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Validation of HUMAN PHENOTYPE ONTOLOGY (HPO) terms and development of an Artificial Intelligence-based diagnostic tool for systemic autoinflammatory diseases using the EUROFEVER Registry: the ODINO project

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INTRODUCTION

Systemic autoinflammatory diseases (SAIDs) are a heterogeneous group of disorders characterized by recurrent or persistent inflammation driven by dysregulation of the innate immune system. In pediatric rheumatology, SAIDs constitute a particularly challenging field, as many of these conditions present early in life and with nonspecific symptoms such as recurrent fever, rash, serositis, and musculoskeletal involvement.

Over the past two decades, advances in molecular genetics have led to the identification of an increasing number of monogenic SAIDs, including Familial Mediterranean Fever (FMF), Cryopyrin-Associated Periodic Syndromes (CAPS), Mevalonate Kinase Deficiency (MKD), and Tumor Necrosis Factor Receptor–Associated Periodic Syndrome (TRAPS). Despite these advances, the diagnostic process remains complex. Clinical phenotypes frequently overlap across diseases, are highly variable, and incomplete penetrance or low-penetrance variants further complicate genotype–phenotype correlations. As a result, many patients experience significant diagnostic delay, with potential consequences for disease management, long-term outcomes, and quality of life.

International registries such as EUROFEVER have been established to collect clinical characteristics, complications, and treatment responses of these diseases, with currently more than 5,000 patients with different SAIDs recorded. Based on the statistical analysis of data catalogued in Eurofever, evidence-based classification criteria have been developed for hereditary recurrent fevers (FMF, MKD, TRAPS, CAPS). Moreover, a registry dedicated to all known pathogenic and benign genetic variants associated with autoinflammatory diseases has been created, the INFEVERS registry. This database is continuously updated and includes also variants of uncertain significance (VUS) reported in scientific studies.

Nonetheless, even with these tools, the diagnosis of SAIDs remains a challenge. Genetic variants are not always easy to interpret, raising the issue of correctly contextualizing genetic findings across multiple diseases with inflammatory phenotypes.

Therefore, new diagnostic strategies have been developed, progressing towards the use of standardized, computerized terminology that allows for the description of phenotypic characteristics, crucial for genotype–phenotype correlation analysis. The Human Phenotype Ontology (HPO) was developed to address this gap by providing a standardized, hierarchical vocabulary for phenotypic abnormalities. HPO is a terminology that enables consistent annotation of clinical features and supports interoperability across datasets, registries, and

analytical tools. Its increasing adoption in rare disease research has facilitated phenotype-driven gene discovery and computational diagnostics.

In 2021, a group of Experts revised and updated the HPO terminology for 73 inborn errors of immunity, including SAIDs. However, the actual accuracy of the new and revised HPO terms in correctly classifying SAIDs has not yet been validated in a cohort of real patients.

In parallel with advances in phenotypic standardization, machine learning (ML) approaches have emerged as powerful tools for analyzing complex, high-dimensional clinical data. ML methods are particularly well suited for rare diseases, where diagnostic reasoning relies on subtle combinations of features rather than single pathognomonic findings. When applied to structured phenotypic data, ML has the potential to support differential diagnosis, identify clinically meaningful subgroups, and uncover hidden genotype–phenotype relationships. Importantly, such approaches can aid, rather than replace, clinical expertise by providing data-driven support to clinicians.

An international project, the "Optimization of the diagnostic approach for inborn errors of immunity leading to hyper-inflammation (ODINO)", is currently ongoing. Its aim is to improve the diagnostic approach to inborn errors of immunity, using SAIDs as proof of concept. This project is one of the 3 working packages in which ODINO is articulated.

The present work aims to integrate clinical expertise, standardized phenotyping, and machine-learning methodologies to improve the characterization and classification of pediatric autoinflammatory diseases. By systematically linking Eurofever clinical variables to HPO terms and evaluating their diagnostic performance through supervised and unsupervised ML approaches, this work seeks to assess the strengths and limitations of current phenotypic ontologies, and to lay the ground for phenotype-driven diagnostic support tools in pediatric rheumatology.

1. SYSTEMIC AUTOINFLAMMATORY DISEASES

Systemic autoinflammatory diseases (SAIDS) are characterized by a sudden, antigen-independent over-activation of the immune response leading to an exaggerate sterile inflammatory reaction. This group defines a new class of diseases for which more than 100 associated genes have been identified over the past 20 years [1]. The first to be identified were the so-called recurrent fever syndromes: Familial Mediterranean Fever (FMF), Mevalonate Kinase Deficiency (MKD) or hyper-IgD syndrome, TNF Receptor-Associated Periodic Syndrome (TRAPS), and Cryopyrin-Associated Periodic Syndromes (CAPS). Over the following years, new diseases were discovered, not necessarily characterized by recurrent fever, but still characterized by an inflammasome hyperactivation with interleukin-1 (IL-1) overproduction, for which they were recently termed "inflammasomopathies"[2]. Alongside these, other diseases have been identified, such as interferonopathies, characterized by type I interferon overproduction, and the most recently described relopathies, related to mutations in the NFκB pathway. To complicate the picture, many polygenic diseases can present with symptoms overlapping those of monogenic SAIDs. Among them, Periodic Fever, Aphthous Stomatitis, Pharyngitis, and Adenitis (PFAPA) stands as a common mimicker of monogenic recurrent fevers with onset in the infancy.

The present thesis will focus on the main monogenic recurrent fevers (FMF, MKD, TRAPS; CAPS) and on PFAPA. In this chapter, an overview of their genetic basis, clinical manifestations, and treatment is provided.

In this first page we provide the EUROFER/PRINTO classification criteria (EPCC) for the genetic (Table 1.1) and clinical (Table 1.2) classification of FMF, MKD, TRAPS, CAPS and PFAPA, which will be widely cited in this chapter.

Table 1.1. Classification criteria for CAPS, FMF, TRAPS and MKD. Reproduced from [3]

CAPS	FMF	TRAPS	MKD
Presence of a *confirmatory NLRP3 genotype** and *at least one* among the following: ► Urticarial rash. ► Red eye (conjunctivitis, episcleritis, uveitis). ► Neurosensorial hearing loss. OR Presence of *not confirmatory NLRP3 genotype*† and *at least two* among the following: ► Urticarial rash. ► Red eye (conjunctivitis, episcleritis, uveitis). ► Neurosensorial hearing loss.	Presence of *confirmatory MEFV genotype** and *at least* one among the following: ► Duration of episodes 1–3 days. ► Arthritis. ► Chest pain. ► Abdominal pain. OR Presence of *not confirmatory MEFV* genotype‡ and *at least two* among the following: ► Duration of episodes 1–3 days. ► Arthritis. ► Chest pain. ► Abdominal pain.	Presence of confirmatory *TNFRSF1A* genotype* and at least one among the following: ► Duration of episodes ≥7 days. ► Myalgia. ► Migratory rash. ► Periorbital oedema. ► Relatives affected. OR Presence of a not confirmatory *TNFRSF1A* genotype† and *at least two* among the following: ► Duration of episodes ≥7 days. ► Myalgia. ► Migratory rash. ► Periorbital oedema. ► Relatives affected.	Presence of a *confirmatory MVK genotype** and *at least one* among the following: ► Gastrointestinal symptoms. ► Cervical lymphadenitis. ► Aphthous stomatitis.
Sensitivity: 1	Sensitivity: 0.94	Sensitivity: 0.95	Sensitivity: 0.98
Specificity: 1	Specificity: 0.95	Specificity: 0.99	Specificity: 1
Accuracy: 1	Accuracy: 0.98	Accuracy: 0.99	Accuracy: 1

A patient with (1) evidence of elevation of acute phase reactants (ESR or CRP or SAA) in correspondence to the clinical flares and (2) careful consideration of possible confounding diseases (neoplasms, infections, autoimmune conditions, other inborn errors of immunity) and a reasonable period of recurrent disease activity (at least 6 months) is classified as having hereditary recurrent fever if the criteria are met.
*Pathogenic or likely pathogenic variants (heterozygous in AD diseases, homozygous or in trans (or biallelic) compound heterozygous in AR diseases).
†Variant of uncertain significance (VUS). Benign and likely benign variants should be excluded.
‡In trans compound heterozygous for one pathogenic *MEFV* variants and one VUS, or biallelic VUS, or heterozygous for one pathogenic *MEFV* variant. See online supplementary table 7 for glossary.
AD, autosomal dominant; AR, autosomal recessive; CAPS, cryopyrin-associated periodic syndromes; CRP, C-reactive protein; ESR, erythocytes sedimentation rate; FMF, familial Mediterranean fever; MKD, mevalonate kinase deficiency; *MVK*, mevalonate kinase; PRINTO, pediatric rheumatology international trial organization; SAA, serum amyloid A; TRAPS, tumour necrosis factor receptor-associated periodic fever syndrome.

Table 1.2. Clinical Classification criteria for CAPS, FMF, TRAPS, MKD and PFAPA (no genetic required). Reproduced from [3]

PFAPA	CAPS	FMF	TRAPS	MKD
At least *seven out of* eight: Presence ► Pharyngotonsillitis. ► Duration of episodes, 3–6 days. ► Cervical lymphadenitis. ► Periodicity. Absence ► Diarrhoea. ► Chest pain. ► Skin rash. ► Arthritis.	Presence of *at least two of five**: ► Urticarial rash. ► Cold/Stress-triggered episodes. ► Sensorineural hearing loss. ► Chronic aseptic meningitis. ► Skeletal abnormalities (epiphysial overgrowth/frontal bossing).	At least *six out of nine*: Presence ► Eastern Mediterranean ethnicity. ► Duration of episodes, 1–3 days. ► Chest pain. ► Abdominal pain. ► Arthritis. Absence ► Aphthous stomatitis. ► Urticarial rash. ► Maculopapular rash. ► Painful lymph nodes.	Score ≥5 points: Presence ► Fever ≥7 days (2 points). ► Fever 5–6 days (1 point). ► Migratory rash (1 point). ► Periorbital oedema (1 point). ► Myalgia (1 point). ► Positive family history (1 point). Absence ► Aphthous stomatitis (1 point). ► Pharyngotonsillitis (1 point).	Presence of *at least three of six*: ► Age at onset <1 years. ► Gastrointestinal symptoms. ► Painful lymph nodes. ► Aphthous stomatitis. ► Triggers. ► Maculopapular rash.
Sensitivity: 0.97	Sensitivity: 0.80	Sensitivity: 0.91	Sensitivity: 0.87	Sensitivity: 0.91
Specificity: 0.93	Specificity: 0.91	Specificity: 0.92	Specificity: 0.92	Specificity: 0.82
Accuracy: 0.99	Accuracy: 0.85	Accuracy: 0.97	Accuracy: 0.96	Accuracy: 0.92

*Modified by Kuemmerle-Deschner *et al*.[14] See online supplementary table 6 for glossary. See table 2 for prerequisite criteria.
CAPS, cryopyrin-associated periodic syndromes; FMF, familial Mediterranean fever; MKD, mevalonate kinase deficiency; PFAPA, periodic fever, aphthosis, pharyngitis and adenitis; PRINTO, pediatric rheumatology international trial organization; TRAPS, tumour necrosis factor receptor-associated periodic fever syndrome.

FAMILIAL MEDITERRANEAN FEVER

Familial Mediterranean Fever (FMF, OMIM 249100) is characterized by recurrent episodes of short-lasting fever (1–3 days) that resolve spontaneously, associated with arthritis, skin manifestations, and serositis. Although the disease was described as a distinct clinical entity as early as 1945[4], the causative gene was only identified in 1997[5,6].

Pathogenesis and genetics

Unlike classic autosomal recessive disorders, FMF is caused by gain-of-function mutations in the *MEFV* (MEditerranean FeVer) gene, located on chromosome 16p13.3[5], which encodes a protein known as pyrin (or marenostrin). Pyrin is part of a multiprotein complex called the "pyrin inflammasome," which activates caspase-1, leading to the production and secretion of pro-inflammatory cytokines such as IL-1β and IL-18 from their precursors, and promotes pyroptosis through the gasdermin D pathway[7,8].

Pyrin senses alterations in cytoplasmic homeostasis induced by noxious stimuli ("homeostasis-altering molecular processes," HAMPs), which under physiological conditions modify the cellular RhoA GTPase. RhoA activates the serine-threonine kinases PKN1 and PKN2, which bind to and phosphorylate pyrin. Phosphorylated pyrin subsequently binds to its inhibitory molecule 14-3-3, thereby maintaining pyrin in an inactive state. In FMF, mutations impair the correct interaction of pyrin with PKNs and the inhibitory 14-3-3 protein, thus facilitating the formation of the pyrin inflammasome even in the absence of external stimuli[9,10]. The release of IL-1β in turn stimulates the expression of genes involved in the IL-1 pathway, creating a vicious cycle and contributing to a baseline inflammatory state.

The *MEFV* gene consists of 10 exons, and more than 370 variants have been described to date (http://fmf.igh.cnrs.fr/infevers/)[11] and, with the advent of next-generation sequencing (NGS) panels and exome analysis, the number of identified mutations is expected to increase further. Most pathogenic or likely pathogenic mutations are located in exon 10, which encodes a domain responsible for caspase-1 activation. In regions where FMF is endemic, approximately 80–90% of mutations are represented by M694V, M694I, V726A, and M608I[12]. These variants are associated with the classic FMF phenotype and with more severe disease[13].

"Benign" and "likely benign" mutations are generally located in exon 2 and typically do not cause a classic FMF phenotype. An exception is the p.S242R mutation in exon 2 of *MEFV*, which has been described in association with PAAND (Pyrin-Associated Autoinflammation with Neutrophilic Dermatosis), a condition characterized by episodes of prolonged fever

associated with neutrophilic dermatoses (acne, pyoderma gangrenosum), arthro-myalgias, and elevated inflammatory markers[14].

Approximately two-thirds of the variants recorded for FMF are currently unclassified or classified as variants of uncertain significance (VUS) (https://infevers.umai-montpellier.fr/web/search.php?n=1)[11].

FMF is considered an autosomal recessive (AR) disease, thus requiring FMF patients to carry two genetic variants, each on a different parental chromosome (biallelic). The concept of an AR transmission has been however challenged. In fact, while about 98% of individuals carrying a single pathogenic mutation are asymptomatic (healthy carriers), about 30% of symptomatic patients carry a single pathogenic mutation in heterozygosity (monoallelic)[15,16]. Moreover, some *MEFV* variants with autosomal dominant behavior have been identified in two papers, even if their phenotype slightly differs from classical FMF in terms of fever duration and skin manifestations[17,18].

For this reason, an expert panel of geneticists categorized the pathogenicity of *MEFV* variants (and other recurrent fevers) reported in the InFevers database, classifying the mutations as (i) pathogenic, (ii) likely pathogenic, (iii) VUS, (iv) likely benign, (v) benign, (vi) unclassified (the INSAID classification criteria)[19].

In 2019, the genetic criteria were also included in the EPCC[3]. A genotype is considered *confirmatory* in case of a *MEFV* homozygous or in trans (or biallelic) compound heterozygous mutations, while *non-confirmatory* in case of one *MEFV* pathogenic trans compound heterozygous variant and one VUS, or biallelic VUS, or one single pathogenic *MEFV* variant (monoallelic). The non-confirmatory phenotype requires at least 2 clinical symptoms for the diagnosis.

On the contrary, asymptomatic patients with two pathogenic mutations are defined as having "phenotype 3" FMF[20].

A recent study was conducted to compare the transcriptomic profiles of symptomatic monoallelic patients, classical biallelic carriers and healthy carriers, to identify differential molecular signatures and potential diagnostic pathways. The Authors highlighted an upregulation of the IFN signalling and inflammasome-related pathways in patients carrying one mutation, with marked upregulation of type I ISGs and the inflammasome sensor Pyrin. Moreover, functional assays demonstrated that type I IFN stimulation increased Pyrin protein levels in PBMCs and isolated monocytes, revealing cross-talk between IFN responses and

inflammasome signalling. These findings suggest that type I IFN signalling acts as a critical 'second hit', amplifying Pyrin expression in monoallelic individuals and enabling disease manifestation despite a single MEFV mutation, thus offering an explanation for the much-debated issue of the carriers expressing disease phenotypes[21].

Among genetic modifiers of disease course, polymorphisms in IL-1B have been associated with disease severity[22], and the α/α genotype of the SAA1 gene has been shown to increase the risk of developing AA amyloidosis without modifying the clinical disease phenotype[23]. The influence of specific environmental factors has not yet been clearly defined; however, the country of residence appears to act as an independent disease modifier, as living in countries such as Turkey, Armenia, and the Middle East has been associated with an increased risk of developing AA amyloidosis[24,25].

Epidemiology and clinical manifestations

FMF shows an ethnic distribution, with a higher prevalence among populations from the Middle East and the Mediterranean basin—particularly Turks, non-Ashkenazi Jews, Arabs, and Armenians. Nevertheless, hundreds of cases have been also reported in patients originating from European regions, the Americas, and Japan[26,27], and these numbers are expected to increase in view of the migration flows observed in recent years from endemic areas[28].

The prevalence of FMF ranges from 1:500 to 1:1,000 in endemic countries[26], where the frequency of heterozygous carriers is extremely high (up to 1:3 in Armenia[25], 1:395 in regions of central Anatolia[29]).

The onset of febrile attacks generally occurs in early childhood, and 80%–90% of patients become symptomatic before the age of 20 years[30]. Patients with FMF often report and recognize disease triggers, such as emotional stress, exposure to cold, menstrual cycle, and travel, and in some cases prodromal symptoms may occur prior to attacks[31].

