AUTOINFLAMMATORY DISEASES: FROM GENES TO BEDSIDE

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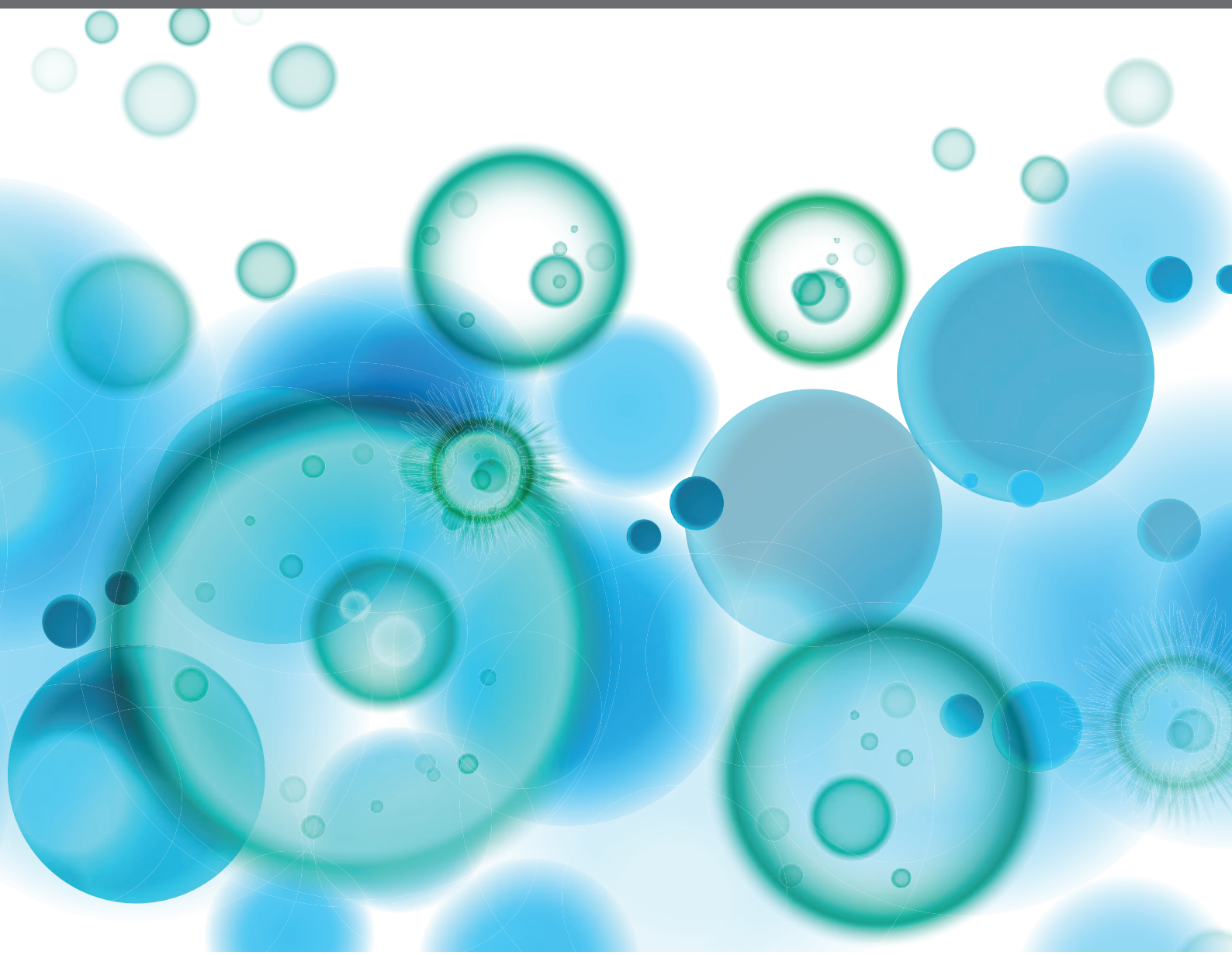

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AUTOINFLAMMATORY DISEASES: FROM GENES TO BEDSIDE

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EDITORIAL
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Editorial: Autoinflammatory Diseases: From Genes to Bedside

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Editorial on the Research Topic

Autoinflammatory Diseases: From Genes to Bedside

The year 2019 marked the 20th anniversary of the formal recognition of autoinflammatory diseases as a distinct group of rheumatological conditions, following the identification of the gene mutated in patients with a dominantly inherited periodic fever known as familial Hibernian fever (FHF) (1). This nosological concept was introduced by one of the founders of the field, Dr. Daniel Kastner. Prior to this time, the only recognized periodic fever disease was familial Mediterranean fever (FMF) and patients presenting with similar symptoms, irrespective of inheritance pattern, were suspected to have a variant FMF. Most patients, with exception for FMF, were treated with NSAID, glucocorticoids alone, or in a combination with immunosuppressive agents. These chronic life-long conditions negatively impacted patients' quality of life and were associated with significant morbidity and mortality, partially due to treatment-related side effects.

The early advances in the field of autoinflammation were driven by the ascertainment of families with inflammatory phenotypes segregating either as a recessive (FMF) or dominantly (FHF) inherited trait. This allowed for linkage mapping, positional cloning and candidate gene screening even before the completion of human genome sequencing project in 2003. These gene-hunting projects were laborious and time-consuming, but nonetheless successful and led to identification of the first three genes associated with autoinflammatory diseases: *MEFV, TNFRSF1A*, and *CIAS1/NLRP3*.

Familial Mediterranean fever was the first disease to be characterized at the molecular level. FMF, being a common illness in multiple Mediterranean populations, was initially noted in the literature by Galen in the second century AD. Although there were reports of cases with cyclic fevers and pains in the nineteenth century, it remained a mysterious disease and its pathogenesis was attributed to the phases of the moon and other environmental factors. Genetic etiology was not suspected until 1958 when Dr. Harry Heller emphasized the genetic nature of the disease and coined its modern name familial Mediterranean fever (2). The first accurate clinical description of FMF was published in 1945 by Dr. Sheppard Siegal, who reported 10 patients suffering from recurrent bouts of abdominal pain and fevers but who between attacks "may enjoy good health" (3). He named the disease "benign paroxysmal peritonitis." The next major break-through was in the early nineties, when Drs. Daniel Kastner and Isabelle Touitou undertook finding the causal gene for FMF (4, 5). Independently, they collected many families with FMF to launch genome-wide linkage mapping. This was incredibly ambitious considering available techniques, Southern blot analysis of DNA by restriction fragment length polymorphism (RLFP) markers not dense enough to cover the entire human genome. Complete sequences of many expressed genes were not available. After 7 years

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of tedious labor, a poorly characterized transcript harboring bi-allelic missense pathogenic mutations associated with FMF was identified. This discovery was celebrated at the First International Meeting on FMF in the summer of 1997 at Jerusalem, which was organized by Dr. Mordechai Pras.

It was not until late nineties that the second periodic fever disease came to attention. Although a five-generation family of the European ancestry with dominantly inherited fevers was described in 1957 by Drs. Bouroncle and Doan (6), most physicians were not aware of the disease. In 1998, the molecular basis of familial Hibernian fever was elucidated with the identification of heterozygous mutations in the *TNFRSF1A* gene (1). The disease was renamed tumor necrosis factor (TNF) receptor-1 associated periodic syndrome (TRAPS) and the term autoinflammation was coined to describe new diseases of the innate immune system (1).

A new era of medical and genetic research in autoinflammation had begun. In 2002, Drs. Hoffman and Kolodner identified heterozygous mutations in the *CIAS1* gene in patients with dominantly inherited cold urticaria (FCAS) and Muckle-Wells (MWS) syndromes, both diseases considered cryopyrin-associated periodic syndromes (CAPS) (7). Another remarkable discovery from this study was that pyrin (encoded by *MEFV*) and the NLRP3 protein share the same N-terminal ∼92aa domain denoted as pyrin domain (PYD). This finding suggested the existence of common pathways in the pathogenesis of autoinflammation. Subsequently, a whole family of proteins with the pyrin domain was identified by *in silico* analysis. Many of these proteins function as intracellular sensing receptors to recognize foreign or self-generated danger-associated molecular patterns. They form a molecular complex known as inflammasome that was discovered and characterized in 2002 by the team of late Dr. Jurg Tschopp (8). The initial observation that gain-of-function mutations in the NLRP3 inflammasome lead to increased production of IL-1β formed the basis for genomically-informed therapies. Over time, numerous studies in human cells and murine models showed that IL-1β plays a major role in the pathogenesis of autoinflammatory diseases. These three discoveries established the basis for an entirely new field of investigation.

The next chapter began around 10 years ago with the development of new genomic technologies, next-gene sequencing (NGS), bioinformatics, and the completion of human genome project. These strategies provided researchers and clinicians a variety of tools for gene-hunting projects. Genetic discoveries are now often made in a matter of months if not weeks. The list of genes associated with monogenic autoinflammatory diseases has grown rapidly and currently includes more than 30 genes. Advanced sequencing technologies revealed unusual inheritance patterns including somatic mutations as the cause of adult-onset diseases and cases with digenic inheritance. Digenic inheritance refers to presence of pathogenic mutations in two interacting proteins as the cause of a disease. High-throughput sequencing has brought to light a number of variants with uncertain clinical significance (VUS). Attempts to clarify the clinical implication of these low frequency (1–5%) variants have been carried out through international collaborations. Despite of major accomplishments in dissecting the genetic basis of autoinflammatory conditions, the genetic cause of disease for many patients remains unknown. To complicate things, many of these patients are sporadic cases and it may require orchestrated efforts between multiple research groups to find causal genes.

The field of molecular diagnostics for autoinflammatory diseases has seen substantial growth, providing physicians with the specific information necessary to diagnose and treat patients. There are close to 100 academic and commercial laboratories across the world that perform genetic by testing through single-gene or targeted gene panel analysis, or whole exome sequencing. An international group of experts has been convened in effort to standardize genetic reports and develop consensus guidelines for interpretation of genetic variants.

We have learned a great deal about a wide spectrum of clinical manifestations in patients with autoinflammation. It is likely, as is the case with most human traits, that these phenotypic differences will be explained by modifying gene alleles, epigenetics effects, and environmental factors. Deficiency of adenosine deaminase 2 (DADA2) is case in point: patients may present with fevers, rash, ischemic strokes or with a sole manifestation of pure red cell aplasia. Mutations in the same protein—often in different domains—may give rise to distinct clinical features, therefore these phenotypes need to be referred to in the context of mutant protein e.g., Pyrin-, NOD2-, NLRP3-, or NLRC4-associated diseases. In contrast, a distinct phenotype, such is the case with cold-induced urticaria, could be caused by pathogenic mutations in different genes (*NLRP3, NLRC4, NLRP12, PLCG2, FXII*). About a decade ago an immunological continuum was proposed to designate patients who present with features of autoinflammation and autoimmunity (9). Recent studies described patients with cell-specific features of autoinflammation and/or immunodeficiency.

Studies of molecular pathways in murine models have shed light on broad aspects of the biology of inflammatory responses. Mice deficient for inflammasome components have become instrumental in dissecting signaling pathways that regulate innate immune responses. Studies utilizing pyrin knock-out mice, showed that the pyrin inflammasome has evolved as an innate immune sensor to detect bacterial-induced modifications, which is the first known example of the "guard mechanism" in mammalian innate immunity (10).

A number of "biologicals" have been developed to treat acute inflammation in patients with a broad spectrum of systemic inflammatory diseases. Targeted cytokine therapies, in particular anti-IL1 and anti-TNF, have been efficacious and with minimal side effects in treating patients with autoinflammation even without a known molecular cause of disease. Non-biological drugs such as JAK-STAT inhibitors have recently emerged and are promising in treating patients with interferon-mediated disorders including CANDLE (Chronic Atypical Neutrophilic Dermatosis with Lipodystrophy and Elevated Temperature) syndrome, STING-associated vasculopathy with onset in infancy (SAVI) and Aicardi-Goutieres syndrome (AGS).

The term autoinflammation has spread beyond the boundaries of internal medicine and rheumatology, and is now used in disciplines of dermatology, immunology, and neurology. Dysregulation of the innate immune system is increasingly considered to have role in the pathogenesis of many human conditions, including common multifactorial cardiovascular, metabolic, neurodegenerative, autoimmune diseases, and in particular in polygenic or systemic inflammatory diseases, such as Behçet disease and periodic fever, aphthous stomatitis, pharyngitis and cervical adenitis (PFAPA) syndrome. A better understanding of the molecular mechanisms underlying dysregulation of the innate immune system will provide a foundation for developing more affordable and effective treatments. Witnessing these developments has been incredibly rewarding for those of us in the field and it will be exciting to see where it goes from here.

In the present issue of Frontiers in Immunology, "Autoinflammatory diseases: from genes to bedside," the first monographic issue about autoinflammatory diseases, several investigators have contributed with original and review articles covering genetic, pathogenic, epigenetic, clinical and therapeutic aspects of different autoinflammatory conditions.

The "genes" part of the topic explores relevant genetic, pathogenic and epigenetic mechanisms implicated in autoinflammatory diseases. Martorana et al.. review the most common mutations and the evidences of genotype/phenotype correlations of the main monogenic autoinflammatory diseases. The role of NLRP3 and pyrin inflammasomes in the pathogenesis of CAPS, and FMF and pyrin-associated autoinflammation with neutrophilic dermatosis (PAAND), respectively, has been addressed by de Torre-Minguela et al.. Aksentijevich and Zhou describe the latest advances on the pathogenic mechanisms of ubiquitinopathies, a new category of autoinflammatory diseases involved in the NF-κB pathway, which include linear ubiquitin chain assembly complex (LUBAC) and OTULIN deficiencies, and haploinsufficiency of A20. Carta et al.. propose two different pathways of inducing abnormal IL-1β production in autoinflammatory diseases depending on the cell type affected, in which the authors postulate that professional inflammatory cells would cause a direct inflammatory response and non-immune cells may participate indirectly in the inflammatory cascade by releasing stress signals that trigger and propagate inflammation. In the same sense, Gül reviews the concept of autoinflammation and uses it for monogenic and polygenic autoinflammatory diseases associated with seemingly unprovoked inflammatory episodes mediated mainly by the innate immune system. In addition, Gül also proposes and expands nomenclature by using the concept of "hyperinflammatory" state for those disorders characterized by episodes of exaggerated inflammatory response only when triggered by certain factors or situations. Álvarez-Errico et al.. review the recent advances on the contribution of epigenetic mechanisms in the disease expression of some autoinflammatory diseases.

The "bedside" part of the topic reviews important clinical and translational research, and therapeutic contributions in autoinflammatory diseases. Özen et al.. wrote a comprehensive overview about what are still considered unsolved problems in FMF, such as the involved mechanism of the disease, inheritance patterns and treatment in colchicine resistant patients. Ruiz-Ortiz et al.. make an original contribution in characterizing clinical manifestations associated with the low-penetrance R92Q variant in *TNFRSF1A* and differentiating disease phenotypes between patients with pediatric and adult onset. In an article about CANDLE syndrome, Torrelo et al. reviews in depth all the pathophysiological, clinical, and biologic features of this complex monogenic interferonopathy. The cytokine signature in patients with Behçet disease is explored by Lopalco et al., who suggest an increased signature of IL-6, TNF-α, and Th17 in patients with mucocutaneous and uveitis manifestations. Cantarini et al.. propose a set of clinical diagnostic criteria for adult-onset PFAPA syndrome with a high-predictive potential for identifying PFAPA patients among subjects with fever of unknown origin. Finally, in two review articles, Figueras-Nart et al.. analyze the most remarkable dermatologic and dermatopathologic features of monogenic autoinflammatory diseases by using a classification based on the predominant cutaneous lesion, and Soriano et al. describe the current treatment of the most frequent monogenic autoinflammatory diseases and PFAPA syndrome based on the best available evidence and also propose a practical guide to their management.

This first monography entirely dedicated to autoinflammatory diseases provides stimulating information on many of the past, present and future advances and challenges in this field.

AUTHOR CONTRIBUTIONS

All authors have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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REVIEW
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The NLRP3 and Pyrin Inflammasomes: Implications in the Pathophysiology of Autoinflammatory Diseases

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Inflammasomes are multiprotein complexes that critically control different aspects of innate and adaptive immunity. Among them we could highlight the release of pro-inflammatory cytokines that induce and maintain the inflammatory response. Usually, inflammasomes result from oligomerization of a nucleotide-binding domain-like receptor (NLR) after sensing different pathogenic or endogenous sterile dangerous signals; however, other proteins such as absent in melanoma 2, retinoic acid-inducible gene I, or pyrin could also form inflammasome platforms. Inflammasome oligomerization leads to caspase-1 activation and the processing and release of the pro-inflammatory cytokines, such as interleukin (IL)-1β and IL-18. Mutations in different inflammasomes are causative for multiple periodic hereditary syndromes or autoinflammatory diseases, characterized by acute systemic inflammatory flares not associated with infections, tumors, or autoimmunity. This review focuses on germline mutations that have been described in cryopyrin-associated periodic syndrome (CAPS) for *NLRP3* or in familial Mediterranean fever (FMF) and pyrin-associated autoinflammation with neutrophilic dermatosis (PAAND) for *MEFV*. Besides the implication of inflammasomes in autoinflammatory syndromes, these molecular platforms are involved in the pathophysiology of different illnesses, including chronic inflammatory diseases, degenerative processes, fibrosis, or metabolic diseases. Therefore, drug development targeting inflammasome activation is a promising field in expansion.

Keywords: inflammation, NLRP3, pyrin, extracellular ATP, P2X7 receptor, cryopyrin-associated periodic syndrome, familial Mediterranean fever

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DANGER SIGNALS, INFLAMMASOMES, AND THE PHYSIOLOGICAL SIGNIFICANCE OF THE INFLAMMATORY RESPONSE

Inflammation is the response of the innate immune system to a noxious stimulus, including infections or tissue damage (1, 2). Characterization of inflammasomes represents a considerable advance in the understanding of the inflammatory molecular events that occur in response to infections, and importantly, to tissue damage in the absence of pathogens. Furthermore, inflammasome activation

has also been attributed to changes on physiological homeostatic parameters, such as changes in extracellular osmolarity (3, 4), and virtually, any perturbation in homeostasis could generate a local or systemic inflammatory response (1, 2). Tissue damage and alteration of the homeostatic parameters induce the release of danger signals from the cells that activate the inflammasome in innate immune cells (5). Danger signals are usually referred as danger or damage-associated molecular patterns (DAMPs). The dual use of the term "danger" or "damage" in the acronym DAMP denotes that danger signals are not only released after damaging conditions but also in response to dangerous situations, such as during cellular environment alterations. In homeostasis, cells in tissues are in a physiological "basal" state maintained by nutrients, oxygen, growth factors, and adherence to other cells and the extracellular matrix. Changes in environmental parameters (temperature, osmolarity, oxygen, or pH) induce a cellular stress response and the subsequent release of DAMPs. Stress is then recognized by tissue-resident macrophages, activating different signaling pathways, including inflammasomes, and inducing an inflammatory response aimed to restore tissue functionality during noxious conditions. This inflammatory response was termed para-inflammation by Medzhitov (1). Deregulation of para-inflammation is intimately related with immunity and involved in the pathogenesis of immune-mediated diseases, being the base for the chronic low-level inflammation associated, for example, to type 2 diabetes (6). If homeostasis imbalance continues or is complicated with infection, cells become necrotic inducing an acute inflammatory response that will damage the tissue (7).

Damage-associated molecular patterns are intracellular components released to the extracellular milieu in response to cell stress or necrosis that activates different inflammatory pathways, such as inflammasomes. Inflammasomes are multimeric complex of innate immune receptors, activating caspase-1 and proteolytic mechanisms involved in pro-inflammatory cytokines [interleukin (IL)-1β and IL-18] (8). During cell stress, plasma membrane becomes permeable to ions, such as K^+, or to intracellular metabolites, such as the nucleotide adenosine triphosphate (ATP) or uric acid (1). One of the best characterized DAMP is ATP, since in physiological homeostatic conditions, ectonucleotidases maintain low extracellular ATP concentration, but during necrosis or inflammatory conditions, a high extracellular ATP concentration is reached, and the purinergic P2X7 receptor is activated in macrophages (9–12). P2X7 receptor is a potent activator of the inflammasome in macrophages and other innate immune cells (9). Leakage of cellular proteins with intracellular functions is another example of DAMPs; the release of these proteins usually follows secretory pathways independent of the endoplasmic reticulum (ER) and Golgi apparatus. Activation of caspase-1 by inflammasomes controls the release of these intracellular proteins by activating different unconventional release pathways, including a particular type of cell death called pyroptosis (1, 13, 14). Caspase-1 ultimately controls the release of inflammasome particles, a signal produced to amplify the release of DAMPs by activating caspase-1 in neighbor cells (11, 15). The high mobility group box 1 (HMGB1) nuclear protein is another example of DAMP released upon caspase-1 activation. HMGB1 presents histone-binding properties in the nucleus, and in the extracellular milieu, HMGB1 engages the advanced glycation end-product-specific receptor in conjunction with toll-like receptors (TLR) to induce an inflammatory response (16). In conclusion, innate immunity mechanisms converge in producing an inflammatory response as a consequence of infection, tissue damage, or loss of homeostasis.

INFLAMMASOME SENSOR PROTEINS

The nucleotide-binding domain-like receptor (NLR) family forms the main group of proteins considered as inflammasome sensors. These proteins contain a pyrin domain (PYD) or a caspase activation and recruitment domain (CARD). The presence of one of these domains in the sensor protein is required to assemble the inflammasome. Additionally, other proteins with some of these structural domains can also form functional inflammasomes, like absent in melanoma 2 (AIM2) protein, interferon-inducible protein 16 (IFI-16), retinoic acid-inducible gene I (RIG-I), and pyrin (17) (**Figure 1**).

There are different inflammasome sensors dedicated to recognize the presence of cytosolic nucleic acids. AIM2 presents an N-terminal PYD and a C-terminal hematopoietic interferon (IFN)-inducible nuclear protein with 200-amino acid repeat (HIN-200) domain. AIM2 is critical to respond against the infection of different pathogens by forming an inflammasome after recognition of double-stranded DNA (dsDNA) in the cytoplasm by the HIN-200 domain (18–20). Interestingly, other nucleic acid sensor protein called IFI-16 has two C-terminal HIN-200 domains and one N-terminal PYD. Upon detection of dsDNA, IFI-16 triggers the IFN response as a component of the signaling pathway (21) and can also induce the assembly of inflammasome with ulterior caspase-1 activation (22). RIG-I is also a sensor for viral RNA that contains two CARD domains and is able to assemble an inflammasome (23). However, it should be noted that additional studies are required to demonstrate that IFI-16 and RIG-I can form an inflammasome.

The structure of the sensor protein family NLR presents a central nucleotide-binding domain (NBD), and most of them have a C-terminal leucine-rich repeat (LRR) domain. The N-terminal protein domain is used to classify this group of proteins in NLRP if it contains a PYD domain or NLRC if it contains a CARD domain (24). Interestingly, the capacity for assembling inflammasome is a feature that has not been described for all members of the NLR family. These sensor proteins are also involved in other aspects of innate immune response by regulating diverse non-inflammasome pathways. Indeed, NLRP12 can play a role as a negative regulator of NF-κB signaling (25) or modulating IL-4 production in T cells (26), and NLRP6 is a negative regulator of mucosal immunity in the gut (27, 28).

The first sensor protein identified to form inflammasome was NLRP1 (29). Interestingly, human NLRP1 contains two additional protein domains compared to the canonical domains of the NLR family, such as a function-to-bind domain and a C-terminal CARD. These domains seem to play a critical role to assemble functional inflammasomes, as proteolytic cleavage of their N-terminal by pathogen components of *Bacillus anthracis*

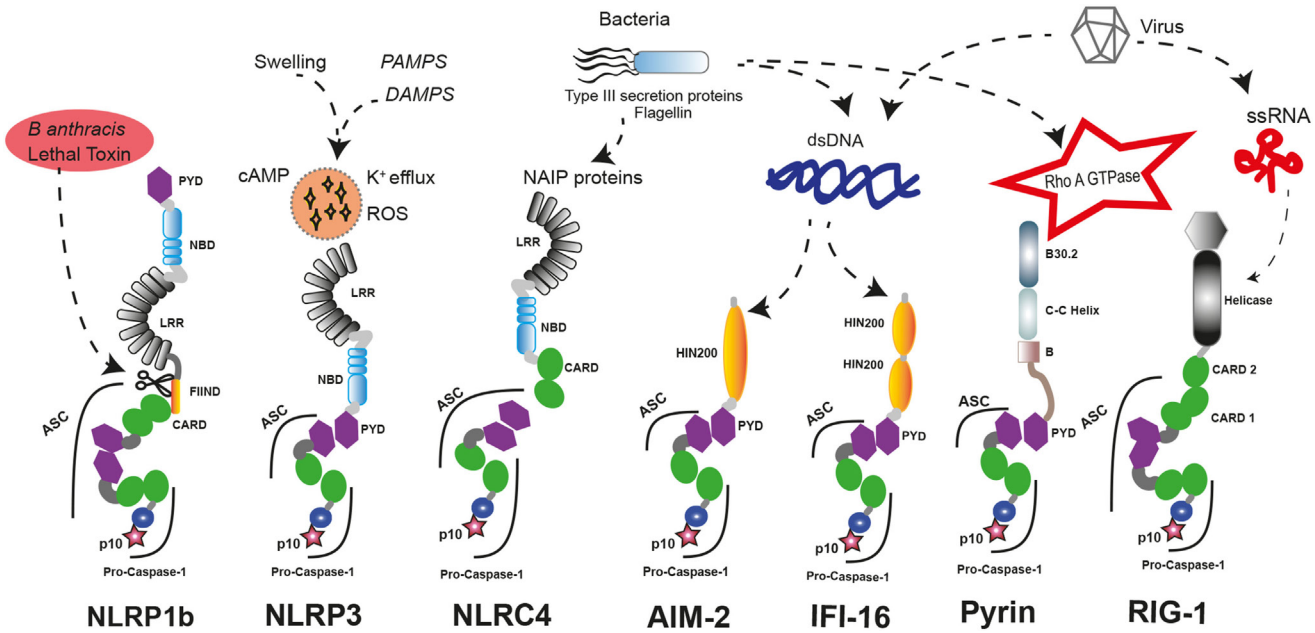

FIGURE 1 | **Inflammasome sensors and activators**. A wide variety of pathogenic ligands and intracellular mediators are involved in inflammasome assembly. NLRP1b responds to proteolytic cleavage on their N-terminal induced by lethal toxin of *Bacillus anthracis*. NLRP3 is a general sensor of cellular damage that responds to intracellular harm induced by pathogenic or sterile insults. NLRC4 recognizes bacterial proteins *via* NLR family-apoptosis inhibitory proteins (NAIPs) and can assemble inflammasomes with or without recruiting ASC, similar to NLRP1b. Absent in melanoma 2 (AIM2) and interferon-inducible protein 16 (IFI-16) sense dsDNA through their HIN-200 domains; meanwhile, RIG-1 activates caspase-1 through an inflammasome assembly after it detects ssRNA. Pyrin inflammasome is induced by bacterial toxins that modify RhoA GTPase. DAMPs, danger-associated molecular patterns; PAMPs, pathogen-associated molecular patterns; ssRNA, single strand RNA, dsDNA, double strand DNA.

is required for their activation (30, 31). Furthermore, the presence of a CARD domain in the C-terminal allows the direct interaction and activation of caspase-1 without the presence of any other adaptor proteins like the apoptosis speck-like protein with a CARD domain (ASC), even though ASC incorporation to the platform enhances the processing of IL-1β (32), and in human THP-1 monocyte cell line, ASC is required for NLRP1 activation (33). In contrast, mouse NLRP1a could form an inflammasome independent of ASC (34).

A genetic study of families with vitiligo with or without other autoimmune diseases has revealed a link between these autoimmune disorders and the presence of polymorphisms in *NLRP1* gene (35). Recently, a novel gain-of-function mutation in *NLRP1* gene that predisposes to inflammasome activation has been associated with NLRP1-associated autoinflammation with arthritis and dyskeratosis autoinflammatory syndrome (36). This syndrome is characterized by diffuse skin dyskeratosis, autoinflammation, autoimmunity, arthritis, and elevated transitional B-cells (36) (**Table 1**). Furthermore, *NLRP1* mutations have been implicated in non-fever inflammasome-related disorders, in particular with two overlapping skin disorders: multiple self-healing palmoplantar carcinoma and familial keratosis lichenoides chronica, demonstrating that NLRP1 has an important role controlling skin inflammation (33).

The most prominent member of NLR family in the study of hereditary autoinflammatory syndromes is NLRP3. Indeed, gain-of-function mutations on *NLRP3* gene have been identified in patients with cryopyrin-associated periodic syndromes (CAPS, see below) (59, 60) (**Table 1**). NLRP3 contains the three canonical domains described in the NLRP family: PYD, NBD, and LRR, and it is able to assemble a functional inflammasome in response to a wide variety of triggers, suggesting that it could be a global sensor of cellular damage and different pathogens (5).

Besides NLRP3, formation of active inflammasomes triggered by a bacterial infection has only been described *in vitro* for other two members of NLRP family: NLRP7 (61) and NLRP12 (62). Interestingly, NLRP12 displays a sequence similar to NLRP3, and it is predominantly expressed in myeloid-monocytic cells (63). In some cases, genetic studies of symptomatic patients with CAPS-like syndrome without mutations in *NLRP3* revealed the presence of mutations in *NLRP12* gene (56, 57). *In vitro* study of these NLRP12 variants has shown an increase in the activity of caspase-1 and the secretion of IL-1β, suggesting the potential role of NLRP12 mutations in CAPS-like syndrome-associated inflammation (**Table 1**) (58, 64).

NLRC4 is another well-known member of the NLR family assembling functional inflammasomes in response to pathogens. NLRC4 is a component of a detection system for bacterial proteins such as flagellin and several components of the type III secretion system (65, 66). As a member of the NLRC subgroup, NLRC4 contains a C-terminal CARD besides of NBD and LRR domains, but unlike other NLR sensor proteins, NLRC4 requires of sensors

TABLE 1 | Molecular and clinical features of autoinflammatory diseases associated with mutations in inflammasome sensor proteins.

Disease	Disease symptoms	Clinical treatment	Inflammasome sensor	Gene	Mutations detected (references)	Mouse model (references)
CAPS	Systemic activation Urticarial rash CNS: deafness, cephalea, meningitis Musculoskeletal Amyloidosis	Anakinra[a] Rilonacept[a] Canakinumab[a]	NLRP3	*NLRP3*	(37–40)	(41–45)
FMF	Periodic fever Serositis/arthritis Myalgia Erysipeloid rash Amyloidosis	Colchicine[a] Anakinra Canakinumab	Pyrin	*MEFV*	(39, 46–48)	(49, 50)
PAAND	Fever Neutrophilic dermatosis Myalgia/myositis	Anakinra[b]	Pyrin	*MEFV*	(51)	–
AIFEC	Early onset recurrent macrophage activation syndrome High levels interleukin (IL)-18	Dexamethasone[b] Cyclosporine[b] IL-18-binding protein[c]	NLRC4	*NLRC4*	(52–54)	(55)
CAPS-like syndrome	Cold triggered Arthralgia–myalgia Fever Urticarial rash	NSAIDS[b] Anti-IL-1[b]	NLRP12	*NLRP12*	(56–58)	–
NAIAD	Recurrent fever Dyskeratosis Arthritis Metaphyseal abnormalities	Acitretin[b] Anti-IL-1[b]	NLRP1	*NLRP1*	(36)	–
FKLC	Symmetric hyperkeratotic lichenoid papules	UVB phototherapy[b]	NLRP1	*NLRP1*	(33)	–
MSPC	Multiple recurrent keratoacanthoma Palmar-plantar-eye Risk of squamous cell carcinoma	Surgery[b]	NLRP1	*NLRP1*	(33)	–

AIFEC, autoinflammation with infantile enterocolitis; CAPS, cryopyrin-associated periodic syndromes; FKLC, familial keratosis lichenoides chronica; FMF, familial Mediterranean fever; MSCP, multiple self-healing palmoplantar carcinoma; NAIAD, NLRP1-associated autoinflammation with arthritis and dyskeratosis; PAAND, pyrin-associated autoinflammation with neutrophilic dermatosis.

[a]*Approved clinical treatment.*

[b]*Clinical treatment approach.*

[c]*Emergency compassionate-use Investigational New Drug authorization.*

co-receptors, termed NLR family-apoptosis inhibitory proteins (NAIPs), that recognize the pathogen proteins in the cytoplasm and oligomerize NLRC4 (67, 68). Similar to NLRP1, NLRC4 could interact directly with pro-caspase-1 through their CARD domain generating an inflammasome with a less efficient state of activation, and the association with the adaptor protein ASC is important to amplify the activation of caspase-1 (69). Gain-of-function mutations in *NLRC4* gene are associated with early onset autoinflammation with enterocolitis or recurrent macrophage activation syndrome depending on the mutation (**Table 1**) (52, 54). These patients are characterized by mutations in the NBD region of NLRC4 and benefits from recombinant human IL-18-binding protein therapy (53). The autoinflammatory-associated NLRC4 mutation H443P is able to constitutively activate caspase-8 and induce apoptosis *via* interaction with the component of the 26S proteasome Suppressor of Gal 1 and with ubiquitinated cellular proteins (70).

All inflammasome sensor proteins are activated in response to different pathogen and danger signals, suggesting that each activator triggers the formation of its own particular inflammasome complex. Interestingly, a recent work describes the recruitment of two sensor proteins (NLRC4 and NLRP3) to the same inflammasome complex as a result of the recognition of different danger signals from the same pathogenic infection (71).

Pyrin is another important inflammasome-forming protein (72). This protein contains an N-terminal PYD domain that is responsible for their interaction with ASC and later activation of caspase-1, a central coiled-coil domain and a C-terminal B30.2/SPRY domain that is not present in the mouse orthologous protein. The pyrin-inflammasome assembly could be triggered after sensing the activity of bacterial toxins from different species that covalently modify switch-I region of Rho family proteins (73). In addition, mutations in the gene that codify pyrin, *MEFV* gene, are

found in symptomatic patients with hereditary autoinflammatory disorders (see below and **Table 1**) (46).

INFLAMMASOME ADAPTOR AND EFFECTOR PROTEIN ASSEMBLY

Inflammasome sensor proteins are involved in the recognition of particular danger stimulus and then initiate the assembly of inflammasome multimeric complex; in most inflammasomes, the interaction with an adaptor protein is required to enhance the activation of caspase-1. The protein ASC (also known as Pycard) is the ubiquitous adaptor for inflammasomes, and its interaction with the active inflammasome sensor protein induces a prion-like oligomerization process essential for the final structural conformation of the inflammasome. ASC is composed by two death-fold domains, a N-terminus PYD and a C-terminus CARD (74, 75). For those inflammasome sensor proteins associated with autoinflammatory disorders, i.e., NLRP3 or pyrin, their PYD domain is responsible for ASC recruitment *via* PYD–PYD homotypic interactions inducing the formation of filamentous structures that assemble into a large protein aggregate (76). Caspase-1 activation occurs within this aggregate, and interestingly, the same process of polymerization for ASC and pro-caspase-1 has been shown independent of the inflammasome sensor protein activated (77).

Recent works have provided additional information about the interactions between the components of the inflammasome, suggesting an initial self-nucleation of the sensor protein (NLRP3 or AIM2) promoting the assembly of helical ASC filaments *via* PYD homotypic interaction (78, 79). These ASC filaments, generated after multiple PYD interactions, expose CARD domains in the outer part of the filament and consolidate the inflammasome aggregation with an appropriated cross-linking between filaments *via* CARD–CARD interactions (80). The multiple oligomerization of pro-caspase-1 with the ASC filaments also occurs *via* CARD–CARD interactions and amplifies the danger signal started by the sensor protein (81).

NLRP3 INFLAMMASOME ACTIVATION PATHWAYS

The activation of NLRP3 inflammasome appears in response to infection and is amplified by danger signals triggered during the infection, or by tissue injury or alterations in tissue homeostasis without infection. As it was described before, the majority of inflammasome sensor proteins are able to recognize different pathogen-associated molecules (bacterial proteins, toxins, and nucleic acids) and therefore activate inflammasome assembly in response to a microbial or viral infection. NLRP3 sensor is particularly able to oligomerize in response to a wide variety of stimuli that include pathogen molecules such as bacterial cell wall components or pore-forming toxins (nigericin and maitotoxin), endogenous danger signals like extracellular ATP, amyloid-β aggregates, uric acid crystals, or metabolic dysfunction, and pollutant particles as silica, asbestos, or alum (5, 82). The direct interaction between this broad range of activators and NLRP3 seems unlikely, and therefore it is suggested that NLRP3 is able to sense the cellular stress associated with the exposition to these agents. The precise molecular mechanism involved in the NLRP3 inflammasome activation remains elusive although recent studies begin to uncover the molecules and the cellular machinery responsible for this process (17, 83, 84).

Maintenance of ion gradients between different cellular compartments and between the cytosol and the extracellular environment is a feature of all living cells. Any alteration of this homeostasis will induce molecular mechanisms to respond and adapt to this aggression. Significant decrease of intracellular K^+ is indeed detected during NLRP3 activation after the treatment with microbial pore-forming toxins or after P2X7 receptor engagement by extracellular ATP (85), where the hemichannel pannexin-1 plays a critical role (86). Interestingly, decrease of intracellular K^+ is also detected during the NLRP3 inflammasome activation along with other sterile inductors as the decrease of osmolarity (3) or metabolic lipids (87), suggesting that intracellular K^+ concentrations could be one of the common mechanisms involved in the activation of the NLRP3 inflammasome; however, its mechanism of function is not well understood (88–90).

In addition to the decrease of intracellular K^+, a mobilization of Ca^{+2} in the cytosol is also detected in most of the stimulus that activates NLRP3. The ER is the main reservoir for intracellular Ca^{+2}, and its mobilization as a consequence of the activation of inositol trisphosphate receptor has been observed during NLRP3 activation induced with different stimuli. The activation of P2X7 receptor also induces an influx of Ca^{+2} from the extracellular space; however, in this cellular context, the blockage of extracellular Ca^{+2} influx does not inhibit NLRP3 inflammasome, and artificial mobilization of Ca^{+2} is not sufficient to trigger the NLRP3 inflammasome activation in absence of K^+ depletion (12, 14, 91). Cell swelling after hypotonic shock activates transient receptor potential cation channels (TRPM7 and TRPV2) involved in the modulation of intracellular Ca^{+2} that is crucial for the transforming growth factor beta-activated kinase 1 activation. These molecular events are required in combination with K^+ efflux for NLRP3 inflammasome assembly (3). In addition, several works show evidences that extracellular Ca^{+2} can trigger mechanisms that activate inflammasome through G protein-coupled receptors (92, 93). The activation of these receptors leads to the mobilization of intracellular Ca^{+2} *via* phospholipase C activation with a concomitant reduction of cyclic AMP (cAMP) (92). The effect of this reduction in cAMP will be discussed later in the context of the negative regulation mechanisms of NLRP3. Interestingly, elevated concentrations of extracellular Ca^{+2} have been detected at infection sites or in ischemic injury, suggesting that extracellular Ca^{+2} would play a role as a DAMP (93).

Alteration of lysosomal function after phagocytosis of molecular crystals has been described as an additional activation process of NLRP3 inflammasome, possibly as a consequence of the activity of released lysosomal proteases altering the integrity of cellular organelles (94). Furthermore, other cellular stress associated with the intracellular ionic mobilization, as the induction of ER stress, is able to activate NLRP3 inflammasome in a K^+ efflux-dependent manner. In this process, the endoribonuclease

inositol-requiring enzyme 1α, an unfolded protein sensor expressed in ER, is required to activate the NLRP3 inflammasome (95, 96). Taken together, these data show that changes in intracellular ion concentration play a key role in the activation of NLRP3 inflammasome, although their precise molecular mechanism remains unclear.

Besides ion fluxes, changes in the cellular oxidative state is a common process detected during NLRP3 inflammasome activation, being mitochondrial damage one of the main source of reactive oxygen species (ROS) (97). Interestingly, several works link mitochondrial ROS production with changes in the intracellular concentration of K^+ and Ca^{+2}, which would induce depolarization of the mitochondrial membrane (91, 98). Mitochondrial ROS production has also been described as a novel NLRP3 activation mechanism involving a decrease of NADH levels after disruption of the glycolytic flux (82). Mitochondria have also been suggested as a cellular platform to assemble the NLRP3 inflammasome. The activation of NLRP3 induces its relocation from the ER to the proximity of the mitochondria in the perinuclear environment (97, 99). This recruitment requires the reorganization of the microtubule system (100). Moreover, the mitochondria may also release other molecules implicated in the activation of NLRP3 inflammasome as cardiolipin (101) or oxidized mitochondrial DNA (102, 103), and it has been shown that mitochondrial antiviral-signaling protein interacts with the PYD of NLRP3, being essential for their activation after the stimulation with ATP or nigericin but not with crystals (99). All these data point out the essential role of mitochondria in NLRP3 inflammasome activation.

Finally, caspase-4, and its mouse orthologous caspase-11, activates NLRP3 after recognition of cytosolic LPS (104, 105). This signaling is known as the non-canonical NLRP3 inflammasome activation pathway, and although the mechanism of caspase-4-inducing NLRP3 activation is not known, it is also dependent on the decrease of intracellular K^+ (106–108).

REGULATORY MECHANISMS OF NLRP3 INFLAMMASOME

Several proteins have been described as positive or negative regulators of the NLRP3 inflammasome assembly (**Figure 2**). Guanylate-binding protein 5 binds *via* its GTPase domain to the PYD of NLRP3 during inflammasome activation by most of the stimuli except crystalline agents. This interaction promotes the oligomerization of NLRP3 with ASC (109). Furthermore, several works have described that, during ATP stimulation, NLRP3 deubiquitination mediated by the Lys63-specific deubiquitinase BRCC3 is an early process essential for inflammasome activation (110–112).

Recent works have revealed a new NLRP3 inflammasome regulatory molecule, the never-in-mitosis A-related kinase 7 (NEK7), a serine, and threonine kinase required for mitosis progression (113). This protein interacts with the LRR domain of NLRP3 upstream of NLRP3 inflammasome assembly independent of their kinase activity (114). This interaction is required for NLRP3 inflammasome oligomerization and introduces a new component of inflammasome regulation, the restriction of NLRP3 inflammasome formation to cells in interphase (115). Moreover, the absence of NEK7 in cellular models harboring frequent CAPS-associated mutations in NLRP3 reduces their ability to activate caspase-1, while the association between NEK7 and mutant NLRP3 is stronger (114, 115). Further investigation is required to elucidate the role of NEK7 in the auto-activation of NLRP3 inflammasome in autoinflammatory syndromes.

Maintenance of low NLRP3 protein levels avoids the auto-assembly of NLRP3 inflammasome in the absence of a danger stimulus; therefore, transcriptional regulation of NLRP3 is an additional control mechanism to avoid unexpected inflammasome activation. Transcriptional regulation of NLRP3 requires NF-κB activation by TLR or IL-1 receptor type I (IL-1RI) signaling to increase NLRP3 protein concentration to certain level that can be activated after sensing a triggering stimulus (116, 117). Furthermore, the amount of NLRP3 mRNA is tightly regulated in myeloid cells through the microRNA miR-223, although this miRNA is not regulated by pro-inflammatory signals (118). In addition, under unstimulated conditions, NLRP3 is inhibited by posttranslational modifications with ubiquitin chains that also target NLRP3 for its degradation through proteasome or autophagy as will be described later (112). Other mechanism involved in the inhibition of the NLRP3 activity is the post-translational modification of NLRP3 generated by the activation of inducible nitric oxide synthase. The increase of nitric oxide leads to the S-nitrosylation of NLRP3 impairing the assembly of the inflammasome, and this mechanism is suggested as a protective mechanism (119, 120). Therefore, the control of functional NLRP3 concentration within the cell is crucial for the activation of the inflammasome.

In addition to these negative regulatory mechanisms, two families of proteins containing CARD (COPs) or PYD (POPs) that could sequester either sensor proteins or effector proteins through PYD–PYD and CARD–CARD interactions have been described (121). In the absence of mutations, pyrin is also suggested to be a key regulator for the degradation of several inflammasome components (caspase-1, NLRP1, and NLRP3), preventing an excessive release of pro-inflammatory cytokines (122, 123). However, a recent work shows that the absence of pyrin in a mouse model leads to an increase in the release of IL-1β without affecting different inflammasome assembly (124). Therefore, the role of pyrin as an inflammasome inhibitor remains to be determined, and different domains among human and mouse pyrin proteins should be taken into account.

Cellular damage implicated in the activation of the NLRP3 inflammasome also activates autophagy, a mechanism involved in the clearance of intracellular pathogens and damaged organelles (102, 125). Autophagy is a negative mechanism to control the induction of the inflammatory response given its involvement in the degradation of damaged mitochondria, including molecular NLRP3 inflammasome inductors as mitochondrial DNA or ROS (102, 126, 127), the clearance of ASC specks (125), and pro-IL-1β (128). Ubiquitinated NLRP3 could be directed to the autophagosome for degradation by a complex with cAMP that recruits the E3 ubiquitin ligase MARCH7 (129, 130). This molecular mechanism can be triggered by activators of the

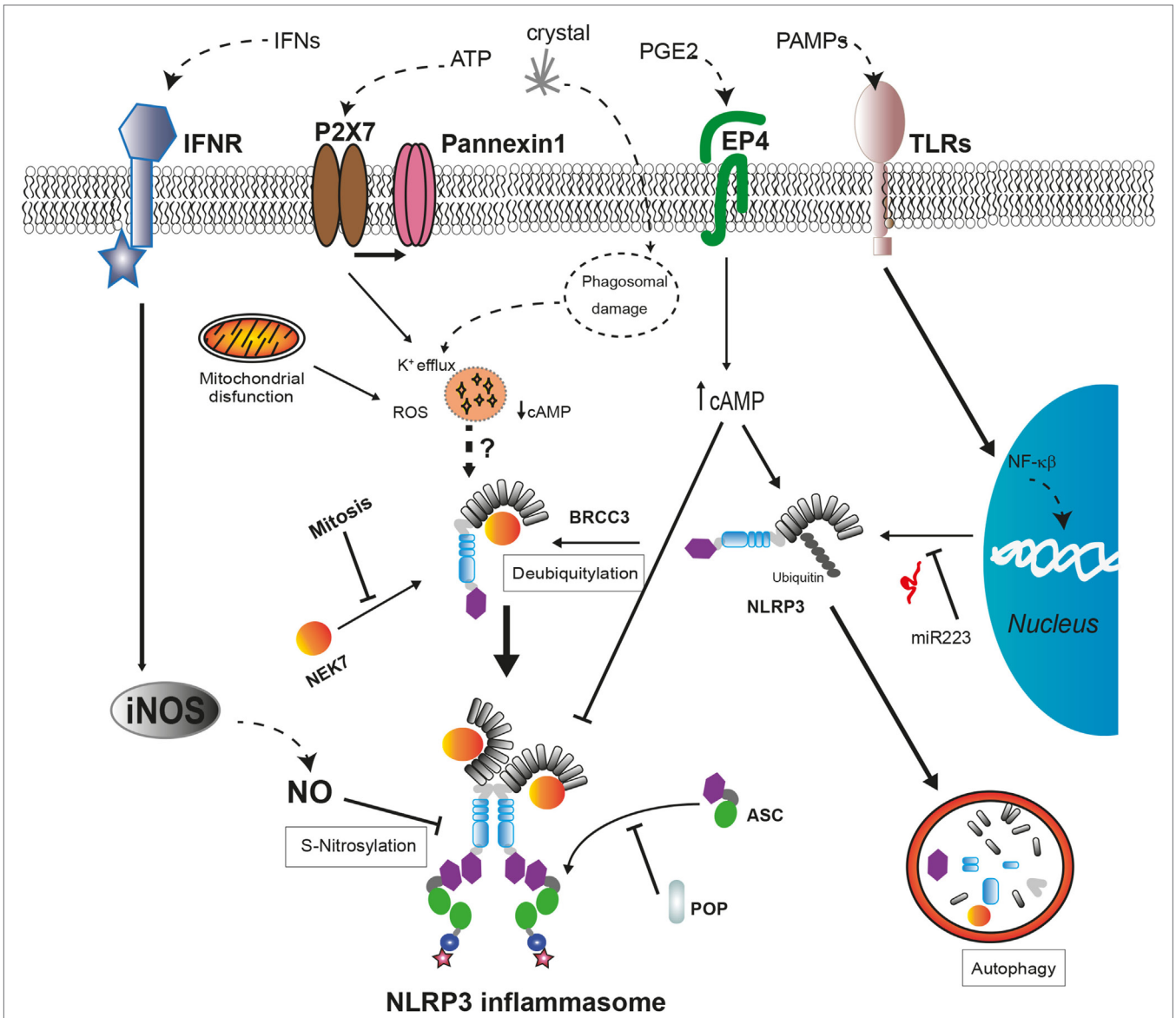

FIGURE 2 | Regulatory mechanisms of NLRP3 inflammasome assembly. The expression levels of *NLRP3* are regulated by miR-223 in basal conditions but can be upregulated after cell recognition of pathogen-associated molecular patterns that induce NF-κB signaling pathway. The activation of NLRP3 by cellular damage signals as intracellular K^+ decrease or reactive oxygen species (ROS) production requires a deubiquitylation of NLRP3 by BRCC3 and also their interaction with NEK7, only available during interphase. External signals, such as interferons (IFNs) or prostaglandin E_2 (PGE2), negatively regulate NLRP3 through different mechanisms. The increase in nitric oxide (NO) produced by inducible nitric oxide synthase (iNOS) leads to the S-nitrosylation of NLRP3 impairing the assembly of the NLRP3 inflammasome. The increase of cyclic AMP (cAMP) induced by PGE2 signaling *via* prostaglandin E_2 receptor 4 (EP4) activates the phosphorylation of NLRP3 reducing its oligomerization and increasing its ubiquitination to be degraded in autophagosomes. The interaction with pyrin domain-only proteins (POPs) can regulate NLRP3 inflammasome assembly by sequestering ASC or NLRP3.

adenylate cyclase as the neurotransmitter dopamine (130). Furthermore, an alternative negative regulatory mechanism has been described for NLRP3 inflammasome involving cAMP. The increase of cAMP induced by prostaglandin E_2 signaling *via* prostaglandin E_2 receptor 4 activates protein kinase A that phosphorylates NLRP3 in their NBD domain reducing its ATPase activity and oligomerization (131). Interestingly, this negative regulation could be disrupted by certain CAPS-associated mutations in the NBD of NLRP3 (114).

PYRIN INFLAMMASOME ACTIVATION PATHWAYS AND REGULATORY MECHANISMS

Recent data begin to unveil the mechanism involved in pyrin-inflammasome activation, as well as a protective mechanism concerned in blocking pyrin-inflammasome assembly. The inactivation of the RhoA GTPase by bacterial modification induces the activation of the pyrin inflammasome (73),

suppressing a protective mechanism that avoids pyrin inflammasome activation through their downstream phosphorylation by serine/threonine-protein kinase N1 and N2 (132). This mechanism requires the phosphorylation of certain amino acids of pyrin (S208 and S242 in human) allowing their binding to regulatory protein 14-3-3 and blocking the formation of the pyrin inflammasome (51, 132). Pyrin inflammasome activation through bacterial toxins is detected in human and mice, indicating that the C-terminal B30.2/SPRY domain, present only in human, is not required for their activation (133). Interestingly, this domain harbors most of the mutations detected in familial Mediterranean fever (FMF) patients, although some mutations affect one of the serine, in other domain of the protein, described as a key amino acid in the protective mechanism against the uncontrolled activation of pyrin inflammasome (see below). The inhibition of microtubule polymerization by colchicine abolishes pyrin inflammasome assembly induced by bacterial toxins, without affecting pyrin dephosphorylation (133, 134). However, this control of pyrin inflammasome activation leaded by microtubules is not effective in FMF patients that harbor mutations in B30.2/SPRY domain (133), suggesting that these mutations may force a protein conformation that aids the assembly of pyrin inflammasome after their dephosphorylation.

