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Curriculum

REUMATOLOGIA PEDIATRICA

Ciclo XXXV

TYPE I INTERFERON ACTIVATION IN A COHORT OF PATIENTS FROM THE EUROFEVER REGISTRY: CLINICAL AND MOLECULAR ANALYSIS

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Abstract

The role of type I interferon (IFN-I) in autoimmune and autoinflammatory diseases has been widely demonstrated, but its diagnostic role in clinical practice remains a controversial topic. We evaluated 214 consecutive patients, enrolled in the Eurofever Registry with defined or undefined pediatric conditions, for IFN-I activation using real-time quantitative PCR. IFN-I signature was calculated as the median of the relative quantification of six IFN-I -inducible genes compared to healthy controls.

Lymphoid involvement was more frequent among patients with activation of the IFN-I pathway (34.5% vs. 20%; $p<0.02$) compared to IFN-I negative patients. All patients presenting with erythema nodosum had a positive IFN-I score.

A significant percentage of patients with activation of the IFN-I pathway had autoantibodies, confirming the strong link between autoimmunity and innate immune activation.

We identified 14 patients with genetically determined interferonopathy, 18 with autoinflammatory diseases, 69 with autoimmune disorders, 5 with immunodeficiency and 108 patients presenting with other conditions, including undifferentiated systemic autoinflammatory diseases (USAID) (69/198) and undifferentiated recurrent fever syndrome (SURF) (7/108).

Most of the patients affected by systemic erythematosus lupus (SLE) revealed a positive IFN-I signature and six patients with interferonopathies showed a phenotype similar to SLE. The latter had a significantly lower median age at onset and high median values of the interferon signature. These findings could be indicators of the presence of a monogenic disease in patients with atypical SLE. Six patients in this cohort received JAK inhibitors, with a good response in the absence of adverse effects.

The possibility of targeted treatment by JAK kinase inhibitors is the real advantage of early genetic diagnosis with the aim of preventing organ damage and reducing the use of steroid therapy or other immunosuppressants.

Introduction

In recent years, the advances in molecular biology and genetics have led to an understanding, at least in part, of the mechanisms underlying the functional defects of the immune system habitually referred to as immune dysregulation.

Unlike what was previously thought, these defects can lead to a complex spectrum that can include autoinflammatory, autoimmune, or true immunodeficiency conditions, either in the case of hypofunction of the affected cellular pathway or in the case of its hyperfunction. The clinical manifestations that characterize these conditions are extremely variable ranging from recurrent fevers to skin or osteoarticular inflammation, systemic autoimmune syndromes to the risk of even fatal infectious events due to the inability to face pathogens.

Notably, even monogenic defects that result in altered immune system cell pathways can lead to a broad clinical spectrum that varies from patient to patient.

A prime example is alterations affecting the IFN-I pathway.

The COVID-19 pandemic has demonstrated how this pathway is essential in protecting the body from infection since monogenic alterations inducing poor or delayed production of this cytokine mediator have been demonstrated in severe or fatal cases of SARS-CoV-2 infection, even in pediatric age(1).

Single gene mutations leading to constitutive and inappropriate activation of IFN-I pathway are known as type I interferonopathies(2–4). As these are diseases with wide clinical heterogeneity, type I interferonopathies should be excluded in the case of pediatric patients with rheumatologic diseases with atypical presentation, especially in the presence of clinical pictures characterized by the presence of chilblains, lipodystrophy, cutaneous vasculopathy, arthralgias and/or arthritis, inflammatory pneumopathy, pulmonary arterial hypertension, and typical symptoms of flu-like and TORCH-like syndromes(5).

The term “type I interferonopathies” was first used in 2011 by the British neurologist and geneticist Yanick Crow to refer to the Aicardi-Goutières syndrome characterized by neurological and autoimmune components and a genetic cause(3,6).

Since then, considerable progress has been made in understanding these diseases.

In a recent review, Crow and Stetson suggests how there are close to 40 discrete genotypes, whose mutation leads to a disease which can be considered among interferonopathies(7) (**Table 1**).

Regardless to the pathogenesis of the single condition, every disease with clinical or serological evidence of inappropriate IFN-I activation could be considered interferonopathy.

Indeed, whether the mutation involves nucleic acid metabolism or results in constitutive signal transduction through constitutive overproduction of IFN-I, the consequence is the activation of a primarily antiviral mechanism, in the absence of viral DNA or RNA and due to misrecognised self-stimuli.

Table 1. Genotypes linked to putative type I interferonopathies

Gene	Protein function	Proposed link to type I interferon signalling	Mutation effect	Phenotypic label/features
TREX1	Deoxyribonuclease	Cytosolic DNA	LOF (autosomal recessive or dominant negative)	AGS, FCL, SLE
DNASE2	Deoxyribonuclease	Cytosolic DNA	LOF (autosomal recessive)	Neonatal anaemia, glomerulonephritis, liver fibrosis, deforming arthropathy
SAMHD1	Control of dNTP pool	Cytosolic DNA	LOF (autosomal recessive)	AGS, FCL, cerebrovascular disease
STING1	Cytosolic DNA signal transduction	Cytosolic DNA	GOF (autosomal dominant)	STING-associated vasculopathy with onset in infancy, FCL
RNASEH2A	Ribonuclease	Cytosolic RNA–DNA hybrids	LOF (autosomal recessive)	AGS
RNASEH2B	Ribonuclease	Cytosolic RNA–DNA hybrids	LOF (autosomal recessive)	AGS, spastic paraparesis
RNASEH2C	Ribonuclease	Cytosolic RNA–DNA hybrids	LOF (autosomal recessive)	AGS
POLA1	DNA polymerase	Cytosolic RNA–DNA hybrids	LOF (X-linked recessive)	X-linked reticulate pigmentary disorder
ADAR1	RNA editing	Cytosolic dsRNA	LOF (autosomal recessive or dominant negative)	AGS, dyschromatosis symmetrica hereditaria, bilateral striatal necrosis, spastic paraparesis
IFIH1	dsRNA sensor	Cytosolic dsRNA	GOF (autosomal dominant)	AGS, spastic paraparesis, Singleton–Merten syndrome
DDX58	dsRNA sensor	Cytosolic dsRNA	GOF (autosomal dominant)	Singleton–Merten syndrome, juvenile open-angle glaucoma
SKIV2L	RNA helicase	Cytosolic RNA (UPR)	LOF (autosomal recessive)	Trichohepatoenteric syndrome
LSM11	RDH pre-mRNA processing	Histone stoichiometry/genomic DNA	LOF (autosomal recessive)	AGS
RNU7-1	RDH pre-mRNA processing	Histone stoichiometry/genomic DNA	LOF (autosomal recessive)	AGS
PNPT1	Polynucleotide phosphorylase	Mitochondrial RNA	LOF (autosomal recessive)	Infantile encephalopathy, bilateral striatal necrosis
NGLY1	N-deglycosylation	Mitochondrial DNA and RNA (indirect)	LOF (autosomal recessive)	Infantile encephalopathy, movement disorder
ATAD3A	Multiple	Mitochondrial DNA	Dominant negative (autosomal dominant)	Global developmental delay, systemic sclerosis, spastic paraparesis
ATM	dsDNA break repair	dsDNA breaks	LOF (autosomal recessive)	Ataxia telangiectasia
DCLRE1C	dsDNA break repair	dsDNA breaks	LOF (autosomal recessive)	Immunodeficiency
BLM	Genome stability	DNA damage	LOF (autosomal recessive)	Bloom syndrome
COPA	Vesicle transport (ER to Golgi apparatus)	STING trafficking	Dominant negative (autosomal dominant)	Interstitial lung disease, pulmonary haemorrhage, arthropathy, glomerulonephritis
ISG15	Inhibition of ISG transcription	IFNAR2 signalling	LOF (autosomal recessive)	Intracranial calcification, Mendelian susceptibility to mycobacterial disease
UPS18	Inhibition of ISG transcription	IFNAR2 signalling	LOF (autosomal recessive)	AGS-like