Fever develops abruptly, with febrile peaks reaching very high temperatures within a few hours, and usually resolves spontaneously within the same day, although it may last 1–3 days. The recurrence of episodes is variable, with no defined periodicity. Although the frequency of manifestations associated with fever varies according to ethnic groups and age, fever is accompanied in approximately 90% of cases by abdominal pain and aseptic peritonitis[13,26]. Serositis may also present as pleuritic chest pain (33–53% of cases), whereas pericarditis is much rarer (1–2%)[32].

The third most common symptom is arthritis/arthralgia (21–76%): arthritis is usually mono- or oligoarticular, asymmetric, and most frequently involves the hip, knee, or ankle. Arthritis generally resolves within 1–3 days, similarly to fever, and prolonged arthritis is observed in less than 5% of patients, with permanent erosive damage occurring only rarely, particularly when the hip joint is involved[33]. Sacroiliitis has also been reported in approximately 10% of patients[34].

Cutaneous manifestations are less common (12–43%) and mainly consist of an erysipelas-like erythema of the lower limbs, which, although not very frequent, is highly pathognomonic of the disease[35]. Leukocytoclastic vasculitis has also been described in FMF patients, presenting as palpable purpura of the lower limbs, with Henoch-Shonnlein-like appearance[36]. Occasionally, cases of polyarteritis nodosa associated to FMF have also been described[37]. Other clinical manifestations may occur during attacks, including myalgias[33,38], which may persist for weeks after the fever[39], and splenomegaly.

During febrile attacks, there is a marked increase in inflammatory markers associated with neutrophilic leukocytosis, and a significant rise in serum amyloid A (SAA), the levels of which may remain elevated even during symptom-free periods[33]. SAA underlies the most feared complication of FMF, as its cleavage products deposit in tissues leading to the development of AA amyloidosis. The organ most commonly affected by amyloidosis in FMF is the kidney, with possible progression to renal failure, which in the pre-colchicine era affected up to 60–80% of FMF patients[30].

A rare FMF phenotype, defined as “phenotype 2,” is characterized by the absence of febrile episodes, with renal amyloidosis as the presenting manifestation of the disease (less than 1% of cases)[40]. In this setting, chronic inflammation—likely unrecognized because of the lack of febrile clinical manifestations—represents the major risk factor for the development of amyloidosis, renal failure, and other disease-related complications[41].

On the contrary, asymptomatic patients with two pathogenic mutations are defined as having “phenotype 3” FMF[20].

The heterogeneity of clinical manifestations and disease course results from a complex interplay between genetic, epigenetic, and environmental factors. The phenotype is primarily determined by the MEFV variants carried[12], with M694V being the most frequent and highest-penetrance mutation (causing increased neutrophil secretion of caspase-1, IL-1, and S100A12[42]). Patients carrying biallelic exon 10 variants, particularly M694V homozygotes, exhibit a disease phenotype characterized by early onset, more frequent attacks, musculoskeletal symptoms,

reduced response to colchicine, and chronic inflammation with an increased risk of secondary amyloidosis [32,43,44].

Heterozygous patients, when symptomatic, generally display a milder clinical phenotype compared with homozygous or compound heterozygous patients, with later disease onset, less frequent serositis attacks, better response to colchicine, and fewer complications[45,46]. Notably, even among patients carrying the same MEFV variant, symptoms and disease severity may vary considerably, including among members of the same family[47], highlighting the possible role of external factors influencing disease presentation and progression.

Diagnosis and classification criteria

The diagnosis of FMF is based on the patient's clinical history, the presence of inflammation (in particular elevated SAA), and, more recently, the use of genetic analyses. Several diagnostic criteria have been proposed, including the Tel Hashomer criteria[30], the Livneh criteria[48], and the Turkish pediatric criteria[49], all of which rely on clinical symptoms, family history, and response to colchicine.

In 2014, diagnostic/classification criteria for monogenic recurrent fevers were proposed, in which scores were assigned based on the presence or absence of specific symptoms, with a disease-specific cut-off allowing patient classification[50].

When relying solely on clinical criteria, the diagnosis of FMF may be challenging, particularly in non-endemic countries and in cases with atypical presentations. In this context, the advent of genetic testing has revolutionized its diagnostic process.

In 2019, new Eurofever/PRINTO classification criteria (EPCC) were proposed, incorporating genetic data in addition to clinical manifestations (Table 1.1 and 1.2). The EPCC were developed to provide highly specific criteria in order to include a homogeneous patient population in clinical or translational studies. Although these criteria are not mandatory for establishing a diagnosis, the inclusion of genetic information allows them to be used as a valuable diagnostic guide[3].

According to these criteria, patients with a *confirmatory genotype* can be classified as FMF in the presence of at least one clinical manifestation (attacks lasting 1–3 days, arthritis, abdominal pain, or chest pain), while patients with a *non-confirmatory genotype*, need at least two disease-related symptoms.

The impact of EPCC with the Infevers *MEFV* classification was evaluated in a recent work by Bustaffa and colleagues. They showed that patients who fulfilled the EPCC (EPCC+) displayed

the typical FMF features, while EPCC- patients had a more variable phenotype and a lower percentage of response to colchicine[51]. Two studies have compared the performances of the three diagnostic criteria (Tel-Hashomer, Livneh, pediatric criteria) and the EPCC, showing a good performance of all the criteria in diagnosing/classifying patients with homozygous or compound heterozygous mutations, but with a lower performance in heterozygous patients. [52,53]. Thus, the issue of FMF diagnosis in heterozygous patients remains unsettled[54].

Therapy

The goal of FMF treatment is to reduce the number of attacks and to decrease the degree of systemic inflammation, thereby preventing the development of AA amyloidosis.

The marked clinical heterogeneity among patients requires treatment to be individualized, with careful monitoring of attack frequency and number, as well as the presence of proteinuria.

Since its first use by Goldfinger in 1972[55], colchicine has represented the first-line treatment for FMF, having proven effective in reducing attack frequency and preventing the development of amyloidosis. Prior to its introduction, up to 50% of patients died from this complication[28].

Colchicine is an ancient drug, used for centuries in the treatment of acute gout. Although its precise mechanism of action is not yet fully understood, it has been shown to exert inhibitory effects on microtubule polymerization, adhesion molecules, neutrophil chemotaxis, and the NLRP3 inflammasome[56].

The EULAR guidelines for the treatment of FMF recommend colchicine as first-line treatment, with a variable dose depending on the patient's weight, that can be increased up to 2 mg/day in pediatric patients and up to 3 mg/day in adults if the response is inadequate and the drug is well tolerated[57].

The use of colchicine has dramatically reduced the incidence of amyloidosis in FMF patients, with a current rate of approximately 10%[58]. About 65% of patients achieve complete disease remission on therapy, and 20–30% experience significant improvement with a reduction in attack frequency and severity. Conversely, 5–10% of patients are non-responsive to colchicine.

In patients receiving the maximum tolerated dose, "colchicine resistance" is defined as the persistence of disease activity, namely the persistence of febrile attacks (on average ≥1 attack per month for at least 3 months), or persistently elevated CRP or SAA levels during attack-free intervals, in the absence of any other plausible explanation (e.g., after verifying adherence)[59].

Nevertheless, poor treatment adherence remains one of the major causes of therapeutic failure. Colchicine is associated with gastrointestinal (GI) side effects, particularly diarrhea and

abdominal pain, which are very common and may limit the ability to reach an adequate dose (colchicine intolerance). Colchicine toxicity is rarer and manifests with severe GI effects (persistent diarrhea), elevated transaminases, leukopenia, myopathy, azoospermia, and, in rare cases, cutaneous manifestations. It should be noted that colchicine often unmasks lactose intolerance; therefore, GI side effects can frequently be resolved by eliminating lactose from the diet[59].

For patients resistant to colchicine, the addition of a biological therapy, is indicated. Several trials have been conducted in FMF patients with anti IL-1 agents (anakinra, canakinumab, and rilonacept)[60–65] and 2 trials with an anti-IL-6 treatment[66,67]. Based on these evidences, a therapeutic algorithm has been proposed[57]. The recent recommendations cite also the JAK-inhibitor tofacitinib. However, other than IL-1 inhibitors, anti-IL-6 and JAK-inhibitors have not been approved for use in colchicine-resistant FMF and thus require expert advice before they can be initiated[57]. Today, the agents most commonly used in colchicine-resistant patients are therefore anakinra and canakinumab which resulted safe and effective in the treatment of colchicine resistant FMF patients[60–65]. No comparative studies between these two drugs are available; however, both have demonstrated the ability to stabilize and improve short-term complications such as proteinuria. Until their efficacy is fully established, colchicine is still recommended in combination with these agents, unless contraindicated[57].

Anti–IL-1 therapies remain strongly indicated in patients with AA amyloidosis and active inflammation (elevated CRP or SAA) despite the maximum tolerated dose of colchicine, with a target SAA level <10 mg/L. This cut-off has been associated with regression of amyloid deposits in approximately 60% of cases and improved survival[68], likely reflecting the ability of these agents to improve renal function in patients with AA amyloidosis[69].

Follow-up and assessment of disease activity

After a diagnosis of FMF has been established, all family members should undergo both clinical and genetic evaluation. Treatment response, adherence, and potential colchicine toxicity should be monitored every 3–6 months, together with laboratory tests including complete blood count, liver and renal function tests, SAA, CRP, and urinalysis with assessment of microalbuminuria.

The most effective way to monitor disease activity remains the use of a diary of febrile episodes, in which the patient records the symptoms experienced, their duration, medications taken, and any potential triggering factors. To this end, a standardized form has been developed for all recurrent fevers—the AutoInflammatory Disease Activity Index (AIDAI)—which includes all possible symptoms and is completed by the patient on a daily basis and reviewed by the

physician, allowing for assessment of the clinical course of the disease[70,71]. An international effort is currently underway, through a Delphi survey, to develop a more comprehensive tool for disease activity assessment that will also incorporate laboratory parameters[72].

MEVALONATE KINASE DEFICIENCY

Mevalonate Kinase Deficiency (MKD, OMIM 260920, or Hyper-IgD Syndrome) is a monogenic autoinflammatory recurrent fever with early onset, characterized by febrile episodes lasting 4–6 days and associated with aphthosis, lymphadenopathy, cutaneous, and gastrointestinal manifestations[73]. The term hyper-IgD derives from the observation that elevated IgD levels were found in all patients in the first reported cases[74]. However, now that the pathogenesis of the disease has been clarified, the term mevalonate kinase deficiency (MKD) is considered more appropriate.

Pathogenesis and genetics

MKD is caused by mutations in the *MVK* gene, which encodes the enzyme mevalonate kinase[75]. This enzyme is the second enzyme in the cholesterol and non-sterol isoprenoid biosynthesis pathway, acting immediately downstream of HMG-CoA reductase and catalyzing the conversion of mevalonic acid to phosphomevalonate. Isoprenoids are involved in protein prenylation through the addition of farnesyl or geranylgeranyl groups, a process that is essential for correct protein function. Defects in *MVK* therefore lead to accumulation of the substrate mevalonic acid and to a deficiency in the production of intermediate and end products of prenylation, particularly geranylgeranyl pyrophosphate (GGPP), leading to compromised protein prenylation. This impairs the post-translational modification of small GTPases (such as RhoA, Rac1, and Cdc42), resulting in their aberrant activation, triggering inappropriate signaling cascades and dysregulation of the innate immune response[76–78]. Moreover, this defect in prenylation promotes the activation of pyrin inflammasome, leading to increased production of IL-1beta, explaining the systemic inflammation and recurrent fever of MKD[78–81].

Depending on the residual levels of enzymatic activity, different clinical manifestations may occur, forming a true disease spectrum, with lower activity resulting in more severe phenotypes. This spectrum ranges from the most severe phenotype, mevalonic aciduria (MA), which occurs when enzymatic activity is below 1% and is characterized by severe neurological involvement with intellectual disability, cerebellar ataxia, facial dysmorphisms, and inflammatory symptoms leading to early death, to a predominantly inflammatory and milder phenotype, MKD[82].

MKD is an autosomal recessive disease. To date, more than 283 substitutions or deletions in the *MVK* gene have been reported (https://infevers.umai-montpellier.fr/web/search.php?n=3). Some variants (e.g., V310M, A334T) are strongly associated with a severe MA phenotype and with markedly reduced cellular MVK activity[83]. The most common mutation in the *MVK* gene is the V377I variant, which is exclusively associated with the milder MKD phenotype and with residual MVK activity[84]. This variant is found in compound heterozygosity in the vast majority of patients with MKD-associated periodic fever. Other mutations, such as H20P and I268T, have also been associated with both MA and MKD phenotypes[83,85].

Some patients may present an intermediate phenotype, characterized by typical febrile attacks associated with neurological manifestations (intellectual disability, cerebellar ataxia) of variable severity[86], highlighting how MA and MKD represent the two extremes of a broad clinical spectrum.

Clinical manifestations

The onset of MKD occurs in childhood, typically within the first years of life. MKD is characterized by recurrent febrile episodes lasting approximately 5–7 days, associated with cervical lymphadenopathy, oral aphthosis, abdominal pain, cutaneous rash, hepatosplenomegaly, and arthralgias[73]. Mucocutaneous manifestations are present in about 70% of patients and consist, in approximately 50% of cases, of aphthosis, which may occasionally also involve the genital area, and of a rash with variable characteristics—most commonly maculopapular, but which may also be morbilliform or purpuric[87].

The frequency of episodes is variable; however, typical disease triggers have been reported, such as vaccinations, intercurrent infections, or trauma[73]. Some patients also experience recurrent infections and may therefore enter the differential diagnosis with immunodeficiencies, despite the absence of an actual immune defect[85,88]. Symptoms of MKD tend to attenuate over time, although adults with persistent febrile episodes have been reported, in whom amyloidosis may also occur[89,90].

During febrile episodes, neutrophilic leukocytosis and elevated inflammatory markers are observed. Elevated IgD levels were historically considered a distinctive feature of the disease, but are difficult to measure; concomitant elevation of IgA is more commonly observed and may further support the diagnostic suspicion[85]. Finally, during febrile episodes, increased urinary excretion of mevalonic acid is detected (urine samples should be collected and promptly frozen due to volatility); when present, this finding is highly specific for the disease.

Diagnosis and classification criteria

The diagnosis is based on the presence of the above-mentioned clinical manifestations together with pathogenic genetic mutations in homozygosity or compound heterozygosity. While awaiting genetic results, the combination of compatible clinical features, very early disease onset, and the detection of mevalonic aciduria during febrile episodes allows a presumptive diagnosis to be made.

According to the Eurofever/PRINTO classification criteria, in the presence of *confirmatory* genetic testing (two pathogenic mutations), a single clinical manifestation among gastrointestinal involvement, cervical lymphadenitis, and aphthous stomatitis is sufficient for classification. In contrast, purely clinical criteria require the presence of at least three of the following features: age at onset <1 year, gastrointestinal manifestations, cervical lymphadenitis, aphthous stomatitis, typical triggers, and maculopapular rash[3].

Treatment

Febrile attacks respond dramatically to corticosteroid administration. However, because of the high frequency of febrile episodes, some patients require almost continuous treatment. For the management of acute attacks, symptomatic medications such as paracetamol and nonsteroidal anti-inflammatory drugs (NSAIDs) are therefore recommended[91].

The currently recommended maintenance therapy consists of IL-1 inhibitors[92]. Although both anakinra and canakinumab have been used successfully in children with MKD[62,93,94], only canakinumab has been evaluated in a randomized controlled trial[62] and has been approved by the FDA and the EMA. The recommended dosage in pediatric patients is 2–4 mg/kg every 4 weeks, while in adult patients it is 150–300 mg every 4 weeks[92]. When IL-1 inhibitor therapy is unavailable or ineffective, a few cases successfully treated with other biologic agents, such as tocilizumab or etanercept, have been reported[95]. It should be noted that colchicine therapy is not effective in patients with MKD[73,96].

None of the above-mentioned approaches addresses the underlying molecular defect of the disease, and gene therapy or enzyme replacement therapies are not yet available. An attempt has been made to administer statins, aiming to reduce the accumulation of the mevalonate substrate; however, this strategy did not yield significant results[97].

For the most severe disease phenotype (mevalonic aciduria), allogeneic bone marrow transplantation has been employed[98–100] and has also proven effective in intermediate phenotypes that are difficult to manage with conventional therapy[101,102].

Follow-up and assessment of disease activity

As already outlined for FMF, it is essential to follow these patients with a symptom diary (AIDAI) and with serial blood tests (every 3–6 months) to assess disease activity and systemic inflammation. Indeed, although cases of amyloidosis are rare, this complication may occur in the presence of persistent inflammation[89,90].

TRAPS (TNF-Receptor Associated Periodic fever Syndrome)

Tumor Necrosis Factor Receptor–Associated Periodic Syndrome (TRAPS, OMIM 142680), also known as familial Hibernian fever, was first described in 1982 in a family of Irish descent[103]. It is characterized by long-lasting episodes (>1 week), with highly variable periodicity, associated with periorbital edema, cutaneous manifestations, and abdominal pain.

Pathogenesis and genetics

TRAPS is caused by mutations in the type 1 tumor necrosis factor receptor (TNFR1), encoded by the *TNF Superfamily Receptor 1A* (*TNFRSF1A*) gene, and is inherited in an autosomal dominant manner[104].

It should be noted that two mutation numbering systems are currently in use for TRAPS. Initially, the first 29 amino acids encoding the signal peptide were not included; however, numbering should start from methionine 1 of the transcript. Consequently, according to the updated nomenclature, mutations differ by 29 amino acids (e.g., R92Q becomes R121Q).

To date, more than 170 missense variants of the *TNFRSF1A* gene have been reported, of which 43 are classified as pathogenic and 56 as likely pathogenic in the Infevers database (https://infevers.umai-montpellier.fr/web/search.php?n=2). Most patients carry missense mutations affecting one of the cysteine residues involved in disulfide bond formation, which play a fundamental role in correct extracellular protein folding[105]. These structural mutations are associated with a more severe disease phenotype and with a higher predisposition to the development of amyloidosis.

