Out of 306 patients, 94 (30.7%) received anakinra as first-line treatment and among the remaining 212 patients, 78 (36.8%) were treated with various combinations of DMARDs and glucocorticoids before starting anakinra and continued with those treatments concomitantly with the IL-1 inhibitor. 134 (63.2%) patients stopped other treatments before starting anakinra. At start of anakinra treatment, 193 (63.1%) patients received at least 1 concomitant SJIA related medication other than NSAIDs, mostly glucocorticoids (161 patients, 52.6%). (Supplementary table S1)

Anakinra treatment

The mean (SD) duration of anakinra treatment for the total study population was 17.0 (21.1) months and the median (IQR) was of 8.9 (3.1-23.5) months. The shortest treatment course was 0.2 months and the longest was 109.9 months (9.1 years). In 92/306 (30.1%) of the patients anakinra treatment was ongoing at the last report in the registry.

Adverse events

A total of 201 AEs was identified with an overall IR of 39.5/100 py (95% CI 30.8-50.6). (Table 2) The overall incidence of AEs decreased over time with the highest IRs during the first 6 months of anakinra treatment. (Table 2 and supplementary table S2) The group of AEs that was the most frequently reported was “Infections and Infestations” (52 AEs, IR=10.2 per 100 py), followed by “Skin and subcutaneous tissue disorders” (25 AEs, IR=4.9 per100 py), “General disorders and administration site conditions” (23 AEs, IR=4.5 per 100 py), and “Gastrointestinal disorders” (18 AEs, IR=3.5 per 100 py).

Among “Infections and Infestations”, respiratory tract infections accounted for 53.8% (28/52). In the group of infections, 3 cases of varicella and 1 case of herpes zoster were also identified. Among “Skin and subcutaneous tissue disorders”, rash (8 occurrences) and urticarial eczema (3 occurrences) were the most frequently reported events. Events related to the injection site were the most common PTs both among “General disorders and administration site conditions” (16 occurrences) and “Injury, poisoning, and procedural complications” (10 occurrences). Among “Gastrointestinal disorders”, constipation (6 occurrences) and abdominal pain (4 occurrences) were the most frequently reported.

By single PTs, MAS, in SOC “Immune system disorders”, was the most frequent event with 12 occurrences and an IR of 2.4 per 100 py, followed by injection-related reactions (2.0/100py), injection site reactions (1.6/100py), rash (1.6/100py), and constipation (1.2/100py). The IRs for the remaining AEs were below 1 per 100 py. This included known adverse reactions of anakinra, such as neutropenia and hepatitis, which accounted for few events among the total (6 AEs of increased liver enzymes and 4 AEs of neutropenia). The majority of the ISRs occurred early after start of anakinra. Three infusion related reactions were connected to tocilizumab, which was administered in close connection to anakinra treatment.

Serious adverse events

56 of the 201 AEs were serious. (Table 3 and supplementary table S3) Overall, the IR of the SAEs was of 11.0 per 100 py (95% CI 7.9-15.2), with events within SOC “Infections and Infestations” being the most reported (a total of 13 SAEs, IR=2.6 per 100 py), followed by “Immune system disorders” (a total of 11 SAEs, IR=2.2 per 100 py, all describing MAS). “Injury, poisoning and procedural complications” covered 9 events, with an IR of 1.8 per 100 py. The remaining SAEs had an IR below 1.0 per 100 py.

Similarly, as for all AEs, the subset of SAEs also occurred primarily during the first 6 months of treatment (IR=28.1 per 100 py during the 1-6 month-time window).

By single PTs, MAS was the most frequently reported serious adverse event (n=11, IR=2.2 per 100 py), primarily reported in the first 6 months of anakinra (IR=6.0/100 py), followed by injection-related reactions (n=6, IR=1.2 per 100 py). All remaining events had an IR below 1 event per 100 py. No malignancies or SAEs leading to death occurred during anakinra exposure. Outside of anakinra exposure 3 patients died, 0.5, 3 and 5 years respectively after anakinra discontinuation, while receiving glucocorticoids and in one case combined with sDMARDs. Death reasons were sepsis, multi-organ failure and sudden death, respectively.

Analysis of AE and SAE by long-term treatment set

The analysis of the total group of AEs with respect to the long-term treatment sets -12, -18 and 24, showed an overall IR of 20.9, 14.3, 13.5 per 100 py for AEs, of which the IR of SAEs were5.1, 3.8, 2.9 per 100 py. In the 86 patients treated continuously for more than 24 months, the IR for AEs per 100 py in the first 6 months of treatment was 21.1 and after 24 months the IR was 13.1 per 100 py. Among AEs, the SOC "Infections and infestations" was the most frequent in the different treatment sets. (Please refer to complete report (33))

In the SAE subset, the SOCs "Infections and infestations" and "Injury, poisoning and procedural complications" were equally represented with an IR of 1.2 per 100 py in the long-term treatment set-12, while the SOC "Immune system disorders", where MAS is included, and "Injury, poisoning and procedural complications" were equally represented with an incidence of 0.8 per 100 py in the long-term treatment set-18. Finally, for the long-term treatment set-24, MAS was the most frequent SAE with only 3 events for an IR of 0.9/100 py.(33)

Incidence proportions of AEs

Among the 306 patients included in the study, 99 patients experienced at least one AE, for an incidence proportion (IP) of 32.4% across an average treatment duration of 1.66 years. Overall, the total number of 201 AEs (Table 2) yielded an average of 0.66 AEs per patient. In the patients who had at least one AE, the average was of 2 AEs per patient.

Considering the SOC categories, 11.8% of patients experienced events in SOC "Infections and infestations", 7.5% of patients experienced events in SOC "Skin and subcutaneous tissue disorders", 6.9% in SOC "General disorders and administration site conditions", 5.2% in the SOCs "Gastrointestinal disorders" and "Injury, poisoning and procedural complications", and 3.9% in SOC "Immune system disorders".

Overall, a higher frequency of patients experiencing AEs was observed during the first 6 months (IP=23.2%) and after 24 months (IP=19.2%) compared to other time windows (9.8% for 7-12 months, 7.6% for 13-18 months, 4.7% for 19-24 months). However, the number of py was much higher in the >24 month-time window compared to the other time windows.

Among the 306 patients of the study, 44 patients experienced at least one SAE, for an IP of 14.4%. The total number of SAEs was 56, (Table 3) meaning an overall average of 0.18 SAEs per patient. Among patients who had at least one SAE the average was 1.3 SAEs per patient.

The risk of experiencing SAEs was higher during the first 6 months (9.2%), too. The overall incidence showed that the most frequently reported SAEs were serious infections in 13 patients (4.2%), followed by 10 patients with MAS (3.3%) and 9 patients with events in the SOC "Injury, poisoning and procedural complications" (2.9%). (Supplementary tables S4-S5)

MAS as an event of special interest

In total, 11 patients experienced 12 events (11 SAEs and 1 non-serious AE) of MAS during anakinra treatment. (Table 4) In one patient MAS was considered non-serious by the reporting physician; there was an isolated increase in ferritin levels (67390 mg/ml), and anakinra was continued. The IR of the first occurrence was 2.2 per 100 py. Ten patients (3.3%) had a previous history of MAS at baseline, 9 of these did not experience any new MAS episode while on anakinra and 1 patient had 2 additional episodes of MAS during anakinra treatment.

The IR for the first occurrence of MAS while on anakinra was lower in patients without a history of MAS (IR (95% CI) = 2.1 per 100 py (1.1-3.9)) compared to those with MAS before anakinra start (IR (95% CI) = 5.6 per 100 py (0.7-42.9)). (Table 4) The average time from start of anakinra to first MAS occurrence was 9 months. 36.4% of MAS events occurred during the first 30 days of anakinra treatment and 36.3% occurred ≥6 months after the first injection. The shortest time from baseline to a MAS event was 4 days. The frequency of MAS did not increase during continued treatment. (Supplementary Table S6). After stopping anakinra, MAS was reported in 8 patients. The earliest recurrence was in the time window 90-180 days, with no indication of an increase in MAS incidence immediately after stopping anakinra. Triggers for the MAS events were recorded as disease flares (4 events, 33.3%), changes of treatments (3 events, 25.0%), infections (2 events, 16.7%), and unknown (3 events, 25.0%). (Supplementary Table S7)

Reasons for anakinra treatment discontinuation

233 out of 306 patients (76.1%) discontinued anakinra at least once. (Table In total, there were 268 discontinuations with 281 reasons recorded. The most frequent reason for anakinra discontinuation was inefficacy (43.1%), followed by remission (30.6%). AEs caused 10.0% of discontinuations, in 8.2% of the cases due to events of moderate intensity, mild in the remaining cases. Intolerance was given as the reason for discontinuation in 5.0% of the cases. Discontinuations due to AEs and intolerance were more frequently reported during the first 6 months of therapy.

We analyzed the long-term safety profile of anakinra used in standard clinical practice to treat patients with SJIA from the international cohort of the Pharmachild registry.(29) The evidence collected in this study with patients coming from different countries provides a safety real-world overview, which could be generalized to the wider general population of patients with SJIA treated with anakinra.

It has been suggested that already after a few months of anakinra treatment in new onset SJIA patients, it is possible to achieve excellent responses (13, 14, 34-37) and after 1 year of therapy 39-76% of the patients achieve clinical remission on therapy, with a glucocorticoid sparing effect.(38) Patients with persistently active SJIA may need continuous treatment with anakinra for one year or more. This study aimed to investigate the long-term safety of anakinra in these patients. In our population, 46% of patients were on continuous anakinra treatment for at least 1 year, and 28% for more than 2 years.

Our results showed that the safety of anakinra after long-term administration is in line with the well-established profile of the drug.(12) A much lower overall incidence of AEs (39.5/100 py) compared to the canakinumab phase III prospective long-term extension study (796.69/100 py) could be observed.(24) This difference may be at least partially explained by different study design and population, as well as inclusion of also mild AEs in the canakinumab trial. The lower number of AEs during anakinra treatment compared to other biologic treatments has also been previously reported.(39)

Additionally, in this study we showed that the rate of reported AEs, including SAEs, was higher in the first 6 months of treatment and then decreased over time. This trend has also been reported in adult patients with rheumatoid arthritis treated with TNF inhibitors (40, 41), and could possibly be explained by immune system dysregulation when adapting to the new immunomodulatory drug. Furthermore, higher doses of glucocorticoids, often given at start of SJIA treatment, may contribute to more AEs reported early in the patient history. In addition, injection-site reactions typically appear early during treatment with anakinra, usually within 2 weeks of therapy initiation, and disappear within 4–6 weeks during continued anakinra treatment.(9) The most frequently reported AEs were related to infections, skin manifestations and injection site reactions, confirming data from previous publications.(13, 14) Among the SAEs, infections and MAS were most frequently reported. In the last decade, rare lung disorders, i.e. pulmonary arterial hypertension, interstitial lung disease, and alveolar proteinosis, have been reported in children with Still's disease.(42-45) These disease manifestations have often been associated with MAS, sometimes with fatal outcome. It has been discussed whether immunosuppressive therapy including IL-1 inhibitors can contribute to the development of these disorders. However, in our study, only one patient presented with a pulmonary SAE, an unspecified interstitial lung disease that occurred after more than 24 months of treatment.

A specific focus in our study was given to the most severe complication of SJIA, MAS. The frequency of MAS in our cohort was in line with what is expected from the literature.(46, 47) One third of the MAS events occurred during the first 30 days of treatment. A possible explanation for

this could be that anakinra was started when symptoms of MAS were already present, the clinical conditions were rapidly deteriorating due to a severe SJIA flare onset, or the anakinra dose was not sufficient to counter the hyperinflammatory state. Events of MAS have previously been described in patients treated with anakinra for SJIA, although a causal relationship between anakinra and MAS has never been established. It should be noted that anakinra has also been reported as an effective treatment for MAS.(20, 48) In our study no increased risk of MAS during or directly after anakinra treatment was identified, and more importantly there was no evidence that the frequency of MAS events increased during continued treatment with anakinra or during the first 90 days after stopping therapy. A higher frequency of MAS prior to anakinra treatment than what we found has been recently reported in the United Kingdom (38); this difference was probably related to a greater severity of the SJIA in the UK population. The demographic and clinical features of the different populations described in the literature and especially the prior and concomitant treatments, should also be considered, as they might influence the frequency of MAS by a long-term or concomitant effect. This may explain the wide range of variability in reporting MAS in the literature, which suggests the development of overt MAS in 7-17% of all patients with SJIA (49, 50).

The main reason for anakinra discontinuation, in our study, was inefficacy, followed by disease remission. In line with the most recent literature, relatively few patients discontinued anakinra due to AEs or intolerance, and most of these events occurred during the first 6 months of treatment.(14, 16)

The study design carries some general limitations inherent to a real-world registry study, e.g. the lack of a control group. In addition, most of the patients received at least 1 concomitant SJIA related medication, other than NSAIDs at start of anakinra treatment, and half of the patients were treated with concomitant glucocorticoids, which may have affected the incidence of AEs. The Pharmachild registry included both prospective and retrospective data, although it has already been showed by Swart et al.(29) that no significant differences were reported in the prospective and retrospective counterpart of the Pharmachild population.

In conclusion, the results of the present study confirm the long-term safety profile of anakinra in SJIA patients and show that the overall incidence of AEs and SAEs declines over time. The study also highlights that there is no evidence that long-term treatment with anakinra increases the risk for MAS.

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Table 1. Demographics and baseline characteristics.

	Complete set	Long-term treatment set-12	Long-term treatment set-18	Long-term treatment set-24
N (%)	306 (100.0)	141 (46.1)	104 (34.0)	86 (28.1)
Male	152 (49.7)	81 (57.4)	59 (56.7)	49 (57.0)
Age yrs (IQR)[a]	8.0 (4.0, 11.8)	8.5 (4.6, 11.9)	8.5 (4.9, 11.4)	8.5 (4.9, 11.1)
Age groups, n (%)				
Infant (< 2 yrs)	22 (7.2)	7 (5.0)	2 (1.9)	1 (1.2)
Child (2 yrs - <12 yrs)	210 (68.6)	100 (70.9)	81 (77.9)	68 (79.1)
Adolescent (12 yrs - <18 yrs)	69 (22.6)	31 (22.0)	19 (18.3)	16 (18.6)
Adult (≥ 18 yrs)	5 (1.6)	3 (2.1)	2 (1.9)	1 (1.2)
Time since SJIA onset (yrs), median (IQR)	0.6 (0.2, 2.2)	1.1 (0.4, 3.4)	1.5 (0.6, 4.3)	1.5 (0.6, 4.3)
Time since SJIA diagnosis (yrs), median (IQR)	0.3 (0.0, 1.9)	0.8 (0.1, 3.0)	1.1 (0.2, 3.8)	1.3 (0.2, 4.0)
Time from SJIA onset to first visit (yrs)[b], median (IQR)	0.2 (0.0, 0.8)	0.2 (0.1, 0.8)	0.2 (0.1, 0.9)	0.2 (0.1, 0.8)
Patients with History of MAS, n (%)	10 (3.3)	6 (4.2)	5 (4.8)	4 (4.6)
Origin, n (%)				
Europe	299 (97.7)	138 (97.9)	101 (97.1)	83 (96.5)
Asia	7 (2.3)	3 (2.1)	3 (2.9)	3 (3.5)
Ethnicity, n (%)				
Caucasian	216 (70.6)	97 (68.8)	75 (72.1)	60 (69.8)
Other	90 (29.4)	44 (31.2)	29 (27.9)	26 (30.2)

Abbreviations: yrs, years;; n, number of patients;; IQR, inter quartile range.

[a]Age at baseline for the complete set; age at baseline or at index date for the long-term treatment set 12,18 and 24. [b]First visit in the clinical center.

Table 2. Number of AEs and incidence rates in the complete set and for 1-6 and 19-24 time windows.

	Time windows[a]	1-6 months		19-24 months		Overall	
	N	306		106		306	
	Patient-time (y)[b]	117.3		47.0		509.3	
SOC	PT	n[c]	Rate (95% CI)[d]	n[c]	Rate (95% CI)[d]	n[c]	Rate (95% CI)[d]
All	All	116	98.9 (75.8-129.0)	7	14.9 (6.0-37.1)	201	39.5 (30.8-50.6)
Infections and infestations	All	23	19.6 (12.4- 31.0)	-	-	52	10.2 (6.7- 15.6)
	Pneumonia	2	1.7 (0.4- 6.8)	-	-	4	0.8 (0.3- 2.1)
	Respiratory tract infection	2	1.7 (0.4- 6.8)	-	-	4	0.8 (0.3- 2.1)
Skin and subcutaneous tissue disorders	All	18	15.3 (9.5- 24.7)	-	-	25	4.9 (3.2- 7.5)
	Rash	6	5.1 (2.0- 12.8)	-	-	8	1.6 (0.7- 3.4)
General disorders and administration site conditions	All	16	13.6 (8.2- 22.7)	1	2.1 (0.3- 15.1)	23	4.5 (2.9- 7.0)
	Injection site reaction	7	6.0 (2.9- 12.4)	-	-	8	1.6 (0.8- 3.1)
Gastrointestinal disorders	All	13	11.1 (6.0- 20.4)	1	2.1 (0.3- 14.9)	18	3.5 (2.1- 5.9)
	Constipation	5	4.3 (1.8- 10.2)	-	-	6	1.2 (0.5- 2.7)
	Abdominal pain	3	2.6 (0.8- 7.9)	-	-	4	0.8 (0.3- 2.1)
Injury, poisoning and procedural complications	All	10	8.5 (4.6- 15.8)	1	2.1 (0.3- 15.1)	16	3.1 (1.9- 5.2)
	Injection related reaction	6	5.1 (2.3- 11.4)	-	-	10	2.0 (1.1- 3.7)
Immune system disorders	All	7	6.0 (2.8- 12.5)	-	-	13	2.6 (1.4- 4.6)
	HLH	7	6.0 (2.8- 12.5)	-	-	12	2.4 (1.3- 4.3)

Events with a frequency >10 by overall SOC and >3 by overall PT are presented in this table. For details on the complete timeline, including time windows 7-18 and >24 months, please consider the supplementary material.
Abbreviations: AE, adverse event; SOC, system organ class; PT, preferred term, MedDRA version 21.1; N, number of patients ever treated with anakinra during the time window irrespectively of the length of any unexposed periods; 95% CI, 95% Confidence Interval; HLH, haemophagocytic lymphohistiocytosis;
[a] in relation to baseline (start of anakinra treatment)
[b] only time (py) while anakinra treatment was ongoing + 2 days after discontinuation was counted
[c] number of events. Only AEs occurring during anakinra exposed periods + 2 days after discontinuation were counted
[d] incidence rate per 100 patient years; number of events/∑patient time.

Table 1. Number of SAEs and incidence rates in the complete set and the 1-6 and 19-24 time windows.

	Time window[a]	1-6 months		19-24 months		Overall	
	N	306		106		306	
	Patient-time (years)[b]	117.3		47.0		509.3	
SOC	PT	n[c]	Rate (95% CI)[d]	n[c]	Rate (95% CI)[d]	n[c]	Rate (95% CI)[d]
All	All	33	28.1 (19.1-41.5)	2	4.3 (1.1-16.9)	56	11.0 (7.9-15.2)
Infections and infestations	All	7	6.0 (2.9- 12.4)	-	-	13	2.6 (1.4- 4.8)
	Pneumonia	2	1.7 (0.4- 6.8)	-	-	4	0.8 (0.3- 2.1)
Immune system disorders	All	7	6.0 (2.8- 12.5)	-	-	11	2.2 (1.1- 4.1)
	HLH	7	6.0 (2.8- 12.5)	-	-	11	2.2 (1.1- 4.1)
Injury, poisoning and procedural complications		5	4.3 (1.8- 10.2)	1	2.1 (0.3- 15.1)	9	1.8 (0.9- 3.4)
PT: Infusion related reaction		1	0.9 (0.1- 6.0)	1	2.1 (0.3- 15.1)	2	0.4 (0.1- 1.6)
Injection related reaction		4	3.4 (1.3- 9.1)	-	-	6	1.2 (0.5- 2.6)
Metabolism and nutrition disorders	All	3	2.6 (0.8- 7.9)	-	-	4	0.8 (0.3- 2.1)
Skin and subcutaneous tissue disorders	All	3	2.6 (0.8- 7.9)	-	-	4	0.8 (0.3- 2.1)
Blood and lymphatic system disorders	All	1	0.9 (0.1- 6.1)	-	-	2	0.4 (0.1- 1.6)
General disorders and administration site conditions	All	1	0.9 (0.1- 6.1)	-	-	2	0.4 (0.1- 1.6)
Investigations	All	2	1.7 (0.4- 6.8)	-	-	2	0.4 (0.1- 1.6)
Nervous system disorders	All	1	0.9 (0.1- 6.0)	1	2.1 (0.3- 14.9)	2	0.4 (0.1- 1.6)
Surgical and medical procedures	All	1	0.9 (0.1- 6.0)	-	-	2	0.4 (0.1- 1.5)

Events with a frequency >1 by overall SOC and >1 by overall PT are presented in this table. For details on the complete timeline, including time windows 7-18 and >24 months, please consider the supplementary material.

Abbreviations: SAE, serious adverse event; SOC, system organ class; PT, preferred term, MedDRA version 21.1; N, number of patients ever treated with anakinra during the time window irrespectively of the length of any unexposed periods; 95% CI, 95% Confidence Interval; HLH, haemophagocytic lymphohistiocytosis

[a] in relation to baseline (start of anakinra treatment).

[b] only time while anakinra treatment was ongoing + 2 days after discontinuation was counted

[c] number of events. Only SAEs occurring during anakinra exposed periods + 2 days after discontinuation were counted

[d] incidence rate per 100 patient years; number of events/∑patient time.

Table 4. Number of first occurrence and recurrence of MAS events and incidence rates, overall and by history of MAS in the complete set of patients

History of MAS at baseline	Patients (N)		10	
	MAS event	n[a]	Patient-time (years)[b]	Rate (95% CI)[c]
	1st occurrence[d]	1	18.0	5.6 (0.7-42.9)
	2nd occurrence	1	1.0	100
No History of MAS recorded at baseline	Patients (N)		296	
	MAS event	n[a]	Patient-time (years)[b]	Rate (95% CI)[c]
	1st occurrence[d]	10	479.5	2.1 (1.1-3.9)
	2nd occurrence	0	5.2	0
Complete study population	Patients (N)		306	
	MAS event	n[a]	Patient-time (years)[b]	Rate (95% CI)[c]
	1st occurrence[d]	11	497.5	2.2 (1.2-4.1)
	2nd occurrence	1	6.2	16.1 (2.6-97.7)

Abbreviations: MAS, macrophage activation syndrome; N, number of patients starting anakinra treatment; 95% CI, 95% Confidence Interval.

[a] number of MAS events. Only MAS occurring during anakinra treatment (including 2 days after discontinuation)

[b] only time during periods with anakinra treatment (including 2 days after discontinuation)

[c] incidence rate per 100 patient years; number of events/∑patient time;

[d] the 1st occurrence of MAS is defined to occur at or after baseline regardless of whether the patient had a history of MAS or not

Table 5. Reasons for discontinuation of anakinra treatment.