INFLAMMASOME-ASSOCIATED SECRETOME

The formation of inflammasome leads to the activation of caspase-1, and this protease triggers a broad number of cellular events as a consequence of its catalytic activity, including the release of cytosolic proteins associated with a specific type of cell death termed pyroptosis. Specifically, the analysis of the secretome associated with caspase-1 has revealed the key role of this protease in the unconventional secretion of multiple essential molecules involved in the inflammatory process. From them, the cytokine IL-1β is one of the most prominent and critical products of inflammasome activation, since it is a key regulator of the inflammatory response and is the most important current target of therapeutic treatments for autoinflammatory syndromes. The synthesis of IL-1β mRNA and the production of the inactive precursor form of IL-1β are strongly induced by microbial products such as LPS signaling *via* TLR or by IL-1 itself signaling through the IL-1RI (135). Caspase-1 is able to process the inactive precursor form of IL-1β to its mature bioactive form and induce its release. Similarly, caspase-1 is also responsible to the maturation of the inactive IL-18 precursor, another IL-1 family cytokine, to its bioactive form (136).

Both IL-1β and IL-18 are the canonical cytokines signaling downstream inflammasome activation, but beyond these caspase-1 substrates, caspase-1 also controls the unconventional release of other cytosolic proteins (FGF-2, thioredoxin, and annexins), lysosomal proteins (cathepsins and cystatins), or nuclear proteins (HMBG1, IL-1α) that are not direct substrates of the protease (13, 14). In addition, caspase-1 also controls the release of large complex protein aggregates as ASC inflammasome oligomers that are now able to spread caspase-1 activation to adjacent cells and maintain inflammasome signaling (11, 15).

Unconventional protein release induced by caspase-1 has been widely studied for IL-1β, a cytokine that does not follow the conventional route of protein secretion through ER or Golgi. Different mechanisms of unconventional secretion have been explored to explain this process including the release through exocytosis of secretory lysosomes (137) or extracellular vesicles released after NLRP3 inflammasomes activation (138, 139). Caspase-1-induced pyroptosis is associated with an increase in plasma membrane permeation that may help a passive release of IL-1β (140). The destabilization of cell membrane integrity during pyroptosis is induced by the cleavage of the cytosolic substrate gasdermin D by caspase-1 or caspase-4; gasdermin D N-terminus integrates into the plasma membrane forming pores (141–143). The application of membrane stabilizing agents, as the complex polyphenol punicalagin, prevents the execution phase of pyroptosis and release of mature IL-1β from macrophages after NLRP3 inflammasome activation, suggesting that loss of plasma membrane is involved in this secretion in parallel with cell death (144). Interestingly, the stabilization of the plasma membrane by punicalagin inhibits the release of IL-1β in neutrophils in the absence of cell death (144). Therefore, the secretion of bioactive form of IL-1β requires membrane permeation and may occur in secreting cells as neutrophils where NLRP3 inflammasome does not induce pyroptosis (145, 146). Initially, these mechanisms are not mutually exclusive and may participate in the secretion of IL-1β depending on the intensity of the stimulus and cell type. The release of other caspase-1-dependent secretome proteins is less studied, and the involvement of pyroptotic cell death in this process is not known, neither its contribution to the pathophysiology of autoinflammatory syndromes.

IMPLICATIONS OF NLRP3 INFLAMMASOME IN CAPS

Cryopyrin-associated periodic syndromes are rare hereditary autosomal-dominant autoinflammatory diseases with an estimated prevalence of 1–3 cases per million of inhabitants (147, 148). They include familial cold urticaria syndrome (FCAS) (59), Muckle–Wells syndrome (MWS) (149), and chronic infantile neurological cutaneous and articular (CINCA) syndrome also known as neonatal onset multisystemic inflammatory disease (NOMID) (150). All three syndromes were independently described and latterly found to be caused by gain-of-function mutations in the *NLRP3* gene, located in the short arm of chromosome 1 (1q44) (37, 38). Mutant NLRP3 drives a constitutive hyperactivity of inflammasome, activation of caspase-1, and an excessive unregulated release of IL-1β, although systemic circulating levels of IL-1β during inflammatory flares are in most cases undetectable (11, 147).

Clinical features of CAPS are related to systemic effects of IL-1β-inducing fever, malaise, fatigue, and chronic pain along with a blood serum rise of acute-phase reactants, such as C-reactive protein and serum amyloid A. CINCA/NOMID is characterized for an almost continuous early onset inflammatory

state with fever and non-pruritic migratory urticaria-like rash; central nervous system symptoms and arthropathy are common. MWS shows a moderate phenotype with latter onset of fever, rash, arthralgia, conjunctivitis, uveitis, sensorineural deafness, and potentially life-threatening amyloidosis. FCAS is a milder familial condition characterized by febrile urticarial rash with headache, arthralgia, and sometimes conjunctivitis but no central nervous system symptoms and is typically triggered by cold exposure. FCAS, MWS, and CINCA/NOMID are considered a clinical continuum than distinct diseases as intermediate phenotypes occur; being FCAS the mildest and CINCA/NOMID the most severe forms (151). Neurologic manifestations in CAPS are common including headache, sensorineural hearing loss, myalgia, chronic aseptic meningitis, increased intracranial pressure, cerebral atrophy, seizures, and mental retardation (152). Musculoskeletal symptoms in CAPS are also very frequent; up to 86% may have any musculoskeletal manifestation during follow up, 30% at onset. In a large cohort, these included arthralgia in 86% and arthritis in 58%; joint destruction and typical knee deformities appeared rarely (9% and 2%, respectively). Tendinopathies occurred in 21.5%, tender points in 16.5%, and myalgia in 33% of patients (153).

To date, a total of 177 variants of *NLRP3* gene have been included in infevers database (39), most of them located in exons 3 or 4 and intron 4. Among them, the most frequent is R260W (40, 148) and are associated with a milder phenotype along with A439V. The variants, T348M and D303N, and low frequency mutations are associated with a severe phenotype; E311K accounts for a high rate of hearing loss. On the other hand, Q703K or V198M variants have little clinical significance and are considered a functional polymorphism and a low penetrance variant, respectively. Clinically affected patients with no germline mutations could have an NLRP3 somatic mosaicism (154–156). The highly heterogeneous phenotypes within identical genotypes show the need for advancing the underlying understanding of the pathophysiological mechanisms.

The relevance of NLRP3 mutations as key players in the induction of these autoinflammatory syndromes has been explored in animal models. Specifically, the development of knock-in mouse strains harboring some of the frequent mutations detected in CAPS syndrome has demonstrated the pivotal role of IL-1β and the innate immunity in the pathogenesis of this syndrome; meanwhile, the adaptive immune system seems not to be involved (41). These animal models exhibit an autoinflammatory disease similar to that in humans associated with an inflammasome hyperactivation and unregulated release of IL-1β (41–43). Humanized mice expressing CAPS-associated mutation D305N present an increased sensitivity to endotoxin and develop progressive and debilitating arthritis (44). Furthermore, the study of these knock-in animal models has revealed the critical role of microbiota as inducer of disease, and myeloid and mast cells as cellular sources of IL-1β in the development of the skin inflammation (42). In addition, the study of these CAPS-like animals has revealed the key role of IL-18 in the early tissue inflammation and suggests the presence of other players beyond IL-1β and IL-18 that are involved in inflammatory activities associated with the pyroptosis and possible by the caspase-1-associated secretome (45). A recent study with blood monocytes from patients affected by CAPS detected a high level of cellular stress including elevated levels of ROS compared with healthy subjects (157). Interestingly, associated with this oxidative stress, there is a reduction in the production of the anti-inflammatory cytokine, IL-1 receptor antagonist (IL-1Ra) (158), as well as a decrease in the threshold of inflammasome activation of CAPS monocytes (159). The exposure of this monocyte to inflammatory stimuli such as LPS induces an increase in the release of ATP that produces an increase in the secretion of IL-1β, IL-18, and IL-1α (159). These data collectively suggest the involvement of genetic and environmental factors beyond a single mutation that needs to be explored to obtain a more accurate clinical picture of this disease.

THE PYRIN INFLAMMASOME AND IMPLICATIONS IN FMF AND PYRIN-ASSOCIATED AUTOINFLAMMATION WITH NEUTROPHILIC DERMATOSIS (PAAND)

The inflammasome sensor protein pyrin is primarily expressed in myeloid cells, and wild-type pyrin negatively modulates NLRP3 inflammasome-dependent IL-1β release (160). However, mutations in the *MEFV* gene (that codify for pyrin) are associated with two clinically different autoinflammatory syndromes: FMF and PAAND (51); in both diseases, mutated pyrin associates with high serum IL-1β levels during febrile episodes.

Familial Mediterranean fever is the most common inherited monogenic autoinflammatory disease worldwide and is caused by loss-of-function mutations in *MEFV* gene, mostly affecting eastern Mediterranean population (161). Classically considered an autosomal recessive condition, it is actually discussed if it should be considered an autosomal-dominant disease with variable penetrance, since heterozygosis mutations are associated with clinical autoinflammatory FMF manifestations (162). Nevertheless, homozygosis is associated with severe FMF phenotypes.

Familial Mediterranean fever patients typically show recurrent self-limited acute febrile attacks of 1–3 days of duration, accompanied by inflammation of serosa and/or synovial linings (90% abdominal pain, 40% arthritis, and 30% thoracic pain), myalgia (40%), and erysipeloid type rash (20%). Onset before the age of 18 is common and has been associated with higher rates of arthritis, arthralgia, myalgia, and erysipeloid-like rash (163). Pleuritis, pericarditis, scrotal pain, aseptic meningitis, thrombosis, and vasculitis may appear during flares, but FMF can also be associated with many other disorders in a non-canonical manner (164). The most severe complication of FMF is amyloidosis as a result of chronically uncontrolled inflammation that occurs in undiagnosed or untreated patients; it is more likely to occur in patients with recurrent arthritis (165). Renal amyloidosis seldom occurs as the first clinical manifestation of FMF, and these individuals are referred as phenotype II patients (166). Homozygosis in serum amyloid A gene 1 (alpha/alpha) and male sex have shown influence on the risk of developing amyloidosis in FMF patients (167, 168).

Over 300 *MEFV* gene variants have been described (39), but only 14 occur frequently in FMF (E148Q, E167D, T267I, P369S, F479L, I591T, M680I, I692del, M694I, M694V, K695R, V726A, A744S, and R761H). The majority of pathogenic mutations are located in exon 10, being M694V the most frequent *MEFV* mutation encountered in FMF patients; its presence in homozygosis or compound heterozygosis is related to severe phenotype. In exon 2, E148Q is the most frequent *MEFV* variant in asymptomatic carriers and in some population subsets, it may even be a benign polymorphism (47); it is present in FMF patients with a mild phenotype (48). Diagnosis of FMF is sometimes elusive and is made under clinical basis. Validated diagnostic criteria include typical clinical manifestations, family history, and response to colchicine therapy (169). Genetic testing leads to higher rates of diagnosis (170, 171), supporting but not excluding clinical diagnosis (172). Inconsistency among similar phenotypes may be explained by major histocompatibility class I chain-related gene A alleles, as shown in a study on homozygous M694V population (173).

Pyrin-associated autoinflammation with neutrophilic dermatosis is an inherited autosomal-dominant autoinflammatory disease characterized by childhood onset. This autoinflammatory syndrome is characterized by recurrent episodes of neutrophilic dermatosis, fever, elevated acute-phase reactants, arthralgia, and myalgia or myositis (51). PAAND is caused by a loss in guard mechanism of pyrin due to S242R mutation that leads to a non-phosphorylated pyrin in S242 (51). Dephosphorylated pyrin loses interaction with the protein 14-3-3 and thus forming a constitutive active inflammasome by recruiting ASC (51). Mutations and clinics of PAAND are distinct from FMF because of a clearly dominant inheritance pattern and for its longer fever episodes (lasting weeks), more prominent cutaneous features, and absence of serositis or amyloidosis (51). Currently, it is not fully understood how mutations in two regions of the same protein can induce different diseases. FMF-related mutations have recently been found to induce a pyrin-inflammasome that could be dephosphorylated by RhoA GTPase and not inhibited by colchicine, questioning the critical dependency on microtubules for ASC aggregation and inflammasome activation (133). PAAND-associated mutations in MEFV gene are associated with a reduction in the binding of pyrin to microtubules, decreasing the threshold to assemble pyrin inflammasome. It is not known if PAAND syndrome-associated pyrin inflammasome is dependent on microtubules, although the use of colchicine has shown partial clinical benefit in this patient (51). Cutaneous manifestations of PAAND resemble other autosomal-dominant monogenic autoinflammatory disease called pyogenic arthritis, pyoderma gangrenosum, and acne (PAPA) syndrome, in which arthritis is the distinct and prominent feature. PAPA is caused by mutations at proline–serine–threonine phosphatase-interacting protein 1 gene (174). This protein is a cytoskeleton-associated adaptor protein that interestingly binds pyrin and regulates IL-1β production (175). The generation of knock-in mice with FMF-associated pyrin mutation (harboring a human C-terminal B30.2/SPRY domain that is absent in mouse *Mefv* gene) has shown data supporting the activation of a pyrin-inflammasome and an increase of IL-1β in this animal model independent of NLRP3 (49). Furthermore, autoinflammation in this animal model is dependent on the ASC-caspase-1 axis and IL-1β, whereas IL-1α and caspase-8 are dispensable for the inflammation observed in this FMF model (50).

CURRENT THERAPEUTICS TARGETING THE INFLAMMASOME PATHWAY

Inflammasomes are main drivers of autoinflammatory diseases as well as important regulators of innate immunity and inflammation. Although specific drugs that directly interfere with inflammasome activation are under development, current treatments used in clinic target upstream regulation process, in the case of colchicine, or downstream IL-1 signaling (176).

Colchicine is the classical mainstay treatment for FMF (177), decreasing attack frequency, improving quality of life, and preventing amyloidosis (178, 179). Clinical response to colchicine is considered a supportive diagnostic criterion for FMF, but it shows no benefit in CAPS patients. Colchicine is known to directly recover activity of the GTPase RhoA and therefore suppresses pyrin oligomerization but is also able to interfere with neutrophil migration and adhesion by downregulating the expression and distribution of selectins on neutrophils and endothelial cells (180). Interestingly, pyrin associates with microtubules and colocalizes with actin filaments (181). Thus, colchicine treatment may also prevent cytoskeletal changes that favor pyrin inflammasome assembly. However, recent data have shown that microtubule polymerization is not a requirement for pyrin inflammasome activation in FMF patients in contrast with wild-type pyrin carriers, providing a new concept for understanding the molecular mechanisms present in the activation of pyrin inflammasome (133). Nevertheless, some FMF patients are resistant to colchicine, and in this subset of patients, IL-1 blocking agents have shown efficacy (182–184). Anakinra therapy was also effective in a patient diagnosed with PAAND (51).

As exposed above, IL-1β is one of the main products of inflammasome and caspase-1 activation and exerts its inflammatory action by binding to the IL-1RI (185), this binding is antagonized by the IL-1Ra, a protein that binds IL-1RI without agonistic activity preventing IL-1β binding and signaling (185).

Therapies blocking IL-1 are available for the treatment of CAPS and other autoinflammatory syndromes (i.e., colchicine-unresponsive FMF patients). Anakinra is the recombinant form of IL-1Ra and was the first anti-IL1 agent clinically available. Due to its short half-life, it has to be administered by subcutaneous injection daily, and side effects are common at the site of injection; also liver enzymes need to be monitored regularly. There is a strong evidence of the effectiveness of anakinra for CAPS treatment (186, 187), with improvement of clinical features like hearing loss or amyloidosis with quick relapse of symptoms after withdrawal, demonstrating the requisite of daily injections in persistent and severe phenotypes. Despite its effectiveness, sore daily injections of anakinra are sometimes unpopular among patients, and in selected cases with mild phenotypes are possible

to use it on demand basis during inflammatory attacks as in other autoinflammatory diseases (188, 189).

Other anti-IL-1 agents have been developed with a better pharmacokinetic profile and are actually approved for the treatment of CAPS. Canakinumab is a humanized monoclonal antibody against IL-1β administered intravenously or subcutaneously at a dose of 2–4 mg/kg every 8 weeks, and it is licensed for treatment of CAPS patients over 4 years of age. It has shown a very rapid and sustained response with little side effects, mainly infections, with stabilization of the majority of sequelae and potential improvement in clinical manifestations such as sensory-neural hearing loss (190). Abnormal bone formation in CAPS patients is unaffected by IL-1 blockage (191), revealing that other pathways downstream NLRP3 inflammasome play important roles in the clinical manifestations. Canakinumab up-titration may be needed and is actually encouraged in partial responders and severe phenotypes, rising the dose and shortening administration up to 8 mg/kg every 4 weeks (192).

Rilonacept is an engineered IL-1 trap that neutralizes circulating IL-1β and IL-1α, and it is administered subcutaneously with a load dose of 320 mg followed by 160 mg weekly (185). After initial pilot study and phase III studies, rilonacept was the first drug approved for treatment of CAPS, including FCAS and MWS in children of 12 years and older, due to its safety and effectiveness (193, 194). Benefits were obtained within hours of its administration with maximal effect within day 6 and 10, with mild or moderate adverse reactions.

Caspase-1 activation precedes IL-1β release after inflammasome activation; therefore, there have been advances in generating specific and clinical relevant caspase-1 inhibitors. The most developed caspase-1 inhibitor for therapeutic use is VX-765, an orally available pro-drug that is rapidly hydrolyzed by plasma and liver esterase into a potent and selective inhibitor of caspase-1 (195). In fact, VX-765 was able to reduce the release of IL-1β and IL-18 in monocytes of patients with FCAS treated with LPS (196). However, its clinical use is still under investigation.

The standard goal to treat autoinflammatory syndromes, specially CAPS patients, will be to directly target NLRP3 inflammasome using small compounds, in this respect a compound developed by Pfizer (CP-456773 or CRID3, recently renamed as MCC950) has been proved to block IL-1β release in CAPS monocytes after LPS treatment, being able to reduce clinical symptoms in an animal model of CAPS (197, 198). Furthermore, this compound has been recently found to reduce inflammation in animal models of renal, dermal, and pulmonary inflammation (199, 200). Therefore, CP-456773 represents a promising drug for the treatment of autoinflammatory syndromes.

CONCLUSION

Mutations in genes coding for inflammasome sensor proteins, such as NLRP3 or pyrin, accomplish for the development of different autoinflammatory diseases by uncontrolled activation of caspase-1 and the aberrant release of pro-inflammatory cytokines. In physiological conditions, the inflammasome pathway is activated in response to dangerous situations provoked by infections, tissue injury, or cellular stress, being the inflammasome formed by the sensor NLRP3 the most promiscuous inflammasome pathway activated in many different situations. Furthermore, non-mutated NLRP3 activation has been involved in different autoinflammatory syndromes, and, for example, patients with mutations in *PLCG2* (autoinflammation and phospholipase Cγ2-associated antibody deficiency and immune dysregulation, APLAID syndrome) present an aberrant cytosolic Ca^{+2} signaling leading to NLRP3 activation, or patients with mutations in the deubiquitinase *OTULIN* (otulipenia) result in aberrant IL-1 production by NLRP3 activation (201, 202). NLRP3 has also been implicated as a key inflammasome sensor protein in different chronic diseases; in these circumstances, different endogenous danger signals activate NLRP3 and could contribute to the inflammatory response in metabolic and degenerative diseases, such as gout, type 2 diabetes, obesity atherosclerosis, or Alzheimer's disease (6, 203, 204). Therefore, inflammasome is central in autoinflammatory diseases, and increasing our understanding on NLRP3 and pyrin activation may lead to development of more potent novel therapies for the treatment of not only autoinflammatory syndromes but also for chronic inflammatory, metabolic, and degenerative diseases.

AUTHOR CONTRIBUTIONS

CT-M: figure preparation. CT-M, PC, and PP: literature search and manuscript preparation.

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Cytokine Signatures in Mucocutaneous and Ocular Behçet's Disease

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Behçet's disease (BD) is a multi-systemic inflammatory disorder consisting of recurrent oral aphthosis, genital ulcers, and chronic relapsing bilateral uveitis; however, many other organs may be affected. Several pro-inflammatory cytokines, mainly derived from Th1 and Th17 lymphocytes, seem to be involved in different pathogenic pathways leading to development of the clinical manifestations. On this basis, the primary aim of our study was to compare a core set of pro-inflammatory cytokines between patients with BD and healthy control (HC). The secondary goal was to evaluate potential correlations between these putative circulating biomarkers, the status of disease activity, and the specific organ involvement at the time of sample collection. Fifty-four serum samples were collected from 46 BD patients (17 males, 29 females, mean age 45.5 ± 11.3 years), and 19 HC (10 males, 9 females, mean age 43 ± 8.3 years). Twenty-five serum cytokines (APRIL/TNFS13, BAFF/TNFSF13B, sCD30/TNFRSF8, sCD163, Chitinase3-like1, gp130/sIL-6Rb, IFNb, sIL-6Ra, IL-10, IL-11, IL-19, IL-20, IL-26, IL-27 (p28), IL-28A/IFN-lambda2, IL-29/IFN-lambda1, IL-32, IL-34, IL-35, LIGHT/TNFSF-14, Pentraxin-3, sTNF-R1, sTNF-R2, TSLP, and TWEAK/TNFSF-12) were simultaneously quantified using a Bio-Rad cytokine bead arrays. Serum concentration of sTNF-R1 ($p < 0.01$) and sTNF-R2 ($p < 0.01$) resulted higher in both active and inactive BD than HC, while Chitinase3-like1 ($p < 0.05$) and gp130/sIL-6Rb ($p < 0.01$) serum levels were significantly higher in inactive BD, and IL-26 ($p < 0.01$) in active BD than HC. No differences were observed between inactive and active BD group. In addition, we observed that gp130/sIL-6Rb, sIL-6Ra, IL-35, and TSLP serum levels were significantly enhanced in patients with mucocutaneous manifestations plus ocular involvement (MO-BD) compared to subgroup with only mucocutaneous involvement (M-BD). Our findings may suggest a signature of IL-6, tumor necrosis factor-α as well as of Th17 response in BD patients due to increased levels of gp130/sIL-6Rb, sTNF-R1, sTNF-R2, IL-26, respectively. This evidence could contribute to improve the knowledge regarding the role of these citokines in the induction of specific BD clinical features.

Keywords: Behçet's disease, cytokines, mucocutaneous involvement, ocular disease, signature

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INTRODUCTION

Behçet's disease (BD) is a rare multisystemic inflammatory disorder clinically characterized by the "triple symptom complex," consisting of recurrent oral aphthosis, genital ulcers, and relapsing bilateral uveitis. Besides this classical clinical pattern, also other organ engagements including gastrointestinal tract, musculoskeletal, cardiovascular, and central nervous system, are documented (1–3). The recent understandings on cellular and molecular biology seem to suggest that an abnormal activation of both innate and adaptive immunity would be able to generate an inflammatory process leading to a CD4+ T lymphocytes clonal expansion which in turn produces high concentrations of both pro-inflammatory cytokines and cytotoxic CD8+ cells (4–11). The link between innate and adaptive immunity in patients with BD has been further clarified demonstrating that T cell immune response is skewed toward Th1 and Th17 polarization with decreased activity of regulatory T cells (12–14). The Th17 cells, play a critical role in the pathogenesis of a variety of autoimmune inflammatory diseases leading to production of Th17 effector cytokines, namely IL-17, IL-22, and IL-26 (15). This latter cytokine, belonging to the IL-10 family proteins, acts on monocytes to produce other pro-inflammatory mediators, such as IL-1β, IL-6, and tumor necrosis factor (TNF)-α which enhance the generation of new Th17 cells (16). Although the contribution of IL-26 to the development of autoimmune diseases is undoubted, its role in BD pathogenesis is still unclear. Additional cytokines involved in mechanisms known to play a critical role in BD pathogenesis were recently associated to disease activity. Indeed, molecules such as Chitinase3-like1 regulating monocytes differentiation, as well as antibacterial and type 17 responses, was observed to be upregulated in BD patients compared to healthy control (HC). Interestingly, this cytokine was also seen to be associated to disease activity in BD, correlating positively with elevated IL-6 serum levels (17–19).

Tumor necrosis factor-α appears to be crucial in promoting the development of the disease. This is documented by an overproduction of soluble TNF-α receptors and TNF-α sera levels spontaneously secreted by monocytes in active BD patients (20). Yet, elevated levels of TNF-α and soluble tumor necrosis factor receptors sTNFR1 and sTNFR2 have been found in BD patients. Moreover, increased systemic and synovial levels of sTNFRs in active BD strongly suggest a central role for the TNF/TNFR pathway in the pathogenesis of skin and joint involvement (21). Even though it is well documented that TNF blockade reduces the expression of different biological mediators and their receptors, the knowledge in BD regarding the role of the receptor gp130/sIL-6Rb is still poor. This shared receptor is utilized by several related cytokines, including IL-6, IL-11, and IL-27, which in turn regulate cellular recruitment to local sites of inflammation, induce differentiation factor for Th17 cells, promote Th2 differentiation, and inhibit multiple T cell subsets (22).

Upregulation of TNF family members involved in T and B lymphocytes activation including APRIL/TNFSF13, BAFF/TNFSF13B, sCD30/TNFRSF8 were also observed in BD patients underling a potential role of these cytokines in the immune response (23, 24).

Although literature data have proven that several cytokines are involved in BD pathogenesis, to date, biologic markers correlating with the disease activity have not yet been well recognized. Moreover, in the context of the same disease, it is difficult to identify a subset of disease signed by a specific cytokine profile. Therefore, the purpose of this work was to investigate the potential role of specific circulating biomarkers of inflammation involved in adaptive and innate immune response in BD, in order to correlate their circulating levels with clinical manifestations and disease activity.

PATIENTS AND METHODS

Patients

Fifty-four serum samples were routinely collected and analyzed from 46 consecutive BD patients (17 males, 29 females, mean age 45.5 ± 11.3 years), who met the International Study Group Criteria (ISGC) (25) and the International Criteria for BD (ICBD) (26) and from 19 HC (10 males, 9 females, mean age 43 ± 8.3 years) who attending the outpatient clinic at the Rheumatology Unit of the University of Bari and who are negative for BD criteria (ISGC and ICBD). These subjects underwent detailed clinical and laboratory workup, in order to rule out any inflammatory, metabolic, and neoplastic disorders (in particular, they all showed inflammatory markers within normal values). All patients and controls were Caucasians of Italian origin. For seven patients, more than one serum sample was obtained during an active phase of disease, resulting in a total of 54 BD samples. Moreover, the samples were collected every 3 to 4 months and in case of disease relapse. **Table 1** summarizes the clinical and demographic characteristics of BD patients. The primary aim of the study was to compare a cytokine profile between BD patients and HC; the secondary aim was to evaluate potential correlations between these putative circulating biomarkers, the status of disease activity, and the specific organ involvement at the time of sample collection. According to several other studies correlating circulating biomarkers with disease activity, BD patients were included in active BD group when they had at least two of the following clinical findings: uveitis, oral aphthosis, genital aphthosis,

TABLE 1 | Demographic, laboratory, and clinical characteristics of patients affected by Behçet's disease (BD) recruited in our study.

	BD patients ($n = 46$)	HLA-B51-positive patients ($n = 24$)
Males, *n* (%)	17 (37)	8 (33)
Disease onset (mean ± SD) in years	32.16 ± 10.56	33.47 ± 11.17
Disease duration (mean ± SD) in months	144.5 ± 91.83	106.9 ± 88.49
Patients fulfilled the International Study Group Criteria in %	100	100
Patients fulfilled the International Criteria for BD in %	100	100
Clinical features (%)		
Uveitis	13/46 (28)	8/24 (33)
Oral aphthosis	29/46 (63)	15/24 (62)
Genital aphthosis	7/46 (15)	4/24 (17)
Cutaneous disease	24/46 (52)	12/24 (50)
Gastrointestinal involvement	10/46 (22)	5/24 (21)

cutaneous disease, and gastrointestinal involvement (6, 9, 27–29). More specifically, anterior and posterior uveitis were observed in 4/13 and 9/13 patients, whereas gastrointestinal involvement was endoscopically characterized by the presence of typical oval ulcers mostly localized in the terminal ileum. Written informed consent was obtained both from patients and HC. The study protocol was reviewed and approved by the Ethical Committee of the Medical University of Bari. Demographic and clinical information was obtained through structured interview, review of medical records, physical examination, and laboratory tests.

Multiplex Bead Analysis

A panel of 25 serum cytokines [APRIL/TNFS13, BAFF/TNFSF13B, sCD30/TNFRSF8, sCD163, Chitinase3-like1, gp130/sIL-6Rb, IFNb, sIL-6Ra, IL-10, IL-11, IL-19, IL-20, IL-26, IL-27 (p28), IL-28A/IFN-lambda2, IL-29/IFN-lambda1, IL-32, IL-34, IL-35, LIGHT/TNFSF-14, Pentraxin-3, sTNF-R1, sTNF-R2, TSLP, TWEAK/TNFSF-12] were simultaneously quantified using a Bio-Rad cytokine bead arrays according to the manufacturers' instructions. Data analysis was performed using the Bioplex manager software 6.0.

Statistical Analysis

Statistical analyses were performed using GraphPad Prism 5 software. Two-tailed Mann–Whitney test (for two non-parametric groups) and Student's *t*-test (for two parametric groups) were used for statistical comparisons between groups. Significance in multiple comparisons was by one-way analysis of variance with a Bonferroni correction or Kruskall–Wallis test with a Dunn's multiple comparison correction. Correlations were calculated using Spearman's correlation (two-tailed *p*-value) as well as Pearson's correlation test when required. Significance was defined as $p < 0.05$.

RESULTS

Clinical Characteristics of BD Patients

Overall, 54 serum samples were obtained from 46 BD patients, and 31 of these (57%) were collected from patients with active disease. The main demographic and clinical characteristics of the subjects involved in this study are shown in **Table 1**. Moreover, 27 serum samples were obtained from HLA-B51-positive BD patients (50%) and at the time of serum collection patients were receiving the following treatments: TNF inhibitors 21/54, DMARDs combined with corticosteroids 14/54, DMARDs alone 10/54, corticosteroids 3/54, anti-IL-1 agents 3/54, and three patients were no treated.

Elevated Cytokine Levels of Chitinase3-like1, gp130/sIL-6Rb, IL-11, IL-26, sTNF-R1, and sTNF-R2 in BD Patients in Comparison to Healthy Controls

Circulating levels of 25 cytokines were measured in serum samples obtained from BD patients ($n = 54$) and HC ($n = 19$). Cytokine levels of IL-10, IL-27 (p28), IL-28A/IFN-lambda2, IL-29/IFN-lambda1, IL-32, IL-34, and LIGHT/TNFSF-14 were found in less than 50% of samples collected; for this reason these cytokines were not included in the analysis. No differences between BD patients and HC, in serum levels of APRIL/TNFS13, BAFF/TNFSF13B, sCD30/TNFRSF8, sCD163, IFNb, sIL-6Ra, IL-19, IL-20, IL-35, Pentraxin-3, TSLP, and TWEAK/TNFSF-12 were observed. In contrast, serum levels of Chitinase3-like1 ($p = 0.009$), gp130/sIL-6Rb ($p = 0.002$), IL-11 ($p = 0.008$), IL-26 ($p < 0.001$), sTNF-R1 ($p < 0.001$), and sTNF-R2 ($p < 0.001$) were significantly higher in BD than HC (**Figure 1**). Correlation study revealed significant correlation between cytokines showed to be upregulated in serum from BD patients. Among these, strong correlation was found between gp130/sIL-6Rb and sTNF-R1 ($r = 0.706$, $p < 0.001$), sTNF-R2 ($r = 0.783$, $p < 0.001$), sCD163 ($r = 0.775$, $p < 0.001$), TWEAK/TNFSF-12 ($r = 0.775$, $p < 0.001$), and sIL-6Ra ($r = 0.705$, $p < 0.001$) serum levels. sTNF-R1 also correlated with TSLP ($r = 0.730$, $p < 0.001$) and sTNF-R2 ($r = 0.739$, $p < 0.001$). Moreover, sTNF-R2 serum levels positively correlated with TSLP ($r = 0.772$, $p < 0.001$) and sCD163 ($r = 0.724$, $p < 0.001$) (Table S1 in Supplementary Material).

Serum Cytokine Profiles and Their Correlation with Disease Activity and Clinical Features

As shown in **Figure 2**, BD patients were divided into two subgroups based on the disease activity. In active BD group were included patients characterized by at least two of the following clinical findings: uveitis, oral aphthosis, genital aphthosis, cutaneous disease, gastrointestinal involvement. Twenty-three serum samples were collected from inactive BD patients (8 males, 15 females, mean age 44.6 ± 11.01 years) and 31 from active patients (12 males, 19 females, mean age 46.1 ± 11.67 years). Serum levels of sTNF-R1 and sTNF-R2 resulted higher in both active BD ($p = 0.002$ and $p = 0.002$, respectively) and inactive BD ($p = 0.0101$ and $p = 0.002$, respectively) subgroup than HC, while Chitinase3-like1 ($p = 0.042$) and gp130/sIL-6Rb ($p = 0.008$) serum levels were significantly higher in inactive BD as well as IL-26 ($p = 0.002$) in active BD than HC (**Figure 2**). Interestingly, among significantly serum cytokines upregulated in inactive BD and active BD patients, gp130/sIL-6Rb strongly correlated with sTNF-R2 ($r = 0.716$, $p < 0.001$), TWEAK/TNFSF-12 ($r = 0.821$, $p < 0.001$), IL-26 ($r = 0.773$, $p = 0.007$) serum levels, and sTNF-R1 was to correlate with sTNF-R2 ($r = 0.877$, $p < 0.001$), IL-26 ($r = 0.773$, $p = 0.007$), and IL-20 ($r = 0.709$, $p = 0.0018$) serum levels. In contrast, active BD subgroup revealed sTNF-R1 and TSLP ($r = 0.769$, $p < 0.001$) serum levels strong positively correlation. Moreover, sTNF-R2 serum levels positively correlated with TSLP ($r = 0.830$, $p < 0.001$), gp130/sIL-6Rb ($r = 0.818$, $p < 0.001$), and sCD163 ($r = 0.725$, $p < 0.001$) (Table S2 in Supplementary Material). To determine the relationship between clinical features and the circulating levels of inflammatory markers, we measured and compared cytokine levels in serum from patients who presented at the time of blood collection mucocutaneous manifestations with (MO-BD) or without (M-BD) ocular involvement (**Figure 3**). Results showed significant enhanced levels of Chitinase3-like1 ($p = 0.039$) in M-BD but no differences were identified in MO-BD subgroup ($p = 0.1035$) compared with

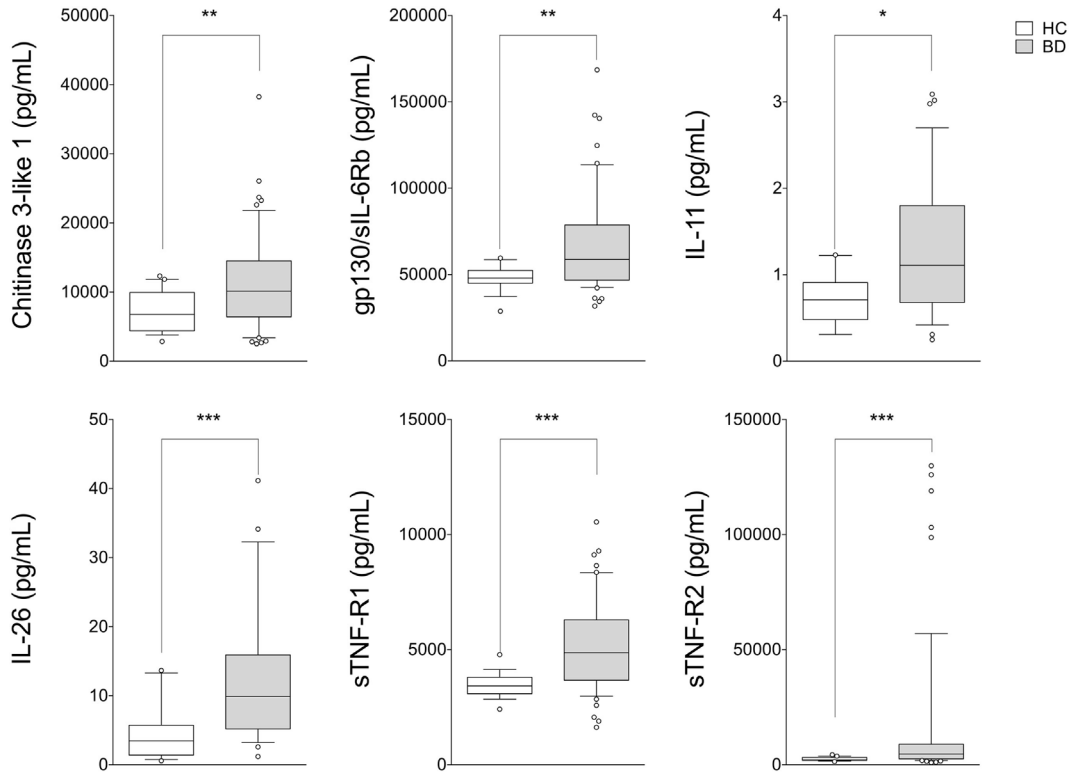

FIGURE 1 | Serum cytokine profile in patients with BD. BD patients ($n = 54$) showed upregulation of serum levels of Chitinase3-like1, gp130/sIL-6Rb, IL-11, IL-26, sTNF-R1, and sTNF-R2 compared with HC ($n = 19$). Mann–Whitney *U*-test and Student's *t*-test were carried out to check for statistical significance between groups when required (***$p < 0.001$, **$p < 0.01$, *$p < 0.05$). The central line represents the distribution median, boxes span 25th to 75th percentiles, and error bars extend from 10th to 90th percentiles. Dots (°) are outlier values, higher than the 90th percentile. Abbreviations: HC, healthy controls; BD, Behçet's disease.

HC. Serum levels of sTNF-R1 and sTNF-R2 were higher in both M-BD ($p = 0.0155$ and $p = 0.0066$, respectively) and MO-BD ($p < 0.001$ and $p < 0.001$, respectively) than HC. In addition, elevated serum levels of gp130/sIL-6Rb ($p < 0.001$), sIL-6Ra ($p = 0.005$), IL-11 ($p = 0.027$), IL-26 ($p = 0.002$), and TSLP ($p = 0.0103$) were also observed in MO-BD compared to HC. Interestingly, we observed that gp130/sIL-6Rb ($p = 0.043$), sIL-6Ra ($p = 0.029$), IL-35 ($p = 0.026$), and TSLP ($p = 0.013$) serum levels were significantly enhanced in MO-BD compared to M-BD subgroup (**Figure 3**). Correlation analysis among all evaluated cytokines was also assessed. Notably, stronger significant correlation was revealed in MO-BD compared with M-BD subgroup (Table S3 in Supplementary Material).

Linear Regression Analysis between Serum Cytokine Levels and Disease Duration

A linear regression analysis of cytokine serum levels in all patients as well as in disease activity and clinical features subgroups as a function of disease duration was assessed. No significant correlations were obtained in all patients and in active BD subgroup (Table S4 in Supplementary Material). In contrast, Chitinase3-like1 was found significant in inactive BD, M-BD, and MO-BD subgroup ($r_s = 0.277$ $p = 0.025$, $r_s = 0.188$ $p = 0.011$, $r_s = 0.274$ $p = 0.038$, respectively) (Table S4 in Supplementary Material). Moreover, significant results were found in both M-BD and MO-BD subgroups for sCD163 ($r_s = 0.124$ $p = 0.041$ and $r_s = 0.473$ $p = 0.003$, respectively) and gp130/sIL-6Rb ($r_s = 0.162$ $p = 0.018$ and $r_s = 0.645$ $p < 0.001$, respectively). In addition, sIL-6Ra revealed significant correlation in M-BD ($r_s = 0.157$ $p = 0.02$) while sTNF-R1 ($r_s = 0.628$ $p < 0.001$), TWEAK/TNFSF-12 ($r_s = 0.723$ $p < 0.001$), and sCD30/TNFRSF8 ($r_s = 0.39$ $p = 0.010$) were significantly correlated to disease duration in MO-BD subgroup (Table S4 in Supplementary Material; **Figure 4**).

DISCUSSION

Our study was aimed at investigating a core set of cytokines in a cohort of patients mainly affected by the most common clinical manifestations of BD, including mucosal, skin, and ocular involvement, in order to assess any potential correlation between these circulating biomarkers, disease activity, and the specific clinical features of disease. First of all, we found elevated levels of several inflammatory markers in BD patients including Chitinase3-like1, gp130/sIL-6Rb, IL-11, IL-26, sTNF-R1, and sTNF-R2 compared with HC. Comparing HC with disease activity, we observed enhanced levels of sTNF-R1 and sTNF-R2 in both active BD and inactive BD subgroups, while Chitinase3-like1 and gp130/

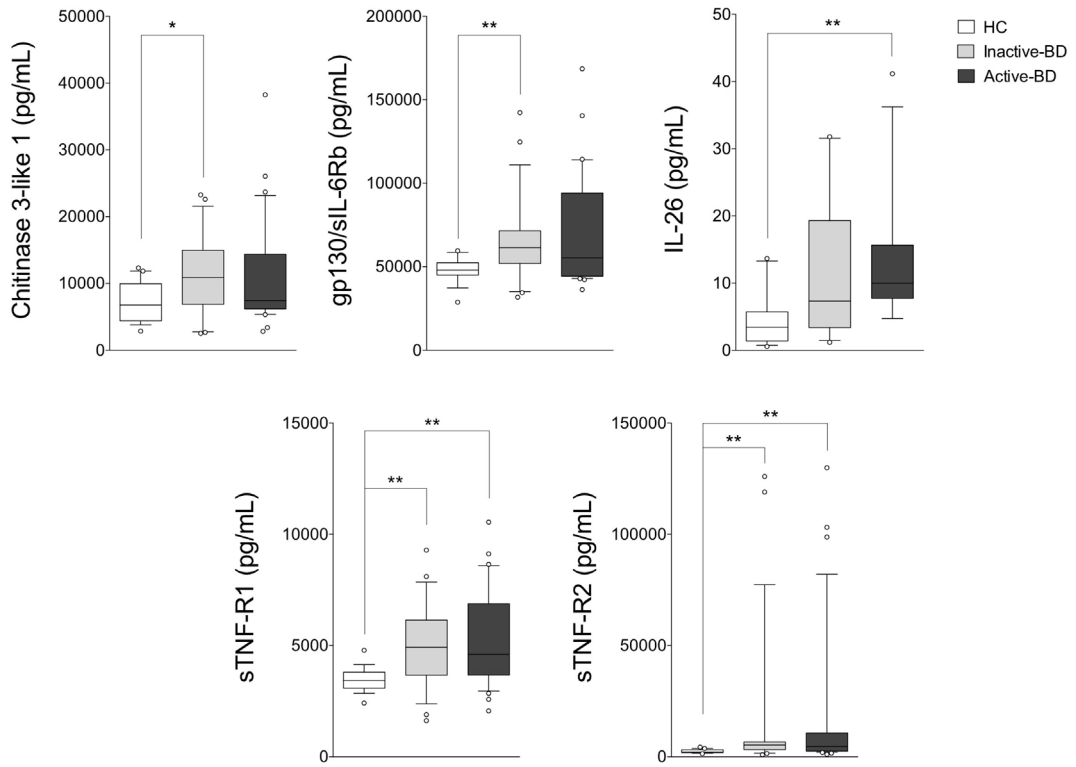

FIGURE 2 | Serum cytokine profile analysis in BD patients with different disease activity. Serum cytokine levels in inactive BD ($n = 23$), active BD ($n = 31$) patients, and HC ($n = 19$). Analysis of variance was used for data comparison (***$p < 0.001$, **$p < 0.01$, *$p < 0.05$). The central line represents the distribution median, boxes span 25th to 75th percentiles, and error bars extend from 10th to 90th percentiles. Dots (°) are outlier values, higher than the 90th percentile. Abbreviations: BD, Behçet's disease; HC, healthy controls.

sIL-6Rb serum levels were significantly higher in inactive BD as well as IL-26 in active BD than HC. Furthermore, M-BD patients showed enhanced levels of Chitinase3-like1, sTNF-R1, and sTNF-R2 compared with HC as well as increased levels of gp130/sIL-6Rb, sIL-6Ra, IL-11, IL-26, IL-35, sTNF-R1, sTNF-R2, and TSLP were found in MO-BD compared with HC. Interestingly, enhanced levels of gp130/sIL-6Rb, sIL-6Ra, IL-35, and sTNF-R1 between MO- and M-BD were also found.

Chitinase3-like1, an inflammatory biomarker of endothelial dysfunction without chitinase activity, is secreted from activated neutrophils and macrophages in several pathological conditions characterized by tissue injury and inflammation (30). In this regard, it has been demonstrated that Chitinase3-like1 correlates with the severity of skin lesions in patients with psoriatic arthritis but not in those with psoriasis vulgaris alone (31), being, however, its serum levels increased in psoriasis vulgaris and in generalized pustular psoriasis (32). Chitinase3-like1 has recently received considerable attention as marker for inflammation in BD patients (33, 34). Indeed a Korean study on 112 patients with BD whose main clinical features were recurrent oral ulcers and skin lesions, has shown increased serum levels of Chitinase3-like1 and a positive correlation with disease activity (17). These results are in agreement with Bilen et al. that showed higher serum levels of Chitinase3-like1 in BD patients compared with HC, albeit they did not correlate with disease activity (35). Similarly, we found enhanced levels of Chitinase3-like1 in M-BD group, even if its values were higher in the inactive BD group. Besides Chitinase3-like1, for the first time we observed the upregulation of IL-11 in our BD patients. This pleiotropic gp130-signaling cytokine, is able to induce anti-inflammatory and mucosal protective effects in a variety of animal models of acute and chronic inflammation, such as mucositis and inflammatory bowel diseases. In particular, IL-11 may exert anti-inflammatory effects by reducing cytokines production by macrophages (36). *In vitro* studies suggest that recombinant human IL-11 inhibits TNF-α, IL-1β, IL-12, IL-6, and nitric oxide production from activated macrophages reducing inflammation and tissue damage and promoting mucosal repair (37). Data from our study suggest that IL-11 does not correlate with disease activity and there are no significant differences between the active and inactive BD groups. Interestingly, we also found a higher level of IL-11 in the MO-BD group rather than in M-BD alone, even though it has been suggested that this cytokine is connected to repair processes of mucosal tissue damage (37).

Regarding gp130/sIL-6Rb, inactive BD showed higher values of this cytokine than HC. Gp130 also known as beta-subunit of the IL-6 receptor (sIL-6Rb) or CD130 is a ubiquitously expressed signal-transducing receptor that forms part of the receptor complex for several cytokines, including IL-6, IL-11, and IL-27

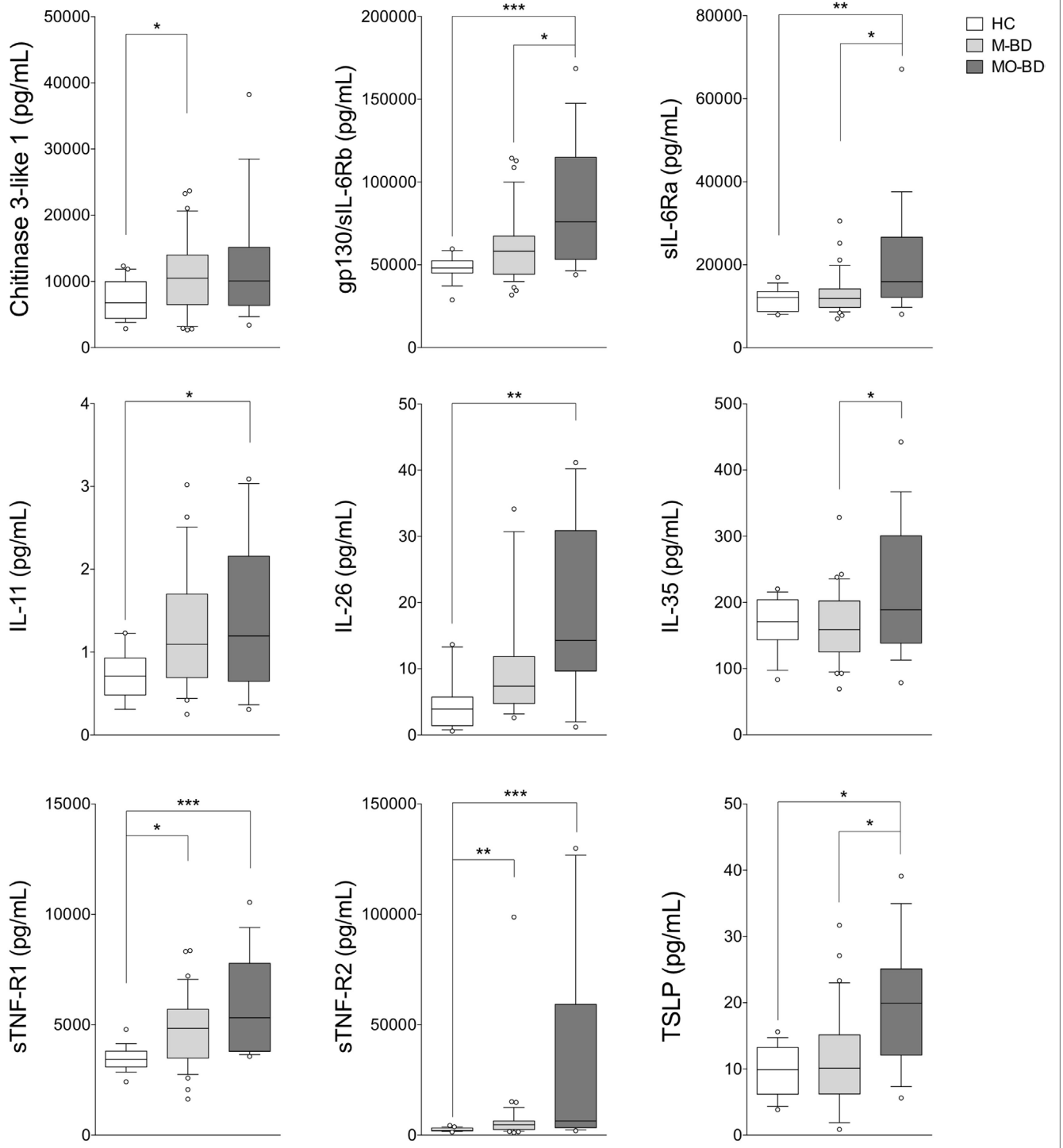

FIGURE 3 | Serum cytokine profile in mucocutaneous patients with (MO-BD) or without (M-BD) ocular involvement. Serum cytokine levels in M-BD ($n = 35$), MO-BD ($n = 17$) patients, and HC ($n = 19$). Analysis of variance was used for data comparison (***$p < 0.001$, **$p < 0.01$, *$p < 0.05$). The central line represents the distribution median, boxes span 25th to 75th percentiles, and error bars extend from 10th to 90th percentiles. Dots (°) are outlier values, higher than the 90th percentile. Abbreviations: HC, healthy controls; BD, Behçet's disease; M-BD, mucocutaneous patients without ocular involvement; MO-BD, mucocutaneous patients with ocular involvement.