In addition, distinct low-penetrance structural variants have been described, the most notable being p.Arg121Glu (R121Q, also known as R92Q) and p.Pro75Leu (P75L, also known as P46L). The P46L variant is found exclusively in individuals of African ancestry, whereas R92Q is predominantly observed in Caucasian populations[106]. Initially, the R92Q variant was believed to be disease-causing and was reported in a large proportion (12–83%) of TRAPS patients[107]. However, this mutation is present in 1–3% of the general population, and compared with the

mutated protein found in classic TRAPS patients, the functional properties of the p.R92Q TNFR1 protein are very similar to those of the wild-type receptor[105,108]. Moreover, numerous studies and clinical observations suggest that the pathogenesis, clinical manifestations, and treatment response of patients carrying the R92Q variant differ from those of patients with classic TRAPS[105,109]. More recently, one study has shown that patients with the R92Q mutation more closely resemble patients with SURF than those with TRAPS in terms of clinical characteristics[110]. In this context, R92Q should therefore be considered as a low-penetrance variant associated with a milder and distinct phenotype. On the contrary, P46L should be considered as a benign polymorphism.

The precise mechanisms by which TNFR1 mutations cause the inflammatory phenotype of TRAPS are not yet fully understood. It is believed, however, that these mutations alter the structure of the receptor within its extracellular domain, interfering with its ability to bind TNF and with receptor shedding from the cell surface to generate soluble TNF receptors, which normally contribute to attenuation of TNFR1-mediated signaling. In addition, mutated proteins misfold and accumulate intracellularly, causing endoplasmic reticulum stress with the release of reactive oxygen species[111], and activating pro-inflammatory pathways[112,113]. Finally, patients with TRAPS exhibit defective autophagy mechanisms that normally serve to remove intracellular TNFR1, further promoting chronic inflammation and excessive interleukin-1β secretion[114].

Both TNF-dependent and TNF-independent mechanisms contribute to the disease: mutant TNFR1 sensitizes cells to TNF stimulation and also drives ligand-independent proinflammatory signaling[115]. Moreover, studies in mice have shown that the concerted action of wild-type and mutant TNFR1 proteins from distinct cellular locations potentiate the inflammatory response[113]. These mechanisms explain the variable efficacy of anti-TNF therapies in these patients.

Clinical features

TRAPS is the second most common recurrent fever syndrome worldwide after FMF. The disease primarily affects individuals of Northern European descent, but it has been described in all ethnic groups, including the Mediterranean basin and Asia[116].

At onset, TRAPS is characterized by recurrent febrile attacks lasting 1–3 weeks, separated by periods of complete wellness, with highly variable periodicity. Febrile episodes are predominantly associated with cutaneous, musculoskeletal, and gastrointestinal manifestations[117,118].

Cutaneous symptoms associated with TRAPS consist of a migrating macular rash that occurs on the extremities and/or trunk. These skin lesions are generally warm and painful and are histologically characterized by deep perivascular mononuclear cell infiltrates. When lesions occur on the arms or legs, they may be associated with myalgia due to underlying monocytic fasciitis. Other types of rashes may also be observed, such as generalized serpiginous plaques or annular patches[35].

Abdominal and chest pain are frequently present, caused by acute peritoneal inflammation or pleuritis. Ocular involvement is common and is characterized by periorbital edema and conjunctivitis[118]. Arthralgia is more frequent than arthritis and generally involves large joints such as the hips, knees, and ankles. During attacks, marked elevation of inflammatory markers (ESR, CRP) and neutrophilic leukocytosis is observed[107].

In adulthood, febrile episodes may become less frequent, and patients may present a subchronic disease course with flare-ups of abdominal pain, arthro-myalgias, ocular manifestations, and a mild but persistent increase in acute-phase reactants, including serum amyloid A (SAA). AA renal amyloidosis represents the most severe long-term complication: its prevalence was initially around 14%[105] but has markedly decreased with the introduction of new therapies. In a few adult patients, central nervous system involvement has been reported, characterized by a demyelinating disorder[119].

Diagnosis

The diagnosis of TRAPS is based on the presence of characteristic clinical symptoms combined with the detection of pathogenically relevant mutations in the TNFRSF1A gene. According to Eurofever/PRINTO criteria, a TRAPS patient is classified in the presence of a pathogenic mutation of TNFRSF1A associated with at least one of the following: fever duration >7 days, myalgias, rash, periorbital edema, or affected family members. In the case of a variant of uncertain significance (VUS), at least two of the above clinical criteria are required[3].

Treatment

Febrile episodes respond very well to steroid therapy. However, due to the potentially long duration of attacks and the tendency toward a chronic course, patients may become steroid-dependent. First-line therapy currently consists of IL-1 inhibitors (anakinra or canakinumab)[92].

Although anakinra was the first anti–IL-1 drug used successfully in TRAPS patients[120–122], the only anti–IL-1 agent currently approved for this condition is canakinumab, which has proven highly effective in controlling disease activity[62,123].

Early therapies for TRAPS included TNF inhibitors, based on the causative mutation. Among these, etanercept was effective in some cases but appears to lose efficacy over time and is indicated only in refractory cases[124–127]. Other TNF inhibitors (adalimumab and infliximab) have shown highly variable efficacy and, in some cases, a worsening of the clinical picture, and are therefore not recommended[128,129].

Follow-up and assessment of disease activity

As already specified for FMF and MKD, it is essential to monitor these patients using a symptom diary (AIDAI) and serial blood tests (every 3–6 months) to evaluate disease activity and systemic inflammation, aiming to prevent the development of amyloidosis.

CRYOPYRINOPATHIES

In 2001/2002, the NLRP3 gene (also known as *CIAS1* or *NALP3*), which encodes the protein cryopyrin[130–132], was discovered. Mutations in this gene cause the **cryopyrin-associated periodic syndromes (CAPS),** a clinical spectrum of different autoinflammatory phenotypes, including a mild phenotype called familial cold autoinflammatory syndrome (FCAS, OMIM 120100), a moderate phenotype known as Muckle-Wells syndrome (MWS, OMIM 191900), and the severe phenotype neonatal-onset multisystem inflammatory disease (NOMID)/chronic infantile neurological cutaneous articular syndrome (CINCA, OMIM 607115). In a recent consensus proposal for a new taxonomy of monogenic AIDs, it was suggested to use the term NLRP3-associated autoinflammatory disease (NLRP3-AID) to describe the CAPS spectrum[133].

Pathogenesis and genetics

The *NLRP3* gene encodes the NLRP3 protein, which is part of an intracellular multimolecular complex called the NLRP3 inflammasome[134]. The NLRP3 inflammasome consists of specific adaptor proteins such as ASC (apoptosis-associated speck-like protein containing a caspase recruitment domain) and various chaperone proteins. Its assembly allows the activation of caspase-1, which cleaves pro-interleukin (IL)-1β and pro-IL-18 into their biologically active forms (IL-1β, IL-18). Once released, IL-1β triggers downstream signaling cascades that ultimately activate nuclear factor κB (NFκB) and promote the production and release of other inflammatory cytokines[135–137]. In non-mutated cells, the NLRP3 inflammasome can be activated by a wide variety of extracellular stimuli linked to pathogens or cellular damage (PAMPs and DAMPs). In contrast, in mutated cells from CAPS patients, the NLRP3 inflammasome is constitutively active, leading to persistent basal inflammation that is further

exacerbated by external stimuli. The inflammasome is normally maintained in an inactive state via an autoinhibitory mechanism through interactions of specific protein domains (LRR and NACHT). Mutations affecting these domains appear to disrupt this autoinhibition, keeping the NLRP3 inflammasome constantly activated and resulting in continuous IL-1β release[138–141].

CAPS are autosomal dominant diseases, so a single mutation is sufficient to cause disease. Most mutations are missense substitutions concentrated in exon 3, which encodes the NACHT domain, while other pathogenic mutations have been identified in exon 5, encoding the LRR domain[142]. Different *NLRP3* mutations have been associated with the three distinct clinical phenotypes, although some overlap may occur.

Notably, there are CAPS patients with acquired somatic NLRP3 mutations. First described in 2005[143], somatic mosaicism has since been confirmed in approximately 70% of CAPS patients without confirmatory germline genetics[144]. To date, 35 different somatic mutations in the *NLRP3* gene have been identified[145]. In a recent paper that evaluated 17 somatic-NLRP3 patients, a marked similarity in terms of clinical manifestations, and treatment response with patients with germline variants was shown[146]. Due to the low or very low frequency of the mutant allele, somatic mutations may not be detected using conventional genetic analysis methods, such as Sanger sequencing. Therefore, technologies with greater depth, such as Next Generation Sequencing (NGS), are required[147].

Clinical features

CAPS is a multisystem inflammatory disorder affecting multiple organs and systems. Some inflammatory signs are commonly associated with distinct phenotypes within the CAPS spectrum, while others are present across all subgroups. Characteristic CAPS symptoms may arise from acute inflammation but can also result from organ damage caused by chronic inflammation.

As they are secondary to mutations in the same gene, FCAS, MWS, and CINCA should be considered a continuum of a single condition, with severity dependent on the type of NLRP3 mutation[147,148].

Familial Cold Autoinflammatory Syndrome (FCAS) represents the mildest clinical phenotype. It is characterized by short episodes (<24 hours) triggered by cold exposure, including fever, non-pruritic urticarial rash, arthro-myalgias, and conjunctivitis, associated with systemic inflammation detectable in laboratory tests that normalizes during intercritical periods. Unlike

cold urticaria, FCAS exhibits a latency between cold exposure and symptom onset (ice cube test negative)[147].

Muckle-Wells Syndrome (MWS) represents the intermediate clinical phenotype. It typically begins in the first months of life, with fever accompanied by urticarial rash, conjunctivitis, arthralgia, and arthritis. Sensorineural hearing loss develops in young adulthood[147]. Systemic inflammation is present during flares and does not normalize in intercritical periods. In untreated patients, renal amyloidosis represents a severe long-term complication[149].

Chronic Infantile Neurologic Cutaneous Articular (CINCA) syndrome represents the most severe clinical phenotype. The onset is neonatal, with intermittent fever and systemic inflammation characterized by urticarial rash, conjunctivitis, lymphadenopathy, hepatosplenomegaly, arthralgia, arthritis, patellar overgrowth, "clubbing" of fingers, widening of metaphyses and epiphyses of long bones, and a typical facies with prominent frontal bossing, mandibular hypoplasia, and saddle-shaped nose. Chronic CNS inflammation causes chronic meningitis with possible sequelae (mental retardation), papilledema, and cochlear inflammation, leading to sensorineural hearing loss[147]. If untreated, this severe form results in generalized impairment with long-term mental retardation and, due to chronic inflammation, carries a risk of developing renal amyloidosis[149].

Diagnosis

Diagnostic criteria for CAPS have been proposed in 2017 for CAPS. According to these criteria, a diagnosis can be made even in the absence of genetic analysis, requiring elevated inflammatory markers as a mandatory criterion, along with at least two of the following: urticarial rash, cold-induced episodes, sensorineural hearing loss, musculoskeletal symptoms, chronic aseptic meningitis, and skeletal abnormalities[150]. According to Eurofever/PRINTO classification criteria, a confirmatory genotype is required, associated with at least one of the following: urticarial rash, red eye (conjunctivitis, scleritis, uveitis), or sensorineural hearing loss[3].

Treatment

Since IL-1 plays a central role in CAPS pathogenesis, anti–IL-1 therapy is recommended across the CAPS spectrum[92], and multiple studies have evaluated its safety and efficacy[151–154,154–159]. However, symptomatic patients with low-penetrance variants may achieve only partial response to anti–IL-1 therapy, as inflammation appears to be mediated not only by NLRP3-specific IL-1β but also by IL-6 or TNF independent of NLRP3[147].

Anakinra and canakinumab are approved by both the European Medicines Agency (EMA) and the Food and Drug Administration (FDA) for CAPS.

The dosage of anakinra ranges from 1–2 mg/kg/day for patients with FCAS to 10 mg/kg/day for severe CINCA patients. Canakinumab is indicated at an initial dose of 2 mg/kg for mild to moderate CAPS, with potential escalation to 3–4 mg/kg[92,160], and doses up to 8 mg/kg every 4 weeks have been described for NOMID/CINCA patients[161].

Notably, anakinra appears to penetrate the central nervous system more effectively than canakinumab and is therefore the treatment of choice for aseptic meningitis in CINCA, as well as for progressive hearing loss despite canakinumab therapy in MWS and CINCA patients[162].

Follow-up and assessment of disease activity

Regular clinical and laboratory monitoring is essential to determine disease activity and organ damage. Monitoring includes proper assessment of growth, periodic blood tests to exclude chronic systemic inflammation, and in MWS/CINCA patients, annual audiometric exams to detect early onset sensorineural hearing loss, with potential inner ear MRI. In CINCA, annual brain MRI and cerebrospinal fluid analysis are also recommended to exclude CNS inflammation. For monitoring disease activity in CAPS, the AIDAI can be used[71]. For MWS specifically, the MWS-DAS is available, dividing disease symptoms into ten domains: nine reflecting organ involvement (fever, headache, eye involvement, hearing disorders, oral ulcers, abdominal pain, renal disease, musculoskeletal disease, and skin rash), and a tenth domain representing the patient global assessment score[163].

PFAPA (*Periodic Fever, Aphthous stomatitis, Pharyngitis and cervical Adenitis*)

PFAPA syndrome is the most common cause of periodic fever in childhood and was first described in 1987 by Marshall et al.[164]. The disease typically begins between ages 2 and 5 and generally resolves by adolescence. It is characterized by fever episodes lasting approximately 3–6 days, associated with aphthous stomatitis, lateral cervical lymphadenopathy, and exudative pharyngitis. The episodes are highly stereotyped, occurring with a very precise periodicity, typically every 3–8 weeks. Patients are asymptomatic between episodes and demonstrate normal growth. During episodes, additional symptoms such as abdominal pain, arthro-myalgias, and headache may occur, though they are less severe than in monogenic periodic fevers. Unlike monogenic periodic fevers, children with PFAPA are in good clinical condition even during febrile episodes[165].

PFAPA is a multifactorial condition, not associated with mutations in genes responsible for monogenic periodic fevers. Although sporadic familial cases have been described, no genetic basis has been demonstrated.

The natural course of PFAPA is generally favorable, as febrile episodes tend to resolve by the age of 10, though cases persisting into adulthood have been reported. Long-term complications are absent[165]. There is currently no evidence that medical treatment alters long-term outcomes, but therapy can be effective in managing episodes, which is crucial for improving the quality of life of patients and their families.

Diagnosis

Diagnostic criteria were established in 1999 (Marshall modified), requiring for diagnosis: recurrent fever with onset <5 years, constitutional symptoms in the absence of airway infections, at least one of the following symptoms: pharyngitis, adenitis, or aphthous stomatitis, normal growth, asymptomatic intercritical periods, and exclusion of cyclic neutropenia[166].

The 2019 Eurofever/PRINTO classification criteria require the presence of at least 7 of 8 criteria: fever duration 3–6 days, fixed periodicity, pharyngotonsillitis, cervical lymphadenitis, and absence of diarrhea, chest pain, rash, and arthritis. These criteria have proven to be highly sensitive and specific, particularly due to the presence of negative diagnostic elements (absence of certain features), allowing PFAPA to be distinguished from other recurrent fevers[3].

Treatment

Fever episodes respond dramatically to a single dose of corticosteroid (betamethasone 0.1 mg/kg or prednisone 1 mg/kg), even if corticosteroid therapy has been shown to shorten the interval between episodes[167].

Tonsillectomy resulted effective in resolving PFAPA episodes in a variable percentage of patients, especially those with significant pharyngotonsillar involvement, although success rates vary across different studies[168,169]. There is no evidence that combining adenoidectomy with tonsillectomy improves outcomes compared to tonsillectomy alone. Considering PFAPA's favorable natural history and potential post-surgical complications, adenotonsillectomy should be offered to selected patients with prominent pharyngotonsillar involvement and in patients with refractory or severe disease impacting quality of life, or when medical therapy fails or is not tolerated[170].

Regarding other therapies, results are controversial for colchicine, cimetidine, and vitamin D. In studies using colchicine, approximately half of patients carried high-penetrance MEFV mutations[171–173].

IL-1 seems to play a central role in PFAPA pathogenesis[174], and anakinra has been administered in some patients with good results[175,176]. However, despite these promising reports, due to the lack of randomized trials and the generally favorable spontaneous course, anti–IL-1 therapy for PFAPA should be limited to highly selected cases.
Finally, probiotics have been used in PFAPA treatment. In a recent study of 20 PFAPA patients treated with Lactobacillus plantarum HEAL9 and Lactobacillus paracasei 8700:2, all but one patient experienced a reduction in attack frequency, and 5 of 11 patients (45%) with ≥1 attack while on probiotics reported reduced symptom severity[177]. Similar results were reported using Streptococcus salivarius K12 (SSK12) in a cohort of 85 PFAPA patient[178]. Although no trials exist, this approach may be considered in selected patients with prominent pharyngotonsillar involvement.

2. THE HPO SYSTEM

The **Human Phenotype Ontology (HPO)** (HPO, https://hpo.jax.org) is an ontology-based standardized vocabulary developed to enable the structured representation of human phenotypic abnormalities in a format suitable for computational analysis. By providing standardized terms for clinical features, HPO allows the annotation of phenotypic characteristics of patients and diseases, facilitating phenotype-driven diagnosis and genotype–phenotype correlation studies. Since its introduction in 2008, the ontology has undergone continuous expansion and refinement through contributions from an international community of clinicians and researchers across different disciplines[179,180].