Time window[a]	Overall	1-6 months	7-12 months	13-18 months	19-24 months	>24 months
N-total number of patients	306	306	194	144	106	104
Total number of reasons for discontinuations	281	109	53	34	13	72
Reasons	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)
Adverse events at least of moderate intensity	23 (8.2)	17 (15.6)	2 (3.8)	3 (8.8)	0 (0.0)	1 (1.4)
Intolerance	14 (5.0)	8 (7.3)	3 (5.7)	1 (2.9)	0 (0.0)	2 (2.8)
Dose change	2 (0.7)	1 (0.9)	1 (1.9)	0 (0.0)	0 (0.0)	0 (0.0)
Inefficacy	121 (43.1)	51 (46.8)	20 (37.7)	8 (23.5)	6 (46.2)	36 (50.0)
Remission	86 (30.6)	20 (18.3)	21 (39.6)	19 (55.9)	4 (30.8)	22 (30.6)
Surgery	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Pregnancy	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Other reason	23 (8.2)	7 (6.4)	5 (9.4)	3 (8.8)	2 (15.4)	6 (8.3)
Mild adverse events[b]	5 (1.8)	4 (3.7)	1 (1.9)	0 (0.0)	0 (0.0)	0 (0.0)
Change therapy	11 (3.9)	2 (1.8)	2 (3.8)	2 (5.9)	1 (7.7)	4 (5.6)
No compliance	4 (1.4)	0 (0.0)	2 (3.8)	0 (0.0)	1 (7.7)	1 (1.4)
Other	3 (1.1)	1 (0.9)	0 (0.0)	1 (2.9)	0 (0.0)	1 (1.4)
Unknown	12 (4.3)	5 (4.6)	1 (1.9)	0 (0.0)	1 (7.7)	5 (6.9)
Number of patients who discontinued[c]	233 (76.1)	102 (33.3)	50 (25.8)	33 (22.9)	13 (12.3)	61 (58.7)
Number of discontinuations[d]	268 (87.6)	103 (33.7)	50 (25.8)	33 (22.9)	13 (12.3)	69 (66.3)

A patient can contribute with multiple discontinuations, if starting a new treatment period after a temporary stop of more than 30 days, and multiple reasons for one single discontinuation.

[a]In relation to baseline (start of anakinra treatment).

[b] Mild adverse events are not reported elsewhere because excluded from the Pharmachild registry

[c,d]The denominator is the total number of patients (N)

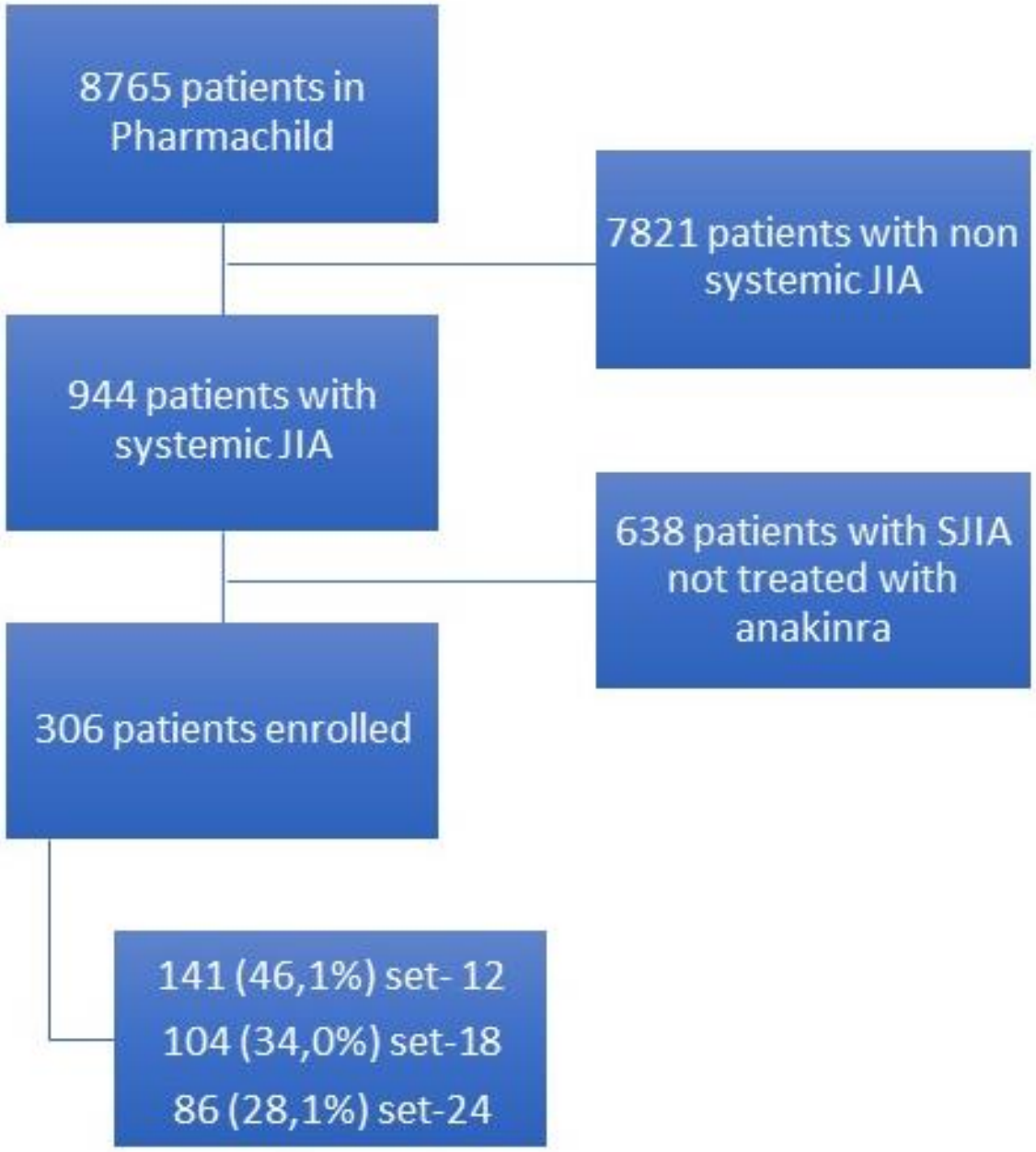

Figure 1. Flow-chart of the study population selected. Patients can belong to different treatment sets. Set-12, -18, -24: patients treated for at least 12, 18 or 24 months with anakinra.

Supplementary table S1. Latest treatment regimen(s) of sDMARDs, bDMARDs and glucocorticoids received at any time from disease onset to the first dose of anakinra and concomitantly to anakinra treatment in the complete set.

N	228	78*	306	306
Treatment regimen(s)	Latest treatment regimen(s) occurring any time before the first dose of anakinra, n(%)	Treatment starting before the first dose of Anakinra but continuing after starting of anakinra, n(%)	Total treatment regimens starting before the first dose of anakinra, n(%)	Concomitant treatment regimen(s) to the first dose of anakinra, n(%)
None	94 (41.2)	-	94 (30.7)	113 (36.9)
Glucocorticoids only	32 (14.0)	42 (53.8)	74 (24.2)	83 (27.1)
bDMARDs[a] only	24 (10.5)	-	24 (7.8)	1 (0.3)
bDMARDs[a]+glucocorticoids	13 (5.7)	-	13 (4.2)	0
MTX+bDMARDs[a]	12 (5.3)	1 (1.3)	13 (4.2)	1 (0.3)
MTX only	11 (4.8)	8 (10.3)	19 (6.2)	25 (8.2)
MTX+glucocorticoids	11 (4.8)	25 (32.0)	36 (11.8)	63 (20.6)
MTX+bDMARDs[a]+glucocorticoids	8 (3.5)	-	8 (2.6)	3 (1.0)
sDMARDs[b]+bDMARDs[a] +MTX	5 (2.2)	-	5 (1.6)	0
sDMARDs[b]+bDMARDs[a]+MTX+glucocorticoids	5 (2.2)	-	5 (1.6)	0
sDMARDs[b]+bDMARDs[a]	3 (1.3)	-	3 (1.0)	1 (0.3)
sDMARDs[b]+glucocorticoids	3 (1.3)	-	3 (1.0)	5 (1.6)
sDMARDs[b]+MTX	2 (0.9)	-	2 (0.7)	0
sDMARDs[b]+bDMARDs[a]+glucocorticoids	2 (0.9)	-	2 (0.7)	1 (0.3)
sDMARDs[b]+MTX+glucocorticoids	2 (0.9)	2 (2.6)	4 (1.3)	6 (2.0)
sDMARDs[b] only	1 (0.4)	-	1(0.3)	4 (1.3)

Abbreviations: MTX: Methotrexate; DMARDs: Disease modifying antirheumatic drugs

[a] biologic other than anakinra

[b] other than MTX Supplementary table S2.

*Among the 306 patients treated with anakinra, 78 patients were treated with concomitant treatment regimens (treatments starting before the first dose of anakinra but continuing after starting of Anakinra)

Supplementary table S2. Complete number of AEs and incidence rates (95% CI) by SOC, PT and time window in the complete set.

	Time window[a]	1-6 months		7-12 months		13-18 months		19-24 months		>24 months		Overall	
	N	306		194		144		106		104		306	
	Patient-time (years)[b]	117.3		80.2		58.1		47.0		206.7		509.3	
SOC	PT	n[c]	Rate (95% CI)[d]	n[c]	Rate (95% CI)[d]	n[c]	Rate (95% CI)[d]	n[c]	Rate (95% CI)[d]	n[c]	Rate (95% CI)[d]	n[c]	Rate (95% CI)[d]
All	All	116	98.9 (75.8-129.0)	26	32.4 (19.6-53.5)	16	27.5 (14.5-52.2)	7	14.9 (6.0-37.1)	36	17.4 (11.1-27.4)	201	39.5 (30.8-50.6)
Blood and lymphatic system disorders	All	3	2.6 (0.8- 7.9)	2	2.5 (0.6- 9.9)	-	-	-	-	4	1.9 (0.6- 6.4)	9	1.8 (0.9- 3.6)
	Febrile neutropenia	-	-	-	-	-	-	-	-	1	0.5 (0.1- 3.4)	1	0.2 (0.0- 1.4)
	Lymphadenitis	-	-	1	1.2 (0.2- 8.8)	-	-	-	-	-	-	1	0.2 (0.0- 1.4)
	Lymphadenopathy	1	0.9 (0.1- 6.1)	-	-	-	-	-	-	1	0.5 (0.1- 3.4)	2	0.4 (0.1- 1.6)
	Neutropenia	2	1.7 (0.4- 6.8)	1	1.2 (0.2- 8.8)	-	-	-	-	1	0.5 (0.1- 3.4)	4	0.8 (0.3- 2.1)
	Pancytopenia	-	-	-	-	-	-	-	-	1	0.5 (0.1- 3.4)	1	0.2 (0.0- 1.4)
Ear and labyrinth disorders	All	1	0.9 (0.1- 6.0)	-	-	-	-	-	-	-	-	1	0.2 (0.0- 1.4)
	Ear pain	1	0.9 (0.1- 6.0)	-	-	-	-	-	-	-	-	1	0.2 (0.0- 1.4)
Endocrine disorders	All	5	4.3 (1.8- 10.2)	-	-	-	-	-	-	-	-	5	1.0 (0.4- 2.4)
	Cushing's syndrome	2	1.7 (0.4- 6.8)	-	-	-	-	-	-	-	-	2	0.4 (0.1- 1.6)
	Cushingoid	2	1.7 (0.4- 6.8)	-	-	-	-	-	-	-	-	2	0.4 (0.1- 1.6)
	Hypothyroidism	1	0.9 (0.1- 6.0)	-	-	-	-	-	-	-	-	1	0.2 (0.0- 1.4)
Eye disorders	All	-	-	1	1.2 (0.2- 8.8)	1	1.7 (0.2- 12.2)	-	-	1	0.5 (0.1- 3.3)	3	0.6 (0.2- 1.8)
	Conjunctivitis allergic	-	-	-	-	1	1.7 (0.2- 12.2)	-	-	-	-	1	0.2 (0.0- 1.4)
	Dry eye	-	-	-	-	-	-	-	-	1	0.5 (0.1- 3.3)	1	0.2 (0.0- 1.4)
	Eyelid ptosis	-	-	1	1.2 (0.2- 8.8)	-	-	-	-	-	-	1	0.2 (0.0- 1.4)
Gastrointestinal disorders	All	13	11.1 (6.0- 20.4)	1	1.2 (0.2- 8.8)	-	-	1	2.1 (0.3- 14.9)	3	1.5 (0.5- 4.3)	18	3.5 (2.1- 5.9)
	Abdominal pain	3	2.6 (0.8- 7.9)	1	1.2 (0.2- 8.8)	-	-	-	-	-	-	4	0.8 (0.3- 2.1)
	Anal pruritus	1	0.9 (0.1- 6.0)	-	-	-	-	-	-	-	-	1	0.2 (0.0- 1.4)
	Constipation	5	4.3 (1.8- 10.2)	-	-	-	-	-	-	1	0.5 (0.1- 3.5)	6	1.2 (0.5- 2.7)
	Gastritis	-	-	-	-	-	-	-	-	1	0.5 (0.1- 3.3)	1	0.2 (0.0- 1.4)
	Nausea	1	0.9 (0.1- 6.0)	-	-	-	-	1	2.1 (0.3- 14.9)	-	-	2	0.4 (0.1- 1.6)
	Odynophagia	-	-	-	-	-	-	-	-	1	0.5 (0.1- 3.3)	1	0.2 (0.0- 1.4)
	Pneumatosis intestinalis	1	0.9 (0.1- 6.1)	-	-	-	-	-	-	-	-	1	0.2 (0.0- 1.4)
	Stomatitis	1	0.9 (0.1- 6.0)	-	-	-	-	-	-	-	-	1	0.2 (0.0- 1.4)
	Vomiting	1	0.9 (0.1- 6.0)	-	-	-	-	-	-	-	-	1	0.2 (0.0- 1.4)
General disorders and administration site conditions	All	16	13.6 (8.2- 22.7)	3	3.7 (1.2- 11.5)	1	1.7 (0.2- 12.2)	1	2.1 (0.3- 15.1)	2	1.0 (0.2- 3.8)	23	4.5 (2.9- 7.0)
	Chest pain	1	0.9 (0.1- 6.0)	-	-	-	-	-	-	-	-	1	0.2 (0.0- 1.4)
	Fatigue	3	2.6 (0.8- 7.9)	-	-	-	-	-	-	-	-	3	0.6 (0.2- 1.8)
	Injection site hypersensitivity	1	0.9 (0.1- 6.1)	-	-	-	-	-	-	-	-	1	0.2 (0.0- 1.4)

	Time window[a]	1-6 months		7-12 months		13-18 months		19-24 months		>24 months		Overall	
	N	306		194		144		106		104		306	
	Patient-time (years)[b]	117.3		80.2		58.1		47.0		206.7		509.3	
SOC	PT	n[c]	Rate (95% CI)[d]	n[c]	Rate (95% CI)[d]	n[c]	Rate (95% CI)[d]	n[c]	Rate (95% CI)[d]	n[c]	Rate (95% CI)[d]	n[c]	Rate (95% CI)[d]
	Injection site inflammation	-	-	-	-	1	1.7 (0.2- 12.2)	-	-	-	-	1	0.2 (0.0- 1.4)
	Injection site pain	-	-	2	2.5 (0.6- 9.9)	-	-	-	-	-	-	2	0.4 (0.1- 1.6)
	Injection site rash	2	1.7 (0.4- 6.8)	-	-	-	-	-	-	-	-	2	0.4 (0.1- 1.6)
	Injection site reaction	7	6.0 (2.9- 12.4)	-	-	-	-	-	-	1	0.5 (0.1- 3.3)	8	1.6 (0.8- 3.1)
	Injection site urticaria	1	0.9 (0.1- 6.0)	-	-	-	-	-	-	-	-	1	0.2 (0.0- 1.4)
	Local reaction	1	0.9 (0.1- 6.0)	-	-	-	-	-	-	-	-	1	0.2 (0.0- 1.4)
	Mucosal erosion	-	-	-	-	-	-	-	-	1	0.5 (0.1- 3.4)	1	0.2 (0.0- 1.4)
	Pain	-	-	-	-	-	-	1	2.1 (0.3- 15.1)	-	-	1	0.2 (0.0- 1.4)
	Pyrexia	-	-	1	1.2 (0.2- 8.8)	-	-	-	-	-	-	1	0.2 (0.0- 1.4)
Hepatobiliary disorders	All	3	2.6 (0.8- 7.9)	-	-	-	-	-	-	1	0.5 (0.1- 3.4)	4	0.8 (0.3- 2.1)
	Hepatotoxicity	-	-	-	-	-	-	-	-	1	0.5 (0.1- 3.4)	1	0.2 (0.0- 1.4)
	Hypertransaminasaemia	3	2.6 (0.8- 7.9)	-	-	-	-	-	-	-	-	3	0.6 (0.2- 1.8)
Immune system disorders	All	7	6.0 (2.8- 12.5)	1	1.2 (0.2- 8.8)	2	3.4 (0.9- 13.6)	-	-	3	1.5 (0.5- 4.4)	13	2.6 (1.4- 4.6)
	Autoimmune disorder	-	-	-	-	1	1.7 (0.2- 12.1)	-	-	-	-	1	0.2 (0.0- 1.4)
	Haemophagocytic lymphohistiocytosis	7	6.0 (2.8- 12.5)	1	1.2 (0.2- 8.8)	1	1.7 (0.2- 12.2)	-	-	3	1.5 (0.5- 4.4)	12	2.4 (1.3- 4.3)
Infections and infestations	All	23	19.6 (12.4- 31.0)	12	15.0 (6.8- 32.9)	5	8.6 (3.6- 20.7)	-	-	12	5.8 (2.4- 14.1)	52	10.2 (6.7- 15.6)
	Conjunctivitis	-	-	1	1.2 (0.2- 8.8)	-	-	-	-	-	-	1	0.2 (0.0- 1.4)
	Cystitis	-	-	-	-	-	-	-	-	1	0.5 (0.1- 3.5)	1	0.2 (0.0- 1.4)
	Ear infection	-	-	1	1.2 (0.2- 8.8)	-	-	-	-	-	-	1	0.2 (0.0- 1.4)
	Epstein-Barr viraemia	1	0.9 (0.1- 6.0)	-	-	-	-	-	-	-	-	1	0.2 (0.0- 1.4)
	Epstein-Barr virus infection	-	-	-	-	-	-	-	-	1	0.5 (0.1- 3.5)	1	0.2 (0.0- 1.4)
	Folliculitis	-	-	1	1.2 (0.2- 8.8)	-	-	-	-	-	-	1	0.2 (0.0- 1.4)
	Fungal skin infection	1	0.9 (0.1- 6.0)	-	-	-	-	-	-	-	-	1	0.2 (0.0- 1.4)
	Gastroenteritis	-	-	1	1.2 (0.2- 8.8)	-	-	-	-	2	1.0 (0.2- 3.9)	3	0.6 (0.2- 1.8)
	Gastroenteritis rotavirus	1	0.9 (0.1- 6.1)	-	-	-	-	-	-	-	-	1	0.2 (0.0- 1.4)
	Gastroenteritis viral	1	0.9 (0.1- 6.0)	-	-	-	-	-	-	-	-	1	0.2 (0.0- 1.4)
	Herpes zoster	-	-	-	-	-	-	-	-	1	0.5 (0.1- 3.4)	1	0.2 (0.0- 1.4)
	Impetigo	1	0.9 (0.1- 6.0)	-	-	-	-	-	-	-	-	1	0.2 (0.0- 1.4)
	Infection	1	0.9 (0.1- 6.0)	-	-	1	1.7 (0.2- 12.2)	-	-	-	-	2	0.4 (0.1- 1.6)
	Influenza	2	1.7 (0.4- 6.8)	-	-	-	-	-	-	-	-	2	0.4 (0.1- 1.6)
	Lower respiratory tract infection	1	0.9 (0.1- 6.0)	-	-	-	-	-	-	-	-	1	0.2 (0.0- 1.4)
	Otitis media	1	0.9 (0.1- 6.0)	-	-	-	-	-	-	-	-	1	0.2 (0.0- 1.4)
	Otitis media acute	1	0.9 (0.1- 6.0)	2	2.5 (0.6- 9.9)	-	-	-	-	-	-	3	0.6 (0.1- 2.6)

	Time window[a]	1-6 months		7-12 months		13-18 months		19-24 months		>24 months		Overall	
	N	306		194		144		106		104		306	
	Patient-time (years)[b]	117.3		80.2		58.1		47.0		206.7		509.3	
SOC	PT	n[c]	Rate (95% CI)[d]	n[c]	Rate (95% CI)[d]	n[c]	Rate (95% CI)[d]	n[c]	Rate (95% CI)[d]	n[c]	Rate (95% CI)[d]	n[c]	Rate (95% CI)[d]
	Parasitic gastroenteritis	-	-	1	1.2 (0.2- 8.8)	-	-	-	-	-	-	1	0.2 (0.0- 1.4)
	Parvovirus B19 infection	1	0.9 (0.1- 6.0)	-	-	-	-	-	-	-	-	1	0.2 (0.0- 1.4)
	Pharyngitis	1	0.9 (0.1- 6.0)	-	-	1	1.7 (0.2- 12.3)	-	-	-	-	2	0.4 (0.1- 1.6)
	Pharyngitis bacterial	-	-	-	-	-	-	-	-	1	0.5 (0.1- 3.5)	1	0.2 (0.0- 1.4)
	Pneumonia	2	1.7 (0.4- 6.8)	1	1.2 (0.2- 8.8)	1	1.7 (0.2- 12.2)	-	-	-	-	4	0.8 (0.3- 2.1)
	Pneumonia viral	1	0.9 (0.1- 6.0)	-	-	-	-	-	-	-	-	1	0.2 (0.0- 1.4)
	Pyelonephritis	1	0.9 (0.1- 6.0)	-	-	-	-	-	-	-	-	1	0.2 (0.0- 1.4)
	Respiratory tract infection	2	1.7 (0.4- 6.8)	-	-	2	3.4 (0.9- 13.7)	-	-	-	-	4	0.8 (0.3- 2.1)
	Rhinitis	-	-	-	-	-	-	-	-	1	0.5 (0.1- 3.3)	1	0.2 (0.0- 1.4)
	Sinusitis	-	-	1	1.2 (0.2- 8.8)	-	-	-	-	-	-	1	0.2 (0.0- 1.4)
	Subcutaneous abscess	1	0.9 (0.1- 6.0)	-	-	-	-	-	-	-	-	1	0.2 (0.0- 1.4)
	Tonsillitis	-	-	-	-	-	-	-	-	1	0.5 (0.1- 3.5)	1	0.2 (0.0- 1.4)
	Tonsillitis streptococcal	-	-	-	-	-	-	-	-	2	1.0 (0.1- 6.8)	2	0.4 (0.1- 2.8)
	Upper respiratory tract infection	2	1.7 (0.4- 6.8)	1	1.2 (0.2- 8.8)	-	-	-	-	-	-	3	0.6 (0.1- 2.6)
	Varicella	-	-	2	2.5 (0.6- 9.9)	-	-	-	-	-	-	2	0.4 (0.1- 1.6)
	Varicella zoster virus infection	-	-	-	-	-	-	-	-	1	0.5 (0.1- 3.3)	1	0.2 (0.0- 1.4)
	Viral infection	1	0.9 (0.1- 6.0)	-	-	-	-	-	-	1	0.5 (0.1- 3.5)	2	0.4 (0.1- 1.6)
Injury, poisoning and procedural complications	All	10	8.5 (4.6- 15.8)	1	1.2 (0.2- 8.8)	2	3.4 (0.9- 13.6)	1	2.1 (0.3- 15.1)	2	1.0 (0.3- 3.7)	16	3.1 (1.9- 5.2)
	Hand fracture	1	0.9 (0.1- 6.0)	-	-	-	-	-	-	-	-	1	0.2 (0.0- 1.4)
	Humerus fracture	-	-	-	-	-	-	-	-	1	0.5 (0.1- 3.3)	1	0.2 (0.0- 1.4)
	Infusion related reaction	2	1.7 (0.4- 6.8)	-	-	-	-	1	2.1 (0.3- 15.1)	-	-	3	0.6 (0.2- 1.8)
	Injection related reaction	6	5.1 (2.3- 11.4)	1	1.2 (0.2- 8.8)	2	3.4 (0.9- 13.6)	-	-	1	0.5 (0.1- 3.3)	10	2.0 (1.1- 3.7)
	Joint injury	1	0.9 (0.1- 6.0)	-	-	-	-	-	-	-	-	1	0.2 (0.0- 1.4)
Investigations	All	6	5.1 (2.3- 11.3)	1	1.2 (0.2- 8.8)	-	-	-	-	1	0.5 (0.1- 3.3)	8	1.6 (0.7- 3.4)
	Biopsy bone marrow	2	1.7 (0.4- 6.8)	-	-	-	-	-	-	-	-	2	0.4 (0.1- 1.6)
	Hepatic enzyme increased	2	1.7 (0.4- 6.8)	1	1.2 (0.2- 8.8)	-	-	-	-	-	-	3	0.6 (0.1- 2.5)
	Transaminases increased	2	1.7 (0.4- 6.8)	-	-	-	-	-	-	1	0.5 (0.1- 3.3)	3	0.6 (0.2- 1.8)
Metabolism and nutrition disorders	All	3	2.6 (0.8- 7.9)	-	-	1	1.7 (0.2- 12.1)	-	-	-	-	4	0.8 (0.3- 2.1)
	Dehydration	1	0.9 (0.1- 6.0)	-	-	-	-	-	-	-	-	1	0.2 (0.0- 1.4)
	Hyperuricaemia	1	0.9 (0.1- 6.0)	-	-	-	-	-	-	-	-	1	0.2 (0.0- 1.4)
	Metabolic acidosis	1	0.9 (0.1- 6.0)	-	-	-	-	-	-	-	-	1	0.2 (0.0- 1.4)
	Overweight	-	-	-	-	1	1.7 (0.2- 12.1)	-	-	-	-	1	0.2 (0.0- 1.4)
Musculoskeletal and connective tissue disorders	All	1	0.9 (0.1- 6.0)	1	1.2 (0.2- 8.8)	1	1.7 (0.2- 12.3)	-	-	1	0.5 (0.1- 3.3)	4	0.8 (0.3- 2.1)