STAT2	Inhibition of ISG transcription	IFNAR2 signalling	LOF (autosomal recessive; separation-of-function)	AGS-like
STAT1	Cytokine signalling	ISG signalling	GOF (autosomal dominant)	Chronic mucocutaneous candidiasis, immunodeficiency, autoimmunity, intracranial calcification
JAK1	Cytokine signalling	ISG signalling	GOF (autosomal dominant)	Eosinophilia, atopy
C1QA	Alternative complement pathway	Immune complexes/CD8+ T cell metabolism	LOF (autosomal recessive)	SLE
C1QB	Alternative complement pathway	Immune complexes/CD8+ T cell metabolism	LOF (autosomal recessive)	SLE
C1QC	Alternative complement pathway	Immune complexes/CD8+ T cell metabolism	LOF (autosomal recessive)	SLE
ACP5	Phosphatase	Phosphorylation of osteopontin	LOF (autosomal recessive)	Spondyloenchondrodysplasia, SLE
PSMB8	Proteasome	Unknown	LOF (autosomal recessive)	PRAAS
PSMB4	Proteasome	Unknown	LOF (autosomal recessive)	PRAAS
PSMA3	Proteasome	Unknown	LOF (autosomal recessive)	PRAAS
PSMB9	Proteasome	Unknown	LOF (autosomal recessive)	PRAAS
POMP	Proteasome	Unknown	Dominant negative (autosomal dominant)	PRAAS
PSMB10	Proteasome	Unknown	LOF (autosomal recessive)	PRAAS
PSMG2	Proteasome	Unknown	LOF (autosomal recessive)	PRAAS
PSMB12	Proteasome	Unknown	LOF (autosomal dominant)	Global developmental delay
SAMD9L	Host antiviral restriction factors targeted by virus-encoded virulence factors	ISG signalling	GOF (autosomal dominant)	SAAD, ATXPS
NRAS	Multiple (proliferation and apoptosis)	Unknown	Somatic mutation	RALD (ALPS-like or SLE-like phenotype)

ADAR1, double-stranded RNA-specific adenosine deaminase 1; AGS, Aicardi–Goutières syndrome; COPA, coatomer subunit-α; dNTP, deoxynucleoside triphosphate; dsDNA, double-stranded DNA; dsRNA, double-stranded RNA; ER, endoplasmic reticulum; FCL, familial chilblain lupus; GOF, gain of function; ISG, interferon stimulated gene; LOF, loss of function; PRAAS, proteasome-associated autoinflammatory syndrome; RDH, replication-dependent histone; SLE, systemic lupus erythematosus; UPR, unfolded protein response; SAMD9L, sterile alpha motif domain containing 9 like; SAAD, SAMD9L-associated autoinflammatory disease; ATXPS, ataxia pancytopenia syndrome; RALD, RAS-associated Autoimmune Leukoproliferative disease; ALPS, Autoimmune lymphoproliferative syndrome.

Adapted and modified from Crow and Stetson 2022, *Nature Reviews | Immunology* (7)

Systemic autoinflammatory diseases (SAIDs) are among the conditions most often likely to be caused by Mendelian single-gene mutations.

SAIDs include a group of disorders characterized by abnormal activation of innate immunity leading to recurrent episodes of systemic inflammation associated with elevation of inflammation markers(8–10).

The clinical picture can be very heterogeneous involving several organs and systems, such as mucocutaneous, osteoarticular, and gastrointestinal. Recurrent flare-up episodes are usually interspersed with periods of variable duration of complete clinical well-being, normal status-ponderal growth, and, in most cases, normalization of serum inflammatory indices.

A proportion of these conditions are due to the presence of mutations in single genes coding for proteins that play a crucial role in the regulation of the inflammatory response. Monogenic SAIDs are very rare diseases that have an early clinical onset, usually in the first decade of life(9,11).

Since the diagnosis of these forms is a challenge for clinicians, delayed recognition could lead to the risk of complications in the long term. With recent advances in identifying the pathogenetic mechanisms of these conditions, it has been possible to identify drugs that can achieve good disease control.

Therefore, identifying clinical determinants and informative serologic markers can facilitate early diagnosis as well as initiate appropriate and tailored therapy, reducing the risk of developing long-term complications.

Recent studies on the pathogenesis of monogenic SAIDs have confirmed that the abnormality of the innate immune response involves proinflammatory cytokines with a key role. With these, the involvement of IFN-I allows some of these conditions to be classified among interferonopathies.

Specifically, some recently defined conditions such as STING-associated vasculopathy with onset in childhood (SAVI) caused by gain-of-function mutations in the STING1/TMEM173 viral sensor (STING) and atypical chronic neutrophil dermatosis with lipodystrophy and elevated temperatures (CANDLE) caused by additive loss-of-function mutations in proteasome genes have shown a significant increase in the IFN-I signature, confirming the pathogenic role for IFN-I in these autoinflammatory diseases authorizing its inclusion among type-1 interferonopathies (12–14).

SAVI syndrome is an interferonopathy caused by heterozygous mutations in the TMEM173 gene, recently renamed STING1, which codes for an endoplasmic reticulum transmembrane protein involved in interferon production. The STING protein is activated by binding to cGAMP (cyclic guanosine monophosphate-adenosine monophosphate), a second messenger synthesized by the enzyme cGAS (cyclic GMP-AMP synthase), which in turn is activated by recognition of dsDNA residues in the cell cytoplasm; the binding between STING and cGAMP leads to the formation of a homodimer which, when phosphorylated by TANK-binding kinase 1 (TKB1), activates Interferon Regulatory Factor 3 (IRF-3), which leads to transcription of the IFNB1 gene and thus to the production of interferon β. The mutations underlying SAVI syndrome led to constitutive activation of STING, independent of cGAMP binding. This results in continuous, unregulated trafficking between the endoplasmic reticulum and the intermediate compartment that lies between it and the Golgi (ERGIC); this leads to uncontrolled hypersecretion of interferon β(15) . The main clinical features of the syndrome are systemic involvement with febrile seizures, malaise, chronic anemia, and growth disturbances; skin involvement; and pulmonary interstitial disease(12,16).

A clinical condition that presents clinical signs similar to SAVI is COPA. The COPA protein is involved in the transport of proteins between the Golgi and the ER, at precisely the same level as the STING mutations linked to the SAVI syndrome. The result of its mutation is the expansion of Th-17 cells, overactivation of IFN-I pathway, and autoimmunity(17).

In the context of inflammatory diseases with involvement of innate immunity, several conditions lead to a clinical picture similar to early-onset SLE, often satisfying the most recent diagnostic criteria(18,19).

Among these, the role of defects in the endonuclease genes is well-known. Some studies have shown reduced DNASE1 endonuclease activity in the serum of patients and mice with SLE and with autoantibodies (especially anti-nucleosomal autoantibodies)(20,21). More recently, the essential role of DNASE1L3 endonuclease in degrading the genomic DNA of circulating microparticles produced by cell apoptosis has been defined. Defect in this gene results in increased exposure of microparticles resulting in the generation of autoantibodies(22).

Moreover, DNASE1L3 mutations are associated with pediatric-onset SLE with lupus nephritis(23). DNASE2 has also recently been studied as being associated with SLE-like signs. In fact, the production of anti-dsDNA antibodies has been demonstrated in Dnase2a/STING double knockout mice(24).

In any case, growing knowledge of the molecular mechanisms underlying immune dysregulation diseases has led to the possibility of modifying the natural course of the disease using targeted therapies.

As well as diseases associated with mutations in IL-1-activating inflammasomes (NLRP3 and pyrin) have been shown to be sensitive to IL-1-inhibiting therapies, a therapeutic role can be hypothesized for agents capable of blocking the pathway leading to IFN-I-induced gene activation.

There are now several descriptions of a good clinical response after administration of JAK inhibitor (ruxolitinib, baricitinib) in patients with an interferonopathy(16,25,26).

The aim of this work is to analyze the clinical, laboratory and molecular characteristics of a large cohort of patients uniformly tested with interferon signature assay.

Methods

Patients

We analyzed data of patients enrolled in the Eurofever registry (Executive Agency for Health and Consumers project no.2007332), which has been retrospectively collecting data from patients with recurrent fever and/or other symptomatology suspicious for an autoinflammatory disease since November 2009.

Data entered up to January 2020 were extracted to conduct this work. We collected patients with at least one interferon signature determination.

The identification and classification of patients was carried out in a two-step process.

By reviewing Eurofever registry patients records, detailed information regarding the clinical presentation at the first visit were collected.

Patients with already established diagnoses were divided into four groups (interferonopathy, autoinflammatory disease, systemic autoimmune disease, immunodeficiency).

A group of patients were identified who presented with symptoms and signs of systemic immune dysregulation disease in the absence of a genetic diagnosis or an already known autoimmune or inborn error of immunity (IEI) condition. This group has been defined “Others”. Among these, patients those suspected for an autoinflammatory disease based on signs of activation of innate immunity (fever, increased indices of inflammation) were defined, in accordance with recent literature as undifferentiated systemic autoinflammatory diseases (USAID) (11,27,28).

Patients who presented recurrent fever episodes with elevated inflammatory markers and negative criteria for PFAPA as well as negative genotype for HRF, were classified as SURF(29).

Eleven macro categories were identified based on the involvement of a specific organ or apparatus: febrile, constitutional, mucosal and cutaneous, musculoskeletal, gastrointestinal, cardiovascular, ocular, lymphoproliferative, neurological, genitourinary, and other.

Details regarding the site involved and the grade of severity were collected for each macro category.