Each HPO term corresponds to a specific phenotypic abnormality and is labeled by a unique identifier (e.g., "recurrent fever", HP:0001954). Terms are organized within a hierarchical structure based on "is-a" relationships, in which more specific phenotypes are linked to broader parent categories. At the top of the hierarchy are general categories (e.g., *Phenotypic abnormality*), which branch into increasingly detailed and specific phenotypic terms. This structure allows individual "child" terms to inherit properties from multiple ancestors, reflecting the complexity and overlap inherent to human phenotypes. This hierarchical organization enables the computational comparison of phenotypic profiles at different levels and allows phenotype-based disease analysis. In **Figure 2.1**, an example of an HPO hierarchical tree regarding the term "Fever" is provided.

```
Phenotypic abnormality (HP:0000118)
└─ Abnormality of metabolism/homeostasis (HP:0001939)
   └─ Abnormal homeostasis (HP:0012337)
      └─ Abnormality of temperature regulation (HP:0004370)
         └─ Fever (HP:0001945)
            ├─ Recurrent fever (HP:0001954)
            ├─ Persistent fever (HP:0004387)
            ├─ Hyperpyrexia (HP:0001955)
            ├─ Low-grade fever (HP:0011134)
            └─ Unexplained fevers (HP:0033829)
```

Figure 2.1 Hierarchical organization of the HPO tree for the term Fever

Each HPO term can be systematically linked to one or more diseases through phenotype–disease annotations. These associations describe which phenotypic abnormalities are observed in a specific disease and are derived from expert curation of the medical literature, clinical

databases, and validated case series. Diseases are typically referenced using standardized identifiers (such as OMIM, Orphanet, or MONDO IDs), ensuring interoperability across different biomedical resources.

For example, the term "recurrent fever" HP:0001954, is linked to 117 diseases that present with recurrent fever. Under the term "recurrent fever" there are two branches: "periodic fever" and "non-periodic fever". If we examine the term "periodic fever" HP:0032323, it is associated to only 8 of the previously included diseases (**Figure 2.2**).

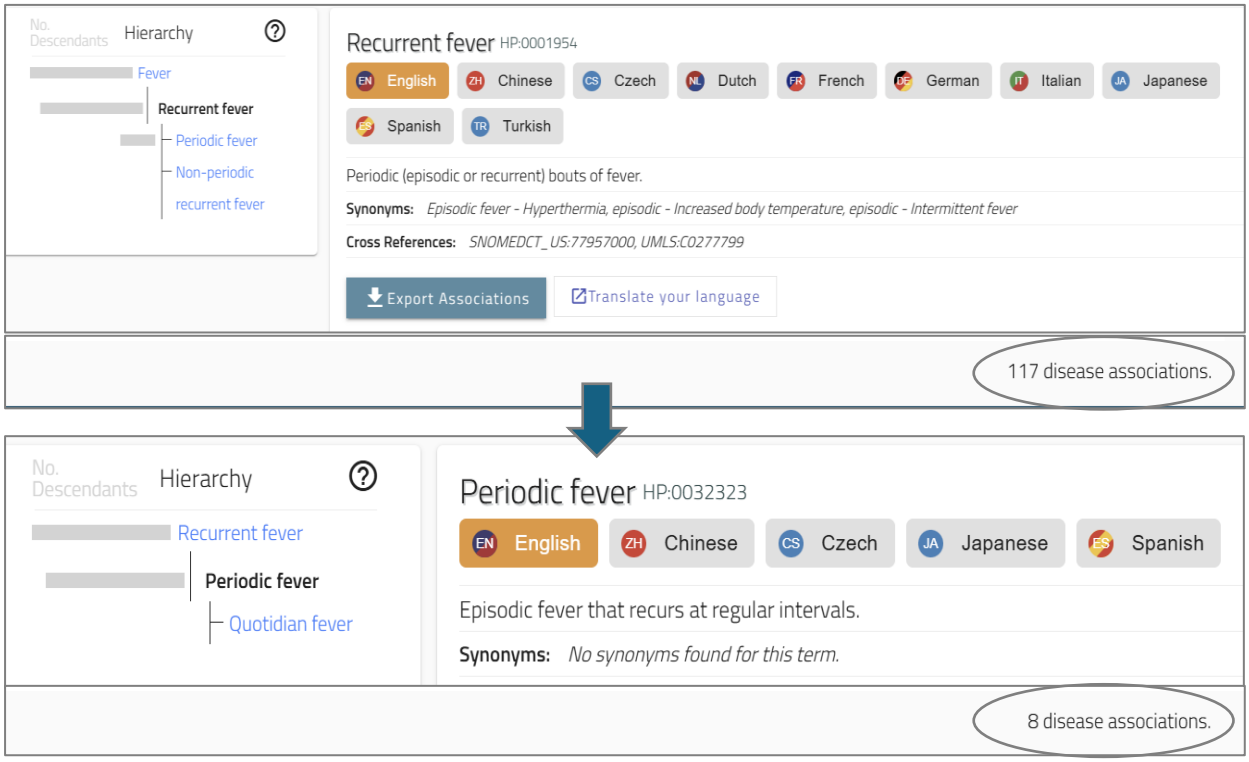


Figure 2.2 Screenshots from the HPO site for the terms "Recurrent fever" and "periodic fever"

For each disease, a set of HPO terms is used to define its phenotypic profile. Each term is than associated with the frequency reported per that term in that disease (**Figure 2.3**). Importantly, these associations are not binary: HPO annotations can incorporate the frequency of information (e.g., very frequent, occasional, rare) and, when available, onset modifiers or clinical qualifiers, allowing a more accurate representation of disease variability. This means that a phenotype is not simply recorded as "present" or "absent" in a disease. Instead, HPO allows additional layers of information that describe *how often*, *when*, and in *what form* a clinical feature occurs in a given condition. Through this structure, patient phenotypes encoded with HPO terms can be computationally compared with known disease profiles, enabling phenotype-driven diagnostic algorithms, differential diagnosis ranking, and improved genotype–phenotype correlation

analyses. This type of information can be used by algorithms to weight findings in the context of clinical differential diagnosis [181].

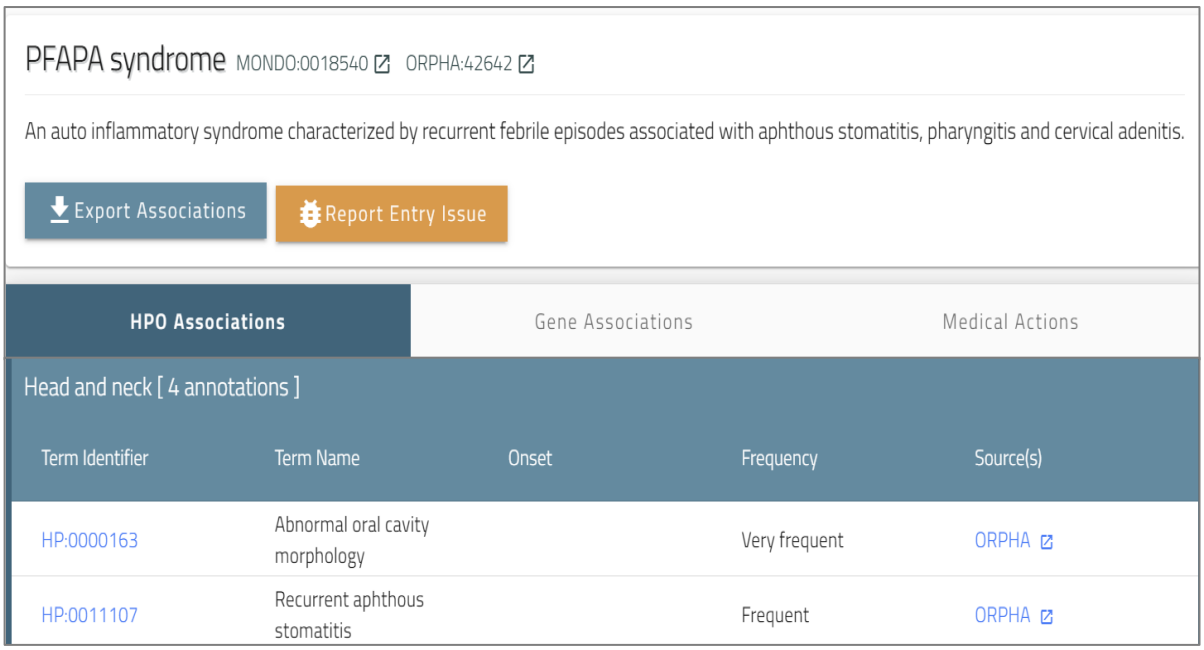


Figure 2.3. An example for the disease PFAPA syndrome: a list of the associated HPOs is provided, with the frequency of the recurrence of each term per that disease.

Importance and utility of the HPO system

Compared with other clinical terminologies, HPO offers several distinctive advantages. Its phenotypic coverage is broader and more granular than that of most existing vocabularies, including those integrated within the Unified Medical Language System (UMLS), which led to HPO subsequent incorporation into UMLS[182]. Moreover, HPO is implemented as a full Web Ontology Language resource, enabling logical reasoning and advanced computational analyses beyond simple term matching[180,183].

HPO-based disease models are now widely adopted in phenotype-driven genomic diagnostic tools (eXtasy, Phen-Gen, Phevor, PhenomeCentral, Simulconsult, DECIPHER, Phenopolis, Exomiser, LIRICAL, SimulCon- sult, PhenoTips and Face2Gene)[184–191], where they are used to encode patient features and prioritize candidate diseases or variants. Through this integration, HPO facilitates data sharing across platforms and institutions, supporting large-scale collaborative initiatives, patient registries, and rare disease matchmaking efforts [192,193].

The HPO is used to annotate diseases listed in Online Mendelian Inheritance in Man (OMIM) [194], which are mostly monogenic Mendelian disorders. In parallel, Orphanet also uses HPO terms to describe rare diseases. These annotations include information on how frequently a clinical feature occurs and whether it represents a major diagnostic criterion or a

pathognomonic sign of the disease. Although there is some overlap between OMIM and Orphanet diseases, Orphanet also includes non-Mendelian rare diseases and defines conditions mainly on the basis of clinical features, thus providing complementary information. Annotations from both OMIM and Orphanet are integrated into a single combined file available on the HPO website. In addition to clinical annotation, HPO is widely used in medical research projects, such as SOLVE-RD, a European Commission–funded initiative aimed at identifying the molecular causes of rare diseases that are still unsolved.

Inborn errors of immunity in the HPO system

Seen the lack of disease-specific HPO terms to accurately describe patients with inborn errors of immunity (IEI) and their corresponding immune profiles[195], in 2018 a collaborative initiative between ERN-RITA (European Network on Rare Primary Immunodeficiency, Autoinflammatory and Autoimmune Dis- eases), ESID (European Society for Immunodeficiencies) and ISSAID (International Society of Systemic Autoinflammatory Diseases) was launched to improve HPO terminology for IEIs, coupling bioinformatic methodologies with expert review. Four main branches of the HPO trees were re-annotated: immunodeficiencies affecting cellular and humoral immunity, antibody deficiencies, immune-dysregulation disorders and autoinflammatory diseases. This effort resulted in the reannotation of 73 IEIs, with the creation of 57 new HPO terms and restructuring of 67 terms [196]. This approach resulted in an increase in information content and a quantitative gain in the number of available HPO terms per each disease. Specifically, in a validation cohort of 30 genetically diagnosed patients, improved HPO annotations led to 47% improvement in patient-to-disease phenotypic similarity, a 14.9-fold improvement in ranking the correct diagnosis (mean rank improved from 285 to 19), and a more frequent placement of the correct diagnosis within the top 10 candidate diseases. Overall, this effort enhanced phenotype-driven disease classification: after reannotation, phenotype-based clustering of IEIs aligned more closely with established clinical classifications, supporting its clinical validity.

In Figure 2.3, an illustration of the work done on HPOs for IEI is provided[196].

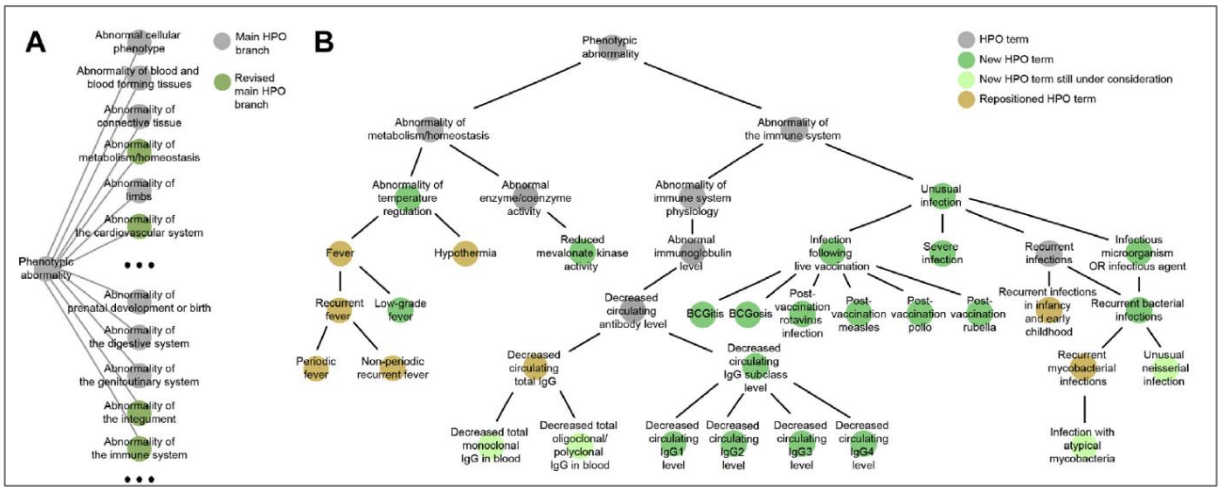


Figure 2.3. Revision and expansion of the HPO tree. A, Schematic representation of the restructuring of the HPO tree. Main branches of the HPO tree where restructuring was performed are marked with light green. B, examples of revised branches of the HPO tree. New additions and suggestions are marked with green, and repositioned terms are marked with yellow Reproduced from Haimel et al[196].

Systemic autoinflammatory diseases in the HPO system

The HPO terms for SAIDs were improved and curated in the previously cited effort[196]. In a recent work, the ability of the new HPO terms to identify and to demonstrate the potential of phenotype-driven genome diagnostics for SAIDs was demonstrated. To do so, the Authors used 42 reannotated SAIDs: 32 curated SAIDs from a previous endeavor and 10 new SAIDs that were curated and submitted to the HPO database during the study[196,197]. To validate the curation, HPO terms from 98 genetically confirmed SAID patients across 8 different European centers were collected and the LIRICAL (Likelihood Ratio Interpretation of Clinical Abnormalities) computational algorithm was used to estimate the effect of HPO curation on the prioritization of the correct SAID for each patient. HPO reannotation led to enhanced homogeneity in the disease clusters and improved the ranking of the true diagnoses in most real-life patients. The curation resulted in more accurate ranking of verified SAIDs, with seven SAIDs prioritized in the highest-ranking category (percentage of correct diagnoses increased from 66% to 86% and number of diagnoses with the highest ranking increased from 38 to 45). In a further pilot study, curation improved HPO based whole-exome sequencing (WES) analysis, diagnosing 10/12 patients before and 12/12 after curation. In addition, the average number of candidate diseases that needed to be interpreted decreased from 35 to 2.[198].

3. MACHINE LEARNING MODELS

Artificial Intelligence (AI) encompasses a broad set of computational methods that aim to develop systems capable of performing tasks that typically require human intelligence, such as learning, reasoning, and problem solving[199,200]. These technologies rely on algorithms designed to replicate selected cognitive functions, including pattern recognition, adaptation, and sensory interpretation[200].

Within this field, **machine learning (ML**) represents a methodological approach in which algorithms identify patterns in large datasets and progressively improve their performance through experience rather than explicit rule-based programming[201,202]. By learning relationships directly from data, ML models can generate predictions or classifications for specific tasks and adapt to complex scenarios, such as image analysis or natural language processing[203].

In medical applications, ML has the potential to support clinical decision-making by enhancing the efficiency and accuracy of data interpretation. These methods can assist physicians in diagnosis, risk assessment, and personalized patient management through the access to large volumes of clinical information[204].

Despite their strong performance in well-defined tasks, current ML models exhibit narrow intelligence, meaning they are optimized for specific applications and lack the ability to generalize reasoning across unrelated domains.

ML models can be trained through supervised and unsupervised learning.

In **supervised learning**, algorithms are trained on labeled datasets, enabling them to learn associations between inputs and known outputs and to apply this knowledge to new data. Depending on the nature of the task, supervised methods are commonly applied to classification problems or to regression analyses[205].

Unsupervised learning refers to a class of ML techniques designed to identify underlying structures and patterns within unlabeled data, without predefined outcome variables. These methods are commonly applied to exploratory tasks such as grouping similar observations, identifying associations among variables, or reducing data dimensionality[206].

Although both supervised and unsupervised approaches are fundamental to ML, they differ substantially in terms of data requirements, objectives, and applications. Supervised learning relies on labeled datasets to train the model toward specific predictive tasks, often achieving higher accuracy at the cost of requiring manual data annotation. In contrast, unsupervised

learning operates independently of labeled data, enabling the discovery of previously unrecognized patterns, while still requiring expert interpretation to assess the clinical relevance of the results.

In the present thesis, four different ML models were applied and their performance compared. In the following paragraph, the difference between the four models is provided.

XGBoost, Random Forest, KNN, and Elastic Net

The selection of an appropriate ML model is strongly influenced by the nature of the available data—such as structured, tabular patient records—and by the trade-off required between predictive accuracy and interpretability. In clinical research, this balance is particularly critical, as models must not only perform well but also support transparent and reliable decision-making.