	Time window[a]	1-6 months		7-12 months		13-18 months		19-24 months		>24 months		Overall	
	N	306		194		144		106		104		306	
	Patient-time (years)[b]	117.3		80.2		58.1		47.0		206.7		509.3	
SOC	PT	n[c]	Rate (95% CI)[d]	n[c]	Rate (95% CI)[d]	n[c]	Rate (95% CI)[d]	n[c]	Rate (95% CI)[d]	n[c]	Rate (95% CI)[d]	n[c]	Rate (95% CI)[d]
	Arthritis	-	-	-	-	-	-	-	-	1	0.5 (0.1- 3.3)	1	0.2 (0.0- 1.4)
	Back pain	-	-	1	1.2 (0.2- 8.8)	-	-	-	-	-	-	1	0.2 (0.0- 1.4)
	Rheumatoid arthritis	-	-	-	-	1	1.7 (0.2- 12.3)	-	-	-	-	1	0.2 (0.0- 1.4)
	Still's disease	1	0.9 (0.1- 6.0)	-	-	-	-	-	-	-	-	1	0.2 (0.0- 1.4)
Nervous system disorders	All	2	1.7 (0.4- 6.8)	-	-	-	-	3	6.4 (2.1- 19.5)	-	-	5	1.0 (0.4- 2.7)
	Amnesia	-	-	-	-	-	-	1	2.1 (0.3- 14.9)	-	-	1	0.2 (0.0- 1.4)
	Headache	1	0.9 (0.1- 6.0)	-	-	-	-	1	2.1 (0.3- 15.1)	-	-	2	0.4 (0.1- 2.8)
	Petit mal epilepsy	1	0.9 (0.1- 6.0)	-	-	-	-	1	2.1 (0.3- 14.9)	-	-	2	0.4 (0.1- 1.6)
Psychiatric disorders	All	-	-	-	-	2	3.4 (0.9- 13.6)	-	-	1	0.5 (0.1- 3.3)	3	0.6 (0.2- 1.8)
	Attention deficit/hyperactivity disorder	-	-	-	-	-	-	-	-	1	0.5 (0.1- 3.3)	1	0.2 (0.0- 1.4)
	Persistent depressive disorder	-	-	-	-	1	1.7 (0.2- 12.1)	-	-	-	-	1	0.2 (0.0- 1.4)
	Psychotic disorder	-	-	-	-	1	1.7 (0.2- 12.1)	-	-	-	-	1	0.2 (0.0- 1.4)
Renal and urinary disorders	All	1	0.9 (0.1- 6.1)	-	-	-	-	1	2.1 (0.3- 14.9)	-	-	2	0.4 (0.1- 1.6)
	Urethral meatus stenosis	1	0.9 (0.1- 6.1)	-	-	-	-	-	-	-	-	1	0.2 (0.0- 1.4)
	Urinary incontinence	-	-	-	-	-	-	1	2.1 (0.3- 14.9)	-	-	1	0.2 (0.0- 1.4)
Reproductive system and breast disorders	All	1	0.9 (0.1- 6.0)	-	-	-	-	-	-	-	-	1	0.2 (0.0- 1.4)
	Balanoposthitis	1	0.9 (0.1- 6.0)	-	-	-	-	-	-	-	-	1	0.2 (0.0- 1.4)
Respiratory, thoracic and mediastinal disorders	All	1	0.9 (0.1- 6.0)	-	-	-	-	-	-	1	0.5 (0.1- 3.5)	2	0.4 (0.1- 1.6)
	Asthma	1	0.9 (0.1- 6.0)	-	-	-	-	-	-	-	-	1	0.2 (0.0- 1.4)
	Interstitial lung disease	-	-	-	-	-	-	-	-	1	0.5 (0.1- 3.5)	1	0.2 (0.0- 1.4)
Skin and subcutaneous tissue disorders	All	18	15.3 (9.5- 24.7)	3	3.7 (1.2- 11.5)	1	1.7 (0.2- 12.2)	-	-	3	1.5 (0.5- 4.4)	25	4.9 (3.2- 7.5)
	Dermatitis atopic	1	0.9 (0.1- 6.0)	-	-	-	-	-	-	-	-	1	0.2 (0.0- 1.4)
	Drug eruption	1	0.9 (0.1- 6.0)	-	-	-	-	-	-	-	-	1	0.2 (0.0- 1.4)
	Eczema	1	0.9 (0.1- 6.0)	-	-	-	-	-	-	2	1.0 (0.3- 3.7)	3	0.6 (0.2- 1.8)
	Erythema	2	1.7 (0.4- 6.8)	-	-	-	-	-	-	-	-	2	0.4 (0.1- 1.6)
	Mucocutaneous rash	1	0.9 (0.1- 6.1)	-	-	-	-	-	-	-	-	1	0.2 (0.0- 1.4)
	Onychomadesis	-	-	-	-	1	1.7 (0.2- 12.2)	-	-	-	-	1	0.2 (0.0- 1.4)
	Pityriasis rosea	1	0.9 (0.1- 6.0)	-	-	-	-	-	-	-	-	1	0.2 (0.0- 1.4)
	Pruritus	1	0.9 (0.1- 6.0)	-	-	-	-	-	-	-	-	1	0.2 (0.0- 1.4)
	Pseudocellulitis	1	0.9 (0.1- 6.0)	-	-	-	-	-	-	-	-	1	0.2 (0.0- 1.4)
	Psoriasis	-	-	1	1.2 (0.2- 8.8)	-	-	-	-	-	-	1	0.2 (0.0- 1.4)
	Rash	6	5.1 (2.0- 12.8)	1	1.2 (0.2- 8.8)	-	-	-	-	1	0.5 (0.1- 3.5)	8	1.6 (0.7- 3.4)
	Urticaria	3	2.6 (0.8- 8.0)	-	-	-	-	-	-	-	-	3	0.6 (0.2- 1.8)

	Time window[a]	1-6 months		7-12 months		13-18 months		19-24 months		>24 months		Overall	
	N	306		194		144		106		104		306	
	Patient-time (years)[b]	117.3		80.2		58.1		47.0		206.7		509.3	
SOC	PT	n[c]	Rate (95% CI)[d]	n[c]	Rate (95% CI)[d]	n[c]	Rate (95% CI)[d]	n[c]	Rate (95% CI)[d]	n[c]	Rate (95% CI)[d]	n[c]	Rate (95% CI)[d]
	Vitiligo	-	-	1	1.2 (0.2- 8.8)	-	-	-	-	-	-	1	0.2 (0.0- 1.4)
Surgical and medical procedures	All	1	0.9 (0.1- 6.0)	-	-	-	-	-	-	1	0.5 (0.1- 3.3)	2	0.4 (0.1- 1.5)
	Hip arthroplasty	-	-	-	-	-	-	-	-	1	0.5 (0.1- 3.3)	1	0.2 (0.0- 1.4)
	Lymphadenectomy	1	0.9 (0.1- 6.0)	-	-	-	-	-	-	-	-	1	0.2 (0.0- 1.4)
Vascular disorders	All	1	0.9 (0.1- 6.0)	-	-	-	-	-	-	-	-	1	0.2 (0.0- 1.4)
	Hypertension	1	0.9 (0.1- 6.0)	-	-	-	-	-	-	-	-	1	0.2 (0.0- 1.4)

Abbreviations: AE, adverse event; SOC, system organ class; PT, preferred term, MedDRA version 21.1; N, number of patients ever treated with anakinra during the time window irrespectively of the length of any unexposed periods; 95% CI, 95% Confidence Interval.

[a] in relation to baseline (start of anakinra treatment)

[b] only time while anakinra treatment was ongoing + 2 days after discontinuation was counted

[c] number of events. Only AEs occurring during anakinra exposed periods + 2 days after discontinuation were counted

[d] incidence rate per 100 patient years; number of events/∑patient time

Supplementary table S3. Number of SAEs and incidence rates (95% CI) by SOC, PT and time window in the complete set

	Time window[a]	1-6 months		7-12 months		13-18 months		19-24 months		>24 months		Overall	
	N	306		194		144		106		104		306	
	Patient-time (years)[b]	117.3		80.2		58.1		47.0		206.7		509.3	
SOC	PT	n[c]	Rate (95% CI)[d]	n[c]	Rate (95% CI)[d]	n[c]	Rate (95% CI)[d]	n[c]	Rate (95% CI)[d]	n[c]	Rate (95% CI)[d]	n[c]	Rate (95% CI)[d]
All	All	33	28.1 (19.1-41.5)	4	5.0 (1.9-13.2)	8	13.8 (5.9-31.9)	2	4.3 (1.1-16.9)	9	4.3 (2.2-8.8)	56	11.0 (7.9-15.2)
Blood and lymphatic system disorders	All	1	0.9 (0.1- 6.1)	-	-	-	-	-	-	1	0.5 (0.1- 3.4)	2	0.4 (0.1- 1.6)
	Febrile neutropenia	-	-	-	-	-	-	-	-	1	0.5 (0.1- 3.4)	1	0.2 (0.0- 1.4)
	Lymphadenopathy	1	0.9 (0.1- 6.1)	-	-	-	-	-	-	-	-	1	0.2 (0.0- 1.4)
Gastrointestinal disorders	All	1	0.9 (0.1- 6.0)	-	-	-	-	-	-	-	-	1	0.2 (0.0- 1.4)
	Abdominal pain	1	0.9 (0.1- 6.0)	-	-	-	-	-	-	-	-	1	0.2 (0.0- 1.4)
General disorders and administration site conditions	All	1	0.9 (0.1- 6.1)	1	1.2 (0.2- 8.8)	-	-	-	-	-	-	2	0.4 (0.1- 1.6)
	Injection site pain	-	-	1	1.2 (0.2- 8.8)	-	-	-	-	-	-	1	0.2 (0.0- 1.4)
	Injection site reaction	1	0.9 (0.1- 6.1)	-	-	-	-	-	-	-	-	1	0.2 (0.0- 1.4)
Hepatobiliary disorders	All	1	0.9 (0.1- 6.0)	-	-	-	-	-	-	-	-	1	0.2 (0.0- 1.4)
	Hypertransaminasaemia	1	0.9 (0.1- 6.0)	-	-	-	-	-	-	-	-	1	0.2 (0.0- 1.4)
Immune system disorders	All	7	6.0 (2.8- 12.5)	1	1.2 (0.2- 8.8)	1	1.7 (0.2- 12.2)	-	-	2	1.0 (0.2- 3.8)	11	2.2 (1.1- 4.1)
	Haemophagocytic lymphohistiocytosis	7	6.0 (2.8- 12.5)	1	1.2 (0.2- 8.8)	1	1.7 (0.2- 12.2)	-	-	2	1.0 (0.2- 3.8)	11	2.2 (1.1- 4.1)
Infections and infestations	All	7	6.0 (2.9- 12.4)	1	1.2 (0.2- 8.8)	2	3.4 (0.9- 13.8)	-	-	3	1.5 (0.3- 6.3)	13	2.6 (1.4- 4.8)
	Epstein-Barr virus infection	-	-	-	-	-	-	-	-	1	0.5 (0.1- 3.5)	1	0.2 (0.0- 1.4)
	Gastroenteritis	-	-	-	-	-	-	-	-	1	0.5 (0.1- 3.5)	1	0.2 (0.0- 1.4)
	Otitis media	1	0.9 (0.1- 6.0)	-	-	-	-	-	-	-	-	1	0.2 (0.0- 1.4)
	Parvovirus B19 infection	1	0.9 (0.1- 6.0)	-	-	-	-	-	-	-	-	1	0.2 (0.0- 1.4)
	Pharyngitis	-	-	-	-	1	1.7 (0.2- 12.3)	-	-	-	-	1	0.2 (0.0- 1.4)
	Pneumonia	2	1.7 (0.4- 6.8)	1	1.2 (0.2- 8.8)	1	1.7 (0.2- 12.2)	-	-	-	-	4	0.8 (0.3- 2.1)
	Pneumonia viral	1	0.9 (0.1- 6.0)	-	-	-	-	-	-	-	-	1	0.2 (0.0- 1.4)
	Pyelonephritis	1	0.9 (0.1- 6.0)	-	-	-	-	-	-	-	-	1	0.2 (0.0- 1.4)
	Tonsillitis	-	-	-	-	-	-	-	-	1	0.5 (0.1- 3.5)	1	0.2 (0.0- 1.4)
	Viral infection	1	0.9 (0.1- 6.0)	-	-	-	-	-	-	-	-	1	0.2 (0.0- 1.4)
Injury, poisoning and procedural complications	All	5	4.3 (1.8- 10.2)	-	-	2	3.4 (0.9- 13.6)	1	2.1 (0.3- 15.1)	1	0.5 (0.1- 3.3)	9	1.8 (0.9- 3.4)
	Humerus fracture	-	-	-	-	-	-	-	-	1	0.5 (0.1- 3.3)	1	0.2 (0.0- 1.4)
	Infusion related reaction	1	0.9 (0.1- 6.0)	-	-	-	-	1	2.1 (0.3- 15.1)	-	-	2	0.4 (0.1- 1.6)
	Injection related reaction	4	3.4 (1.3- 9.1)	-	-	2	3.4 (0.9- 13.6)	-	-	-	-	6	1.2 (0.5- 2.6)
Investigations	All	2	1.7 (0.4- 6.8)	-	-	-	-	-	-	-	-	2	0.4 (0.1- 1.6)
	Biopsy bone marrow	1	0.9 (0.1- 6.0)	-	-	-	-	-	-	-	-	1	0.2 (0.0- 1.4)
	Hepatic enzyme increased	1	0.9 (0.1- 6.1)	-	-	-	-	-	-	-	-	1	0.2 (0.0- 1.4)

	Time window[a]	1-6 months		7-12 months		13-18 months		19-24 months		>24 months		Overall	
	N	306		194		144		106		104		306	
	Patient-time (years)[b]	117.3		80.2		58.1		47.0		206.7		509.3	
SOC	PT	n[c]	Rate (95% CI)[d]	n[c]	Rate (95% CI)[d]	n[c]	Rate (95% CI)[d]	n[c]	Rate (95% CI)[d]	n[c]	Rate (95% CI)[d]	n[c]	Rate (95% CI)[d]
Metabolism and nutrition disorders	All	3	2.6 (0.8- 7.9)	-	-	1	1.7 (0.2- 12.1)	-	-	-	-	4	0.8 (0.3- 2.1)
	Dehydration	1	0.9 (0.1- 6.0)	-	-	-	-	-	-	-	-	1	0.2 (0.0- 1.4)
	Hyperuricaemia	1	0.9 (0.1- 6.0)	-	-	-	-	-	-	-	-	1	0.2 (0.0- 1.4)
	Metabolic acidosis	1	0.9 (0.1- 6.0)	-	-	-	-	-	-	-	-	1	0.2 (0.0- 1.4)
	Overweight	-	-	-	-	1	1.7 (0.2- 12.1)	-	-	-	-	1	0.2 (0.0- 1.4)
Musculoskeletal and connective tissue disorders	All	-	-	-	-	1	1.7 (0.2- 12.3)	-	-	-	-	1	0.2 (0.0- 1.4)
	Rheumatoid arthritis	-	-	-	-	1	1.7 (0.2- 12.3)	-	-	-	-	1	0.2 (0.0- 1.4)
Nervous system disorders	All	1	0.9 (0.1- 6.0)	-	-	-	-	1	2.1 (0.3- 14.9)	-	-	2	0.4 (0.1- 1.6)
	Amnesia	-	-	-	-	-	-	1	2.1 (0.3- 14.9)	-	-	1	0.2 (0.0- 1.4)
	Petit mal epilepsy	1	0.9 (0.1- 6.0)	-	-	-	-	-	-	-	-	1	0.2 (0.0- 1.4)
Psychiatric disorders	All	-	-	-	-	1	1.7 (0.2- 12.1)	-	-	-	-	1	0.2 (0.0- 1.4)
	Psychotic disorder	-	-	-	-	1	1.7 (0.2- 12.1)	-	-	-	-	1	0.2 (0.0- 1.4)
Respiratory, thoracic and mediastinal disorders	All	-	-	-	-	-	-	-	-	1	0.5 (0.1- 3.5)	1	0.2 (0.0- 1.4)
	Interstitial lung disease	-	-	-	-	-	-	-	-	1	0.5 (0.1- 3.5)	1	0.2 (0.0- 1.4)
Skin and subcutaneous tissue disorders	All	3	2.6 (0.8- 7.9)	1	1.2 (0.2- 8.8)	-	-	-	-	-	-	4	0.8 (0.3- 2.1)
	Drug eruption	1	0.9 (0.1- 6.0)	-	-	-	-	-	-	-	-	1	0.2 (0.0- 1.4)
	Pityriasis rosea	1	0.9 (0.1- 6.0)	-	-	-	-	-	-	-	-	1	0.2 (0.0- 1.4)
	Psoriasis	-	-	1	1.2 (0.2- 8.8)	-	-	-	-	-	-	1	0.2 (0.0- 1.4)
	Urticaria	1	0.9 (0.1- 6.1)	-	-	-	-	-	-	-	-	1	0.2 (0.0- 1.4)
Surgical and medical procedures	All	1	0.9 (0.1- 6.0)	-	-	-	-	-	-	1	0.5 (0.1- 3.3)	2	0.4 (0.1- 1.5)
	Hip arthroplasty	-	-	-	-	-	-	-	-	1	0.5 (0.1- 3.3)	1	0.2 (0.0- 1.4)
	Lymphadenectomy	1	0.9 (0.1- 6.0)	-	-	-	-	-	-	-	-	1	0.2 (0.0- 1.4)

Abbreviations: SAE, serious adverse event; SOC, system organ class; PT, preferred term, MedDRA version 21.1; N, number of patients ever treated with anakinra during the time window irrespectively of the length of any unexposed periods; 95% CI, 95% Confidence Interval.

[a]in relation to baseline (start of anakinra treatment).

[b]only time while anakinra treatment was ongoing + 2 days after discontinuation was counted

[c]number of events. Only SAEs occurring during anakinra exposed periods + 2 days after discontinuation were counted

[d]incidence rate per 100 patient years; number of events/∑patient time.