Patients with undefined conditions were re-evaluated at the end of the follow-up period and after the diagnostic workup that included a genetic investigation with a targeted NGS panel based on clinical signs or by exome.

After about three years since the start of the evaluations of the data collected in the registry, a re-evaluation of the patients and a new classification was performed based on the newly obtained diagnostic elements.

All clinical investigations were conducted according to Declaration of Helsinki principles.

Interferon signature

Interferon-induced gene expression was determined using quantitative Real-Time PCR (RT-PCR).

Total RNA was extracted from whole blood cells using the PAXGene blood kit (QIAGEN, http://www.qiagen.com/us/products/catalog/sampletechnologies/rna-sample-technologies/total-rna/paxgene-blood-rna-kit-ivd/).

The concentration of total RNA was determined by spectrophotometry. Total RNA was subjected to retrotranscription, allowing for the synthesis of complementary DNA (cDNA) using the SuperScript® VILO cDNA Synthesis Kit (Life Technologies).

One nanogram of cDNA obtained from retro transcription was used in the real-time PCR reaction to determine the relative amount of mRNA of the 6 interferon-induced genes.

Quantitative RT-PCR was performed by standard methods using the probes of Universal Probe Library from Roche. The samples were analyzed in triplicate copy and two housekeeping genes (HPRT1 and G6PD) were used.

The Interferon Signature is calculated as the median of the relative quantification of the six genes compared to a healthy control, named calibrator, using as reference 19 healthy donors. The cut-off value is 0.8, which represents the median and two standard deviations.

Statistical analysis

All data analyses were performed using Stata® version 13 software (StataCorp LLP, Texas, USA). A p-value less than 0.05 was considered as statistically significant. Continuous variables were presented as the median and 1st and 3rd quartile (interquartile range, IQR), or minimum and maximum values, and calculated using the Mann-Whitney U test. Categorical variables were presented as number and percentage.

Fisher exact test or chi-square test were used to evaluate differences in categorical variables between groups.

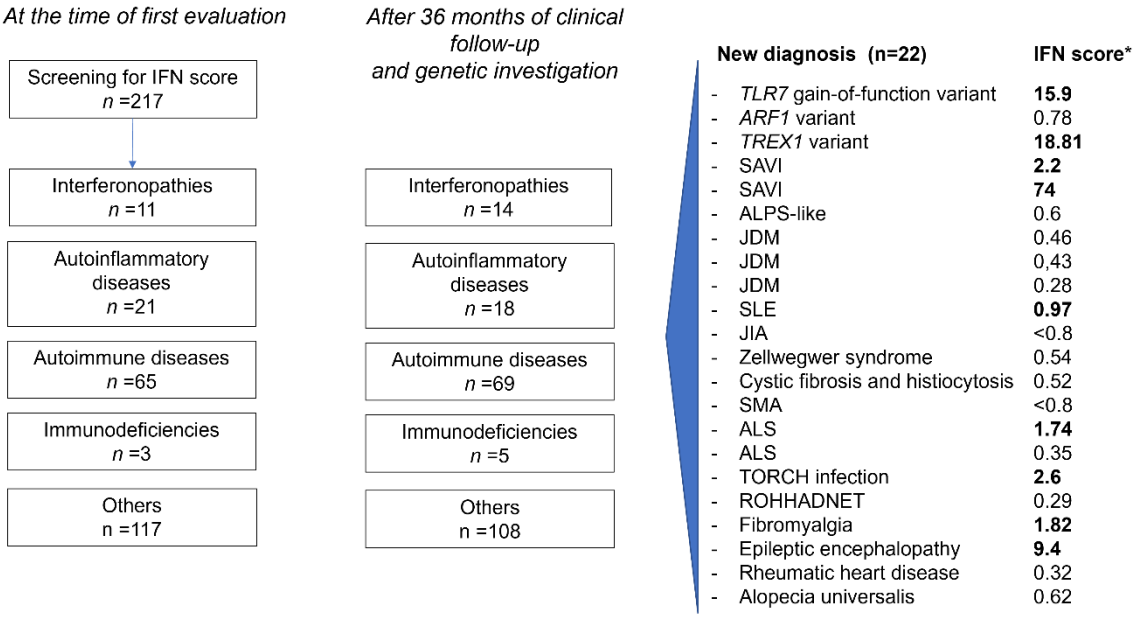
Results

Between 2015 and 2021, 214 consecutive patients included in the Eurofever registry were evaluated with an IFN-I signature test.

At the end of the follow up the patients were divided into 5 groups (**Figure 1**). Specifically, 14 were affected by an interferonopathy, 18 had an autoinflammatory disease, 69 presented with a systemic autoimmune disease, and 5 present an immunodeficiency.

A total of 108 patients presented signs and symptoms suspected of immune dysregulation condition without a defined diagnosis. An USAID was the diagnosis in 69 patients.

Figure 1. Study overview



TLR7: Toll-like receptor 7; ARF1: ADP Ribosylation Factor 1; TREX1:Three Prime Repair Exonuclease 1; SAVI: STING-associated vasculopathy with onset in infancy; ALPS: Autoimmune lymphoproliferative syndrome; JDM: Juvenile dermatomyositis; SLE: systemic lupus erythematosus; JIA: Juvenile idiopathic arthritis; SMA: Spinal muscular atrophy; ALS: Amyotrophic lateral sclerosis; ROHHADNET: rapid-onset obesity, hypothalamic dysfunction, hypoventilation, and autonomic dysregulation associated with a neuroendocrine tumor;
***In bold are the positive IFN score values (cut-off value: 0.8)**

Table 2. Clinical phenotype, diagnosis and the IFN-I positivity rate for each group

Group	Patients	Phenotype	IFN-I positivity	
Interferonophaties	**14**		11/14	**78.5%**
	3	SLE-like syndrome (*DNASE1L3*)	1/3	
	1	SLE-like syndrome (*TREX1*)	1/1	
	1	Type I interferonopathy (*DNASE2*)	1/1	
	2	SAVI (*TMEM173*)	2/2	
	3	Aicardi-Goutières syndrome (*TREX1, ADAR1, RNASEH2B*)	3/3	
	1	RALD (*NRAS)*	1/1	
	1	COPA (*COPA*)	1/1	
	1	TLR7 (*TLR7*)	1/1	
	1	ARF1-related disorder (*ARF1*)	0/1	
Autoinflammatory diseases	**18**		8/18	**44.4%**
	6	DADA2 (*ADA2*)	4/6	
	2	MKD (*MVK*)	0/2	
	3	FMF (*MEFV*)	1/3	
	4	CINCA [*CIAS1, NLRP3* (2)]	2/4	
	3	SjIA	1/3	
Autoimmune systemic diseases	**69**		45/69	**65.2%**
	22	SLE	19/22	
	8	JIA	4/8	
	12	JDM	5/12	
	5	ALPS	3/5	
	1	ALPS (*FAS*)	0/1	
	1	ALPS (*UNC13D*)	0/1	
	2	Sclerodermia	1/2	
	2	Sjogren	2/2	
	4	Mixed connettivitis	2/4	
	2	Polyarteritis nodosa	2/2	
	2	ANCA vasculitis	1/2	
	1	Lupus discoide	1/1	
	1	Antiphospholipid syndrome	1/1	
	1	Chronic nonbacterial osteomyelitis	1/1	
	1	Wegener Granulomatosis	0/1	
	1	Autoimmune hepatitis and arthropathy	0/1	
	1	Long lasting autoimmune neutropenia	1/1	
	1	Immune thrombocytopenia with livedo reticularis	1/1	
Others	**108**		**40/107**	**37.3%**
	69	USAID	23/69	33.3%
	5	Lupus pernio	3/5	
	4	Suspected SLE	2/3	
	8	Vasculopathy	3/8	
	7	Acrocianosis	1/7	
	3	Suspected AGS	1/3	
	1	Suspected mixed connettivitis	0/1	
	3	Cromosomopathy	3/3	
	7	SURF	4/7	

	1	Rohhad-net	0/1	
	1	Noonan like syndrome (*KRAS*)	1/1	
Immunodeficiencies	**5**		**4/5**	**80%**
	1	Immune dysregulation syndrome (STAT1 Gain of Function)	1/1	
	1	ARPC1B related combined immunodeficiency	1/1	
	1	Lymphoproliferation, thrombocytopenia, leukopenia (*STAT5* Heterozigous)	1/1	
	1	SAMD9L-associated autoinflammatory disease (SAAD)	1/1	
	1	Trichohepatoenteric syndrome (TTC37)	0/1	

IFN-I positive group vs IFN-I negative group

No significant difference in age and sex were found between IFN-I positive and negative groups.

Clinical features of patients based on IFN-I signature are detailed in **Table 3**. Concerning the muco-cutaneous involvement, a significant difference was detected in patients with positive IFN-I signature for erythema nodosum. This sign was detected in 6 patients, whose 3 was categorized as USAID (P1395, P1400, P1625), 2 had a diagnosis of ADA2 deficiency (P1519, P1520) and one patient was diagnosed as Polyarteritis nodosa (P1566).