Extreme Gradient Boosting (XGBoost) represents one of the most advanced and widely used implementations within the Gradient Boosting Decision Tree (GBDT) family. The method constructs an ensemble of decision trees in a sequential manner, where each subsequent tree is trained to correct the errors of the preceding ones. This iterative optimization process, combined with built-in regularization strategies, allows XGBoost to achieve high predictive performance while mitigating overfitting. Owing to its ability to capture complex, non-linear interactions among variables and to handle class imbalance, XGBoost is often regarded as a state-of-the-art approach for tabular datasets commonly encountered in healthcare applications[207,208].

Random Forest (RF) is another ensemble-based learning algorithm, sharing the use of decision trees but differing in its training strategy. Instead of sequential learning, RF constructs a large number of independent trees in parallel, each trained on a bootstrapped subset of the data and a randomly selected subset of features. The final prediction is obtained by aggregating the outputs of all trees, typically through averaging or majority voting. This approach effectively reduces variance and improves robustness, making RF particularly suitable for medical datasets characterized by complex patterns and non-linear relationships, where strong predictive accuracy is required without the computational overhead of boosting methods[209].

K-Nearest Neighbors (KNN) is among the simplest and most intuitive machine learning algorithms. It operates as a distance-based method, assigning class labels to new observations based on the labels of the most similar instances in the training data. One of the primary advantages of KNN lies in its inherent interpretability, as predictions can be directly traced back

to specific neighboring samples. However, the method is highly sensitive to noise, feature scaling, and high dimensionality, which often limits its reliability and scalability in complex clinical settings[210].

Elastic Net (EN) is a regularized regression technique that combines the properties of Lasso (L1) and Ridge (L2) penalties. By integrating both regularization terms, EN addresses key limitations of traditional linear regression, particularly in the presence of multicollinearity among predictors. The method encourages grouped selection of correlated variables while simultaneously performing feature selection, resulting in more stable and generalizable models. EN is especially advantageous in high-dimensional scenarios where the number of predictors exceeds the number of observations, a common situation in biomedical research[211,212].

Key Differences between the four models and clinical considerations

Across a wide range of medical diagnostic tasks, gradient boosting models such as XGBoost often outperform traditional approaches like KNN and even sophisticated deep learning models when applied to tabular data. Although KNN and single decision trees are appreciated for their interpretability, ensemble techniques, including Random Forest and XGBoost, typically achieve markedly higher predictive accuracy.

However, the literature emphasizes that in high-stakes clinical settings, performance alone is insufficient. Clinicians must be able to understand the rationale behind a model's predictions to ensure decisions are not driven by confounding factors or biased data. Consequently, despite XGBoost's strong performance, its relative "black-box" behavior necessitates the use of complementary interpretability methods to build clinical trust.

In summary, while simpler models such as KNN offer greater transparency, ensemble methods like Random Forest and XGBoost generally deliver the level of predictive accuracy needed to minimize diagnostic errors and enhance patient outcomes, provided their predictions can be adequately explained.

Summary of Differences between the four models: XGBoost, RF, KNN and EN

Model	Type	Learning Style	Primary Strength
XGBoost	Ensemble (GBDT)	Sequential	High accuracy and strong optimization for tabular data
Random Forest	Ensemble	Parallel	Robustness against overfitting; handles complex non-linear patterns
KNN	Instance-based	Distance-based	Simple method with natural interpretability
Elastic Net	Regularized Regression	Optimization-based	Combines L1 and L2 penalties; effective for correlated features and feature selection

GBDT gradient boosting decision tree, KNN K-Nearest Neighbors

4. AIM OF THE STUDY

The main aim of this project is to validate the diagnostic accuracy of HPO terms in a real-life cohort of patients with SAIDs, through a machine learning approach.
The secondary objectives are to implement the HPO terminology for SAIDs and to develop a novel diagnostic tool for SAIDs.

5. METHODS

Patients were extracted from the Eurofever Registry (EF). The main characteristics of the Registry have been previously described[213,214]. The data in EF are encrypted, and patients' names are not identifiable, except by the center that entered the data. Each center participating in EF must have obtained local Ethics Committee approval for data entry.

1. Patient selection

From the EF Registry, all monogenic SAIDs were selected. As confounder, also patients with Periodic Fever, Aphthous Stomatitis, Pharyngitis, Adenitis (PFAPA), a frequent polygenic autoinflammatory disease, were included. All the patients were screened for missing/non-clear clinical data. If needed, the enrolling centers were contacted to fill in the missing information. When possible, missing data were re-integrated in the system, otherwise patients were excluded from the analysis. Genetic data of the included patients were thoroughly screened. Specifically, for recurrent monogenic fevers (FMF, CAPS, MKD, TRAPS), a confirmatory/non confirmatory genotype was considered based on the classification criteria for recurrent fevers (Gattorno et al., 2019). For FMF, also monoallelic patients (one pathogenic mutation with a corresponding clinical phenotype) were retained for the analysis, and later compared in a subanalysis with biallelic patients (homozygous or compound heterozygous). According to the criteria, patients with isolated VUS that did not fulfill the EF clinical classification criteria were excluded. For TRAPS, also patients carrying low-penetrance structural variants (R92Q, P46L) were initially included in the analysis, even if a separate sub-analysis was later conducted in these patients. For PFAPA, only patients without genetic mutations were included.

2. Coding of clinical variables according to HPO terms

From EF, all clinical variables were extracted and coded using HPO terminology. Missing terms were annotated for later integration. Each patient was then re-coded according to the HPO terms describing their clinical symptoms.

3. Dataset preparation

After harmonization of EF variables into HPO terms, a total of 223 HPO-coded variables were initially obtained (207 clinical, 12 laboratory, and 4 demographic). Non-informative "other" categories were removed and redundant mappings were addressed by collapsing selected pairs of highly overlapping symptoms into a single binary feature using a logical OR rule. Additionally, HPO terms that were not present in any patient were excluded from the analysis. The resulting feature set comprised 168 binary HPO-derived clinical features describing disease manifestations. Demographic and clinical variables (age at onset, fever duration, sex, and ethnicity encoded via dummy variables) were appended, yielding a total of 174 predictors used for modelling.

In order to better exploit the hierarchical structure of the HPO, an extended version of the dataset was subsequently generated by incorporating ancestor terms. Specifically, for each annotated HPO term, its parent terms within the ontology hierarchy were added using the *ontology Index* R package, allowing the representation of both specific and more general phenotypic concepts. This approach was adopted to capture broader clinical patterns and improve phenotype comparability across patients.

Following this hierarchical expansion, the dataset included 429 HPO terms, together with the same set of additional clinical and demographic variables (age, fever duration, sex, and ethnicity), for a total of 435 features. As in the original dataset, the diagnosis was retained as the target variable for the supervised analysis.

Each dataset was divided into a training set (70%) and a test set (30%), ensuring that the distribution of diagnoses was preserved across the two subsets. The test set was held out and used exclusively for the final evaluation of model performance. Both dataset versions (with and without ancestors) were used for downstream supervised analyses in order to assess the impact of hierarchical phenotype enrichment on diagnostic performance. In all subsequent analyses, models trained on the dataset including HPO ancestor terms were considered as the primary reference, while models trained without hierarchical enrichment were used for sensitivity analyses.

4. Evaluation of HPO terms performance using machine learning models

A supervised machine learning (ML) framework was implemented to evaluate the diagnostic performance of HPO-based phenotypic representations. Several classification strategies and algorithms were systematically explored using the same preprocessing and evaluation pipeline.

Four ML classifiers were considered: Elastic Net regression (EN), k-Nearest Neighbors (kNN), Random Forest (RF), and eXtreme Gradient Boosting (XGBoost). These models were selected to cover different methodological approaches, including linear, distance-based, and ensemble tree-based methods.

Two different classification strategies were investigated.

First, a multiclass classification setting was implemented, in which each patient was directly assigned to one of the diagnostic categories included in the study.

Second, a one-vs-rest (OVR) approach was adopted. In this setting, a separate binary classifier was trained for each disease to distinguish patients affected by the target condition from all other diagnoses grouped together as "Other".

Both multiclass and OVR approaches were applied consistently across all evaluated ML models.

Model training and hyperparameter tuning were performed exclusively on the training set (70%) using a stratified 5-fold cross-validation framework. Hyperparameter tuning was carried out using a grid search strategy. Within each cross-validation fold, model performance was evaluated using the Fβ-score, with β set to 2 (F2-score) in order to prioritize recall over precision, and the configuration yielding the best average cross-validation score was selected and subsequently refitted on the full training set. For XGBoost models, hyperparameter tuning was performed using a predefined grid search strategy. The grid included the number of trees (n_estimators = 100, 500), maximum tree depth (max_depth = 3, 5, 7), learning rate (learning_rate = 0.3, 0.1), column subsampling rate (colsample_bytree = 0.5, 1.0), and minimum child weight (min_child_weight = 1, 5). This grid was designed to balance model expressiveness and computational efficiency, while limiting overfitting. These analyses were conducted using the XGBClassifier from the Python XG-Boost library[215]. In the OVR setting, class imbalance was addressed during model training through the use of class-specific sample weights, whereby samples belonging to the positive class were assigned higher weights proportional to the imbalance between positive and negative cases. Furthermore, feature importance analyses were performed for models allowing direct interpretability. For XGBoost models, feature importance was quantified using the gain metric, which reflects the average improvement in model performance contributed by a feature when used for splitting. The independent test set (30%) was used only for final performance evaluation. In the OVR framework, five disease-specific binary classifiers were trained (CAPS, FMF, MKD, PFAPA, and TRAPS). During the test phase, each patient was independently evaluated by all disease-specific classifiers, producing a set of disease-specific predicted probabilities.

Performance results obtained with different ML models, dataset representations, and classification strategies were systematically compared.
Error analyses were performed on the test set to investigate systematic patterns of misclassification, including subgroup-specific enrichments.
In addition, unsupervised dimensionality reduction using Uniform Manifold Approximation and Projection (UMAP)[216] was applied to selected disease subsets to explore phenotypic similarity patterns independent of diagnostic labels.

Finally, in order to facilitate the practical use of the proposed model, we developed a web-based application implemented using R Shiny[217], which allows users to input patient-specific HPO terms and obtain real-time disease probability predictions generated by the final XGBoost OVR model.

6. RESULTS

6.1 Patients' inclusion

From the EF Registry, 18 monogenic SAIDs plus PFAPA patients were identified, for a total of 2716 patients. In **Figure 6.1.1**, the selected diseases and the correspondent patients' numbers are provided. Since only few patients were enrolled for some of the rarer diseases (i.e. Majeed syndrome 5 patients, Deficit of IL-1Receptor Antagonist 3 patients, etc) we decided to include only those diagnoses with larger patient numbers. Specifically, the analysis was restricted to patients with FMF (n=1182), CAPS (n=303), TRAPS (n=277), MKD (n=204), and PFAPA (n=610), for a total number of 2576 patients included (**Figure 6.1.2**)

A descriptive summary of the main demographic and clinical characteristics of the cohort, including sex, age, ethnicity, and fever duration, is reported in Table 6.1.

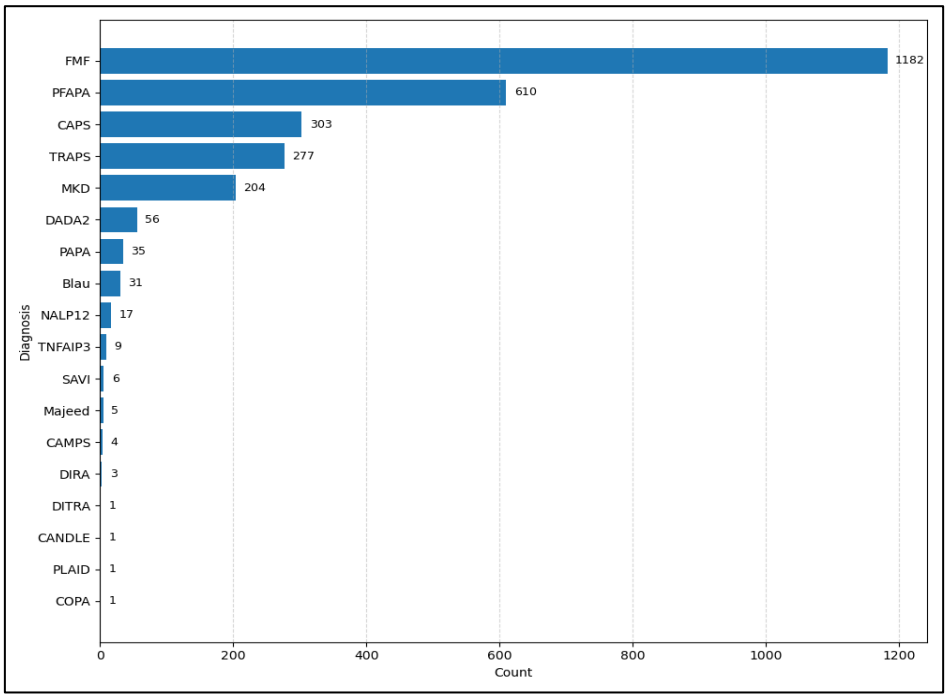


Figure 6.1.1 Initial Number of patients included per each disease

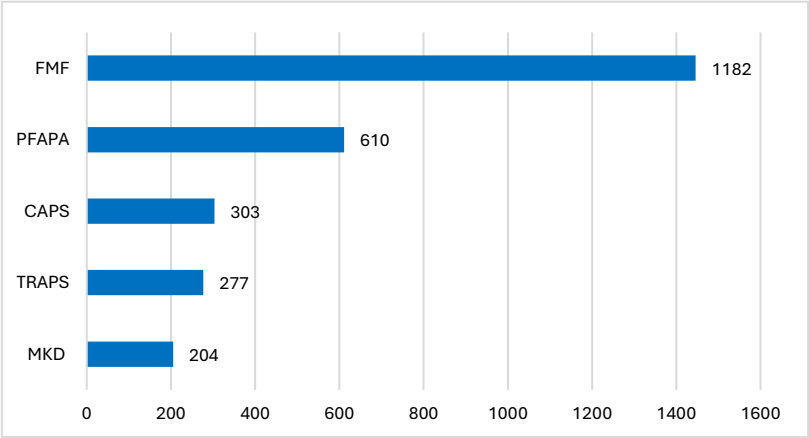


Figure 6.1.2. Final number of the patients included in the analysis

	FMF (n=1182)	PFAPA (n=610)	CAPS (N=303)	TRAPS (N=277)	MKD (N=204)
# of Females (%)	563 (48)	263 (43)	153 (50)	136 (49)	107 (52)
Age, mean (SD)	6.18 (7.91)	2.58 (2.90)	7.13 (12.32)	10.44 (13.77)	2.51 (5.48)
Fever length, days mean (SD)	2.54 (1.69)	3.88 (2.00)	2.06 (3.61)	7.35 (5.89)	4.63 (4.66)

Table 6.1. Descriptive characteristics of the dataset

6.2 Coding of clinical variables according to HPO terms

From EF a total of 223 variables (207 clinical, 12 laboratory, 4 demographic) were codified into HPO codes. An absolute correspondence was found for 185 EF terms, a partial correspondence for 26 terms (of which in 6 cases more than 1 HPO term was found corresponding to 1 specific EF term), while no corresponding HPO terms were found for 13 EF terms. The majority of the absent terms were present only in few patients, whereas the terms "gender", "ethnicity" and "length of the episodes", were present for each patient. The term "age at onset", also present in all patients, presented a "partial correspondence", with several corresponding terms -neonatal, juvenile, pediatric, adult onset- retrieved. A redundance of 5 EF terms was also highlighted (Table 6.2.1 in red).

As explained in the methods paragraph, after data preprocessing, 168 HPO terms plus 6 demographic and clinical variables were retained, for a total of 174 variables.

In the following pages the complete tables of the terms with absolute, partial, and absent correspondence between EF and HPO are provided (**Table 6.2.1-3**).