Supplementary table S4. Number of patients and incidence proportions of AEs (non-serious AEs of at least moderate intensity and all serious AEs) by SOC, PT and time window in the complete set

	Time window[a]	1-6 months		7-12 months		13-18 months		19-24 months		>24 months		Overall	
	N[b]	306		194		144		106		104		306	
	Patient-time (years)	117.3		80.2		58.1		47.0		206.7		509.3	
SOC	PT	n[c]	Incidence proportion[d]	n[c]	Incidence proportion[d]	n[c]	Incidence proportion[d]	n[c]	Incidence proportion[d]	n[c]	Incidence proportion[d]	n[c]	Incidence proportion[d]
All	All	71	23.2	19	9.8	11	7.6	5	4.7	20	19.2	99	32.4
Blood and lymphatic system disorders	All	3	1.0	2	1.0	-	-	-	-	3	2.9	8	2.6
	Febrile neutropenia	-	-	-	-	-	-	-	-	1	1.0	1	0.3
	Lymphadenitis	-	-	1	0.5	-	-	-	-	-	-	1	0.3
	Lymphadenopathy	1	0.3	-	-	-	-	-	-	1	1.0	2	0.7
	Neutropenia	2	0.7	1	0.5	-	-	-	-	1	1.0	4	1.3
	Pancytopenia	-	-	-	-	-	-	-	-	1	1.0	1	0.3
Ear and labyrinth disorders	All	1	0.3	-	-	-	-	-	-	-	-	1	0.3
	Ear pain	1	0.3	-	-	-	-	-	-	-	-	1	0.3
Endocrine disorders	All	5	1.6	-	-	-	-	-	-	-	-	5	1.6
	Cushing's syndrome	2	0.7	-	-	-	-	-	-	-	-	2	0.7
	Cushingoid	2	0.7	-	-	-	-	-	-	-	-	2	0.7
	Hypothyroidism	1	0.3	-	-	-	-	-	-	-	-	1	0.3
Eye disorders	All	-	-	1	0.5	1	0.7	-	-	1	1.0	3	1.0
	Conjunctivitis allergic	-	-	-	-	1	0.7	-	-	-	-	1	0.3
	Dry eye	-	-	-	-	-	-	-	-	1	1.0	1	0.3
	Eyelid ptosis	-	-	1	0.5	-	-	-	-	-	-	1	0.3
Gastrointestinal disorders	All	11	3.6	1	0.5	-	-	1	0.9	3	2.9	16	5.2
	Abdominal pain	3	1.0	1	0.5	-	-	-	-	-	-	4	1.3
	Anal pruritus	1	0.3	-	-	-	-	-	-	-	-	1	0.3
	Constipation	5	1.6	-	-	-	-	-	-	1	1.0	6	2.0
	Gastritis	-	-	-	-	-	-	-	-	1	1.0	1	0.3
	Nausea	1	0.3	-	-	-	-	1	0.9	-	-	2	0.7
	Odynophagia	-	-	-	-	-	-	-	-	1	1.0	1	0.3
	Pneumatosis intestinalis	1	0.3	-	-	-	-	-	-	-	-	1	0.3
	Stomatitis	1	0.3	-	-	-	-	-	-	-	-	1	0.3
	Vomiting	1	0.3	-	-	-	-	-	-	-	-	1	0.3
General disorders and administration site conditions	All	15	4.9	3	1.5	1	0.7	1	0.9	2	1.9	21	6.9

	Time window[a]	1-6 months		7-12 months		13-18 months		19-24 months		>24 months		Overall	
	N[b]	306		194		144		106		104		306	
	Patient-time (years)	117.3		80.2		58.1		47.0		206.7		509.3	
SOC	PT	n[c]	Incidence proportion[d]	n[c]	Incidence proportion[d]	n[c]	Incidence proportion[d]	n[c]	Incidence proportion[d]	n[c]	Incidence proportion[d]	n[c]	Incidence proportion[d]
	Chest pain	1	0.3	-	-	-	-	-	-	-	-	1	0.3
	Fatigue	3	1.0	-	-	-	-	-	-	-	-	3	1.0
	Injection site hypersensitivity	1	0.3	-	-	-	-	-	-	-	-	1	0.3
	Injection site inflammation	-	-	-	-	1	0.7	-	-	-	-	1	0.3
	Injection site pain	-	-	2	1.0	-	-	-	-	-	-	2	0.7
	Injection site rash	2	0.7	-	-	-	-	-	-	-	-	2	0.7
	Injection site reaction	7	2.3	-	-	-	-	-	-	1	1.0	8	2.6
	Injection site urticaria	1	0.3	-	-	-	-	-	-	-	-	1	0.3
	Local reaction	1	0.3	-	-	-	-	-	-	-	-	1	0.3
	Mucosal erosion	-	-	-	-	-	-	-	-	1	1.0	1	0.3
	Pain	-	-	-	-	-	-	1	0.9	-	-	1	0.3
	Pyrexia	-	-	1	0.5	-	-	-	-	-	-	1	0.3
Hepatobiliary disorders	All	3	1.0	-	-	-	-	-	-	1	1.0	4	1.3
	Hepatotoxicity	-	-	-	-	-	-	-	-	1	1.0	1	0.3
	Hypertransaminasaemia	3	1.0	-	-	-	-	-	-	-	-	3	1.0
Immune system disorders	All	7	2.3	1	0.5	2	1.4	-	-	3	2.9	12	3.9
	Autoimmune disorder	-	-	-	-	1	0.7	-	-	-	-	1	0.3
	Haemophagocytic lymphohistiocytosis	7	2.3	1	0.5	1	0.7	-	-	3	2.9	11	3.6
Infections and infestations	All	20	6.5	9	4.6	5	3.5	-	-	7	6.7	36	11.8
	Conjunctivitis	-	-	1	0.5	-	-	-	-	-	-	1	0.3
	Cystitis	-	-	-	-	-	-	-	-	1	1.0	1	0.3
	Ear infection	-	-	1	0.5	-	-	-	-	-	-	1	0.3
	Epstein-Barr viraemia	1	0.3	-	-	-	-	-	-	-	-	1	0.3
	Epstein-Barr virus infection	-	-	-	-	-	-	-	-	1	1.0	1	0.3
	Folliculitis	-	-	1	0.5	-	-	-	-	-	-	1	0.3
	Fungal skin infection	1	0.3	-	-	-	-	-	-	-	-	1	0.3
	Gastroenteritis	-	-	1	0.5	-	-	-	-	2	1.9	3	1.0
	Gastroenteritis rotavirus	1	0.3	-	-	-	-	-	-	-	-	1	0.3
	Gastroenteritis viral	1	0.3	-	-	-	-	-	-	-	-	1	0.3
	Herpes zoster	-	-	-	-	-	-	-	-	1	1.0	1	0.3
	Impetigo	1	0.3	-	-	-	-	-	-	-	-	1	0.3

	Time window[a]	1-6 months		7-12 months		13-18 months		19-24 months		>24 months		Overall	
	N[b]	306		194		144		106		104		306	
	Patient-time (years)	117.3		80.2		58.1		47.0		206.7		509.3	
SOC	PT	n[c]	Incidence proportion[d]	n[c]	Incidence proportion[d]	n[c]	Incidence proportion[d]	n[c]	Incidence proportion[d]	n[c]	Incidence proportion[d]	n[c]	Incidence proportion[d]
	Infection	1	0.3	-	-	1	0.7	-	-	-	-	2	0.7
	Influenza	2	0.7	-	-	-	-	-	-	-	-	2	0.7
	Lower respiratory tract infection	1	0.3	-	-	-	-	-	-	-	-	1	0.3
	Otitis media	1	0.3	-	-	-	-	-	-	-	-	1	0.3
	Otitis media acute	1	0.3	2	1.0	-	-	-	-	-	-	2	0.7
	Parasitic gastroenteritis	-	-	1	0.5	-	-	-	-	-	-	1	0.3
	Parvovirus B19 infection	1	0.3	-	-	-	-	-	-	-	-	1	0.3
	Pharyngitis	1	0.3	-	-	1	0.7	-	-	-	-	2	0.7
	Pharyngitis bacterial	-	-	-	-	-	-	-	-	1	1.0	1	0.3
	Pneumonia	2	0.7	1	0.5	1	0.7	-	-	-	-	4	1.3
	Pneumonia viral	1	0.3	-	-	-	-	-	-	-	-	1	0.3
	Pyelonephritis	1	0.3	-	-	-	-	-	-	-	-	1	0.3
	Respiratory tract infection	2	0.7	-	-	2	1.4	-	-	-	-	4	1.3
	Rhinitis	-	-	-	-	-	-	-	-	1	1.0	1	0.3
	Sinusitis	-	-	1	0.5	-	-	-	-	-	-	1	0.3
	Subcutaneous abscess	1	0.3	-	-	-	-	-	-	-	-	1	0.3
	Tonsillitis	-	-	-	-	-	-	-	-	1	1.0	1	0.3
	Tonsillitis streptococcal	-	-	-	-	-	-	-	-	1	1.0	1	0.3
	Upper respiratory tract infection	2	0.7	1	0.5	-	-	-	-	-	-	2	0.7
	Varicella	-	-	2	1.0	-	-	-	-	-	-	2	0.7
	Varicella zoster virus infection	-	-	-	-	-	-	-	-	1	1.0	1	0.3
	Viral infection	1	0.3	-	-	-	-	-	-	1	1.0	2	0.7
Injury, poisoning and procedural complications	All	10	3.3	1	0.5	2	1.4	1	0.9	2	1.9	16	5.2
	Hand fracture	1	0.3	-	-	-	-	-	-	-	-	1	0.3
	Humerus fracture	-	-	-	-	-	-	-	-	1	1.0	1	0.3
	Infusion related reaction	2	0.7	-	-	-	-	1	0.9	-	-	3	1.0
	Injection related reaction	6	2.0	1	0.5	2	1.4	-	-	1	1.0	10	3.3
	Joint injury	1	0.3	-	-	-	-	-	-	-	-	1	0.3
Investigations	All	6	2.0	1	0.5	-	-	-	-	1	1.0	7	2.3
	Biopsy bone marrow	2	0.7	-	-	-	-	-	-	-	-	2	0.7

	Time window[a]	1-6 months		7-12 months		13-18 months		19-24 months		>24 months		Overall	
	N[b]	306		194		144		106		104		306	
	Patient-time (years)	117.3		80.2		58.1		47.0		206.7		509.3	
SOC	PT	n[c]	Incidence proportion[d]	n[c]	Incidence proportion[d]	n[c]	Incidence proportion[d]	n[c]	Incidence proportion[d]	n[c]	Incidence proportion[d]	n[c]	Incidence proportion[d]
	Hepatic enzyme increased	2	0.7	1	0.5	-	-	-	-	-	-	2	0.7
	Transaminases increased	2	0.7	-	-	-	-	-	-	1	1.0	3	1.0
Metabolism and nutrition disorders	All	3	1.0	-	-	1	0.7	-	-	-	-	4	1.3
	Dehydration	1	0.3	-	-	-	-	-	-	-	-	1	0.3
	Hyperuricaemia	1	0.3	-	-	-	-	-	-	-	-	1	0.3
	Metabolic acidosis	1	0.3	-	-	-	-	-	-	-	-	1	0.3
	Overweight	-	-	-	-	1	0.7	-	-	-	-	1	0.3
Musculoskeletal and connective tissue disorders	All	1	0.3	1	0.5	1	0.7	-	-	1	1.0	4	1.3
	Arthritis	-	-	-	-	-	-	-	-	1	1.0	1	0.3
	Back pain	-	-	1	0.5	-	-	-	-	-	-	1	0.3
	Rheumatoid arthritis	-	-	-	-	1	0.7	-	-	-	-	1	0.3
	Still's disease	1	0.3	-	-	-	-	-	-	-	-	1	0.3
Nervous system disorders	All	2	0.7	-	-	-	-	3	2.8	-	-	4	1.3
	Amnesia	-	-	-	-	-	-	1	0.9	-	-	1	0.3
	Headache	1	0.3	-	-	-	-	1	0.9	-	-	1	0.3
	Petit mal epilepsy	1	0.3	-	-	-	-	1	0.9	-	-	2	0.7
Psychiatric disorders	All	-	-	-	-	2	1.4	-	-	1	1.0	3	1.0
	Attention deficit/hyperactivity disorder	-	-	-	-	-	-	-	-	1	1.0	1	0.3
	Persistent depressive disorder	-	-	-	-	1	0.7	-	-	-	-	1	0.3
	Psychotic disorder	-	-	-	-	1	0.7	-	-	-	-	1	0.3
Renal and urinary disorders	All	1	0.3	-	-	-	-	1	0.9	-	-	2	0.7
	Urethral meatus stenosis	1	0.3	-	-	-	-	-	-	-	-	1	0.3
	Urinary incontinence	-	-	-	-	-	-	1	0.9	-	-	1	0.3
Reproductive system and breast disorders	All	1	0.3	-	-	-	-	-	-	-	-	1	0.3
	Balanoposthitis	1	0.3	-	-	-	-	-	-	-	-	1	0.3
Respiratory, thoracic and mediastinal disorders	All	1	0.3	-	-	-	-	-	-	1	1.0	2	0.7
	Asthma	1	0.3	-	-	-	-	-	-	-	-	1	0.3
	Interstitial lung disease	-	-	-	-	-	-	-	-	1	1.0	1	0.3
Skin and subcutaneous tissue disorders	All	17	5.6	3	1.5	1	0.7	-	-	3	2.9	23	7.5
	Dermatitis atopic	1	0.3	-	-	-	-	-	-	-	-	1	0.3

	Time window[a]	1-6 months		7-12 months		13-18 months		19-24 months		>24 months		Overall	
	N[b]	306		194		144		106		104		306	
	Patient-time (years)	117.3		80.2		58.1		47.0		206.7		509.3	
SOC	PT	n[c]	Incidence proportion[d]	n[c]	Incidence proportion[d]	n[c]	Incidence proportion[d]	n[c]	Incidence proportion[d]	n[c]	Incidence proportion[d]	n[c]	Incidence proportion[d]
	Drug eruption	1	0.3	-	-	-	-	-	-	-	-	1	0.3
	Eczema	1	0.3	-	-	-	-	-	-	2	1.9	3	1.0
	Erythema	2	0.7	-	-	-	-	-	-	-	-	2	0.7
	Mucocutaneous rash	1	0.3	-	-	-	-	-	-	-	-	1	0.3
	Onychomadesis	-	-	-	-	1	0.7	-	-	-	-	1	0.3
	Pityriasis rosea	1	0.3	-	-	-	-	-	-	-	-	1	0.3
	Pruritus	1	0.3	-	-	-	-	-	-	-	-	1	0.3
	Pseudocellulitis	1	0.3	-	-	-	-	-	-	-	-	1	0.3
	Psoriasis	-	-	1	0.5	-	-	-	-	-	-	1	0.3
	Rash	5	1.6	1	0.5	-	-	-	-	1	1.0	7	2.3
	Urticaria	3	1.0	-	-	-	-	-	-	-	-	3	1.0
	Vitiligo	-	-	1	0.5	-	-	-	-	-	-	1	0.3
Surgical and medical procedures	All	1	0.3	-	-	-	-	-	-	1	1.0	2	0.7
	Hip arthroplasty	-	-	-	-	-	-	-	-	1	1.0	1	0.3
	Lymphadenectomy	1	0.3	-	-	-	-	-	-	-	-	1	0.3
Vascular disorders	All	1	0.3	-	-	-	-	-	-	-	-	1	0.3
	Hypertension	1	0.3	-	-	-	-	-	-	-	-	1	0.3

Abbreviations: AE, adverse event; SOC, system organ class, PT, preferred term, MedDRA version 21.1.
[a] in relation to baseline (start of anakinra treatment).
[b] number of patients ever treated with anakinra in respective time window.
[c] number of patients experiencing the event. Only AEs occurring during anakinra exposed periods (including 2 days post stop) are counted.
[d] incidence proportion (%), n/N.

Supplementary table S5. Number of patients and incidence proportions of SAEs by SOC, PT and time window in the complete set.

	Time window[a]	1-6 months		7-12 months		13-18 months		19-24 months		>24 months		Overall	
	N[b]	306		194		144		106		104		306	
	Patient-time (years)	117.3		80.2		58.1		47.0		206.7		509.3	
SOC	PT	n[c]	Incidence proportion[d]	n[c]	Incidence proportion[d]	n[c]	Incidence proportion[d]	n[c]	Incidence proportion[d]	n[c]	Incidence proportion[d]	n[c]	Incidence proportion[d]
All	All	28	9.2	4	2.1	6	4.2	2	1.9	8	7.7	44	14.4
Blood and lymphatic system disorders	All	1	0.3	-	-	-	-	-	-	1	1.0	2	0.7
	Febrile neutropenia	-	-	-	-	-	-	-	-	1	1.0	1	0.3
	Lymphadenopathy	1	0.3	-	-	-	-	-	-	-	-	1	0.3
Gastrointestinal disorders	All	1	0.3	-	-	-	-	-	-	-	-	1	0.3
	Abdominal pain	1	0.3	-	-	-	-	-	-	-	-	1	0.3
General disorders and administration site conditions	All	1	0.3	1	0.5	-	-	-	-	-	-	2	0.7
	Injection site pain	-	-	1	0.5	-	-	-	-	-	-	1	0.3
	Injection site reaction	1	0.3	-	-	-	-	-	-	-	-	1	0.3
Hepatobiliary disorders	All	1	0.3	-	-	-	-	-	-	-	-	1	0.3
	Hypertransaminasaemia	1	0.3	-	-	-	-	-	-	-	-	1	0.3
Immune system disorders	All	7	2.3	1	0.5	1	0.7	-	-	2	1.9	10	3.3
	Haemophagocytic lymphohistiocytosis	7	2.3	1	0.5	1	0.7	-	-	2	1.9	10	3.3
Infections and infestations	All	7	2.3	1	0.5	2	1.4	-	-	3	2.9	13	4.2
	Epstein-Barr virus infection	-	-	-	-	-	-	-	-	1	1.0	1	0.3
	Gastroenteritis	-	-	-	-	-	-	-	-	1	1.0	1	0.3
	Otitis media	1	0.3	-	-	-	-	-	-	-	-	1	0.3
	Parvovirus B19 infection	1	0.3	-	-	-	-	-	-	-	-	1	0.3
	Pharyngitis	-	-	-	-	1	0.7	-	-	-	-	1	0.3
	Pneumonia	2	0.7	1	0.5	1	0.7	-	-	-	-	4	1.3
	Pneumonia viral	1	0.3	-	-	-	-	-	-	-	-	1	0.3
	Pyelonephritis	1	0.3	-	-	-	-	-	-	-	-	1	0.3
	Tonsillitis	-	-	-	-	-	-	-	-	1	1.0	1	0.3
	Viral infection	1	0.3	-	-	-	-	-	-	-	-	1	0.3
Injury, poisoning and procedural complications	All	5	1.6	-	-	2	1.4	1	0.9	1	1.0	9	2.9
	Humerus fracture	-	-	-	-	-	-	-	-	1	1.0	1	0.3
	Infusion related reaction	1	0.3	-	-	-	-	1	0.9	-	-	2	0.7
	Injection related reaction	4	1.3	-	-	2	1.4	-	-	-	-	6	2.0
Investigations	All	2	0.7	-	-	-	-	-	-	-	-	2	0.7
	Biopsy bone marrow	1	0.3	-	-	-	-	-	-	-	-	1	0.3

	Time window[a]	1-6 months		7-12 months		13-18 months		19-24 months		>24 months		Overall	
	N[b]	306		194		144		106		104		306	
	Patient-time (years)	117.3		80.2		58.1		47.0		206.7		509.3	
SOC	PT	n[c]	Incidence proportion[d]	n[c]	Incidence proportion[d]	n[c]	Incidence proportion[d]	n[c]	Incidence proportion[d]	n[c]	Incidence proportion[d]	n[c]	Incidence proportion[d]
	Hepatic enzyme increased	1	0.3	-	-	-	-	-	-	-	-	1	0.3
Metabolism and nutrition disorders	All	3	1.0	-	-	1	0.7	-	-	-	-	4	1.3
	Dehydration	1	0.3	-	-	-	-	-	-	-	-	1	0.3
	Hyperuricaemia	1	0.3	-	-	-	-	-	-	-	-	1	0.3
	Metabolic acidosis	1	0.3	-	-	-	-	-	-	-	-	1	0.3
	Overweight	-	-	-	-	1	0.7	-	-	-	-	1	0.3
Musculoskeletal and connective tissue disorders	All	-	-	-	-	1	0.7	-	-	-	-	1	0.3
	Rheumatoid arthritis	-	-	-	-	1	0.7	-	-	-	-	1	0.3
Nervous system disorders	All	1	0.3	-	-	-	-	1	0.9	-	-	2	0.7
	Amnesia	-	-	-	-	-	-	1	0.9	-	-	1	0.3
	Petit mal epilepsy	1	0.3	-	-	-	-	-	-	-	-	1	0.3
Psychiatric disorders	All	-	-	-	-	1	0.7	-	-	-	-	1	0.3
	Psychotic disorder	-	-	-	-	1	0.7	-	-	-	-	1	0.3
Respiratory, thoracic and mediastinal disorders	All	-	-	-	-	-	-	-	-	1	1.0	1	0.3
	Interstitial lung disease	-	-	-	-	-	-	-	-	1	1.0	1	0.3
Skin and subcutaneous tissue disorders	All	3	1.0	1	0.5	-	-	-	-	-	-	4	1.3
	Drug eruption	1	0.3	-	-	-	-	-	-	-	-	1	0.3
	Pityriasis rosea	1	0.3	-	-	-	-	-	-	-	-	1	0.3
	Psoriasis	-	-	1	0.5	-	-	-	-	-	-	1	0.3
	Urticaria	1	0.3	-	-	-	-	-	-	-	-	1	0.3
Surgical and medical procedures	All	1	0.3	-	-	-	-	-	-	1	1.0	2	0.7
	Hip arthroplasty	-	-	-	-	-	-	-	-	1	1.0	1	0.3
	Lymphadenectomy	1	0.3	-	-	-	-	-	-	-	-	1	0.3

Abbreviations: SAE, serious adverse event; SOC, system organ class; PT, preferred term, MedDRA version 21.1.
[a] in relation to baseline (start of anakinra treatment).
[b] number of patients ever treated with anakinra in respective time window.
[c] number of patients experiencing the event. Only SAEs occurring during anakinra exposed periods (including 2 days post stop) are counted.
[d] incidence proportion (%), n/N.

Supplementary table S6. Number of first occurrence of MAS events by history of MAS and time since first injection with anakinra.

	History of MAS at baseline		No History of MAS recorded at baseline		Total	
N	1		18		19	
Time since first injection with Anakinra	n	%	n	%	n	%
MAS during anakinra treatment						
1 -30 days	0	0.0	4	40.0	4	36.4
31-180 days	0	0.0	3	30.0	3	27.3
181-365 days	1	100.0	0	0.0	1	9.0
>365 days	0	0.0	3	30.0	3	27.3
Total	1	100.0	10	55.6	11	57.9
Mean time[a] (sd;min;max)	358		270 (395; 4; 968)		278 (375; 4; 968)	
MAS after anakinra is stopped						
1 -30 days	0	0.0	0	0.0	0	0.0
31-180 days	0	0.0	0	0.0	0	0.0
181-365 days	0	0.0	2	25.0	2	25.0
>365 days	0	0.0	6	75.0	6	75.0
Total	0	0.0	8	44.4	8	42.1
Mean time[a] (sd;min;max)	-		873 (725; 220; 2377)		873 (725; 220; 2377)	
Overall						
1 -30 days	0	0.0	4	22.2	4	21.0
31-180 days	0	0.0	3	16.7	3	15.8
181-365 days	1	100.0	2	11.1	3	15.8

	History of MAS at baseline		No History of MAS recorded at baseline		Total	
N	1		18		19	
Time since first injection with Anakinra	n	%	n	%	n	%
>365 days	0	0.0	9	50.0	9	47.4
Total	1	100.0	18	100.0	19	100.0
Mean time[a] (sd;min;max)	358		538 (628; 4; 2377)		528 (612; 4; 2377)	

Supplementary table S7. Trigger events for MAS events during simultaneous anakinra treatment.

	Overall	
N	**19**	
Number of MAS events during anakinra	**12**	
Trigger event	**n**	**%**
Disease flare	4	33.3
Infection	2	16.7
Changes of treatment	3	25.0
Unknown	3	25.0
Total	12	100.0

Abbreviations: MAS, macrophage activation syndrome; N, number of patients, n, number of MAS events.

Safety of anakinra in patients with cryopyrin associated periodic syndromes (CAPS) using a graduated pre-filled syringe

Cryopyrin associated periodic syndromes (CAPS) are ultra-rare, monogenic autoinflammatory diseases, caused by autosomal dominant mutation of the NLRP3 gene, which leads to overproduction of interleukin (IL)-1β.[1,2] Patients with CAPS present a large severity spectrum of clinical manifestations, historically classified in three disorders: familial cold autoinflammatory syndrome (FCAS), Muckle-Wells syndrome (MWS) and neonatal-onset multisystem inflammatory disease/chronic infantile neurological cutaneous articular syndrome (NOMID/CINCA).[3,4]
Anakinra is a human IL-1 receptor antagonist with a well known safety profile.[5,6] To meet the demands for using anakinra in children needing smaller and varying doses, a graduated pre-filled syringe was introduced. Due to the potential risk of overdosing or underdosing, carefully designed instructions are provided in the package leaflet. In addition, educational materials were made available to both healthcare providers and patients/caregivers.
Eurofever is an international registry aimed to collect information on the clinical presentation, outcome and response to treatments of patients affected by autoinflammatory diseases,[7–9] promoted by the Autoinflammatory Diseases' Working Group of the Pediatric Rheumatology European Society (PReS) and managed by the Pediatric Rheumatology International Trials Organisation (PRINTO).

We utilized the Eurofever registry to find and prospectively follow the CAPS patients post anakinra authorization in order to evaluate the safety of the graduated pre-filled syringe.

By September 2013, 225 CAPS patients had been enrolled in the Eurofever registry. Investigators managing CAPS patients were asked by PRINTO for their interest and capability to recruit patients according to the following inclusion criteria: 1) a signed informed consent by the patient and/or caregiver; 2) anakinra treatment according to the approved recommendation for CAPS. No specific exclusion criteria were applied.
The primary study endpoint was the occurrence of adverse events (AEs) with focus on serious infections, malignancies, injection site reactions (ISRs), allergic reactions and medication errors, including re-use of the pre-filled syringe. The secondary objectives were to evaluate the anakinra dosage over time, the proportions and reasons for discontinuations and switch to another IL-1 blocking treatment. The duration of the enrollment period was 1 year and 11 months and the follow

up duration for each patient was at least 3 years. All endpoints were summarized using 95% confidence intervals and descriptive statistics. No formal statistical comparison was to be done.