This difference was not observed in any other skin sign although a higher frequency is present among patients with IFN-I activation for maculopapular rash (43.4% vs 27.6%; p=0.07) and urticarial rash (15.2% vs 11.3%; p=0.06)

The lymphoid involvement was more frequent among patients with an IFN-I pathway activation. A bilateral generalized lymph nodes enlargement characterized the 22.4% of patients of positive group. In particular, the presence of at least one among splenomegaly, hepatomegaly, and generalized adenomegaly was found in 37/107 (34.5%) of positive patients compared to 22/107 (20 %) of patients with negative IFN-I signature; $p<0.02$.

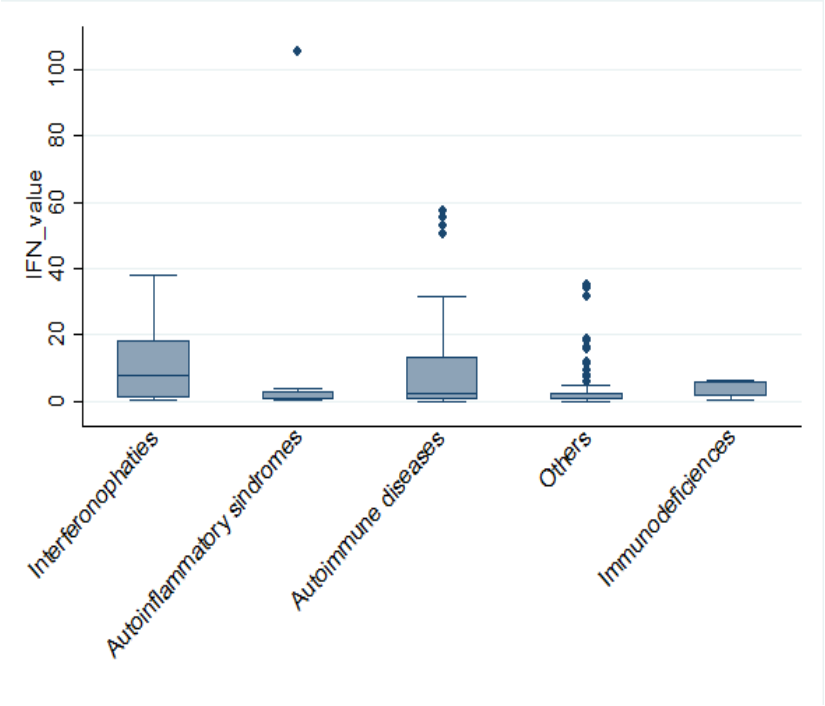
No difference was found in the C-reactive protein (CRP) and serum amyloid a (SAA) positivity, while erythrocyte sedimentation rate (ESR) was more frequently positive in the IFN-I positive group.

Table 3. Clinical features of patients based on IFN-I signature.

	Positive IFN-I	Negative IFN-I	*p*-value
Demographics			
Sex (M)	33/107	44/107	0.1
Age of onset (median and IQR)	7.3 (1.7-10-9)	5.1 (1.8-9.3)	0.1
Signs and symptoms			
Mucocutaneous	74/108	73/106	1
Maculopapular rash	42/105	29/106	0.07
Urticarial rash	16/105	12/106	0.06
Migratory rash	5/105	4/105	0.7
Palpable purpura	9/106	5/107	0.4
Erythema nodosum	6/106	0/107	**0.02**
Erythematous plaque	8/106	8/107	1
Erysipelas-like erythema	1/105	3/107	0.6
Aphtae	12/106	17/107	0.5
Musculoskeletal	74/108	61/106	0.12
Ocular	11/108	10/106	1
Gastrointestinal	34/108	27/106	0.36
Lymphoid	42/108	29/106	0.06
Splenomegaly	18/107	14/107	0.1
Generalized lymph nodes enlargement	24/107	10/107	**0.002**
Enlarged cervical lymphnodes	19/107	13/107	**0.02**
Bilaterality	18/97	13/106	0.2
Hepatomegaly	17/107	15/107	0.6
Cardiorespiratory	31/108	29/106	0.8
Pulmunar fibrosis	10/106	4/107	0.2
Pericarditis	5/106	5/107	1
Neurological	39/108	41/106	0.7
Seizures	12/106	8/106	0.48
Peripheral neuropathy	3/106	1/107	0.3
Intellectual impairment	10/106	11/107	1
Cerebellar syndrome	2/106	1/107	0.6
Headache	18/106	21/107	0.2
Genito-urinary	14/107	12/107	0.7
Constitutional (fatigue, malaise)	53/104	53/107	0.8
Fever	30/44	30/48	0.5
Low fever	23/44	22/48	0.4
Laboratory			
CRP	57/106	46/102	0.2
ESR	74/104	58/100	**0.049**
SAA	40/71	35/73	0.3
ANA	45/81	29/82	**0.01**
AutoAb	45/77	12/74	**0.0001**

Low IgG	7/98	7/98	1
Low IgA	6/98	4/97	0.7
Low IgM	6/98	2/98	0.3
High IgA	4/98	2/97	0.7
High IgG	7/98	4/98	0.5
High IgM	5/98	0/97	0.06
Genetic diagnosis	26/63	17/53	0.3

Figure 2. Quantitative IFN-I signature for each group.



Clinical description of patients with a genetically defined interferonopathy

Over the total cohort, 43/116 patients analyzed for autoinflammatory or IEI genes variants had a genetic diagnosis without significant difference between IFN-I positive or negative patients.

The genetic characterization of these patients is reported in **Supplementary table** (Appendix).

Aicardi-Goutières syndrome

Among patients with type 1 interferonopathies three were diagnosed with Aicardi-Goutières syndrome, whose one (P1589) treated with JAK inhibitors.

Patient 1589 presented bilateral nystagmus since birth and a psychomotor delay was noticed at 6 months of age.

In the following months, the patient presented paroxysmal epileptic episodes (up to 10/day). An electroencephalogram detected bilateral frontal irritative abnormalities with inconstant amplitude asymmetry. An encephalic MRI and a CT scan confirmed the presence of cerebral calcifications with multiple foci in the nucleus-basal site and at the level of the frontal white matter, both deep and subcortical, as well as at the level of the bilateral parieto-occipital and right posterior temporal white matter.

Subsequent imaging reported a progressive brain atrophy and lack of progression of supratentorial white matter myelination process. Suspecting Aicardi-Goutières syndrome, interferon signature was performed, with positive results. Genetic analysis found a dominant negative heterozygous mutation in the ADAR1 gene (p.Gly1007Arg). Therefore, an off-label treatment with Ruxolitinib was started at a dosage of 7.5 mg daily, later increased to 10 mg. Following the introduction of the drug, the child presented an improvement in clinical condition, with disappearance of the seizures.

SAVI

Two patients had a diagnosis of SAVI:

Patient 1405 presented from the age of 8 months with the appearance of necrotic-ulcerative skin lesions, with cicatricial evolution, on the limbs, the topical treatment of which with antibiotic-steroid creams is reported to be ineffective.

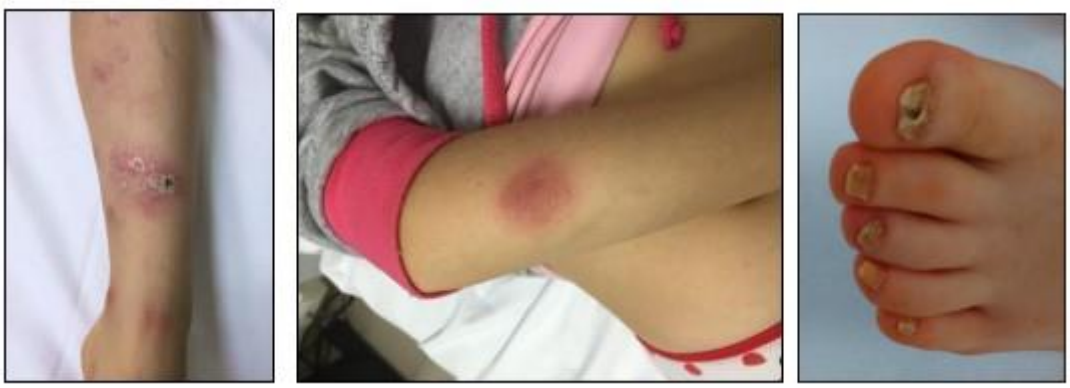
From the age of about 3 years, the appearance of nail dystrophy is also reported, mainly affecting the feet, initially interpreted as a lesion of a psoriatic, congenital dyskeratosis was ruled out. She subsequently presented with recurrent episodes of bronchospasm and from the age of 8 years repeated febrile episodes and finding of pulmonary interstitial disease with progressive worsening of respiratory function.