Table 6.2.1. EUROFEVER terms with an absolute correspondence (in red, the EF redundant terms)

EUROFEVER TERMS	HPO CODE	DESCRIPTION
Muco-cutaneous manifestations	HP:0000951	Abnormality of the skin
Musculoskeletal system	HP:0033127	Abnormality of the musculo-skeletal system
Ocular manifestation	HP:0000478	Abnormality of the eye
Gastrointestinal system	HP:0011024	Abnormality of the GI tract
Lymphoid organs	HP:0100763	Abnormality of the lymphatic system
Disease Course	HP:0011008	Temporal pattern
Cardio-respiratory system	HP:0001626	abnormality of the cardiovascular system
Neurological manifestations	HP:0000707	Abnormalities of the nervous system
Genito - Urinary manifestations	hp:0000119	Abnormality of the genito-urinary system
Constitutional symptoms	HP:0025142	Constitutional symptom
Hematological alteration	HP:0001871	Abnormality of blood and blood-forming tissues
Chest pain	HP:0100749	Chest Pain
Dyspnoea	HP:0002094	Dyspnea
Pericarditis	HP:0001701	Pericarditis
Pleurisy	HP:0002102	Pleuritis
Pneumonia -	HP:0011949	Acute Infectious Pneumonia
Interstitial lung	HP:0006530	Abnormal pulmonary interstitial morphology
Persistent cough	HP:0034315	Chronic cough
Pulmonary vasculitis	HP:0034705	Pulmonary vasculitis
Hemoptysis	HP:0002105	Hemoptysis
Venous thrombosis	HP:0004936	Venous thrombosis
Arterial thrombosis	HP:0004420	Arterial thrombosis
Myocarditis	HP:0012819	Myocarditis
Large vessel vasculitis	HP:0005310	Large vessel vasculitis
Myocardial ischemia, infarction	HP:0001658	Myocardial infarction
Cardiac hypertrophy	HP:0001639	Hypertrophic cardiomyopathy
Arterial aneurysm/stenosis	HP:0100545 HP:0002617	Arterial stenosis Aneurysmal dilatation
Large vessel vasculopathy	HP:0005310	Large vessel vasculitis
Medium/small vessels vasculitis	HP:6000658 HP:0011944	Medium vessel vascuilitis Small vessels vasculitis
Cardiomyopathy	HP:0001638	Cardiomyopathy
Pulmonary fibrosis	HP:0002206	Pulmonary fibrosis
Fever (>38)	HP:0001945	fever
Low Grade fever (<38)	HP:0011134	Low grade fever
Fatigue	HP:0012378	Fatigue
Malaise	HP:0033834	Malaise
Mood disorders	HP:0000712	Emotional lability
Sensation of fever, chills without fever	HP:0025143	Chills
Weight loss	HP:0001824	Weight loss
Fever >38º C	HP:0001945	Fever
Low grade fever <38º	HP:0011134	Low-grade fever
Chills at fever onset	HP:0025143	Chills
Triggers	HP:0025204	Triggered by

Cold	HP:002526	Triggered by cold
Emotional stress	HP:0025226	Triggered by stress
Vaccination	HP:0025219	Triggered by vaccination
Infection	HP:0033184	Triggered by infection
Exercise	HP:0025377	Triggered by exertion
Trauma	HP:0031135	Triggered by physical trauma
Food	HP:0033793	Triggered by food ingestion
Menstruation	HP:0025220	Triggered by menstruation
Fatigue	HP:0012378	Fatigue
Vomiting	HP:0002013	Vomiting
Abdominal pain	HP:0002027	Abdominal pain
Constipation	HP:0002019	Constipation
Diarrhea	HP:0002014	Diarrhea
Gastrointestinal ulcers	HP:0034274	Gastrointestinal ulcer
GI bleeding	HP:0002239	Gastrointestinal hemorrhage
Mesenteric aneurysm	HP:0011934	Dilatation of mesenteric artery
Hepatitis	HP:0012115	Hepatitis
Portal hypertension	HP:0001409	Portal hypertension
Gastritis	HP:0005263	Gastritis
Gut perforation	HP:0031368	Intestinal perforation
Peritoneal adhesions	HP:0033134	Abdominal adhesions
Intestinal occlusion/sub-occlusion	HP:0005214	Intestinal obstruction
Gonadal pain without proven ischaemia	HP:0033839	Testicular pain
Gonadal infarction	HP:0033403	testicular ischemia
Epididymitis	HP:0000031	Epididymitis
Essential hypertension	HP:0000822	Hypertension
Renovascular hypertension	HP:0100817	Renovascular hypertension
Renal infarction	HP:0032618	kidney infarction
Renal aneurysm	HP:0033261	Renal artery aneurysm
Kidney amyloidosis	HP:0001917	Renal amyloidosis
Glomerulonephritis	HP:0000099	Glomerulonephritis
Glomeruloscerosis	HP:0000096	Glomerular sclerosis
Pancytopenia	HP:0001876	Pancytopenia
Leucopenia	HP:0001882	Leukopenia
Thrombocytopenia	HP:0001873	Thrombocytopenia
Aplastic anemia	HP:0001915	Aplastic anemia
Neutropenia	HP:0001875	Neutropenia
Lymphopenia	HP:0001888	Lymphopenia
Hypogammaglobulinemia	HP:0004313	Decreased circulating antibody level
Hypercoagulability	HP:0100724	Hypercoagulability
Recurrent infections	HP:0002719	Recurrent infections
Severe infections (hospitalization)	HP:0032169	Severe infection
Opportunistic infections (bacterial, viral, elimintic, other)	HP:0031690	Opportunistic infection
Leukemia	HP:0001909	Leukemia
Hydiopatic myelofibrosis	HP:0011974	Myelofibrosis
Generalized lymphnodes enlargement	HP:0008940	Generalized lymphadenopathy

Enlarged cervical lymphnodes	HP:0025289	Cervical lymphadenopathy
Hepatomegaly	HP:0002240	Hepatomegaly
Splenomegaly	HP:0001744	Splenomegaly
Splenic infarct	HP:0034336	Splenic infarction
Parotitis	HP:0011850	Parotitis
Aphthous stomatitis	HP:0000155	Oral ulcers
Exudative pharyngitis	HP:0034035	Pharyngeal exudate
Maculo-papular rash	HP:0040186	Maculopapular exanthema
Urticarial rash	HP:0001025	Urticaria
Palpable purpura	HP:0031363	Palpable purpura
Erysipelas-like erythema	HP:0001055	Erysipelas
Pseudo-folliculitis	HP:0025084	folliculitis
Acne	HP:0001061	Acne
Psoriasis	HP:0003765	Psoriasiform dermatitis
Ulcers at genitalia	HP:0003249	Genital ulcers
Pyoderma gangrenosum	HP:0025452	Pyoderma gangrenosum
Palmo-plantar pustulosis	HP:0100847	Palmoplantar pustulosis
Erythema nodosum EN	HP:0012219	Erythema nodosum
Other Panniculitis non-EN	HP:0012490	Panniculitis
Positive pathergy test	HP:0025532	Positive pathergy test
Erythematous/violaceous plaques	HP:0025474	Erythematous plaque
Lipodystrophy	HP:0009125	lipodystrophy
Eyelid violacoeus swelling	HP:0100540	Palpebral edema
Cutaneous ulceration	HP:0200042	Skin ulcer
Alopecia	HP:0001596	Alopecia
Livedo racemosa/reticularis	HP:0033260 HP:0033505	Livedo racemosa Livedo reticularis
Eczema	HP:0000964	Eczema
Subcutaneous nodules	HP:0001482	Subcutaneous nodule
Photosensitivity	HP:0000992	Cutaneous photosensitivity
Raynaud’s phenomenon	HP:0030880	Raynaud phenomenon
Chilblains	HP:0009710	Chilblains
Nail distrophy	HP:0008404	Nail dystrophy
Arthralgia	HP:0002829	Arthralgia
Myalgia	HP:0003326	Myalgia
Muscle weakness	HP:0001324	Muscle weakness
Myositis	HP:0100614	Myositis
Fasciitis	HP:0100537	Fascitiis
Bone pain	HP:0002653	Bone pain
Osteomyelitis - infectious	HP:0002754	Osteomyelitis
Oligoarthritis - ≤4 joints	HP:0040313	Oligoarthritis
Polyarthritis - ≥5 joints	HP:0005764	Polyarticular arthritis
Dactylitis	HP:0031090	Finger dactylitis
Flexion contractures	HP:0001371	Flexion contracture
Bone alteration	HP:0003330	Abnormal bone structure
Patellar overgrowth	HP:0033308	Patellar overgrowth
Frontal bossing	HP:000207	Frontal bossing

Clubbing	HP:0001217	Clubbing
Camtpodactily	HP:0012385	Camptodactily
Osteolytic lesions	HP:0002797	Osteolysis
Hyperostosis	HP:0100774	Hyperostosis
Osteoporosis	HP:0000939	Osteoporosis
Muscular atrophy	HP:0003202	Skeletal muscle atrophy
Headache - anytime	HP:0002315	Headache
Optic neuritis	HP:0100653	Optic neuritis
Seizures	HP:0001250	Seizure
Cranial nerve palsy	HP:0006824	Cranial nerve paralysis
Vertigo	HP:0002321	Vertigo
Ataxia	HP:0001251	Ataxia
Peripheral neuritis	HP:0009830	Peripheral neuropathy
Transient Ischemic Attack - TIA	HP:0002326	Transient ischemic attack
Stroke	HP:0001297	Stroke
Intracranial hemorrhage	HP:0002170	Intracranial hemorrhage
Hydrocephalus	HP:0000238	Hydrocephalus
Neurosensorial hearing loss	HP:0000407	Sensorineural hearing impairment
Peripheral neuropathy	HP:0009830	Peripheral neuropathy
Tetra/paraplegia	HP:0002445 HP:0010550	Tetraplegia Paraplegia
Mental retardation	HP:0001249	Intellectual disability
Periorbital oedema	HP:0100539	Periorbital edema
Conjunctivitis	HP:0000509	Conjunctivitis
Uveitis anterior	HP:0012122	Anterior uveitis
Uveitis posterior	HP:0012123	Posterior uveitis
Keratitits	HP:0000491	Keratitis
Scleritis	HP:0100532	Scleritis
Episcleritis	HP:0100534	Episcleritis
Lacrymal gland involvement	HP:0000620	Dacryocystitis
Papilledema	HP:0001085	Papilledema
Papillitis	HP:0001085	Papilledema
Optic nerve atrophy	HP:0000648	Optic atrophy
Retinal vasculitis	HP:0025188	Retinal vasculitis
Cataract	HP:0000518	Cataract
Glaucoma	HP:0000501	Glaucoma
Band keratopathy	HP:0000585	Band keratopathy
Optic nerve atrophy	HP:0000648	Optic atrophy
Impaired vision	HP:0000505	Impaired vision
Blindness	HP:0000618	Blindness
AA- Amyloidosis	HP:0011034	Amyloidosis
Macrophage activation syndrome	HP:0012156	hemophagocytosis
Infertility	HP:0000789	Infertility
Tumors	HP:0002664	Neoplasm
Failure to thrive	HP:0001508	Failure to thrive
Gender / height related hypertension	HP:0000822	Hypertension
CRP	HP:0032436	Abnormal circulating C-reactive protein concentration

ESR	HP:0025021	Abnormal erythrocyte sedimentation rate
Serum amyloid A	HP:0033332	Elevated circulating amyloid A
Urine mevalonic acid measurement	HP:0032638	Elevated urine mevalonic acid level
WBC	HP:0001974	Leukocytosis

Table 6.2.2. Eurofever terms with partial HPO correspondence

EUROFEVER TERMS	HPO 1	DESCRIPTION	HPO 2	DESCRIPTION
Lymphatic dysplasias	HP:0100766	Abnormal Lymphatic vessel morphology	HP:0003759	Hypoplasia of lymphatic vessels
Pattern of frequency	HP:0040279	Frequency	HP:0025304	Periodic
Anal/perianal ulcers	HP:0012390	Anal fissure		
Aseptic Peritonitis	HP:0002586	Peritonitis		
Mesenteric infarct	HP:0005244	Gastrointestinal infarctions	HP:0033404	Intestinal ischemia
Intestinal amyloidosis	HP:0011034	Amyloidosis		
Urethritis/ cistitis	HP:0500006	Urethritis	HP:0100577	Urinary bladder inflammation; Cystitis of the urinary bladder
Necrotising pneumonia	HP:0033243	Pulmonary necrosis		
Erythematous pharyngitis	HP:0025439	Pharyngitis		
Migratory rash	HP:0033622	Migratory erythematous plaque		
Papulopustular lesions	HP:0033605	Pustular rash		
Ichthyosiform rash	HP:0008064	Ichthyosis		
Digital/peripheral ischaemia (gangrenous)	HP:0033401	Digital ischemia		
Digital/peripheral ischaemia (non-gangrenous)	HP:0033402	Digital ischemia		
Discoid skin lesion	HP:0033120	Nummular eczema	HP:0007417	Discoid lupus rash
Monoarthritis	HP:0001369	Arthritis		
Tenosynovitis	HP:0025230	Tendonitis	HP:0100769	Synovitis
Digit(s) amputation	HP:0007460	Autoamputation of digits		
Limb amputation	HP:0001868	Autoamputation of foot		
Bone Deformity	HP:0002814	Abnormality of lower limbs		
Bone erosions	HP:0003106	Subperiosteal bone erosions		
Aseptic meningitis	HP:0001287	Meningitis		
Cranial neuropathy	HP:0006824	Cranial nerve palsy		
Headache (morning)	HP:0002315	Headache		
Culture proven severe infections	HP:0032169	Severe infection		
Death	HP:0011420	Age of death		
Age at onset		HP:0003623 HP:0410280 HP:0003581	Neonatal Pediatric Adult	

Table 6.2.3. EUROFEVER TERMS without correspondence in the HPO. Specifics of why an adequate correspondent term is not present are given in the second column when appropriate

Gender	
Ethnicity	
Mean duration of episodes (fever length)	
Number of episodes/year	
Seasonal changes	
Travel (triggered by)	
Intestinal vasculitis	The term vasculitis in general is present, with specificity about skin, muscle, cerebral, nasal, retinal and renal vasculitis
Painful lymph nodes	Lymphadenopathy HP:0002716 present, but no indication regarding any pain
Osteitis - non-infectious	Only the term "osteomyelitis" **HP:0002754** present in HPO and labeled as infectious osteomyelitis, even if the term is associated to CRMO
Cerebellar syndrome	
Periorbital pain	Only periorbital oedema present
Periorbital erythema	"
Coronaries' arteritis	No terms referring to coronary vasculitis

6.2.2 Problems encountered within the HPO system

Within the coding activity we highlighted some problems and redundances within the HPO system.

First, some terms present an overlap. For example, both the terms “oral ulcer HP:0000155” and “aphthous ulcer HP:0032154” are present in HPO, and belong to the same family tree under the general term “Abnormality of oral mucosa”. These terms are synonyms in clinics, and are therefore to be considered one the duplicate of the other. Moreover, they are not linked to the same diseases: for example, only the term “aphthous ulcer HP:0032154” is linked to FMF, while only the term “oral ulcer HP:0000155” is linked to MKD, while none of them is linked to PFAPA, in which the term “Recurrent aphthous stomatitis HP:0011107” is present. This latter term, is a child term present both under “erosion of oral mucosa” and under “stomatitis” with the same HP code. This is exemplified in Figure **6.2.2.1**.

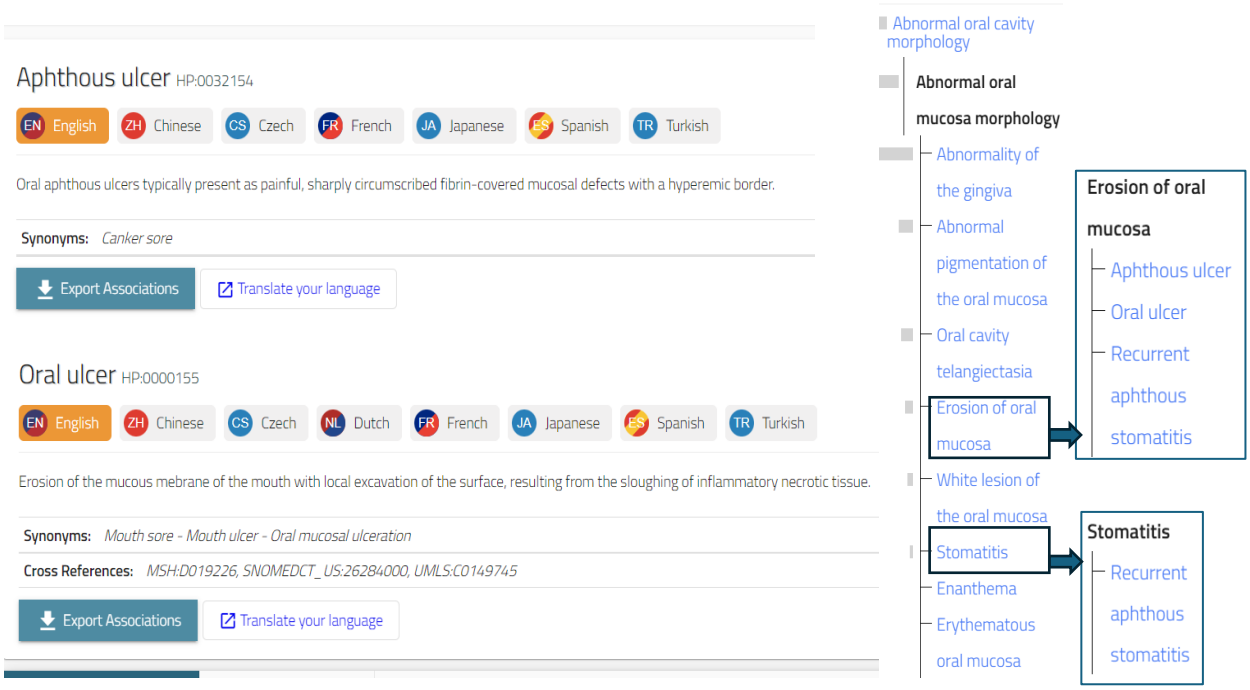


Figure 6.2.2.1 Different Screenshots of the HPO site, highlighting the redundance of the term aphtous and oral ulcer.

Second, more than one disease entry is sometimes present for each disease.

For FMF, three different terms exist on the HPO site, two of them linked to OMIM and one to Orphanet (ORPHA). The two OMIM terms refer: one to the “classic” FMF recessive type, while the other to the AD type described in some families[18,218]. These three terms present different HPOs, and different frequencies for each associated HPO term (Figure 6.2.2.2).

Disease Identifier	Disease Name	Matching String
OMIM:134610	Familial Mediterranean fever, AD	**Familial Mediterranean fever**, AD
ORPHA:342	Familial Mediterranean fever	**Familial Mediterranean fever**
OMIM:249100	Familial Mediterranean fever, AR	**Familial Mediterranean fever**, AR

Figure 6.2.2.2 Screenshot of the HPO site for the three FMF terms

This is true also for TRAPS and CAPS. Under the gene TNFRSF1A, two different terms are reported: one ORPHA term "TNFR1-associated periodic syndrome", and one OMIM entry: "Periodic fever, familial autosomal dominant". Also in this case, different HPOs are associated to the two disease-related terms. Under the gene NLRP3 there are one OMIM and one ORPHA code for each of the three CAPS diseases (FCAS, MWD and CINCA). On the contrary, for MKD only an OMIM entry is available while for PFAPA there is only an ORPHA entry (due to the fact that it is not a monogenic mendelian disorder and it is not enlisted in OMIM).