A total of 12 CAPS patients were included, 8 with MWS, 2 with FCAS and 2 with CINCA/NOMID (Table 1). The majority were male (75%) and white (83.3%). Of these, only 1 patient started anakinra treatment at baseline and 11/12 (91.7%) patients were already using the pre-filled syringe at baseline. 5 patients required a median dose of 2 to 3 mg/kg/day and 6 patients below 2 mg/kg/day. During a total of 26.1 patient years of treatment, there were 7 AEs with rate of 26.8 (95% CI 4.2-169.6) per 100 patient years.

All AEs were infections observed in 1 patient with CINCA/NOMID and considered unrelated to anakinra by the investigator. 2/7 (28.6%) AEs were considered serious due to the required hospitalization (1 tonsillitis and 1 urinary tract infection). No AEs were severe, 6 of the AEs were of moderate severity and 1 was of mild severity.

In total, 6/12 (50%) patients permanently discontinued anakinra, of those 2 during the first year of treatment, 3 during the second years, and 1 after the third year. The reasons of permanent discontinuations included the switch to canakinumab (5 patients), inefficacy (1 patient) and non-compliance (1 patient). The remaining 5 patients continued anakinra until the end of the study. Only 1 patient temporarily discontinued anakinra during the second year of treatment due to non-compliance. No deaths, malignancies, ISRs, allergic reactions or medication errors, including re-use of the pre-filled syringe, were observed.

This study was designed to address the safety of anakinra in CAPS patients and the effectiveness of the risk minimization measures for medication errors in routine clinical care, including re-use of syringe with potential infection risk at the injection site, and over- or underdosing. An uncontrolled study design was deemed acceptable for this intention. Accordingly, the study design carried the general limitations inherent in an uncontrolled design regarding statistical analyses, interpretation, generalizability and conclusiveness, in addition to the threat of inherent bias. However, all the primary endpoints are variables easy to measure objectively and do not require a subjective interpretation from the investigator, which minimizes the threat of bias although the study did not have a control group.

Although the 12 patients included in this study should be representative of this ultra-rare disease, generalizing should be done with caution due to the low number of included patients. In addition, patients who received anakinra outside of the approved treatment recommendations for CAPS were not included. Furhtermore, the previous use of the graduated syringe was not to be an exclusion

criterion at enrollment. As a result, only 1 patient started anakinra at baseline. Thus, the study did not capture data from the patient's first time use of the graduated syringe. It is known that the development of ISRs and allergic reactions in patients who had not previously experienced ISRs is uncommon after the first month of therapy.[6] The fact that only 1 patient started anakinra at baseline might be reflected in the absence of reported ISRs and allergic reactions.

On the other hand, the inclusion of patients with anakinra treatment previous baseline allows to further explore the potential oncogenicity of long term treatment with anakinra. The patients had been treated with anakinra for a median of 1154.5 days (interquartile 167, 2556). None of the patients reported any malignancies or other long term related AEs, which strengthens the safety profile of anakinra.

In conclusion, the AEs observed in this study are in line with the known safety profile for anakinra treatment in CAPS patients. Based on these data, the introduction of the pre-filled syringe with a graduated label for single-use injections don't seems to alter the risks for medication errors, including re-use of syringe. No new safety concerns about anakinra have been identified.

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Table 2. Study results.

Patients	12 (100)
Gender, M:F	9:3 (75:25)
Ethnicity, white:black	10:2 (83.3:16.7)
Age at baseline, median (range), years	25.3 (1.4-54.9)
Age at disease onset, median (range), years	2.5 (0-29.5)
Age at diagnosis, median (range), years	24.4 (0.6-54.6)
Disease duration, median (range), years	15.5 (1.4-51.3)
Patients already using anakinra at baseline	11 (91.7)
Patients with history of other IL-1 blocking treatments at baseline	3 (25)
Anakinra dose at baseline, median (range), mg/kg/day	1.7 (1.1-2.5)
Anakinra dose during follow-up years 1:2:3:>3, median (range), mg/kg/day	1.6:1.6:2.6:1.4 (1.1-3.9:1-4.4:1.7-3.7:1-3.2)
Patients with AEs	1 (8.3)
Patients with permanent discontinuation of anakinra	6 (50)
Patients with continuation of anakinra until the end of the study	5 (41.7)
Patients with temporary discontinuation of anakinra	1 (8.3)
Total duration of anakinra exposure, median (range), years	1.3 (0.5-7)
Any AE	7 (100)
Urinary tract infection	1 (14.3)
Upper respiratory tract infection	1 (14.3)
Tonsillitis	5 (71.4)

Results are shown as number (%) unless stated otherwise.

Successful treatment of refractory hyperferritinemic syndromes with canakinumab: a report of two cases

Hyperferritinemic syndromes (HFS) are systemic inflammatory disorders characterized by a dysfunctional immune response, leading to excessive activation of the monocyte-macrophage system with hypercytokinemia, and pronounced hemophagocytosis [1]. Serum ferritin level higher than 500 ng/ml is the common laboratory feature of this heterogeneous group of disorders, which ranges from rheumatic to non-rheumatic diseases, including primary immunodeficiencies, chronic infections, and malignancies.

The main condition within this disease spectrum is hemophagocytic lymphohistiocytosis (HLH), which is sub-classified into primary (or familial) HLH and secondary (or acquired or reactive) HLH [2]. The HLH associated with rheumatic illnesses is termed macrophage activation syndrome (MAS) [3, 4]. Among pediatric rheumatic diseases, MAS is encountered most commonly in children with systemic juvenile idiopathic arthritis (sJIA) [5]. Although many patients with active sJIA without MAS have ferritin levels exceeding 1000 ng/ml [6], when MAS develops ferritin usually increases sharply. Beside the known conditions associated with HFS, in several instances no evident cause or underlying disease is observed. Timely recognition of HFS and prompt institution of an appropriate therapy are fundamental to avoid progression toward overt MAS.

In the past two decades, several well established criteria that can help to identify MAS in its early stage have been published [7-10]. Conversely, the management of the syndrome is not standardized and no universally agreed therapeutic protocols exist. Although high-dose glucocorticoids and cyclosporine A (CSA) still represent the mainstay of the treatment, instances of MAS that are refractory to these therapies are often encountered. Recently, a number of cases of sJIA-associated MAS dramatically benefiting from the interleukin (IL)-1 receptor antagonist anakinra (ANK) after inadequate response to glucocorticoids and CSA have been reported [11-17]. However, most patients needed dose escalation, up to 10 mg/kg/day, to control symptoms [18]. Based on these data, there is now wide agreement that ANK is a valuable medication for MAS.

The role of the IL-1β antibody canakinumab (CNK) is less clear, due to both the lack of experience with its use as interventional therapy in MAS and the occurrence of instances of MAS, recorded as adverse event, in the randomized clinical trials that led to its registration in sJIA [19, 20]. However, the incidence of MAS in the trials was similar to the incidence of MAS in sJIA patients reported from a tertiary care pediatric rheumatology center in the US, which suggested that IL-1 inhibition with CNK does not have a major effect on the risk of developing MAS. In addition, many of the instances of MAS appeared to be triggered by an infection [21]. Recently, three patients with sJIA

associated MAS which was refractory to conventional therapies or could not be controlled with standard doses of ANK or CNK, but responded dramatically to one single injection of CNK at higher doses (7,5 to 15 mg/kg) have been reported in a meeting abstract [22].

In the present paper, we describe two patients with HFS, one with sJIA-like illness and impending MAS and one with sJIA and overt MAS, who were resistant or intolerant to conventional therapies, but improved rapidly with the administration of CNK.

Patient 1

A previously healthy 11-year-old boy was admitted to his local hospital with a 1-week history of fever (maximum temperature 39.4 °C), urticarial rash and arthralgia, which did not improve with nonsteroidal anti-inflammatory, antihistamine and antibiotic therapy. On physical examination, he had generalized lymphadenopathy, but no evidence of overt arthritis. Body temperature was 38.7 °C. Laboratory tests showed increased acute phase reactants, anemia, and marked hyperferritinemia. Liver and kidney function tests, triglycerides, serum complement fractions, rheumatoid factor and antinuclear antibodies were all normal or negative. Abdominal ultrasound revealed diffuse lymph nodes enlargement and positron-emission tomography increased concentration of the radioactive tracer in the supraclavicular, mediastinal and abdominal lymph nodes. Chest radiograph, echocardiography, extensive infectious serology and autoantibodies were negative. Bone marrow aspirate disclosed expansion of the myeloid cell line and cervical lymph node biopsy a nonspecific reactive B and T lymphocyte hyperplasia. Next generation sequencing panel for monogenic autoinflammatory diseases and primary HLH as well as interferon signature were all negative. On the fifth day of admission, glucocorticoid therapy with 2 mg/kg/day prednisone was started, which led to defervescence of fever in 3 days and rapid improvement in clinical and laboratory abnormalities.

Six months later, after the decrease of prednisone dose to 0.15 mg/kg/day, there was recurrence of urticarial rash and diffuse arthralgia, without fever. The boy was then admitted to our hospital. Laboratory tests disclosed leukocytosis with neutrophilia, anemia, thrombocytosis, and increased erythrocyte sedimentation rate, C-reactive protein (CRP), aspartate aminotransferase (AST), lactate dehydrogenase and ferritin (Table 1, T0). Because the illness did not meet both the International League for Associations of Rheumatology (ILAR) criteria for sJIA [23], due of the absence of arthritis and fever, and the 2016 classification criteria for MAS complicating sJIA [9], due to the lack of fever, a diagnosis of systemic juvenile idiopathic arthritis (JIA)-like hyperferritinemic syndrome was made. Treatment with intravenous methylprednisolone at 3 mg/kg/day administered in three divided doses and ANK at 5 mg/kg/day (i.e. 100 mg twice a day) was initiated. However,

ANK had to be first tapered to 100 mg/day and then discontinued due to the development of severe, intractable injection site reactions during the first week of treatment. In substitution of ANK, oral CSA at 4 mg/kg/day in two daily doses was started on the 7th day of hospitalization. Over the subsequent week, there was improvement in laboratory changes (Table 1, T+14) and methylprednisolone dose was, then, gradually diminished.

However, on the 45th day of continuous hospitalization, although the boy had remained afebrile, there was worsening of his clinical conditions, with malaise, fatigue and diffuse arthralgia, and laboratory tests showed sharp increase in white blood cells, neutrophil count and ferritin, together with decreased hemoglobin and elevated AST (Table 1, T+45). At this stage, the 2016 classification criteria for MAS were still not fulfilled, but the child was thought to have impending MAS. We, then, decided to increase methylprednisolone dose to 3 mg/kg/day and to start CNK at 4 mg/kg subcutaneously, while continuing CSA. After the first injection of CNK, there was quick and striking improvement of laboratory abnormalities, with decrease in ferritin value from 18,000 to 4099 ng/ml and normalization of white blood cell and neutrophil count within 5 days (Table 1, T+50). One month later, the blood cell counts, the acute phase reactants and the AST had returned to normal and the level of ferritin was markedly reduced (Table 1, T+80). The boy was, then, discharged with monthly CNK administration, in association with CSA and tapering oral prednisone.

Patient 2

A 9-year-old girl was diagnosed with sJIA and MAS at her local hospital based on the presence of high-spiking fever, erythematous rash and polyarthritis, together with increased acute phase reactants, AST, triglycerides and ferritin, and decreased platelet count and fibrinogen. She was given high-dose intravenous methylprednisolone at 30 mg/kg for 3 consecutive days, which led to improvement in clinical manifestations and laboratory abnormalities. However, shortly after glucocorticoid therapy had been switched to oral prednisone there was a recurrence of MAS features and the girl was transferred to the regional tertiary care hospital.

On admission, a full-blown clinical and laboratory picture of MAS was detected. Three additional pulses of intravenous methylprednisolone at 30 mg/kg were, then, administered and CSA at 4 mg/kg/day, with ANK at 6 mg/kg/day, were started. This treatment led to rapid improvement in MAS features. However, during hospitalization the girl developed a thrombophlebitis in the right arm in the site of a venipuncture and developed fever, malaise and chest pain in spite of antibiotic therapy. A few days later, a chest radiograph disclosed marked cardiomegaly, which was found on echocardiography to be due to massive pericardial effusion. This complication was followed by

recurrence of MAS abnormalities, with increase in ferritin to 20,350 ng/ml (Table 2, T-4). The girl was given 3 additional pulses of intravenous methylprednisolone at 30 mg/kg and was, then, transferred to our hospital for further care.

On admission, the girl was afebrile and laboratory tests revealed an overall improvement of MAS abnormalities as compared with previous assessment (Table 2, T0). Treatment was, then, continued with intravenous methylprednisolone at 2 mg/kg/day, together with CSA and ANK at unchanged doses. In the meantime, pericardial drainage revealed the purulent nature of the accumulated fluid, whose culture led to the isolation of a methicillin-resistant Staphylococcus Aureus. Therapy with large-spectrum antibiotics was started. However, on the third week of hospitalization there was a sudden worsening of clinical conditions, with recurrence of fever and malaise. Repeated laboratory tests showed a full-blown picture of MAS, with sharply elevated ferritin (Table 2, T+20), which met the 2016 classification criteria for the syndrome. Treatment consisted of 3 consecutive days of methylprednisolone at 30 mg/kg plus intravenous immunoglobulin at 2 gr/kg. Cyclosporine A and ANK were continued, but the dose of the latter medication was raised to 8 mg/kg/day. The laboratory picture of MAS improved (Table 2, T+30), but after the shift of intravenous methylprednisolone to the conventional regimen of 2 mg/kg/day and surgical pericardiotomy, which led to the evacuation of 350 ml of purulent fluid, there was another flare of MAS, with a further increase in ferritin to 28,634 ng/ml (Table 2, T+35). Due to the presence of major signs of corticosteroid toxicity and the inefficacy of ongoing treatment in preventing MAS recurrences, we decided to start CNK at 5 mg/kg. Anakinra was stopped and shortly afterward also CSA was discontinued. The first injection of CNK was followed by prompt improvement in all MAS biomarkers and the girl could be discharged 2 weeks later in good general condition, with normal ferritin level (Table 2, T+50) and on tapering oral prednisone. Nine months later she was receiving low-dose prednisone and monthly CNK and had experienced no relapses of either sJIA or MAS.

We have described two patients with HFS, which were refractory or intolerant to conventional therapies, but responded dramatically to the administration of CNK. The first patient had a long-standing inflammatory illness closely resembling sJIA, but without overt arthritis. Although the 2016 classification criteria for MAS [9] were not met, he had a sharp increase in ferritin level, which was thought to herald the potential occurrence of full-blown MAS. Although initial ANK therapy, in conjunction with intravenous methylprednisolone and CSA, was followed by improvement in laboratory abnormalities, especially hyperferritinemia, the subsequent discontinuation of ANK because of serious and intractable injection site reaction was followed by worsening of the condition, which was reversed quickly after a single injection of CNK. The second

patient had recurrent episodes of MAS, the last of which was precipitated after surgical pericardiotomy to evacuate purulent pericardial fluid and was not controlled by the combination of high-dose intravenous methylprednisolone, CSA and ANK. The administration of CNK led to rapid recovery of all clinical and laboratory features of MAS.

The rationale for the use of cytokine blockers in the management of MAS was provided by the demonstration of the prominent role of the pro-inflammatory cytokines targeted by these agents, namely TNF-α, IL-1 and IL-6, in the pathophysiology of the syndrome [4, 18]. The first cytokine inhibitors used to treat MAS were TNF blockers. However, after initial encouraging reports, subsequent experiences indicated that these agents may trigger MAS in some instances. Nowadays, TNF inhibition is not considered the ideal therapy for MAS, whereas there is much more interest for therapy directed at two other pro-inflammatory cytokines, IL-1 and IL-6 [18].

Thus far, the information on the use of IL-6 blockade in the management of MAS is still limited, given the reports from the tocilizumab clinical trials and post-marketing surveillance, which suggest that IL-6 inhibition does not provide full protection against MAS [21]. By contrast, substantial benefit from ANK treatment in sJIA-associated MAS after inadequate response to glucocorticoids and CSA has been described in many case reports [24, 25]. Notably, occurrence of MAS was occasionally seen in children treated with doses of 1–2 mg/kg daily [26, 27]. In some of these patients, however, features of MAS improved after the dose of ANK was escalated. There is now widespread consensus that ANK, particularly at higher doses, might be effective at least in some patients with sJIA-associated MAS [18].

Our report suggests that the monoclonal IL-1β antibody CNK may represent an alternative therapeutic option for children with MAS who are refractory or intolerant to conventional therapies and for select critically ill children with HFS who do not meet neither sJIA nor MAS criteria. The efficacy of CNK in ANK-resistant patients with HFS has been previously reported [28]. The better effectiveness of CNK may be due to differences in molecular targets (i.e. ANK blocks both IL-1α and IL-1β) or pharmacokinetics (i.e. longer half-life of CNK). Further clinical experience is needed to confirm our observations and to clarify whether a dose of CNK higher than the 4 mg/kg used in the routine management of sJIA is needed to control HFS, including MAS.

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Table 1. Course of laboratory tests over time in patient 1.

Days from admission at Authors' center	0	+14	+45*	+50	+80
White blood cells ($x10^3/mm^3$)	17.9	10.4	44.5	11.4	8.9
Neutrophil count ($x10^3/mm^3$)	15.0	8.9	41.7	6.4	5.0
Hemoglobin (g/dL)	10.3	10.6	9.3	9.8	12.5
Platelet count ($x10^3/mm^3$)	482	320	418	459	315
Erythrocyte sedimentation rate (mm/h)	59	37	57	61	18
C-reactive protein (mg/dL)	8.2	4.9	11.5	1.5	< 0.46
Aspartate aminotransferase (U/L)	229	68	170	127	12
Lactate dehydrogenase (U/L)	1752	951	956	862	853
Triglycerides (mg/dL)	112	158	133	226	107
Fibrinogen (mg/dL)	341	442	415	251	287
Ferritin (ng/mL)	13200	8550	18000	4099	581

*Start of canakinumab administration

Table 2. Course of laboratory tests over time in patient 2.

Days from admission at Authors' center	-4	0	+20	+30	+35*	+40	+50
White blood cells ($x10^3/mm^3$)	14.2	10.6	3.4	5.4	3.7	18.9	5.5
Neutrophil count ($x10^3/mm^3$)	11.9	6.6	1.9	3.4	1.7	15.1	2.9
Hemoglobin (g/dL)	9.2	11.2	10.8	10.8	10.5	9.9	11.1
Platelet count ($x10^3/mm^3$)	285	424	105	233	145	164	253
Erythrocyte sedimentation rate (mm/h)	75	68	43	55	63	50	36
C-reactive protein (mg/dL)	2.5	< 0.46	1.83	<0.46	0.6	< 0.46	< 0.46
Aspartate aminotransferase (U/L)	61	40	350	111	113	35	31
Lactate dehydrogenase (U/L)	1673	1048	4960	1541	2122	1116	468
Triglycerides (mg/dL)	209	173	273	-	157	-	-
Fibrinogen (mg/dL)	356	418	234	297	424	312	267
Ferritin (ng/mL)	20350	5065	49700	17580	28634	12340	201

*Start of canakinumab administration

MERITA project: A metadata registry for the ERN RITA

List of Applicants

Applicant No	Applicant Organisation Name	Country
1 (Coordinator)	Clinica Pediatrica e Reumatologia, IRCCS Istituto Giannina Gaslini (**IGG**)	Italy
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3	General University Hospital, Prague (**GUH**)	Czech republic
4	Hippokration General Hospital, Thessaloniki University School of Medicine (**AUTH**)	Greece
5	University Children's Hospital, University Medical Centre Ljubljana (**UKCL**)	Slovenia
6	University of Latvia (**UL**)	Latvia
7	Warsaw Children's Memorial Health Institute (**IPCZD**)	Poland
8	Hospital of Lithuanian University of Health Sciences, Kaunas (**LSMU**)	Lithuania
9	Institute for Immunodeficiency, Center for Chronic Immunodeficiency, Medical Center - University of Freiburg (**CCI**)	Germany

1 Problem Analysis Including Evidence Base

The current state of the art

Rare diseases (RDs) affect a small number of people compared to the general population and specific issues are raised in relation to their rarity. In Europe, a disease is considered to be rare when it affects not more than 1 person per 2000. To date, six to eight thousand RDs have been discovered and new RDs are regularly described in medical literature. About 30 million people living in the European Union (EU) suffer from a RD. Specific issues are equally raised among patients with RDs regarding access to quality health care, overall social and medical support, effective liaison between hospitals and general practices, as well as professional and social integration and independence.

The **RD field suffers from a deficit of medical and scientific knowledge**. For a long time, physicians, researchers and policy makers were unaware of RDs and until recently there was little real research or public health policy concerning this issue. A multidisciplinary care using complex diagnostic evaluation and highly specialized therapies are required for patients with RDs. For example, genome-wide analysis platforms and functional immune assays represent rapidly developing diagnostic tests usually not available in all centres. Providing high-quality and cost-effective treatments is particularly difficult for RDs. No country alone has the resources to address this issue. The European Reference Networks (ERNs) are networks involving healthcare providers (HCPs) and patient organizations across Europe. They aim to facilitate discussion on RDs that require highly specialised treatments and a concentration of knowledge and resources. The ERNs are part of a broader strategy of the European Commission (EC) to make the national and European health systems more efficient, accessible and resilient. ERNs offer the potential to give patients and physicians access to the best expertise and timely exchange of life-saving knowledge, without having to travel to another country.

Identified causes and their evidence

Though appropriate treatment and medical care may improve the quality of life of patients with RD and extend their life expectancy, **there is no cure for most RDs**. One of the reasons is that companies are less likely to recover the development cost for medicines for such small numbers of patients. The European Medicines Agency (EMA) plays a central role in facilitating the development and authorisation of medicines for RDs, which are termed 'orphan medicines' in the medical world. The incentives laid down in the orphan legislation aim to stimulate sponsors to develop medicinal products for RDs. Medicinal products eligible for these incentives are identified

through the EU procedure of orphan designation. The orphan designations cover a wide variety of RDs, including genetic diseases and rare cancers, for which there are limited treatment options. Since the year 2000, over 2121 orphan designations have been issued by the EC, of which so far 164 have resulted in authorised medicinal products.

In this view, **RD registries can be helpful** in understanding the course of the diseases, providing necessary information for the design and implementation of clinical trials for orphan medicines. The term 'registry' can denote any type of data collection. However, not all data collections that are presented as disease registries are suitable for clinical trial design or contain necessary information for regulatory evaluation. Currently, a vast amount of data on patients with RD is scattered across Europe in very high numbers of registries and databases which hold information on patients with specific conditions. Individual hospitals, research institutions, pharmaceutical companies or patient advocacy groups often collect data locally. The types of data collected vary widely.