He also presented with arthralgias at the wrists and ankles. Inflammation markers, ANAs, pANCAs were consistently positive and several treatments were ineffective (Prednisone, Azathioprine, Etanercept).

Based on the interferon signature found to be positive, therapy with JAK inhibitor (Ruxolitinib) was initiated at an initial dosage of 2.5 mg twice a day, later increased to 5 mg twice a day. This therapy resulted in improvement of the pulmonary picture (demonstrated by improved exercise tolerance and improved spirometry and chest CT data) and reduction of steroid therapy.

Sequencing of the TMEM173 gene identified the presence of the V155M mutation in the heterozygous state, which confirmed the diagnosis of SAVI syndrome.

Fig.2 Skin lesion of patient with SAVI (1405)



Patients with SLE-like phenotype

Patient 1413, previously healthy, from the age of 7 years developed complex symptoms: hyperpyrexia, vomiting, anuria, signs of dehydration. He presented with left laterocervical lymphadenopathy, hepatosplenomegaly, erythematous-macular lesions on the upper and lower limbs, and necrotic lesions on the third toe, sole and dorsum of the right foot. p-ANCA were positive.

Renal involvement was diagnosed as necrotizing glomerulonephritis with positive ANCA, needing systemic therapies including cyclophosphamide, steroid, mycophenolate, azathioprine, tacrolimus, rituximab. During the follow up, the child developed pulmonary fibrosis. Overall, the skin, lung, and kidney involvement demonstrated the presence of a disseminated vasculitis process, and the presence of innate immunity involvement was suggested by the high IFN-I signature (36.6). Molecular analysis found a frame-shift mutation in the gene encoding for the enzyme DNase1L3 (c.289_290delAC), leading to protein truncation. This mutation is described in the literature associated with Urticaria Vasculitis Syndrome with Severe Hypocomplementemia (HUVS) in a family in which three children were affected(30).

More than 50% of patients with HUVS develop SLE(31), so it is unclear whether this is the spectrum of a single disease or the association between two distinct conditions.

Patient 1662 developed from the age of 7 years, a nephrotic syndrome, diagnosed as membranous glomerulonephritis (stage II), treated with steroids and Cyclosporine, then with Mycophenolate mofetil and Chlorambucil, with no efficacy. The child progressed to chronic renal failure. At the age of 15 started peritoneal dialysis, and at 17 underwent renal transplantation. However, the function of the transplanted kidney gradually deteriorated, and hemodialysis was started. In the following months, the patient developed recurrent episodes of fever, ascites, pericarditis, pleurisy, with positive ANA findings (1:160) and hypocomplementemia (C3 86.4 mg/dl). The worsening of renal function led to the need for renal artery embolization, followed by nephrectomy, on suspicion of Graft Intolerance Syndrome. The interferon signature was negative, while genetic investigation

demonstrated a homozygosity mutation in the DNASE1L3 gene (c.289_290delAC), leading to truncation of the normally encoded protein.

Patient 1658 is a girl presented at birth with a condition of acute liver failure. Biopsy showed a histiocytic infiltrate at the portal spaces with signs of cholestasis.

At age 3 years, liver biopsy found an early cirrhosis. Arthralgias and joint stiffness, fever, poor growth, and nonselective proteinuria due to glomerulonephritis appeared at the age of 5 years. In addition, leukopenia and anemia were detected with increased inflammatory indices. The child shows steroid dependence, despite initiation of other immunosuppressive therapies (Methotrexate), Etanercept, Anakinra and Infliximab. At 12 years of age, she was diagnosed with immune complex nephritis compatible with lupus nephritis, with positive ANA and anti-dsDNA. Mycophenolate mofetil (250 mg x 2 vv/day) and hydroxychloroquine (150 mg/day) were also ineffective. A course of injections of Canakinumab (antibody humanized monoclonal antibody against Interleukin-1) 100 mg s.c. resulted in steroid reduction. Nevertheless, this therapy was also ineffective. Skin involvement with erythematous-desquamative lesions at the elbows and on the extensor surface of the fingers of the hands and progressive pathology of the pulmonary interstitium also appeared. Metabolic and genetic investigations for major autoinflammatory diseases (CANDLE, MKD, FMF, TRAPS, CIAS1), were negative. Finally, interferon signature was positive (14,92) and further genetic testing by NGS method revealed a DNASE2 mutation (c.362A>T p.Asp121Val)

Patient 1548 is a 13-year-old girl adopted from Cambodia. She presented with a complex picture with malar rash, leukopenia (WBC 2620/mmc, L 1130/mmc, N 1130/mm3), hypocomplementemia, hypergammaglobulinemia, increased ESR, positive anti-dsDNA, anti-Sm and anti-SSA/Ro antibodies.

In accordance with ACR1997(18) and SLICC-2012(19) criteria, the diagnosis of SLE was made.

The child was treated with oral prednisone (2 mg/kg/day) and hydroxychloroquine.

However, some recurrences led to initiation of mycophenolate mofetil (MMF), monthly IVIG infusions, and tacrolimus without being able to control the skin involvement characterized by severe infiltrative erythematous plaques on the face and yasculitic lesions.

A NGS gene panel analysis revealed a previously unreported novel heterozygous missense mutation on Three Prime Repair Exonuclease 1 (*TREX1)* gene (c.374A>G; p.Asn125Ser). Furthermore, INF-I signature resulted strongly positive (18.81).

Tacrolimus was thus replaced with the JAK 1/2 inhibitor baricitinib (2 mg 3 times daily).

Baricitinib resulted in significant improvement of skin lesions in the following weeks. However, one month after discontinuation of tacrolimus, persistent proteinuria appeared, and renal biopsy showed stage IV lupus nephritis with the need to restart MMF, which, combined with baricitinib, led to renal and skin improvement.

Other Interferonopathies

Patient 0105, previously healthy, presented at the age of 3 years with very severe arthritis similar to polyarticular juvenile idiopathic arthritis, followed by the onset of persistent cough.

Chest CT showed interstitial thickening with micronodular pattern and bronchoalveolar lavage revealed lymphocytic alveolitis without atypical cells or CD1a-positive histiocytes. Mantoux and Quantiferon tests were both negative. Total body MRI with STIR sequence excluded the presence of bone lesions. The patient was treated with high-dose steroids (3 boluses of i.v. methylprednisolone at a dosage of 30 mg/kg), oral prednisone (2 mg/kg) for one month and then methotrexate without any results. Joint and lung involvement worsened. The use of Abatacept was also unsuccessful.

She was then lost from follow-up for three years.

Following the first report of COPA syndrome(32), Sanger sequencing was performed on the patient's DNA and the c.698G>A mutation in the *COPA* gene was found in both the patient and her mother.

Based on the clinical findings and genetic testing, the diagnosis of COPA was formulated(17).

After the girl's return to care, a combination treatment with mycophenolate mofetil, hydroxychloroquine as well as steroids was started. This resulted in controlling the progression of the pulmonary disease and arthritis but, due to the continuing disease activity and the previous transient discontinuation of treatment, the osteoarticular scars persisted.

Patient 1398 was followed since the age of 13 years for suspected autoimmune lymphoproliferative syndrome (ALPS). During follow-up, he developed autoimmune manifestations, recurrent infections, and progressive hypogammaglobulinemia. Functional testing showed a B-cell defect with increased proliferation activity of CD4+ T cells and decreased function of regulatory T cells.

The interferon signature was strongly positive (18.01).

Genetic analysis, by WES, showed a de novo pathogenic variant of NRAS. The somatic nature of this mutation was confirmed on oral brush and urinary sediment. The patient is treated with mycophenolate and immunoglobulin infusions after first-line steroid treatment.