Another problem is represented by the different reported frequencies for each HPO term. These frequencies are expressed in numbers (e.g. 1/100) for OMIM entries, with a direct link to the Pubmed articles from which the frequencies were extracted, whereas are expressed as "occasional, frequent, very frequent" etc for ORPHA entries, with a direct link to the correspondent Orphanet page. Moreover, the frequencies reported as numbers very often report small numbers of patients (e.g. 11/25) (**Figure 6.2.2.3**).

Periodic fever, familial, autosomal dominant MONDO:0007727 OMIM:142680

Term Identifier	Term Name	Onset	Frequency	Source(s)
HP:0030953	Conjunctival hyperemia		11/25	PubMed
HP:0000509	Conjunctivitis		35/158	PubMed
HP:0100539	Periorbital edema		43/183	PubMed PubMed

Tumor necrosis factor receptor 1 associated periodic syndrome MONDO:0007727 ORPHA:32960

HP:0100776	Recurrent pharyngitis	Occasional	ORPHA
HP:0000509	Conjunctivitis	Occasional	ORPHA
HP:0100539	Periorbital edema	Occasional	ORPHA

Figure 6.2.2.3. Screenshot of the HPO pages, showing the different frequencies for the OMIM and ORPHA entries for TRAPS.

These differences in terms of reported frequencies not only generate confusion, but do not allow for a direct comparison between the used terms. Moreover, seen that in certain cases very low total numbers are reported (i.e. 8/13), these frequencies fail to really capture the effective frequency of the disease features.

Overall, these inconsistencies—ranging from redundant terminology and fragmented disease entries to heterogeneous frequency annotations—limit the internal coherence of the HPO system and may reduce the reliability of phenotype-driven analyses unless carefully addressed during data curation**.**

6.3 Evaluation of HPO terms performance using machine learning models

i) Models' comparison and selection

Four ML models (EN, KNN, RF, and XGB) were evaluated under both Multiclass and OVR classification strategies, using the F2-score as the primary optimization metric in order to prioritize recall. Overall, the OVR strategy achieved higher predictive performance than the multiclass approach for most diseases (**Table 6.3.1; Figure 6.3.1**), with the only exception of CAPS, for which the multiclass formulation yielded slightly better results.

Across both classification frameworks, XGBoost consistently emerged as the top-performing model.

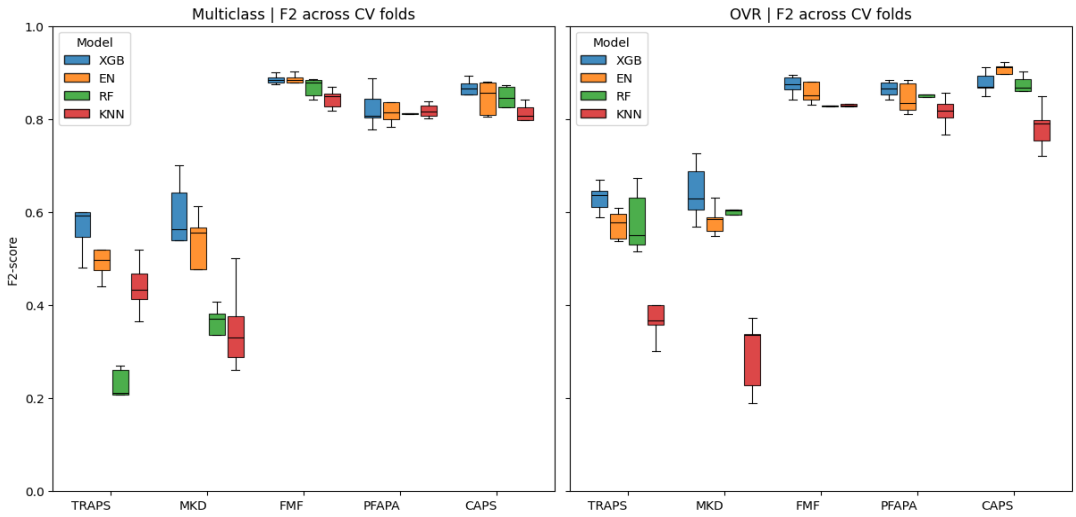
Model comparison was first performed on the training set using a 5-fold CV framework (70% of the data).

Table 6.3.1. Comparison of the best-performing models for each disease under Multiclass and One-vs-Rest (OVR) classification strategies on the validation set.

Disease	Best model (Multiclass)	F2-score Multiclass	Best model (OVR)	F2-score OVR
CAPS	XGB	0.854 ± 0.044	EN	0.897 ± 0.033
FMF	XGB	0.886 ± 0.01	XGB	0.873 ± 0.021
MKD	XGB	0.565 ± 0.121	XGB	0.644 ± 0.063
PFAPA	EN	0.826 ± 0.043	XGB	0.864 ± 0.018
TRAPS	XGB	0.583 ± 0.079	XGB	0.63 ± 0.032

Performance is reported as the mean ± standard deviation of the F2-score across the five cross-validation folds. For each disease, the best performance achieved between the two classification strategies is highlighted in red.

Figure 6.3.1. Comparison of model performance across diseases under Multiclass and One-vs-Rest (OVR) classification strategies on the validation set.



The advantages of the OVR strategy were particularly evident for diseases characterized by lower baseline performance in the multiclass setting. On the test set (30%), recall in the multiclass analysis was markedly lower for TRAPS (50.6%) and MKD (53.7%) compared with FMF (90.8%), PFAPA (90.4%), and CAPS (77.9%) (**Figure 6.3.2**). When reformulated as independent OVR tasks, recall substantially improved for TRAPS (65.8%) and MKD (70.4%), while remaining high for the remaining diseases (**Figure 6.3.3; Table 6.3.2).**

Based on these results, the OVR strategy combined with XGBoost was selected as the final modeling framework for all subsequent analyses.

Table 6.3.2 reports the detailed performance metrics of the XGBoost model under the OVR strategy for each disease, highlighting consistently high recall and balanced accuracy for FMF, PFAPA, and CAPS, and moderate performance for MKD and TRAPS.

Disease	F2-score	Bal. acc.	MCC	Recall	Precision
CAPS	0.8544	0.9168	0.8373	0.8526	0.8617
FMF	0.8982	0.9030	0.8055	0.8997	0.8882
MKD	0.6031	0.8095	0.4722	0.7037	0.4000
PFAPA	0.8943	0.9203	0.7869	0.9361	0.7586
TRAPS	0.5895	0.7766	0.4551	0.6582	0.4160

Table 6.3.2. XGBoost performance metrics for each disease in the OVR approach evaluated on the test set (30% hold-out data). From left to right: F2-score, Balanced accuracy, MCC, Recall, Precision.

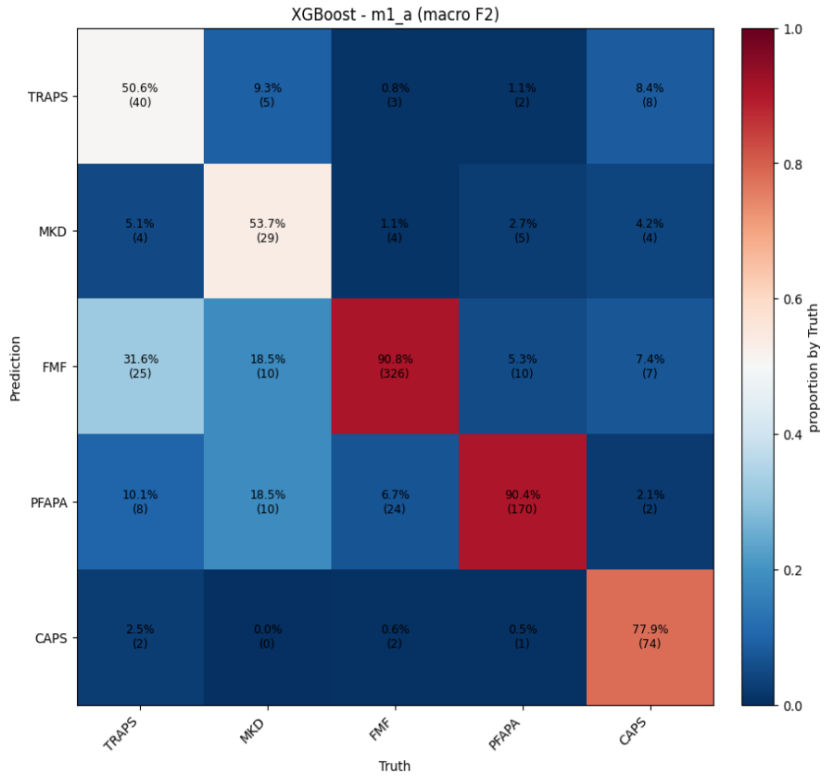


Figure 6.3.2. Confusion matrix shows results of the multiclass classification analysis on the test set. Red values indicate high recall, blue values indicate low recall.

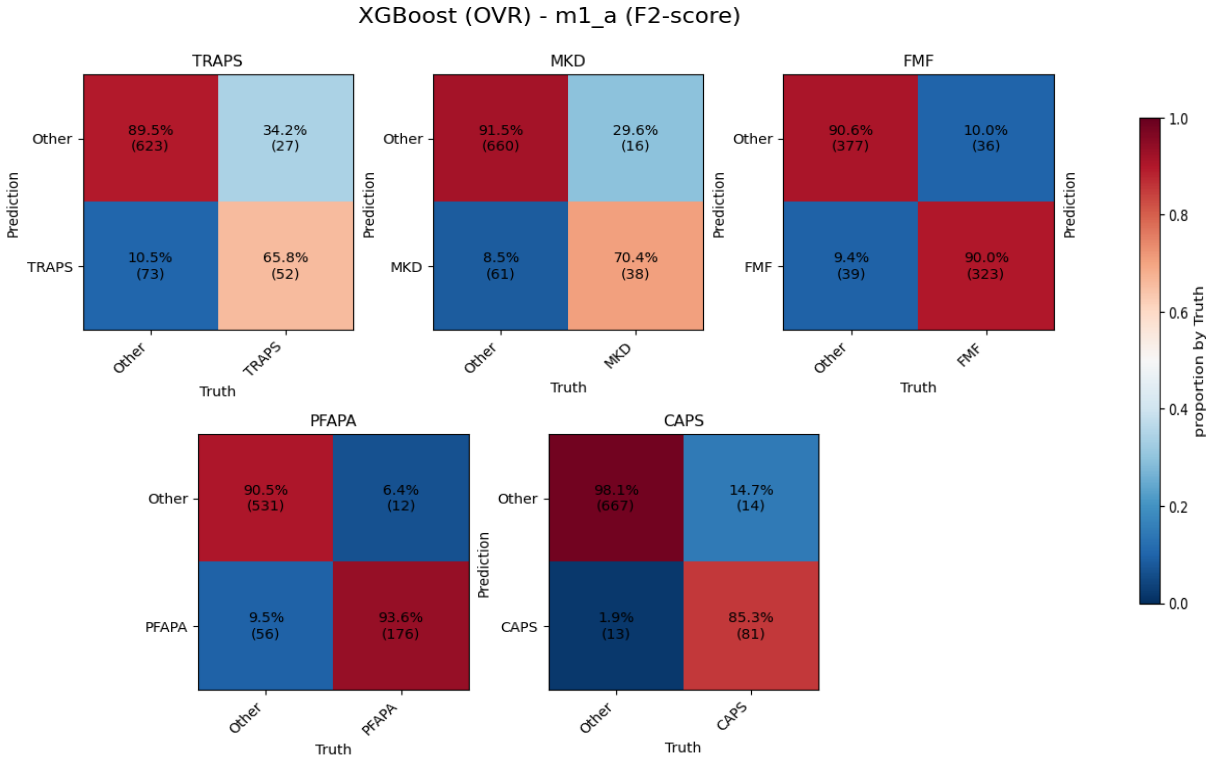


Figure 6.3.3. Confusion matrices for each disease show results of the OVR classification analysis on the test set. Red values indicate high recall, blue values indicate low recall.

Having identified XGBoost under the OVR framework as the final model, we next investigated the features driving disease-specific predictions by analyzing XGBoost feature importance scores computed on the training set (Figure **6.3.4**). Feature importance was quantified using the *gain* metric, which reflects the contribution of each feature to improving node purity across the ensemble. This analysis provides a global view of the most informative clinical features for discriminating each disease within the OVR framework.

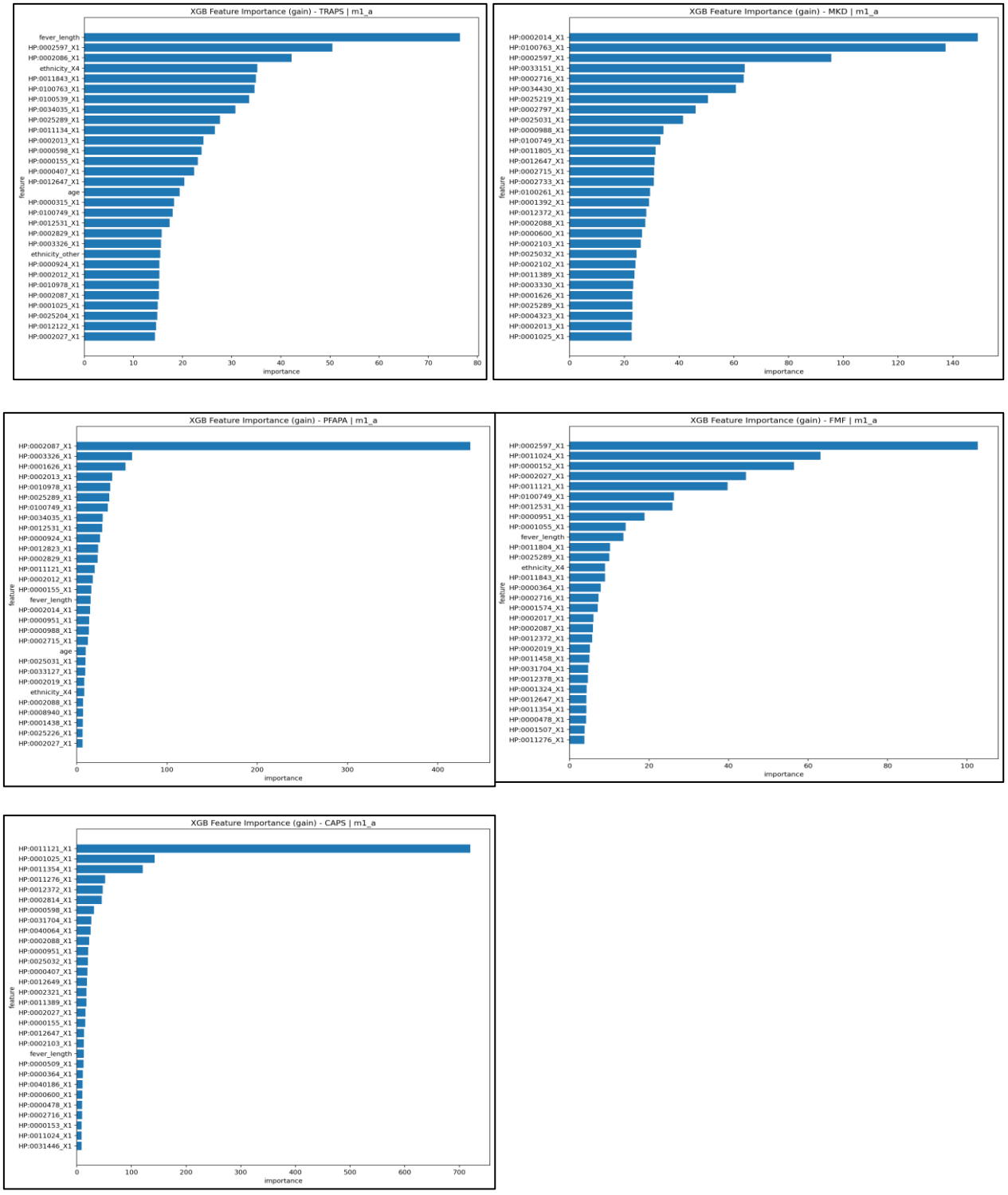


Figure 6.3.4.XGB features importance for FMF, CAPS, TRAPS, MKD and PFAPA

ii) Sensitivity analysis

All results presented above rely on models trained using HPO ancestor terms. To support this design choice, we performed a sensitivity analysis comparing XGBoost models trained with and without hierarchical phenotypic information. Across all diseases, performance remained highly comparable between the two settings, with only marginal differences observed **(Figure 6.3.5**). Given the comparable performance and the improved clinical interpretability of hierarchical phenotypic representations, the ancestor-based strategy was retained for all analyses.