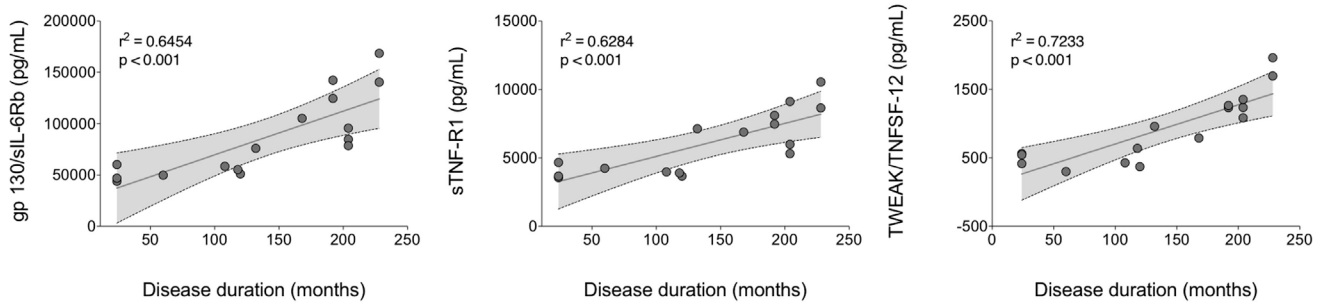

FIGURE 4 | **Linear regression analysis of serum cytokine levels versus disease duration**. A positive strong correlation was found between gp130/sIL-6Rb, sTNF-R1, and TWEAK/TNFSF-12 circulating levels and disease duration in MO-BD (mucocutaneous patients with ocular involvement) subgroup.

(38). Classically, IL-6 activates gp130 by binding a non-signaling cognate IL-6 receptor, which then leads to the initiation of JAK/STAT signaling, a pathway that is often constitutively switched on in several inflammatory processes (39). However, IL-6 responses can also be elicited through IL-6 trans-signaling mediated *via* a naturally occurring soluble IL-6R (40). Several biological processes, including the switch from neutrophil to mononuclear cell recruitment during inflammation, the leukocyte trafficking, activation, and apoptosis (41, 42), are due to IL-6 trans-signaling which is inhibited by a soluble form of gp130, in turn able to effectively bind the IL-6/sIL-6R complex and to prevent activation of membrane-bound gp130, modulating the severity of inflammatory responses (43, 44). The ability of soluble gp130 to downregulate the severity of inflammation and joint destruction in murine antigen induced arthritis has been demonstrated by a significant reduction in inflammatory infiltrate within the affected joints (45). Convincing proofs regarding the inflammatory role of the IL-6/sIL-6R complex derive also from the study of Curnow et al. aimed at proving an insufficient lymphocytes apoptosis in uveitis able to induce an inflammatory process through the trans-signaling pathway (46). In this regard, in our study, we found enhanced levels of gp130/sIL-6Rb, especially in MO-BD group than M-BD, although no correlation with disease activity was observed. Finally, a strong correlation between gp130/sIL-6Rb circulating levels and disease duration in MO-BD subgroup was also observed.

To the best of our knowledge, no studies have focused on the role of IL-26 in BD. In our study, serum concentration of IL-26 was significantly higher in BD, especially in active BD, than in HC. IL-26, a member of the IL-10 cytokine family, capable of inducing the production of several pro-inflammatory cytokines, such as IL-1β, IL-8 and TNF-α (16), is released in large amount in response to classic pro-inflammatory stimuli and enhances chemotaxis of neutrophils (47). Interestingly, this cytokine may impair the responsiveness to itself in certain structural cells such as colon epithelial cell line suggesting its pathogenic role in inflammatory bowel diseases. Indeed, increased infiltration of IL-26-positive Th17 cells was found in the colon of Crohn's disease patients (48) and elevated expression of IL-26 mRNA was observed in the colon of pediatric-onset ulcerative colitis (49) as well as in tonsils and Payer's patches in response to microbial stimuli, thus suggesting a pivotal role in mucosal immunity for this cytokine (50). Moreover, in some dermatological diseases, such as psoriasis, IL-26 has been found more highly expressed in lesions than in normal skin, proving an important function in regulating the innate immunity of epithelial cells (51). Despite this cytokine would seem more related to a mucocutaneous disease subset, in our experiment, IL-26 serum levels were found higher in MO-BD group than M-BD alone, consequently its increased values are not discriminating for the mucocutaneous involvement.

A reasonable explanation of this discrepancy may lie in the fact that IL-26 expression should be directly sought in the skin lesions rather than in the serum from BD patients since the major source of IL-26 is provided by infiltration of Th17 lymphocytes in inflamed tissue (52).

Tumor necrosis factor-α is the main cytokine involved in acute inflammatory responses and stimulates the release of other pro-inflammatory mediators. Endogenous mechanisms mediated through two distinct TNF receptor types 1 and 2 which are shed from cell surface as soluble forms (sTNF-R1 and sTNF-R2) may limit the systemic inflammation (53). As previously reported, increased serum concentrations of the soluble forms of membrane receptor for TNF-α, sTNF-R1, and sTNF-R2, have been found in active BD (54) and demonstrated in several rheumatic diseases (55–58), however, controversial are data regarding their putative role as markers of disease activity. In particular, Turan et al. reported that increased sTNF-R2 may serve as a marker of disease activity in BD, especially in those patients with arthritis, furthermore an increased TNF-R2 expression was found in mucosal and cutaneous ulcers where mast cells were identified as the major source for this receptor (21). On the contray, in line with our study, Düzgün et al. found significantly increased serum levels of sTNF-R1 in BD patients compared with HC, even though they did not reflect active disease (20). Interestingly, higher levels of sTNF-R were observed in our cohort of BD patients than in HC; however, no differences were observed between the patients with mucocutaneous plus ocular involvement and those with the sole mucocutaneous symptoms. More recently, Ke et al. have proven that sTNF-R1 released by skin-derived mesenchymal stem cells is critical for inhibiting the differentiation of Th17 cells, which are the major contributor of experimental murine models of autoimmune disease leading to IL-17A, IL-17F, IL-21,

and IL-22 production (59). Similarly to what was observed for serum levels of gp130/sIL-6Rb, important results were obtained analyzing the values of sTNF-R1 and sTNF-R2 versus disease duration: in particular a positive strong correlation was found between sTNF-R1 circulating levels and disease duration in MO-BD subgroup, thus suggesting as previously reported that BD appears to have a less aggressive clinical course over time related to disease duration (60).

The main limitations of our study are represented by the sample size that did not allow us to ascertain the actual correlation of specific cytokines with the different subsets of disease as well as the absence of a disease control group. Moreover, at the time of samples collection, all of the patients were already taking immunosuppressive agents, which might have affected cytokine serum levels. Finally, we believe that not evaluating disease activity with outcome measures, due to the incompleteness of data, could represent another limitation of our study.

In conclusion, our findings were in agreement with several studies that showed that the immune response in BD is skewed toward a Th1 as well as Th17 pathway (61). Moreover, we observed the upregulation of chitinase3-like1 and IL-11 in BD. These molecules are produced by innate immune cells and lead to monocytes dendritic cell maturation and inhibition of TNF-α and IL-6 signaling (62, 63). Finally, this IL-6, TNF and Th17 signature could discriminate mucocutaneous patients with ocular involvement from mucocutaneous patients without ocular involvement, even if further studies in a larger cohort of patients as well as a comparison with disease group are necessary. Our preliminary data could contribute to improve the knowledge regarding the role of specific target for novel therapies or for a different and more suitable use of biologic drug currently available, suggesting a possible role of these cytokines in the induction of specific BD clinical manifestations.

AUTHOR CONTRIBUTIONS

GL (Giuseppe Lopalco), OL, and AL wrote the manuscript; LC, CF, and FI designed the study and finally revised the manuscript; OL, GL (Giovanni Lapadula), and CF performed the data analysis; VV, BF, MG, GL (Giuseppe Lopalco), LC took care of patients enrollment, follow-up of the patients, and data collection.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at http://journal.frontiersin.org/article/10.3389/fimmu.2017.00200/full#supplementary-material.

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Genetic and Epigenetic Determinants in Autoinflammatory Diseases

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The concept of autoinflammation has evolved over the past 20 years, beginning with the discovery that mutations in the Mediterranean Fever (*MEFV*) gene were causative of Familial Mediterranean Fever. Currently, autoinflammatory diseases comprise a wide range of disorders with the common features of recurrent fever attacks, prevalence of hyperreactive innate immune cells, and signs of inflammation that can be systemic or organ specific in the absence of pathogenic infection of autoimmunity. Innate immune cells from the myeloid compartment are the main effectors of uncontrolled inflammation that is caused in great extent by the overproduction of inflammatory cytokines such as IL-1β and IL-18. Defects in several signaling pathways that control innate immune defense, particularly the hyperreactivity of one or more inflammasomes, are at the core of pathologic autoinflammatory phenotypes. Although many of the autoinflammatory syndromes are known to be monogenic, some of them are genetically complex and are impacted by environmental factors. Recently, epigenetic dysregulation has surfaced as an additional contributor to pathogenesis. In the present review, we discuss data that are currently available to describe the contribution of epigenetic mechanisms in autoinflammatory diseases.

Keywords: autoinflammatory diseases, epigenetics, DNA methylation, non-genetic factors, cryopyrin-associated periodic syndromes, Familial Mediterranean Fever

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INTRODUCTION

Autoinflammatory diseases are a growing group of debilitating and chronic conditions characterized by overt inflammation that is often systemic and manifests as recurrent fever episodes. Hyperreactive innate immune cells contribute largely to the pathogenesis of these diseases, and patients who display this pathology correlates with increased levels of acute-phase proteins and inflammatory cytokines in the plasma. Originally, the term autoinflammation was coined to describe the occurrence of apparently unprovoked episodes of inflammation in the absence of self-reactive T cells and/or high titers of autoantibodies, as well as in the absence of any detectable infectious agent (1). Many of the autoinflammatory syndromes display systemic and/or organ-specific inflammatory features such as recurrent and episodic periodic fever, serositis, arthritis and/or cutaneous inflammation, overproduction of IL-1β, and activation of innate immune cells, particularly monocytes (2). Albeit initially the term autoinflammatory diseases only applied to those prototypical hereditary monogenic periodic fever syndromes, such as cryopyrin-associated periodic syndromes (CAPS) and Familial Mediterranean Fever (FMF), the list has now expanded as a consequence of the application of emerging technologies, such as next-generation sequencing, and comprises an increasing number of newly described monogenic disorders caused by mutations of inflammation-related genes. There

is also increasing evidence that epigenetic dysregulation participates in the pathogenesis of these diseases [**Table 1**; reviewed by Stoffels and Kastner (3)]. In addition, autoinflammatory diseases also include a few multifactorial and complex diseases, such as Behçet's disease and Crohn's disease (CD), which not only involve the participation of multiple alleles but also a number of environmental factors (2, 4). Also, it is now accepted that there is a continuum of disorders in the inflammatory spectrum that ranges from autoimmune diseases at one end to autoinflammatory at the other, with several mixed complex conditions that display both features of innate and adaptive immune dysregulation (5, 6). This growing spectrum of conditions indicates the existence of a highly complex etiology and pathophysiology of inflammatory diseases, even in the case of monogenic diseases, where additional agents, besides the causative gain-of-function mutations, may have a relevant impact on the clinical course of the disease. In autoinflammatory diseases, a failure in the regulation of the defense mechanisms of innate immune cells, which responds to pathogen-expressed molecules or molecules signaling cellular stress, and the orchestration of a response to such insults with the production of proinflammatory cytokines such as IL-1β or IL-18 (2) are central to pathology. Genetic inheritance in autoinflammatory disorders varies depending on the specific disease and has been a subject of controversy. FMF is mostly transmitted in an autosomal recessive manner, which requires mutations in both alleles of the Mediterranean Fever (*MEFV*) locus, encoding the sensor protein pyrin that is expressed in neutrophils, eosinophils, and cytokine-activated monocytes (7). Interestingly, there have been reports of several cases of FMF patients that are heterozygous for the *MEFV* allele, with only one allele displaying a mutation or, in even rarer cases, no detectable mutation, and yet still associate with the development of disease (8–10). Several groups studying the FMF phenotype in *MEFV* mutation-negative patients found the phenotype to be milder, with a late disease onset and a lower rate of familiar history of FMF. However, the unequivocal existence of such mutation-free patients suggests the existence of additional causes for disease development including mutation in alternative genes, and perhaps the occurrence of epigenetic dysregulation. Identification of those alterations is essential for patient diagnosis and management.

In the case of CAPS, inherited dominant autosomal gain-of-function mutations of NOD-like receptor, *NLRP3* gene encoding cryopyrin, are responsible for the overactivation of the inflammasome (11–13). In fact, CAPS is a spectrum disorder that includes, in increasing order of severity, the familial cold autoinflammatory syndrome (FCAS), Muckle–Wells syndrome, and neonatal-onset multisystem inflammatory disease (NOMID), caused by sporadic *de novo* mutations in the same gene (otherwise termed chronic infantile neurologic cutaneous and articular syndrome/CINCA) (14). Several lines of evidence, including the existence of mutations with different degrees of penetrance leading to a gradient of disease severity and heterogeneous phenotypes in terms of disease progression that arises from identical germline mutations, suggest that additional factors contribute to pathophysiology of hyperinflammation. Moreover, a great number of cases (as much as 40% in the case of NOMID/CINCA for conventional sequencing) are considered "genetic orphans," i.e., patients without any identified associated mutations, which further supports this notion. In some of these cases, the existence of mosaicism restricted to the myeloid compartment has been reported; however, there is the possibility that, in some cases, non-genetic mechanisms could lead to autoinflammation. For example, it is plausible that, in addition to the occurrence of specific mutations, certain amplification loops establish vicious circles that increase IL-1β production and inflammation. The complexity of genome regulation in autoinflammatory diseases is reflected in CAPS, where it is extensively agreed that the lack of genetic confirmation for some patients does not exclude their diagnosis (15). In the cases of complex autoinflammatory disorders where heritability models are not well established, it is entirely possible that, although there may be a genetic component that contributes to certain parts of disease, both genetic and environmental/epigenetic factors may define pathogenicity, and this applies to disorders like Behçet's disease, inflammatory bowel disease (IBD), and chronic recurrent multifocal osteomyelitis (CRMO) among others. It has been long recognized that environmental factors contribute to the establishment of pathological immune responses as well as the development and severity of inflammatory immune disorders, and twin studies have been valuable to determine the extent of genetic and non-genetic contributions, such as in the case of IBD (16). Since epigenetic mechanisms establish a diversity of links between the environment and the regulation of the genome, understanding epigenetic control within the innate immune compartment is crucial to fully grasp the etiology of autoinflammatory disorders.

TABLE 1 | Autoinflammatory disorders and evidence of epigenetic contribution to pathogeny.

Mutated gene	Disease	Effector cytokine	Data on epigenetic regulation
Hereditary monogenic periodic fever syndromes			
MEFV	Familial Mediterranean Fever	IL-1β	Yes (38)
TNFRSF1A	TRAPS	IL-1β	No
MVK	Hyper IgD syndrome	IL-1β	No
NLRP3	Cryopyrin-associated periodic syndromes [familial cold autoinflammatory syndrome (FCAS), Muckle–Wells syndrome, neonatal-onset multisystem inflammatory disease/CINCA]	IL-1β	Yes (40)
NLRC4	NLRC4-MAS	IL-1β/IL-18	No
PSTPIP1	PAPA	IL-1β	No
NLRP12	FCAS2	IL-1β	No
Antagonist deficiencies			
IL1RN	DIRA	IL-1β	No
IL36RN	DITRA	IL-36	No
Complex autoinflammatory disorder			
	Behçet	IL-6/IL-1β	Yes (41, 48, 49)
	CRO/chronic recurrent multifocal osteomyelitis	IL-10/IL-1β	Yes (42, 43)
	Crohn	IL-19/IL-3/IL-27	Yes (50, 51)

CONTROL OF INNATE IMMUNE CELL FUNCTION

The acquisition of full host protection requires proper orchestration and balance between resistance and tolerance, with the former being necessary to maximally reduce pathogen burden and the latter to minimize self-tissue damage by inflammation (17). Innate immune cells, including monocytes, macrophages, and neutrophils, are in the first line of defense and hence are equipped with very specialized molecular machinery aimed at sensing and destroying invading pathogens and restoring homeostasis. Pattern-recognition receptors that recognize pathogen-associated molecular patterns, and non-microbial stress signals, known as danger-associated molecular patterns, constitute the sensors that trigger upon recognition of their substrates during an inflammatory response. Many of these receptors, including the toll-like receptors and C-type lectin receptors, are located on the cell membrane in contact with the extracellular milieu, whereas others are cytoplasmic, such as the inflammasome-participating NOD (nucleotide-binding oligomerization domain)-like receptors and AIM2 (absent in melanoma) family of receptors (18). Inflammasomes are a key component of such defensive machinery that consist of multimeric cytoplasmic platforms that ensemble upon recognition of an insult and respond by activating pro-caspase-1, leading to proteolytic processing and release of IL-1β and IL-18, and pyroptosis (**Figure 1**) (19, 20).

Innate immune cells, in particular monocytes and macrophages, rely on epigenetically controlled functional reprogramming in order to coordinate a proper response once stimuli are detected. During the differentiation of monocytes to macrophages, whole transcriptome and epigenome studies have shown that substantial changes affect ~19 Mbp, which is equivalent to 0.6% of the human genome. Epigenetic changes affect the activity of promoters (H3K4me3/H3K27ac) and distal regulatory elements that are presumed enhancers (H3K4me1/H3K27ac) to a similar extent (21).

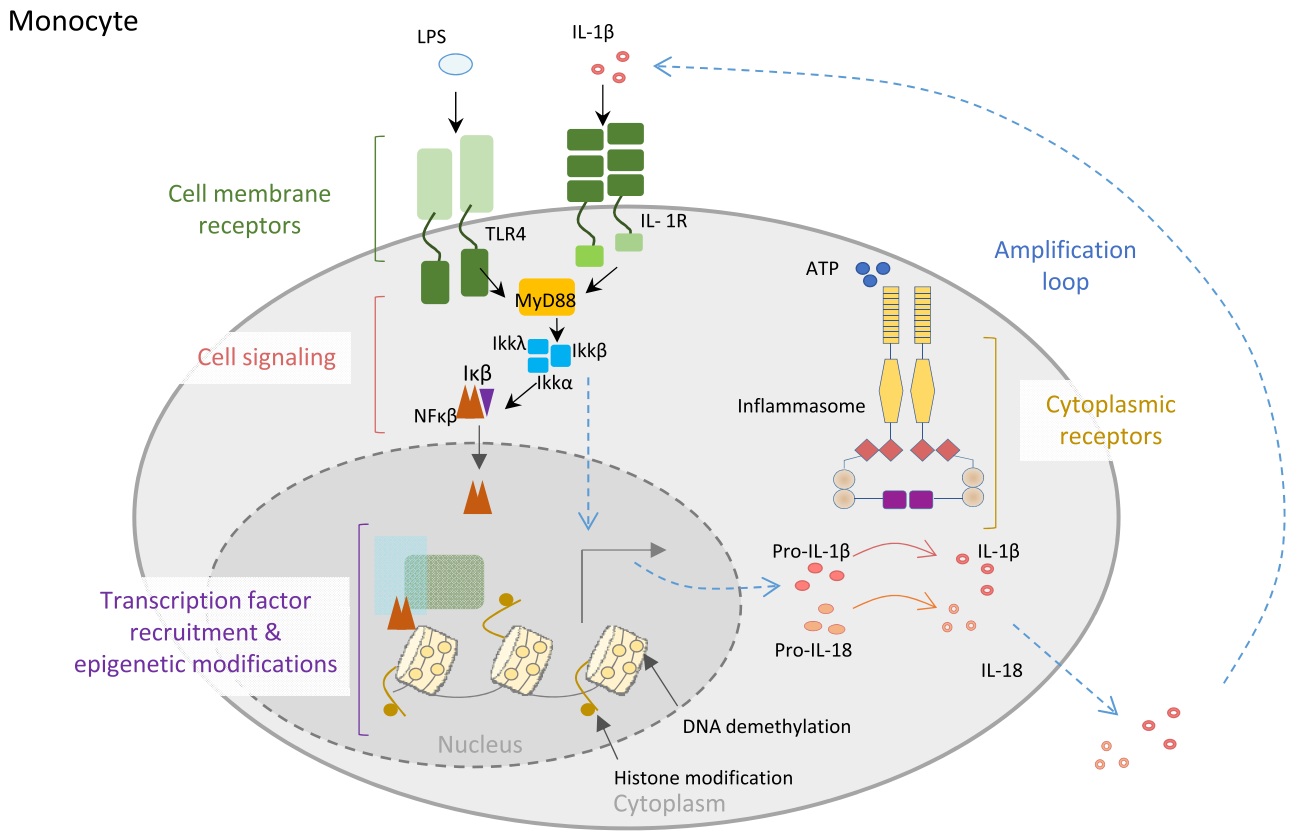

FIGURE 1 | Control of innate immune cell function. Monocyte cell membrane receptors as TLR4 and IL-1R allow communication between the cell and the environment. Engagement of the receptors by their ligands (LPS or IL-1β) triggers cell signaling cascades, allowing transcription factors, particularly NF-κB translocation into the nucleus, where it recognizes specific regions of the DNA and recruits other transcription factors, as well as epigenetic enzymes, like TET2 (protein involved in DNA demethylation in myeloid cells). Both the binding of transcription factors to the DNA and the epigenetic modifications of the DNA will increase the expression of inflammatory genes, like the inflammasome complex components. Posttranscriptional modifications of inflammasome proteins play a crucial role in the formation of the inflammasome complex, leading to the activation of caspase-1, which then is able to process the proinflammatory cytokines IL1-β and IL-18 into mature bioactive IL-1β and IL-18 cytokines that are secreted to the external media, creating an inflammatory microenvironment. Importantly, IL-1β is able to amplify its own signal through the binding to IL-1R.

The acquisition of a trained or tolerant state in macrophages upon encounter with an external stimulus of microbial origin is associated with changes in around 0.12% of the entire monocyte/macrophage epigenome. There is also around 12% difference in the expression of transcription factors, which dictates the specific antimicrobial response, which also results in an immunological memory coded in the chromatin that will have an impact on how the cell reacts to future challenges. Furthermore, transcriptomic analysis of the acquisition of functional memory by macrophages reveals that it relies on ~200 transcription factors, ~100 kinases, and ~20 epigenetic enzymes that are differentially expressed in differentiated macrophages compared to their monocytic precursors [reviewed in Ref. (22)].

DYSREGULATION IN INNATE IMMUNE CELLS

Inflammatory responses aiming at destroying invading pathogens consist of very potent effector mechanisms that, if not properly regulated, could potentially be harmful to the host, as illustrated by the appearance of autoinflammatory disorders. In order to provide specificity to the innate immune response, different inflammasomes are defined by the sensor protein that triggers the assembly, such as the NLRP1 that recognizes muramyl dipeptide and anthrax lethal toxin (mouse NLRP1b) (23), NLRP3 that is triggered by several stress-induced molecules including monosodic urate crystals or ATP, NLRC4 that recognizes cytosolic flagellin inflammasomes (24), and the AIM2 inflammasome that assembles in response to cytoplasmic DNA (**Figure 2**) (25–28). All these cytoplasmic innate immune receptors signal through the adaptor ASC (apoptosis-associated speck-like protein containing a caspase recruitment domain) that recruits caspase-1, leading to the activation of IL-1β and the processing of IL-18 (20). The group of autoinflammatory disorders caused by dysregulation of the inflammasomes is referred to as "inflammasomopathies" (29). Gain-of-function mutations of *NLRP3* leading to aberrant activation of such inflammasomes are the cause of the CAPS spectrum disorders (30). For example, ATP, which is a very well

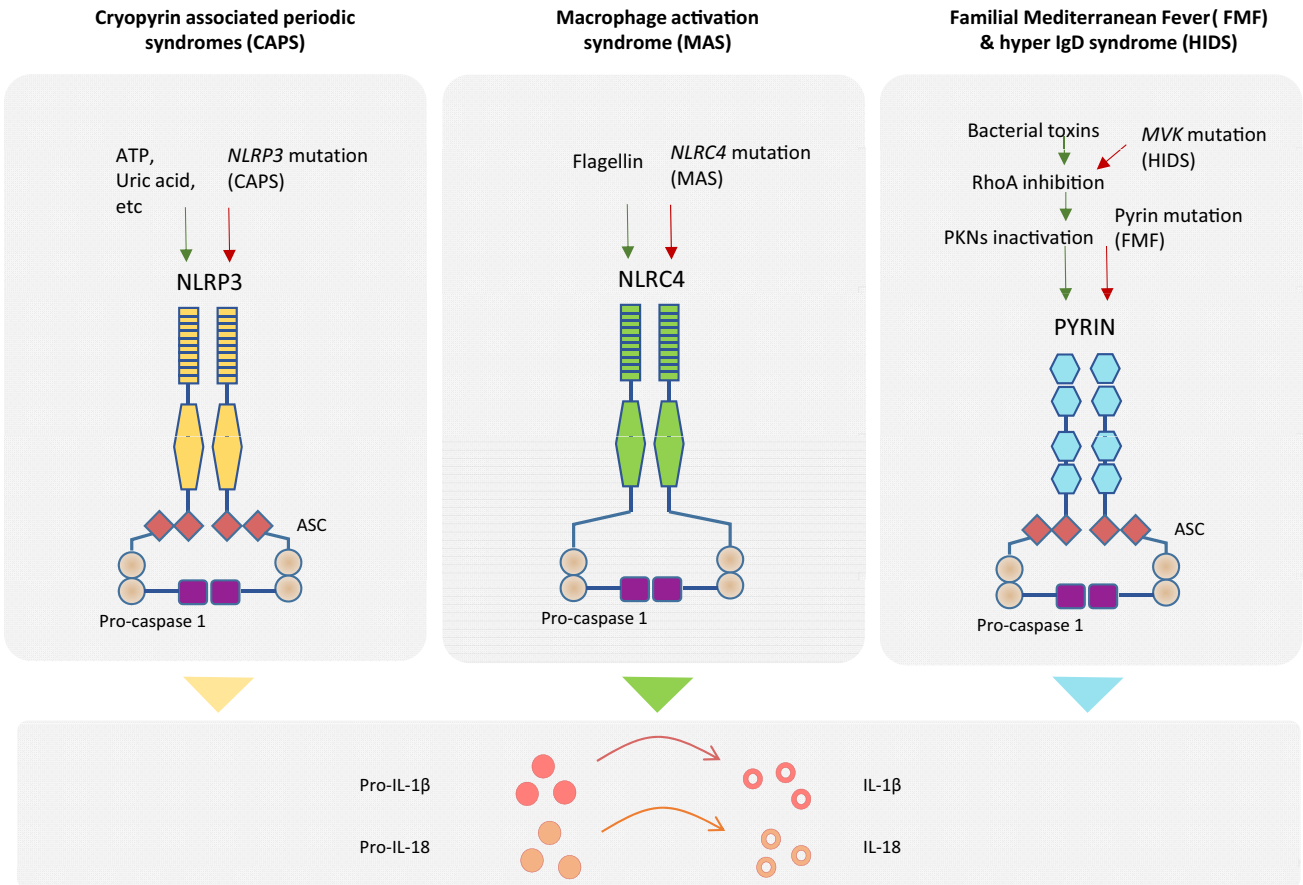

FIGURE 2 | Genetics of autoinflammatory diseases. Different inflammasome complexes are activated by different stimulus recognized by specific sensor molecules. Mutations of genes coding inflammasome proteins have been identified in autoinflammatory disorders. Gain-of-function mutations in NLRP3 gene have been detected in cryopyrin-associated periodic syndromes (CAPS), and mutations in NLRC4 gene have been observed in macrophage activation syndrome (MAS). The mechanistic explanation of the exacerbated inflammatory response for patients with Familial Mediterranean Fever (FMF) has recently been described. Mutation of pyrin in FMF patients causes a decrease in pyrin phosphorylation and deregulation of the inflammasome assembly. Higher amounts of inflammasome complex in the different diseases are associated with increased production of mature IL-1β and IL-18 inflammatory cytokines.

known NLRP3 inflammasome activator and a signal of cellular stress, is released in great amounts by CAPS monocytes when exposed to minute concentrations of inflammatory stimuli that do not induce a reaction in healthy counterparts. CAPS monocytes appear to be more sensitive and prone to generate a stronger reaction to same amounts of LPS compared to monocytes from healthy donors, hence producing an inflammatory feedback loop by secreting large amounts of ATP that will further activate the inflammasome and aberrantly augment the production IL-1β and IL-18 (31). Mutations in *NLRC4* have been shown to cause life-threatening autoinflammatory macrophage activation syndrome with systemic overproduction of IL1-β and IL-18 as well as uncontrolled macrophage activation (32, 33).

In the case of FMF, it was not until this year that a mechanistic explanation of exacerbated IL-1β release by mutated pyrin (coded by *MEFV*) was reported by Park and colleagues. Although pyrin does not seem to bind directly to bacterial products, it is phosphorylated by PKN1 and PKN2 in a RhoA GTPase-dependent manner, leading to inactivation of the pyrin inflammasome formation in the absence of pathogen infection. By contrast, either the presence of several bacterial toxins or the mutation of pyrin in FMF patients results in lack or diminished pyrin phosphorylation, reduced regulation of inflammasome assembly and hyperproduction of cleaved IL-1β (34). Moreover, a molecular link between pyrin (but not NLRP3, AIM2, and NLRC4) inflammasome regulation and the mevalonate kinase pathway has also been recently reported. Mevalonate kinase deficiency generates systemic inflammation with recurrent fever and lymphadenopathy, namely, the hyper IgD syndrome (HIDS) and the more severe mevalonate aciduria. Mevalonate kinase contributes to the inhibition of pyrin expression in an NF-κB-dependent manner through the production of geranyl pyrophosphate, which is necessary for repression of pyrin. As a consequence of absent mevalonate kinase pathways in HIDS patients, *MEFV* is overexpressed, and pyrin is abnormally activated leading to exacerbated inflammatory cytokine release and autoinflammation (35).

EPIGENETICS OF AUTOINFLAMMATORY DISORDERS

Epigenetic is broadly defined as the mitotically heritable changes that affect gene expression without affecting genome DNA sequence. More specifically, epigenetics encompass mechanisms that register, mark or perpetuate gene activity states. It is accepted that, due to their upstream connections with transcription factors and signaling pathways, epigenetic factors sense and mediate interactions between environment (extracellular signals) and the genome. The main epigenetic mechanisms comprise DNA methylation, histone modifications, non-coding RNAs, and chromatin remodeling. DNA methylation occurs by the addition of a methyl group to the 5′ position of a cytosine followed by guanine (CpG dinucleotide). Subsequent demethylation results from the oxidation of 5-methylcytosine catalyzed by ten–eleven translocation enzymes, which forms intermediates (5-hydroxymethylcytosine; 5-formylcytosine; and 5-carboxylcytosine) to yield the final unmethylated cytosines; however, recently, it has been described that these oxidized intermediates may have independent functional roles on their own merit. Posttranslational modifications of different histone amino acid residues are a vast group of epigenetic modifications. The functional role of all these epigenetic modifications depend on various factors including genomic location, and it can be very different in promoters, enhancers, and other genomic sites. Myeloid cells are very plastic, and they display vast changes in epigenetic modifications in response to a variety of environmental stimuli and under pathological inflammatory conditions (36).

In monogenic disorders, such as FMF, studies comparing patients with the same ancestry living in Turkey or in Germany have allowed the determination of the impact of the environment on the severity of FMF, in which environmental factors may contribute to as much as 12% of the phenotypic variation (37). In addition, it has been reported that gains of DNA methylation of the FMF causative gene *MEFV* lead to reduced *MEFV* expression in FMF peripheral leukocytes from 51 FMF patients compared to 21 healthy controls (38).

In the case of other classical monogenic disorders, evidence of epigenetic dysregulation is also starting to emerge from recent studies. Analysis of skin biopsies from NOMID patients, comparing skin lesions with both non-lesional skin and normal skin, is suggestive of epigenetic regulation, as genes that encode histones and enzymes that modify histones were differentially regulated in lesional skin. Moreover, two microRNAs, miR-29c and miR 103-2, were significantly downregulated in lesions, whereas some other skin specific miRNAs including miRNAs miR 9-1, miR 199a-2, miR 203, and miR 320a, were upregulated (39). Nevertheless, a more cell-specific and systematic analysis of the contribution of epigenetics in NOMID pathology is required. Our group has recently described that activation of monocytes and macrophages by inflammatory stimuli, such as cytokines GM-CSF and IL-1β, drives TET2-mediated demethylation of several inflammasome-related molecules including PYCARD, AIM2, IL-1α, and IL-1β. These data led us to further investigate the methylation status of inflammasome genes in a cohort of CAPS and FMF patients. We found that demethylation of such genes is exacerbated in untreated CAPS patients and that this demethylation was reverted by anti-IL-1β treatment (40). We provided evidence for the first time that an epigenetic mechanism, in this case DNA methylation, may contribute the decrease in IL-1β production threshold in CAPS patients, and provide the basis for the discovery of novel biomarkers that could complement the diagnosis of autoinflammatory disorders (**Figure 3**). Evidence for epigenetic dysregulation has also been provided in the case of complex autoinflammatory disorders such as Behçet's disease. Genome-wide DNA methylation studies in monocytes and CD4+ cells of BD patients, during flares and remission, comparing to healthy counterparts have revealed significant differences in methylation levels throughout the genome. Moreover, BD monocytes displayed 383 differentially methylated CpGs in 228 genes, whereas CD4+ showed 125 differential CpGs in 62 genes. Both hypermethylation and hypomethylation were represented in equivalent levels, and GO analysis of affected genes revealed

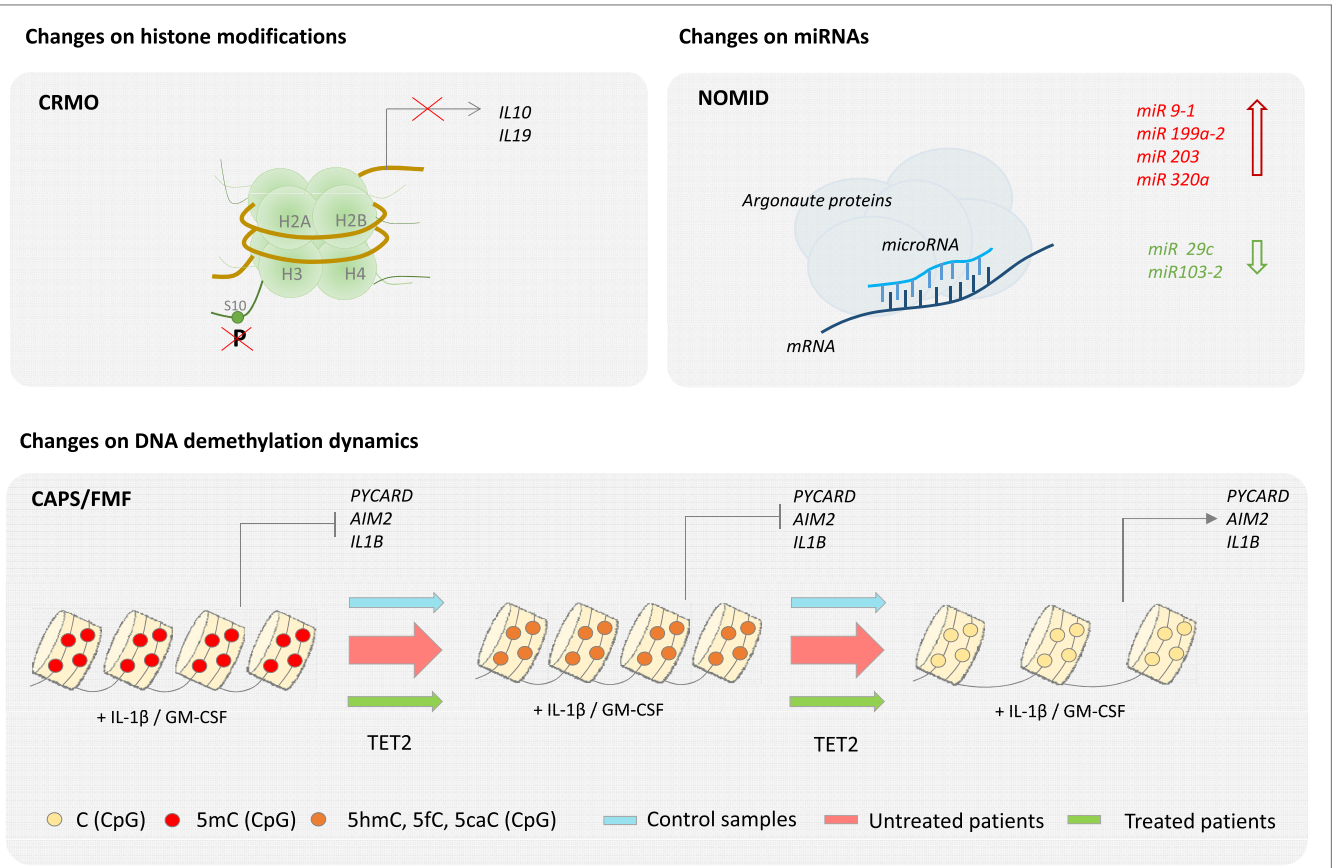

FIGURE 3 | Epigenetics of autoinflammatory diseases. Epigenetic changes have been described in several autoinflammatory diseases. For example, in the case of chronic recurrent multifocal osteomyelitis (CRMO), a failure of histone H3 phosphorylation at serine residue 10 (H3S10p) in the promoter region impairs IL-19 and IL-10 expression. Also, neonatal-onset multisystem inflammatory disease (NOMID) patients are associated with an increase of miR 9-1, miR 199a-2, miR 203, and miR 320a and a decrease of miR 29c and miR 103-2 in their skin. Finally, changes on DNA demethylation dynamics have been recently described in cryopyrin-associated periodic syndromes (CAPS) and Familial Mediterranean Fever (FMF).

an overrepresentation of cytoskeletal remodeling genes in monocytes and antigen processing and antigen presentation in CD4+ lymphocytes. Interestingly, BD patients in remission showed similar DNA methylation patterns as healthy controls, suggesting that changes in global DNA methylation patterns directly reflect disease pathology (41). In the case of CRMO, an autoinflammatory disease affecting the bone, an imbalance of proinflammatory and regulatory signals has been described. In particular, decreased expression of *IL10* has been shown to be directly attributed to epigenetic dysregulation. CRMO monocytes fail to produce IL-10, and related anti-inflammatory cytokine IL-19, upon LPS stimulation, which in turn leads to IL-1β overproduction and inflammation within the bone. *IL10* repression is suggested to occur through impaired chromatin remodeling caused by altered histone H3 phosphorylation at serine residue 10 at the *IL10* proximal promoter, which also encompasses the regulatory elements of the *IL19* (*CNS1*) gene and partially the *IL20 gene* (*CNS2*). In addition, differential DNA methylation of the *IL10* intronic enhancer element (I-SRE) and the *IL19* CNS1 was also observed. This strongly suggests that epigenetic regulation contribute to the overall proinflammatory imbalance and pathophysiology in CRMO (42, 43). Another set of multifactorial, complex disorders are the group of IBDs, typically CD and ulcerative colitis (UC), in which genetic predisposition, environmental microbiota, and immune responses are the main contributing factors to its pathology. Regarding to genetic contribution, twin studies show a 50% concordance for monozygotic and 10% for dizygotic twins for disease development (16). Using methylation bead arrays to compare whole blood from 21 CD adults versus 19 sex-matched controls, as well as 16 CD pediatric patients, a specific methylation profile for CD was determined, which includes differential methylation in several immune related genes such as *MAPK13*, *FASLG*, *PRF1*, *S100A13*, *RIPK3*, and *IL21R* in patients compared to healthy controls (44). Interestingly, the DNA methyltransferase gene *DNMT3A* has been identified by GWAS as a CD susceptibility gene, which suggests that aberrant DNA methylation may be participating in CD etiology (45). Although specific profiles of miRNA expression in UC and CD have been described in both target tissues and blood, cell type-specific miRNA expression data to unambiguously assess causality are still lacking (46).

Altogether, epigenetic dysregulation is emerging as a relevant contributing factor of autoinflammatory development, and

further investigation would provide valuable insight into their pathogenesis that could hint for molecular-tailored treatment.

CONCLUSION

The possibility of additional causative mechanisms leading to exacerbated autoinflammation in both mutated and non-mutated pathogenic genes increases the complexity of how autoinflammatory diseases manifest and evolve. It is conceivable that different gene variants could behave in a differential manner depending on its association with non-genetic background, which in turn is able to shape disease presence and severity, ranging from being a true causative mutation, a functional polymorphisms or remaining silent (47). In this respect, in addition to more in-depth genetic studies using massively parallel sequencing techniques (such as whole-exome sequencing and targeted deep resequencing), epigenetic genome-wide profiling studies could be of great value as they would inform of non-genetic landscapes that contribute to pathogenicity. Moreover, current genetic diagnosis of a few candidate genes would expand potential biomarkers taking into account clinical and molecular traits other than described mutations. Overall, the identification of epigenetic dysregulation contributing to autoinflammation will allow us to address environmental contribution to autoinflammatory syndromes.

AUTHOR CONTRIBUTIONS

DÁ-E and EB wrote the manuscript. RV-T wrote the manuscript and made figures.

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REVIEW
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Familial Mediterranean Fever: Recent Developments in Pathogenesis and New Recommendations for Management

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Familial Mediterranean fever (FMF) is the most common monogenic autoinflammatory disease (AID) affecting mainly the ethnic groups originating from Mediterranean basin. The disease is characterized by self-limited inflammatory attacks of fever and polyserositis along with elevated acute phase reactants. FMF is inherited autosomal recessively; however, a significant proportion of heterozygotes also express the phenotype. FMF is caused by mutations in the *MEFV* gene coding for pyrin, which is a component of inflammasome functioning in inflammatory response and production of interleukin-1β (IL-1β). Recent studies have shown that pyrin recognizes bacterial modifications in Rho GTPases, which results in inflammasome activation and increase in IL-1β. Pyrin does not directly recognize Rho modification but probably affected by Rho effector kinase, which is a downstream event in the actin cytoskeleton pathway. Recently, an international group of experts has published the recommendations for the management of FMF. Colchicine is the mainstay of FMF treatment, and its regular use prevents attacks and controls subclinical inflammation in the majority of patients. Furthermore, it decreases the long-term risk of amyloidosis. However, a minority of FMF patients fail to response or tolerate colchicine treatment. Anti-interleukin-1 drugs could be considered in these patients. One should keep in mind the possibility of non-compliance in colchicine-non-responders. Although FMF is a relatively well-described AID and almost 20 years has passed since the discovery of the *MEFV* gene, there are still a number of unsolved problems about it such as the exact mechanism of the disease, symptomatic heterozygotes and their treatment, and the optimal management of colchicine resistance.

Keywords: familial Mediterranean fever, *MEFV*, pyrin, colchicine, Rho GTPases

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INTRODUCTION

Familial Mediterranean fever (FMF) is the most common monogenic autoinflammatory disease (AID) over the world. Its prevalence is very high among people from the eastern Mediterranean such as Jews, Turks, Armenians, and Arabs (1, 2). However, patients from different ethnicities (such as Japan) are being increasingly recognized (3, 4). Self-limited inflammatory attacks of fever and polyserositis along with high acute phase response are the typical phenotype expected in FMF (5). The most significant complication of FMF is amyloidosis, and it is responsible for long-term

morbidity and mortality (6). Although it is known to be inherited autosomal recessively, a substantial number of heterozygotes are present expressing the phenotypic characteristics (7).

Since the definition of *MEFV* gene mutations underlying FMF in 1997, around 310 sequence variants in *MEFV* gene have been detected (8). *MEFV* gene, located on chromosome 16 encodes for pyrin protein (9, 10). Pyrin, exists mostly in neutrophils and macrophages, has a key role in apoptosis and inflammatory pathways (9, 11). Mutated pyrin causes an exaggerated inflammatory response by uncontrolled interleukin-1 (IL-1) secretion (11). Recent studies have supplied information about the importance of the role of pyrin as a pattern recognition receptor (PRR), as well (12).

Colchicine is the mainstay of FMF treatment, and its regular use prevents attacks and suppresses chronic subclinical inflammation (13–15). Anti-IL-1 drugs emerged as promising treatment options in patients who fail to response or tolerate colchicine. Compliance to this orally administered drug is a problem. In resistant cases, the clinicians should also keep in mind whether the patient is compliant to the therapy (16). Recently, a group of international experts has published the recommendations for the management of FMF to guide physicians taking care of these patients (17).

In this review, we will discuss the new findings in the pathogenesis of FMF and the new recommendations for management.

GENETICS OF FMF

In 1997, mutations in the *MEFV* gene, composed of 10 exons and located on chromosome 16 (16p13.3), were found to be associated with FMF (9, 10, 18). The gene encodes a 781 amino acid protein termed pyrin or marenostrin (9, 10, 18). Only a few mutations had been defined in selected families when the genetic association was first described (10, 19). Up to date, according to the INFEVERS database, more than 310 *MEFV* sequence variants have been reported (http://fmf.igh.cnrs.fr/infevers/). However, all variants are not associated with a disease phenotype and are termed "variants of uncertain significance." With the description of new mutations, concerns emerged for the adequacy of checking only the common mutations. Booty et al. sequenced the *MEFV* gene in FMF patients and showed that screening the most common mutations instead of sequencing the whole gene appears sufficient to diagnose FMF in presence of clinical symptoms (20). In 2012, a group of clinical and molecular experts reached a consensus to test for a total of 14 *MEFV* variants if possible (21). These include nine clearly pathogenic variants (M694V, M694I, M680I, V726A, R761H, A744S, I692del, E167D, and T267I) and five variants of unknown significance (E148Q, K695R, P369S, F479L, and I591T) (21).

In the Eastern Mediterranean, the distribution of *MEFV* mutations is quite similar. M694V is the most common mutation in Turk (5), Armenian (22, 23), Arab (24), and Jewish populations (25); however, it is less common in Arabs (26). The second most common mutation is M680I in Turks (5); and V726A in Armenians (22, 23), Arabs (24), and Jews (25). M680I is the third most common mutation in Armenians (23). M694I is mostly seen in the Arabic population (24). On the other hand, in populations where FMF is a rare disease, the aforementioned mutations are less common, and other mutations are also seen. For example, in Japanese patients, E148Q is the most common variant followed by M694I and L110P (3). The clinical variability in FMF could be partly explained by genetic heterogeneity. For instance, most experts agreed that M694V was associated with a severe disease phenotype (8).

Recently, evidence-based recommendations have been developed for genetic diagnosis of FMF by the Single Hub and Access point for pediatric Rheumatology in Europe (SHARE) initiative (8). These recommendations are presented in **Table 1**. According to these, patients homozygous for M694V should be considered at higher risk of early disease onset and developing a severe phenotype (8). Furthermore, the patients carrying two mutated alleles in position 680–694 on exon 10 are also considered at risk of having a more severe disease (8).

Another area of debate is E148Q variant. E148Q, the most frequent sequence alteration in the *MEFV* gene (27), is the result of the substitution of glutamine for glutamic acid at codon 148 in exon 2 (28, 29). E148Q is a common variant in the general population; however, the pathogenic role of E148Q is still uncertain (30).

In 2000, in a case–control study, Ben-Chetrit et al. found a similar frequency for E148Q mutation both in patients and healthy controls and in patients and their asymptomatic relatives (27). Tchernitchko et al. also demonstrated that E148Q allele frequency was comparable among patients and asymptomatic relatives and they concluded E148Q as a benign polymorphism (31). However, other studies (32, 33) showed that patients with homozygous E148Q variant might have an FMF-like phenotype

TABLE 1 | Recommendations for familial Mediterranean fever (FMF) genetic diagnosis [adapted from Ref. (8)].

Recommendation	Strength of evidence
1. FMF is a clinical diagnosis, which can be supported but not excluded by genetic testing	B
2. Consider patients homozygous for M694V at risk of developing, with very high probability, a severe phenotype	B
3. FMF patients carrying two of the common mutated alleles (homozygotes or compound heterozygotes), especially for M694V mutation or mutations at position 680–694 on exon 10, must be considered at risk of having a more severe disease	B
4. The E148Q variant is common, of unknown pathogenic significance, and as the only *MEFV* variant does not support the diagnosis of FMF	B
5. Patients homozygous for M694V mutation are at risk of early onset disease	C
6. Individuals homozygous for M694V who are not reporting symptoms should be evaluated and followed closely in order to consider therapy	A
7. For individuals with two pathogenic mutations for FMF who do not report symptoms, if there are risk factors for AA amyloidosis (such as the country, family history, and persistently elevated inflammatory markers, particularly serum amyloid A protein), close follow-up should be started and treatment considered	B
8. Consultation with an autoinflammatory disease specialist may be helpful in order to aid in the indication and interpretation of the genetic testing and diagnosis	C

requiring colchicine treatment. In a recent study, it has been suggested that the disease was less severe, the disease onset was later, and the ratio of patients responding completely to colchicine was higher in—at least a portion of—patients homozygous for E148Q when compared to the patients with exon 10 mutations (34).

Shinar et al. defined E148Q as a variant of unknown significance (21) and according to the SHARE recommendations, E148Q, as the only *MEFV* variant, does not support the diagnosis of FMF (8).

Although FMF is considered as an autosomal recessive disease, it was recognized that a significant portion of the patients had only one mutation in the *MEFV* gene (25, 35). Marek-Yagel et al. examined heterozygote FMF patients and performed haplotype studies in FMF families (36). They concluded that in some cases, the disease in heterozygotes could not be distinguished from that of homozygous patients, and FMF could be viewed as a dominant condition with low penetrance. Booty et al. searched for a second *MEFV* mutation in heterozygote patients who had a clinical diagnosis of FMF (20). However, re-sequencing the entire *MEFV* gene did not yield a second mutation in any of these cases (20). A recent study demonstrated that the frequency of FMF-like symptoms increased from patients carrying a single low penetrance mutation toward patients with two high-penetrance mutations suggesting a "dose effect" associated with mutations (37). One other explanation for heterozygote FMF patients may be the effect of the other modifier genes such as serum amyloid A (SAA) gene. *SAA* polymorphisms have been shown to contribute the severity of FMF phenotype inducing the expression of pro-interleukin-1β (IL-1β) and activating NLRP3 inflammasome resulting in the secretion of active IL-1β (4, 38). In the same lines, recently, Atoyan et al. have shown that SAA1 α allele was strongly associated with amyloidosis in FMF patients (39). On the other hand, environmental factors also have effect on the disease phenotype. Touitou et al. examined the characteristics of 2,482 FMF patients (260 of whom had amyloidosis) from 14 different countries (40). They demonstrated that the country of recruitment (roughly the same as the country of residence) was the most important determinant of amyloidosis risk.

We had shown that Turkish children with FMF in Germany expressed a less severe disease phenotype in comparison with the ones living in eastern Mediterranean (41). In addition, when we examined the Eurofever registry, we have seen that patients with a European ancestry have a milder disease than the Eastern Mediterranean patients (41). Furthermore, patients living in eastern Mediterranean countries had a higher frequency of fever episodes per year, and more frequent arthritis, pericarditis, chest pain, abdominal pain, and vomiting compared to the patients living in Western Europe (41). It was noteworthy that Western European patients had less frequent abdominal pain, pericarditis, and arthritis than eastern Mediterraneans (41). All of the above studies suggest the effect of environment on the phenotype of this monogenic disease.

Another issue that deserves a mention is autosomal dominant FMF. Different mutations (H478Y, T577S, T577A, T577N, M694del, M694I, E148Q, and L110P) in *MEFV* have been reported to cause dominant FMF in patients from different populations; Spanish, Turkish, Dutch, British, Indian, and Japanese (18, 41–45). These reports have shown that FMF could also be inherited autosomal dominantly, and these patients may have different disease phenotypes. Rowczenio et al. reported that symptoms may develop later in life in autosomal dominant FMF with p.M694Vdel than in classical recessive FMF (45). Stoffels et al. have demonstrated that these patients may have different symptoms during attacks such as urticarial rash and conjunctivitis overlapping with other AID (18).

Although this is a monogenic disease, epigenetic factors and microbiota may play role in the pathogenesis of FMF or phenotypic expression. It is tempting to speculate that host–microbe interactions may be important in this innate immune system disease. Khachatryan et al. demonstrated that the composition and divergence of microbiota were different during attack and attack-free periods as well as between FMF patients and healthy controls (46).

DISEASE PATHOGENESIS

Pyrin, encoded by *MEFV*, has been suggested to interact with ASC (the inflammasome adaptor protein). The subsequent assembly of the inflammasome was suggested to activate caspase-1 leading to the cleavage and activation of IL-1β (47).