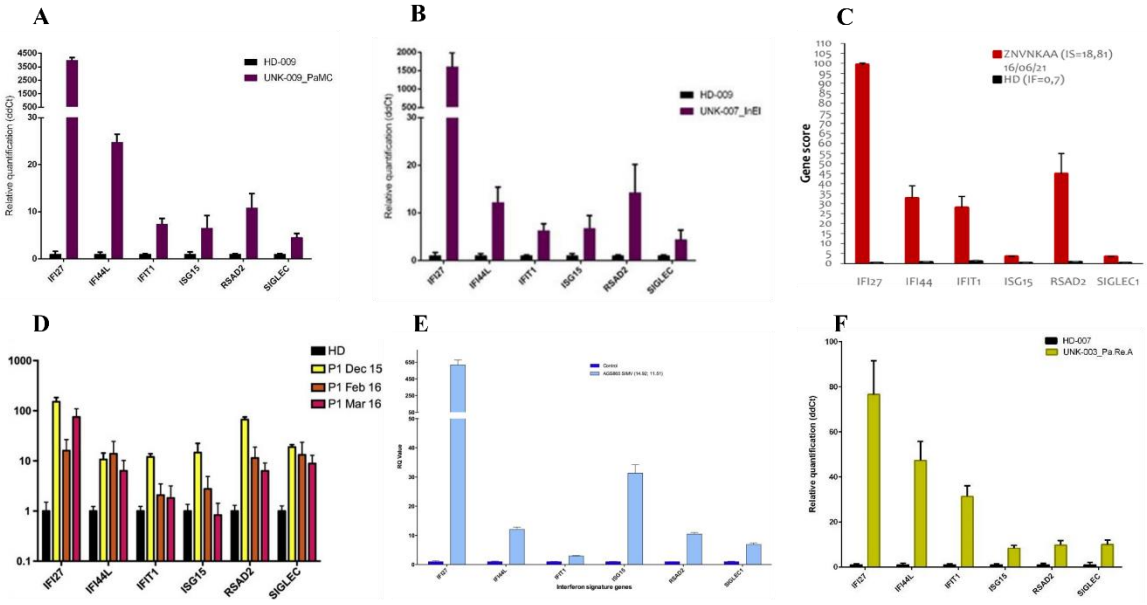
Patient 1610 presented a clinical history that began at birth with cleft lip-palate, cryptorchidism, inter atrial heart defect type ostium secundum, micrognathia, and strabismus.

The diagnosis of Pierre-Robin sequence was initially hypothesized. His development was complicated by severe mental retardation associated with self-aggression. Skin lesions (edema and hyperemic patches on the extremities and ear pinnae) appeared from the first days of life during fever without obvious infectious causes.

Over the years the skin lesions worsened with a tendency to ulcerative evolution.

In recent months, a variant of the *ARF1* gene in de novo heterozygosity, known in 3 other patients described in the literature with very similar neurological picture (delay especially of speech, with communication difficulties)(33), was found by WES method. Recently, the patient started baricitinib treatment.

Figure 3. Positive IFN-I signatures for patients with Interferonopathies. A: SAVI (1405), B: SAVI (1407) C: TREX1 (1548), D COPA (0105), E DNASE2 (1658), F DNASE1L3 (1413).



Autoinflammatory syndromes

Of a total of 6 patients with ADA2 deficiency, 4 had a positive IFN-I signature in one case with very high values (P1355, 105.5). All had typical skin signs of the disease (livedo reticularis and, in two cases, subcutaneous nodules) and constitutional signs (asthenia). Five manifested musculoskeletal signs or symptoms, mainly arthralgias, myalgias, and in two cases bone lesions. A stroke episode with neurological outcomes had occurred in three patients.

In particular, a 1-year-old boy (P1355) presented with an ischemic injury of the internal capsule resulting in hemiplegia. While in one case (P1519) the outcome of the ischemic event was ataxia.

One patient (P1355) had autoantibodies (p-ANCA) while patient 1584 had ANA positivity.

Complete hypogammaglobulinemia was present in one case, whereas one patient had low IgG and IgM and one had low IgA and IgM with increased IgG.

Patients with a diagnosis of monogenic recurrent fever, mevalonate kinase deficiency (MKD) and familial mediterranean fever (FMF), revealed an inconstant IFN-I positivity with low median values, 0.27 (min 0.19 - max 0.34) and 0.33 IQR (min 0.15 - max 1.48). Half of the patients with

Chronic, Infantile, Neurologic, Cutaneous and Articular syndrome (CINCA) tested positive for IFN-I with a median value of 1.7 (min. 0.57, max 2.78).

Autoimmune diseases

Patients with a well-defined autoimmune systemic disease showed a high prevalence (45/69; 65.2%) of positive IFN-I signature.

SLE is the most represented disease in our cohort. Median age at the onset was 10.53 years (IQR 8.57-12.82). The main clinical manifestations of patients with SLE were cutaneous and musculoskeletal signs. Autoantibodies were found in 19/21 evaluable cases being anti-dsDNA (16) and ENA (17) the most detected. The interferon signature among these patients shows a median value of 5.97 (min. 0.16, max. 57.5) and was positive in 19/22 cases (86.4%).

Twelve patients had a diagnosis of juvenile dermatomyositis (JDM). Median IFN-I value was 0.49 (min. 0, max 15.5). Four out eight patients with juvenile idiopathic arthritis (JIA) showed IFN-I positivity with an overall median of 1.38 (min. 0.1, max 20).

Eight children showed ALPS-like clinical phenotype with clinical and biochemical signs of lymphoproliferation. In two cases, the diagnosis of ALPS was confirmed by the finding of pathogenic genetic variants on FAS and UNCN13D.

Table 4. Comparison between patients with interferonopathy and SLE phenotype and patients with typical SLE.

	Interferonopathy with SLE phenotype	SLE	*p*-value
Demographic			
Sex (M)	3/6	4/22	0.1
Age of onset (median in years and IQR)	5.77 (3.4-7.65)	10.2 (8.57-12.82)	**0.006**
Signs and symptoms			
Mucocutaneous	4/6	18/22	0.6
Musculoskeletal	4/6	19/22	0.3
Gastrointestinal	3/6	8/22	0.6
Lymphoid	3/6	9/22	1
Splenomegaly	3/6	1/22	**0.02**
Generalized lymph nodes enlargement	1/6	6/22	1
Enlarged cervical lymphnodes	1/6	4/22	1
Bilaterality	1/6	4/20	1
Hepatomegaly	3/6	2/22	**0.05**
Cardiorespiratory	3/6	4/22	0.1
Neurological	2/6	4/22	0.6
Genito-urinary	3/6	6/16	0.3
Constitutional (fatigue, malaise)	3/6	14/22	0.6
Laboratory			
IFN signature (positive)	4/6	19/22	0.3
IFN signature (median values and IQR)	15.4 (0.7-36.6)	5.9 (1.7-16.6)	0.7
CRP	5/6	7/21	0.06
ESR	5/5	15/21	0.3
ANA	6/6	17/21	0.5
AutoAb	4/5	19/21	0.5
Genetic diagnosis	6/6	0/6	**0.002**

Other undefined conditions

Four patients of this group presented with signs and symptoms suspected for SLE. Musculoskeletal (all the patients), mucocutaneous (3/4), lymphoid (2/4), cardiovascular (2/4), neurological (2/4) signs were the most frequent. None presented with recurrent fever. Median age at the onset was 11.14 years (IQR 9.77- 14.62).

The median IFN-I value for this subgroup was 0.72 (min. 0.2, max. 34). Two patients had IFN-I signature positive.

Among the patients with skin manifestations, 5 patients with lupus pernio classified in this group among undefined diseases while one patient with discoid lupus reported instead in the group of autoimmune diseases.

Eight patients presented with vasculopathy without any other diagnosis. One presented a complex picture with necrotizing granulomatous osteoperiostitis, eosinophilic pneumonia and obliterative vasculopathy (P1605).

Two patients with unconfirmed suspicion of Wegener's granulomatosis (P1388, P1592); one with Moya-Moya Syndrome (P1650), two patients with stroke (P1602,P1629), two patients with systemic vasculopathy still undergoing diagnostic investigation (P1588, P1612).

Patient 1576 presented, at the first evaluation, with serositis (pericarditis and pleurisy) and a seizure episode with positive anti-DNA and anticardiolipin antibodies. The patient had selective hypogammaglobulinemia for IgM and IgA with normal IgG value. The interferon signature was positive (2.91) and a germline mutation of KRAS gene (Ala146Ser) was detected leading to the diagnosis of Noonan syndrome.

108 patients presented with presumed undefined autoinflammatory diseases in absence of a genetic diagnosis. For these patients, onset symptoms included fever (31%), lymphoproliferation signs (28%), musculoskeletal (60%), cardiac (15%), gastrointestinal (14%), ocular (6%), neurologic (56%), mucocutaneous involvement (71%). 39% of these patients showed positivity for IFN-I signature with a median of 0.5 (min. 0, max. 4).

Seven patients had a diagnosis of SURF at the end of diagnostic investigations. The median IFN-I value for this subgroup is 2 (min 0.18, max 18.2).

Immunodeficiencies

Five patients had a diagnosis of immunodeficiency and elevated IFN-I levels was detected in 4/5 cases. Median IFN-I value was 1.85 (IQR 1.4-5.7).

Lymphoproliferative signs were present in all the cases except for patient 1604 presenting with a diagnosis of Trichohepatoenteric syndrome, which was the sole with IFN-I negativity in this group.

Gastroenteric, mucocutaneous and musculoskeletal symptoms were described in 4, 3 and 2 cases, respectively.

Patient 1389 is a male who started at 2 months of age with a macrophage activation syndrome (MAS) triggered by CMV infection. He was treated with steroids and antiviral treatment (ganciclovir). During follow up he presented with systemic symptoms such as enterorrhagia, enlarged lymph nodes and spleen, cutaneous vasculitis rash, with an overall growth delay.