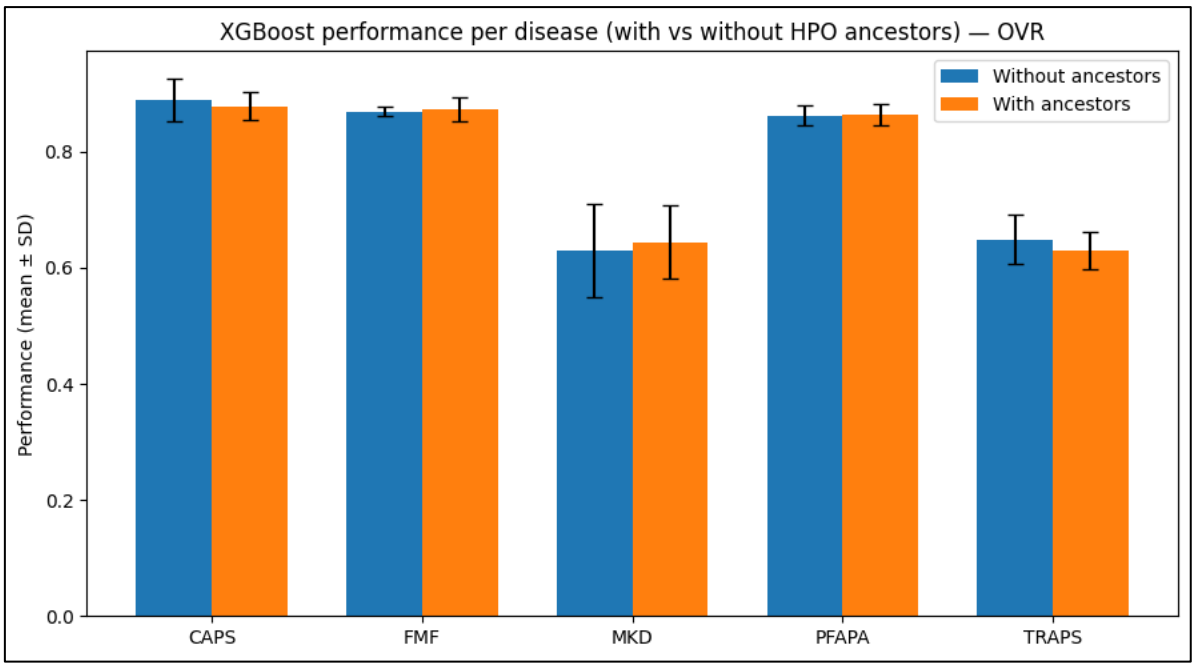


Figure 6.3.5. Sensitivity analysis of XGBoost performance with and without HPO ancestor terms. Comparable performance across diseases supports the use of hierarchical phenotypic representations for improved clinical interpretability.

iii) Predictions' evaluation

In the test phase, disease prediction was performed using a sequential OVR approach. Specifically, each patient was evaluated independently by the five disease-specific XGBoost classifiers (CAPS, FMF, MKD, PFAPA, and TRAPS). The resulting probabilities were used to estimate the likelihood of each disease, and the final prediction was assigned based on the highest predicted probability (**Figure 6.3.6**). This probabilistic formulation enables a more nuanced interpretation of model outputs, particularly in clinically ambiguous cases.

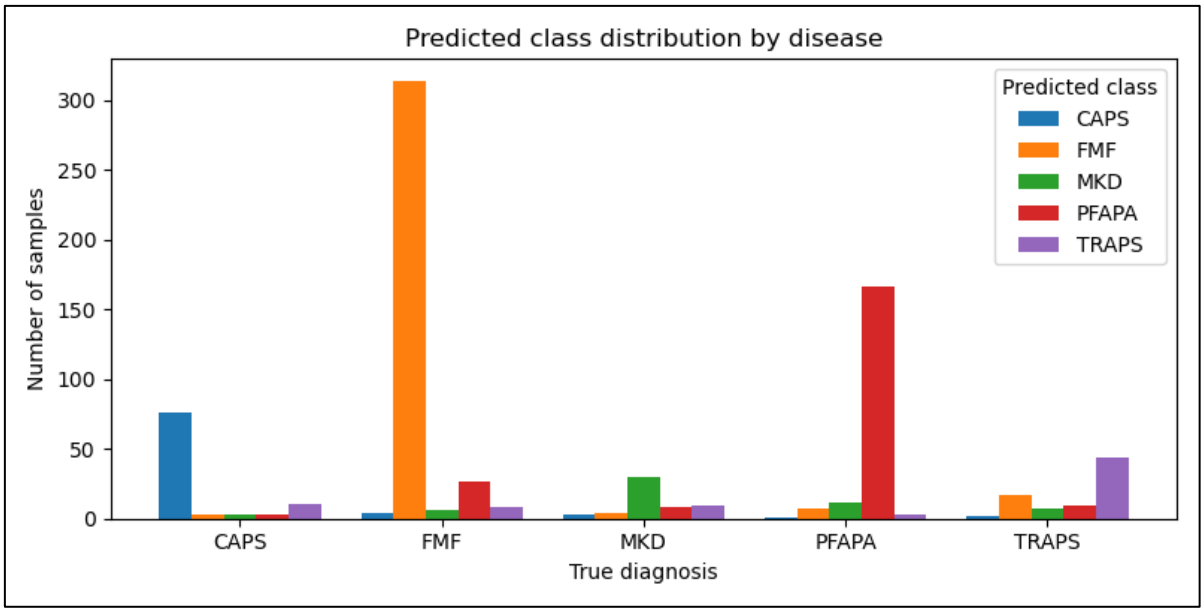


Figure 6.3.6. Schematic overview of the sequential OVR prediction framework. Each patient is independently evaluated by five disease-specific XGBoost classifiers (CAPS, FMF, MKD, PFAPA, and TRAPS), yielding disease-specific probabilities that are used to assign the final predicted diagnosis.

iv) Comparison between classic TRAPS and R92Q, P46L, D12E patients

Patients with Variants of Unknown significance (VUS) such as R92Q, P46L and D12E, were initially included in the general TRAPS group when fulfilling the EF clinical criteria. Seen the lower accuracy of the algorithm in assigning TRAPS patients compared to other diseases, we performed a subanalysis in order to evaluate a possible causality with the inclusion in this group of the patients carrying VUS. Error analysis revealed a statistically significant enrichment of the TRAPS R92Q subgroup among misclassified patients compared with correctly classified cases (Fisher's exact test p = 0.009; **Figure 6.3.7**). Specifically, 60% of R92Q ended in the "false negative" group, while 40% in the true positive group. Regarding patients with P46L and D12E, they were instead all allocated in the true positive group.

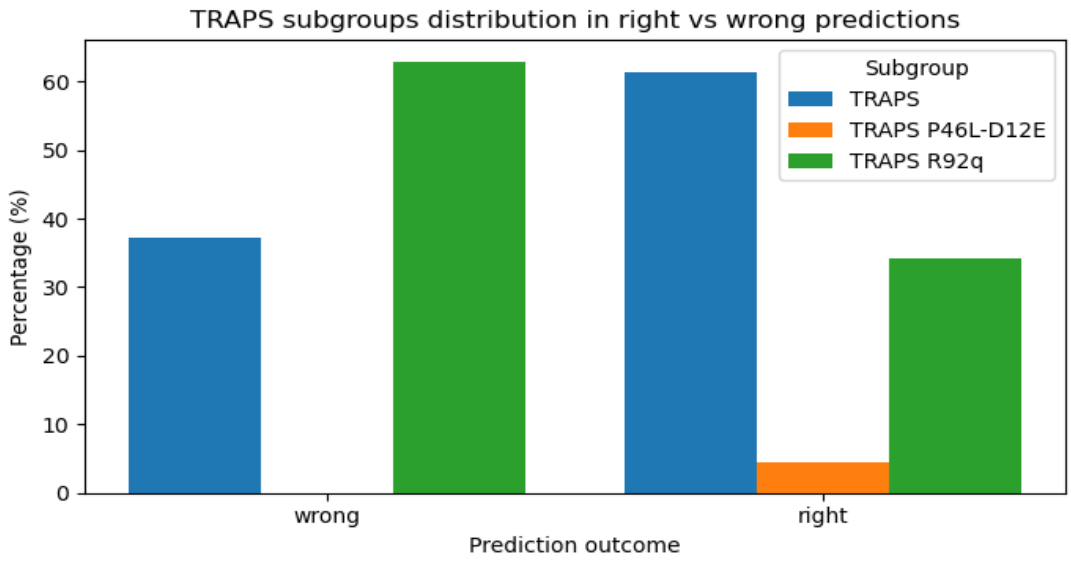


Figure 6.3.7. Error analysis of model predictions. Misclassified patients show a significant enrichment of the TRAPS R92Q subgroup compared with correctly classified cases (Fisher's exact test, p = 0.009).

v) Comparison between monoallelic *versus* biallelic FMF patients

In the FMF group, we included both monoallelic (one pathogenic mutation who fulfilled the EF classification criteria) and biallelic patients (homozygous or compound heterozygous). In order to investigate any potential difference between these two groups, an unsupervised UMAP analysis was conducted[216]. **Figure 6.3.8** shows two partially overlapping clusters, suggesting a high degree of similarity between the two groups based on the analyzed features.

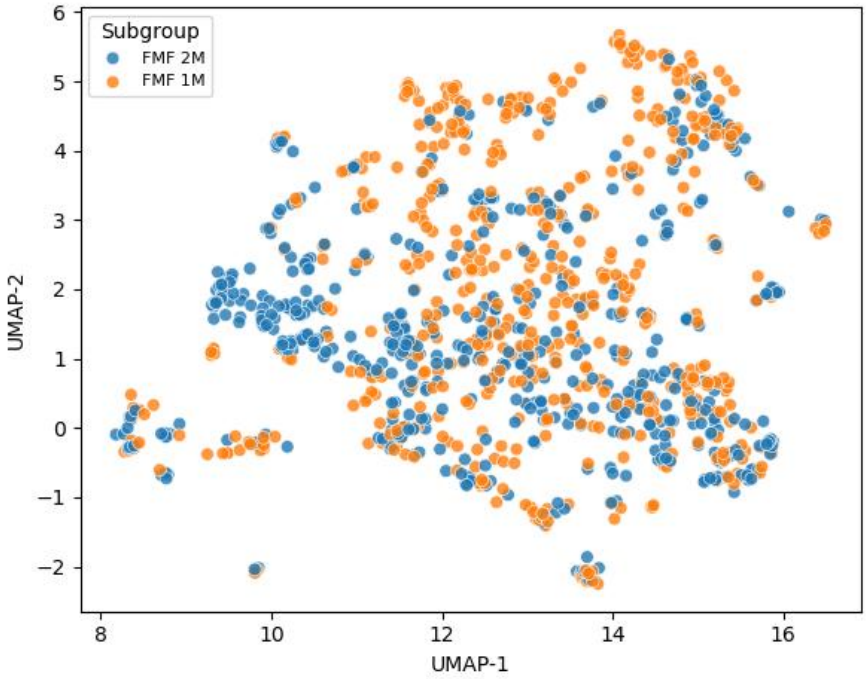


Figure 6.3.8. Unsupervised UMAP projection of FMF patients stratified by the presence of one or two mutations. The two groups show partially overlapping distributions, indicating a high degree of similarity based on the analyzed phenotypic features. FMF 1M = monoallelic patients, 2M biallelic patients

6.4 Creation of a web-based app

The final XGBoost OVR model was integrated into an interactive Shiny-based[217] web application enabling real-time inference. Users can enter the set of HPO terms describing an individual patient through a dedicated interface, after which the system computes disease-specific probability scores across the five target conditions. This application provides an intuitive proof-of-concept tool for translating the proposed machine learning framework into a clinically oriented diagnostic support setting (**Figure 6.4.1**).

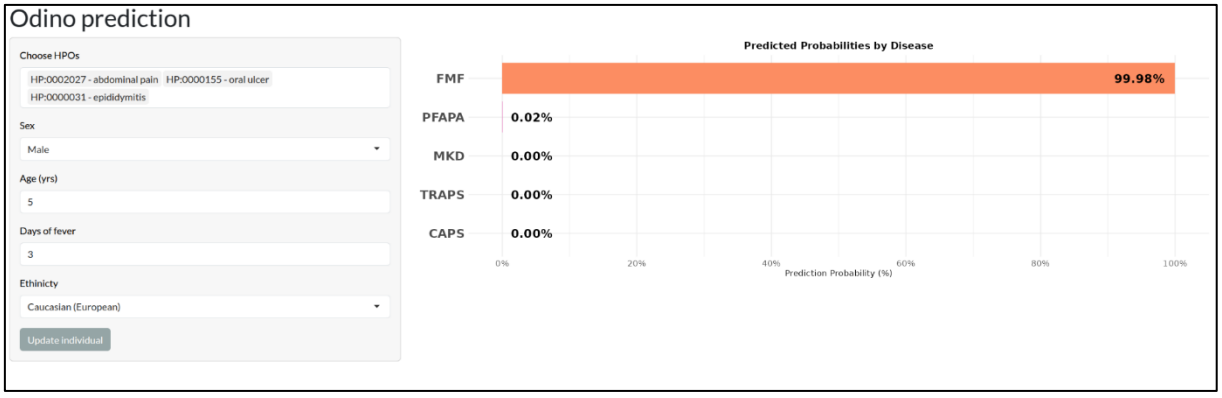


Figure 6.4.1. Screenshot of the Shiny-based web application developed as a proof-of-concept diagnostic support tool. Users can input patient-specific HPO terms, and the system returns real-time probabilistic predictions for each of the five autoinflammatory diseases based on the trained XGBoost OVR model.

7. DISCUSSION

This project has linked the Eurofever clinical terms to the correspondent HPO, highlighting the missing terms in the HPO system. Through a machine learning approach, the accuracy of the HPO terms in discriminating between different recurrent fevers, when derived from a real-world Registry, was assessed. In parallel, we have developed a novel ontology-driven diagnostic tool for the diagnosis of the 5 evaluated periodic fevers, applicable to both clinical research and computational pipelines.

The first major achievement is the systematic coding of the EF clinical variables into HPO terms. The majority of the EF variables could be successfully mapped onto existing HPO codes, supporting the overall adequacy of the HPO terms in describing periodic fevers. However, this coding exercise highlighted several critical gaps that have direct implications for both clinical interpretation and computational analyses. Notably, some of the most informative variables for the differential diagnosis of periodic fever syndromes are absent in HPO: namely “Fever duration”, and “ethnicity”, while a partial correspondence is present for “age at disease onset”. These terms are consistently recorded in EF for all periodic fevers and are also part of the classification criteria of the 5 analysed recurrent fevers: FMF, TRAPS, MKD, CAPS and PAFPA[3]. Nonetheless, these terms were highly relevant at both descriptive and predictive levels. The term “fever duration”, resulted a feature of importance for the algorithms, especially for TRAPS, for which it resulted the first term, but also within the other fevers. This is in line with the fact that TRAPS patients present a longer fever duration when compared to the other recurrent fevers, thus underscoring its diagnostic relevance. Similarly, “age at onset” and “ethnicity” reflect well-known epidemiological and genetic patterns in recurrent fevers, for example, “ethnicity” resulted an important feature in the FMF algorithm. The absence of these terms limits the expressiveness of purely ontology-based phenotypic profiles.

Beyond the missing HPO terms, some redundances of the EF Registry were also highlighted and are currently being revised.

Indeed, the extensive revision work on the HPO system has highlighted also some disease-specific structural limitations of the HPO site, as the presence of more than one disease-term per each disease and the problem of the reported frequencies. To this regard, in order to improve both clinical accuracy and computational use, we propose to update the HPO frequencies with those present in the EF Registry, thus providing for each disease patients-based frequencies that capture the real prevalence of each symptom.

The supervised ML analyses demonstrated that HPO-derived phenotypic representations have strong diagnostic potential. Among the evaluated approaches, the one-vs-rest (OVR) strategy consistently outperformed the multiclass framework. This finding reflects the complexity of SAIDs and suggests that disease-specific classifiers may be better suited to capture subtle phenotypic features than a single multiclass model.

Across all models, XGBoost emerged as the top-performing algorithm, achieving high recall, balanced accuracy, and Matthews correlation coefficients, particularly for FMF, PFAPA, and CAPS. The prioritization of recall through the use of the F2-score proved appropriate in this clinical context, where minimizing false negatives is critical. Importantly, sensitivity analyses showed that the inclusion of HPO ancestor terms did not substantially alter performance metrics but improved the interpretability of the models, supporting the use of hierarchical phenotypic enrichment as a clinically meaningful representation.

Taken together, these results indicate that the developed algorithm is accurate, making it suitable for use by bioinformaticians and for integration into larger diagnostic or research pipelines focused on recurrent fevers.

One of the most informative results arises from the detailed error analysis of model predictions. Patients carrying the TRAPS R92Q variant were significantly enriched among false-negative cases, with the majority of these patients being misclassified as false negatives despite fulfilling EF clinical criteria. This datum provides strong support for the biological and clinical distinctiveness of R92Q-associated disease. These patients appear to display a phenotypic profile that only partially overlaps with that of classical TRAPS caused by high-penetrance *TNFRSF1A* mutations. This result reinforces the concept that R92Q-associated disease represents a distinct clinical entity or a low-penetrance variant with unique characteristics[110], and highlights how ML-based approaches can uncover meaningful genotype–phenotype relationships beyond traditional categorical diagnoses. Moreover, the fact that the majority of R92Q patients were classified as false negatives explains the lower performance of the algorithm for TRAPS.

Another key finding of this work concerns the phenotypic comparison of FMF patients carrying one *versus* two pathogenic MEFV mutations. The unsupervised UMAP analysis demonstrated a strong overlap between these two groups, indicating a high degree of phenotypic similarity. This observation confirms the recent theories that challenge the traditional view of FMF as a strictly autosomal recessive disorder and support a disease model in which patients with a single high-penetrance mutation and a compatible clinical phenotype can be considered affected as

biallelic patients[21,54,218]. These results have important implications not only on disease classification, but also on clinical management and genetic counseling.

Several limitations of this thesis should be acknowledged. First, the analysis was intentionally restricted to five diseases with sufficient patient numbers in the EF Registry. While this choice ensured robust numbers, it limits the application of the findings to the broader spectrum of SAIDs, many of which remain extremely rare. Extending our work to other SAIDs, beyond recurrent fevers, will be essential to fully exploit its diagnostic potential.

Second, although the HPO mapping was systematic and comprehensive, it remains constrained by the current structure and content of the ontology. Some of the most relevant limitations identified—such as missing terms and suboptimal disease-level organization—require community-wide efforts and cannot be fully addressed within a single project.

In conclusion, this thesis demonstrates that the HPO terms, when applied to large, real-life clinical registries, can accurately capture disease-specific patterns and support high-performing diagnostic algorithms for SAIDs. Moreover, by identifying critical gaps in the current HPO structure, this work provides both methodological and conceptual advances.

Future studies might expand this methodological approach to other SAIDs and contribute to the refinement and restructuring of HPO disease annotations. Eventually, such effort will provide a more precise, data-driven diagnosis and a deeper understanding of the heterogeneity of autoinflammatory diseases.

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