Until recently, it was a debate whether the disease-causing mutations in the *MEFV* gene were loss-of-function or gain-of-function mutations. There were different results depending on the different experimental settings. Supporting the loss-of-function model, Papin et al. demonstrated an increase in caspase-1 activation and IL-1β secretion as a result of pyrin knockdown (48). Hesker et al. showed that in response to inflammatory stimuli in a mouse line lacking the *MEFV* gene, IL-1β release by macrophages was enhanced (49).

On the other hand, in compliance with the gain-of-function model, Booty et al. demonstrated a significant increase in pyrin expression in FMF patients compared to healthy controls (20). Yu et al. have shown that activated pyrin forms a trimolecular complex by interacting with ASC and PTSPIP1, and this complex directly activates caspase-1 and leads to secretion of IL-1β (50). In 2011, Chae et al. have demonstrated that homozygous knock-in mice with the mouse pyrin protein fused to the human B30.2 domain containing FMF-associated mutations secrete large amounts of IL-1β in an NLRP3-independent manner (51). These data confirmed that the mutations associated with FMF were gain-of-function mutations and suggested that FMF was a pyrin inflammasomopathy (51).

Almost 20 years after defining the genetic basis of FMF and learning the role of pyrin in its pathogenesis, we now have some new data elaborating the role of pyrin in pathogenesis (12, 52). The detection of pathogenic microorganisms by PRRs triggers the formation of inflammasome (53). Recent data suggest that pyrin is also a PRR (12).

Two major virulence factors of *Clostridium difficile*, namely, TcdA and TcdB (54, 55) inactivate Rho GTPases *via* monoglycosylating a threonine residue in the GTPase switch I region of the protein (12). Recent studies have also shown that TcdB could trigger caspase-1 activation and IL-1β production; thus, it can activate the inflammasome (12, 56, 57). Furthermore, the C3 toxin of *Clostridium botulinum* and type VI secretion system

(T6SS) of *Burkholderia cenocepacia* inactivate RHO by modifying the GTPase switch I region with different chemical groups and both trigger inflammasome activation (12). These results suggest that the bacterial toxins modifying RHO could trigger caspase-1 activation and IL-1β production; thus induce the inflammasome (12). The inflammasome activation by these toxins (TcdB, C3, and T6SS) was independent of NLRP3 and NLRC4 but was decreased in $ASC^{-/-}$ and $MEFV^{-/-}$ bone marrow-derived macrophages (12). In addition, small interference RNA knockdown of pyrin inhibited TcdB-induced caspase-1 activation (12). These results also support the "gain-of-function" model in the pathogenesis of FMF. Since different inhibiting modifications in RHO proteins all result in caspase-1 activation, pyrin probably senses a downstream event in the actin cytoskeleton pathway (12).

The study by Park et al. has enlightened these mechanisms further (52). They have demonstrated that staurosporine, a potent inhibitor of protein kinase C that is an effector of RhoA, induced IL-1β release independent of the NLRP3, NLRC4, or AIM2 inflammasomes but dependent on the pyrin inflammasome (52). This shows that RhoA effector kinases suppress pyrin inflammasome activation. PKNs, RhoA effector kinases, bind to human pyrin and phosphorylate Ser208 and Ser242 units. The binding of PKN1 to the pyrin of FMF-knock-in mice (with B30.2 mutations; $Mefv^{M680I/M680I}$, $Mefv^{M694V/M694V}$, and $Mefv^{V726A/V726A}$) was substantially decreased in comparison with the binding of PKN1 to wild type ($Mefv^{+/+}$) mouse pyrin, which lacks a B30.2 orthologous domain (52). The binding of PKN1 to the pyrin of wild type B30.2 domain knock-in mice ($Mefv^{B30.2/B30.2}$) was also decreased relative to wild-type mouse pyrin (but not as much as in FMF knock-in mice) (52). These suggest that the human B30.2 domain has a role in the regulation of PKN1 binding to pyrin. It was also shown that 14-3-3 protein binds to phospho-pyrin (phosphorylated from Ser208 and Ser242 units by PKNs) to inhibit inflammasome activation. Furthermore, the binding of 14-3-3 to mutant pyrin (M680I, M694V, and V726A) was decreased relative to wild-type human pyrin (52). All aforementioned results show that active RhoA signals through PKNs, which phosphorylate pyrin from Ser208 and Ser242 units. Then, 14-3-3 proteins bind to phospho-pyrin and inhibit the activation of pyrin inflammasome. Pyrin is activated when dephosphorylated at Ser208/Ser242. The binding of PKN1 to pyrin is decreased with the B30.2 domain where most of the common and severe *MEFV* mutations are clustered.

These data enlighten the effect of mutations on pyrin function and the downstream event of RhoA inhibiting pyrin. Active pyrin promotes ASC oligomerization and forms a caspase-1 activating complex resulting in IL-1β production. Wild-type pyrin relies selectively on microtubules for inflammasome activation and microtubules control pyrin signaling downstream of pyrin dephosphorylation (52). Recently, Van Gorp et al. have observed that colchicine pretreatment augments the TcdA-induced IL-1β secretion from FMF peripheral blood mononuclear cells (58). The microtubule assembly inhibition with nocodazole also had the same effect. Thus, FMF-associated mutated pyrin does not require microtubules for ASC speck assembly. *MEFV* mutations in B30.2 domain probably remove the critical reliance on intact microtubules for pyrin-based nucleation of ASC specks and inflammasome signaling (58).

To make the story even more complex, in a recent study, Kimura et al. have demonstrated that pyrin (referred as TRIM20 in the article) recognizes the inflammasome components, NLRP1, NLRP3, and procaspase-1 and leads to their autophagic degradation (59). Diminished autophagic degradation of NLRP3 was shown in single (M694V), double (M680I and M694V), and triple (M680I, M694V, and V726A) mutants (59).

When we look at the cellular level, we know that neutrophilia and influx of neutrophils to the inflamed sites occur in FMF attacks (60). Gohar et al. demonstrated that *in vitro*, unstimulated neutrophils from M694V positive patients spontaneously secreted more S100A12, IL-18, and caspase-1 compared to neutrophils from healthy controls (61). In another study, it has recently been shown that FMF attack is characterized by release of neutrophil extracellular traps (NET) including active IL-1β (60). These NET structures are observed in the first hours of FMF attacks, and subside as the inflammatory attack is resolved. They have demonstrated that NETs restrict their own generation by a negative feedback mechanism, which may be an explanation for the self-limited nature of FMF attacks. Of note, in this study, neutrophils from FMF patients in remission were resistant to induction of NET release. They have shown that reduced basal autophagy levels in these cells could be responsible for this since autophagy induction is needed for NET formation. Thus, lower basal autophagy levels of neutrophils may protect from attacks by attenuating the release of pro-inflammatory NETs.

Manukyan et al. have recently shown that the *ex vivo* spontaneous apoptotic rate of neutrophils from FMF patients in remission is significantly higher compared to control (62). The accelerated apoptosis of neutrophils in FMF may be important for successful resolution of inflammation and prevention of tissue damage. This may be another explanation for the self-limited nature of FMF attacks. Pyrin modulates the susceptibility to apoptosis; however, the effect of the mutant pyrin on apoptotic processes is poorly understood.

Although now we know more about the function of pyrin, the role of neutrophils, and the disease pathogenesis, there are still questions waiting to be answered such as the exact reason for the episodic and short-term nature of the inflammatory attacks and the phenotypic variability in FMF.

TREATMENT

Familial Mediterranean fever can be well controlled with optimum standard management. Recently, with the international collaboration of experienced experts from different countries, the European League Against Rheumatism (EULAR) recommendation set for the management of FMF has been published supported by the best available evidence (17). These recommendations are presented in **Table 2**.

The EULAR recommendations emphasize that the aim of FMF treatment is obtaining the control of acute attacks, minimizing the chronic and subclinical inflammation, preventing complications, and providing an acceptable quality of life.

It is also emphasized that colchicine is the main treatment of FMF since 1972 (63). It is generally a safe and well-tolerated drug, but its mechanism of action in FMF has not been completely

TABLE 2 | The European League Against Rheumatism recommendations for the management of FMF with grade of recommendation [adapted from Ref. (17)].

Recommendation	Grade
01. Ideally, FMF should be diagnosed and initially treated by a physician with experience in FMF	D
02. The ultimate goal of treatment in FMF is to reach complete control of unprovoked attacks and minimizing subclinical inflammation in between attacks	C
03. Treatment with colchicine should start as soon as a clinical diagnosis is made	A
04. Dosing can be in single or divided doses, depending on tolerance and compliance	D
05. The persistence of attacks or of subclinical inflammation represents an indication to increase the colchicine dose	C
06. Compliant patients not responding to the maximum tolerated dose of colchicine can be considered non-respondent or resistant; alternative biological treatments are indicated in these patients	B
07. FMF treatment needs to be intensified in AA amyloidosis using the maximal tolerated dose of colchicine and supplemented with biologics as required	C
08. Periods of physical or emotional stress can trigger FMF attacks, and it may be appropriate to increase the dose of colchicine temporarily	D
09. Response, toxicity, and compliance should be monitored every 6 months	D
10. Liver enzymes should be monitored regularly in patients with FMF treated with colchicine; if liver enzymes are elevated greater than twofold the upper limit of normal, colchicine should be reduced and the cause further investigated	D
11. In patients with decreased renal function, the risk of toxicity is very high, and therefore signs of colchicine toxicity, as well as CPK, should be carefully monitored and colchicine dose reduced accordingly	C
12. Colchicine toxicity is a serious complication and should be adequately suspected and prevented	C
13. When suspecting an attack, always consider other possible causes. During the attacks, continue the usual dose of colchicine and use NSAID	C
14. Colchicine should not be discontinued during conception, pregnancy, or lactation; current evidence does not justify amniocentesis	C
15. In general, men do not need to stop colchicine prior to conception; in the rare case of azoospermia or oligospermia proven to be related to colchicine, temporary dose reduction or discontinuation may be needed	C
16. Chronic arthritis in a patient with FMF might need additional medications, such as DMARDs, intra-articular steroid injections, or biologics	C
17. In protracted febrile myalgia, glucocorticoids lead to the resolution of symptoms; NSAID and IL-1-blockade might also be a treatment option; NSAIDs are suggested for the treatment of exertional leg pain	C
18. If a patient is stable with no attacks for more than 5 years and no elevated APR, dose reduction could be considered after expert consultation and with continued monitoring	D

APR, acute phase reactants; CPK, creatinine phosphokinase; DMARDs, disease-modifying antirheumatic drugs; FMF, familial Mediterranean fever; IL-1, interleukin-1; NSAID, non-steroidal anti-inflammatory drugs.

elucidated. However, we know that it prevents microtubule elongation by binding to tubulin monomers and inhibiting polymer formation (64, 65). Thus, the link between pyrin and colchicine could be through the organization of actin cytoskeleton.

Previously, it was claimed that colchicine is an activator of RhoA (66). It binds to tubulin, depolymerizes microtubules and causes release of the RhoA activator guanine-nucleotide-exchange factor-H1, which is inactive when bound to microtubules (66). Park et al. demonstrated that colchicine inhibited the constitutive IL-1β release from bone-marrow-derived macrophages (BMDMs) of $Mefv^{V726A/V726A}$ mice and C3-toxin-induced IL-1β release from primed BMDMs. In addition, colchicine inhibited IL-1β release from PBMCs of FMF patients (52). In the same lines, Van Gorp et al. demonstrated that microtubule-depolymerizing drugs selectively inhibited the pyrin inflammasome (58). Thus, colchicine may be inhibiting pyrin inflammasome through RhoA activation by releasing RhoA activator from depolymerized microtubules.

Certain other pharmacological anti-inflammatory effects of colchicine have been enlightened such as preventing activation of neutrophils by forming β-tubulin–colchicine complexes and inhibiting the microtubule assembly and mitotic spindle formation, suppressing caspase-1 gene expression, and inhibiting the synthesis of tumor necrosis factor alpha (TNF-α) (65, 67–70).

It is suggested that colchicine should be started as soon as the patient is clinically diagnosed as having FMF. If the patient lacks clinical manifestations or subclinical inflammation, genetic diagnosis is not a precise indication to start treatment; however, these patients should be followed-up closely for clinical symptoms or signs of subclinical inflammation (17). In countries where amyloidosis has high frequency, the physician may consider treatment in these patients especially when the patient has homozygous M694V mutation, which is more frequently associated with the development of amyloidosis (9, 21, 71–77).

The optimal dosage of colchicine varies between studies and different clinical practices. The recommendation of the starting dose of colchicine in FMF is ≤0.5 mg/day for children <5 years of age; 0.5–1 mg/day for children 5–10 years of age; and 1–1.5 mg/day in children >10 years of age and in adults (in case tablet contains 0.6 mg; ≤0.6 mg/day; 1.2 mg/day; and 1.8 mg/day, respectively) (17). Higher starting doses could be used in patients with high disease activity or disease complications such as amyloidosis (17). However, in most patients, it is started at the subtherapeutic dose of 0.5 mg/day and adjusted according to disease activity and tolerance in the follow-up. While escalating colchicine dose in patients with active disease, monitoring C-reactive protein (CRP) and SAA, or both is required at least every 3 months (17). Both increase in attack frequency and presence of subclinical inflammation are indications to increase colchicine dose. The maximum dose is 2 mg/day in children and 3 mg/day in adults (14, 78). Dosing can be in single or divided doses. The dose can be divided to decrease side effects; however, a single daily dose may increase the compliance (17). Polat et al. have recently shown that using colchicine with either once- or twice-daily dosage provides similar clinical and laboratory improvement as well as the similar rate of drug side effect (79).

Colchicine treatment is lifelong in FMF. However, in EULAR recommendations, FMF experts recommend the consideration of colchicine dose reduction by an experienced center under certain circumstances with very careful and close follow-up (17).

Colchicine is a safe drug in the range of doses used for FMF treatment (80). The most common side effects of the drug and toxicity are also reviewed in the aforementioned recommendations. The most common side effect is gastrointestinal disturbance, which may be seen in up to 10% of patients during the

first month of the treatment (81, 82). It was shown that jejunal lactase, sucrase, and maltase activities decreased in patients on long-term colchicine treatment (83). In these patients, increased fecal excretion of starch, fat, and bile acids and decreased absorption of D-xylose and vitamin B12 occur, as well. These may be the explanation for diarrhea and lactose intolerance, and a symptomatic relief can be provided with a lactose-free diet (83, 84). Dose reduction may also improve the gastrointestinal symptoms (85). There are also some rare side effects of colchicine, such as vitamin B12 deficiency, reversible peripheral neuritis and myopathy, bone marrow suppression, and alopecia (86–89). In addition, some animal studies and case reports suggested its association with azoospermia (90, 91); however, this was only in very high doses. Thus, in general, men need not stop colchicine prior to conception (17). Colchicine use is safe during pregnancy and lactation, as well (92–94). However, it should be used cautiously in patients with impaired renal or hepatic functions (95).

Compliance with colchicine is very important for proper management of FMF. One study showed that proteinuria that is usually the first sign of renal amyloidosis, developed after a period of 9–11 years in 1.7% of 960 adult patients who properly used colchicine versus 49% in 54 patients who were not compliant (96). There is a surprisingly high rate of incompliance with colchicine especially among adolescent patient (17). Thus, in the case of patients not responding to colchicine, the physician should keep in mind the possibility of incompliance. Overall, up to 5% of FMF patients may not respond to colchicine treatment and another 2–5% is colchicine intolerant (85).

Anti-IL-1 therapy seems to be a promising second-line therapy in refractory or intolerant patients. However, one should keep in mind that colchicine should be coadministered with biologic therapies since it may reduce the risk of amyloidosis (17). There are three types of anti-IL-1 agents in clinical use; anakinra, a recombinant homolog of the human IL-1 receptor (97); canakinumab, a fully human immunoglobulin G1 monoclonal antibody (98); and rilonacept, a dimeric Fc-fusion protein capturing IL-1 (97); all administered subcutaneously.

The most recent systematic review of the literature (99) has yielded 24 case reports/series, 2 open-label prospective trials (100, 101), and 1 placebo-controlled prospective trial (102) on anti-IL-1 use in FMF. Eighteen reports were on treatment with anakinra (103–120), four on canakinumab (100, 101, 121, 122), four on patients treated with either anakinra or canakinumab (123–126), and the only placebo-controlled prospective trial was on treatment with rilonacept (102). A complete response to therapy was reported in 76.5% of patients on anakinra, and 67.5% of patients on canakinumab treatment (99). In addition, IL-1 blockade can reverse proteinuria in patients with renal AA amyloidosis (99, 127). However, we do not know whether anti-IL-1 therapies could prevent amyloidosis. A new study on efficacy/safety of canakinumab in patients with hereditary periodic fevers including FMF is also underway (http://ClinicalTrials.gov identifier NCT02059291).

Anti-IL-1 drugs may be used "on demand" (starting at first symptom of attacks) in mevalonate kinase deficiency (128). We need further data on whether this would be an option for selected cases in FMF or on certain occasions.

Besides IL-1 blockade, FMF patients with chronic arthritis and/or sacroiliitis could benefit from disease-modifying antirheumatic drugs or anti-TNF agents (129, 130).

Treatment of protracted febrile myalgia syndrome (PFMS) has also been addressed. PFMS is a very rare manifestation of FMF and is defined as severe, disabling myalgia of at least 5 days duration (108, 112). It is associated with fever, the presence of at least one M694V mutation, and elevated inflammatory markers while creatine kinase levels are usually normal (131, 132). Corticosteroid treatment is required to suppress symptoms (17, 131, 133, 134). Non-steroidal anti-inflammatory drugs may also be beneficial (131). In addition, anakinra has been used successfully in two patients with PFMS associated with FMF (112).

Treatment in Heterozygotes

Familial Mediterranean fever is a clinical diagnosis, and we have many patients who are heterozygous for *MEFV* mutations. How patients with one mutation only can express the disease is still not clear (135). We give colchicine treatment to patients who express the typical FMF phenotype. However, some heterozygotes can sometimes "outgrow" the phenotype (30). Ben-Zvi et al. previously demonstrated that their patients (not using colchicine) experienced years of symptom-free interval where 22 out of these 33 were heterozygotes (136).

The data on remission of the disease in heterozygotes are limited. Recently, we have reported our experience on heterozygote patients with transient FMF clinic (7). We discontinued colchicine treatment in 22 heterozygote FMF patients who had an inflammation- and attack-free period for a long duration. The median follow-up after colchicine cessation was 22.5 months, and we restarted colchicine in only two patients because of the recurrence of attacks. However, after colchicine cessation, close follow-up is crucial every 3–6 months to evaluate whether they have recurrence of attacks or subclinical inflammation.

Refractory FMF and Outcome

There is no standard definition for refractory FMF patients. However, in the recent guideline, we stated that patients who continue to have ≥1 attacks per month despite receiving the maximally tolerated dose for ≥6 months might be considered non-responder or resistant to colchicine (17). Another issue is ongoing subclinical inflammation, which leaves the patients at risk of developing amyloidosis (17). In addition, in the case of AA amyloidosis, the FMF treatment should be intensified with biologics and maximal tolerated dose of colchicine (17).

There are mainly two tools to evaluate outcome and disease activity in FMF; FMF50 score and autoinflammatory disease activity index, respectively.

In FMF50, the items are percentage change in the frequency and duration of attacks, arthritis attacks, physician's and patient's/parents' global assessment of disease severity (0–10 cm visual analog scale; 10 the worst), and in ESR, CRP, or SAA level with the treatment (137). At least 50% improvement in five out of six criteria by 3–6 months with no worsening in any one means FMF50 response. It is noteworthy that compliance with the maximum dose of drug is essential for evaluating the patients with FMF50 score.

Autoinflammatory disease activity index is a disease activity assessment tool for AID including FMF, and it is composed of 13 items: overall symptoms, nausea/vomiting, abdominal pain, diarrhea, chest pain, arthralgia or myalgia, swelling of the joints, headaches, eye manifestations, skin rash, and pain relief (138). Each item except pain relief is scored by the patients/parents for a total score of 0–34 in a single day and 0–1,054 in a month of 31 days. A cutoff score of ≥9 discriminates active from inactive patients with a sensitivity of 89% and specificity of 92% (138).

There is also one recent tool for AID including FMF to quantify damage in patients and to compare disease outcomes in clinical studies; autoinflammatory disease damage index (ADDI) (139).

In ADDI, damage is defined as "persistent or irreversible change in structure or function that is present for at least 6 months" (139). ADDI contains 18 items, and these items are categorized by organ systems as follows: reproductive, renal/amyloidosis, developmental, serosal, neurological, ears, ocular, and musculoskeletal. The renal/amyloidosis and neurological damage categories were assigned to have the highest number of points while serosal damage got the lowest. This index provides a universal instrument to measure damage by chronic inflammation in FMF.

These tools could aid us to form a standard definition for refractory FMF patients and standardize the outcome measurement in different studies.

UNSOLVED ISSUES IN FMF

As we mentioned above in the relevant parts, there are still gaps in knowledge about the pathogenesis and treatment mechanisms in FMF. We need further research on the following:

- the significance of the E148Q variant,
- exact roles of modifier factors (microbiota, microRNAs, etc.) on disease pathogenesis, phenotypic expression, and severity of the disease,
- the effects of mutant pyrin on apoptosis,
- the exact reason for the self-limited and episodic nature of disease attacks,
- whether anti-IL-1 treatment prevents amyloidosis,
- the definition of colchicine resistance,
- why certain rheumatic diseases are more common in heterozygotes, and why they sometimes express the disease phenotype,
- the duration of treatment in heterozygous patients,
- more biomarkers for secondary amyloidosis.

CONCLUSION

When the mutated protein for FMF was described 20 years ago, we thought that everything was resolved. However, this monogenic disease continues to be of interest to clinical and basic researchers. We still need to address the above questions and the cause of the phenotypic heterogeneity in this disease. On the other hand, the experts on FMF have worked on compiling recommendations to guide physicians in the diagnosis, management, and treatment of FMF. It is hoped that these recommendations may be of practical use while the work on solving the pathogenesis continue.

AUTHOR CONTRIBUTIONS

EB and SD prepared the first draft of the article. SÖ made the critical revision of the article. All the authors have seen and approved the final version of the manuscript.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at http://journal.frontiersin.org/article/10.3389/fimmu.2017.00253/full#supplementary-material.

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Disease Phenotype and Outcome Depending on the Age at Disease Onset in Patients Carrying the R92Q Low-Penetrance Variant in *TNFRSF1A* Gene

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Background: Tumor necrosis factor receptor-associated periodic syndrome (TRAPS) is an autosomal-dominant autoinflammatory disease caused by mutations in the *TNFRSF1A* gene. R92Q, a low-penetrance variant, is usually associated with a milder TRAPS phenotype than structural or pathogenic mutations. No studies differentiating R92Q-related disease in patients with pediatric and adult onset have been performed to date.

Objective: To analyze clinical features and disease outcomes in patients diagnosed with TRAPS associated with R92Q variant and to investigate differences between patients with pediatric and adult disease onset.

Methods: A retrospective review of patients with R92Q-related disease from four reference centers for autoinflammatory diseases was performed. Clinical and laboratory features, family history of autoinflammatory diseases, treatments received, and outcomes during follow-up were recorded and separately analyzed in pediatric and adult patients. Our results were included in the analysis with other reported pediatric and adult R92Q-related disease series.

Results: Our series encompassed 18 patients (9 females and 9 males) with R92Q variant. In 61% of patients, disease onset occurred during infancy and in 39%, during adulthood, with a median diagnostic delay of 5 years and a follow-up of 5.4 years. A positive family history of autoinflammatory disease was detected in 28% of patients. All patients presented with febrile recurrent episodes. Other common symptoms included arthralgia/arthritis (61%), myalgia (39%), asthenia/fatigue (44%), abdominal pain (39%),

Abbreviations: TNF, tumor necrosis factor; TRAPS, tumor necrosis factor receptor-associated periodic syndrome.

headache (33%), odynophagia (33%), skin rash (28%), and chest pain (22%). During attacks, 80% of patients increased acute phase reactants levels. No patient had developed amyloidosis during the study period. At the end of follow-up, 28% of patients were asymptomatic and treatment free, 50% were receiving non-steroidal anti-inflammatory drugs or glucocorticoids on demand, and 22% were being treated with biologic agents. When differences between pediatric and adult patients were globally analyzed, adults tended to have longer attacks duration and presented more frequently with chest pain and headache, while abdominal pain, vomiting, cervical adenitis, and pharyngitis predominated in pediatric patients. No differences in outcomes and treatment requirements were observed in both age groups.

Conclusion: This study has contributed to characterize R92Q-related disease by identifying trends in disease phenotypes depending on the age at disease onset.

Keywords: tumor necrosis factor receptor-associated periodic syndrome, R92Q, low-penetrance variants, autoinflammatory diseases, pediatric onset, adult onset

INTRODUCTION

The term autoinflammatory diseases was first coined in 1999 by Kastner et al. to encompass a group of clinical syndromes characterized by an increased systemic inflammatory reaction, mediated predominantly by cells and molecules of the innate immune system, and caused by mutations in genes involved in the control of inflammatory pathways (1). Among autoinflammatory diseases, tumor necrosis factor receptor-associated periodic syndrome (TRAPS; OMIM 142680), described as familial Hibernian fever in 1982 (2), was defined as an autosomal-dominant disease caused by mutations in the *TNFRSF1A* gene (located on chromosome 12p13) in 1999 (1).

Although classically TRAPS affects mostly children below 10 years of age, it can also occur in adult patients (3–9). No sex dominance has been reported (6–11). Clinical features of TRAPS include recurrent fever episodes associated with musculoskeletal symptoms, migratory rash, and ocular manifestations (5–14). Raised acute phase reactants during attacks are also typical (5, 8, 10, 11). About 10–15% of patients with TRAPS may develop amyloidosis (6–8, 10, 11, 15). Psychological stress, physical exercise, infections, menstruation, or vaccinations have been occasionally identified as trigger factors of the attacks (5).

Most of the sequence variants identified in TRAPS patients are located in the exons 2–4 (see Infevers database in http://fmf.igh.cnrs.fr/ISSAID/infevers) (16). Those missense substitutions disrupting structurally important cysteine–cysteine disulfide bonds in the extracellular domain and other mutations, such as T50M, are known as structural or pathogenic variants (5, 14). Moreover, two frequent variants, R92Q (the common name for p.Arg121Gln, located in exon 4) and P46L (also known as p.Pro75Leu, located in exon 3), are known by causing a variable TRAPS phenotype in some patients. In addition, these mutations can be observed in asymptomatic first-degree relatives and in healthy individuals (8, 14). For these reasons, R92Q and P46L have been recently classified as variants of uncertain significance (17). However, the potential pathogenic role of low-penetrance mutations causing TRAPS or TRAPS-like phenotypes still generates controversy among investigators (5, 6, 9, 12, 13).

Several studies have reported that patients carrying R92Q tend to present with milder disease phenotype and better long-term prognosis compared with those carrying structural or pathogenic *TNFRSF1A* mutations, who usually suffer from more severe manifestations and long-term complications (e.g., amyloidosis) (4–11, 18). While structural mutations are typically observed in children, those patients with adult onset of TRAPS more often carry the R92Q variant (4, 8).

Studies focused on R92Q-related disease are scarce. In addition, no studies on TRAPS associated with R92Q variant (or R92Q-related disease) differentiating disease phenotype, treatment requirements, and outcomes according to the age at disease onset (pediatric and adult) have been performed to date. Therefore, we aimed to investigate clinical and laboratory features, therapeutic approaches, and long-term outcomes in a cohort of pediatric and adult patients carrying the R92Q low-penetrance variant in *TNFRSF1A* gene, with special interest in the analysis of differences between the two age groups. Previous pediatric and adult case series of patients with R92Q-related disease reported in the literature were also reviewed and used for final comparisons.

MATERIALS AND METHODS

Patients' Selection and Data Collection

From January 2006 to June 2016, we retrospectively reviewed medical charts of pediatric and adult patients diagnosed with an autoinflammatory disease attributed to R92Q variant in *TNFRSF1A* gene, in four reference centers for autoinflammatory diseases (Clinical Unit of Autoinflammatory Diseases, Departments of Autoimmune Diseases and Immunology, Hospital Clínic of Barcelona, Barcelona, Spain; Pediatric

Rheumatology Unit, Department of Pediatrics, Hospital Sant Joan de Déu, Barcelona, Spain; Rheumatology Unit, Department of Internal Medicine, Arcispedale Santa Maria Nuova – IRCCS, Reggio Emilia, Italy; and Autoimmune and Systemic Diseases Unit, Department of Internal Medicine, Hospital Vall d'Hebron, Barcelona, Spain).

Patients were included if an autoinflammatory disease was suspected after ruling out autoimmune, infectious, or malignant causes, and R92Q low-penetrance variant in *TNFRSF1A* gene was found. Patients with structural variants in *TNFRSF1A* gene or with concomitant mutations in *MEFV*, *MVK*, and *NLRP3* genes were excluded to avoid potential confounding factors for a more accurate diagnosis.

The recently published provisional Eurofever classification criteria for autoinflammatory diseases (9) were used to assess the level of agreement with TRAPS diagnosis in our patients. Variables (and their values) for TRAPS classification included: duration of episodes more than 6 days (19 points), presence of periorbital edema (21 points) or migratory rash (18 points), and also absence of vomiting (14 points) or oral aphthae (15 points). In patients with structural mutations, a cut-off value ≥43 was reported to yield 80% sensitivity and 91% specificity for TRAPS classification (9). Of note, in the same study, these criteria were associated with lower sensitivity and specificity (59 and 84%, respectively) for patients carrying the R92Q variant, although 52% of them could be still classified as TRAPS (9).

Clinical features recorded included frequency and duration of attacks, and presence of fever, arthralgia/arthritis, myalgia, abdominal and chest pain, cutaneous rash, ocular symptoms, and other less frequent manifestations (**Table 1**). Type of medications used and response to them, at disease onset and during follow-up, as well as their continuous or on-demand administration, were also collected. Laboratory parameters included complete blood cell counts, C-reactive protein (CRP) and/or serum amyloid A (SAA) levels, erythrocyte sedimentation rate (ESR), urinalysis with proteinuria, and markers of autoimmunity, such as antinuclear antibodies, rheumatoid factor, and complement levels. Genetic testing of the most common genes causing monogenic autoinflammatory diseases (*TNFRSF1A* for TRAPS, *MEFV* for familial Mediterranean fever, *MVK* for mevalonate kinase deficiency, and *NLRP3* for cryopyrin-associated periodic syndromes) was carried out.

This retrospective study was approved by the Research Ethics Committee of the Hospital Clínic of Barcelona. Patients' information was dissociated prior to analysis, and all procedures were performed in accordance with the ethical principles expressed in the 2013 Declaration of Helsinki.

Groups Based on Age at Disease Onset and Review of the Literature

Based on previous studies, patients aged <16 and ≥16 years at disease onset were considered to have pediatric and adult disease onset, respectively (4, 11, 13). In addition, those cohort series of patients with R92Q-related disease reported until 2016 (identified through PubMed search) with consistent data about clinical manifestations and outcomes during the follow-up, which also provided separate information with regard to the age of disease onset (pediatric and adult), were compared with our case series and used for global calculations.

Statistical Analysis

Results (in text and tables) are expressed as mean ± SD or median plus range, where applicable. Chi-square or Fisher's exact tests were used for contingency tables. Quantitative differences between groups were analyzed by using Student's unpaired *t*-test. Data were analyzed with the SPSS PC statistical package (version 20.0). Differences with a value of $p < 0.05$ were considered statistically significant.

RESULTS

Overall Characteristics of Patients of All Ages with R92Q-Related Disease

A total of 18 patients with R92Q variant in *TNFRSF1A* gene following inclusion criteria were analyzed. Seven patients were excluded because of carrying the R92Q variant and other concomitant mutations in *NLRP3* and *MEFV* genes, or they presented with a disease phenotype permitting a different definite diagnosis. None of the included patients met diagnostic criteria for periodic fever, aphthous stomatitis, pharyngitis, and adenitis syndrome (19).

When provisional Eurofever classification criteria for TRAPS (9) were applied in our patients, 10 (56%) of them reached the cut-off for TRAPS classification. Among these, four (36%) were children and six (86%) adult patients ($p = 0.066$).

Nine (50%) patients were female and nine (50%) were male. In 11 (61%) patients, disease onset occurred during infancy, at a mean age of 7.6 years (median 8 years; range 1–15 years); and in seven (39%) patients, symptoms started during adulthood, at a mean age of 25 years (median 23 years; range 16–43 years). Seven (39%) and 11 (61%) patients were diagnosed during pediatric and adult age, respectively. Overall, mean diagnostic delay was 5 years (median 3 years; range 4 months–25 years).

All patients presented with febrile recurrent episodes with disease-free intervals. A remarkable intersubject and intrasubject variability was observed with regard to duration and frequency of attacks (**Table 1**). The most common symptoms accompanying fever episodes were arthralgia/arthritis (61%), myalgia (39%), asthenia/fatigue (44%), abdominal pain (39%), headache (33%), odynophagia (33%), skin rash (29%), and chest pain (22%). Other less frequent manifestations included facial/periocular edema, oral aphthae, cervical adenitis, and conjunctivitis. A positive family history of an autoinflammatory (R92Q-related) disease was detected in five (28%) patients (four children and one adult).

During attacks, 80% of patients had increased CRP or SAA levels and 50% of them showed high ESR values. However, hemoglobin levels and leukocyte and platelet counts were abnormal in less than half of patients during attacks. At the end of follow-up, proteinuria or other AA amyloidosis signs were not observed in any of our patients. With regard to genetic results, all patients included in the study carried R92Q low-penetrance variant in *TNFRSF1A* gene (16 of them as heterozygous mutations and two in homozygosity), and no other mutations were identified in *MEFV* (17 patients), *MVK* (16 patients), or *NLRP3* (14 patients) genes.

TABLE 1 | Demographic, clinical, and laboratory features of patients with R92Q variant in our study series.

	A	B	C	*p* Value (A vs. B)
	R92Q patients with pediatric onset (*n* = 11)	R92Q patients with adult onset (*n* = 7)	All R92Q patients (*n* = 18)	
Demographic data				
Sex (female/male)	4/7	5/2	9/9	0.34
Age at symptoms onset (years)	7.6; 8 (1–15)	25; 23 (16–43)	14.3; 12 (1–43)	0.004
Age at diagnosis (years)	12; 12 (5–16)	31; 25 (16–48)	19; 16 (5–48)	0.015
Time from disease onset to diagnosis (years)	4.1; 4 (0.3–9)	5.8; 2 (0.3–25)	5; 3 (0.3–25)	0.65
Follow-up (years)	6.2; 6 (2–10)	4; 5 (1–8)	5.4; 5.5 (1–10)	0.18
Positive family history	4 (36)	1 (14)	5 (28)	0.60
TRAPS Eurofever classification criteria[a]	4 (36)	6 (86)	10 (56)	0.066
Clinical features[b]				
Fever (≥38°C)	11 (100)	7 (100)	17 (100)	1
Asthenia/fatigue	6 (55)	2 (29)	8 (44)	0.37
Arthralgia/arthritis	6 (55)	5 (71)	11 (61)	0.64
Myalgia	4 (36)	3 (43)	7 (39)	1
Abdominal pain	5 (46)	2 (29)	7 (39)	0.63
Vomiting	1 (9)	0 (0)	1 (6)	1
Chest (pleuro-pericardial) pain	1 (9)	3 (43)	4 (22)	0.25
Skin rash	2 (18)	3 (43)	5 (28)	0.33
Headache	3 (27)	3 (43)	6 (33)	0.63
Conjunctivitis	2 (18)	1 (14)	3 (17)	1
Periorbital edema	1 (9)	1 (14)	2 (11)	1
Cervical adenitis	3 (27)	0 (0)	3 (17)	0.25
Pharyngitis/odynophagia	4 (36)	2 (29)	6 (33)	1
Oral aphthae	2 (18)	1 (14)	3 (17)	1
Attacks characteristics[c]				
Duration (days)	22; 4 (2–160)	35; 21 (4–90)	27; 11 (2–160)	0.056
Frequency (per year)	12; 6 (1.5–50)	5; 6 (0.3–8)	9; 6 (0.3–50)	0.20
Laboratory (during attacks)[d]				
CRP >1.5 mg/dL and/or SAA >6.4 mg/dL	6/8 (75)	6/7 (86)	12/15 (80)	1
ESR >20 mm first hour	4/8 (50)	2/4 (50)	6/12 (50)	1
Leukocyte count >11,000/mm^3	3/8 (38)	2/5 (40)	5/13 (38)	1
Hemoglobin <120 mg/L	2/8 (25)	0/5 (0)	2/13 (15)	0.5
Platelets count >350,000/mm^3	0/7 (0)	2/5 (40)	2/12 (17)	0.15
Proteinuria (absence) at the end of follow-up	10/10 (100)	6/6 (100)	15/15 (100)	1
Other studied genes (negative/performed)				
MEFV	11/11 (100)	6/6 (100)	17/17 (100)	1
MVK	9/9 (100)	7/7 (100)	16/16 (100)	1
NLRP3	8/8 (100)	6/6 (100)	14/14 (100)	1

CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; MEFV, Mediterranean fever gene; MVK, mevalonate kinase gene; NLRP3, nod-like receptor family pyrin domain containing 3 gene; TRAPS, tumor necrosis factor receptor-associated periodic syndrome.

Continuous values are given as mean; median (range).

[a]*Patients achieving provisional Eurofever classification criteria for TRAPS (9).*

[b]*Values as total number of patients and %.*

[c]*Because a remarkable intrasubject variability with regard to duration and frequency of attacks was found in the majority of patients, only the highest values were used for calculations.*

[d]*Abnormal values during attacks, later normalized (from available results).*

The main demographic, clinical, and laboratory features of all patients with R92Q variant from our patients are depicted in **Table 1**. Data from the main (all retrospective) series with mixed pediatric and adult results published to date are shown in **Table 2**.

Global results from series with patients of all ages with R92Q-related disease (5, 8–10, 12), including ours, confirmed no sex preference and a family history of an autoinflammatory (R92Q-related) disease was recorded in 7–28% of cases (5, 8, 12). The age at disease onset ranged from less than 1 year to the fourth to sixth decades. The mean/median of attacks duration varied between 4.7 and 16 days (8, 10, 12) and the frequency of attacks between 6 and 11 episodes per year (10) (with marked inter- and intra-study variability for both parameters). Fever was present in almost 100% of patients (5, 9, 10, 12), except in one study that showed lower prevalence (8). Arthralgia/arthritis was present in about half to two-thirds of patients, myalgia in 39–66%, abdominal pain in 39–66% (5, 8–10, 12), vomiting in 6–40% (5, 9, 12), chest/pleuro-pericardial pain in 22–33%, skin rash in 20–36%, headache in 16–53%, conjunctivitis in 6–20%, periorbital edema in 7–19%, cervical adenitis or lymphadenopathy in 17–26%, odynophagia or pharyngitis in 12–33%, and oral aphthosis in 14–40% of cases (5, 8–10, 12). Of note, the study by Hull et al. (10) reported a higher frequency of joint, muscular, skin, and ocular involvement than the other studies (**Table 2**). Between 80 and 100% of patients

TABLE 2 | Demographic, clinical, and laboratory features of the main series combining adult and pediatric patients with R92Q-related disease.

	Hull et al. (10)	Ravet et al. (8)	Gattorno et al. (12)[b]	Lachmann et al. (5)[c]	Federici et al. (9)[d]	Ruiz-Ortiz et al. (present series)
Demographic data						
N	9	34	15	54	78	18
Sex (female/male)	3/6	17/17	–	–	38/40	9/9
Age at symptoms onset (years)[a]	22 (<1–53)	19	58 ± 64	6 (0–53)	6 (3–19)	12 (1–43)
Age at diagnosis (years)[a]	–	–	–	–	–	16 (5–48)
Time from disease onset to diagnosis (years)[a]	–	–	–	–	6.4 (3.4–25.9)	3 (0.3–25)
Follow-up (years)[a]	–	–	–	–	13	5.5 (1–10)
Positive family history (%)	–	21	7	19	–	28
Clinical features (%)						
Fever (≥38°C)	100	48	100	94	100	100
Asthenia/fatigue	–	–	–	–	72	44
Arthralgia/arthritis	89	48	17	66	65	61
Myalgia	89	48	53	66	28	39
Abdominal pain	56	39	60	66	59	39
Vomiting	–	–	40	26	26	6
Chest (pleuro-pericardial) pain	33	32	13	22	24	22
Skin rash	78	36	33	30	20	28
Headache	–	16	53	39	5	33
Conjunctivitis	100	6	13	17	20	17
Periorbital edema	78	12	7	17	19	11
Cervical adenitis/lymphadenopathy	–	19	60	25	26	17
Pharyngitis/odynophagia	–	12	67	24	22	33
Oral aphthae	–	–	40	14	15	17
Attacks characteristics						
Duration (days)	16 (6–30)	7.4	4.7 ± 3.7	–	–	11 (2–160)
Frequency (per year)	11 (9–>12)	–	–	–	–	6 (0.3–50)
Increased inflammatory markers during attacks (%)	100	100	–	–	–	80
Other studied genes (negative/performed)	–	MEFV (some positive)	MEFV, MVK	MEFV (22/22), MVK (11/11), NLRP3 (2/2)	–	MEFV (17/17), MVK (16/16), NLRP3 (14/14)
Amyloidosis development (%)	0	6.2	–	0	–	0

[a]*Continuous results as mean or median, plus SD or range (when available).*

[b]*Data from 15 patients with TNFRSF1A low-penetrance variants; among them, 13 (87%) patients carried R92Q (12).*

[c]*Data from 59 patients with TNFRSF1A P46L and R92Q variants; among them, 54 (91.5%) patients carried R92Q (5).*

[d]*Data from 78 patients with TNFRSF1A low-penetrance mutations, no mutations or genetic test not done or P46L and R92Q variants; among them, 57 (73%) patients carried R92Q (9).*

had elevated acute phase reactants (mainly CRP and/or SAA) during attacks (8, 10). Overall, results from our study group did not differ with those from the previously reported series.

Differences between Patients with Pediatric and Adult Onset of R92Q-Related Disease

No statistically significant differences with regard to clinical and laboratory features at disease presentation were found in the present series between pediatric and adult patients (**Table 1**). However, when previous studies on R92Q-related disease including patients with pediatric (7, 11, 13) and adult onset (6, 7) were analyzed together with our results (**Table 3**), no sex predominance was observed and a positive family history of R92Q-related disease tended to be higher in pediatric (4–50%) than in adult patients (6–17%). The mean/median age at disease onset was of 3.6–8 years in children and 23–28.8 years in adults. Duration of attacks tended to be longer in adult patients (mean/median from 7 to 21 days) than in children (from 4 to 9 days). Frequency of attacks was similarly heterogeneous in both groups. Among clinical features, fever was equally present in almost all patients, in all studies. Arthralgia/arthritis, myalgia, skin rash, ocular symptoms, and oral aphthae occurred in a similar proportion in both groups. Chest pain was consistently reported in almost half of adult patients and less frequently in most pediatric studies. Headache was also observed in a higher proportion in adults (42–43%) than in children (20–30%). Conversely, abdominal pain was more often presented by children (40–67%) than by adults (25–33%). Vomiting was not reported in adult series but occurred in 9–30% of pediatric patients. Cervical adenitis or lymphadenopathy (27–65 vs. 0–19%) and pharyngitis or odynophagia (13–65 vs. 11–29%) were predominantly observed in children (6, 7, 11, 13).

Disease Outcomes and Therapeutic Approaches during Disease Course

Patients in our study were followed for a mean of 5.4 years (median 5.5 years; range 1–10 years). Only two recent studies have also reported information from patients with R92Q-related disease after a long-term follow-up period (6, 11) (**Table 3**). Therapeutic strategies in ours and those previous studies similarly included the use of non-steroidal anti-inflammatory drugs (NSAIDs),

TABLE 3 | Characteristics of patients with R92Q low-penetrance *TNFRSF1A* variants in studies including patients with pediatric and adult onset.

	Pediatric-onset series				Adult-onset series		
	Dodé et al. (7)	Lainka et al. (13)[b]	Pelagatti et al. (11)	Ruiz-Ortiz et al. (present series)	Dodé et al. (7)	Cantarini et al. (6)[c]	Ruiz-Ortiz et al. (present series)
Demographic data							
Patients (*N*)	6	15	20	11	6	25	7
Sex (female/male)	3/3	–	9/11	4/7	1/5	17/19	5/2
Age at symptoms onset (years)[a]	7.3 (2–15)	5 (1–14)	3.6 (0.6–13)	8 (1–15)	28.8 (22–36)	26.6 ± 15	23 (16–43)
Age at diagnosis (years)[a]	–	7 (1–16)	6.1 (1.2–15)	12 (5–16)	–	–	25 (16–48)
Time from disease onset to diagnosis (years)[a]	–	–	–	4 (0.3–9)	–	–	2 (0.3–25)
Follow-up (years)[a]	–	–	7.3 (1.7–14.3)	6 (2–10)	–	12.7 ± 11.3	5 (1–8)
Positive family history (%)	50	–	4	36	17	6	14
Clinical features (%)							
Fever (≥38°C)	100	100	100	100	100	97	100
Asthenia/fatigue	–	–	–	55	–	–	29
Arthralgia/arthritis	17	53	40	55	17	55	71
Myalgia	–	27	35	36	–	55	43
Abdominal pain	67	40	40	46	33	25	29
Vomiting	–	20	30	9	–	–	0
Chest (pleuro-pericardial) pain	50	20	4	9	50	50	43
Skin rash	50	33	20	18	17	19	43
Headache	–	20	30	27	–	42	43
Conjunctivitis	–	13	10	18	–	19[d]	14
Periorbital edema	–	–	0	9	–	19[d]	14
Cervical adenitis/lymphadenopathy	–	40	65	27	–	19	0
Pharyngitis/odynophagia	–	13	65	36	–	11	29
Oral aphthae	–	–	35	18	–	25	14
Attacks characteristics							
Duration (days)	6 (1–20)	9 (2–24)	5.9 (3–15)	4 (2–160)	7.5 (2–20)	>7 (69%)	21 (4–90)
Frequency (per year)	20 (6–30)	–	10.3 (3–20)	6 (1.5–50)	27.6 (6–48)	7 ± 3.9	6 (0.3–8)
Increased inflammatory markers during attacks (%)	–	100	100	75	–	100[e]	86
Other studied genes (negative)	MEFV	MEFV, MVK	MEFV, MVK	MEFV, MVK, NLRP3	MEFV	MEFV, MVK, NLRP3, NLRP12	MEFV, MVK, NLRP3
Amyloidosis development (%)	17	–	0	0	0	0	0

[a]*Continuous results as mean or median, plus SD or range (when available).*

[b]*Data obtained from 15 patients with R92Q variants in TNFRSF1A gene (13).*

[c]*Data from 36 patients with TNFRSF1A low-penetrance variants; among them, 25 (69%) patients carried R92Q (6).*

[d]*Overall value for ophthalmological abnormalities (which included the presence of conjunctivitis and/or periorbital edema) in this series was 19%.*

[e]*All patients had high serum amyloid A values but normal C-reactive protein levels.*

colchicine, oral glucocorticoids (usually at a dose equivalent to ≥0.5 mg/kg/day of prednisone), and biologic agents (anakinra and etanercept, as IL-1 and TNF blockers, respectively).

Medications used in our cohort are illustrated in **Table 4**. As starting treatment, NSAIDs on demand were used in 6 patients, colchicine in 2 patients, glucocorticoids in 11 patients (5 with continuous and 6 with on-demand administration), and etanercept and anakinra, in 1 and 2 patients, respectively. At the end of follow-up, five (28%) patients were asymptomatic and treatment free (representing 18% of pediatric and 43% of adult patients), five (28%; 36% of pediatric and 14% of adult patients) continued receiving on-demand NSAIDs, four (22%; 27% of pediatric and 14% of adult patients) were on glucocorticoids on demand, and four (22%; 18% of pediatric and 28% of adult patients) were treated with biologic agents (one with weekly etanercept, and two and one with anakinra, daily and on demand, respectively).

Previous data on disease outcomes and treatment used at the end of follow-up in a series of pediatric patients with R92Q-related disease revealed that 25% of cases had shown spontaneous resolution of symptoms, 12 and 44% were being treated with NSAIDs and glucocorticoids on demand, respectively, and 18% with continuous glucocorticoids or biologic agents (11). In a previous adult series, at the end of the study, about 8% of cases were receiving only NSAIDs, 46% glucocorticoids on demand, 23% continuous glucocorticoids, 3% colchicine, and 19% were being treated with cytokine blockers (6).

Although these studies analyzed small number of patients, all of them showed similar trends regarding disease outcomes and therapeutic strategies utilized, without clear differences between pediatric and adult patients.

DISCUSSION

R92Q has been recently classified by a panel of expert clinicians and geneticists in autoinflammatory diseases as a variant of uncertain significance because its common presence in the general population and by the fact that this variant does not segregate with the phenotype in members of the same family

TABLE 4 | Treatment characteristics at baseline and at the end of follow-up.

	R92Q patients with pediatric onset ($n = 11$)	R92Q patients with adult onset ($n = 7$)	All R92Q patients ($n = 18$)
Initial treatment			
NSAIDs	3 (OD)	3 (OD)	6 (OD)
Colchicine	1 (C)	1 (C)[c,d]	2 (C)
Glucocorticoids	5 (OD); 2 (C)[a,b]	1 (OD); 3 (C)[c,d,e]	6 (OD); 5 (C)
Biological agents	1 AN (C)[a]; 1 ET (C)[b]	1 ET/AN (C)[d]	1 AN (C); 1 ET (C); 1 ET/AN (C)
Treatment at the end of follow-up			
No treatment	2	3	5
NSAIDs	4 (OD)	1 (OD)	5 (OD)
Colchicine	0	0	0
Glucocorticoids	3 (OD)	1 (OD)	4 (OD)
Biological agents	1 AN (OD)[a]; 1 ET (C)[b]	1 AN (OD)[d]; 1 AN (C)[e]	3 AN (2 OD, 1 C); 1 ET (C)

AN, anakinra; C, continuous; ET, etanercept; OD, on demand; NSAIDs, non-steroidal anti-inflammatory drugs.

Patients [a] and [b] initially received glucocorticoids and a biological agent; and at the end of follow-up, patient [a] is receiving anakinra on demand and patient [b], etanercept 50 mg/week. Patient [c] received colchicine and glucocorticoid therapy. Patient [d] was treated with colchicine, prednisone, etanercept, and finally, anakinra (on an initial continuous administration, which could be switched to on demand during the disease course). Patient [e] started with continuous glucocorticoids and was later switched to anakinra.

(17). Compared to patients with *TNFRSF1A* structural or pathogenic mutations, patients carrying the R92Q variant exhibit milder disease presentation and disease outcome, shorter febrile episodes, lower intensity and frequency of typical symptoms, a considerably lower or inexistent risk for developing amyloidosis, and a later (even during adulthood) disease onset (4, 6, 8, 10, 11, 14). Despite of this milder phenotype, almost all patients with R92Q variant are usually treated with (on demand or continuous) NSAIDs or glucocorticoids, and a remarkable proportion (up to 20%) of them may also require biologic therapy (4, 6, 11, 13). In addition, R92Q variant has been found in a higher proportion of patients with autoinflammatory symptoms than in several control populations (6, 7, 13–15). For these reasons, many expert clinicians still consider R92Q a low-penetrance mutation rather than a polymorphism (4, 6–8, 10, 12–15).

Definite diagnosis of TRAPS usually relies on the presence of suggestive clinical features supported by the existence of functional mutations in the *TNFRSF1A* gene. In this regard, the recent TRAPS provisional Eurofever classification criteria for patients with structural or pathogenic mutations yielded reasonable sensitivity and specificity (9). The same validation study showed a remarkable lower sensitivity and specificity for patients carrying the R92Q variant, but at least 52% of R92Q patients reached the cut-off for TRAPS classification (9). Similarly, 56% of our patients with R92Q-related disease could be also classified as having TRAPS. Interestingly, this proportion was considerably higher for our adult patients (86%) and lower for our children (30%). The good concordance with the original TRAPS validation study for our patients carrying R92Q makes our results reliable, particularly for those patients with adult disease onset.