At the age of 5 years, he was diagnosed with immunodeficiency due to the presence of a missense variant of ARPC1B. In the same year, he received a TCRab+CD19+-depleted HSCT from a haploidentical parent after a myeloablative conditioning regimen, without adverse events.

Patient 1653 was a little girl presenting at birth with diffuse maculo-papular erythema, which evolved into a fine cutaneous desquamation, associated with fever. Microcephaly, axial hypotonia, hepatosplenomegaly and nail dystrophy were also revealed. Magnetic resonance imaging (MRI) of the brain was normal, except for a small T2-hypointense in the left globus pallidus, consistent with a calcific lesion (Figure 1B).

Congenital infectious diseases were excluded but the immunophenotype showed lymphopenia with NK-cell deficiency. Immunoglobulin levels, NK-cell activity, T-cell proliferation and clonality were normal. A histological diagnosis of leucocytic vasculitis was made for the skin lesions.

The bone marrow aspirate was negative for atypical cells, the karyotype was normal. The peripheral blood type I interferon signature showed slightly increased values. The disease was only partially controlled by increasing doses of steroids, the recombinant interleukin-1 receptor antagonist (anakinra) and sirolimus, despite high dosages.

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 manifested a Staphylococcus aureus central catheter infection and Pneumocystis carinii pneumonia with severe respiratory distress despite antibiotic prophylaxis.

Thus, the child received a haploidentical alpha/beta/CD19-depleted HSCT at the age of 7 months. She subsequently developed neurological symptomatology (multiple absence-type seizures) with new lesions on brain MRI depicting progressive leukoencephalopathy, cerebral atrophy and

increased calcifications. In addition, the child developed bronchiolitis obliterans (temporarily treated with imatinib) complicating the complex picture of delayed psychomotor development. Whole-exome sequencing analysis of the patient revealed a de novo mutation (c.2658-2659del, p.F886Lfs*11) of the SAMD9L gene, confirmed by Sanger sequencing.

Treatments

The median number of drugs administered for the undefined disease group was very low; these patients had received a median of only one drug over the entire follow-up.

For the rest of the cohort, the number of therapies did not differ significantly between groups with a median number of drugs of 3/patient.

Children with interfernopathies most often received steroids (9/14) followed by MMF (9/14), JAK inhibitors (4/14), hydroxychloroquine (5/14), azathioprine (4/14), methotrexate (3/14), cyclophosphamide (3/14).

Among autoinflammatory diseases cases 11/18 had received steroids, 10/18 NSAIDs, 8/18 anakinra, 5/18 colchicine, 5/18 canakinumab, 3/18 etanercept, 3/18 thalidomide.

In the patients diagnosed with systemic autoimmune diseases, steroids were administered in 53 different cases, Methotrexate in 26/69, Hydroxychloroquine in 25/69, NSAIDs in 19/69, MMF in 16/69, immunoglobulins in 13/69, cyclosporine in 11/69, cyclophosphamide in 9/69, etanercept in 7/69, rituximab in 5/69, rapamycin in 4/69.

For patients with immunodeficiency, the most frequently administered drugs were steroids (4/5), then immunoglobulins (3/5) and rapamycin (2/5).

Six patients in this cohort received JAK inhibitor therapy. Specifically, 2 patients (SAVI) benefited from ruxolitinib treatment.

One patient with Aicardi was treated with baricitinib. One patient with *TREX1* mutation benefited from baricitinib therapy. Patient 1524 diagnosed with *STAT1* GOF received ruxolitinib.

Patient 1610 recently started baricitinib for an *ARF1* induced interferonopathy.

Discussion

This work analyzes, from a clinical and molecular perspective, a multicenter cohort whose data were collected in the Eurofever registry. The use of interferon signature was systematically applied in a group of heterogeneous patients presenting at onset with a suspicious phenotype for immunodysregulation disease. Clinical and molecular investigations led to the classification of 4 groups of patients with well-defined diseases and a group including patients without a defined diagnosis or with conditions that could not be defined among IEIs. Specifically, the IFN-I signature allowed the diagnosis of type I interferonopathies in 14 cases (COPA, SAVI, Aicardi Goutières, DNASE1L3 and DNASE2 deficiency, RALD, TLR7 and TREX1).

Overall, the results of the comparative analysis between IFN-I positive and IFN-I negative patients showed some significant differences. Patients with signs of lymphoproliferation show more frequent activation of the IFN-I pathway. Also significantly higher is the finding of autoantibodies among patients with positive IFN-I signature. This confirms the well-known link between innate immunity and autoimmunity. IFN-I signature is often found positive in the context of autoimmune rheumatologic diseases and is considered a marker of disease activity(34).

However, compared with monogenic interferon pathway-related diseases where alterations in the IFN pathway determine disease pathogenesis, in autoimmune diseases it is unclear whether this pathway is pathogenetically relevant or only indirectly involved.

The presence of a direct proportionality between IFN-I level and signs of autoimmunity was recently observed in a cohort of patients with JIA. Interestingly, in these patients ESR correlated with positive IFN-I values, as observed in our case series(35) (**Table 3**).

Another significant clinical difference found between patients with activation of the type 1 interferon pathway is the presence of erythema nodosum. This sign was not detected among IFN-I negative patients. This is not surprising as it is known that patients with erythema nodosum show hyperactivation of this pathway(36).

Some patients diagnosed with interferonopathy had an early onset SLE like picture. Patients with *DNASE1L3* deficiency or *TREX1* mutation show an aggressive renal disease and those with SAVI syndrome suffered from a skin involvement. In both cases systemic symptoms (serositis, inflammation, autoimmunity) mimic a SLE clinical presentation.

These overlapping clinical manifestations between interferonopathies and typical SLE make diagnostic work a clinical challenge(37). In the case of SAVI, the detection of a positive interferon signature could help for the diagnosis and for the treatment's choice. In literature, there are several reports attesting to the effectiveness of JAK inhibitors in these patients (38–41) and both patients of this cohort received ruxolitinib with a positive response.

On the other hand, patients with DNASE1L3 have a more heterogeneous IFN-I pattern, probably reflecting the activity of disease at the time of sampling: in fact, unlike the other interferonopathies, these patients seem to be more affected by background therapy in the activation of the interferon pathway, probably being the mutation involved in more upstream regulation of the pathway(23).

SLE is the most represented disease in our cohort as a documented diagnosis of SLE was present in 22 patients. This well-known systemic disease is characterized by a pathogenetic spectrum that varies between autoinflammation and autoimmunity(42) and the range of clinical manifestations is therefore broad and variable from patient to patient.

The age of onset may also vary and forms occurring before the age of 18 years, known as childhood-onset systemic lupus erythematosus (cSLE)(43), usually have a more severe clinical course than adult-onset SLE(44,45). Furthermore, the probability of monogenic forms is inversely proportional to the age of onset.

Since six patients diagnosed with monogenic interferonopathy (DNASE1L3, DNASE2, TREX, TLR7) had a clinical phenotype compatible with SLE, a comparison with SLE patients without a monogenic condition revealed interesting findings (**Table 4**). Analysis of the IFN-I signature shows that IFN-I pathway activation can be demonstrated in most of these cases. This activation shows a kind of progression in which monogenic forms show a higher activation in terms of median IFN-I values than SLE cases (15.4 vs. 5.97), but without statistical significance.

Moreover, a condition suspected for SLE but in the absence of diagnostic criteria was found in 5 patients that can be considered on the other side of the activation spectrum manifesting lower median IFN-I values (0.72).

Interestingly, the median age of onset of symptoms is significantly lower for monogenic forms (6.6 years, IQR 4.95-7.65) than for typical SLE forms (10.3 years, IQR 8.6-12.8) and suspected cases (11.1 years, IQR 9.8-14.6) (**Table 4**).

Overall, data from this work demonstrate a close link between SLE and IFN-I as already reported in the literature(46).

The recent approval of the type I interferon-blocking antibody, anifrolumab, by the US Food and Drug Administration for the treatment of patients with SLE demonstrates the value of targeting this pathway (47,48).

Several clinical conditions reported in this case report showed clinical and molecular peculiarities related to the IFN-I pathway.

In our cohort, one patient were diagnosed with RALD and positive interferon signature, and has already been described in a recent paper(49).

While germinal NRAS mutations lead to Noonan syndrome, somatic mutations are responsible for RALD. RALD is usually characterized by chronic lymphoproliferation, hypergammaglobulinemia, and monocytosis. The onset commonly occurs during childhood, and the clinical features resemble autoimmune lymphoproliferative syndrome.

Interestingly, the patient show low immunoglobulin levels, whereas usually patients with RALD show hypergammaglobulinemia(50,51).