The European Platform on Rare Diseases Registration (EU RD Platform, https://eu-rd-platform.jrc.ec.europa.eu/) has the main objective to cope with this enormous fragmentation of RD registries across Europe. The Platform makes RD registries' data searchable and findable, thus increasing visibility for each registry, maximising the value of each registry's information and enabling extended use and re-use of registries' data. This is ensured by the European RD Registry Infrastructure (ERDRI), which supports existing registries and the creation of new registries. The EU RD Platform has developed a set of Common Data Elements (CDE) stabilizing the EU-level standards for RD data collection and data exchange. It contains 16 essential data elements to be registered by each RD registry across Europe. These contain data on patient's personal identifiers, diagnosis, disease history and care pathway, information for research purposes and disability. CDE is aimed for all ERN registries that exist and are under development, as well as for the national, regional, and local registries of the Member States, HCPs or patient organizations. In addition, the EU RD Platform includes a registry infrastructure consisting of:

I. the European Directory of Registries (EDR), which gives an overview of each participating registry;
II. the Central Metadata Repository (CMR), which stores a core list of variables used by the registries;
III. the European Patient Identity Management (EUPID, https://eupid.eu/#/home), a data protection tool that makes sure patient data is pseudonymised, yet cannot be traced back to the individual.

RITA proposal (evidence and methods)

The Rare Immunodeficiency, AuToinflammatory and Autoimmune Disease (RITA) network is an ERN that brings together the leading centres with expertise in diagnosis and treatment of rare immune disorders (RID) across Europe. These RID, including monogenic inborn errors of immunity, include well over 400 conditions (see the list at http://rita.ern-net.eu/), generally classified as primary immunodeficiencies (PID) when predisposing to infections, autoinflammatory disorders (AID), if associated with disturbed immune regulation, and autoimmune diseases (AI) in case of defective tolerance to self-antigens. Some errors in the immune system can result not only in specific clinical entities, but also in conditions with two or three features of these pathologic processes.

Polyvalent immunoglobulin therapy has revolutionised the outlook for antibody deficient patients, specific anti-cytokine (*e.g.* anti-TNF, anti-IL-1) treatments have transformed the lives of patients with features of AID and AI, and stem cell and gene-based therapies, originally for PID, are now being applied for all the RID and for the first time enable patients to be completely cured with no need for ongoing medical care.

The ERN-RITA full member and affiliated HCPs, collaborating institutions and individual teams include 126 health professionals/institution of whom 45 are HCPs and eight are patient and family organizations. The member HCPs involved in RITA and related patient organisations are also listed in the RITA website (http://rita.ern-net.eu/). The foundation of RITA has been developed from the already existing successful and highly specialized resources in the field and, in particular, European Society for Immunodeficiencies (ESID) with ESID registry, Paediatric Rheumatology International Trials Organisation (PRINTO) and Paediatric Rheumatology European Society (PRES) with EUROFEVER and PHARMACHILD projects, Paediatric Rheumatology European Society (PRES), International Society for Systemic AID (ISSAID), European Vasculitis Society (EUVAS), and BEHCET International.

The main aims of RITA are:

i) to provide state of the art for comprehensive clinical care for children and adults with RID, harmonizing diagnostic and therapeutic guidelines across Europe, thus ensuring for every patient an equal access to excellent expertise and care and reducing patient search of cross-border healthcare;

ii) to establish sustainable alliances within European centres to accelerate diagnosis, improve access to treatment, and develop transitional care for patients with RID, maximizing the cost-effective use of resources and facilitating mobility of expertise;

iii) to ensure the proper accreditation of quality control on diagnostic tests and targeted therapy, establishing a common tool for pharmacovigilance in these rare conditions;

iv) to reinforce epidemiological surveillance and basic research on RID;

v) to train future leaders in the field, securing their sustainability in the international perspective, and promote the awareness of RID between clinicians, carers, patients, family organizations and politicians, advocating better resources and measure to ensure early diagnosis by screening and enhanced symptom recognition.

RITA registries survey

In April 2018, an online survey was sent to all RITA sites in order to obtain a census of the registries and researches networks among the ERN. [1]

Data from 50 registries were collected across 14 European countries (Table 1), of which 30 are dedicated primarily to AI, 14 to PID and 12 to AID. Fifteen registries (30%) enrolled patients with a single specific rare immune disorder.

More than 55,000 patients with RID are enrolled in a disease registry in Europe. The majority of registries (35; 70%) enrols patients within national boundaries and only one registry collects data from two countries (UKIVAS registry). Among the international registries, five collect data on PID (ESID, EBMT, SCETIDE, PCID and HLH registry), four on AI (Pharmachild, BrainWorks, EULAR web library, and JIR cohort), and three are devoted to AID (Eurofever, Infevers, and ImmunAID). The ESID registry and JIR cohort also collect data on AID. Other international registries are devoted to a single specific disorder. It is also anticipated that clinical data gathered via case consultations using the ERN platform Clinical Patient Management System (CPMS) will form a specific data resource that may directly feed in the shared RID registry.

Collectively, 27 RITA sies are coordinating 25 registries; 53 sites are participating in 38 registries, and 27 sites know the existence of 16 registries without participating. Data usually collected in these registries are demography, diagnosis, clinical manifestations, laboratory tests and treatment, while genetic and imaging data are less frequently reported (respectively in 40% and 10% of registries). A treatment safety profile is reported in 29 registries (58%). Collectively, fifteen biobanks are counted.

The survey of registries clearly shows how the RITA network needs a common plan for inventory of the clinical data about patients with RID. Furthermore, registration of key parameters as patient safety, including the use of medicines and medical technologies, communication issues and breaches in continuity of care, as well as outcome data (i.e. mortality, morbidity and disease complications) or quality of life measures can be improved among the network.

Table 1. RITA registries identified with the survey.

N°	RITA registries
1	AID-Registry
2	ATTRA Clinical Registry
3	Czech National Guillain-Barrè Syndrome Registry
4	Banque nationale de données Maladies Rares (BNDMR)
5	Blau cohort study registry
6	Brainworks: Childhood CNS Vasculitis and Inflammatory Brain Diseases
7	Spanish Registry of Juvenile Dermatomyositis (Registro Nacional Dermatomiositis Juvenil)
8	Centre de Référence Déficits Immunitaires Héréditaires (CEREDIH)
9	Centre de Référence National Cytopénies auto-immunes de l'enfant (CEREVANCE)
10	CID and PIDs registry
11	Czech Registry of ANCA-associated vasculitis
12	Dutch PID registry
13	European Society for Blood and Marrow Transplantation (EBMT) registry
14	European Society for Immunodeficiencies (ESID) registry
15	EULAR WEB Library
16	French Vasculitis Study Group (FVSG) registry
17	Hemophagocytic lymphoistiocytosis (HLH) registry
18	IMMUNAID
19	Inception Cohort for juvenile Systemic sclerosis
20	Infevers
21	Innsbruck registry for Behcet disease: Retrospective and prospective data collection
22	IPINET
23	Irish Rare Kidney Disease registry and biobank
24	JIR cohort
25	JULES: Registro nacional Lupus eritematoso sistémico
26	Kawanet
27	Kawa-race
28	Kidbiosep Cohort
29	Malattie Rare Lazio V 1.3
30	Italian National Biobank
31	Orbis Maladies Rares
32	Paediatric MS database (OPTIMISE)
33	PCID registry
34	Paediatric Behçet's Disease (PEDBD) registry
35	PEDOLUP
36	PIDcare
37	PID-NET
38	Rare Disease Cohort (RADICO)-AcoStill

39	Registro AIJ sistémica y enfermedad de Still del adulto
40	Registro Nazionale Malattie Rare (RNMR)
41	Registro Lombardia Malattie Rare (ReLMaR)
42	Registro de Uvéitis Asociada a Artritis idiopatica juvenil
43	Rational Use of Biologics in rare Refractory Immune mediated inflammatory diseases Consortium (RUBRIC)
44	Sarcoidosis UK
45	Stem Cell Transplant for primary Immune Deficiencies in Europe (SCETIDE)
46	UK JSLE Cohort Study and Repository
47	UK and Ireland Vasculitis Rare Disease Group (UKIVAS) Registry
48	UKPIN Registry
49	Pharmachild registry
50	Eurofever registry

PRINTO Registry as an example

PRINTO (www.printo.it) is a not for profit, non-governmental, international research network founded in 1996 that initially included 14 European countries and now represents a network of 640 centres with 1350 members from almost 90 countries worldwide. The goal of our organization is to foster, facilitate and co-ordinate the development, conduct, analysis, and reporting of multi-centre, international clinical trials and/or outcome standardisation studies in children with paediatric rheumatic diseases. The International Coordinating Centre is based in Genoa at the Istituto Giannina Gaslini (IGG), one of the biggest children's hospitals in Italy. The chief function of the International Coordinating Centre is to facilitate the flow of logistic and scientific aspects needed to design, launch and manage multi-centred, multi-national, collaborative studies.

PRINTO has collected for not for profit collaborative studies as of June 2019 about 38,600 patients' data from 316 centres in 67 countries and, for profit registrative clinical trials, in collaboration with several pharmaceutical companies, over 3,700 children from 248 centres in 39 countries. Of note, the work in collaboration with pharmaceutical companies has led to a paediatric indication for all biologic agents currently used to treat children with Juvenile idiopathic arthritis (JIA).

Conducting clinical trials in paediatric rheumatology has been difficult mainly because of the lack of funding for academic studies and the lack of interest by pharmaceutical companies in the small and non-rewarding paediatric market. The situation changed dramatically a few years ago with the introduction of the Best Pharmaceuticals for Children Act in the USA and of a specific legislation for the development of paediatric medicines (Paediatric Regulation) in the EU.[2-5]

To this regard, it could be noted that paediatric rheumatology is referred "as prime example" in paediatrics for the successful implementation of the paediatric regulation, as recognized by the 10-year report of the EMA to the European Parliament. [6] For this application, it is worthwhile to notice

that PRINTO has already gained great scientific expertise in the harmonization of the two large registries, namely PHARMACHILD and EUROFEVER that collectively have enrolled more than 12,000 children.

PHARMACHILD is a pharmacovigilance project that aims at observing children with JIA for 3-10 years undergoing treatment with methotrexate or biologic agents in order to collect the occurrence of moderate, severe or serious adverse events. Data collection is performed online via the secured PRINTO website on a dedicated server with a username and timely password on an https-encrypted platform according to the recent EU General Data Protection Regulation (GDPR).[7] English is the official language used for all forms completed by the physicians, and a form for patient-reported outcomes (PRO) is available in the appropriate language spoken by parents/patients for use in any electronic personal device. The web system is designed to be user-friendly, modular, and upgradable. During data entry, several hundred automatic checks are in place to ensure data quality and consistency. A designated paediatric rheumatologist acts as medical monitor by performing an electronic check and revision of the adverse events and events of special interest; in addition, for some safety events of special interest, adjudicating committees are in place. As of today PHARMACHILD has enrolled over 8,800 children from 94 centres in 33 countries.[8]

EUROFEVER project started in 2008 by the work group of AID of the PRES and was supported by the Executive Agency for Health and Consumers (EACH, Project No2007332, http://ec.europa.eu/eahc/projects/database.html). The general aims of the project are to:

I. sensitize paediatricians and paediatric rheumatologists to the prompt recognition of AID;
II. provide proper information to families affected by these conditions;
III. increase the knowledge on the clinical presentation, response to treatment and complications of theses RDs.

The main objective of the project has been the creation of a registry of AID. In 2015, a new section dedicated to “efficacy and safety” has been implemented and the registry is now able to collect also longitudinal information.[9-21] As of today, EUROFEVER has enrolled over 4,000 children from 113 centers in 41 countries, collecting information on almost 30 disorders. Under the term of AID a number of inherited disorders secondary to mutations of genes coding for proteins that play a pivotal role in the regulation of the innate inflammatory response are gathered. Most of these disorders have generally an early onset, ranging from the first hours to the first decade of life. The clinical spectrum of these disorders is extremely variable, but can be summarized in five groups:

1) Recurrent Fevers: Familial Mediterranean Fever (FMF), Mevalonate-kinase deficiency (MKD), and Tumor necrosis factor (TNF) Receptor-Associated Periodic Syndrome (TRAPS) are the three monogenic disorders gathered under the term of recurrent

fevers. These diseases are characterized by periodic flares of systemic inflammation presenting as sudden fever episodes associated with a dramatic elevation of acute phase reactants and with a number of clinical manifestations, such as rash, serositis (peritonitis, pleuritis), lymphadenopathy, arthritis. Disease flares are usually separated by symptom-free intervals of variable duration, characterized by a complete well-being, normal growth and complete normalization of acute phase reactants.

2) Inflammasomopathies: in other disorders, a characteristic urticarial rash associated with a number of other clinical manifestations dominates systemic inflammation. Familiar Cold Autoinflammatory Syndrome (FCAS), Muckle-Wells Syndrome (MWS) and Chronic Infantile Neurological Cutaneous and Articular Syndrome (CINCA) represent the clinical spectrum associated to different mutations of a gene named *NLRP3* (or *CIAS1*) coding for a protein called Cryopyrin. These three disorders are also gathered under the term of Cryopyrinopathies or cryopyrin-related periodic syndromes (CAPS). Mutations of other member of the NLRP family, such as the *NLRP12* and *NLRP1* have been associated with new AID.
3) Granulomatous AID: other diseases are characterized by typical granulomatous formations. Blau's syndrome is characterized by non-caseating granulomatous inflammation affecting the joint, skin, and uveal tract and is associated with mutations of the *CARD15* (or *NOD2*) gene. The LACC1 deficiency and diseases caused by variants of the *PLCG2* gene belong in this group.
4) Pyogenic disorders: a further group of diseases is dominated by the presence of sterile pyogenic abscesses affecting skin, joints and bones. This include the Pyogenic Sterile Arthritis, Pyoderma Gangrenosum and Acne (PAPA) syndrome secondary to mutations of the *PSTPIP1* gene and the Majeed syndrome, characterized by chronic recurrent multifocal osteomyelitis, congenital dyserythropoietic anemia, and neutrophil dermatosis caused by mutations of the *LPIN2* gene. Finally, a recently identified autosomal recessive autoinflammatory syndromes, due to the deficiency of the interleukin-1–receptor antagonist (DIRA) and the deficiency of the interleukin-36–receptor antagonist (DITRA), are characterized by a neonatal onset of generalized pustular psoriasis, and in case of DIRA, multifocal osteomyelitis and periostitis.
5) Interferonopathies: hypersecretion of interferon (IFN) alfa/beta is related to a group of disorders characterized by brain calcification, interstitial lung disease, lipodystrophy and autoimmunity. These conditions initially included i) Aicardi-Goutières syndrome (AGS), ii) familial chilblain lupus, iii) spondyloenchondrodysplasia with immunodeficiency

(SPENCDI) and iv) monogenic forms of systemic lupus erythematosus (SLE). An increasing number of genetic diseases belonging to this family have later been discovered, including the Proteasome Associated Autoinflammatory Syndromes (PRAAS), IFN-stimulated gene 15 (ISG15) deficiency, Singleton-Merten syndrome (SMS) and its atypical presentation, and stimulator of IFN genes (STING)-associated vasculopathy with onset in infancy (SAVI).

EUROFEVER project is originally addressed to the Centers referring to the PRES/PRINTO network, but is later open to a direct functional integration to other already existent initiatives involving also adult patients, such as Eurotraps Project (http://fmf.igh.cnrs.fr/ISSAID/EUROTRAPS/public.php) and the Hyper-IgD registry (http://hids.net).

In addition, PRINTO developed a website for families of children with rheumatic diseases (www.pediatric-rheumatology.printo.it) which contains in more than 50 different languages information on pediatric rheumatic diseases in the format of frequently answered questions, the list of pediatric rheumatology centers and the list of family associations worldwide. The website is visited daily by more than 6,000 people from over 180 countries.

In conclusion, PRINTO has gained specific information technology (IT) expertise from the development of the PHARMACHILD and EUROFEVER international registries. Indeed these two registries have already a common database structure for demographic and safety information, which allow their automatic interoperability to research or clinical needs. This specific expertise for database merging will constitute the main benefit of the partners of this application, providing the working model for future harmonisations of all registries identified in the ERN RITA.

ESID Registry as an example

ESID Registry is the key component of European Society for Primary Immunodeficiencies. ESID was founded first as an informal group in 1983 in Rome, Italy. Society's first constitution was established in 1994 and has been revised in 2000 and 2008 (https://esid.org/About-ESID/ESID-Constitution). ESID's current, officially accepted mission emphasizes its values. ESID believes that every child, adolescent and adult with a defective immune system has the right to benefit from both clinical and scientific knowledge. Therefore, the mission of ESID is to enable patients with primary immunodeficiency diseases to live their lives to its full potential by improving awareness, diagnosis, treatment, education and understanding of these diseases. As the leading society in the field, ESID is committed to promote collaboration between healthcare professionals, patient organizations, industry and governmental bodies and to foster education and research.

ESID is clearly the world's largest immunodeficiency organization, with plenty of members from outside Europe. ESID has been striving to improve the knowledge in the field of Primary Immunodeficiency (PID) by encouraging research, developing educational programs and fostering cooperation among all those involved in the diagnosis, treatment and management of these diseases. It hosts the world's largest, widely attended PID meetings biennially, as well as focused meetings around Europe. ESID has two sister societies: The International Nursing Group for Immunodeficiencies (INGID) and the International Patient Organization for Primary Immunodeficiencies (IPOPI).

ESID is organized in 7 working parties – Inborn Errors, Clinical, Education, Genetics, Registry, ESID Juniors, PID Care in Development. All ESID Board with President, former/elect President, Secretary, Treasurer and Working Party Chairs are democratically elected and serve in their position for 4 years.

ESID Registry is ESID's most valued asset and an essential tool for all PID doctors contributing to ESID. The finances of the Registry are secure until 2020, and all in the current ESID Board are highly committed in keeping it alive. Not only are the numbers of known PIDs growing, so is the ESID Registry. It represents the largest PID database in the world, with almost 30,000 PID patients. Also, this network is growing steadily, with more documenting centres joining frequently. The Registry currently comprises >200 documenting centres across Europe and its neighbouring areas. The Registry contains data on patients belonging to all hereditary inborn errors of immunity subgroups (susceptibility to infectious diseases or malignancy, autoimmunity and autoinflammation), i.e. data on patients suffering from some 400 different hereditary diseases.

The web-based The web-based ESID Registry was founded in 2004, with a major upgrade in 2014. The Registry is hosted by the Medical Centre, University of Freiburg, that is also an ERN-RITA HCP. For the technical structure of the ESID registry see Scheible R et al. Bioinformatics 2019.[22] The ESID registry Working Party is responsible for the maintenance and further development of the ESID registry. ESID Registry is governed by its Steering Group (SG) with the head of the Registry Team being a member of the SG (https://esid.org/Working-Parties/Registry-Working-Party/News-and-Events/Registry-WP-Newsletter-February-2019), with further contributions from its Advisory Board with members from national registries. Several important national registries, e.g. in the UK, France, The Netherlands, Spain, Italy and Germany share their data with the ESID registry, thus providing an important backbone for this common European effort. SG has representation from around Europe, with an IPOPI representative belonging to the SC. The ESID Registry (https://esid.org/Working-Parties/Registry-Working-Party/Registry-publications) and the national registries sharing data with ESID Registry have been active in producing peer-reviewed articles for

decades, forming the backbone of much we currently know about PIDs, for example of their epidemiology and life-time burden. Ongoing studies are listed on the ESID Registry website (https://esid.org/Working-Parties/Registry-Working-Party/Studies).

All major decisions, like taking part in the mission to build an ERN RITA Registry, are subjected to the ESID Board (https://esid.org/About-ESID/ESID-Board), which decides by voting.

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2 Goals and Objectives of the Project

General Objectives of the Project

The general objective of the "Metadata registry for the ERN RITA" (MERITA) project is promoting the interoperability of the RITA network registries so far identified and potentially with the other ERNs. This will maximize the adherence of RITA members to the ERDRI platform and foster the set-up of a new registry for sharing clinical data provided by RITA registries according to the European Commission's Joint Research Centre (JRC) standards with the unique expertise gained by the PRINTO network inin the interoperability of its own registries. Moreover, the ERN RITA clinical consultation platform CPMS will be adapted so that the data collected, similarly to the other RITA registries, will be directly transferable into the MERITA registry.

Specific Objective(s) of the Project

ID	Title and Description
1	**ERDRI registration.** To complete the enrolment of RITA registries in ERDRI.
Process Indicator(s)	**Target value**
RITA registries to be registered in the European Directory of Registries (EDR) of ERDRI.	
RITA registries data dictionaries will be registered in the Central Metadata Repository (CMR) of ERDRI.	
Output Indicator(s)	**Target value**
List of RITA registries to be registered in ERDRI.	
Outcome/Impact Indicator(s)	**Target value**
Type of data collected in RITA registries as per ERDRI requirements.	

ID	Title and Description
2	**MERITA registry**. To create a metadata registry collecting Common Data Elements (CDE) from existing (and future) RITA registries.
Process Indicator(s)	**Target value**
MERITA registry to be designed.	
CDE of RITA registries to be collected.	
Output Indicator(s)	**Target value**
CDE of RITA registries are available in MERITA registry.	

Outcome/Impact Indicator(s)	Target value
Future possibility of transnational research on RID based on data collected in the MERITA registry.	

ID	Title and Description
3	**MERITA consensus conference**. To reach consensus among RITA registries for future merging/interoperability of other common database fields.

Process Indicator(s)	Target value
MERITA registry consensus.	
Consensus on further standard data from RITA registries in addition to those required by the JRC.	

Output Indicator(s)	Target value
List of additional common RITA fields to be provided in MERITA registry.	

Outcome/Impact Indicator(s)	Target value
Availability of additional information from MERITA registry in addition to the CDE.	

Stakeholder - Target Groups

MERITA project will potentially affect quality of life of all the patients with RID across Europe and beyond. MERITA project will support the activity of the RITA network that includes 126 sites of whom 45 are HCPs and eight are patients and family organizations. In the ERN RITA disease spectrum more than 50 national and international registries enrol more than 55,000 patients from 14 European countries and many others from countries outside Europe. The MERITA project will also confront with the project of the other ERNs in order to increase the harmonisation of the different initiatives. The overall outcome will also serve as reference tools for pharmaceutical companies (large and small) aiming to develop new targets for the treatment of patients with RID and to evaluate the efficacy of any experimental drugs.

The overall aim of the MERITA project is therefore to reach different stakeholders including - but not limited - to health professionals, patients, Patient Organisations, other ERNs and industries.

Political Relevance

Contribution to the Annual Work Plan

Priorities defined in the Annual Work Programme highlight the importance of patient registries and databases as key instruments to develop clinical research in the field of RDs. Registries serve as a recruitment tool for the launch of studies focusing on diseases aetiology, pathogenesis, diagnosis or therapy.

The MERITA project will contribute reaching these priorities supporting the ERDRI initiative and creating a common web-based platform, called the MERITA registry, where data about patients with RID from several rare disease registries will be pooled in order to achieve a sufficient sample size for epidemiological and/or clinical research in the field and healthcare quality evaluation and planning. The MERITA registry will promote the interoperability between RITA registries according to the JRC standards. During the MERITA consensus conference, RITA members will compile additional sets of data in order to follow up the natural course of these diseases, building a European cohort of patients with RID. The MERITA registry will collect data on patients affected by all diseases considered as related to an immune dysregulation and covered by the ERN RITA, with specific focus on PID, AID and AI, as listed in the RITA website. Furthermore, the MERITA project will establish the first RITA infrastructures in order to demonstrate its efficient and validated organization.

It is envisioned that once the MERITA registry system will be in place with the first data transfer of anonymous data all the following longitudinal update, in case no further funding will be available, will be done on a volunteer base by the registry holders.