Clinical and laboratory features from previous series including R92Q patients of all ages (5, 8–10, 12) are equivalent to those found in our series. When patients with R92Q-related disease are compared with those carrying structural *TNFRSF1A* mutations, a family history of an autoinflammatory (R92Q-related) disease and the presence of myalgia, abdominal pain, and ocular symptoms are more frequently observed in the group with structural variants, and pharyngitis/odynophagia and oral aphthosis predominate in patients with R92Q variant (5, 9). The remaining symptoms are similarly presented by patients with structural and R92Q variants (5, 9). While a variable proportion of patients carrying structural variants may develop amyloidosis over time, those carrying R92Q are at a very low (or absent) risk for developing this complication (5).

When patients with R92Q-related disease with onset during childhood (7, 11, 13) are compared with those initiating symptoms during adulthood (6, 7), a positive family history of an autoinflammatory disease seems to predominate in pediatric patients. No sex dominance exists. Although the age at disease onset shows wide dispersion, most children usually present with disease-related symptoms at 3–7 years of age, and among adults, symptoms often start during the second decade of life. Duration of attacks seems to be longer in adults, but heterogeneity in duration and frequency of attacks is equally observed in both groups. With regard to clinical manifestations, fever is shared by almost all patients; and musculoskeletal, cutaneous, and ocular symptoms are common features similarly present in both age groups; oral aphthosis occurs in a lower proportion, with no differences between children and adults. However, pleuro-pericardial/chest pain and headache are more frequently observed in adult patients than in children. Conversely, other features, such as abdominal pain, vomiting, cervical adenitis/lymphadenopathy, and pharyngitis/odynophagia, seem to be predominant in pediatric patients (6, 7, 11, 13).

Although no treatment guidelines for autoinflammatory diseases have been elaborated yet, therapeutic approaches for all these conditions aim to control symptoms and prevent attacks and long-term complications. Previous investigations have documented differences in disease outcomes and treatments used in TRAPS patients carrying structural variants compared to those carrying R92Q (or other low-penetrance mutations) (6, 11). While patients with structural mutations usually have a chronic and relapsing course, with an increased risk for developing amyloidosis, and the majority of them also require biologic therapy (mainly IL-1 and TNF blockers) (6, 11), most patients with R92Q-related disease can be treated with NSAIDs or glucocorticoids on demand only (6, 11), and in about 25% of patients (pediatric and adults), symptoms may evolve to spontaneous resolution during the course of the disease, without requiring any treatment (11). However, continuous or on-demand administration of biologic agents can be indicated in about 20% of cases (6, 11).

The molecular mechanisms responsible for clinical phenotypes of TRAPS and the role of structural or pathogenic mutations and those low-penetrance variants, such as R92Q, still remain to be completely elucidated. While forms of *TNFRSF1A* cysteine mutations clearly destabilize the protein structure and produce defects in cell surface expression and TNF binding, R92Q mutants, which share structural similarities with the wild-type protein, also share similar mechanisms of action with

wild-type *TNFRSF1A* (20). In this sense, structural mutations have also shown to produce deeper disturbances in T-cell function than R92Q and other low-penetrance variants (21). However, recent investigations have demonstrated a constitutive activity of R92Q mutants associated with TRAPS, which might be explained by configurational changes induced after ligand binding that can act as the trigger for TNFR1 signaling. Indeed, these structural changes are not present in wild-type receptors (22). Other data, in patients with multiple sclerosis, point toward the enhancement of the interaction between the receptor and its ligand by the R92Q variant, resulting in the potentiation of TNF-mediated pathways (23). In addition, several authors have postulated a potential role of low-penetrance *TNFRSF1A* variants (including R92Q, P46L, and other recently reported, such as V95M, D12E, and R104Q) in causing different autoinflammatory phenotypes (6, 21). These low-penetrance variants might also contribute, as a possible susceptibility factor in the development of multifactorial polygenic inflammatory or autoimmune conditions, such as idiopathic recurrent acute pericarditis, Behçet's disease, juvenile idiopathic arthritis, and other autoinflammatory diseases (6, 14, 24–27).

This study has several limitations that include (a) the present study and all the previous series on TRAPS and R92Q-related disease published to date are retrospective and included a relatively small number of patients; (b) very few of them differentiated patients with pediatric or adult disease onset; (c) most TRAPS series studied patients with R92Q together with other low-penetrance variants, thus providing mixed results; (d) the small number of patients analyzed, and the heterogeneity in some variables and results, make comparisons mainly estimative between patients carrying structural and low-penetrance mutations and between R92Q carriers of different ages; and (e) pharmacological treatments used in all the studies were guided by personal experience, and since no evidence-based therapeutic protocols have been elaborated yet, no strong recommendations can be made in this regard. However, we consider that the present study also has several strengths: (a) our results were in concordance with general R92Q-related disease series including patients of all ages, and with those studies focused in patients with pediatric and adult onset; (b) our R92Q patients achieved criteria for TRAPS classification in a similar proportion than the R92Q patients included in the original Eurofever validation study (9), which was also comparable with TRAPS caused by structural mutations for our adult patients with R92Q variant.

In summary, this study has contributed to characterize TRAPS associated with R92Q variant, particularly in differentiating clinical phenotypes according to the age at disease onset. Adult patients tend to have longer duration of attacks and exhibit more frequently chest pain and headache, than pediatric patients. Abdominal pain, vomiting, cervical adenitis/lymphadenopathy, pharyngitis/odynophagia, and a family history of an autoinflammatory disease seem to predominate in pediatric patients. With independence of the age at disease onset, most patients with R92Q-related disease usually receive on demand NSAIDs and glucocorticoids, and about a quarter part of them may have a resolution of symptoms over time, without requiring treatment. However, up to 20% of patients may still need biologic agents (IL-1 or TNF blockers) at the end of follow-up to control disease activity.

The present results evidence the level of clinical and genetic complexity of TRAPS phenotype caused by the R92Q variant and also lead to emphasize the core importance of interpreting genetic results in an appropriate clinical context. In order to corroborate these findings and to achieve a better understanding of TRAPS spectrum, further studies including a large number of patients with TRAPS, caused by structural mutations, and also by R92Q and other low-penetrance *TNFRSF1A* variants, are granted. In addition, whether or not this subset of patients should undergo whole exome sequencing to search for concomitant disease-causing mutations in unknown genes may be a matter of discussion.

AUTHOR CONTRIBUTIONS

Category 1: conception and design of study: ER-O and JH-R; acquisition of data: ER-O, EI, AS, SB, ME-R, RC-M, AT, and JH-R; analysis and/or interpretation of data: ER-O, ME-R, and JH-R. *Category 2*: drafting the manuscript: ER-O and JH-R; revising the manuscript critically for important intellectual content: ER-O, EI, AS, SB, JY, JA, and JH-R. *Category 3*: approval of the version of the manuscript to be published: ER-O, EI, AS, SB, ME-R, RC-M, AT, JY, JA, and JH-R.

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REVIEW
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Monogenic Autoinflammatory Diseases with Mendelian Inheritance: Genes, Mutations, and Genotype/Phenotype Correlations

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Autoinflammatory diseases (AIDs) are a genetically heterogeneous group of diseases caused by mutations of genes encoding proteins, which play a pivotal role in the regulation of the inflammatory response. In the pathogenesis of AIDs, the role of the genetic background is triggered by environmental factors through the modulation of the innate immune system. Monogenic AIDs are characterized by Mendelian inheritance and are caused by highly penetrant genetic variants in single genes. During the last years, remarkable progress has been made in the identification of disease-associated genes by using new technologies, such as next-generation sequencing, which has allowed the genetic characterization in undiagnosed patients and in sporadic cases by means of targeted resequencing of a gene panel and whole exome sequencing. In this review, we delineate the genetics of the monogenic AIDs, report the role of the most common gene mutations, and describe the evidences of the most sound genotype/phenotype correlations in AID.

Keywords: autoinflammatory diseases, hereditary periodic fevers, familial Mediterranean fever, mevalonate-kinase deficiency, tumor necrosis factor receptor-associated periodic syndrome, cryopyrinopathies, inflammasome, whole exome sequencing

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INTRODUCTION

The term autoinflammatory disease (AID) was proposed in 1999 to describe a group of disorders of the innate immune system characterized by recurrent episodes of inflammation without a known origin (1). AIDs are frequently caused by genetic mutations in genes encoding proteins involved in the pathways of the inflammasome, with a crucial role of proinflammatory interleukin-1 (IL-1), which is an important cytokine of the systemic inflammatory response.

Autoinflammatory diseases have in the most of cases a genetic background, with highly penetrant mutations of single genes, but in some cases are polygenic, with a strong environmental influence that can modulate the phenotype (2).

The first AID described was the familial Mediterranean fever (FMF), which is also the most prevalent AID in the world. After FMF, other two AIDs were described: TNF-receptor associated periodic syndrome (TRAPS) (1) and hyperimmunoglobulinemia D with periodic fever syndrome [hyper-IgD syndrome (HIDS)/mevalonate kinase (MVK)] (3–5). These three forms of AID were grouped in the hereditary periodic fever syndromes, because they share fever episodes. After these,

other AIDs were identified, such as familial cold autoinflammatory syndrome (FCAS), Muckle–Wells syndrome (MWS), and neonatal onset multisystem inflammatory disease (NOMID), also known as chronic infantile neurologic cutaneous and articular (CINCA) syndrome, then grouped in the cryopyrinopathies. These disorders are also known as cryopyrin-associated periodic syndromes (CAPS).

Recently, mutations in single genes involved in IL-1 processing have been demonstrated in the deficiency of IL-1 receptor antagonist (DIRA) (6). After this, evidences of drugs able to block IL-1 in the Majeed syndrome (MS), an AID bone disease with clinical similarities to DIRA, demonstrated the pivotal role of IL-1 in this disorder (7). The same drug was used for cryopyrinopathies and DIRA, further expanding its use to other monogenic AID disorders. Other granulomatous disorders are characterized by typical granulomatous formations: Blau syndrome (familial juvenile systemic granulomatosis) is characterized by granulomatous inflammation of joint, skin, and uvea.

In this review, we will focus only on monogenic AIDs, which are mostly represented by early-onset conditions and a clear pattern of autosomal dominant or recessive transmission, at least in some of the families (**Table 1**) and the clinical significance of exonic variants according with pathogenic criteria (**Table 2**). The review will analyze the evidences about the mutations in the genes involved in the pathogenesis of the disease and the genotype/phenotype correlations (8).

Familial Mediterranean Fever

Familial Mediterranean fever is the most common AID, which is inherited as autosomal recessive disease, although features of autosomal dominant pattern of transmission have been demonstrated in several families (9). This different pattern might have conferred an evolutionary advantage in the resistance to an endemic pathogen; in fact, Clostridium, Yersinia, *Vibrio parahaemolyticus* VopS, *Histophilus somni* IbpA, Burkholderia, and other microbes that modify RhoGTPases are able to stimulate pyrin inflammasome. Pyrin play a role in sensing pathogen modification and inactivation of Rho GTPases (10,11). Furthermore, in some populations, as Sephardic Jews, Turks, Arabs, and Armenians, the carrier rate for a mutant *MEFV* allele is high, ranging from 1/3 to 1/6; this represents the highest carrier rates reported for an autosomal recessive disorder.

To estimate prevalence in FMF is difficult, because of the wide range of differences in areas of diffusion of the disease. In particular, the most affected patients belong to Middle Eastern living around the Mediterranean Sea areas.

The causing gene, *MEFV*, was identified in 1997 by two International Consortia, who named the encoded protein pyrin/marenostrin (11, 12), an intracellular regulator of IL-1 production (13, 14). The disease-causing mutations spread all over the gene, even if the exon 10 carries the most typical and severe mutation; in fact, this exon encodes for the B30.2/SPRY domain at the C-terminal end of pyrin, which is demonstrated to interact

TABLE 1 | Classification of monogenic autoinflammatory diseases (AIDs).

Disorder (abbreviation)	#OMIM	*Gene* (Locus)	Protein involved	Inheritance
Familial Mediterranean fever	249100	*MEFV* (16p13.3)	Pyrin (marenostrin)	Autosomal recessive
Hyper-IgD syndrome	260920	*MVK* (12q24.11)	Mevalonate kinase	Autosomal recessive
Mevalonate kinase deficiency	260920	*MVK* (12q24)	Mevalonate kinase	Autosomal recessive
Tumor necrosis factor receptor-associated periodic syndrome	142680	*TNFRSF1A* (12p13)	Tumor necrosis factor receptor type-1	Autosomal dominant
Familial cold autoinflammatory syndrome (FCAS)	120100	*NLRP3* (1q44)	Cryopyrin	Autosomal dominant
Muckle–Wells syndrome	191900	*NLRP3* (1q44)	Cryopyrin	Autosomal dominant
Neonatal onset multisystem inflammatory disease	607115	*NLRP3* (1q44)	Cryopyrin	Autosomal dominant
Deficiency of interleukin (IL)-1 receptor antagonist	612852	*IL1RN* (2q)	IL-1 receptor antagonist	Autosomal recessive
Blau syndrome	186580	*NOD2/CARD15* (16q12.1-13)	Nucleotide-binding oligomerization domain-containing protein 2	Autosomal dominant
Deficiency of the IL-36 receptor antagonist	614204	*IL36RN* (2q14)	IL-36 receptor antagonist	Autosomal recessive
Chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperature syndrome	256040	*PSMB8* (6p21)	Inducible subunit β of the proteasome	Autosomal recessive
Majeed syndrome	609628	*LPIN2* (18p11.31)	Lipin 2	Autosomal recessive
CARD14-mediated pustular psoriasis	177900	*CARD14* (17q25.3)	Caspase recruitment domain family member 14	Autosomal dominant
NLRP12-autoinflammatory disease	609648	*NLRP12* (19q13.42)	Monarch 1	Autosomal dominant
Pyogenic arthritis, pyoderma gangrenosum, and acne syndrome	604416	*PSTPIP1* (15q24-25)	CD2 antigen-binding protein 1	Autosomal dominant
Deficiency of adenosine deaminase 2	615688	CECR1 (22q11.1)	Adenosine deaminase 2	Autosomal recessive
STING-associated vasculopathy	615934	*TMEM173* (5q31.2)	Transmembrane protein 173	Autosomal dominant
TNFRSF11A-associated disease	603499	*TNFRSF11A* (18q21.33)	Tumor necrosis factor receptor 11A	Autosomal dominant
NLRC4-associated diseases (NLRC4-MAS, SCAN4, NLRC4-FCAS)	606831	*NLRC4* (2p22.3)	NLR family CARD domain-containing Protein 4	Autosomal dominant
Sideroblastic anemia, B-cell immunodeficiency, periodic fevers, developmental delay	616084	*TRNT1* (3p26.2)	CCA-adding enzyme	Autosomal recessive
Monogenic form of systemic juvenile idiopathic arthritis	613409	*LACC1* (13q14.11)	Laccase (multicopper oxidoreductase) domain containing 1	Autosomal recessive

TABLE 2 | Clinical significance of exonic variants according with the pathogenic criteria of ClinVar database (https://www.ncbi.nlm.nih.gov/clinvar/) for genes *MEFV*, *MVK*, *TNFRSF1A*, and NLRP3.

Gene	Clinical significance					
	Conflicting interpretation	**Benign**	**Likely benign**	**Uncertain significance**	**Likely pathogenic**	**Pathogenic**
MEFV	Leu110Pro Glu148Gln Glu148Val Gly196Trp Gly304Arg Pro369Ser Arg408Gln Ile591Thr Lys695Arg	Leu110Pro Glu148Gln Gly196Trp Arg202Gln Pro369Ser Arg408Gln	Glu148Gln Arg202Gln Gly304Arg Arg408Gln Ile591Thr	Ser6Arg Val33Leu Arg42Trp Asn78Ile Asn78Ser Glu84Gln Glu93Gln Gln97Ter Asp103His Ser108Arg Leu110Pro Pro115Thr Asp122Gly Gly136Glu Gln146Ter Pro147Ala Glu148Gln Glu148Alafs Glu148Val Arg151Thr Glu163Gln Ala171Thr Gln172Pro Pro183Thr Ala193Thr Gly196Trp Glu230Lys Lys266Glu Gly304Arg Thr309Met Ala311Val Arg314His Gly320Ala Arg329His Ser339Phe Arg348His Gln356Glu Pro369Ser Pro383Arg Arg480Gln Gln440Glu Glu446Ala Lys447Asn Ala457Val Arg461Gln Val469Ala Asp505Tyr Arg579His Ile591Thr Asn599Asp Lys625Gln Pro630Alafs Arg653Cys Gly678Glu Lys695Arg Pro714Leu Lys716Glu Phe743Leu Ile772Val Pro780Thr	Gly304Arg Pro369Ser Arg408Gln Met680Ile Lys695Arg Ala744Ser	Glu148Gln Glu148Val Glu167Asp Pro180Gln Thr267Ile Glu276Ter Leu367Val Pro369Ser His404Arg Thr577Asn Arg478Gln His478Tyr Phe479Leu Ile591Thr Arg653His Met680Ile Gly687Asp Tyr688Ter Ile692del Met694Val Met694del Lys695Arg Val726Ala Ala744Ser Arg761His
MVK	Val80Ile	Arg19His	Arg19Gln	Pro11Leu	Ile268Val	Met1Thr

(*Continued*)

TABLE 2 | **Continued**

Gene	Clinical significance					
	Conflicting interpretation	**Benign**	**Likely benign**	**Uncertain significance**	**Likely pathogenic**	**Pathogenic**
		Ser52Asn	Ser52Asn Val180Ile	Val15Ala Cys21Ser Leu27Phe Val80Ile Cys101Tyr Arg106His Ala111Thr Pro200Ser Pro286Leu Gln302Ter Arg388Gln		Leu6Glyfs His20Pro Gly25Trpfs Leu41Pro Tyr116His Gly140Argfs Ala141Glyfs Ala148Thr Pro165Leu Pro167Leu Trp188Ter Gly202Arg Val203Ala Arg215Ter Leu255Pro Ile268Thr Asn301Thr Val310Met Ala334Thr Phe365Ser Val377Ile Arg388Ter
TNFRSF1A	Arg121Gln	None	Pro75Leu	Leu96Pro Val112Met Arg121Gln Val124Met Asn145Ser Glu178Lys Ile199Thr Pro269Arg Pro275Ser Pro412Ala Ser452Arg	Asp41Glu Phe89Leu Asn94Lys Arg106Gln	Cys59Arg Cys59Ser Cys62Gly Cys62Tyr Thr79Met Cys81Phe Cys99Ser Cys99Arg Cys117Arg Cys117Tyr Arg121Pro Arg121Gln
NLRP3	Val198Met Pro315Leu Arg488Lys Gln705Lys Ser728Gly Thr954Met	Gln705Lys	Met70Thr Val72Met Ser196Asn Val198Met Pro315Leu Arg488Lys His713Leu Ser728Gly Thr954Met	Ala67Glu Ala77Glu Ala77Val Arg100Cys Lys131Arg Arg137His Asn165Ser Thr195Met Val198Met Asp212Asn Ala225Val Gln250Arg Pro315Leu Lys357Arg Thr435Ala Leu447Phe Gly456Glu Lys615Asn Gln705Lys Ser728Gly Gly769Ser Leu800Met Gly811Ser Leu832Ile Ala848Pro Ala873Thr Lys880Glu Thr915Met	Leu305Pro Arg488Lys Gln602Arg	Val198Met Arg206Trp Asp303Asn Glu304Lys Phe309Ser Thr348Met Ala352Val Leu353Pro Thr405Pro Ala439Val Gly569Arg Gly571Arg Phe573Ser Glu627Gly Tyr859Cys

(*Continued*)

TABLE 2 | **Continued**

Gene	Clinical significance					
	Conflicting interpretation	**Benign**	**Likely benign**	**Uncertain significance**	**Likely pathogenic**	**Pathogenic**
				Thr923Ala Lys930Asn Thr954Met Cys990Ser Cys998Ser Lys1015Glu		

with the protein caspase 1 (15). Although five mutations represent more than 85% of all disease-associated mutations, many other mutations with different clinical penetrances have been reported so far (in the Infevers website more than 300 mutations are described) (16). Genetic test can support clinical diagnosis, confirming the presence of two mutations in the *MEFV* gene, although patients with a heterozygous mutation can show clinical pictures of FMF, even if with an incomplete phenotype (17).

In fact, there are evidences that the mutation in the second allele is not demonstrated in 20–25% of the patients with the clinical picture of FMF and a positive response to colchicine therapy (18). The reduced diagnostic accuracy of the genetic tests in terms of mutation finding is common to most of the genes studied for diagnostic purposes, since the diagnostic yield of the tests is never complete, due to factors like genetic heterogeneity, incorrect diagnosis, or phenocopies. When a heterozygous is found, the most obvious hypothesis is that the second disease allele lies in other genic regions not explored by the test (i.e., deep intronic regions); however, the second mutation has not been found also in studies analyzing the promoter and intron regions of the *MEFV* gene. In FMF, however, the evidence of autosomal dominant transmission, segregating a heterozygous mutation (17), or a complex allele (personal data) is consistent with a dosage effect, which is dependent on the type of the mutation, in analogy to models already described in other disease genes (i.e., *GJB2*, in which biallelic mutations cause autosomic recessive nonsyndromic deafness and dominant mutations in specific domains of the gene cause syndromic forms of deafness with palmoplantar keratoderma). Another possible pathogenetic model for the dominant forms could involve the interaction of genetic and environmental factors in the pathogenesis of the disease, in analogy with hemochromatosis, in which heterozygotes for the C282Y in the *HFE* gene are bona fide healthy carriers unless other factors like alcohol or viral infections induce the onset of the clinical phenotype by contributing to the accumulation of iron in the liver. In a genomic study of 22 Belgian individuals, 12 of whom had clinical pictures of AID, the pattern of Mendelian inheritance was autosomal dominant. The phenotype, different from FMF, was characterized by childhood-onset recurrent episodes of neutrophilic dermatosis, fever, elevated acute-phase reactants, arthralgia, and myalgia/myositis. The disease was named pyrin-associated autoinflammation with neutrophilic dermatosis (PAAND). Genomic analysis revealed a mutation in the *MEFV* gene, S242R. This mutation causes a loss of a binding motif in the pyrin protein different from the B30.2/SPRY domain. Interestingly, the loss of the S242 domain was observed in bacterial effectors able to activate the pyrin inflammasome, such as Clostridium difficile toxin B (TcdB). As a result, the S242R mutation has the same effect of pathogen sensing, acting as a trigger of the inflammasome activation and IL-1b production. Based on this fact, the affected patients were successfully treated with therapy targeted on IL-1b, resolving autoinflammation and neutrophilic dermatosis (19).

Another intriguing aspect in FMF and, more in general AID, is the different influences of certain mutations or polymorphisms on the phenotype. For example, an E148Q mutation is sometimes referred to as a functional polymorphism because of the high carrier rate (more than 10%) and the lack of phenotype in some homozygous patients. However, some patients may have severe disease expression as well. Thus, the suggestions put forward for the carrier state may apply to these states as well.

Environmental factors have been investigated in relation with disease severity in FMF (19). The strongest association correlated with amyloidosis was with the country of origin, not with the genotype. Another interesting study showed that Turkish children who are born and live in Turkey have a higher disease severity score compared with Turkish children living in Germany (20); this study demonstrated that the different patterns of infections influence the expression of the phenotype acting as a trigger of the weak innate immune pathway via pathogen-recognition factors.

In order to search for possible mutation in the *MEFV* gene, a multiplex ligation-dependent probe amplification was setup in 216 FMF patients (21). No copy number variants were identified, suggesting that deletion/duplication is not a mutational mechanism in MEFV. The possible functional explanation relies on the pyrin function, which is crucial in the immune surveillance, even if with genetic variants such as point mutations and functional polymorphisms able to modulate its function.

An interesting study aimed at investigating pyrin function during primate evolution analyzed the domain which contains the most MEFV mutations, named ret finger protein domain. Amino acids involved in MEFV mutations (653, 680, 681, 726, 744, and 761 residues) in human are frequently present as wild type in other primates. In some cases, the mutant may be considered the reappearance of an ancestral amino acid state. As a result, an episodic positive selection was postulated. These changes in pyrin sequence could be caused by selective pressures driven from environmental agents (22).

Regarding the therapy, both a single mutation and two mutations, supported by clinical pictures of FMF, support the use of

colchicine (23). Amyloidosis type AA is frequently correlated with the MEFV mutation M694V and the SAA1.1/SAA1.1 genotype (24).

In analogy with most of the disorders with pleiotropic expression, also for FMF, strict clinical criteria have been proposed for the diagnosis (25), taking into account the phenotypic manifestations (the recurrence of fever with serositis), the histological picture (idiopathic AA amyloidosis), and the response to therapy (in typical FMF, the colchicine test results in a favorable response). Two major criteria are necessary for a definite diagnosis of FMF. Recurrent fever without serosal involvement, cutaneous, erysipelas-like manifestations, and a positive family history for the disease in a first-degree relative are considered minor criteria for the lower specificity of the features and take part in the algorithm for diagnosis only if a major criterion is present. The presence of two mutations in *MEFV* gene is generally achieved in patients fulfilling the clinical criteria, but also, to a much lesser extent, also in subjects with atypical phenotypic features, whereas also heterozygous mutation carriers can suffer from an incomplete and even typical disease (17). For all these reasons, the detection of a single heterozygous mutation, in the presence of clear clinical symptoms, appears to be a sufficient prerequisite for a colchicine trial (23).

HIDS/Mevalonate Kinase Deficiency (MKD)

Periodic fever associated with MKD was originally identified in 1984 in patients of Dutch ancestry; they reported recurrent attacks of fever of unknown origin and a high serum IgD level (26). After this first description, the disease was named Dutch fever or HIDS. After the first report, MKD was then described in other European countries around the Mediterranean basin (27) and Asia (28).

Because of the low sensitivity and specificity of the raised IgD serum levels, the term HIDS has been replaced by periodic fever associated with MKD after the discovery of the causing mutations in the *MVK* gene (OMIM *251170) located on chromosome 12q24 (29).

Mevalonate kinase deficiency, also known as hyperimmunoglobulinemia D syndrome (OMIM 260920) is characterized by an autosomal recessive Mendelian inheritance pattern (5) and is allelic to another disorder, mevalonic aciduria (MA, OMIM 610377), characterized by a very low activity of the enzyme MVK.

As in other AIDs, the carrier rate of 1/350 in normal population allowed to hypothesize a selective advantage for heterozygous carriers; a possible explanation postulates that countries with a diet at high consumption of saturated animal fats rich in cholesterol could have selected heterozygous carriers of the most frequent *MVK* mutations (30); nevertheless, this theory has not been demonstrated and other possible explanations are possible.

So far more than 130 substitutions or deletions of the *MVK* gene have been reported (16), even if a small number of mutations (V377I, I268T, H20P, and P167L) represent the 71.5% of the whole mutation spectrum in MKD patients (29).

Several genotype–phenotype associations have been described. The most common mutation is the V377I variant, which is associated with a mild phenotype of MKD and some residual MVK activity. V377I is frequently found in a compound heterozygous state in most MKD patients (5, 31). At the opposite hand, some variants (i.e., V310M, A334T) are closely associated with a severe MA phenotype and severely impaired cellular MVK activity (32).

Interestingly, the H20P and I268T mutations have been described in intermediate phenotype, either with MA and MKD clinical signs, such as fever attacks associated with some neurological manifestations (mental retardation, cerebellar ataxia) of variable severity (33), leading to the hypothesis that the two diseases may represent the two extremities of the phenotypic spectrum which depends on the type (truncating vs non-truncating) of the mutations or the degree of impairment of the MVK enzyme activity. For example, mutations resulting into a MKD phenotype are exclusively missense, associated with a mild reduction of the enzymatic activity whereas in the MA phenotypes, frameshift and nonsense mutations are commonly reported (16), which completely inactivate the gene function. In fact, the *MVK* gene encodes the enzyme MVK, involved in the ATP-dependent phosphorylation of mevalonic acid into 5-phosphomevalonate. Mutations affecting this gene alter the MVK activity, with an overproduction of proinflammatory isoprenoids, reduced synthesis of cholesterol, and accumulation of mevalonic acid in plasma and urine. Fever rushes may be caused by high release of IL-1β as a consequence of insufficient geranylgeranyl pyrophosphate generation (32). The development of fever may be caused by a dysregulation of the MVK pathway, but the pathogenetic mechanisms leading to the autoinflammation remain to be clarified.

Tumor Necrosis Factor Receptor-Associated Periodic Syndrome

Tumor necrosis factor receptor-associated periodic syndrome (OMIM 142680) is the most common autosomal dominant AID in Europe; it was initially named "familial Hibernian fever" from the ancient Latin name "Hibernia" given to Ireland. In fact, in 1982, a large family from Scotland and Ireland was described with a new disorder, characterized by recurrent fevers, skin rashes, monocytic fasciitis, and abdominal pain (2). Since the first description, several cases have been identified in many other populations, such as Black Americans, Japanese, and patients of Mediterranean ancestry (34).

In 1998, the genetic basis of this condition was discovered and the name became TRAPS due to its relationship with the p551A receptor of TNF (TNFR1), encoded by the TNF super family receptor 1A (*TNFRSF1A*) gene (1, 2), whose mutations cause the disease. The *TNFRSF1A* gene is composed of 10 exons with the disease causing mutations, all missense and heterozygous, all concentrated into exons 2, 3, 4, and 6 (2). Based on the mutation position, they can be distinguished as high- or low-penetrance missense mutations. The high-penetrance mutations are located in cysteine-rich N-terminal domains, which are important for the assembly of the receptor's three-dimensional structure (35, 36); furthermore, they cause an early disease onset and more severe clinical manifestations; the substitutions result in single

amino acid substitutions in the cysteine rich domains (CRDs) 1, 2, or 3 of the ectodomain of the mature TNFR protein (37). These CRDs are involved in disulfide bond formation and in the folding of the extracellular portion of the protein. On the other hand, the *TNFRSF1A* low-penetrance mutations, such as R92Q and P46L, are associated with lower risk of amyloidosis and adult-onset, milder and/or atypical clinical features (16, 38–40); for instance, the P46L substitution occurs in up to 20% of clinically asymptomatic West African individuals, which suggests that it represents a polymorphism rather than a disease-causing mutation, whereas the R92Q substitution, relatively common in the Caucasian population, is a low-penetrance variant, which could have a weak contribution to disease expression. Moreover, *TNFRSF1A* mutations affecting TNF receptor shedding from cell membranes might potentially generate a selective advantage related to an increased antibacterial capacity (41). More in general, it can be assumed that, on the contrary to other fully penetrant autosomal dominant disorders, like neurofibromatosis type I (OMIM #162200), in which the new cases of the disease are all caused by new mutations in the *NF1* gene, the new cases of TRAPS belong to families in which the mutation segregates through the generations without giving manifest signs of disease. For this reason, in each proband, a careful family history for the cardinal signs of recurrent fevers, fasciitis, and cutaneous rash should always be collected.

Based on the difficulties with a clinical diagnosis of AID, a genetic test is useful in case of patients with clinical TRAPS phenotype, and a genetic diagnosis of TRAPS can be performed in the presence of a mutation in *TNFSRF1A*. Routine diagnostic analysis is limited to the exons 2, 3, 4, and 6, whereas the expansion of the analysis to the remaining coding regions of the gene is recommended only for cases with extremely suggestive phenotypes yet without a definitive diagnosis. In the absence of a family history and with borderline phenotypes, the probability of mutation finding with the extension of the genetic analysis to the whole gene remains very low and the decision should be carefully discussed with the clinician.

At pathogenic level, several mechanisms may be responsible for the disease onset, such as impaired TNF receptor shedding, defective intracellular TNF receptor trafficking to the cell surface, and subverted TNF-independent cell activation with increased production of IL-1 and IL-6, altered NF-κB pathway, increased activation of mitogen-activated protein kinases, and upregulated production of reactive oxygen species (42, 43).

The molecular link between TRAPS and IL-1 is not clear: the pathogenesis may vary with each mutation, but it is possible that IL-1 might act as a proinflammatory mediator downstream of TNF, or that aggregates of misfolded TNF receptors stimulate intracellular signals resulting in enhanced production of IL-1 and other chemokines (44).

Cryopyrinopathies (FCAS, MWS, and NOMID)

Familial cold autoinflammatory syndrome (OMIM 120100), MWS (OMIM 191900), and CINCA syndrome (OMIM 607115), also known as neonatal onset multisystem inflammatory disease (NOMID), are autosomal dominant disorders (45–47) caused by mutations in the *NLRP3* (NOD-like receptor 3, cold-induced autoinflammatory syndrome 1, also named *CIAS1*) gene, encoding for the cryopyrin protein, an important inflammasome protein that directly activates IL-1β (48). Until 2001, these diseases were considered as three different diseases. Since 2001, mutations in the NACHT domain of the *NLPR3*/*CIAS1* gene were linked to FCAS and MWS (49, 50), whereas mutations in the same gene were identified in 2002 in sporadic cases with NOMID/CINCA (48, 51); after these evidences, the three disorders were grouped under the family of CAPS. FCAS and MWS are usually familial (49), while NOMID/CINCA is sporadic (52, 53).

Cryopyrin-associated periodic syndromes are rare diseases, with an estimated prevalence of approximately 1–2 patients per 1,000,000 people in Europe and in the USA (54).

Familial cold autoinflammatory syndrome, MWS, and NOMID/CINCA share a significant symptom overlap (55), with the latter described across the world as the most severe expression of CAPS (56).

In Infevers database, 175 different nucleotide variants and more than 90 heterozygous mutations on the *NLRP3* gene have been described to date (16, 57). Mutations in *NLRP3* gene are described in approximately 60% of CAPS patients, causing the constitutive activation of the inflammasome and dysregulation with IL-1 overproduction; excessive IL-1 signaling appears to be a constant feature in the background of CAPS, driven by gain-of-function *NLRP3* mutations, even in the absence of a second signal (58). As in all the AIDs, genetic testing is confirmatory, even if the diagnosis needs to be made on clinical symptoms.

More than 40% of NOMID/CINCA patients and a less percentage of FCAS and MWS patients do not carry germ-line mutations in *NLRP3*. In those patients, somatic mosaicism occurring during fetal development may explain the variation in disease onset (59); this mechanism can only be demonstrated by cell cloning and next-generation sequencing (NGS).

Genotype–phenotype correlations were demonstrated in CAPS, with some mutations associated only with a mild clinical phenotype, and others with severe clinical pictures. However, several cases were reported in which patients with the same mutations present different phenotypes (60, 61). Furthermore, some *NLRP3* mutations are described in healthy subjects with no signs of CAPS, such as V198M and Q703K genetic variants, even if there is no apparent selective advantage demonstrated for CAPS (62). Nevertheless, when patients who carry these polymorphisms show CAPS symptoms, the IL-1β-inhibition response is diminished. As in the other AIDs, functional polymorphisms may be considered low penetrance mutations, able to influence the activity of the gene product (58, 63).

In conclusion, CAPS onset may be influenced by environmental factors and genetic determinants, which are also able to modulate the disease phenotype.

Deficiency of Interleukin-1 Receptor Antagonist

Deficiency of IL-1 receptor antagonist is a recently described autosomal recessive disease due to mutations of *IL1RN* that

lead to non-expression of the encoded protein, IL-1 RA, causing unopposed IL-1 receptor activation and increased response to IL-1α and IL-1β stimulation (64).

The disease was first described in 2009 in nine patients presented with sterile multifocal osteomyelitis, periostitis, and pustulosis since the neonatal period, without fever. *IL1RN* gene was sequenced in those DIRA patients (6): either homozygous for mutations in *IL1RN* or heterozygous parents were identified. A patient was homozygous for two nucleotides deletion (c.156_157delCA) that caused a frame-shift mutation named N52KfsX25, followed by the incorporation of 24 aberrant amino acids and a termination codon. Both parents were heterozygous carriers of the same mutation. In other patients, three were homozygous for a nonsense variant affecting the amino acid residue at position 77 (c.229G>T; p.E77X). Patients from a consanguineous Lebanese family were homozygous for a nonsense mutation (c.160C>T, p.Q54X). Patient 9, from Puerto Rico, was homozygous for a deletion of approximately 175 kb on chromosome 2q that includes six genes from a cluster of IL-1-related genes: IL1RN and the genes encoding IL-1 family, members 9 (*IL1F9*), 6 (*IL1F6*), 8 (*IL1F8*), 5 (*IL1F5*), and 10 (*IL1F10*). The *IL1RN* mutations are present in founder populations in Newfoundland, the Netherlands, Puerto Rico, and possibly Lebanon and further founder mutations have since been found in other populations (65). None of these mutations were found in DNA specimens obtained from a panel of 364 controls from the New York Cancer Project.

In 2011, two unrelated Brazilian patients whose clinical phenotype was consistent with the DIRA syndrome were described (66). Both were homozygous for the same 15-bp (in-frame) deletion on *IL1RN*. This novel mutation of *IL1RN* produces a protein that does not bind the IL-1 receptor, and thus lacks functional activity. The authors hypothesize that this variant is likely to be a possible founder mutation in the Brazilian population.

In 2012, a novel nonsense mutation (p.Q119X) in *IL1RN* gene was identified in two Turkish patients with consanguineous parents (67).

Blau Syndrome (BS)

Blau syndrome is an autosomal dominant granulomatous inflammatory disease caused by mutations in the *NOD2/CARD15* gene. This gene is located on chromosome 16q12 and encodes the three domain cytosolic protein of almost 1000 amino acids, the nucleotide-binding oligomerization domain containing 2 (NOD2). The protein contains two N-terminal CARDs for downstream signaling through CARD–CARD interactions, a central nucleotide binding and oligomerization domain (NACHT) with ATPase activity, and nine C-terminal LRRs for pathogen-associated molecular patterns (68).

Mutations in the *NOD2/CARD15* gene cause alteration in single amino acids in the NOD2 protein, resulting in an overactive version, which may lead to abnormal inflammatory reaction.

More details on the pathogenic aspects of BS were obtained from the identification in four European families of three missense mutations in 2001. Two of these families shared the same mutation, encoding an amino acid substitution of arginine to tryptophan in position 334 (R334Q), one family had an R334W and another L469F substitution (69).

The following year, another study on the genetic analysis of NOD2 coding regions based on 10 families with BS was published (70). In five of the families, two sequence variants at position 334 of the gene product (R334W and R334Q) were identified. Affected family members from the original BS kindred, included in this study, were heterozygous for the R334W missense mutation; mutations at the same position were also observed in several unrelated BS families, some of whose phenotypes included large-vessel arteritis and cranial neuropathy. The missense mutations were segregated with the disease phenotype in the families and were not identified in 104 healthy controls.

To date, on a total amount of almost 220 patients with BS carrying CARD15/NOD2 mutations, missense substitutions of R334Q/R334W account for more than 80%, causing a genetic hot spot for mutations in codon 334. E383K has been found in almost 5% of patients, whereas other mutations have been described most rarely (71).

The number of NOD2 variants associated with BS has expanded greatly. In fact, up to 2016, the number of sequence variants of *NOD2* gene is 144 (140 substitutions, 3 deletions, and 1 insertion) (16). There are no known mutations involving the untranslated and the intronic regions of the gene, even though this has not been extensively studied.

Despite the striking clinical similarities between them, for many years BS was considered a distinct entity from early onset sarcoidosis (EOS). Genetic analyses showed that many patients with EOS carry mutations in *CARD15/NOD2* gene; hereafter, some authors proposed that BS and EOS are the familial and sporadic forms of the same disease (71). Moreover, *CARD15/NOD2* gene mutations described in BS and EOS are mainly located in the NACHT domain of the protein. The discovery of *CARD15* mutations in BS families encouraged to investigate similar *CARD15* mutations in EOS patients.

Among 10 EOS cases retrospectively collected in Japan, heterozygous missense mutations were found in nine cases; four showed a c.1000C>T (p.R334W in amino acid change) that has been reported in BS, four showed novel c.1487A>T (p.H496L), c.1538T>C (p.M513T), c.1813A>C (p.T605P), and c.2010C>A (p.N670K), and one case showed double c.1146C>G (p.D382E)/c.1834G>A (p.A612T) mutations on different alleles. The study concluded that EOS is closely related with *CARD15* mutations causing constitutive NF-B activation and shares the common genetic etiology with BS (72).

Deficiency of the IL-36 Receptor Antagonist (DITRA)

Deficiency of the IL-36 receptor antagonist is a recently described autosomal recessive autoinflammatory syndrome caused by mutations in the *IL36RN* gene, characterized clinically by recurrent episodes of generalized skin pustulation, fever, systemic inflammation, and leukocytosis. Other phenotypes of the *IL36RN* mutation include related pustular disorders, palmoplantar pustulosis, acrodermatitis continua of Hallopeau (ACH), and acute generalized exanthematous pustulosis. Histology shows

spongiform pustules, acanthosis, and parakeratosis and an abundance of CD3+ and CD8+ T cells and macrophages (73).

This gene encodes IL-36 receptor antagonist (IL-36Ra), a protein belonging to the IL-1 cytokine family responsible for the tight regulation of IL-36 signaling. The IL-36 pathway is activated after binding of one of the three IL-36 agonists (IL-36b α, β, and γ) to a common specific receptor IL-1Rrp2, leading to the recruitment of the co-receptor IL-1 receptor accessory protein (IL-1RacP) and subsequent activation of intracellular NF-κB and mitogen activated protein kinase pathways (74).

Several mutations were highlighted in *IL36RN* gene with different effects. All *IL36RN* null mutations, such as c.28C>T (p.Arg10X), c.41C>A (p.Ser14X), c.80T>C (p.Leu27Pro), c.227C>T (p.Pro76Leu), c.280G>T (p.Glu94X), c.368C>G (p.Thr123Arg), c.368C>T (p.Thr123Met), and c.420_426del (p.Gly141MetfsX29) were totally unable to antagonize the IL-36 mediated activation of the NF-κB signaling pathway (75–83).

Among the most frequent genetic alterations is one Tunisian founder missense mutation: c.80C>T (p.Leu27Pro1); one European recurrent missense mutation: c.338C>T (p.Ser113Leu2); and one Japanese founder nonsense mutation: c.28C>T (p. Arg10*3).

The mutations c.95A>G (p.His32Arg), c.142C>T (p.Arg48-Trp), and c.308C>T (p.Ser113Leu) only partly reduced the expression level of the corresponding IL-36Ra and consequently the capacity to repress the IL-36 mediated NF-κB signaling cascade. The detection of c.104A>G (p.Lys35Arg) and c.304C>T (p.Arg102Trp) mutations do not produce evident alterations in either protein expression and function, raising doubt about the actual pathogenic contribution of these genetic variants. They are classified as damaging by the pathogenicity prediction tools, such as SIFT and/or PolyPhen. For these mutations, additional functional studies are warranted to understand whether these variants truly have an effect on disease development or if they are polymorphisms (74).

Recently, Cordoro et al. showed a homozygous mutation within the *IL36RN* gene at position c.115+6T>C in a male adolescent with generalized pustular psoriasis (GPP) since infancy. This mutation has been shown to lead to a splicing defect resulting in exon skipping and a premature stop codon, leading to a truncated IL36Ra protein (81).

In summary, the c.28C>T (p.Arg10X) and c.115+6T>C (p.Arg10ArgfsX1) transitions are known to be founder mutations in cases reported in Japan (84). The c.115+6T>C transition is also recurrently found in Chinese and Malaysian patients (81, 85). The c.80C>T (p.Leu27Pro) transition is a recurrent mutation in Africa, and the c.338C>T (p.Ser113Leu) transition is a recurrent mutation in Europe (79–81). In contrast, the c.368C>T and c.368C>G transitions have been reported in one case in Japan (75, 76), the c.104A>G (p.Lys35Arg), c.142C>T (p.Arg48Trp), and c.304C>T (p.Arg102Trp) have been reported in one and two cases in Europe, respectively.

More in general, the null mutations are consistently associated with the more severe phenotypes of GPP and acute exanthematous generalized pustulosis (86, 87), whereas the hypomorphic alleles usually show a milder phenotypic expression featured by localized variants of palmoplantar pustular psoriasis (PPP) and ACH, although generalized pustular phenotypes can be observed as well in carriers of mild variants. The detection of the same gene variants in generalized and localized pustular phenotypes suggests the pathophysiological contribution of other factors such as disease-modifying genes, environmental factors, and epigenetic events, which may all influence disease onset, expression, and severity.

Chronic Atypical Neutrophilic Dermatosis with Lipodystrophy and Elevated Temperature (CANDLE) Syndrome

Chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperature syndrome is a rare autosomal recessive AID, with less than 100 cases described worldwide (88).

The CANDLE syndrome is caused, in the majority of cases, by homozygous mutations in PSMB8 gene, which encodes for a proteasome protein (89). There are evidences that an increase of modified and oxidated proteins occurring in fat and tissue cells, due to mutations of PSMB8 lead to an augmentation of cellular stress and apoptosis (88).

In 2012, a genome-wide analysis of nine CANDLE syndrome affected patients in eight families suggested that mutations in *PSMB8* gene may cause the CANDLE syndrome. In this cohort, four patients were homozygous and two were heterozygous for a missense mutation (p.T75M), two patients were homozygous for a nonsense mutation (p.C135X), and one patient showed no mutations. None of these gene variants were observed in 750 healthy controls. Furthermore, only two of the four patients with the same mutation shared the same haplotype, indicating a possible mutational hot spot (90). Later, in 2015, a diagnosis of CANDLE syndrome was performed by targeted sequencing of one 3-year-old CANDLE syndrome Hispanic male patient, born to consanguineous healthy parents. A homozygous c.280G>C, p.A94P mutation in *PSMB8* gene was not observed in any public genetic databases and was predicted to be pathogenic by several prediction tools (91).

Recently, it was demonstrated that CANDLE syndrome can also be caused by mutations in genes that encode other proteasome subunits, such as PSMB4, PSMB9, and PSMA3 (92); these mutations affect transcription, protein expression, protein folding, proteasome assembly, and, eventually, proteasome activity.

Majeed Syndrome

Majeed syndrome is a congenital predisposition to develop early-onset multifocal osteomyelitis (CRMO), congenital dyserythropoietic anemia (CDA), congenital anemia, and inflammatory dermatosis, resulting from the infiltration of neutrophils into the dermis (93). It is transmitted with an autosomal recessive pattern of inheritance and is caused by mutations in the *LPIN2* gene, which maps on chromosome 18p. The genomic sequence of *LPIN2* is approximately 95 kb and comprises 20 exons, of which exon 1 and the majority of exon 20 are non-coding (5′ and 3′ untranslated regions). The mRNA is approximately 6245 bp and encodes a protein of 896 amino acids, which is expressed in almost all tissues (94). LIPIN2 derives its name from its highly conserved

N-terminal and C-terminal LIP domains. LIPIN-1, -2, and -3 are phosphatidate phosphatases (PAPs), which are important in glycerolipid biosynthesis and as transcription co-activators regulating lipid metabolism genes (95). In addition, lipin-2 regulates increased IL-1β formation in primary human and mouse macrophages by several mechanisms, including activation of the inflammasome NLRP3. In macrophages, reduced levels of lipin-2 cause a decrease of cell cholesterol levels. In conclusion, lipin-2 is able to down-regulate NLRP3 inflammasome (91).

Several mutations have been identified in *LPIN2* gene. One of them, the c.540-541delAT (p.Cys181Ter), is a frameshift mutation that produces a premature stop codon producing a truncated protein that is 180 amino acids long; a second variant, the c. 2201C>T (p. Ser734Leu), is a missense mutation that replaces a highly conserved serine with a leucine (96). Al-Mosawi et al. report a third unique mutation in *LPIN2* in an Arabic female with CRMO and CDA. The c. 2327+1G>C (p.Arg776SerfsTer66) nucleotide change affects a highly conserved nucleotide residue at the 5′ (donor) splice site of exon 17 and it is predicted to introduce a frameshift mutation resulting in a premature stop codon being encountered in intron 17, which would be predicted to produce a truncated message (97). A novel homozygous 2 bp deletion (c.1312_1313delCT) resulting in a premature stop codon (p.Leu438fs+16Ter) and consequently in a truncated LIPIN2 protein was recently described in two Turkish brothers with MS who were treated successfully with IL-1 inhibitors (7).

LPIN2 shares homology with *LPIN1*, which has been shown to play a role in murine lipodystrophy (98). The role of *LPIN2* mutations in producing the inflammatory phenotype of MS is not clear and does not appear to involve a disturbance in lipid metabolism. *LPIN2* has an amino-terminal lipin domain, a Lipin/Ned1/Smp2 domain, and a putative nuclear localization signal. Lipin2 also has PAP type-1 activity and may play a role in lipid biology (99).

Although the number of individuals reported with MS is too small to study genotype–phenotype correlations, the affected individuals with a frameshift variant appear to have a more severe course and complications than individuals with other classes of pathogenic variants (100). More recent observations, however, have indicated that an affected individual with a splice site variant (97) and two affected Turkish brothers with a frameshift variant (7), who were all diagnosed and treated early, had a less complicated course. It is unclear whether their milder clinical course is attributable to the earlier detection and treatment.

CARD14-Mediated Pustular Psoriasis (CAMPS)

CARD14 encodes caspase recruitment domain family member 14 (CARD14). It is known to be specifically expressed in the skin and to be localized mainly to keratinocytes. CARD14 is a scaffolding protein that regulates NF-κB activation. The NF-κB family of transcription factors plays a crucial role in cell activation, survival, and proliferation and results in cancer, immunodeficiency, or autoimmune disorders (e.g., psoriasis). Hence, the presence of the CARD14 mutations may result in greater amplitude of inflammatory response upon epidermal activation. The skin disease in patients with *CARD14* mutations can be limited or generalized. Autosomal dominant or sporadic gain-of-function mutations in the *CARD14* gene cause GPP (101), familial pityriasis rubra pilaris (PRP) (102), psoriatic arthritis (PA) (103), PPP (104), and even pustular psoriasis suggesting a large disease severity spectrum. Fever and other systemic manifestations are generally not present but can occur with superinfections of the skin.

Three variants, c.349G>A (p.Gly117Ser), c.205C>T (p.Arg69Trp), and c.589G>A (p.Glu197Lys), affect the N-terminal region of the protein harboring its caspase recruitment domain or coiled-coil domain but with different effects. The c.589G>A (p.Glu197Lys) and c.349G>A (p.Gly117Ser) lead to upregulation of NF-κB activity, whereas the c.205C>T (p.Arg69Trp) leads to a sevenfold downregulation. In particular, the c.349G>A (p.Gly117Ser) variant described in a family of European descent altered the splicing between *CARD14* exons 3 and 4. One Tunisian patient was reported with a c.1356+5G>A splice alteration which is predicted to lead to the skipping of exon 9, which encodes part of the coiled-coil domain (105). Mutations in *CARD14*, including p.Glu138del and p.Leu156Pro, have been associated with autosomal-dominant pityriasis rubra pilaris, which is phenotypically related to psoriasis (102).

Several gain-of-function variants/mutations in *CARD14* have been reported to be a predisposing factor for psoriasis vulgaris (PV) in a large family with PV and PA. Jordan et al. identified the rare *de novo CARD14* gain-of-function variant p.Glu138Ala in a child with severe early-onset GPP. They also found rare *CARD14* gain-of-function variants in large PV cohorts by the NF-κB assay, which revealed that compared to the wild-type CARD14, the p.Gly117Ser, p.Glu138Ala, and p.Asp176His variants were associated with increased levels of the luciferase reporter. Additional rare variants within CARD14 are c.424G>A (p.Glu142Lys), c.511C>A (p.His171Asn), c.536G>A (p.Arg179His), and c.571G>T (p.Val191Leu) (106).