Among patients with immunodeficiency an actin-related protein complex-1 (*ARPC1B)* mutation was found in a child (P1389) with clinical features classifiable as combined immunodeficiency with symptoms of immune dysregulation (hepatosplenomegaly, myositis, recurrent urticaria rash, a life-threatening episode with macrophage activation syndrome)(52).

ARPC1B is one of the 5 regulatory subunits of the Arp2/3 complex, which is one of the major protein complexes involved in actin polymerization and cell motility. Mutations in this gene can

therefore cause defective actin polymerization in blood cells and have recently been associated with predominantly hematologic immune alteration pictures(53–55).

One patient presented with an immunodeficiency caused by STAT1 gain-of-function (GOF) variant was treated with JAK inhibitors. This is a well-known primary immunodeficiency characterized by chronic mucocutaneous candidiasis (CMC), recurrent infections, autoimmunity, and less frequently X-linked immunoregulation polyendocrinopathy (IPEX)-like syndromes(56). However, the clinical spectrum is very broad(57).

At the basis of the symptoms are not only defective development of Th17 cells and increased apoptosis of T lymphocytes, but also hyperactivation of the IFNα-mediated response(58–60). It is therefore not surprising that an elevated IFN-I signature was found in the patient in this cohort.

The literature data thus support the use of the JAK inhibitors in STAT1 GOF patients.

However, it has recently been suggested that epigenetic factors underlying the hyperactivation of the IFN-I pathway might be responsible for the failure of treatment with JAK inhibitors in some cases(58).

For some patients in this cohort, it was difficult to find the right diagnostic classification (e.g., STAT1 and SAMD9L among immunodeficiencies, NRAS among interferonopathies). In any case, this decision was made on the basis of the prevailing symptomatology for each patient.

However, it is possible that in the coming years, several monogenic conditions currently included generically among immune dysregulation syndromes may find a place among interferonopathies. This reclassification should correspond to a better understanding of pathogenetic mechanisms and consequently to the optimization of therapies.

Finally, the six patients who received JAK inhibitor therapy showed improved disease control with the possibility of reducing or discontinuing steroid therapy, although the follow-up period after starting the drug is still short in some cases.

This study has several limitations. First, the primarily retrospective nature of the analysis does not allow for accurate comparisons. The heterogeneity of the cohort, although it allows for a significant number of cases, may generate analytical bias.

Conclusions

In recent years, the development of new genetic sequencing techniques (Next Generation Sequencing, NGS, and Whole Exome Sequencing, WES) has radically revolutionized the field of immune dysregulation diseases, among which the class of interferonopathies has recently been defined.

However, to optimize access to genetic investigations thus reducing response time and examination costs, it is necessary to find reliable clinical tools to target diagnostic suspicion.

The Interferon signature, based on the measurement of activation of genes involved in the type I interferon pathway, can be a useful tool for defining the most appropriate diagnostic work up for each patient.

Our work demonstrates a strong association between activation of IFN-I pathway and autoimmunity confirming the close link between innate immunity and some monogenic or multifactorial immune disorders.

Some signs and symptoms are significantly associated with activation of IFN-I pathway, especially erythema nodosum and clinical signs of chronic lymphoproliferation.

Patients with a clinical phenotype similar to early-onset SLE show consistent activation of the IFN-I pathway. In these patients, elevated interferon signature values may suggest the presence of monogenic disease.

The real benefit of early genetic diagnosis is the possibility of targeted treatment through JAK kinase inhibitors that are effective in preventing organ damage and reducing the use of steroid therapy or other immunosuppressants. Further studies are needed to understand the pathogenesis of certain conditions that appear to be closely linked to IFN-I activation. Furthermore, a larger observation period will be necessary to better understand the long-term effects of JAK inhibitors.

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Appendix

Supplementary table. Phenotype and genotype of the genetically diagnosed patients in this cohort.

Group	Patient	Phenotype	Gene	Transmission	Pathogenic variant	IFN signature
Interferonophaties						
	IT0100105	COPA Syndrome	COPA	Heterozygous, aploinsufficiency	c.698G>A p.Arg233His	12,3
	IT0101569	Aicardi-Goutières syndrome	TREX1	Homozygous		0.93
	IT0101589	Aicardi-Goutières syndrome	ADAR1	Heterozygous	c.3019G>A Gly1007Arg	3.39
	IT0101663	Aicardi-Goutières syndrome	RNASEH2B	Homozigous	c.529G>A Ala177Thr	1.66
	IT0101660	Early-onset SLE-like syndrome	DNASE1L3	Heterozygous	c290_291 delCA; exon 5 deletion p.Thr97llefs*2	0.04
	IT0101662	Early-onset SLE-like syndrome	DNASE1L3	Homozygous	c.289_290delAC p.Thr97llefs*2	0.69
	IT0101409	Early-onset SLE-like syndrome	TLR7 gain of function variant	Heterozygous	c.1520T>C; p.Phe507Ser	15.9
	IT0101413	Early-onset SLE-like syndrome	DNASe1L3	Homozygous	c.289_290delAC p.Thr97llefs*2	36.6
	IT0101548	Early-onset SLE-like syndrome	TREX1	Heterozygous	c.374A>G; p.Asn125Ser	18.81
	IT0101658	Type I interferonopathy due to DNase II deficiency	DNASE2	Homozygous	c.362A>T ; p.Asp121Val	14.92
	IT0101398	RALD	NRAS	Somatic mutation	c.38G>A; p.gly13Asp	18.01
	IT0101610	ARF1-related disorder	ARF1	Heterozygous, de novo	c.295C>T p.(Arg99Cys)	0.78
	IT0101405	SAVI	TMEM173	Heterozygous GOF	c.463G>A p.Val155Met	24.9
	IT0101407	SAVI	TMEM173	Heterozigous	c.842G>A p.Arg281Gln	74
Autoinflammatory diseases						
	IT0101355	ADA2 deficiency	ADA2	Homozygous	E328D; R312X	105.5
	IT0101357	ADA2 deficiency	ADA2	Homozygous	E328D; R312X	2.03
	IT0101487	ADA2 deficiency	ADA2	Homozygous	p.(Leu183Pro)	0.5
	IT0101519	ADA2 deficiency	ADA2	Homozygous	p.(Gly47Val); p.(Ser479Pro)	2.65
	IT0101520	ADA2 deficiency	ADA2	Homozygous	p.(Gly47Val); p.(Ser479Pro)	4.0
	IT0101584	ADA2 deficiency	ADA2	Homozygous	p.(Gly47Arg)	0.39
	IT0101173	Familial Mediterranean fever	MEFV	Homozygous	p.(Glu148Gln); p.(Arg761His)	0.33

	IT0101425	Familial Mediterranean fever	MEFV	Homozygous	p.(Met694Val)	1.48
	IT0101598	Familial Mediterranean fever	MEFV	Homozygous	p.(Met694Val)	0.15
	IT0101207	CINCA	NLRP3	Heterozygous	p.(Met406Ile)	0.76
	IT0101358	CINCA	CIAS1	Heterozygous		2.78
	IT0101415	CINCA	NLRP3	Heterozygous	p.(Asp303Asn); p.(Gln703Lys)	0.56
	IT0101505	CINCA	NLRP3	Heterozygous	p.(Phe523Leu)	2.63
	IT0101311	Mevalonate kinase deficiency	MVK	Homozygous	p.(Val377Ile); p.(Arg202Gln)	0.19
	IT0101659	Mevalonate kinase deficiency	MVK	Homozygous	p.(Thr237Ser)	0.34
Autoimmune systemic diseases						
	IT0101467	ALPS	FAS	Heterozygous		0.0
	IT0101568	ALPS	UNCN13D	Heterozygous	p.L864V	0.15
Immunodeficiencies						
	IT0101524	Immune dysregulation syndrome	STAT1 Gain of Function	Heterozygous GOF		5.7
	IT0101389	ARPC1B related combined immunodeficiency	ARPC1B	Homozygous	c.622G>T; p.Val208Phe	6.08
	IT0101656	Lymphoproliferation, thrombocytopenia, leukopenia	STAT5	Heterozygous		1.85
	IT0101653	SAMD9L-associated autoinflammatory disease (SAAD)	SAMD9L	Heterozygous GOF	c.2658-2659del; p.F886Lfs*11	1.44
	IT0101604	Trichohepatoenteric syndrome	TTC37	Homozygous	exon42: c.4497-1G>A	0.4
Other						
	IT0101576	Noonan-like syndrome	KRAS	Germline	Ala146Ser	2.91
	IT0101386	Multicentric carpo tarsal Osteolysis (MCTO)	MAFB	Heterozygous (AD)		0.41
	IT0101408	Mixed connective tissue disease	-	del 16p112		11.65
	IT0101477	Arterial tortuosity syndrome (ATS)	SCL2A10	Homozygous		0.31