Added Value

The MERITA project will provide a replicable, transferable and sustainable primary example for future larger harmonisations of registries among ERNs. The harmonization of RITA registries will be performed according to the JRC standards, implementing the EU legislation and contribute to the complementarity, synergy and compatibility of RITA registries with the EU Member State policies and programmes, including compatibility with the European Platform on Rare Diseases and the ERDRI. The MERITA project will also promote new cross-sectional research about RID with data re-use opportunities.

Pertinence of Geographic Coverage

The MERITA project will potentially involve all HCPs and patient organizations related to the RITA network as well as those providing data for member registries not formally included in RITA. Each RITA member and others with expertise in the diagnosis and treatment of patients with RID will provide clinical data according to the local public health practices and policies, taking into account the geographical, cultural, legislative and social diversity. Transnational integration of Centers from Member States whose gross national income per inhabitant is less than 90% of the EU average will be encouraged. The RITA survey served as a tool to identify the partners of this application that involve the following countries: Czech Republic, Finland, Germany, Greece, Latvia, Lithuania, Netherlands, Poland and Slovenia, . In addition, it is expected that also registries in countries that are not official partners of this application, but members of RITA will also be involved such as the following countries: France, Sweden, Belgium, Spain, and United Kingdom.

Context of the project work

Research in RID is constrained by the lack of sufficiently large cohorts and data resources in any single country. Web access to the MERITA registry will overcome this important obstacle and enable larger evidence-based research. Research on the utility and validity of clinical diagnostic tests and efficacy of different treatment strategies will implement local protocols dedicated to patients with RID. The confidentiality and protection of personal data will be secured in the MERITA registry by full anonymisation of data. Regarding data already present in existing registries, each local registry committee assures the compliance of all ethical aspects. In this view, we will take into account the experience derived from the EUROFEVER and PHARMACHILD registries. One of the main aims of the EUROFEVER project was to improve the possibility of an early diagnosis in children suspected of AID in all European countries. The project supported the possibility of sharing among the project members the DNA samples of patients with suspected AID, in order to perform genetic tests even in patients living in countries where it was not possible to obtain a molecular analysis. For patients with undefined recurrent fevers, and thus suspected of *MEFV*, *TNFRSF1A*, *NLRP3*, and *MKD* gene mutations, a validated classification score is available at the EUROFEVER website (https://www.printo.it/eurofever/scoreCriteria.asp), supporting and harmonizing the decision process of performing molecular tests. Furthermore, patients with AID were longitudinally followed, having the opportunity to extend the molecular analysis according late-onset manifestations and outcomes. Similarly, PHARMACHILD now constitutes a benchmark for industries for pharmacovigilance issues such as comparison of adverse events of specific drugs toward the general safety events available in the registry.

Methods and Means

ERDRI registration

In order to complete the enrolment of RITA registries in ERDRI, we will create a group of registry holder members, each one involved in a different disease registry detected by the RITA registries survey (MERITA members). We will choose the participants according to the covered role in the registry committee, giving preference to coordinators. Five applicants of the MERITA project (AUTH, UKCL, UL, IPCZD, and LSMU) will provide the full ERDRI registration of 10 RITA registries. Additionally, the RITA CPMS registry will be adapted so that the data collected will be directly transferable into the MERITA registry.

MERITA registry

To create a metadata registry collecting Common Data Elements (CDE) from existing (and future) RITA registries we will use as starting model the metaregistry already implemented by PRINTO with the Pharmachild and the Eurofever registry.

In particular PHARMACHILD and EUROFEVER were two databases developed by PRINTO sharing some data elements: demographic, drug therapy, safety, and code anonymization system. During the last year, PRINTO decided to merge the two registries in a new database, called PRINTO registry, with the scope to simplify data entry for PRINTO members since they can use the same application for several projects. Currently, the new registry manages six projects with more than 17,000 enrolled patients.

PRINTO registry is a web application written in php and javascript that uses MS SQL server as backend database. Personal patient data, as name, surname, date of birth, and country of birth, are pseudonymized. A user password allows to encrypt/decrypt patient data only by the users belonging to a specific center. Center password is a second private password provided to all PRINTO members. All the passwords are stored in a server of the Istituto Giannina Gaslini certified by different commercial provider: ISO 9001:2015, ISO 27001:2013, ISO 27018:2014 and ISO 27035:2016. The International Coordinating Centre communicates with PRINTO members using a private code as reference for subjects and manages only the server running the application. Only authorized users belonging to each center can enter, modify or view personal data of their patients. PRINTO registry application is developed following part 11 of the FDA 21 CFR and ICH-E6 GCP indications. IGG and GUH will send a survey to MERITA members in order to collect data about the presence of CDE in each RITA registry. IGG and GUH will design MERITA registry based on the specific expertise for database merging developed during the PRINTO registry project and

define IT standards for importing CDE in MERITA registry. A service for importing files will be evaluated during the MERITA consensus meeting when the final version of the new database will be presented to MERITA members. Furthermore, additional fields that the MERITA registry could import from existing RITA registries besides those required by the JRC will be identified. Finally, IGG and GUH will prepare a set of SOP to import procedures to MERITA and work on a web service for MERITA members to import their data to MERITA registry.

MERITA consensus conference.

Several Delphi survey(s) will be implemented among holders of RID registries in order to solicit by each of them, which additional elements, besides the CDE might be useful for research purposes in the field of RID. All participants to the Delphi survey will then prioritise these potential common additional elements. Finally, a consensus conference will be organised to reach consensus among a selected group of RITA related registry holders for final and future merging/interoperability of other common database fields.

Expected Outcomes And Benefits of the Project

Thanks to the MERITA project, all RITA registries which will agree to participate will be enrolled in ERDRI increasing the interoperability with other ERNs. The MERITA registry will collect data from RITA registries according the JRC standards supporting research and interoperability in the field. The MERITA registry provides an example of harmonization/interoperability of clinical data from different registries supporting similar projects in the future.

Different stakeholders will be the target of the MERITA project. In particular, health professionals working in the field of RID will have a clear picture of where exactly patients with a certain RID were diagnosed and followed. Similarly, patients and family organisation will have an international reference of health professionals to whom redirect their members in search of adequate expertise. Other ERNs will share experience and knowledge about patients with other immune-mediated diseases, enlarging the interoperability of the networks in Europe. Research aiming at a comparison of patient characteristics and diagnosis and treatment strategies between Europe and other countries or international organizations will be possible, in order to develop comparable and reproducible health strategies for patients with RID. Finally, industries with specific interests in developing drugs and diagnostic tests for patients with RID will be able to share with a group of experts their strategies, supported by an adequate amount of data derived from the compilation of the MERITA registry. It is also envisioned that once the MERITA tool will be up and running in the future might be used by new stakeholders not currently part of this endeavour.

Work Packages

Overview of Work Packages (WPs)

WP No.	WP Title	WP Description
WP.1	MERITA-C	MERITA Coordination team (MERITA-C) will orchestrate the scientific and administrative aspects of the MERITA project, ensuring cooperation between applicants, support internal communications and meetings, secretariat and logistics.
WP.2	MERITA-D	MERITA Dissemination team (MERITA-D) will guarantee the dissemination of the MERITA project through the ERN RITA and beyond. MERITA-C will organize MERITA consensus conference.
WP.3	MERITA-E	MERITA Evaluation team (MERITA-E) will organize conference calls between RITA coordinator, RITA registry WP coordinator, RITA patient organizations and MERITA-C to discuss project development and criticism.
WP.4	ERT	ERDRI Registration Team (ERT) will be responsible of the ERDRI registration, acquiring data dictionaries from each RITA registry and entering data in ERDRI.
WP.5	MERITA-R	MERITA Registry team (MERITA-R) will design the MERITA registry; acquire CDE from each RITA registry; and merge data coming from the different registries.

Work Packages (WP) Description

WP No.	WP.1											
WP Title	MERITA Technical Coordination team (MERITA-C)											
Starting month	M0			**Ending month**			M36					
Leading Applicant	IGG											
Applicants No.	1											
Applicants Acronym	IGG											
Person Months	0,2/36											
Objectives MERITA-C will orchestrate the scientific ad administrative aspects of the MERITA project, ensuring cooperation between working groups, support internal communications and meetings,												

secretariat and logistics based on PM2 open project management methodology.
Description of work MERITA-C will provide bi-annual internal meeting between the responsible of each working group and the MERITA Steering Committee, in association with the midterm and final internal reviews.
Deliverables linked to this WP D1.1 Midterm report (M18) D1.2 Final report (M36)
Milestones to be reached by this WP M1.1-7 Internal meeting/call conference (M0, M6, M12, M18, M24, M30, M36)

WP No.	WP.2											
WP Title	MERITA Dissemination team (MERITA-D)											
Starting month	M0			**Ending month**			M36					
Leading Applicant	IGG											
Applicants No.	1	2	3									
Applicants Acronym	IGG	UMCU	CCI									
Person Months	0,15/36	0,15/36	0,15/36									

Objectives MERITA-D will guarantee the dissemination of the MERITA project through the ERN RITA and beyond, sharing the concept and progress of the project with the wider research community throughout the project lifetime. Key audiences will be professional networks (e.g. PRINTO, ESID, etc.) and patient-led organisations (e.g. RIPAG, IPOPI, etc.).
Description of work MERITA-D will be responsible for the publication of mandatory and other deliverables, the communication within the ERN RITA, the sharing of project results with target audience including other ERNs, academia, pharmaceutical companies, patients, participating scientific organizations, advocacy organizations, policy makers and the public.
Deliverables linked to this WP MD.1 MERITA leaflet (M3) MD.2 MERITA registry dashboard (M3) MD.3 Layman version of the final report (M36)

D2.1 MERITA consensus conference (M12)
Milestones to be reached by this WP M2.1 MERITA results publication (M36)

WP No.	WP.3											
WP Title	MERITA Evaluation team (MERITA-E)											
Starting month	M0			**Ending month**			M36					
Leading Applicant	UMCU											
Applicants No.	1	2	3									
Applicants Acronym	IGG	UMCU	CCI									
Person Months	0,1/36	0,1/36	0,1/36									
Objectives The RITA coordinator, the RITA registries working group coordinator, the ESID registry working group coordinator, MERITA members and RITA patient organizations will participate in the MERITA-E. Their roles include: - validation of ethical, privacy and confidentiality approach; - review and endorsement of study design; - input into analysis of research results; - communication and dissemination to patients, policy makers and healthcare providers; - collaboration in the design and delivery of the MERITA registry and associated training materials and events.												
Description of work MERITA-E will organize periodic meetings/conference calls to discuss project development and criticism. A midterm and final report will be produced. A plan will be agreed for the long-term sustainability of the MERITA registry.												
Deliverables linked to this WP D3.1 Plan for the long-term sustainability of MERITA registry (M36)												
Milestones to be reached by this WP M3.1-7 Evaluation meetings/call conferences (M0, M6, M12, M18, M24, M30, M36)												

WP No.	WP.4		
WP Title	ERDRI Registration Team (ERT)		
Starting month	M0	**Ending month**	M12

Leading Applicant	UKCL											
Applicants No.	5	6	7	8	9							
Applicants Acronym	AUTH	UKCL	UL	IPCZD	LSMU							
Person Months	0,25/12	0,25/12	0,25/12	0,25/12	0,25/12							

Objectives

To complete the enrolment of RITA registries in the ERDRI platform.

Description of work

ERT will acquire descriptive data and data dictionaries from each RITA registry, upfit data for the ERDRI platform and register data in EDR and CMR. Each applicant of the ERT will be responsible of the full ERDRI registration of 10 RITA registries.

Deliverables linked to this WP

D4.1 ERDRI registration complete leaflet (M12)

Milestones to be reached by this WP

M4.1 EDR registration complete (M6)

M4.2 CMR registration complete (M12)

WP No.	WP.5											
WP Title	MERITA Registry team (MERITA-R)											
Starting month	M0			**Ending month**	M30							
Leading Applicant	IGG											
Applicants No.	1	2	10									
Applicants Acronym	IGG	GUH	CCI									
Person Months	0,25/36	0,25/36	0,25/36									

Objectives

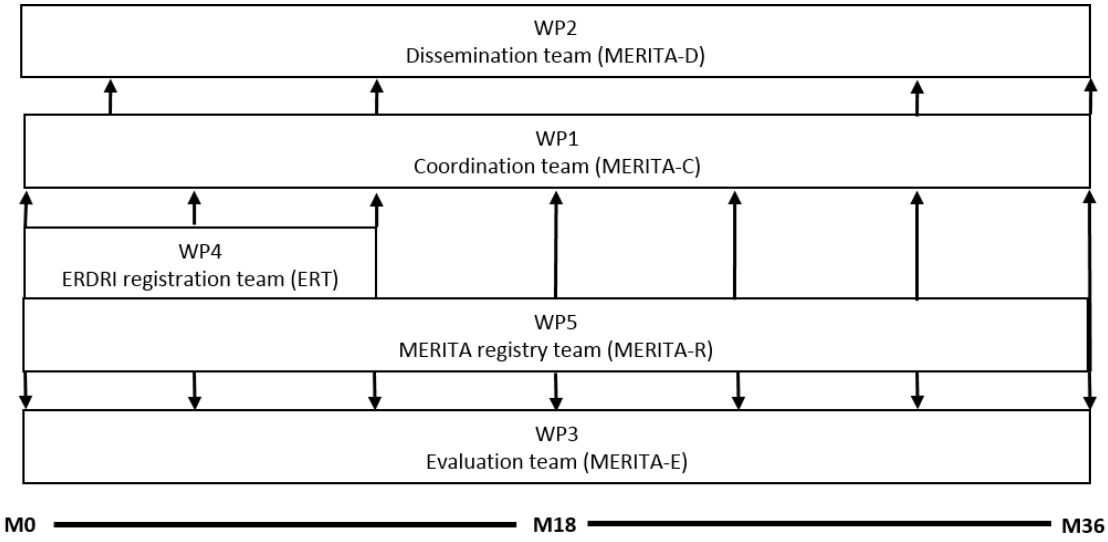
To create a metadata registry collecting CDE from RITA registries.

Description of work

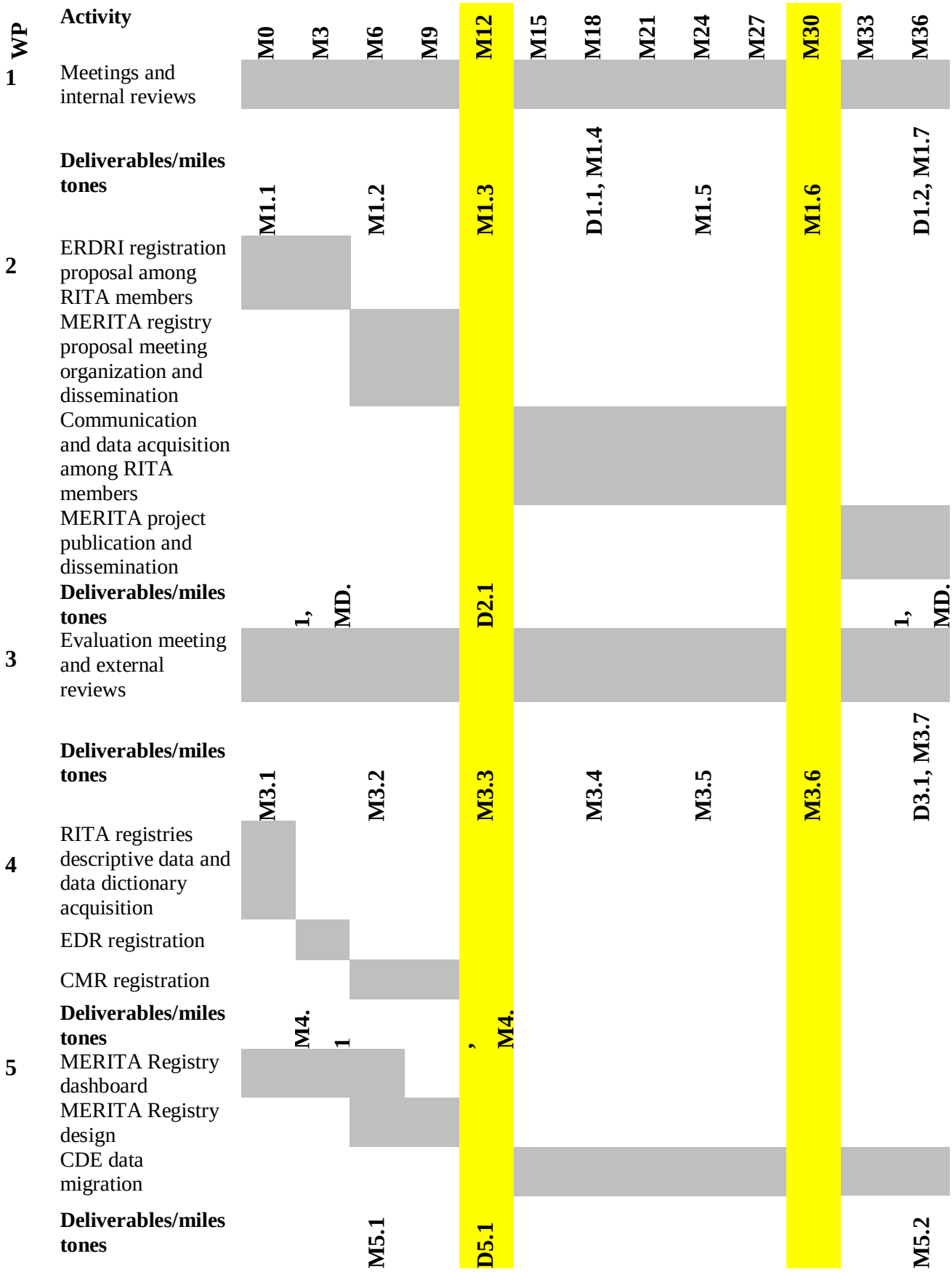
IGG, GUH and CCI will agree on the structure of the MERITA registry based on the PRINTO and ESID registries and CDE. MERITA-C and MERITA-E will validate the MERITA registry draft. MERITA-R will acquire the CDE from each RITA registry in a complete and fully anonymous format and organize the raw data migration from each individual RITA registry (up to 50) to the MERITA registry.

The MERITA registry will be fully operative for the MERITA final consensus conference to be held before the end of the project.
Deliverables linked to this WP D5.1 MERITA registry (M12)
Milestones to be reached by this WP M5.1 MERITA registry draft (M6) M5.2 MERITA registry complete (M36)

Figure 1. Timetable of the MERITA project.



Timetable and Milestones



Deliverables

Dlvrbl No.	Deliverable Name	WP No.	Leading Applicant	Content Specification	Dissemination Level	Delivery Month
D2.1	MERITA proposal meeting	WP.2	IGG	A meeting among RITA members to present the registry.	C	M12
D5.1	MERITA registry	WP.5	GUH	Development of a web-based registry to collect JRC standards data from RITA registries.	C	M12
D4.1	ERDRI registration leaflet	WP.4	ERT	A leaflet informing RITA members about ERDRI registration on the RITA web site.	C	M12
D1.1	Midterm report	WP.1	IGG	Midterm project report.	C	M18
D1.2	Final report	WP.1	IGG	Project final report.	C	M36
D2.2	Results publication	WP.2	IGG	A published peer-reviewed brief report presenting results of the project.	P	M36
Administrative Deliverables (ADs) & Mandatory Deliverables (MDs)						
MD.1	MERITA Leaflet	WP.2	IGG	A leaflet promoting the MERITA project will be available on the RITA web site.	P	M3
MD.2	MERITA website	WP.2	IGG	MERITA registry dashboard will be available on the RITA web site.	P	M3
MD.3	Layman version of the final report	WP.2	IGG	Short version of the final report for RITA members will be available on the RITA web site.	P	M36

Project management

Methods

All members of this application will be involved in the identification and facilitation of issues/risks/changes/decisions management during the MERITA project according the PM2 management methodology, guaranteeing the achievement of specific objectives and the quality of generated deliverables.

Governance, Roles, Responsibilities

We provided the Governance Structure of the MERITA project according the PM2 Methodology of the European Commission (Figure 2) and assigned Roles and Responsibilities according the RASCI model.

The ERN RITA Network Board will act as Appropriate Governance Body (AGB) and consists of:

1. the RITA network coordinator;
2. the RITA subtheme coordinators for PID, AI, AID, and paediatric rheumatology;
3. two RITA Patient Advocacy Group (RIPAG) representatives, and in particular:
    a. the representative of the International Patient Organization for Primary Immunodeficiencies (IPOPI);
    b. the representative of the European Patient Advocacy Group (ePAG).

The MERITA Project steering committee (PSC) consists of:

1. The MERITA grant coordinator;
2. the RITA network coordinator:
3. the ESID registry representative;

The MERITA project manager will act a secretary of the Steering Committee with no voting duties.

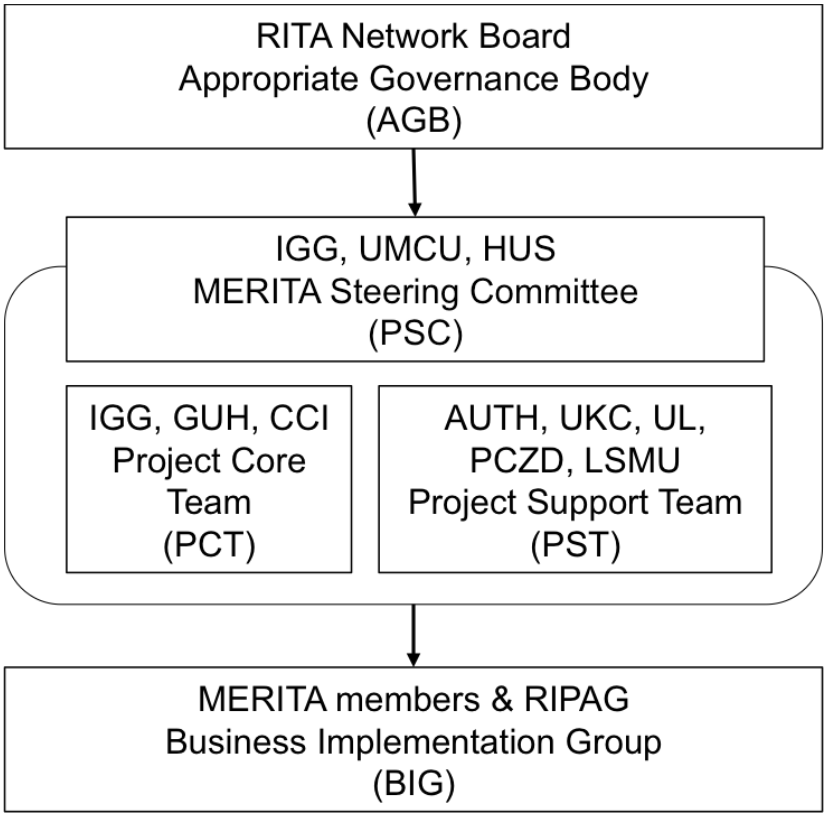
The MERITA Project Core Team (PCT) consists of the IT developers (IGG, CCI and GUH).

The MERITA Project Support Team (PST) consists of one representative from each applicant involved in the ERDRI registration Team (ERT).

All the fifty MERITA members involved in a RITA registry and the RIPAG Council will act as Business Implementation Group (BIG).

RAM (RASCI)	AGB	PSC	PCT	PST	BIG
Meetings and internal reviews	R	A	S	C	I
ERDRI registration proposal among RITA members	I	R	A	S	C
MERITA registry proposal meeting organization and dissemination	I	R	A	S	C
Communication and data acquisition among RITA members	I	R	A	S	C
MERITA project publication and dissemination	S	R	A	C	I
Evaluation meeting and external reviews	R	A	S	C	I
RITA registries descriptive data and data dictionary acquisition	I	R	S	A	C
EDR registration	I	R	S	A	C
CMR registration	I	R	S	A	C
MERITA Registry dashboard	I	R	A	S	C
MERITA Registry design	I	R	A	S	C
CDE data migration	I	R	A	S	C

Figure 2. Governance Structure of the MERITA project.