In conclusion, the above-reported data concur to reach the following conclusions: (1) differences in the genetic background among different geographic populations account for significant variances observed in psoriasis populations, both in terms of frequency and of severity (localized palmoplantar versus generalized forms) of pustular psoriasis (107, 108), (2) despite the dramatic *in vitro* effects of some CARD14 variants on the keratinocytes, there is a wide range of phenotypes, even among individuals who carry the same substitution, suggesting that in many instances, the variable phenotypes are likely to multiple other factors besides the genetic background.

NLRP12-AID

NLRP12 is an NLR encoded by *NLRP12* (also known as *NALP12*, Monarch-1, or PYPAF7) and functions as a negative regulator of NF-κB activation (109). NLRP12 interacts via its pyrin domain with the pyrin domain of apoptosis-associated speck-like (ASC) protein (110) leading to the formation of an intracellular aggregate called speck to active IL-1B (111). Sequencing of *NLRP12* revealed a heterozygous nonsense mutation c.850C>T (p.Arg284X) in

identical twin brothers presenting with symptoms overlapping FCAS and MWS. A second NLRP12 mutation (c.2072+3insT) was identified in a patient presenting with a periodic fever syndrome, including clinical manifestation of FCAS. This mutation, affecting the donor splice site of intron 3, activates a cryptic splice site located upstream in exon 3 and results in a frameshift, followed by a premature stop codon (112). These two mutations were demonstrated to be functionally associated with high levels of NF-κB activity, thus accounting for the autoinflammatory phenotype. Jèru et al. identified a missense mutation c.1054C_T (p.Arg352Cys) within the NBS domain of the protein in two unrelated patients. This missense mutation is associated with a gain of function of caspase 1 processing (110). The c.882C>G (p.Asp294Glu) mutation was found to mostly segregate with a particular sensitivity to cold exposure (especially arthralgias and myalgia), even in the absence of urticarial rash, fever, or elevation in the levels of acute-phase reactants. In any case, the clinical manifestations presented by the carriers were generally mild, although quality of life was affected, especially during the winter season (112–114). Several reports identified the NLRP12 variant F402L (c.1206 C>G) (115–117).

Pyogenic Arthritis, Pyoderma Gangrenosum, and Acne (PAPA) Syndrome

Pyogenic sterile arthritis, pyoderma gangrenosum, and acne syndrome is an autosomal dominant AID caused by mutations in the *PSTPIP1* gene, which is located in chromosomal position 15q24–q25.1. Pyrin protein is a cytosolic receptor for PSTPIP1. Ligation between pyrin and PSTPIP1 induces pyrin to interact with ASC protein, inducing the creation of an active ASC pyroptosome. A possible explanation of PAPA syndrome is a constitutive ligation and consequent activation of pyrin with the mutated PSTPIP1 proteins (112). The disease is extremely rare, with less than 10 families described worldwide. The first case of PAPA syndrome was reported in 1975 (118) and in 1997, in the same family, PAPA syndrome was described as a heritable disease (119).

In 2000, 93 genomic loci were investigated in patients with a pleiotropic inflammatory syndrome characterized by pyoderma gangrenosum, cystic acne, and erosive arthritis, demonstrating PAPA syndrome maps in chromosomal position 15q (120).

There are two hot-spots mutations, c.688G>A (p.A230T) and c.748G>C (p.E250Q), which occur in exons 10 and 11 and have been found in many familial (121–126) and sporadic cases (127, 128). Mutations are thought to disrupt the binding of PSTPIP-1 with protein tyrosine phosphatase–PEST, a regulatory phosphatase, increasing its avidity for pyrin in the cytosol, thereby causing dysregulation of IL-1β production (129).

Until 2016, a total of 27 genetic variants were reported for *PSTPIP1* gene (23 substitutions, 1 insertion, 2 deletions, and 1 duplication), 17 of which are PAPA phenotype associated (16).

Deficiency of Adenosine Deaminase 2

Searching for mutations in systemic inflammation and vasculopathy and/or necrotizing vasculitis polyarteritis nodosa patients, *CECR1* (cat eye syndrome chromosome region, candidate 1) gene mutations were discovered by two independent groups. Pattern of inheritance was autosomal recessive. Subsequent studies described another case with a fatal vasculopathy (130). Common clinical signs are early onset recurrent stroke, neurologic manifestations, and fever. Being the *CECR1* gene highly polymorphic, as other AID causative genes, the correlation between clinical signs and familial ancestry is important. The *CECR1* gene encodes the adenosine deaminase 2 (ADA2) protein, which has partial homology with ADA1 protein. Both ADA1 and ADA2 act as intracellular enzymes that regulate the purinergic signaling pathway. Mutations in ADA1 are known to cause severe combined immunodeficiency disease, characterized by a defect in T- and B-lymphocytes. ADA2 mutations, conversely, cause only mild hypogammaglobulinemia due to a defect in terminal differentiation of B-cells (131).

NLRC4-Associated Inflammatory Diseases (SCAN4, NLRC4-MAS, and NLRC4-FCAS)

Patients with macrophage activation syndrome or a milder phenotype like FCAS may carry gain of function mutations in the *NLRC4* (IPAF; CARD12) gene (132–134). Two novel causal mutations, p.T337S and p.V341A, have been diagnosed by whole exome sequencing (WES) in two sporadic patients (trios); the reported clinical symptoms were early onset fever, failure to thrive, rash, joint pain, and elevated inflammatory markers, including hyperferritinemia (133). The two mutations map in a highly conserved HD1 region of the NLRC4 nucleotide-binding domain and may decrease the function of NLRC4 to maintain itself in an auto-inhibited state. A third mutation, p.H443P, was identified in a Japanese family with milder symptoms including cold induced rash, fever, and arthralgia, even if the patient has a different phenotype from those other two previously patients reported. Mutations are supposed to regulate the mutant proteins into a constitutively active state, with the influence of environmental factors such as cold and stress which act as trigger, causing inflammasome activation. Further studies are necessary to explore full spectrum of monogenic inflammasome-related diseases. Interestingly, the *NLRC4* gene (IPAF, CARD12) is supposed to initiate inflammation in response to bacterial ligands, such as flagellin (135, 136).

Stimulator of Interferon Genes (STING)-Associated Vasculopathy with Onset in Infancy

In a trio with a patient affected by early onset symptoms of systemic inflammation, cutaneous rash, and pulmonary manifestations, and his unaffected parents, WES was performed, identifying a *de novo* mutation, p.N154S, in the TMEM173 gene; this gene encodes for a STING (138). Extending the analysis to other five sporadic cases of different ancestries but with similar phenotype led to the identification of missense mutations in the TMEM173 gene. Functional studies showed that a particular missense mutation, p.V155M, has been previously associated with a phenotypically different disease like systemic lupus erythematosus (139). Most of the mutations are located in the exon 5 of TMEM173 gene, which encodes for a domain important for the STING dimerization site.

The second study highlights that in families with dominantly inherited traits, the possibility of reduced penetrance should not be ignored. Sting knockout mice are prone to viral infections, because of the lacking of the ability to upregulate IFN-beta (137). As a result, all these data support the evidence that TMEM173-associated mutations are gain of function (138, 139).

TNFRSF11A-Associated Hereditary Fever Disease

Patients with this disease have a phenotype similar to those affected by TRAPS. In a single patient with complex phenotype including neonatal onset of systemic inflammation and congenital abnormalities, a *de novo* genomic duplication containing the TNFRSF11A gene was identified (140). The TNFRSF11A gene is one of the 30 genes in the 10-Mb genomic duplication. Another study, using a different approach based on candidate gene screening, identified two other patients (mother and daughter) with a novel heterozygous 1-bp deletion (p.Met416Cysfs*110) in exon 9 of *TNFRSF11A* gene. The resulting protein lacks the C-terminal intracellular domain. As TNFRSF1A, also TNFRSF11A gene encodes for a protein member of the TNF-receptor superfamily. *TNFRSF11A* (RANK, PDB, ODFR) gene encodes a signaling receptor that functions in osteoclast differentiation and bone remodeling (141, 142). RANK-ligand (RANKL) mediates essential signal for osteclastogenesis (143).

Rank-deficient mice present osteopetrosis caused from a block in osteoclast differentiation and the lack of peripheral lymph nodes (144). The pathogenesis of this disorder is unclear; in fact, the *TNFRSF11A* gene duplication suggests a gain of function mutation, while the heterozygous deletion is more consistent with a haploinsufficiency or a dominant negative effect.

TRNT1 Deficiency

Patients presenting with a variable phenotype of congenital sideroblastic anemia, B cell immunodeficiency, and developmental delay have been termed SIFD (145). SIFD is characterized by an early onset, with frequently associated neurological symptoms, and metabolic abnormalities. SIFD has been associated to AID because it is characterized with a pediatric onset with periodic fevers and gastrointestinal involvement with sideroblastic anemia. Genetic cause of SIFD has been found in the *TRNT1* gene in an autosomal recessive inheritance (146, 147). The *TRNT1* gene encodes the ubiquitously expressed CCA-adding enzyme, essential for template-independent maturation of nuclear and mitochondrial transfer RNAs (148). Functional studies in yeast showed deficiency of TRNT1 homolog causes partial loss of function of TRNT1 affecting variable degrees of enzyme activity (146). Knock-out yeast for *TRNT1* was fully restored with human TRNT1 and partially rescued by human mutant proteins.

Monogenic Form of Systemic Juvenile Idiopathic Arthritis

Systemic-onset juvenile idiopathic arthritis is a polygenic inflammatory disease characterized by fever, rash, and symmetrical polyarthritis, with persistent systemic inflammation that seems to be linked to altered innate immune system (149, 150). Mendelian inheritance is autosomal recessive. Studying five consanguineous families with 13 affected patients from the Saudi Arabia with several genomic approaches, such as linkage analysis, homozygosity mapping, and WES, a homozygous missense mutation, p.C284R, located in exon 4 of the laccase domain containing 1 (LACC1) gene was identified (151). This private mutation is highly conserved during evolutionary scale and was not described in more than 2,000 Arab controls, suggesting its role in the pathogenesis.

Laccase domain containing 1 gene belongs to a family of Laccases, multi-copper oxidoreductases able to catalyze the oxidation of a variety of phenolic and non-phenolic compounds. Protein function is largely unknown, even if may regulate the innate immune responses. In a recent study, the gene product of LACC1 gene has been named FAMIN (fatty acid metabolism–immunity nexus), important for the synthesis of endogenous fatty acids and their mitochondrial oxidation, controlling the glycolytic activity and ATP regeneration (145).

Genetic variants in the LACC1 gene have been previously associated with susceptibility to leprosy (152–154). LACC1 gene belongs to a family of Laccases, multi-copper oxidoreductases able to catalyze the oxidation of a variety of phenolic and non-phenolic compounds. Protein function is largely unknown, even if may regulate the innate immune responses.

GENETIC DIAGNOSIS WITH NEW TECHNOLOGIES

In recent years, with the increased use of instruments for NGS, it has become feasible to analyze several genes in a single experiment. This method is of great interest for AID, because of the increasing number of genes associated with the different forms of AID. In some cases, it is difficult to differentiate the different diseases, in particular, in those patients with intermediate phenotypes or a difficult clinical diagnosis. The analysis of a panel of several candidate genes is feasible with targeted sequencing investigating causative mutations, rare variants, or regions associated with the disease.

With the large number of genetic variants found with NGS, it is extremely important to find information about their possible role in the development of a specific disease. Several softwares have been developed in recent years and most of them have been based on the assumption that protein sequences derived from living organisms have survived a natural selection. The goal is to find if a genetic variant is a causative mutation or a common polymorphism.

There is also the possibility to analyze all the 18,000 genes of the human genome. WES is a recent strategy designed to sequence only the coding regions of the genome (which represents 1% of the human genome, about 30 megabases); this is an effective method alternative to whole genome sequencing, cheaper and less complicated in the bioinformatical/statistical analysis. Exons are generally short, functional sequences of DNA which represent the portion of the genome translated in protein. The WES has

the potential to identify the coding variants responsible for both Mendelian and common diseases.

In recent years, with the use of WES, several undiagnosed Mendelian genetic conditions have been investigated in order to search the involved gene (155, 156). WES has been applied to the study of trios (unaffected parents and the sporadic case) and unrelated patients with phenotypic similarities. Identification of causal gene in a single sporadic patient can then be confirmed in other patients with similar phenotypes. The potential of such strategies is high in polygenic human disorders such as AID. The emerging genetic technologies complemented by the development of public databases of human variation can allow discovery of disease causal genes in sporadic and unrelated patients.

Whole genome sequencing allows to sequencing the entire genomic DNA, both chromosomal and mitochondrial. Unlike WES, this method allows the sequencing of both exons and introns, for a total amount of 3 Gigabases.

In a single center study (157), more than 2,000 diagnostic patients have been analyzed with Sanger sequencing for the NLRP3, MVK and TNFRSF1A genes, and other AID gene portions, failing to find mutations in 86% of samples. Possible explanations of this high failure are the restricted number of tested genes, clinical misdiagnosis, genetic heterogeneity, and/or a complex mode of inheritance. In order to improve the sensitivity of the genetic tests, 50 patients were re-analyzed with a gene panel of 10 genes for NGS procedure. The 10 genes were *MEFV, MVK, TNFRSF1A, NLRP3, NLRP12, NOD2, PSTPIP1, IL1RN, LPIN2,* and *PSMB8* (157). In order to better understand the possible role of the detected variants, allele frequencies have been compared with those of 1,000 Genomes Project, and searching for a possible genotype–phenotype correlation. Some genes, such as *NOD2, LPIN2*, and *NLRP12*, showed a high frequency of genetic variants, which in theory may alter the clinical phenotype with mild or atypical symptoms. In the next future, NGS data combined with clinical information may help diagnosis for those patients with intermediate phenotype (156); in fact, the interaction between geneticists and physicians will allow to improve the diagnosis of AID patients (157).

CONCLUSION

In AID, genetic and environmental factors act modulating the clinical presentation of a specific disorder. The knowledge of the biological pathways at the basis of different AIDs is very important; the elucidation of these novel factors may have clinical relevance, because it may be included in genetic-risk modeling approaches. The genetic variants previously identified as playing a role in the same pathway represent new potential therapeutic targets. The new age of the *-omics* has allowed the improvement of the knowledge of AID. By means of genetic fine mapping, targeted sequencing, transcriptomics, proteomics, and metabolomics, physicians may improve treatment and therapy tailored on the single patient.

AUTHOR CONTRIBUTIONS

DM contributed in conception or design of the work, drafting the article, and final approval of the version to be published. FB and PM contributed in drafting the article and final approval of the version to be published. AV contributed in drafting the article, critical revision of the article, and final approval of the version to be published. AP contributed in conception or design of the work, drafting the article, critical revision of the article, and final approval of the version to be published.

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Dysregulated IL-1β Secretion in Autoinflammatory Diseases: A Matter of Stress?

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Infectious and sterile inflammation is induced by activation of innate immune cells. Triggering of toll-like receptors by pathogen-associated molecular pattern or damage-associated molecular pattern (PAMP or DAMP) molecules generates reactive oxygen species that in turn induce production and activation of pro-inflammatory cytokines such as IL-1β. Recent evidence indicates that cell stress due to common events, like starvation, enhanced metabolic demand, cold or heat, not only potentiates inflammation but may also directly trigger it in the absence of PAMPs or DAMPs. Stress-mediated inflammation is also a common feature of many hereditary disorders, due to the proteotoxic effects of mutant proteins. We propose that harmful mutant proteins can induce dysregulated IL-1β production and inflammation through different pathways depending on the cell type involved. When expressed in professional inflammatory cells, stress induced by the mutant protein activates in a cell-autonomous way the onset of inflammation and mediates its aberrant development, resulting in the explosive responses that hallmark autoinflammatory diseases. When expressed in non-immune cells, the mutant protein may cause the release of transcellular stress signals that trigger and propagate inflammation.

Keywords: autoinflammatory syndromes, endoplasmic reticulum stress, IL-1β, inflammation, monocytes, NLRP3 inflammasome, oxidative stress, toll-like receptor

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INTRODUCTION

The term "autoinflammation" (1) groups syndromes with different etiologies characterized by systemic inflammation in the absence of detectable infections and/or autoimmunity. Autoinflammatory diseases are disorders of the innate immune system, sharing recurrent episodes of fever, rash, joint pain, neutrophilia, and increased inflammatory markers. Most of them are monogenic, and the causative gene relates to the innate immune system. Examples are MEFV/pyrin in familial Mediterranean fever (FMF), TNFRSF1A/TNF receptor type 1 in TNF receptor-associated periodic syndrome (TRAPS), and nucleotide-binding domain, leucine-rich-containing family, pyrin domain-containing 3 (NLRP3) in cryopyrin-associated periodic syndromes (CAPS) (2).

Abbreviations: CAPS, cryopyrin-associated periodic syndromes; DAMPs, damage-associated molecular patterns; ER, endoplasmic reticulum; IL, interleukin; NLRP3, nucleotide-binding domain, leucine-rich-containing family, pyrin domain-containing 3; NOX, NADPH oxidases; PAMPs, pathogen-associated molecular patterns; ROS, reactive oxygen species; TLRs, toll-like receptors; TRAPS, TNFRSF1A/TNF receptor type 1 in TNF receptor-associated periodic syndrome; UPR, unfolded protein response.

The reversal of clinical symptoms in CAPS patients upon treatment with recombinant IL-1 receptor antagonist (*Anakinra*) or with IL-1β blocking agents (e.g., *Canakinumab*, a neutralizing antibody) provided compelling *ex adjuvantibus* evidence for the key role of IL-1β (3). The efficacy of anti-IL-1 drugs suggested that "gain-of-function" mutations in NLRP3, a central component of the inflammasome, cause uncontrolled IL-1β production, in turn responsible for the severe inflammatory symptoms (4, 5). Less expectedly, the same drugs displayed strong therapeutic effects also in autoinflammatory diseases, where the causative gene is not directly involved in IL-1β production and regulation (2, 3). A representative case is TRAPS, a disease characterized by recurrent episodes of long-lasting fever, pain, and fasciitis. Despite TRAPS is caused by mutations in p55 TNF receptor type I, patients showed no or modest response to TNFα inhibition (6), whereas IL-1β-blocking agents have high efficacy (7). These observations suggest that the presence of a mutated protein in inflammatory cells, independently from its function, activates mechanisms converging on dysregulated IL-1β secretion.

In this perspective article, we propose a pro-inflammatory role for cell stress and the responses it elicits in some hereditary diseases, and suggest that stress is a central player in the pathophysiology of autoinflammatory disorders, due to its presence in innate immune cells.

STRESS AND INFLAMMATION

Inflammation is traditionally defined as a reaction to infectious or sterile injuries, aimed at recruiting molecules and cells of the immune system to the tissue where the damage is taking place and restoring homeostasis. Inflammation is initiated by activation of pattern recognition receptors on inflammatory cells, by two subclasses of ligands responsible for infectious and sterile inflammation, respectively (8, 9): pathogen-associated molecular patterns and damage-associated molecular patterns (PAMPs and DAMPs). The former are part of pathogens, while the latter are components of cells or extracellular matrix released or degraded upon cell and tissue damage. Additional factors concur in determining the onset, duration, and intensity of inflammatory responses. Among these, particularly important is cell stress due to starvation, enhanced metabolic demand, cold or heat, altered proteostasis. The most common and well studied cell stresses are endoplasmic reticulum (ER) stress and oxidative stress that are counteracted by highly conserved responses. These responses share common traits, for example, eIF2α phosphorylation, with transient translational inhibition and transcriptional activation of chaperones and antioxidants (10). This integrated stress response prevents the toxicity caused by misfolded proteins [named "proteotoxicity" (10)] and limits reactive oxygen species (ROS)-based vicious circles. If excessive or prolonged, however, virtually all stress responses become maladaptive and induce inflammation due to activation of chemokine genes or, in case of cell damage, release of DAMPs that recruit inflammatory cells (11).

Oxidative stress is due to excessive production and/or deficient detoxification of ROS. These can be abundantly generated by mitochondria during oxidative phosphorylation (12) and by flavoenzymes like NADPH oxidases (NOX) (13). In cells of the innate immune system, phagocytosis and toll-like receptor (TLR) triggering activate NOX to produce abundant H_2O_2 (14). H_2O_2 is released into phagosomes to clear microorganisms and induces pro-inflammatory cytokines and inflammation: however, it may generate oxidative stress (13, 14). ROS are also produced in the ER as a by-product of oxidative protein folding, particularly in conditions of ER stress, which elicit the unfolded protein response (UPR) (15, 16). ER stress occurs when misfolded proteins accumulate in the secretory pathway, and also during infections, lipid unbalance, and other metabolic defects (15). UPR, a complex set of intracellular signaling pathways, has evolved to respond to protein misfolding and restore ER homeostasis. In addition, UPR signaling has a recognized role in immunity and inflammation (16). Oxidative and ER stresses are intimately linked: the former can induce misfolding of secretory proteins impacting disulfide bond formation. On the other hand, ER stress leads to ROS production (17). In concert with ROS, a prolonged UPR can induce NF-κB-mediated chemokine production and recruit inflammatory cells. In turn, PAMP or DAMP can potentiate the UPR (16).

These vicious circuits are evident in many chronic disorders such as type 2 diabetes (18), obesity (19), lung respiratory disease (20), inflammatory bowel disease (21), non-alcoholic fatty liver disease (22), and cancer (23).

Also in many hereditary diseases, the mutant protein may alter proteostasis: if this occurs, stress and inflammation are induced. For example, in cystic fibrosis, different mechanisms contribute to the inflammatory lung disease that is the major cause of morbidity and mortality in patients affected by this disease. Firstly, the mutated cystic fibrosis transmembrane conductance regulator (CFTR) protein cannot fold properly into the ER lumen, causing accumulation of misfolded CFTR aggregates, ER stress, and UPR. In turn, UPR activates NF-κB inducing production of chemokines, such as IL-8, that recruit polymorphonuclear leukocytes (PMN). PMN increase the oxidative burden in the lung, with generation of ROS that amplify the production of IL-8 thus locally increasing PMNs (24). Moreover, upregulation of ROS inhibits autophagy with consequent accumulation of protein aggregates and lung inflammation (25). Finally, the mutant CFTR transporter is unable to channel antioxidants into the airways: oxidative stress is worsened and concurs to the hyperinflammatory phenotype (24).

In Duchenne muscle dystrophy, due to the defect of dystrophin, oxidative stress and UPR-activated NF-κB interactively promote fiber necrosis. Recruited macrophages generate inflammatory cytokines and ROS, thereby triggering vicious inflammatory waves (26, 27).

Differently from autoinflammatory disorders, in these cases, the mutant protein, being synthesized by epithelia or muscle, determines the release of stress signals that recruit leukocytes ultimately causing inflammation. These signals include small molecules like ROS and antioxidants, and proteins such as thioredoxin (28) and chemokines (as described above for cystic fibrosis, 24), which induce inflammation transcellularly, i.e., by recruiting and activating other cells (**Figure 1A**). When instead it is a professional inflammatory cell that produces a proteotoxic mutant, inflammation is generated in a cell-autonomous way and

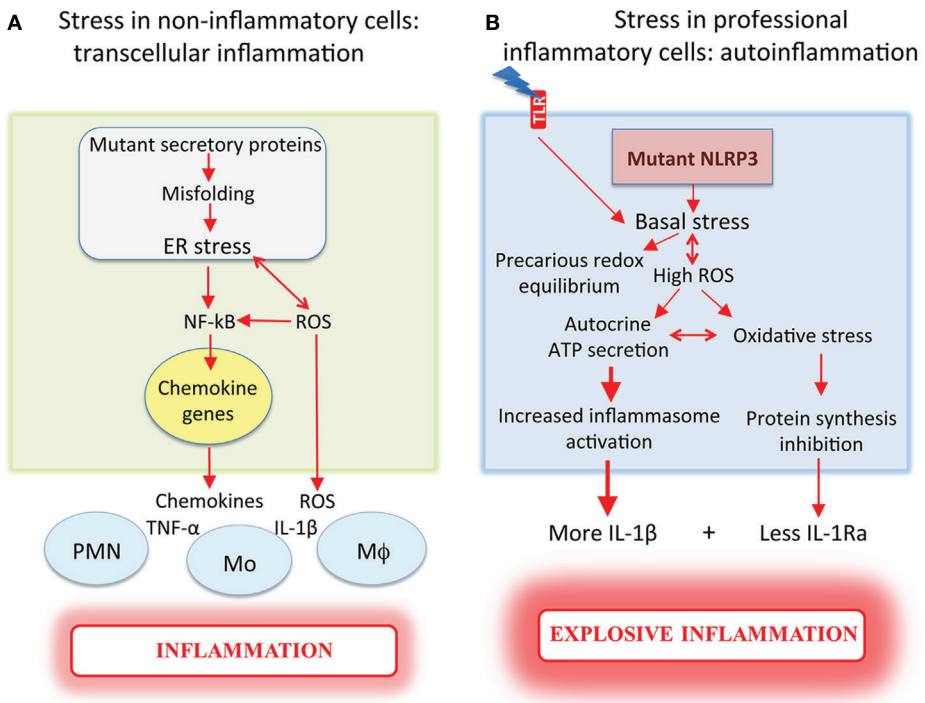

FIGURE 1 | **Mutant proteins induce more severe inflammation when expressed in professional inflammatory cells**. **(A)** Non-inflammatory cells (e.g., epithelia or muscle), which express mutant proteins that undergo aberrant folding in the ER, exhibit ER stress and increased ROS, and promote NF-κB-mediated chemokine induction. The release of chemokines recruits inflammatory cells that secrete pro-inflammatory cytokines, ultimately causing inflammation. **(B)** Inflammatory cells from cryopyrin-associated periodic syndrome patients, which express mutated NLRP3, display cell stress with high reactive oxygen species (ROS) and antioxidant levels resulting in a precarious redox equilibrium that is deranged by toll-like receptor (TLR) stimulation. The high ROS levels facilitate autocrine ATP secretion, with increased and accelerated IL-1β secretion. When the antioxidant responses collapse, oxidative stress occurs with inhibition of protein synthesis responsible for the decrease of IL-1Ra secretion. Dysregulated cytokine production results in explosive inflammation. ROS are released in both conditions, triggering loops of amplification of stress and inflammation.

the onset, development, and outcome of it will be much worse for the host (**Figure 1B**).

STRESS IN CAPS MONOCYTES

This hypothesis is supported by the observation that monocytes from CAPS patients, which express mutated NLRP3 molecules, display redox distress even before PAMP stimulation. Why mutant NLRP3 causes stress is unclear. A possible explanation is that it changes the affinity for the other components of the inflammasome complex (4), causing a disruption of the cytosolic homeostasis with induction of stress and integrated stress responses (10). Whatever the reason of NLRP3-induced stress, CAPS monocytes have higher basal ROS levels than monocytes from healthy donors but also higher expression of antioxidant systems (29–31) that allow them to maintain the redox homeostasis despite their stressed state. This equilibrium is, however, precarious, and CAPS monocytes can easily be induced to overreact, through pathways that largely depend on extracellular ATP, the most common inflammasome-activating signal (32). ATP is released by injured tissues, activated platelets, and other cells through pathways that are still ill defined (32). Unlike other pro-inflammatory cells, however, human monocytes do not need ATP from external sources. The accumulation of ROS upon TLR triggering (33) induces them to secrete ATP (34) that autocrinally or paracrinally stimulates cognate purinergic receptors (P2X7R) at the cell surface (32, 34). The ensuing lower intracellular [K^+] induces inflammasome assembly and IL-1β secretion (35). The higher ROS levels in CAPS monocytes following TLR triggering facilitate ATP release that increases and accelerates IL-1β secretion (31) (**Figure 1B**).

Cell stress also decreases the threshold for IL-1β processing and secretion: minute amounts of TLR agonists, that in healthy monocytes are sufficient to trigger pro-IL-1β synthesis but not its processing and secretion, drive large amounts of IL-1β release in CAPS monocytes (31). Probably owing to their "pre-activated state," small doses of TLR agonists increase ROS, inducing abundant ATP release, and IL-1β processing and secretion (31). This circuit explains why small traumas or infections that go undetected in healthy subjects can cause severe inflammatory manifestations in CAPS patients.

The effects described above occur soon after TLR stimulation. In later phases, the precarious redox equilibrium of CAPS monocytes is broken as antioxidant responses collapse. CAPS monocytes display damaged mitochondria (30), a further indication of the presence of oxidative stress [(12), **Figure 2**].

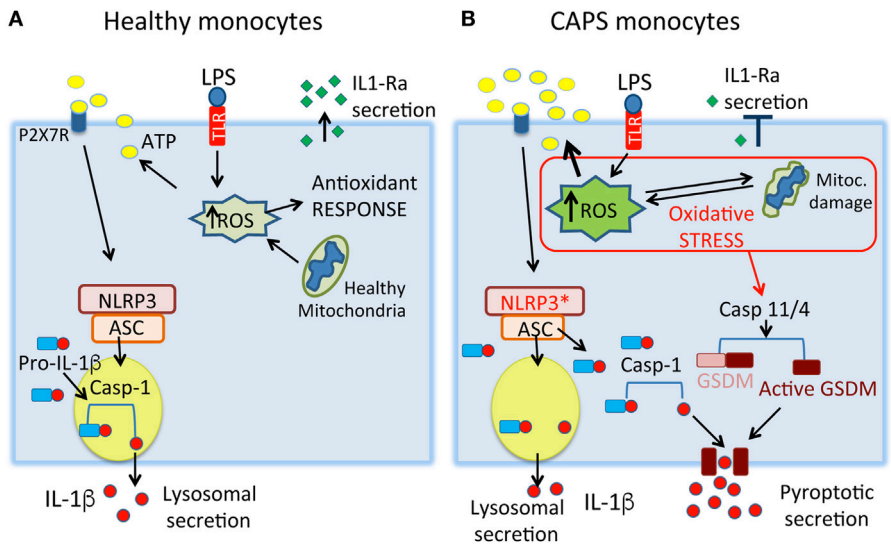

FIGURE 2 | A model for stress-mediated cytokine secretion in cryopyrin-associated periodic syndromes (CAPS) monocytes. **(A)** In healthy monocytes, toll-like receptor (TLR) stimulation induces the production of low amounts of reactive oxygen species (ROS), rapidly neutralized by the antioxidant response. The ROS-induced ATP release is low, resulting in processing and secretion of little amounts of IL-1β through secretory lysosomes. The anti-inflammatory cytokine IL-1Ra is produced, contributing to switch off the inflammatory response. **(B)** In CAPS monocytes, small doses of TLR agonists induce a strong increase of ROS resulting in release of large amounts of endogenous ATP and IL-1β. The state of stress may trigger pyroptotic secretion of IL-1β through activation of caspase-11/4 that cleaves gasdermin D (GSDMD) generating a toxin-like N-terminal peptide that forms pores on the plasma membrane. Mature IL-1β, cleaved by the NLRP3 inflammasome, will be released through gasdermin D-formed pores. Later, failure of antioxidant response and mitochondria dysfunctions lead to severe oxidative stress, with impaired production of IL-1Ra. NLRP3*, mutated NLRP3.

Interestingly, mitochondria are normal in CAPS lymphocytes, which do not express NLRP3, and in monocytes from healthy donors, which express wild-type NLRP3 (30), suggesting that mutant NLRP3 is indeed the causative agent of the oxidative stress. In this crucial phase, stress impacts also the production of IL-1Ra, normally secreted by activated monocytes a few hours after IL-1β to limit inflammation [(30), **Figure 1**]. Thus, deficient IL-1Ra production likely concurs in increasing the severity of the disease. Highlighting the dangerous stress-inflammation liaisons, insufficient IL-1Ra production may depend on eIF2α phosphorylation. Indeed, TLR-activated monocytes from CAPS patients, but not from healthy donors, display attenuated protein translation (30). Thus, IL-1ra mRNA is transcribed but stress prevents translation. Once more, oxidative and ER stress appear to be linked because IL-1Ra secretion is restored by antioxidants.

HYPER-STIMULATED HEALTHY MONOCYTES RECAPITULATE THE BEHAVIOR OF CAPS MONOCYTES

The above observations suggest that the increased IL-1β/IL-1Ra ratio in CAPS depends on the synergistic effects of NLRP3 mutations and stress. Combinations of PAMPs that stimulate surface and intracellular TLRs (LPS, R848, zymosan) were then used to induce a CAPS-like stress state in healthy monocytes (36). When given alone, each TLR agonist triggered the secretion of IL-1β and IL-1Ra by healthy monocytes. When provided simultaneously, however, they induced a superstimulation resulting in enhanced secretion of IL-1β but impaired release of IL-1Ra (36). The underlying molecular mechanisms are similar to those described in CAPS monocytes (29–31): super-stimulation induces ROS accumulation, responsible of the massive ATP release and IL-1β secretion, and of the consequent oxidative stress leading to inhibition of IL-1Ra production, despite normal IL-1Ra mRNA levels. Antioxidants restore IL-1Ra release by super-stimulated healthy monocytes, confirming the role of oxidative stress and recapitulating the phenotype of CAPS monocytes (36). However, the latter are constitutively stressed by the mutation (37) so that stimulation with low doses of a single TLR agonist strongly increases stress that drives prompt and abundant IL-1β secretion and, in a second phase, lowers IL-1Ra (30, 31). In healthy monocytes with balanced basal redox state (29, 36), instead, multiple TLR co-stimulation is needed to cause cell stress and derange the normal cytokine network (36). These observations may suggest that, in CAPS, mutations in NLRP3 are more important indirectly, triggering and enduring stress, than directly activating inflammasome.

DIFFERENT MECHANISMS FOR IL-1β SECRETION: DOES STRESS DETERMINE THE PATHWAYS OF SECRETION?

Since IL-1β is a potent and potentially dangerous mediator of inflammation, its production is tightly controlled virtually at all levels, including post-translationally (38, 39). IL-1β is synthesized as an inactive precursor, pro-IL-1β, and processed mainly by

caspase-1, which in turn must be activated by the inflammasome. Only mature, 17 kDa IL-1β is then secreted. The underlying mechanisms are still poorly understood. Indeed, IL-1β secretion has been a problem for cell biologists, since it was shown that the cytokine lacks a secretory signal sequence (40). Initially, a popular view was that the cytokine was released by dying cells. However, further studies demonstrated that secretion of mature IL-1β is an active process, requires living cells, avoids the ER-Golgi route, and involves secretory lysosomes (41–44). In addition to this pathway, recent studies revealed another route for IL-1β release, involving pyroptosis. This is a highly inflammatory form of programmed cell death, which has been proposed to mediate IL-1β secretion under condition of strong stimulation such as infection with intracellular pathogens (45–48). According to this model of secretion, stressful stimuli (e.g., intracellular LPS) activate caspase-11 (the mouse homologous of human caspase-4). In turn, caspase-11 cleaves gasdermin D, generating toxin-like peptides that form pores on the plasma membrane, which allow secretion of mature IL-1β, but not of the 33 kDa precursor (48, 49). It remains to be determined how the pores guarantee transport selectivity.

The two pathways are not mutually exclusive, and the choice of lysosomal or pyroptotic secretion may depend on the strength of pro-inflammatory signals (**Figure 2**). Mild stimuli, such as low amounts of PAMPs triggering surface bound TLRs, would induce the less efficient but more regulated lysosomal pathway. Accordingly, low doses of LPS induce pro-IL-1β synthesis, but not ATP secretion (31): in the absence of a second trigger, therefore, pro-IL-1β is degraded by lysosomal proteases (31, 42) preventing unnecessary inflammation. Stronger stimuli, such as intracellular infections with gram-negative bacteria (45) could instead induce pyroptosis, causing massive release of IL-1β and possibly DAMPs, and dysregulated cytokine production (36).

Support to this hypothesis comes from our preliminary observations that human monocytes display more IL-1β-containing lysosomes when stimulated with LPS alone than with three agonists simultaneously triggering extra- and intracellular TLRs (unpublished results). Moreover, only in monocytes stimulated with extracellular LPS alone, do drugs interfering with lysosomal function modulate IL-1β secretion. Conversely, caspase-4 inhibitors block IL-1β release only in super-stimulated monocytes (unpublished results).

It is possible that the secretory lysosome-mediated mechanism is more active in low pathogen load or small trauma, as a way to restore the homeostasis. Differently, the pyroptosis-mediated secretion would intervene in severe inflammatory responses, characterized by strong or multiple stimuli such as it may occur in sepsis (50), diabetes (51) or cancer (52).

The ongoing stress could also determine the route of IL-1β secretion. Owing to the high ROS levels that favor ATP release, we predict that CAPS monocytes utilize preferentially the pyroptotic pathway. Accordingly, caspase-4 inhibition blocks IL-1β secretion by CAPS monocytes stimulated with a single TLR agonist, a condition that neither involves caspase-4 nor induces pyroptosis in healthy monocytes (unpublished) (**Figure 2**).

CELL-AUTONOMOUS PROTEOTOXIC STRESS IN MONOCYTES INCREASES IL-1β SECRETION IN AUTOINFLAMMATORY DISEASES

Increased IL-1β secretion has been reported *in vitro* by monocytes from other autoinflammatory diseases, including FMF (53), TRAPS (54) hyperimmunoglobulinemia D syndrome (55), pyogenic sterile arthritis, pyoderma gangrenosum and acne (PAPA) (56), and also in the milder NLRP-12-associated periodic syndrome (57). As introduced above, anti-IL-1β therapies are the standard of care in these syndromes (58), suggesting that IL-1β is a key culprit. Nonetheless, the links between the mutated gene and IL-1β secretion are elusive. Remarkably, in these diseases, the mutant genes are expressed by monocytes that are under stress (53, 57, 59–61). It is tempting to speculate that stress and the ensuing responses converge to induce excessive IL-1β secretion, possibly switching from lysosomal to pyroptotic secretion (**Figure 2**). The consequences on disease severity are many, since pyroptosis-mediated secretion would alter the networks of pro- and anti-inflammatory cytokine production.

Stress-induced hyperinflammatory response may occur in other inherited diseases that are not (yet) classified as autoinflammatory diseases. This is the case of chronic granulomatous disease (CGD), a disorder linked to mutations in NOX2. Because of these mutations, phagocytes of CGD patients fail to produce ROS with consequent deficiency in bactericidal activity and increased susceptibility to infections (62). In addition, and consistent with the evidence that CGD is associated with increased inflammasome activation (63–65), patients often develop hyperinflammatory traits. Moreover, *Anakinra* induced significant clinical improvement in two cases with colitis (66). Thus, CGD was defined as a potentially lethal combination of immunodeficiency and excess inflammation (67), most likely due to cell-autonomous stress responses. Likewise, evidence is accumulating for a role of stress and inflammation in the pathogenesis of Gaucher disease, the inherited deficiency of lysosomal glucocerebrosidase (68). Monocyte/macrophages from these patients display increased secretion of IL-1β that depends on increased inflammasome activation, in turn due to the impaired autophagy secondary to the lysosomal enzyme deficiency (68). A further example is mucopolysaccharidosis type I, where, in innate immune cells, stress induced by lysosomal storage defects can upregulate immunity-related genes. In turn, these may be responsible for the severe inflammation-dependent pathologies observed in patients (69).

CONCLUSION AND PERSPECTIVES

In essence, we propose that stress hallmarks monocytes from patients affected by autoinflammatory syndromes (and possibly other inherited diseases) that express mutant proteins not necessarily directly involved in IL-1β production. Stress induces inflammation and is, therefore, a key pathogenetic factor in these diseases. The stress levels contribute to determine the severity of the disease, and so do individual differences in resistance to

oxidative stress, as proposed for chronic inflammation-mediated diseases such as diabetes (70). Accordingly, we showed clear correlations among basal stress, ongoing antioxidant responses, and disease severity in two CAPS patients sharing the same NLRP3 mutation (31). Extending these concepts, we suggest that a similar stress-related mechanism may be operative in other genetic diseases, where the mutant protein is present in monocytes and inflammation participates to disease progression. Considering that individual tolerance plays a major role (71), improving the responses to stress represents a promising therapeutic opportunity for these serious diseases.

AUTHOR CONTRIBUTIONS

SC, CS, RS, and AR designed, wrote, and approved the final manuscript.

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REVIEW
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NF-κB Pathway in Autoinflammatory Diseases: Dysregulation of Protein Modifications by Ubiquitin Defines a New Category of Autoinflammatory Diseases

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Autoinflammatory diseases are caused by defects in genes that regulate the innate immunity. Recently, the scope of autoinflammation has been broadened to include diseases that result from dysregulations in protein modifications by the highly conserved ubiquitin (Ub) peptides. Thus far these diseases consist of linear ubiquitin chain assembly complex (LUBAC) and OTULIN deficiencies, and haploinsufficiency of A20. The LUBAC is critical for linear ubiquitination of key signaling molecules in immune response pathways, while deubiquitinase enzymes, OTULIN and TNFAIP3/A20, reverse the effects of ubiquitination by hydrolyzing linear (Met1) and Lys63 (K63) Ub moieties, respectively, from conjugated proteins. Consequently, OTULIN or A20-deficient cells have an excess of Met1 or K63 Ub chains on NEMO, RIPK1, and other target substrates, which lead to constitutive activation of the NF-kB pathway. Mutant cells produce elevated levels of many proinflammatory cytokines and respond to therapy with cytokine inhibitors. Patients with an impairment in LUBAC stability have compromised NF-kB responses in non-immune cells such as fibroblasts, while their monocytes are hyperresponsive to IL-1β. Discoveries of germline mutations in enzymes that regulate protein modifications by Ub define a new category of autoinflammatory diseases caused by upregulations in the NF-kB signaling. The primary aim of this review is to summarize the latest developments in our understanding of the etiology of autoinflammation.

Keywords: TNFAIP3/A20, linear ubiquitin chain assembly complex, OTULIN, haploinsufficiency of A20, LUBAC deficiency, otulipenia/otulin-related autoinflammatory syndrome

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INTRODUCTION

Autoinflammatory diseases are a diverse group of inherited conditions characterized by early-onset systemic inflammation and are accompanied by a range of organ-specific manifestations. The genetic etiology involves abnormalities in molecules such as inflammasomes, cytokine inhibitors, cytokine receptors, enzymes, and proteasome complex. The excessive secretion of proinflammatory cytokines can lead to chronic morbidity and may be life-threatening. Therapies with cytokine inhibitors are efficacious in most patients; however, considering the high cost of biologics there is a need to develop more affordable treatment options for these lifelong conditions. The discovery of germline mutations

linked to autoinflammation offer an opportunity to search for new therapeutic targets.

UBIQUITIN (Ub) PATHWAY

Posttranslational protein modification by ubiquitination (also known as ubiquitylation) is critical for the regulation of many biological processes including DNA repair, endocytosis, transcription, protein degradation, and preservation of cellular homeostasis. Ubiquitination involves the attachment of evolutionarily conserved 76-aa Ub molecules to target proteins in the form of a monomer or polymers (Ub chains). The type of conjugation determines the fate of the modified protein by directing protein localization and regulating protein interactions, activity, and degradation (1).

The ubiquitination process is initiated by the attachment of a single Ub molecule to a target protein through a three-step enzymatic pathway that includes Ub-activating enzymes (E1), Ub-conjugating enzymes (E2), and Ub-ligating enzymes (E3) (2). Ub chains are generated by the sequential addition of Ub monomers through one of seven lysine residues that serve as a linker, thus there are seven types of Ub Lys-linkages. Ub can be also conjugated through an N-terminal methionine residue (Met1 linkage; linear linkage). Lys11-, Lys48-, Lys63-, and Met1-linked chains are the best known and most studied. Significance of four other Ub chains Lys6-, Lys27-, Lys29-, and Lys33- is poorly understood. In addition, there is increasing evidence that more than one linkage type can exist on modified proteins either in the form of mixed (hybrid) Ub chains or branched Ub chains, and this may provide a new layer of complexity to the Ub-mediated modifications (2, 3). Ub proteins are also subject to modifications by acetylation, phosphorylation, and ubiquitin-like (UBL) molecules (SUMO or NEDD8) (4). Ub-conjugated proteins are recognized by Ub sensors, Ub-binding proteins (Ub receptors) that can translate each linkage type of Ub modifications into specific functional outcomes. For example, proteins conjugated with Lys (K11) or Lys48 (K48) Ub chains are targeted for proteosomal degradation *via* the ubiquitin–proteasome system (UPS) (5). In addition, Lys11 Ub chains have a role in cell cycle control and may have other functions in the context of mixed Ub chains (6). Lys63 (K63) Ub chains are involved in cell signaling and are essential for DNA damage response (7). Linear (Met1) Ub chains regulate a wide range of immune signaling pathways (8–10). Ubiquitination is a highly dynamic and reversible process whereby Ub chains are removed from modified substrates by a class of enzymes known as deubiqutylases or deubiquitinases (DUBs) (11). There are close to 100 proteases that have DUB activity with different degrees of specificity for Ub linkages. Several DUBs, including A20, OTULIN, CYLD, and Cezanne, function as negative regulators of NF-kB signaling (12).

Alterations in various components of the Ub–proteasome machinery have been linked to many human conditions including immune diseases. Recently, deregulations in the UPS were reported in patients with autoinflammatory disorders including chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperature (13), linear ubiquitin chain assembly complex (LUBAC) deficiency (14, 15), haploinsufficiency of A20 (HA20) (16), and otulipenia/otulin-related autoinflammatory syndrome (ORAS) (17, 18). This review will primarily focus on two diseases caused by malfunction in DUB enzymes, TNFAIP3/A20 and OTULIN, which are known to hydrolyze Lys63- and Met1-linked Ub chains, respectively. In both conditions, HA20 and otulipenia/ORAS, a defect in DUB activity results in excessive ubiquitination and increased activity of key signaling molecules in the canonical NF-kB pathway. LUBAC-associated diseases will be briefly discussed in the context of LUBAC–OTULIN interactions.

LINEAR Ub CHAINS IN IMMUNE SIGNALING

Linear ubiquitin chain assembly complex (LUBAC)-mediated Met1 ubiquitination has emerged pivotal for regulation of innate and adaptive immune responses and regulation of cell death (9, 19). The E3 ligase complex, LUBAC, has been shown to maintain the stability of the TNF receptor 1 (TNFR1), TLRs, IL-1R, CD40, RLR, and inflammasome receptor signaling complexes (RSCs). Upon stimulation with proinflammatory signals, LUBAC is recruited to attach linear Ub chains to target substrates such as IKK (NEMO), RIPK1, RIPK2, IRAKs, MyD88, and ASC (8, 20, 21). Attachment of linear Ub chains is critical for the assembly of RSCs. LUBAC depletion leads to attenuation of NF-kB and the mitogen-activated protein kinases (MAPK)-mediated signaling and increases cell death.

Linear ubiquitin chain assembly complex consists of HOIP (HOIL-1 interacting protein; *RNF31*) and two accessory proteins: HOIL-1 (heme-oxidized IRP2 ubiquitin ligase 1; *RBCK1*) and SHARPIN (SHANK-interacting protein like 1; *SIPL1*) (**Figure 1**). The catalytic subunit HOIP is auto-inhibited until the complex is fully assembled. Depletion of any subunits greatly reduces the stability of LUBAC in cells (14, 15). As the immune responses must be tightly regulated to avoid chronic inflammation, LUBAC activity is counter-regulated by the specific DUB OTULIN (also known as *gumby*; *FAM105B*).

OTULIN is a highly conserved protease with a specific activity to hydrolize linear (Met1)-linked Ub chains (22) (**Figure 1**). OTULIN interacts with the N-terminal PUB domain of HOIP *via* an evolutionarily conserved PUB-interacting motif (23). Loss of HOIP–OTULIN interaction reduces OTULIN capacity to restrict LUBAC-induced NF-kB activation (24). A recent study showed that the activity of LUBAC is also negatively regulated by its interaction with tumor necrosis factor receptor-associated factor 1 (TRAF1). TRAF1 directly interacts with LUBAC to interfere with the activation of IKK/NEMO. Reduced expression of TRAF1 could explain the association of susceptibility alleles in *TRAF1* with rheumatoid arthritis (RA) and other autoimmune diseases (25).

The importance of the linear ubiquitination in regulation of inflammatory pathways has been demonstrated in murine models. Genetic ablation of the catalytic HOIP subunit results in embryonic lethality at day 10.5 due to TNF-mediated endothelial cell death and vascular abnormalities (26). Mice deficient for non-catalytic subunits have variable degrees of inflammation manifesting with chronic proliferative dermatitis in the case

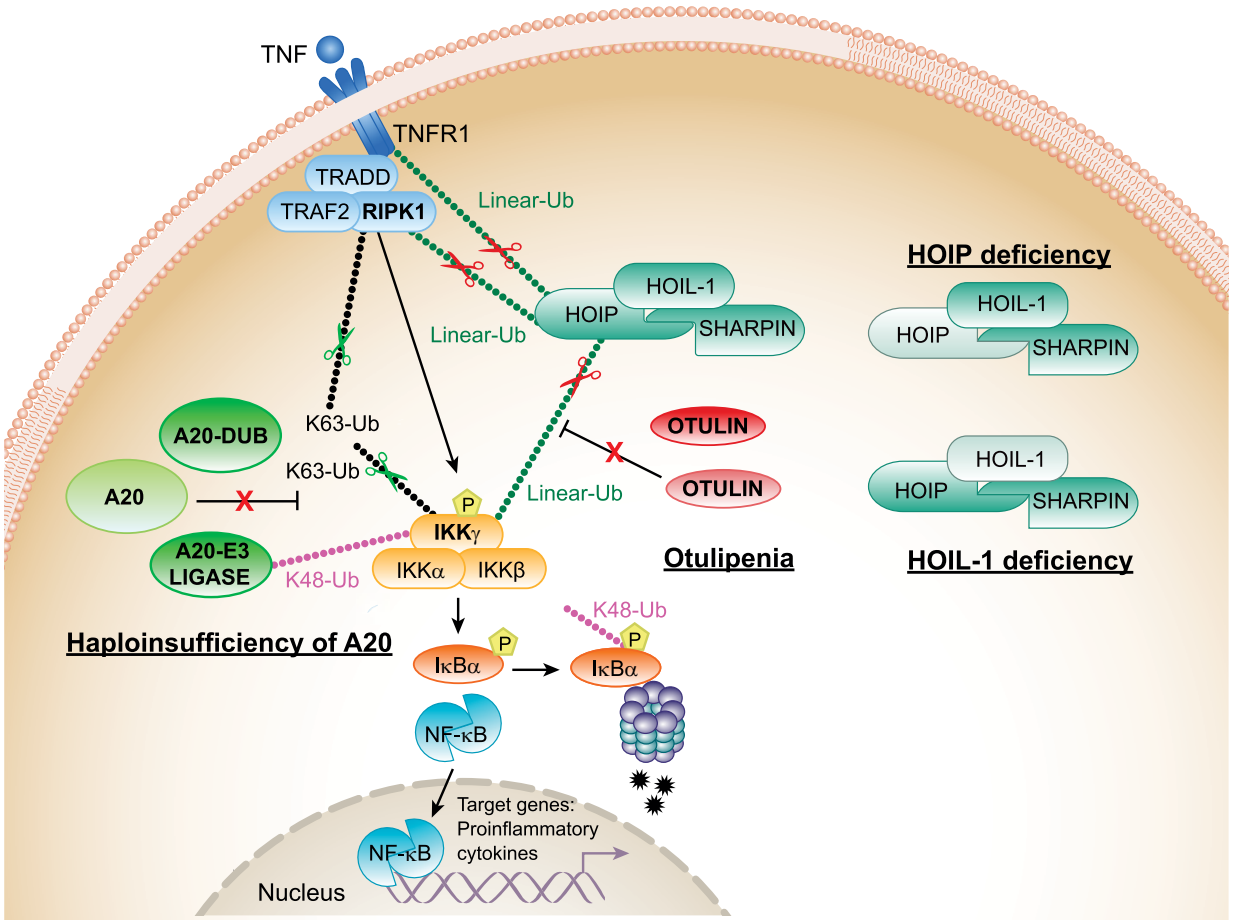

FIGURE 1 | Proposed mechanisms of pathogenesis in haploinsufficiency of A20 (HA20), otulipenia/otulin-related autoinflammatory syndrome (ORAS), and linear ubiquitin chain assembly complex (LUBAC) deficiencies. The canonical NF-κB pathway is regulated both by K63 (Lys63)-linked and linear (Met1)-linked ubiquitin (Ub) chains. RIPK1 is the central adaptor for assembly of the TNFR1 receptor signaling complex and is a predominant target for ubiquitination by K63 and linear Ub chains. Polyubiquitylated RIPK1 mediates recruitment of IKK complex that is also target for ubiquitination. The activated IKK complex phosphorylates inhibitor of kappa B (IκBα) and targets IκBα for proteasome-mediated degradation. Linear Ub chains are added to RIPK1 and IKKγ by LUBAC. A20 and OTULIN negatively regulate the NF-κB pathway by cleaving K63 and linear Ub chains from target molecules, RIPK1 and IKKγ. In addition, A20 through its E3 ligase activity adds K48 Ub chains to IKKγ (and TRAF6, not shown in the figure) targeting them for proteasomal degradation. Decreased expression of A20 in patients with HA20 or OTULIN in patients with otulipenia/ORAS will lead to activation of the NF-κB pathway, increased expression of proinflammatory transcripts in immune cells, and systemic inflammation. Decreased expression of LUBAC complex subunits, in patients who carry mutations in HOIP or HOIL-1, results in inhibition of the NF-κB pathway in fibroblasts and B-cells causing immunodeficiency. However, their monocytes hyperproduce proinflammatory cytokines. TNFR1, TNF receptor 1; TRADD, TNFR1-associated death domain protein; RIPK1, the death domain-containing protein kinase receptor-interacting protein1; IKKγ/NEMO, inhibitor of nuclear factor kappa B kinase subunit gamma.

of SHARPIN-deficient mice (*cpdm*) (27, 28) or with no overt inflammation in mice lacking HOIL-1 (21). Skin inflammation in Sharpin-KO mice is largely dependent on the TNFR1-induced apoptosis (19, 29, 30). LUBAC activity is also important for proper B and T cell development, activation, and maintenance of adaptive immune responses (31, 32). Genetic loss of *Otulin* (*gumby*/*gumby*) causes embryonic lethality (E12.5–E14) due to compromised angiogenesis and defects in neuronal development (33). Tamoxifen-induced *Otulin* deficiency in immune cells results in an acute severe multiorgan inflammatory phenotype (18). Targeted ablation of *Otulin* in myeloid cells leads to chronic inflammation with features of autoimmunity, while *Otulin* deficiency in adaptive immune cells does not produce overt phenotype (18). Together, these data show critical and cell-specific function of OTULIN in the maintenance of immune homeostasis.

LUBAC DEFICIENCIES

Patients with defects in the LUBAC components develop immunodeficiency, autoinflammation, muscular amylopectinosis, and die in early childhood (**Tables 1** and **2**). HOIL-1 and HOIP deficiencies are recessively inherited diseases caused by mutations

TABLE 1 | Comparison of genetics and mechanisms of disease in otulipenia, haploinsufficiency of A20 (HA20), and linear ubiquitin chain assembly complex (LUBAC) deficiencies.

		Otulipenia/ORAS	**HA20**
Gene	Gene name	*OTULIN* (*FAM105B*)	*TNFAIP3*
	Exons	7 exons	9 exons
	Chromosome	Chr.5	Chr.6
Protein	Protein name	OTULIN	A20
	Protein length	352aa	790aa
	Protein domains	PUB-interacting motif domain, ovarian tumor (OTU) domain	OTU domain, 7 ZnF domains
	Protein function	Met1 linear deubiquitinase (DUB)	K63 DUB
	Involved pathway	NF-κB	NF-κB, NLRP3
	Substrate molecules	NEMO, TNF receptor 1 (TNFR1), RIPK1, ASC	NEMO, RIPK1, TRAF6, pro IL-1β
Genetics	Inheritance	Recessive	Dominant
	Type of mutations	Loss-of-function mutations (missense, INDELS)	Loss-of-function mutations (stop gain mutation, missense, INDELS)
	Frequency of mutant alleles	Rare or novel	Novel
	Location of the mutations	OTU domain (linear DUB activity)	OTU domain (k63 DUB activity) or ZnF4 domain
	Number of mutations	Biallelic (compound heterozygous or homozygous)	Heterozygous
Mechanisms	Mechanism	Loss-of-function (reduced protein expression)	Haploinsufficiency (50% decrease in protein expression)
	Protein Interactions	Instability of LUBAC	Decreased association with TNFR1, TRAF2, and RIPK1
	Effect of mutant proteins	Impaired linear deubiquitination of NEMO, TNFR1, RIPK1, ASC	Impaired K63 deubiquitination of NEMO, RIPK1, and TRAF6
	Involved pathway	Increased signaling in NF-κB and mitogen-activated protein kinases (MAPK) pathways	Increased activity of NF-κB, MAPKs, and NLRP3
	Cytokines	IL-1β, TNF, IL-6, IL-12, IL-18, IFNγ	IL-1β, TNF, IL-6, IL-9, IL-17, IL-18, IFNγ
		HOIL-1 deficiency	**HOIP deficiency**
Gene	Gene name	*RBCK1*	*RNF31*
	Exons	12 exons	21 exons
	Chromosome	Chr.20	Chr.14
Protein	Protein name	HOIL-1	HOIP
	Protein length	510aa	1,072aa
	Protein domains	Ubiquitin-like (UBL), novel zinc finger (NZF), RING1, IBR, RING2	PUB, ZnF, NZF1, NZF2, UBA, RING1, IBR, RING2, LDD
	Protein function	Subunit of the LUBAC	Catalytic subunit of the LUBAC
	Involved pathway	NF-κB signaling pathway	NF-κB signaling pathway
Mutations	Type of mutations	Loss-of-function mutations (stop gain mutation, INDELS)	Loss-of-function mutations (missense)
	Frequency of mutant alleles	Rare or novel	Novel
	Location of the mutations	UBL domain (interacts with HOIP UBA domain), NZF domain (ubiquitin binding)	PUB domain (interacts with OTULIN)
	Number of mutations	Biallelic (compound heterozygous or homozygous)	Biallelic (homozygous)
Mechanisms	Inheritance	Recessive	Recessive
	Mechanism	Loss-of-function (decreased protein expression, instability of LUBAC, impaired linear ubiquitination)	Loss-of-function (decreased protein expression, instability of LUBAC, impaired linear ubiquitination)
	Effect of mutant proteins	Defect in linear ubiquitination NEMO, RIPK1, IRAK-1	Defect in linear ubiquitination
	Involved pathway	Impaired NF-κB activation in fibroblasts, increased NF-κB activity in monocytes	Impaired NF-κB activation in fibroblasts and CD40L stimulated B cells, increased NF-κB activity in monocytes
	Cytokines	Impaired expression of IL-6 in fibroblasts upon IL-1β and TNF stimulation; hyperproduction of IL-6 upon in IL-1β stimulated monocytes; high serum IL-1, IL-6	Hyperproduction of IL-6 in IL-1β stimulated monocytes

that either create truncating proteins or affect a highly conserved PUB domain of HOIP (14, 15) (**Figure 2**). Pathogenic mutations in one subunit destabilize the expression of the entire LUBAC complex. As LUBAC is important for activation of immune signaling, stimulated patient fibroblasts and B cells fail to upregulate NF-kB activity, which manifests in recurrent bacterial infections. In contrast to immunodeficient fibroblasts, peripheral blood mononuclear cells (PBMCs) of HOIP and HOIL-1-deficient patients are highly responsive to IL-1 stimulation and produce high levels of proinflammatory cytokines IL-6 and MIP-1α. One patient was noted to have severe T cell lymphopenia and impaired antibody production. Muscular amylopectinosis/myopathy appear to be unrelated to a defect of linear ubiquitination in immune cells and its mechanism remains to be investigated. Abnormalities in the lymphatic system have been observed in the HOIP-deficient patient and HOIP-deficient mice, which suggests

TABLE 2 | Clinical manifestations in patients with otulipenia, haploinsufficiency of A20 (HA20), and linear ubiquitin chain assembly complex deficiencies.

Clinical manifestations	Otulipenia (17)		HA20 (16, 35–37)					HOIL-1 deficiency (14)		HOIP deficiency (15)
	Yes or no	Patients ($n = 3$)	Yes or no	Patients (16) ($n = 11$)	Patients (37) ($n = 6$)	Patients (36) ($n = 3$)	Patients (35) ($n = 1$)	Yes or no	Patients ($n = 3$)	Yes or no ($n = 1$)
Early age onset	Yes (1–4.5 months)	3/3	Yes (7 months–16 years)	11/11	6/6	3/3	1/1	Yes	3/3	Yes
Fevers	Yes (fever lasting 2–3 weeks)	3/3	Yes	2/11	3/6	1/3	1/1	Yes	3/3	Yes
Skin rash	Yes (erythematous with skin nodules, pustular rash)	3/3	Yes (erythematous papules, folliculitis, skin abscesses)	4/11	6/6	0/3	1/1	Yes (eczema; erythroderma, desquamative dermatitis)	1/3; 1/3	No
CNS	No	1/3	Yes (CNS vasculitis, chorea, migraine)	2/11	/	/	0/1	No	0/3	No
GI	Yes (abdominal pain, diarrhea)	1/3	Yes (colitis)	4/11	1/6	1/3	0/1	Yes (abdominal pain, diarrhea, vomiting, blood and mucus in the stools)	3/3	Yes (recurrent episodes of fatty diarrhea, intestinal lymphangiectasia)
Arthritis Arthralgias Myalgias	Yes (arthralgias, myalgias)	3/3	Yes (arthralgia, polyarthritis)	6/11	/	2/3	0/1	No	0/3	Yes
Elevated CRP, ESR	Yes	3/3	Yes	6/6	1/1	/	1/1	Yes	3/3	Yes
Immunodeficiency	No obvious primary immunodeficiency	2/3	Yes (IgG2 and 4 subclass deficiency, low anti-polysaccharide antibodies lymphopenia)	2/11	/	/	1/1	Yes (recurrent bacterial infections, memory B-cell deficiency, and hyper-IgA syndrome)	3/3	Yes (recurrent viral and bacterial infections, lymphopenia, antibody deficiency, hypogammaglobulinemia)
Oral ulcers	No	0/3	Yes	11/11	6/6	2/3	0/1	Yes	1/3	Yes
Genital ulcers	No	0/3	Yes	10/11	6/6	1/3	0/1	No	0/3	No
Ophtho	No	0/3	Yes (uveitis, retinal vasculitis)	3/11	/	1/3	/	No	0/3	No
Pathergy	No	0/3	Yes	3/11	/	/	/	No	0/3	No
Autoantibodies	No	0/3	Yes (RNP, ANA, lupus anticoagulant)	5/11	/	/	1/1	No	0/3	No
Panniculitis	Yes	3/3	No	0/11	0/6	0/3	0/1	No	0/3	No
Failure to thrive	Yes	3/3	No	0/11	0/6	0/3	0/1	Yes	3/3	Yes
Lipodystrophy	Yes	3/3	No	0/11	0/6	0/3	0/1	No	0/3	No
Lymphadenopathy	Yes	2/3	Yes	0/11	0/6	0/3	1/1	Yes	2/3	Yes
Systemic lymphangiectasia	No	0/3	No	0/11	0/6	0/3	0/1	No	0/3	Yes

(*Continued*)

TABLE 2 | Continued

Clinical manifestations	Otulipenia (17)		HA20 (16, 35–37)				HOIL-1 deficiency (14)		HOIP deficiency (15)
	Yes or no	Patients (n = 3)	Yes or no	Patients (16) (n = 11)	Patients (37) (n = 6)	Patients (36) (n = 3)	Patients (35) (n = 1)	Yes or no	Patients (n = 3)	Yes or no (n = 1)
Weakness in lower extremities	No	0/3	No	0/11	0/6	0/3	0/1	Yes	2/3	Yes
Cardiomyopathy	No	0/3	No	0/11	0/6	0/3	0/1	Yes	3/3	Yes
Respiratory distress	No	0/3	No	0/11	0/6	0/3	0/1	Yes	1/3	Yes
Amylopectinosis	No	0/3	No	0/11	0/6	0/3	0/1	Yes	3/3	Yes
Systemic edema	No	0/3	No	0/11	0/6	0/3	0/1	No	0/3	Yes
Effective treatment	anti-TNF, anti-IL-1	2/3; 1/3	anti-IL-1, anti-TNF; colchicine	1/11; 4/11; 2/11	0/6; 0/6; 1/6	0/6; 0/6; 0/6	0/1; 0/1; 0/1	anti-TNF, steroids; BMT	2/3; 1/3	Naproxen, antibiotic prophylaxis, IVIG

/, not reported.
Source of data: Ref. (14–18, 35–37).

that HOIP, independently of other LUBAC components, may have a function in the regulation of angiogenesis. LUBAC appears to be important for toll-like receptor 3 (TLR3)-mediated innate immune responses to influenza A virus infection, by enabling TLR3-mediated activation of NF-kB, MAPK, and IRF3 (34).

In summary, the identification of patients with LUBAC deficiencies showed the critical role of LUBAC in regulation of immune responses.

OTULIPENIA/ORAS

Recessively inherited loss-of-function mutations in the linear (Met1)-specific DUB OTULIN have been linked to the early-onset severe inflammatory disease, named otulipenia/ORAS (**Table 1**) (17, 18). Patients present with prolonged recurrent fevers, joint swelling, GI inflammation/diarrhea, and failure to thrive (**Table 2**; **Figure 3**). The cutaneous manifestations include painful erythematous rash with skin nodules, lipodystrophy, and episodes of pustular rash in one patient (**Figure 3B**). Skin biopsy showed evidence for neutrophilic dermatitis, mixed type panniculitis, and vasculitis of small and medium-sized blood vessels (**Figure 3C**) (17). In contrast to patients with the LUBAC deficiency, OTULIN-deficient patients have no obvious immunodeficiency, although some of them suffered from iatrogenic infections induced by immunosuppressive therapies. Initial analyses showed adequate specific antibody responses to vaccines, adequate T and B cell proliferative responses, normal levels of immunoglobulins, and normal T, B, and NK cell counts.

The four patients identified carry novel homozygous mutations in the *FAM105B* gene that encodes OTULIN. Heterozygote carriers are asymptomatic, which suggests that reduced protein expression of OTULIN may not be critical for maintenance of immune homeostasis. OTULIN is a 352-residue protein that consists of N-terminal LUBAC-binding domain and C-terminal ovarian tumor (OTU) domain. Disease-associated mutations affect the OTU domain and binding of OTULIN to linear Ub chains (**Figure 2**).

OTULIN functions as a negative regulator of the canonical NF-kB pathway and as such is essential for resolving inflammation (**Table 1**; **Figure 1**). Mutant OTULIN proteins cannot restrict the accumulation of Met1 Ub chains on target substrates IKK/NEMO, RIPK1, and ASC. Patients' mononuclear leukocytes and fibroblasts have a strong inflammatory signature as evidenced by increased degradation of IkBα and increased phosphorylation of IKKα/IKKβ and IkBα, the hallmarks of the activated NF-kB pathway. Mutant cells overproduce many proinflammatory cytokines, including cytokines associated with activation of adaptive immune cells, and therapy with TNF inhibitors is very effective in controlling disease activity. Tamoxifen-induced *Otulin* ablation in murine immune cells (CreERT2-$Otulin_{LacZ/flox}$ chimeras) resembles the phenotype described in patients with otulipenia/ORAS including responsiveness to therapy with TNF inhibitors (18).

In summary, combined data from human and murine studies suggest that deficiency of the linear DUB, OTULIN, leads to amplification of the Met1 Ub regulated signaling in the canonical NF-kB pathway, most notably in myeloid cells.

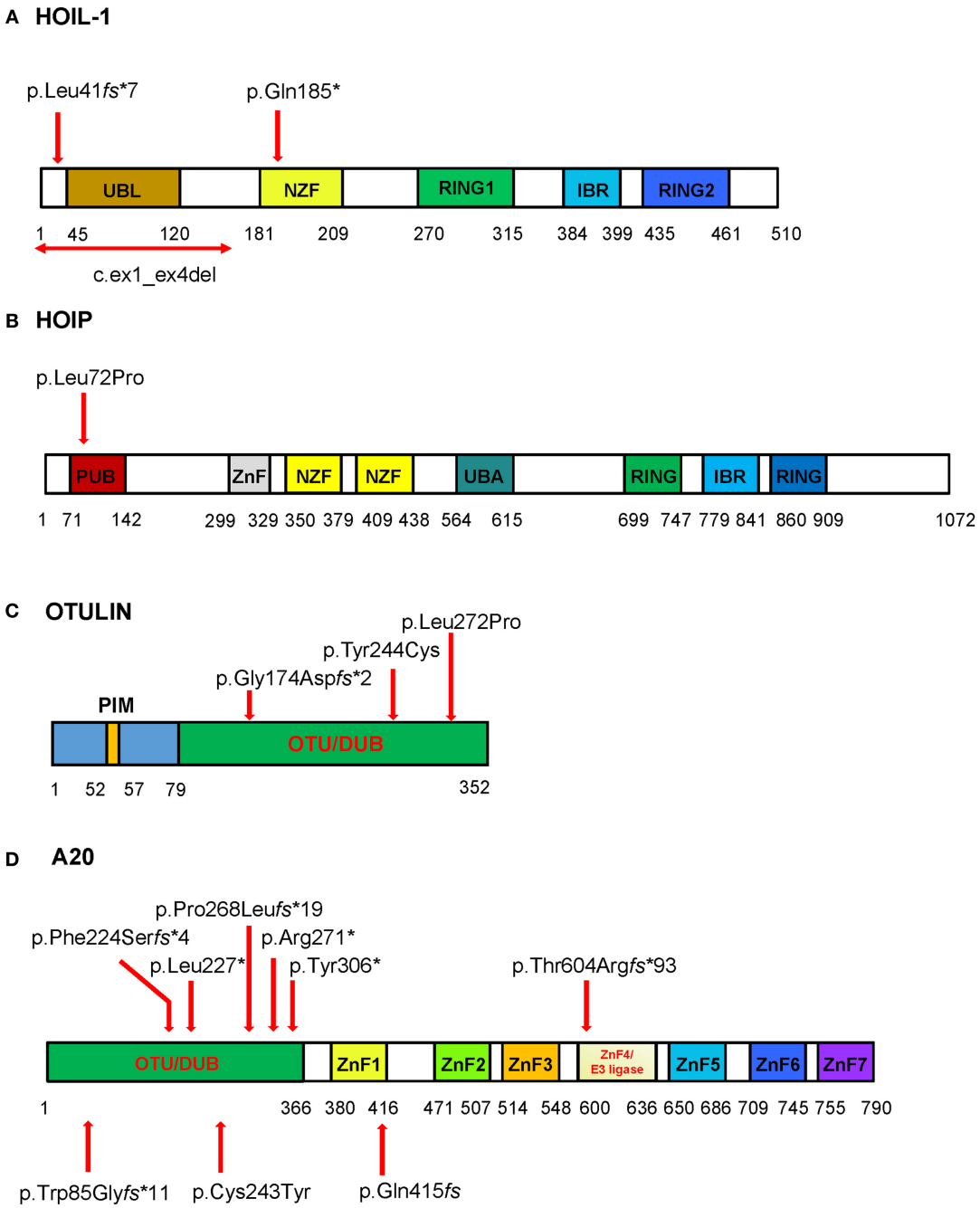

FIGURE 2 | Schematic of the domains and locations of mutations in respective proteins HOIL-1, HOIP, OTULIN, and TNFAIP3/A20. The domains identified are depicted as boxes. The locations of the mutations are indicated with red arrows. **(A)** HOIL-1 contains the ubiquitin-like (UBL), NPL4 zinc-fingers (NZF) domain, really interesting new gene (RING) domain, and in-between RING (IBR) domain. The mutations are located in UBL and NZF domains. The UBL domain is required for linear ubiquitin chain assembly complex (LUBAC) formation and linear ubiquitination. **(B)** HOIP has PNGase/UBA or UBX (PUB), zinc finger (ZnF), NZF, ubiquitin-associated (UBA), RING domain, and IBR domain. The homozygous disease-associated mutation is located in the PUB domain that is critical for interaction with OTULIN and stability of LUBAC. **(C)** OTULIN consists of N-terminal LUBAC-binding PUB-interacting motif (PIM) and C-terminal ovarian tumor (OTU) domain that mediates deubiquitinase (DUB) activity of OTULIN (79–352aa). All three mutations are located in the OTU domain. **(D)** The DUB activity of A20 is mediated by the OTU domain, and the ZnF domains mediate A20 ubiquitin (Ub) E3 ligase activity, binding to Lys63-linked Ub chains and dimerization. Mutations reported by Zhou at al. are shown on the top of the diagram, while three mutations reported in Japanese patients are shown bellow the diagram. To stay consistent with the Human Genome Variation Society nomenclature the reported p.Gln415fs (35) should be described as p.Lys417Serfs*4. The four nucleotide deletion in the repeat sequence (reported as c.1245_1248del) should be assigned as the most 3′ position in the repeat, i.e., c.1249_1252del; therefore, the proposed nomenclature for the mutation is p.Lys417Serfs*4.

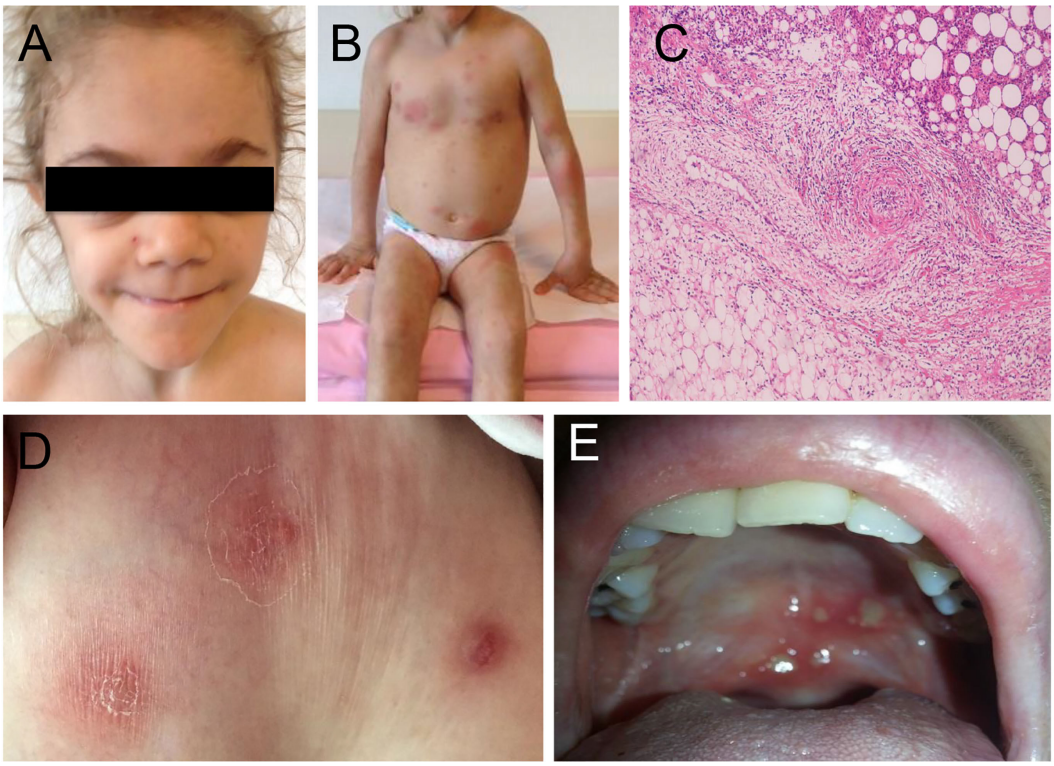

FIGURE 3 | Clinical manifestations of the patients with haploinsufficiency of A20 (HA20) and otulipenia/otulin-related autoinflammatory syndrome. **(A)** Prominent fat loss (lipodystrophy) and **(B)** erythematous skin lesions and subcutaneous nodules in a patient with otulipenia. **(C)** Skin biopsy showed dense inflammatory infiltrate throughout the subcutaneous lobules (right upper corner of the image) and subcutaneous lobular atrophy or lipodystrophy (similar findings have been reported in chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperature patients) on the left lower corner of the image. The middle part of the image showed vasculitis affecting a medium-sized artery, characterized by dense intramural and perivascular inflammation with endothelial proliferation and vascular occlusion. Adjacent the affected artery is a medium-sized vein (left lower) showing mild inflammation of the vessel wall. **(D)** Dermal abscesses in a patient with HA20. **(E)** Recurrent aphthous (oral) ulcers in a patient with HA20.

Lys63-LINKED Ub CHAINS IN IMMUNE SIGNALING

Lys(K)63-Ub modification was first described as a mechanism for the activation of the canonical NF-κB pathway (38). K63-linked Ub chains are generated following cell stimulation with inflammatory cytokines, and they function as a scaffold for the formation of receptor signaling complexes. Molecules such as NEMO/IKK, TRAF6, and RIPK1 are ubiquitinated both by linear and K63 Ub chains, which suggest a substantial regulatory redundancy in immune signaling (39–42). TNFAIP3/A20 restricts inflammatory responses *via* its dual yet synergistic functions: its deubiquitinase activity by hydrolyzing K63 linkages and its E3 ligase activity by conjugating substrates with K48 Ub chains to target them for proteasomal degradation (43, 44) (**Figure 1**). A20 is also subject to regulations as it undergoes posttranslational phosphorylation (45) and is cleaved by mucosa-associated lymphoid tissue lymphoma translocation protein 1 (46). In tumor cells, A20 acts as a tumor-suppressor gene and is frequently inactivated by somatic mutations and deletions in diffuse large B-cell and Hodgkin lymphomas (47, 48).

The *TNFAIP3* gene is highly conserved and intolerant to genetic variations, in particular to loss-of-function mutations. Common low-penetrance mostly non-coding variants in *TNFAIP3* have been associated with many autoimmune diseases including systemic lupus erythematosus (SLE) (49–51), RA (52), psoriasis (53), type 1 diabetes (54), celiac disease (55), coronary artery disease (56), inflammatory bowel disease (57), and more recently with protection to allergy and asthma (58, 59). Given the potent anti-inflammatory function of A20, these susceptibility alleles are predicted to decrease A20 expression and function, although that has been experimentally demonstrated only for a single non-coding variant associated with SLE. The dinucleotide functional variant downstream of *TNFAIP3*, TT > A, likely alters the binding of transcription factors in response to proinflammatory signals (51, 60).

Genetic ablation of A20 leads to spontaneous inflammation in mice with a range of cell-specific phenotypes. A20-deficient ($A20^{-/-}$) mice exhibit multiorgan inflammation, cachexia, and early lethality (61). Although A20 was initially described as required for termination of TNF-induced signals, the excessive inflammation observed in double-deficient mice, A20-TNF or

A20-TNFR1, suggested that A20 might be critical for the regulation of TNF-independent signals including the termination of TLR-induced activity of NF-kB (62). Cell-specific deletions of A20 resemble human autoimmune diseases, from a mild autoimmune phenotype in *Tnfaip3 Cd19* (B-cell) KO mice to severe spontaneous inflammation in mice with A20-deficient dendritic cells. Loss of A20 in macrophages mimics human RA, although the phenotype appears to be TNF-independent. Deficiency of A20 in keratinocytes leads to hyperkeratosis, while loss of A20 in intestinal epithelial cells causes DSS-induced TNF-dependent colitis (63). Aging heterozygous mice ($A20^{+/-}$) develop spontaneous autoantibodies. In summary, multiple murine models with cell-specific ablation of A20 demonstrate closer approximation of human diseases than the complete knock-out mice.

This past year, Zhou et al. reported 11 patients from 6 families with a new dominantly inherited autoinflammatory disease, termed haploinsufficiency of A20, characterized by childhood-onset episodic fevers, arthralgia/arthritis, oral and/or genital ulcers, skin pathergy, GI, and ocular inflammation (16) (**Table 2**; **Figures 3D,E**). These symptoms resemble Behcet's disease (BD). One patient was initially diagnosed with SLE and presented with CNS vasculitis and idiopathic thrombocytopenic purpura. Subsequently, two families of Japanese ancestry diagnosed with entero-BD and one Japanese patient diagnosed with autoimmune lymphoproliferative syndrome (ALPS) were reported to carry novel LOF mutations in the gene (**Figure 2**) (35–37). A patient with ALPS presented with fevers, bilateral cervical lymphadenopathy, extensive skin rash, and massive hepatosplenomegaly. The patient's immunophenotyping revealed an increased percentage of DNT cells and a decreased number of IgM memory B cells, which is characteristic of ALPS. However, unlike in ALPS the patient's central memory (TCM), naïve, TEMRA, and effector memory (TEM) subpopulations of $CD^{3+}CD^{8+}$ cells were normal (35).

TNFAIP3/A20 is a 790-residue protein that consists of an amino-terminal OTU followed by seven zinc finger (ZnF) domains. HA20-associated mutations create truncated mutant proteins, and most of them are located in the OTU domain (**Figure 2**). In addition to its OTU domain-mediated DUB activity, A20 can downregulate IKK activation by blocking IKK phosphorylation (64). Two pathogenic mutations have been identified in the ZnF domains 1 and 4. The ZnF4 domain is essential for A20 E3 ligase activity and dimerization (65).

Similar to patients with otulipenia/ORAS, mutant A20 cells have enhanced NF-kB activity as demonstrated by increased phosphorylation of IKKα/β and increased degradation of IkBα. Stimulated patient PBMCs and fibroblasts failed to hydrolyze K63 Ub chains from NEMO/IKK, RIPK1, and TNFR1 (16). Accumulation of K63 Ub proteins on these molecules triggers activity of the NF-kB and the MAPK pathways (**Table 1**; **Figure 1**). In murine models, A20/Tnfaip3 was shown to downregulate the activity of NLRP3 inflammasome (40, 66). Zhou et al. demonstrated constitutive NLRP3 activity in PBMCs of HA20 patients. Stimulated PBMCs and serum samples of HA20 patients have highly elevated levels of many proinflammatory cytokines produced by myeloid cells (IL-1, TNF, IL-6, IL-18, and IP-10) and T cells (IL-9, IL-17, and IFNγ). Therapies with cytokine inhibitors, anti-TNF, or anti-IL-1, have been very efficient in suppressing systemic inflammation.

Taken together, human genetic studies and murine models of A20 deficiency provide strong evidence that the reduced expression of A20 is associated with a range of inflammatory phenotypes.

CONCLUSION

Maintenance of immune homeostasis is a highly balanced act that requires coordinated action of many proteins to allow optimal and efficient immune responses. Discovery of otulipenia/ORAS, HA20, and LUBAC-associated diseases has reiterated the importance of ubiquitination in regulation of immune signaling and revealed cell-specific functions of these proteins.

Despite similar function of OTULIN and A20 in restricting immune responses, the DUB activity of A20 appears to be less critical than the one of OTULIN (22). This may explain a milder inflammatory phenotype in HA20 than in patients with otulipenia. In addition, patients with otulipenia have more profound protein deficiency (less than 50%) than the patients with HA20 who retain one functional allele of the gene (50% protein deficiency).

Given the importance of the ubiquitination in cellular physiology, the UPS system has elicited a significant interest for drug development. The list of human diseases related to abnormalities in UPS has been steadily increasing and includes neurodegenerative diseases, cancer, and immune diseases. Ub-mediated protein degradation is critical for homeostasis in aging neuronal cells (67). Deficiency of A20 has been linked to lymphomas, and its most aggressive subtype is associated with constitutive activation of NF-kB. Reconstitution of A20 in mutant cell lines induced apoptosis and suppressed tumor growth (47). In immune diseases, A20 and OTULIN might be new therapeutic targets for development of immunomodulatory drugs that can potentially increase or stabilize their expression.

A key challenge for finding effective drugs will be in developing cell-based therapies. The ubiquitination process is regulated at multiple levels: generation, recognition, and removal. Targeting more components of the Ub–proteasome pathway may provide new opportunities for therapeutic exploitation and drug discovery (68, 69).

AUTHOR CONTRIBUTIONS

IA reviewed the literature and wrote the manuscript. QZ reviewed the literature to make figures and tables and helped with writing the manuscript.

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REVIEW
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CANDLE Syndrome As a Paradigm of Proteasome-Related Autoinflammation

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CANDLE syndrome (*C*hronic *A*typical *N*eutrophilic *D*ermatosis with *L*ipodystrophy and *E*levated temperature) is a rare, genetic autoinflammatory disease due to abnormal functioning of the multicatalytic system proteasome–immunoproteasome. Several recessive mutations in different protein subunits of this system, located in one single subunit (monogenic, homozygous, or compound heterozygous) or in two different ones (digenic and compound heterozygous), cause variable defects in catalytic activity of the proteasome–immunoproteasome. The final result is a sustained production of type 1 interferons (IFNs) that can be very much increased by banal triggers such as cold, stress, or viral infections. Patients start very early in infancy with recurrent or even daily fevers, characteristic skin lesions, wasting, and a typical fat loss, all conferring the patients a unique and unmistakable phenotype. So far, no treatment has been effective for the treatment of CANDLE syndrome; the JAK inhibitor baricitinib seems to be partially helpful. In this article, a review in depth all the pathophysiological, clinical, and laboratory features of CANDLE syndrome is provided.

Keywords: CANDLE, neutrophilic dermatosis, proteasome, immunoproteasome, interferonopathy, autoinflammation

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DEFINITION

CANDLE is an acronym standing for *C*hronic *A*typical *N*eutrophilic *D*ermatosis with *L*ipodystrophy and *E*levated temperature (1–3). CANDLE syndrome is an autoinflammatory disease (AID) characterized by the appearance of recurrent fever in the first months of life, along with characteristic skin lesions, lipodystrophy, and manifestations of multisystem inflammation. Mutations in different genes encoding protein subunits of the proteasome–immunoproteasome system are the cause of CANDLE syndrome.

HISTORY

CANDLE syndrome was described in 2010 by Torrelo et al. (1). They reported on four children collected in two centers in Madrid and Chicago, two of whom were siblings, who showed striking skin lesions which on histopathology displayed an infiltration of immature, myeloid, mononuclear cells, resembling leukemia cutis. Because in many parts of the skin biopsies there was some maturation into polymorphonuclears and karyorrhexis, a type of yet undescribed "neutrophilic dermatosis" was suspected. The skin lesions had appeared very early in infancy, in attacks that occurred after common triggers (especially cold and viral infections), but some degree of skin involvement was present all the time. Furthermore, the patients had recurrent, almost daily, fevers or temperature

elevations below 38.3°C, also since very early in life. The disease seemed to cause some general growth delay, and after more than 10 years of follow-up most patients looked wasted, with a striking loss of fat. With all these features, the chronic eruption with skin neutrophilic and mononuclear immature infiltration, the fevers and the lipodystrophy, an acronym was coined. The dermatological aspects were emphasized in this description, because they had been the most constant features, but it was recorded that the patients had suffered unexplained inflammatory attacks in many different organs of the body, such as the central nervous system (CNS), cartilage, joints, testes, and many others. One of the patients died of an attack of "carditis," but autopsy was not performed.

Also, in 2010, Garg et al. (4) reported three adult patients with a disease they named JMP (*J*oint Contractures, *M*uscle atrophy, microcytic anemia, and *P*anniculitis-induced lipodystrophy syndrome). The authors emphasized the lipodystrophic features that were related to panniculitis, and the joint features, but did not mention on the skin manifestations of the disease. However, they anticipated that their patients would suffer a disease of the innate immune system.

A similar constellation of signs had been reported in the Japanese literature. It had been reported by Nakajo in 1939 and Nishimura in 1950, under the names "secondary hypertrophic osteoperiostosis with pernio," a syndrome with nodular erythema, elongated and thickened fingers, and emaciation, and "hereditary lipomuscular atrophy with joint contracture, skin eruptions and hyper-γ-globulinemia" (5). An eponym for the syndrome was proposed by the Japanese authors as "Nakajo–Nishimura syndrome." Overall, patients described started in early infancy with a pernio-like rash, periodic high fever, nodular erythema-like eruptions, and myositis. Lipoatrophy and joint contractures occurred progressively in life, mainly on the upper part of the body, leading to a very characteristic facial appearance.

After the description of CANDLE and JMP, and the appearance of new cases from Japan, a number of cases from different parts of the world were published. It was later disclosed that most patients described under these descriptions had homozygous or compound heterozygous mutations in the gene *PSMB8*, encoding the subunit β5i of the immunoproteasome (2, 6–8). However, some patients with CANDLE syndrome did not bear mutations in *PSMB8* (2). Further genetic studies disclosed that some patients have homozygous and compound heterozygous mutations in other subunits of the proteasome–immunoproteasome, as well as digenic heterozygous mutations in two different genes encoding subunits (9). Finally, mutations in the proteasome maturation protein (*POMP*) gene have also been reported in a patient of Lebanese origin that had been reported by Mégarbané et al. (9, 10) in 2002 under the name "unknown autoinflammatory syndrome associated with short stature and dysmorphic features."

Several terms have been proposed to encompass the three denominations, such as PRAAS (proteasome-associated autoinflammatory syndrome) (11) or ALDD (autoinflammation, lipodystrophy, and dermatitis) (12). However, all of them represent the same entity, and it seems that the most accepted nomenclature is CANDLE syndrome. The general appearance of consumption in the patients, like a burntout candle, emphasizes the name of the syndrome.

PATHOPHYSIOLOGY

Overall, proteasome–immunoproteasome dysfuntion causes a continuous state of inflammation with exacerbations in CANDLE syndrome. Proteasome–immunoproteasome dysfunction leads to constitutional hypersecretion of type 1 Interferons (IFNs), which by several mechanisms will lead to accumulation of waste proteins within the cells (13). Thus, further proteasome–immunoproteasome activity is required, which cannot be achieved. Accumulation of waste proteins within the cell causes cellular stress, which in turn stimulates type 1 IFN, and finally closes the circle of inflammation. Common triggers, such as cold, physical or psychical stress, banal infections or others cause stimulation of type 1 IFN secretion, and thus provoke severe inflammatory attacks that can occur in any organ of the body.

The Proteasome–Immunoproteasome System

The proteasome is a multiprotein structure present both in the nucleus and the cytoplasm of all eukaryotic cells (**Figure 1**) (14). It is constitutively expressed, and has multicatalytic activity. It has a cylindrical shape, and there are several types. All of them contain at least two different complexes: the 20S or core complex, which contains the proteolytic activity, and the 19S or regulatory complex, responsible for recognizing ubiquitinated proteins and transporting them into the 20S complex. The 20S complex is composed of two α-rings flanking two β-rings; each ring is composed of seven different protein subunits, named α-1 to α-7 and β-1 to β-7.

The immunoproteasome is very similar to the constitutive proteasome (**Figure 1**). The 19S complex of the proteasome is replaced with the PA289 (or 9S) complex, and the β1, β2, and β5 subunits in the beta rings are substituted by specialized units, named β1i, β2i, and β5i (i = inducible) (14). The β1 subunits have caspase-like activity, β2 subunits have trypsin-like activity, and β5 subunits have chemotrypsin-like activity (9). Immunoproteasome formation is mainly induced by IFNs, to account for an increased demand of catalytic activity within the cell.

The assembly and maturation of proteasome and immunoproteasomes are governed by facilitating proteins. POMP is essential for proteasome formation and is strongly involved in the incorporation of the β5i subunit into the immunoproteasome. Proteasomal β subunits but β3 and β4, are synthesized as preforms that require autocatalytic cleavage during assembly to liberate the active-site threonines (9, 15, 16).

The proteasome is mostly involved in the removal of waste intracellular proteins. The immunoproteasome is also responsible for the degradation of foreign proteins. Whereas the proteasome is constitutively expressed in every cell, only proinflammatory cytokines and mostly type 1 IFNs induce the immunoproteasome formation. However, immunoproteasomes are constitutively expressed in hematopoietic cells (17, 18). The catalytic activity of the proteasome and the immunoproteasome cleaves protein substrates to generate smaller peptides that can be easily removed

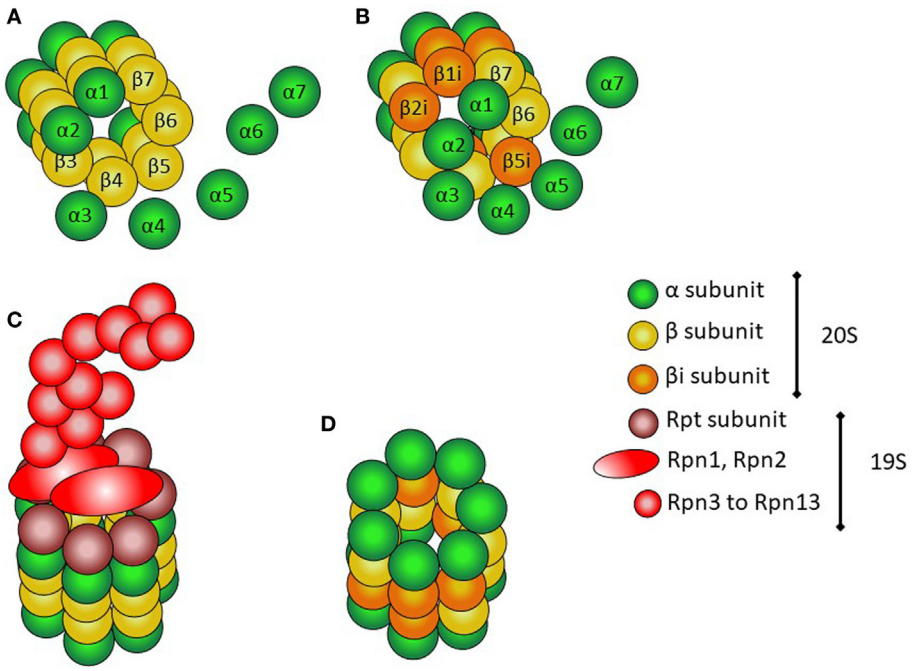

FIGURE 1 | Structure of proteasomes. **(A)** Assembly of α and β subunits to form the 20S (core) complex of the constitutive protesome. **(B)** Assembly of the inducible β subunits to form the 20S (core) of the immunoproteasome. **(C)** 26S proteasome with the 20S core particle and the 19S regulatory complex. **(D)** 20S (core) immunoproteasome.

from the cell or may act as antigens that can be presented through the MHC type I molecules to the adaptive immune system. In this way, more specifically the immunoproteasome acts as a link between innate and adaptive immunity.

Normal Functioning of the Proteasome–Immunoproteasome System

Cellular proteins destined for degradation or cleavage should be first marked with ubiquitin. Ubiquitinization allows for recognition by the 19S complex. The ubiquitinized protein is entered into the 20S particle, which degrades the protein through enzymatic proteolysis (**Figure 2**). The small peptides resulting from degradation can thus be easily removed or enter the endoplasmic reticulum to be eventually presented to T lymphocytes by means of the MCH type I on the cell surface (19, 20).

Viral infections or other triggers such as cellular stress or cold can induce secretion of type 1 (α or β) IFNs. When IFNs are recognized by their cell surface receptor, transphosphorylation of JAK1 and TYK2 occurs (21), as the first step of JAK/STAT signaling pathway activation. As a result of activated JAKs, the STAT proteins STAT1 and STAT2 dimerize and enter the nucleus, leading to transcription of type 1 IFN genes (**Figure 3A**). On the other hand, danger signals such as an irritant or infection provoke inflammation, which is associated with the production by immune cells of microbiocidal reactive oxygen and reactive nitrogen species during the innate immune response (22). Such increased oxidative stress conditions have profound consequences for the functional integrity of proteins.

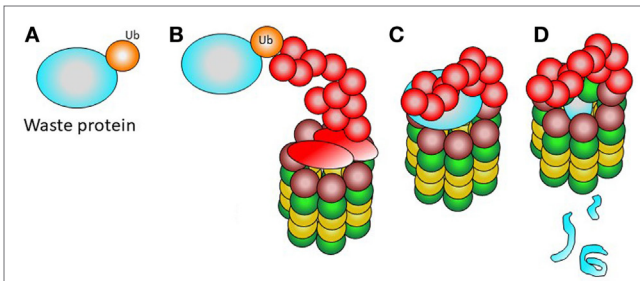

FIGURE 2 | Normal function of the proteasome. **(A)** A waste protein fated to elimination is marked with ubiquitin. **(B)** The 19S complex recognizes the ubiquitinated protein. **(C)** The ubiquitinated waste protein is entered into the proteasome. **(D)** The catalytic activity of the 20S complex renders small products, easy to remove from the cell.

During JAK/STAT pathway activation and increased oxidative stress, many waste proteins are generated, as well as many irreversibly oxidant-damaged and potentially toxic proteins. All these require increased proteolytic degradation machinery for their degradation to preserve cell viability and basic cellular functions (22, 23). The constitutive proteasome may not be sufficient to accomplish this extra effort, and hence the immunoproteasomes are recruited and assembled, mainly induced by type 1 IFNs themselves.

Proteasome Dysfunction and CANDLE Syndrome

In CANDLE syndrome (**Figure 3B**), proteasome system dysfunction leads to an inability of the cell to get rid of waste proteins. This

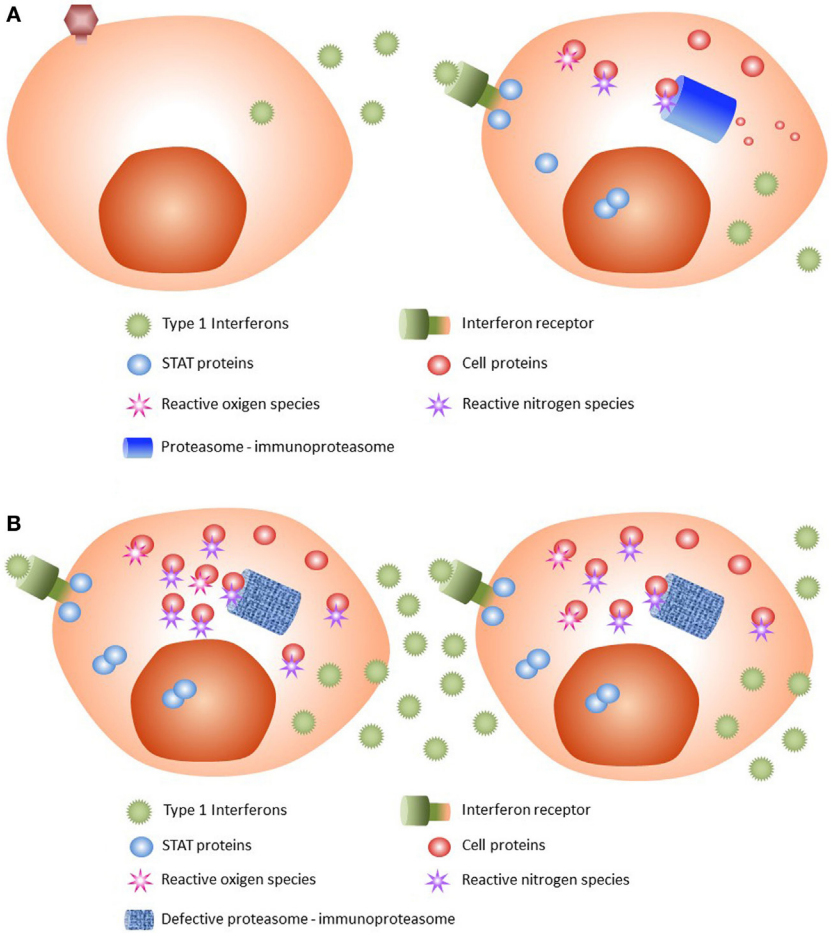

FIGURE 3 | Pathophysiology of CANDLE syndrome. **(A)** Normal state. After viral infection, type 1 interferons (IFNs) are released by the infected cell. IFNs are recognized by their receptor, with activation of JAK/STAT pathway and subsequent dimerization of STAT proteins. The STAT dimers enter the nucleus and stimulate transcription of type 1 IFNs. JAK/STAT activation produces reactive oxygen and nitrogen species that are damaging to cell proteins. These damaged proteins, and others generated by cell catabolism, are removed by the proteasome and the immunoproteasome; the latter is stimulated by type 1 IFNs. **(B)** CANDLE syndrome. After IFN activation, cells with mutated proteasome–immunoproteasome will not be able to remove all waste proteins, which will accumulate and be poly-ubiquitinized. As a result, cellular stress occurs, leading to increased type 1 IFNs production. The high levels of secreted type I IFNs recruit inflammatory cells that will cause tissue damage.

situation may lead to a weak or moderate state of proinflammation in the absence of triggers, but under situations of stress, such as cold, viral infections, or physical stress, higher requirements of removing waste proteins are not met.

When a cell is infected by a virus, viral genetic material is sensed in the cytoplasm and activation of the central protein STING (stimulator of interferon genes) ensues. As a result, type 1 IFN genes are transcripted, and IFNs are released (24, 25). As stated above, when the IFN receptor is activated by IFNs, many waste proteins are produced, which must be removed by the proteasome and the IFN-induced immunoproteasome. On the other hand, cells infected by viruses produce viral proteins that are also a substrate for the proteasome system for their removal. Other triggers causing cellular stress also produce type 1 IFN release. If the proteasome system does not work properly, waste proteins accumulate in the cell and are also further marked with more ubiquitin (poly-ubiquitinization) (7, 8). The accumulation of poly-ubiquitinized proteins causes further cellular stress and more type 1 IFN production, thus feeding a vicious cycle of inflammation (2, 7–9).

Type 1 IFNs are virtually produced by every cell in the body, but the plasmacytoid dendritic cells are the most potent producers of type 1 IFNs and are involved in the pathophysiology of CANDLE (26). Type 1 IFNs exert many different actions (27). They increase release of proinflammatory substances and recruit inflammatory cells, including neutrophils and myeloid cells (28). Due to the enhanced and continuous type 1 IFN release, myeloid cells are rapidly mobilized from the bone marrow, and thus "atypical" or immature cells reach the target organs, contributing to the "atypical" skin infiltrate (26).

The IFN signature is very strong in CANDLE syndrome. Microarray analyses have shown an intense expression of IFN

signature genes, and the serum of patients with CANDLE contains high levels of proteins of the IFN pathway such as IFN-derived protein 10 (2).

GENETIC BACKGROUND

The first gene mutations detected in patients with CANDLE syndrome were located in the gene *PSMB8* (proteasome subunit, beta-type, 8) in chromosome 6p21.32, encoding for the β5i (i = inducible) subunit of the immunoproteasome (2, 6–8). Mutations in PSMB8 were responsible for CANDLE, JMP, and Nakajo–Nishimura syndromes. However, mutations in other genes, encoding other proteasome–immunoproteasome subunits or the regulatory protein *POMP*, were later discovered in patients with CANDLE syndrome, thus expanding the CANDLE genotype (9). All mutations were located in highly conserved sites in vertebrates and were thus predicted to be pathogenetic. It was proven that CANDLE syndrome is a disease of proteasome–immunoproteasome dysfunction, which could be inherited as a recessive homozygous, compound heterozygous or digenic trait, or less commonly in a dominant fashion (9). The following mutations have been so far identified in patients with CANDLE (9):

- *PSMB4 mutations*. The gene *PSMB4* (proteasome subunit, beta-type, 4), located in chromosome 1q21, encodes for the β7 subunit of the proteasome. It seems to be important for proteasome assembly and stabilization.

  The c.-9G>A mutation originates a β7 subunit protein with lower expression than that of wild type, which is therefore less incorporated into proteasome complexes than the wild-type counterpart. It is also possible that this mutant β7 subunit also impairs propeptide cleavage of the β5i subunit. A deletion of three aminoacids in β7, p.D212-V214, affects the N terminus of an α-helix forming an intramolecular hydrogen-bonding network that stabilizes its C-terminal extension. The C-terminal extension is essential for proteasome assembly (29). Two other mutations affect the C-terminal extension: (1) the c.44insG insertion, which causes a frameshift mutation (p.P16Sfs*45) and leads to non-expression of the mutant allele; and (2) the missense p.Y222X, which causes the loss of the C-terminal extension of the β7 subunit; although β7 subunit is expressed, it fails to incorporate into the 20S or 26S proteasome complexes.