Processes & Artefacts

According the PM2 management methodology, MERITA-C will set up a Project Handbook in the format of a study protocol that will summarise for each WP the specific task for each partner in a comprehensive manner. In addition a project log will be regularly updated every month in order to provide a progressive report with the outcome reached by a certain date including all deliverables. Any unforeseen risk will be solved by the project steering committee through dedicated web teleconferences.

Quality & Capacity of the Partnership

The MERITA project is the direct expression of the Working Group on Registries and Biobanks of the ERN RITA that gathers HCPs involved in the care of RID belonging to most European countries. The main objective of the Working Group is to provide a census of already running registries in Europe, potentially from local initiatives to international realities, in order to give a precise picture of the number of registries, number of patients, conditions examined, type of information collected, to encourage rationalization and efficiency of already existing and future registries.

In particular, the coordinator is the Chairman of the Working Group and all associated partners are members with a relevant specific expertise in RID. All these individuals are actively involved in the care of affected patients and in many aspects concerning basic and clinical research of RID. The other members of the ERN that are not directly involved in the Working Group will participate to the collection of data on already diagnosed and suspected patients and will actively collaborate to the dissemination of the information in their national context.

All the participants are strongly motivated toward the present project that could represent an extraordinary tool for a better understanding of these disorders.

The cooperation with the already existing PRINTO and ESID networks guarantees the maximum coverage of this initiative on a European basis and beyond.

Capacity of the staff to carry out the project

App. No	App. Name	Name	Website	Competence, expertise, leadership quality and authority
1	IGG	Clinica Pediatrica e Reumatologia, IRCCS Istituto Giannina Gaslini	www.gaslini.org	IGG is the coordinator of this application. IGG is a multidisciplinary, highly specialized reference centre with national and international catchment area. It is the only hospital in Italy providing specialist and sub-specialist care, both medical and surgical, for mothers and children. Therefore, it is a national referral centre for rare and complex diseases affecting newborns, children, and adolescents requiring highly specialized treatment. The hospital staff members are over 1,800, including 264 physicians and 845 nurses. On average, hospitalizations are over 29,800/year and outpatient admissions over 536,000/year. More than 42% of patients come from all regions of Italy, mainly Sicily, Piedmont, Apulia, Campania, Lombardy, and Tuscany. Annually, 4,4% of patients come from abroad. The Gaslini meets the international excellence standards for safe health care delivery. The Institute has been accredited by Joint Commission International since 2007. It counts over 25 reference and highly specialized centres. The Gaslini hosts facilities and staff of the University of Genoa under a formal agreement. The Gaslini has established collaboration agreements with over 250 national and international institutions and HCPs all over the world. The annual Impact Factor, an international bibliometric indicator, is over 1,550 with over 300 publications in international journals. The Paediatric Rheumatology Clinic received from the EULAR (European League Against Rheumatisms), the accreditation to Center of Excellence in Rheumatology (years 2008-2023), the only one in Italy and one of the few for Paediatric Rheumatology in Europe. IGG host the international coordinating centre of PRINTO (www.printo.it) and Eurofever (https://www.printo.it/eurofever/index.asp).
2	UMCU	Department of Pediatrics, Section Pediatric Rheumatology, Wilhelmina	www.hetwkz.nl	Our research group is a product of both the clinical department of pediatric rheumatology and the translational research groups from the Laboratory for Translational Immunology (LTI). The first one is the largest academic center for juvenile arthritis and other pediatric rheumatic diseases in the

		Children's Hospital, University Medical Centrum Utrecht		Netherlands. Since 2016, we are listed as EULAR Center of Excellence as only the 2nd pediatric rheumatology department worldwide. We diagnose around 40 patients / year and follow around 600 patients in our clinic. Our translational research group combines both fundamental and clinically orientated projects and conducts several investigator initiated clinical trials. We have coordinated multiple international research projects including EUTRAIN (2012-2015, on translational research in juvenile arthritis), SHARE (2012-2015, developing international best practices on diagnosis and treatment for rare pediatric rheumatic diseases). Currently we are 1 of the 2 leading centers in UCAN-CANDU, a Dutch Canadian Collaborative research project aiming to develop personalized treatment regimes in JIA. Importantly, we also manage the UCAN-U platform, developing novel research techniques, standardizations and operation procedures allowing for international collaborative efforts in pediatric rheumatology.
3	GUH	Centre for paediatric rheumatic and autoinflammatory diseases, General University Hospital, PragueCharles University	www.vfn.czen.lf1.cuni.cz	The General University Hospital in Prague is a major medical facility designated by the Ministry of Health of the Czech Republic. It provides highly specialized outpatient and inpatient care for children and adults with a wide disease spectrum including rare diseases covering 5 ERNs where it is a full member. In addition to providing health care, GUH is the main teaching base of the First Faculty of Medicine of Charles University in Prague and one of the most important scientific institutes in the field of therapeutic and diagnostic methods in the Czech Republic. It hosts the national PRINTO coordinating centre and leads the ERN RITA IT/eHealth working group and Operational Helpdesk. The paediatric rheumatology team has been involved in multiple international projects with the leading role in some of them including paediatric vasculitis outcome project and one of the SHARE workpackages. It is a leading paediatric rheumatology, autoinflammatory diseases and adult vasculitis centre in the country following over 800 patients with RDs within the RITA spectrum. First Faculty of Medicine with almost 1 200 staff members and 3 400 students represents the largest medical faculty in the Czech Republic. It is one of the 17 faculties and an integral part of Charles University from its foundation by the King of Bohemia and Emperor of the Holy Roman Empire Charles the Fourth in 1348. As such, it is

				also the oldest medical faculty in Central Europe.
4	AUTH	Hippokration General Hospital, Thessaloniki University School of Medicine	www.auth.gr	The School of Medicine of the Aristotle University of Thessaloniki is one of the most important and well-established Schools of Greece, both quantitatively and qualitatively, with 4,000 registered students and 82 departments and laboratories.
5	UKCL	University Children's Hospital, University Medical Centre Ljubljana	www.kclj.si	University Medical Centre Ljubljana (UKC) is the largest and most research-intensive hospital in Slovenia. Children's Hospital Ljubljana is an integral part of the UKC and is the largest centre dedicated to improving children's health in the country with a long history of clinical and research excellence serving as the national and regional tertiary referral centre for paediatric patients from Central and South Eastern European countries. Department of Allergology, Rheumatology and Clinical Immunology is part of the Children's Hospital Ljubljana and provides comprehensive multidisciplinary services for children with inflammatory paediatric rheumatic diseases. The department is the only referral centre for patients with paediatric rheumatic diseases in Slovenia and is integrated within the national, regional and international networks including PRES, PRINTO, SHARE, Pharmachild, Eurofever and UCAN-U.The Ljubljana University Medical Centre is the largest hospital centre in Slovenia. It was officially opened on 29 November 1975 and has over 2,000 beds and over 7,800 employees, making it one of the largest hospital centers in Central Europe. UKCL'Cntral Europetps://en.wikipedia.org/wiki/Central_Europe" \o "" tion and research. UKCL is the only hospital in Slovenia providing specialized care for some diseases, including the rare diseases with particular interest in genetic inherited disorders like inborn errors of metabolism and primary immune deficiencies.
6	UL	University of Latvia	www.lu.lv	UL study directions are represented by 131 accredited study programmes, including 53 undergraduate programmes, 54 graduate and 24 doctoral study programmes. Total student count, including P. Stradins Medical College of the University of Latvia and UL RMC in 2016/2017 is 14290, including 9807 undergraduate and 4483 graduate programme students. The number of students at the UL and its colleges constitutes 17% of the total student population in Latvia. The total number of recipients of professional qualifications,

				academic and scientific degrees in 2016/2017 stands at 3615, including 2279 graduates of undergraduate programmes and 1336 graduates of graduate programmes.
7	IPCZD	Warsaw Children's Memorial Health Institute	www.czd.pl	The Children's Memorial Health Institute is the largest and best-equipped institute of paediatric healthcare in Poland. Located in Warsaw and directly subordinate to Poland's Ministry of Healthcare, the center employs roughly 2,000 physicians and staff, and includes 17 ward and 29 disease-specific out-patients clinics. It collaborates with Poland's leading medical schools as well as non-governmental organizations.
8	LSMU	Hospital of Lithuanian University of Health Sciences, Kauno Klinikos, Kaunas	www.lsmuni.lt	Hospital of Lithuanian University of Health Sciences Kauno klinikos is the largest health care institution in Lithuania. Established in 1940 the hospital has grown into one of the most prominent hospitals in the country. Hospital of Lithuanian University of Health Sciences Kauno klinikos provides emergency medical care, in-patient hospital services and outpatient testing and services. The highest quality of health care services is available to patients from all the country and abroad. Health care is delivered according to European standards, evidence-based medicine is provided by highly qualified medical staff: at present more than 1200 medical doctors and 2500 nursing specialists are working at the Hospital. Besides, close cooperation with Lithuanian University of Health Sciences, Kaunas klinikos has established the Rare diseases management coordination center. This center consolidates 19 centers for rare diseases. The main peculiarity of center organization is that leading Kauno klinikos physicians are brought together in each center in order to co-operate in rare disease diagnosis and treatment. A total of 35 departments with different clinical profiles are situated in 15 buildings. More than 2000 patients can be treated at inpatient departments at a time. There are 15 departments for outpatient care. In 2009 there were about one million outpatients and 84 000 inpatients.
9	CCI	*Institute for Immunodeficiency, Center for Chronic Immunodeficiency, Medical Center - University of*	https://www.uniklinik-freiburg.de/institut-fuer-immundefizienz/institut-fuer-immundefizienz.htmlims.uniklini	The Center for Chronic Immunodeficiency (CCI) is an internationally recognized institution in Germany where immunologists, infectious disease specialists, hematologists and transplant physicians collaborate to provide optimal care for immunodeficient patients of all age groups. The excellent translational research program allows rapid transfer of research results into state-of-the-

		Freiburg ***(CCI)***Center for Chronic Immunodeficiency, Clinical Research Unit, University of Freiburg Medical Center	k-freiburg.de	art diagnosis and treatment of patients with immunodeficiency. The CCI provides clinical and laboratory services to pediatric and adult patients with diseases of the immune system associated with infection susceptibility, inflammation or autoimmunity. This includes an active program of stem cell transplantation for pediatric and adult patients with severe immunodeficiencies. CCI hosts the international ESID Registry.

App. No	**App. Name**	**Key staff**	**Academic qualification**	**Professional experience, competence, expertise, leadership quality and authority**
1	IGG	Nicola Ruperto	*MD, MPH*	Senior Scientist of PRINTO (*www.printo.it*) with over 20 years of research experience. Dr. Ruperto is among the founders of the Paediatric Rheumatology International Trials Organisation (PRINTO at www.printo.it), where he has managed all collaborative international academic research projects conducted by the network with over 42,000 data collected from children with pediatric rheumatic diseases. He was awarded the 2005 Gerolamo Gaslini Prize for Excellence in Research from the Gerolamo Gaslini Foundation. Metrics: Over 300 peer reviewed manuscripts: h-index 73, citation > 20,000. Listed in the top Italian scientist (www.topitalianscientists.org/). Over 200 oral presentation in international or national meeting.
1	IGG	Riccardo Papa	*MD*	Paediatrician at AID and PID Centre, IGG, Genoa, Italy. Dr. Papa works at the EUROFEVER project since May 2011 with particular focus on TRAPS, genotype-phenotype correlation (*https://www.printo.it/eurofever/correlation.asp*) , and undefined AID.
2	UMCU	Nico M. Wulffraat	*MD, Prof.*	Nico Wulffraat is professor of pediatric Rheumatology at the department of pediatrics, University Medical Center, Utrecht, The Netherlands. Since 2010 he is the head of this subunit. From 1997-2004 he was also coordinator of the pediatric allogeneic and autologous stem cell transplantations for immune deficiencies, metabolic disorders and autoimmune diseases. He is PI of several investigator initiated trials of immunisations in children with rheumatic diseases including MMR, HPV and Men C, prevention of MTX

				side effects, and application of Mesenchymal Stem cells in refractory GvHD and JIA. He is coordinator of a FP7 consortium for pharmacovigilance of biologics used in JIA (PHARMACHILD). Also he leads the EHHC project SHARE that aims at documenting standards of care and treatment recommendations for pediatric rheumatic diseases throughout Europe. Since 2008 he became supervisor of the postgraduate clinical fellowship pediatric rheumatology and since 2009 head of pediatric immunology and rheumatology. In 2014, he was appointed as head of pediatric Rheumatology, Immunology, hematology and infectious diseases. He has co-authored 220 Pubmed cited papers in international journals on pediatric rheumatology and immunology and 12 chapters in books.
3	GUH	Dolezalova Pavla	MD, Prof.	Professor of paediatrics and consultant in paediatric rheumatology, head of the Centre for paediatric rheumatic and autoinflammatory diseases and of the GUH ERN RITA team. National PRINTO coordinator, chair of the IT/eHealth working group of RITA, second Czech representative in the Board of Member States, past chair of the Clinical Affairs committee of the Paediatric Rheumatology European Society council, chair of the national paediatric rheumatology working group and of the Czech Ministry of Health accreditation board for paediatric rheumatology, responsible for paediatric rheumatology clinical fellowship.Pediatric Rheumatology and General Pediatric and Adolescent Medicine, 1st Faculty of Medicine and General Faculty Hospital, Prague, Assistant Professor of Pediatrics (consultant level) since 1997. Associate Professor of paediatrics, Head of the Paediatric Rheumatology Unit, since 2005. Professor of Paediatrics, Charles University in Prague, 1st Faculty of Medicine, since June 2013. Research interests: Paediatric vasculitis, dermatomyositis, methotrexate mechanism of action and pharmacokinetics, epidemiology of paediatric vasculitis, vasculitis outcome measures, autoinflammatory diseases.
4	AUTH	Maria Trachana	*MD, Prof.*	Pediatrician, Pediatric Rheumatologist and Associate Professor in the Aristotle University of Thessaloniki.
5	UKCL	Tadej Avcin	*MD, Prof.*	Tadej Avcin is Head of Department of

				Allergology, Rheumatology and Clinical Immunology at the Children's Hospital, University Medical Center Ljubljana and Professor of Pediatrics at the University of Ljubljana, Faculty of Medicine. His clinical and research focus are systemic autoimmune and auto-inflammatory diseases with an emphasis on juvenile idiopathic arthritis, systemic connective tissue diseases and diseases of immune dysregulation. He has published more than 130 indexed publications and 14 book chapters on pediatric rheumatology and immunology. He is currently Chairman of the PReS Paediatric Rheumatology Academy, past Chairman of the European League Against Rheumatism Standing Committee on Pediatric Rheumatology, and Member of the Advisory Council of Paediatric Rheumatology International Trials Organisation.Tadej Avcin studied medicine and pediatrics at the Faculty of Medicine, University of Ljubljana (Slovenia) and completed fellowship in pediatric rheumatology at the Hospital for Sick Children, University of Toronto (Canada). His clinical and research focus are systemic autoimmune and auto-inflammatory diseases with an emphasis on juvenile idiopathic arthritis, systemic connective tissue diseases and diseases of immune dysregulation. He has published more than 125 indexed publications and 14 book chapters on pediatric rheumatology and immunology. He is currently Chairman of the Education and Training Committee of Paediatric Rheumatology European Society, past chairman of the European League Against Rheumatism Standing Committee on Pediatric Rheumatology, and Member of the Advisory Council of Paediatric Rheumatology International Trials Organisation.
6	UL	Ingrida Rumba-Rozenfelde	*MD, Prof.*	My major research fields are paediatric chronic diseases, rheumatology and autoimmune diseases studying epidemiology, genetic markers, viruses in pathogenesis, outcomes and prognostic factors. I am very involved into medical education so I am doing research in this area although. I am supervisor of 3 research students.
7	PCZDI PCZD	Beata Wolska Kusnierz	MD, PhD	A graduate from the Medical University of Warsaw in 1996. Board certified pediatrician since 2005 and clinical immunologist since 2010.Research assistant, since 1997 and Senior

				Research Assistant in the Clinic of Immunology at the Institute "Memorial – Children's Health Center" since 2008. A member of the Polish Society of Experimental and Clinical Immunology, the European Society for Immunodeficiencies, Polish Society of Vaccinology. Dr. Wolska-Kuśnierz is focused in her clinical work on diagnosing and treatment of primary immunodeficiency diseases, autoimmune diseases, particularly recurrent fever syndromes, guidance on the implementation of mandatory and recommended vaccinations.
8	LSMU	Ausra Snipaitiene	MD	Pediatrician rheumatologist and Pediatric cardiologist specialised in Cardiology and Rheumatology, with an interest in JIA and in ultrasound assessment. Highly interested in pediatric rare rheumatologic conditions as neurolupus, CRMO and Kawasaki disease.
9	CCI	Gerhard Kindle and Mikko Seppanen	MD, MSCSPhD and Prof.PhD	Gerhard Kindle is the IT and Database coordinator at the CCI in Freiburg,.f ormer Chairperson of the ESID Registry Working Party and curator of the. Responsible for the ESID Registry since 2006database. Mikko Seppänen is the Director of the Rare Disease Center. Hospital for Children and Adolescents, Helsinki University Hospital (HUH), Hospital District of Helsinki and Uusimaa (Finland). Main organizer in 19 Finnish Conferences on primary immunodeficiency diseases. Invited expert on IgG in PIDDs of Finnish National Reimbursement Agency (KELA), of Finnish Medicines Agency (Fimea), and of European Commission (Wildbach, Kreuth). He is the current chairperson of the ESID Registry working partydatabase.

Risk Analysis and Treatment

ID	Risk Title/Description	Likelihood	Impact	Proposed Risk Treatment and contingency plan
R1	RITA registries not participate in the ERDRI registration project.	1	5	We will increase interoperability with ERDRI giving IT support to collect data dictionary and descriptive data from each registry. We will create a list of RITA registries not enrolled in ERDRI for future proposal and discuss with JRC other possibility to increase the participation in ERDRI.
R2	RITA registries not participate in the MERITA project.	2	5	We will support centres to increase interoperability with MERITA. At least, we will create a full merge/interoperability between PRINTO and ESID registries. Based on this example, future registries merging may be motivated.
R3	RITA registries not participate in the MERITA consensus conference.	2	1	We will organize call conferences for RITA members not participating in the MERITA kick-off meeting.
R4	CDE missing in RITA registries.	3	2	Each registry owner will be supported to fit his registry according the JRC standards and HCPs will be stimulated to fulfil CDE information of their enrolled patients. At least, we will import in MERITA those CDE that are present in the registry.
R5	Ethics	2	3	We will support RITA registries about the ethical issues regarding data migration from each registry. We will avoid migration of data with evident ethical conflicts.

Financial management

Financial management will be implemented by the IGG administration in collaboration with key personnel from PRINTO, which currently employs 15 full-time staff (research assistants, web-developers, pharmacist, statistician). The PRINTO group has overseen financial, administrative and scientific issues related to the coordination of the PRINTO research projects that collected over 42,000 patients' data from children with paediatric rheumatic diseases from over 300 centres in more than 60 countries .

IGG will take care, in collaboration with UMCU to monitor each WP and prepare the related required financial and scientific reports. IGG will ensue that co-funding will be transmitted in time to all partners of this application.

Budget

Content description and justification

Summary of Effort

	WP.1	WP.2	WP.3	WP.4	WP.5	Total Person Months
1. IGG	0,2/36	0,15/36	0,1/36	0	0,25/36	0,7/36
2. UMCU	0	0,15/36	0,1/36	0	0	0,25/36
4. GUH	0	0	0	0	0,25/36	0,25/36
5. AUTH	0	0	0	0,25/12	0	0,08/36
6. UKCL	0	0	0	0,25/12	0	0,08/36
7. UL	0	0	0	0,25/12	0	0,08/36
8. IPCZD	0	0	0	0,25/12	0	0,08/36
9. LSMU	0	0	0	0,25/12	0	0,08/36
10.CCI	0	0,15/36	0,1/36	0	0,25/36	0, 5/36
Total Person Months	0,2/36	0,45/36	0,3/36	0,4/36	0,75/36	2,1/36

Third-Party Contributions

No third party contribution is foreseen for this application.

Previous and current grants relevant to the programme

App. Owner	Name	Grant number	Description
Newcastle upon Tyne Hospitals NHS Foundation Trust (United Kingdom)	CEF Telecom ERN RITA Core Services IT Helpdesk	2017-EU-IA-0090	This Action aims at supporting the members of ERN RITA to ensure adequate and efficient use of the core service platform (i.e. the European Reference Networks Collaborative Platform (ECP) and CPMS. The Action will set-up an Operational Helpdesk, adjusted to the specific needs of ERN RITA, including purchase of the necessary hardware and software. The Helpdesk will support the multidisciplinary healthcare teams in the organisation of their daily work regarding cases reviewed by the ERN. Through the Operational Helpdesk emerging problems will be discussed and sorted in collaboration with the Core Services IT Helpdesk provided by DG SANTE. The Action will also provide necessary training for the use of ERN Core Services to relevant staff.

Current applications relevant to the programme

App. Owner	Name	Grant number	Description
None			

Exceptional Utility

MERITA project fulfills the criteria of exceptional utility because: 1) at least 60 % of the total budget of the action is used to fund staff and 2) at least 30 % of the budget of the proposed action is allocated to six different Member States whose gross national income (GNI) per inhabitant is less than 90 % of the EU average.

C**ollaborating stakeholders**

Collaborating stakeholders of the MERITA project are RITA members involved in a RID registry coordination or participation (see Table 1). Furthermore, all other RITA members not directly involved in a RID registry will support the project and act as collaborating stakeholder and external experts.

Institution	**Contact Person**	**Country**	**City**
University Hospital Leuven	Carine Wouters	Belgium	Leuven
Hôpital Bicêtre	Isabelle Koné-Paut	France	Paris
Hôpital Cochin	Xavier Puechal	France	Paris
Hôpital Necker-Enfants Malades	Pierre Quartier	France	Paris
Hôpitaux Universitaires de Strasbourg	Anne Sophie Korganow	France	Strasbourg
Klinikum der Universität	Fabian Hauck	Germany	München
Universitätsklinikum	Dirk Foell	Germany	Münster
Universitätsklinikum	Stephan Ehl	Germany	Freiburg
Foundation IRCCS Polyclinic San Matteo	Laura Obici	Italy	Pavia
Pediatric Hospital Bambino Gesù	Fabrizio De Benedetti	Italy	Rome
San Raffaele Hospital	Alessandro Aiuti	Italy	Milan
Spedali Civili	Alessandro Plebani	Italy	Brescia
University Medical Center	Abraham Rutgers	Netherlands	Groningen
Erasmus University Medical Center	Martin van Hagen	Netherlands	Rotterdam
Hospital Universitari Vall d'Hebron	Laura Alonso Garcia	Spain	Barcelona
Karolinska University Hospital	Edvard Smith	Sweden	Solna
Barts Health NHS Trust	Jawad Ali	United Kingdom	London
Teaching Hospitals NHS Trust	Jacqueline Andrews	United Kingdom	Leeds
Hospitals NHS Foundation Trust	Andrew Cant	United Kingdom	Newcastle upon Tyne
Great Ormond Street Hospital for Children in consortium with Royal Free Hospital London NHS Foundation Trusts	Paul Brogan	United Kingdom	London