  Finally, the deletion causing the p.D212_V214del mutant of β7 leads to a poor maturation of the β7 subunit. Although the mutant protein is detected in proteasome assembly intermediates, it is poorly incorporated into 20S or 26S proteasomes.
- *PSMA3 mutations*. The gene *PSMA3* (proteasome subunit, alpha-type, 3), located in chromosome 14q23.1, encodes for the α7 subunit of the proteasome.

  Two mutations in *PSMA3* have been reported in patients with CANDLE syndrome. A p.R233del deletion (c.696_698delAAG) most likely affects the subunit folding and prevents incorporation of the subunit to the mature proteasomes. Overall reduced proteasome content is thus resulting (9). On the other hand, a c.404+2T>C mutation affects a splice site and causes an unstable transcript due to exon 5 skipping.
- *PSMB8 mutations*. The gene *PSMB8* (proteasome subunit, beta-type, 8), located in chromosome 6p21.32, encodes for the β5i subunit of the immunoproteasome. Incorporation of β5i subunit to the maturing immunoproteasome requires proteolytic removal of a prosequence by proteolytically active subunits. The β5i subunit has chemotrypsin-like activity, crucial for the immunoproteasome function.

  Mutations in PSMB8 in CANDLE syndrome may affect the chemotrypsin activity or impair immunoproteasome assembly or maturation. The most common mutation in CANDLE syndrome is T75M; when found in homozygosis, it causes selective impairment in chemotryptic-like activity. The A92T mutation produces a similar effect, as well as the mutations K105Q and M117V.

  The K105Q mutation is also associated with defects in incorporation and/or maturation of proteasome subunits and with inability to completely trim the β5i propeptide (9). The common T75M and the G201V mutations also cause decreased proteasome assembly (7, 8). Finally, the C135X mutation leads to truncation and non-expression of the protein (2, 9); when found in homozygosis, the subunit β5i is absent in all immunoproteasomes and most likely impairs immunoproteasome assembly, thus showing reduction in all three proteasome activities (trypsin-like, caspase-like, and chemotrypsin-like) (9).
- *PSMB9 mutations*. The gene *PSMB9* (proteasome subunit, beta-type, 3), located in chromosome 6p21.32, encodes for the β1i subunit of the immunoproteasome. The β1i subunit has a caspase-like proteolytic activity.

  The only β1i variant described so far is a missense mutation, p.G165D, located in a loop interconnecting 2 α-helices that define the position of a β1i/caspase-like activity conferred by threonine (9).
- *POMP mutations*. The gene *POMP*, located in chromosome 13q12.3, encodes for the POMP, which is key for the maturation and assembly of the proteasome subunits. POMP associates specifically with proteasome precursor intermediates and facilitates the sequential assembly of β subunits onto the preformed α subunit rings (15).

  A single patient with CANDLE has been found to bear no mutations in proteasome subunit genes, but a heterozygous, dominant, insertion in POMP causing a frameshift, p.E115Dfs*20 (c.344_345insTTTGA) and a truncated protein, which is likely unstable. POMP insufficiency causes proteasome precursor accumulation, reduced mature proteasome formation, and reduced overall proteasome activity (9).

Patients with CANDLE have shown variable combinations of these mutations (9). Most frequently, patients are homozygous or compound heterozygous for *PSMB8* mutations, but others are compound heterozygous for *PSMB4*, or are heterozygous for combinations such as *PSMA3*/*PSMB8*, *PSMB9*/*PSMB4*, or *PSMB8*/*PSMB4*. In the latter situation, a digenic inheritance is suggested causing additive proteasome defects. Patients with digenic inheritance have variable proteolytic defects. For example, a combination of *PSMB8*/*PSMA3* causes impairment in all three proteolytic activities, somewhat similar to patients' compound heterozygous for *PSMB4* in whom proteasome assembly

is severely impaired. Patients with combined *PSMB9*/*PSMB4* mutations have reduced caspase-like activity, which is conferred by subunit β1i. Finally, patients with double *PSMB8* mutations experience a severe decrease in chemotrypsin-like activity.

CLINICAL FEATURES

Onset and Course

CANDLE syndrome usually starts in the first months of life (1, 30). The most common presenting sign is fever or temperature elevations below 38.3°C. These appear daily or almost daily, but the general state is minimally affected or even normal. Sometimes, cold exposure can trigger temperature elevation and skin lesions. Skin lesions are the first clinical sign to appear in CANDLE, and usually are present all along the disease course, although they may be less conspicuous after puberty. Lipodystrophy usually starts in early childhood and is usually well established before puberty. Finally, disabling joint manifestations usually occur in the long term. During patients' life, different acute attacks of disease may ensue, spontaneously or after common triggers, which may affect virtually every organ in the body.

Skin Manifestations of CANDLE

The skin lesions of CANDLE syndrome are very characteristic and should raise the diagnosis. The combination of fever, typical skin lesions, and classic histopathologic features should allow for a rapid diagnosis of CANDLE (**Figures 4–6**). The skin lesions in CANDLE are of three types (1):

1. Acral, perniotic lesions. These usually appear in newborns and infants and are not regularly seen in childhood or later. They consist of intense, red or purplish, edematous plaques mostly located on the nose, ears, fingers, or toes. Cold may be a trigger for these lesions, but often there is no history of cold exposure.
2. Annular plaques. These lesions usually start in infancy or childhood and consist of erythematous or purpuric edematous lesions, often with annular shape with raised borders and a flat, purpuric center. They may appear in crops or individually and tend to fade within days or weeks, leaving a purpuric macule. New, active lesions coexist all over time with residual, purpuric macules, which confers a very typical appearance to the patients. These lesions are very conspicuous during childhood, but in adult life they may be less visible and may be absent in long-standing disease.
3. Perioral and periocular edema. Patients with CANDLE develop in infancy or childhood a persistent erythematous to violaceous edema affecting the periorbital and less commonly the perioral area. It may be less visible after puberty and in long-standing disease.

The histological features of the skin lesions in CANDLE are very characteristic and may permit a diagnosis in early stages of the disease (1, 27). The papillary and reticular dermis contains a perivascular and interstitial infiltrate of varying intensity, extending to the subcutaneous fat as lobar panniculitis. The infiltrate is predominantly composed of mononuclear cells, many of which have large, irregularly shaped nuclei; the atypical appearance of the infiltrate may lead to a diagnosis of skin malignancy.

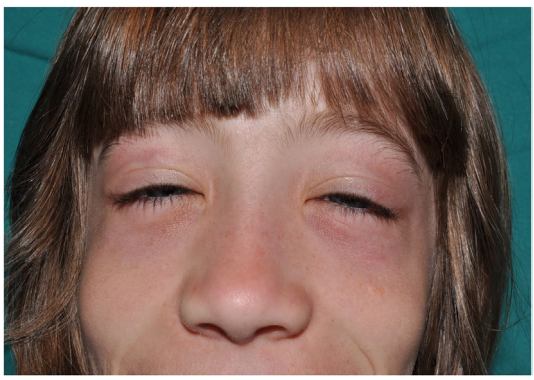

FIGURE 4 | Periorbital erythema and edema and flat nose in a patient with CANDLE.

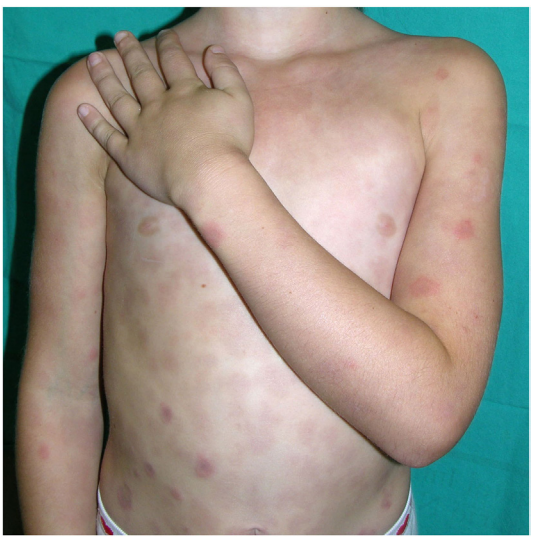

FIGURE 5 | Annular, purpuric plaques of CANDLE syndrome.

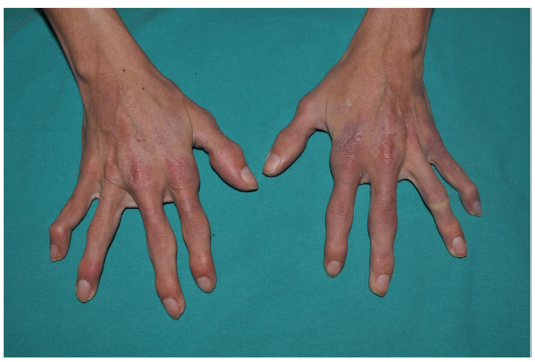

FIGURE 6 | CANDLE hands: skin lesions and swollen joints.

The infiltrate also contains mature neutrophils, some eosinophils, and a few mature lymphocytes. Leukocytoclasia is often seen, without fibrinoid necrosis of the vessels (1, 27). Immunohistochemistry shows that the infiltrate is mixed, with an important presence of myeloid cells (positive for myeloperoxidase) and a prominent cell population of macrophages (positive for CD163 and CD68/PMG1) (27). CD123-positive plasmacytoid dendritic cells are seen in clusters (27).

Lipodystrophy

Fat loss is a key manifestation of CANDLE (1, 3, 30–32). It can be seen in most patients before the age of two, but it is progressive and may take some years to become fully developed. The loss of subcutaneous fat usually starts on the face and progresses to the trunk and upper limbs. The lower limbs are usually less affected. The cause of lipodystrophy is not well known, but it can be related to chronic inflammation involving the fatty tissue (33, 34). Alternatively, increased expression of proinflammatory cytokines in adipose tissue and reduced secretion of adiponectin and leptin may be involved (35, 36). An intense type 1 IFN signature is believed to be associated with fat loss in children with lipoatrophic panniculitis (37), which reinforces the role of IFN in CANDLE syndrome. Type 1 IFNs may be toxic to adipocytes, as is suggested by the development of lobar panniculitis with lipophagia and lipoatrophy in patients treated with intramuscular injections of IFN-β (38, 39).

Lipodystrophy and the typical skin lesions confer to CANDLE patients a unique phenotype. On the face, the loss of fat on the cheeks and the periorbital and periocular edemas are pathognomonic (1). In adulthood, the eyelids and the lips are retracted, causing a false proptosis and exposure of the teeth; coupled with the severe fat loss, these features cause an unmistakable appearance (4). On the limbs, a progressive fat loss, coupled with muscle wasting (see later), is seen. A prominent abdomen may be related to increased visceral fat, which remains the only fat storage capability of the patient. An increased distance between nipples is also typical in CANDLE (1). Metabolic disturbance due to absence of body fat can also impair hydrocarbon metabolism and lead to acanthosis nigricans and hirsutism.

General Examination

Patients with CANDLE have a mild to moderate growth delay and show low weight and height (1, 30–32). Chronic inflammation may explain this physical delay, but muscle wasting and lipoatrophy are also major contributors. However, most patents with CANDLE do not show mental retardation.

CANDLE patients show variable degrees of hepatomegaly, which can be related to a secondary metabolic disturbance due to extensive lipoatrophy. Splenomegaly and generalized lymphadenopathy are common findings, reflecting the persistent autoinflammatory activity.

Musculoskeletal Signs

Arthralgias are very common in children with CANDLE, but patients do not show radiologic features of arthritis. Some joint swelling can appear in the interphalangeal joints, but the absence of overt arthritis distinguishes CANDLE from rheumatoid arthritis or juvenile idiopathic arthritis (1, 30–32). With time, hyperextensibility of interphalangeal joints can occur, and during adulthood, most patients will develop variable degrees of joint contractures on the hands and feet, which are often severely disabling (4).

Cartilage inflammation has been reported in CANDLE patients (1). Recurrent and also chronic chondritis of the ears and nose causes partial loss of auricles and a flat, saddle-like nose. Because both ears and nose are usually exposed, a role of triggering by cold has been suspected.

Muscle involvement is also a feature of CANDLE (4). Acute attacks of inflammatory myositis have been reported that can be demonstrated by MRI. Possibly, there is also a role for chronic muscle inflammation in the development of severe muscular wasting.

Nervous System

As stated above, mental delay is not a common feature in CANDLE (1). However, patients may suffer attacks of aseptic meningitis, meningoencephalitis, and possibly some degree of chronic inflammation in the CNS. Basal ganglia calcifications have been reported (1), most likely as a result of encephalitis; these are similar to those present in Aicardi–Goutières syndrome, which also features a high type 1 IFN production.

Other Organ Involvement

Episodes of inflammation may occur in any organ, as well as some degree of persistent generalized inflammation. Attacks of acute sterile epididymitis, conjunctivitis and nodular episcleritis, parotitis, pneumonitis, nephritis, carditis, and otitis have been reported. The clinical manifestations are related to the organs involved. Some of these complications have been reported to be fatal (1).

LABORATORY INVESTIGATIONS

As is the case with other AIDs, laboratory findings are not very striking (1, 3, 32). The most common features are elevation of acute phase reactants (ESR, CRP, and thrombocytosis) and a chronic, hypochromic anemia. Liver enzymes are usually moderately elevated, but this may be caused by lipodystrophy itself; also, increased triglyceride levels can occur in relation to metabolic disturbance by lipodystrophy. Less frequently, elevated muscle enzymes (CPK and aldolase) reveal chronic muscle involvement (1). Studies for autoimmunity and autoantibodies are usually negative, but some patients show increased levels of antinuclear antibodies. Serum levels of immunoglobulins are regularly normal. Bone marrow and lymph node biopsies have been unconspicuous, revealing only reactive changes.

Other laboratory and imaging anomalies are seen during acute inflammatory attacks; these are dependent on the organs affected by inflammation.

DIAGNOSIS AND DIFFERENTIAL DIAGNOSIS

The diagnosis of CANDLE is suspected by the early onset of fevers, skin lesions, and lipodystrophy. A skin biopsy with

immunohistochemistry studies can be characteristic enough to permit an accurate diagnosis. The genetic study of the genes involved establishes a confirmation diagnosis.

Other AIDs may share some features with CANDLE syndrome, including NOMID syndrome, TRAPS, or hyper-IgD syndrome. Lipodystrophy in CANDLE syndrome is a very characteristic feature of the disease, but other causes of loss of fat must be considered, including generalized congenital lipodystrophy, partial familial lipodystrophy, leprechaunism, or acquired partial lipodystrophy of Barraquer–Simons. Aicardi–Goutières syndrome and other type 1 interferonopathies (such as SAVI syndrome, familial chilblain lupus, or C1q deficiency) may show features similar to CANDLE syndrome (28, 40, 41). Sweet syndrome in infants may present with violaceous ring lesions reminiscent of CANDLE syndrome, and histology may be misleading in some cases. Fat loss and skin lesions are clinical manifestations of a recently described autoinflammatory syndrome named otulipenia, due to loss-of-function mutations in OTULIN, encoding a deubiquitinase that cleaves Met1-linked chains (42).

PROGNOSIS AND FOLLOW-UP

CANDLE patients have a variable outcome. Some patients have had a lethal course due to acute attacks of inflammation in important organs of the body. In other patients, a long survival is possible, with variable degrees of disability (1, 3, 4).

Regular clinical follow-up is mandatory. A protocol has not been established, but attention must be paid to identify inflammatory attacks as early as possible. Regular skin, eye, and joint exams are recommended. Endocrinologist consultation is mandatory for diet and metabolic control because of lipodystrophy. Basic laboratory follow-up must include CBC (with special attention to anemia, leukocytosis with increased neutrophil count, and thrombocytosis), ESR, CRP, serum liver enzymes, muscle enzymes, and metabolic profile (including glucose, triglyceride, and cholesterol levels). Ultrasound may be helpful in detecting enlarged liver, spleen, or lymph nodes. Specific tests for organ involvement must be considered in patients with abnormal clinical examination.

TREATMENT

So far, no individual treatment has been consistently effective in CANDLE syndrome. Oral corticosteroids and methotrexate can provide some improvement. Methotrexate can be considered the first line therapy. NSAIDs may provide partial control of fevers. Dapsone or colchicine has been ineffective. Cyclosporine, azathioprine, or intravenous immunoglobulins have achieved minimal improvements, if any. Anti-TNF drugs such as etanercept have not been helpful and have even been the cause of disease exacerbations (1). Acute attacks may need systemic corticosteroids as well as organ-specific therapy.

A compassionate use treatment protocol has been started for CANDLE syndrome with the selective JAK1/2 kinase inhibitor baricitinib. Oral baricitinib was used in patients who failed to achieve control or required high doses of corticosteroids. Eight patients treated with this drug showed clinical and analytical improvement (43), but these results still await confirmation.

Finally, physical therapy to prevent joint contractures and specific organ therapy must be provided.

CONCLUDING REMARKS

CANDLE syndrome is an AID due to gene mutations leading to protesome–immunoproteasome dysfunction. CANDLE syndrome can be diagnosed very early in life because the skin signs and their histopathology are very characteristic. Genetic confirmation is necessary. Early therapy to prevent disabling manifestations is desirable, but still no agent has been truly effective. Prevention and treatment of acute inflammatory attacks will permit longer life expectancy in these patients.

AUTHOR CONTRIBUTIONS

There is one single author for this article. The manuscript has been completely written by AT.

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Diagnostic Criteria for Adult-Onset Periodic Fever, Aphthous Stomatitis, Pharyngitis, and Cervical Adenitis (PFAPA) Syndrome

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Objective: To identify a set of variables that could discriminate patients with adult-onset periodic fever, aphthous stomatitis, pharyngitis, and cervical adenitis (PFAPA) syndrome from subjects with fever of unknown origin (FUO).

Methods: We enrolled 74 adults diagnosed with PFAPA syndrome according to the currently used pediatric diagnostic criteria and 62 additional patients with FUO. After having collected clinical and laboratory data from both groups, univariate and multivariate analyses were performed to identify the variables associated with PFAPA diagnosis. Odds ratio (OR) values, their statistical significance, and corresponding 95% confidence interval (CI) were evaluated for each diagnostic factor both at the univariate and multivariate analyses. Diagnostic accuracy was evaluated by the area under receiver operating characteristic (ROC) curve, while the leave-one-out cross-validation procedure was used to ensure that the model maintains the same diagnostic power when applied to new data.

Results: According to the multivariate analysis, the clinical variables that discriminated PFAPA patients were: fever episodes associated with cervical lymphadenitis (OR = 92; $p < 0.0001$), fever attacks associated with erythematous pharyngitis (OR = 231; $p < 0.0001$), increased inflammatory markers during fever attacks (OR = 588; $p = 0.001$), and the lack of clinical and laboratory signs of inflammation between flares (OR = 1202; $p < 0.0001$). These variables were considered for a diagnostic model which accounted for their OR values. The diagnostic accuracy of the proposed set of criteria corresponded to an area under ROC curve of 0.978 (95% CI 0.958–0.998), with a model sensitivity and specificity equal to 93.4% (95% CI 87.5–96.5%) and 91.7% (95% CI 82.8–96.7%), respectively.

Conclusion: we have provided herein a set of clinical diagnostic criteria for adult-onset PFAPA syndrome. Our criteria represent an easy-to-use diagnostic tool aimed at identifying PFAPA patients among subjects with FUO with a high-predictive potential, as shown by its very high sensitivity and specificity.

Keywords: PFAPA syndrome, autoinflammatory disease, differential diagnosis, diagnostic criteria, adults, fever of unknown origin

INTRODUCTION

Periodic fever, aphthous stomatitis, pharyngitis, and cervical adenitis (PFAPA) syndrome belongs to the spectrum of multifactorial autoinflammatory diseases (AIDs) and is characterized by spontaneous flares of systemic inflammation characterized by fever and other clinical manifestations, especially cardinal signs described by the PFAPA acronym (1).

To date, the pathogenesis of this syndrome remains still obscure, but studies aimed at assessing immunological mechanisms, also supported by therapeutic evidences (2, 3), have highlighted an abnormal interleukin-1 release in response to many environmental triggers, which associates PFAPA syndrome to other hereditary periodic fever disorders (4, 5). However, unlike other AIDs characterized by recurrent fever attacks, no genetic mutations have been clearly associated with PFAPA syndrome (6, 7).

In addition to fever (often achieving and overcoming 40°C), aphthous stomatitis, pharyngitis, and cervical adenitis, many other clinical manifestations may enrich the clinical framework of PFAPA patients, including abdominal pain, headache, nausea, skin manifestations, and arthralgia (1, 8–10). Inflammatory flares arise every 3–8 weeks with no premonitory symptoms and generally last 3–6 days. Patients are typically healthy between febrile episodes and the overall growth of children affected by this syndrome is not stunted (11, 12). Although during the last decades diagnosis of PFAPA syndrome has been relegated to children aged under 5 years, increasing evidence has recently shown that the disease can also arise in older children as well as during adulthood (2, 3, 9, 10, 13–18). The treatment of PFAPA patients is based on intermittent corticosteroid administration, as patients are generally responsive to a single dose of a corticosteroid given at the onset of febrile flares (1, 12, 14, 19, 20).

No laboratory or instrumental tools are available to support the diagnosis of PFAPA syndrome, which is currently based on the fulfillment of clinical diagnostic criteria. In particular, to date, clinical criteria proposed by Marshall et al. in 1986 (21) and later modified by Thomas et al. in 1999 (11) represent the most used set of criteria in the clinical practice. However, these criteria are tailored on pediatric patients and their application on adults is categorically excluded by the first item that requires the presence of recurrent fever in patients under 5 years of age. In addition, the fifth item imposes the lack of normal growth and development for patients affected, which is not applicable to adult-onset PFAPA patients. In this context, Padeh et al. employed a further set of inclusion criteria valid for both children and adult patients (8). This set included the presence of monthly fever attacks, exudative tonsillitis, possibly oral ulcers, cervical lymph node enlargement, negative throat cultures, and failure of antibiotic treatment during the acute episodes or as prophylactic treatment, while normal growth/development and a rapid response to a single corticosteroid administration were later added as further items (8, 22). However, to the best of our knowledge, no statistical procedures were employed to identify variables useful in discriminating PFAPA patients among subjects presenting with recurrent fever attacks. In addition, recent evidences have proved that erythematous pharyngitis is more typical than sterile exudative pharyngitis in adult-onset PFAPA patients (10). Therefore, the need for a new set of diagnostic criteria for patients experiencing PFAPA syndrome during adulthood has prompted our group to evaluate a set of variables on both clinical and statistical basis that could discriminate such patients from subjects with fever of unknown origin (FUO).

MATERIALS AND METHODS

Patients

Seventy-four consecutive adult patients who had been referred to our Units from September 2007 to December 2016 because of recurrent fever attacks and other clinical manifestations consistent with PFAPA syndrome were classified as suffering from adult-onset PFAPA syndrome (PFAPA group) according to the Marshall criteria modified by Thomas et al. (11, 21), which are the most frequently used diagnostic tool in the clinical practice. As this set of criteria is tapered on pediatric patients, the item requiring a disease onset before the age of 5 was neglected, while the item requiring a normal growth and development was retrospectively applied, as previously made in other studies (9, 10, 13). Two patients out of 74 were included in the PFAPA group despite the lack of symptom-free intervals. In both cases, the patients showed the resolution of fever and of cardinal symptoms as well as the normalization of acute phase reactants. Conversely, the sole arthralgia and myalgia persisted in both cases and were attributed to the presence of concomitant degenerative, not inflammatory musculoskeletal diseases.

Sixty-two additional adult subjects admitted in our Units between September 2016 and March 2017 for recurrent FUO were consecutively enrolled in the study as control group (control group). FUO diagnosis was based on the currently available diagnostic criteria (23). As for patients with adult-onset PFAPA syndrome, any specific disease related to fever or inflammatory manifestations had been ruled out at the time of enrollment in this study. The control group was included into a follow-up protocol aimed at early identify any sign or symptom potentially useful for a prompt specific diagnosis; patients were treated with

non-steroidal anti-inflammatory drugs or low-to-high dosage corticosteroids.

Assessment Parameters

Each patient's medical record was reviewed for demographic and clinical data. In particular, we looked for the age at disease onset, characteristics of the febrile pattern (peak temperature, duration of flares, frequency of fever episodes per year), clinical manifestations accompanying fever (oral and/or genital aphthosis, exudative and/or erythematous pharyngitis, cervical and/or widespread lymphadenitis, abdominal pain, vomiting, diarrhea, thoracic pain, arthralgia, arthritis, myalgia, urticarial-like rash, maculopapular rash, erysipelas-like rash, erythematous rash, periorbital edema, conjunctivitis, asthenia, and headache), any increase of inflammatory markers (erythrocyte sedimentation rate and/or C-reactive protein and/or serum amyloid A) during attacks and the presence or absence of clinical manifestations and positive laboratory inflammatory markers during fever-free intervals.

None of the patients with adult-onset PFAPA syndrome showed upper respiratory infections, while the throat swab was negative in all cases presenting with pharyngitis or cervical lymphadenitis. Both PFAPA group and control group patients underwent detailed laboratory and instrumental screening tests to rule out potential underlying diseases, such as infections, autoimmune diseases, and malignancies. In all patients enrolled, previous antibiotic therapies administered during flares did not change the progression of clinical manifestations. Monogenic periodic fever syndromes were ruled out by performing molecular analysis of *MEFV*, *MVK*, *TNFRSF1A*, and *NLRP3* genes, respectively responsible for familial Mediterranean fever (FMF), mevalonate kinase deficiency, tumor necrosis factor receptor-associated periodic syndrome, and cryopyrin-associated periodic syndrome (CAPS). Moreover, neither PFAPA patients nor subjects included in the control group fulfilled clinical diagnostic criteria for FMF or CAPS as well as for Still's disease, Schnitzler's syndrome, and Behçet's disease (24–33).

The study was approved by the local Ethics Committee of Azienda Ospedaliera Universitaria Senese, Siena (Italy) and each patient provided a written consent for both genetic testing and clinical data processing, in accordance with the Declaration of Helsinki.

Statistical Analysis

Descriptive statistics are expressed as mean and SD for quantitative variables as well as frequency counts and percentages for quantitative binary variables.

Multivariate stepwise logistic regression analysis was performed to identify, among all possible diagnostic factors (predictive variables), a statistically significant minimum subset of factors with the highest possible accuracy to establish a diagnosis of PFAFA syndrome. In the stepwise process, one independent variable was added to or removed from the discriminant model at each step, on the basis of maximum likelihood-ratio statistics. The process stops when no statistical significant variables can be more entered or removed. We used the leave-one-out (LOO) cross-validation procedure to ensure that the model maintains the same diagnostic power when applied to new data. LOO uses all available data to train and test model: it executes a number of training sessions equal to the sample size (N) and in each of them it classifies each patient (LOO testing case) in turn by using all other patients as training set.

Diagnostic accuracy was evaluated by the area under receiver operating characteristic (ROC) curve (AUC) along with its 95% confidence interval (95% CI). Model sensitivity and specificity together with their 95% CIs were also estimated by selecting a probability threshold giving comparable sensitivity and specificity values, along with their 95% CIs. The Hosmer–Lemeshow goodness-of-fit test was used to evaluate model calibration, that is its prognostic ability.

Finally, the odds ratio (OR), its statistical significance, and corresponding 95% CI were evaluated for each diagnostic factor, taken singularly (univariate analysis), and for the model selected factors, taken together (multivariate analysis). The SPSS software, version 10, was used for all statistical computations, always considering a significance level of 95% (p value < 0.05).

RESULTS

Both patients with adult-onset PFAPA syndrome and subjects belonging to the control group experienced a disease onset over the age of 16. Specifically, the mean age at disease onset was 26.55 ± 10.03 years for PFAPA patients and 27.94 ± 17.67 for those with FUO. **Table 1** summarizes demographic and clinical features of patients enrolled.

Univariate analysis performed on patients with PFAPA syndrome and subjects with FUO recognized clinical variables positively or negatively associated with PFAPA syndrome by an OR significantly different from 1, i.e., with a 95% CI not including 1.0. The results of univariate analysis are summarized in **Table 2**.

As reported in **Table 3**, according to multivariate analysis performed on the two groups of patients, clinical variables that showed a statistical significant ($p < 0.05$) discriminant power to identify PFAPA patients were: recurrent fever accompanied by cervical lymphadenitis (OR = 92), recurrent fever with concomitant erythematous pharyngitis (OR = 231), increased inflammatory markers during attacks (OR = 588), and symptom-free intervals corresponding to the lack of clinical manifestations and laboratory abnormalities between flares (OR = 1,202). These variables were then considered for a diagnostic model that accounts for their OR values. In particular, the occurrence of symptom-free intervals and the increase of inflammatory markers during attacks, which have higher OR values, represent mandatory items in the proposed diagnostic model. Conversely, on the basis of their lower OR values, only one between fever associated with erythematous pharyngitis and fever with cervical lymphadenitis is required for the diagnosis of PFAPA syndrome. **Table 4** shows the resulting set of criteria proposed in this study.

The diagnostic accuracy of the proposed diagnostic criteria corresponded to an AUC of 0.978 (95% CI 0.958–0.998), with sensitivity and specificity equal to 93.4% (95% CI 87.5–96.5%) and 91.7% (95% CI 82.8–96.7%), respectively. **Figure 1** represents the ROC curve assessing the performance of the criteria for our PFAPA patients and the control group with FUO.

TABLE 1 | Demographic and clinical features of patients diagnosed with periodic fever, aphthous stomatitis, pharyngitis, and cervical adenitis (PFAPA) syndrome (PFAPA group) and patients with fever of unknown origin (control group).

	PFAPA group	Control group
Age (years)	34.00 ± 11.86	40.56 ± 16.45
Males (%)/females (%)	48 (64.9)/26 (35.1)	23 (37.1)/39 (62.9)
Age at disease onset (years)	26.55 ± 10.03	27.94 ± 17.67
Mean temperature at attacks (°C)	39.31 ± 0.92	38.9 ± 1.02
Attacks per year	15.2 ± 8.44	9.45 ± 7.03
Duration of flares		
≤2 days	2 (2.7%)	14 (22.6%)
3–5 days	46 (62.2%)	12 (18.2%)
6–9 days	9 (12.2%)	8 (12.9%)
≥10 days	11 (14.9%)	22 (35.5%)
PFAPA cardinal symptoms during attacks		
Pharyngitis	70 (94.6%)	39 (62.9%)
Cervical lymphadenitis	61 (82.4%)	14 (22.6%)
Oral aphthosis	48 (64.9%)	21 (33.9%)
Other associated symptoms during attacks		
Generalized lymphadenitis	4 (5.4%)	14 (22.6%)
Asthenia	62 (83.8%)	51 (82.3%)
Abdominal pain	33 (44.6%)	20 (32.3%)
Diarrhea and/or vomiting	16 (21.6%)	13 (21%)
Thoracic pain	13 (17.6%)	24 (38.7%)
Arthralgia	53 (71.6%)	42 (67.7%)
Arthritis	11 (14.9%)	16 (25.8%)
Myalgia	47 (63.5%)	37 (59.7%)
Urticaria-like rash	4 (5.4%)	10 (16.1%)
Erythematous rash	9 (12.2%)	0 (0.0%)
Erysipelas-like rash	0 (0.0%)	3 (4.8%)
Maculo-papular rash	3 (4.1%)	9 (14.5%)
Periorbital edema	6 (8.1%)	5 (8.1%)
Conjunctivitis	8 (10.8%)	18 (29%)
Headache	43 (58.1%)	37 (59.7%)
Genital aphthosis	3 (4.1%)	2 (3.2%)
Increased inflammatory markers during attacks	72 (97.3%)	46 (74.2%)
Symptom-free intervals	72 (97.3%)	33 (53.2%)

Quantitative data are referred as mean ± SD values; qualitative data are reported as frequency counts and percentages.

DISCUSSION

Despite the increasing evidence on the possible delayed onset of PFAPA syndrome during adulthood, current diagnostic criteria are tailored on pediatric patients (34) and their application on adults requires specific adjustments not yet validated. On this basis, we looked for clinical variables that can identify patients with adult-onset PFAPA syndrome among patients presenting with FUO. Therefore, we analyzed the occurrence of inflammatory features in patients with a clinical picture consistent with adult-onset PFAPA syndrome, being excluded all the known causes of recurrent fever, as well as in patients consecutively visited in our Units because of FUO during a 6-month period. Multivariate analysis allowed to identify a set of clinical variables capable of discriminating adult-onset PFAPA patients. These variables were then rearranged into a diagnostic model in which items with a higher OR were considered mandatory for the diagnosis of PFAPA, while just one out of the two variables with a lower OR value had to be fulfilled.

Noteworthy, these proposed diagnostic criteria should be applied after having ruled out the known causes of fever in terms of infective, autoimmune, and neoplastic diseases. Monogenic AIDs should be also excluded on the basis of clinical presentation, as required by the clinical classification criteria recently proposed by Federici et al. to drive genetic analysis for patients with periodic fevers (35). According to Federici et al., we have also reported that the diagnosis of monogenic AIDs in adulthood is not unworkable when patients' symptoms are carefully classified (36, 37). Therefore, a correct evaluation of the patients' clinical picture integrated by familiar and laboratory data may allow the identification of adult-onset monogenic AIDs by specifically performing genetic testing. In addition to this, specific clinical diagnostic and classification criteria, when available, should also be applied to preventively recognize both monogenic (i.e., FMF and CAPS) and multifactorial AIDs (i.e., Behçet's disease, Still's disease, and Schnitzler's disease) (24–33). Moreover, our present criteria should not be applied in patients with positive throat swab during fever episodes and in patients responsive to antibiotics, as for previous diagnostic and classification criteria (11, 22).

Among the cardinal signs of PFAPA syndrome, the occurrence of recurrent fever with erythematous pharyngitis represented the variable most strongly associated with diagnosis of PFAPA syndrome in adulthood, while exudative pharyngitis and oral aphthosis during attacks were not included in the model. Accordingly, we had previously found that the exudative form of pharyngitis is almost rare in patients with a delayed onset of PFAPA syndrome (9, 10), while univariate analysis performed in this study even highlights a protective role of exudative pharyngitis against the diagnosis of PFAPA syndrome, further remarking a less important role of this clinical manifestation in adults. In relation to lymph node involvement, the specific observation of cervical lymphadenitis was strongly correlated to PFAPA syndrome both when considered individually and at the overall multivariate assessment. Conversely, at the univariate analysis, generalized lymphadenitis represented a variable tending to exclude the diagnosis of PFAPA syndrome in adults.

Regarding oral aphthosis, although significantly discriminant when considered singularly, it was not included into the multivariate model as its diagnostic information resulted to be absorbed by "recurrent fever accompanied by erythematous pharyngitis" and "symptom-free intervals." Therefore, most patients with oral aphthosis also presented at least one out of these two variables included in the model, thus minimizing the diagnostic value of oral aphthosis as an additional item. Interestingly, according with our results, Padeh had already suggested oral ulcers just as "possible" among the classification items proposed in 2005 (22). Furthermore, other authors have also highlighted that oral aphthosis is less frequently encountered in adult-onset PFAPA patients than among children (9, 11, 14). These observations seem to corroborate that aphthous stomatitis, although important for clinical evaluation, does not necessarily have to be included for diagnostic purposes in adults.

In relation to non-cardinal symptoms, beyond generalized lymphadenitis, also thoracic pain, conjunctivitis, maculopapular, and urticarial-like skin rash appeared to be protective factors against PFAPA diagnosis when considered individually. Consequently, the

TABLE 2 | Results of univariate logistic regression analysis performed on adult-onset periodic fever, aphthous stomatitis, pharyngitis, and cervical adenitis (PFAPA) patients and subjects with fever of unknown origin by evaluating clinical manifestations described in both groups.

Clinical variable	*p*-Value	Sensitivity (%)	Specificity (%)	OR	95% CI
Age	0.010	47.5	81.1	0.968	0.944–0.992
Age at onset	0.591	1.7	98.6	0.994	0.971–1.017
Frequency of flares	0.001	51.1	81.2	1.108	1.046–1.174
Duration of flares					
≤2 days	0.003	23.0	97.1	0.099	0.021–0.455
3–5 days	<0.0001	80.0	65.7	7.667	3.437–17.102
6–9 days	0.936	0.0	100	0.959	0.345–2.664
≥10 days	0.006	37.3	84.3	0.314	0.136–0.721
Increased inflammatory markers during attacks	0.065	11.5	97.3	4.696	0.909–24.268
Symptom-free intervals	<0.0001	45.9	97.3	30.545	6.867–135.878
Oral aphthosis	0.001	65.6	64.9	3.516	1.726–7.166
Pharyngitis	0.010	64.5	86.5	1.943	1.169–3.229
Erythematous pharyngitis	<0.0001	91.9	78.4	41.325	14.194–120.315
Exudative pharyngitis	0.005	27.4	91.9	0.234	0.086–0.637
Laterocervical lymphadenitis	<0.0001	76.3	82.4	15.082	6.463–35.199
Generalized lymphadenitis	0.006	22.6	94.6	0.196	0.061–0.631
Asthenia	0.813	0.0	100	1.114	0.454–2.736
Abdominal pain	0.163	67.2	44.6	1.650	0.816–3.337
Diarrhea/vomiting	0.965	0.0	100	1.019	0.446–2.326
Thoracic pain	0.006	39.3	82.4	0.329	0.149–0.723
Arthralgia	0.624	0.0	100	1.202	0.577–2.504
Arthritis	0.104	26.2	85.1	0.491	0.208–1.158
Myalgia	0.647	0.0	100	1.176	0.588–2.354
Skin rash	0.531	0.0	100	0.776	0.350–1.718
Urticaria-like rash	0.047	16.4	94.6	0.291	0.087–0.982
Erythematous rash	0.999	0.0	100	NE	0.000–NE
Maculo-papular rash	0.041	14.8	95.9	0.244	0.063–0.946
Erysipelas-like rash	0.999	4.9	100	0.000	0.000–NE
Periorbital edema	0.993	0.0	100	1.006	0.292–3.469
Conjunctivitis	0.009	29.0	89.2	0.296	0.119–0.741
Headache	0.764	0.0	100	0.900	0.451–1.795
Genital aphthosis	0.787	0.0	100	1.286	0.208–7.952

NE, not evaluable; CI, confidence interval; OR, odds ratio.

TABLE 3 | Estimations derived from multivariate logistic regression analysis performed on adult periodic fever, aphthous stomatitis, pharyngitis, and cervical adenitis (PFAPA) patients and patients with fever of unknown origin, representing the control group.

Clinical variable	*p*-Value	OR	95% CI
Erythematous pharyngitis	<0.0001	231	14.463–3,715.288
Cervical lymphadenitis	<0.0001	92	8.865–953.279
Increased inflammatory markers during attacks	0.001	588	3,534.3–40,879.463
Symptom-free intervals	<0.0001	1202	12.631–27,937.885

CI, confidence interval; OR, odds ratio.

TABLE 4 | Clinical diagnostic criteria resulting from the multivariate analysis.

Diagnostic criteria for adult-onset periodic fever, aphthous stomatitis, pharyngitis, and cervical adenitis (PFAPA) syndrome

Recurrent fever accompanied by

(a) Erythematous pharyngitis
and/or
(b) Cervical lymphadenitis

Increased inflammatory markers during attacks
Symptom-free intervals

Diagnostic items accounted for their odds ratio (OR) values: variables with a higher OR value (increased inflammatory markers during attacks and symptom-free intervals) were established as mandatory in the final diagnostic model; conversely, on the basis of a lower OR value, only one item between erythematous pharyngitis during fever and cervical lymphadenitis during fever is required for the diagnosis of PFAPA syndrome. These diagnostic criteria should be applied on patients aged at least 16 years and after having excluded infective, autoimmune, and neoplastic diseases as well as monogenic autoinflammatory diseases (AIDs) and febrile polygenic AIDs. In addition, throat swab performed during fever have to be negative and antibiotic therapy ineffective.

observation of these manifestations in patients with a suspected PFAPA syndrome should call for caution before assigning the diagnosis. In addition, univariate analysis shows that PFAPA syndrome is mostly connected with a fever duration ranging between 2 and 5 days, while fever attacks lasting less than 48 h and longer than 10 days should point to other diagnoses than PFAPA syndrome.

Although results obtained by univariate analysis are clinically interesting and potentially useful to identify or exclude adult-onset PFAPA syndrome, we aimed at creating a set of diagnostic criteria easy to be applied in the clinical practice and reproducible for further studies. Therefore, we deliberately avoided a longer list of diagnostic items as well as concomitant exclusion criteria, without decreasing the predictive potential of the model. Indeed, as demonstrated by the very high level of sensitivity and specificity obtained at ROC analysis, 93.4% of all patients fulfilling the diagnostic criteria would be correctly identified as having PFAPA syndrome and only 8.3% (100% − specificity) would be incorrectly classified as PFAPA patients.

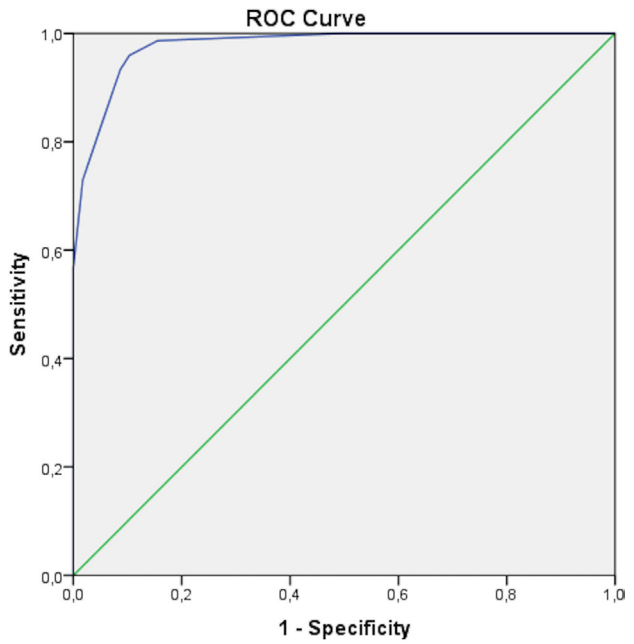

FIGURE 1 | Receiver operating characteristic (ROC) curve obtained for adult periodic fever, aphthous stomatitis, pharyngitis, and cervical adenitis (PFAPA) patients and subjects with fever of unknown origin as control group. The area under curve is of 0.978 (95% CI 0.958–0.998), corresponding to sensitivity of 93.4% (95% CI 87.5–96.5%) and specificity of 91.7% (95% CI 82.8–96.7%) for the proposed diagnostic model.

In our casuistry, a male preponderance was observed among patients with adult-onset PFAPA syndrome, while the number of females was higher in the control group. Looking at the past data on adult-onset PFAPA syndrome, a gender imbalance was not clearly observed as the male/female ratio was 1 according to a review evaluating all the cases published until 2015 (38). However, more recently, we have already reported 30 patients characterized by a male preponderance (9). As this trend has been confirmed again in this study, the male preponderance could represent a non-random finding. Nevertheless, future observational studies are required to clarify whether the higher number of males is a stochastic event related to the consecutive enrollment of patients or a specific feature of the disease.

Of note, we did not take into account the complete response to a single dose of corticosteroid as a possible diagnostic item to provide a set of criteria immediately applicable at the first clinical assessment, also in patients never treated with steroids. In addition, the complete resolution of flares after a single-steroid administration has proved to be less pronounced in adults than among pediatric patients. In this regard, we have recently highlighted that 98.8% of 85 pediatric PFAPA patients and only 88.2% out of 17 adult patients with PFAPA syndrome experienced total resolution of flares after a single-corticosteroid administration (10). Since this is probably explained by inadequate corticosteroid dosages in adults, *ad hoc* dosage trials should be conducted on late-onset PFAPA patients before including this variable as an additional diagnostic item.

Although we performed genetic testing in all patients to exclude subjects carrying mutations in genes related to the most frequent monogenic AIDs, we did not perform a testing for myeloid restricted somatic mutations that have recently been described in adult patients and could explain autoinflammatory manifestations in some cases (39–41). This represents a potential limit of the genetic screening strategy adopted in our cohort of patients. Also, the sample size of our study is relatively small due to the rarity of adult-onset PFAPA syndrome. Nevertheless, we have reported herein the largest cohort of patients ever described, adequate for performing a reliable statistic computation aimed at creating diagnostic criteria.

Our diagnostic criteria have been tested on adult patients and should be applied only to subjects aged at least 16 years. Their ability in differentiating adult-onset PFAPA patients from patients with late-onset monogenic AIDs could be tested in future studies.

In conclusion, we provide a set of clinical diagnostic criteria focused on adult patients presenting with suspected adult-onset PFAPA syndrome. They have been designed as an easy-to-use diagnostic tool aimed at identifying PFAPA patients from subjects with FUO with a high-predictive potential as shown by its very high sensitivity and specificity.

ETHICS STATEMENT

The study was approved by the local Ethics Committee of Azienda Ospedaliera Universitaria Senese (AOUS), Siena (Italy) and each patient provided a written consent for both genetic testing and clinical data processing, in accordance with the Declaration of Helsinki.

AUTHOR CONTRIBUTIONS

LCa and AV designed the study; LCa and RM finally revised the manuscript; LCa, RM, AV, GE, DR, LLS, GE, EV, IP, LCe, CF, BF, and MG final approval of the manuscript; LCa, AV, and DR drafting of the manuscript; GC and AV data analysis; LLS, GE, EV, IP, LCe, CF, BF, and MG patients enrollment, follow-up of the patients, and data collection.

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Dynamics of Inflammatory Response in Autoinflammatory Disorders: Autonomous and Hyperinflammatory States

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Autoinflammatory diseases were originally defined as a group of monogenic disorders associated with seemingly unprovoked inflammatory episodes mediated mainly by the innate immune system and without direct involvement of adaptive immunity. The renewed concept encompasses a larger group of disorders including multifactorial diseases, which share the same inflammatory and clinical features with the monogenic disorders. Coining of the "auto" prefix to these inflammatory diseases suggests a constitutively active and self-augmenting innate immune response, but only a subgroup of them including cryopyrin-associated periodic syndrome (CAPS), associated with dominantly inherited gain-of-function *NLRP3* variants, fits well with the definition of the "autonomous" inflammatory conditions. However, the "autoinflammation" concept also includes another group of disorders characterized by episodes of exaggerated inflammatory response only when challenged by certain triggers. The dynamics of this latter group can be better defined as a "hyperinflammatory" state, which shares similar characteristics with the innate memory or trained immunity. Differentiation of "autonomous" and "hyperinflammatory" states of autoinflammatory disorders can provide additional insights to understand their pathogenesis and develop better management strategies since both conditions may have different inflammatory dynamics affecting the severity and frequency of clinical findings and treatment responses.

Keywords: inflammation, autoimmunity, autoinflammatory disorders, innate immunity, hyperinflammatory response, autonomous inflammation, trained immunity, innate tolerance

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"Heat not a furnace for your foe so hot
That it do singe yourself."
Henry VIII
William Shakespeare

INTRODUCTION

Inflammation is a physiologic process aiming to protect the integrity of organisms against exogenous or endogenous dangerous insults (1–3). This process involves the recognition of pathogen—or danger associated molecular patterns by relevant receptors, which leads to the development of a response involving different cells and mediators to eliminate or limit the threat; and this response resolves with the repair of damages to restore the homeostasis.

The inflammatory response is well regulated to ensure a controlled reaction limited only to the pathogens or dangerous insults. During the turn of the twentieth century, Paul Ehrlich used the "*horror autotoxicus*" term to describe organisms' ability to recognize the self and not develop a harmful response against self-tissues (4). Although the regulatory mechanisms involved in inflammatory response work perfectly well in general, the problems in these mechanisms are considered to be responsible for the development of so-called "inflammatory" disorders (5). Autoimmune diseases are the most widely known examples of the "*autotoxic*" inflammatory disorders, and they do develop as a result of failures in the immune tolerance mechanisms causing the persistent activity of pathogenic self-reactive T and B cells.

On the other hand, regulatory problems in the innate immune response have been identified as the underlying pathology of another subset of inflammatory disorders, and uncontrolled or disproportionate innate inflammatory response triggered by the recognition of pathogen—or danger-associated molecular patterns has been shown to be responsible for the clinical and pathologic findings (6). Hereditary periodic fever syndromes constitute the best examples of this subgroup, and these conditions have been named as "*autoinflammatory disorders*" to differentiate them from "*autoimmune diseases*", following the identification of genetic basis of familial Mediterranean fever (FMF) and tumor necrosis factor receptor associated periodic syndrome (TRAPS). Autoinflammatory disorders have originally been described as pathological conditions associated with seemingly unprovoked episodes of inflammatory response mainly involving the innate immune system with excessive production of proinflammatory cytokines and chemokines and without direct role of pathogenic autoantibodies or cellular immunity against self-antigens (6–8). An updated definition of this group encompasses a larger spectrum of disorders including multifactorial diseases, which share the same inflammatory characteristics and clinical features with the monogenic disorders (8, 9).

Following the identification of several new members of the autoinflammatory disorders, different approaches have been used to classify them. Classification attempts have mainly been based on the mechanisms affecting the regulation of innate immune response or the types of over-produced cytokines/inflammatory mediators involved in their pathogenesis (6, 8). Regulatory defects may rely on the constitutive activation of intracellular pathogen—or danger-associated signal sensing, intracellular accumulation of signals triggering innate sensors, loss of the negative regulatory function of innate response proteins, or up-regulation of post-receptor signaling mechanisms in innate immunity (8). Depending on the dysregulated pathways, inborn errors result in increased production of particular cytokines, such as interleukin 1 beta (IL-1β) or type 1 interferon, or up-regulated secretion of several proinflammatory cytokines and chemokines in a more complex way (6, 8).

This review aims to discuss the dynamics of inflammatory response during the course of the autoinflammatory disorders within the context of trained immunity.

AUTONOMOUS VS. HYPERINFLAMMATORY STATES IN AUTOINFLAMMATORY DISORDERS

In monogenic autoinflammatory disorders, increased inflammatory response develops as a result of gain-of-function or loss-of-function mutations in the genes involved in the innate immune response (8). Cryopyrin-associated periodic syndromes (CAPS) typically represent the autoinflammatory mechanisms associated with gain-of-function mutations in the *NLRP3* gene, which result in increased constitutive activity of the intracellular sensor protein (10). Mutation-dependent conformational changes in the NLRP3 protein result in increased production of IL-1β and a clinical spectrum ranging from the self-limited inflammatory episodes to the persistent severe inflammation (10, 11). In the mildest end of the spectrum, CAPS patients develop a "hyperinflammatory" response only when they are exposed to cold (**Table 1**). However, in the severe end, which was previously called as *Neonatal Onset Multisystem Inflammatory Disorder* (NOMID), patients start to have an "autonomously" increased IL-1β production starting within the first year of life (**Table 1**). Somatic mosaicism in myeloid cell lineages for the *NLRP3* gene mutations may be enough for the development of disease manifestations associated with moderate to severe inflammation, and expansion of the mutated clone with the passage of time may be the cause of the late onset of clinical findings in some patients (12–17).

On the other hand, Familial Mediterranean Fever (FMF), the most common form of the autoinflammatory disorders, is associated with autosomal recessively inherited variants in exon 10 of the *MEFV* gene, which encodes the pyrin protein. Pyrin has been linked to different roles in the regulation of the inflammasome complex, and monocytes of FMF patients produce increased amount of IL-1β depending on the number of penetrant exon 10 mutations, only when

TABLE 1 | Possible contributions of autonomous and hyperinflammatory states to the clinical findings of common monogenic autoinflammatory disorders with putative scores based on the characteristics of clinical findings.

Disease	Gene	Hyperinflammatory state	Autonomous inflammatory state
CAPS (FCAS)	*NLRP3*	++	+
CAPS (NOMID)	*NLRP3*	++	++++
FMF	*MEFV*	+++	+
crFMF	*MEFV*	+	+++
PAAND	*MEFV*	+	++++
Blau syndrome	*NOD2*	+	+++
Crohn disease	*NOD2*	+++	+
MKD	*MVK*	++	+++
TRAPS	*TNFRSF1A*	+++	++

Abbreviations. CAPS, Cryopyrin associated periodic syndrome; FCAS, Familial cold autoinflammatory syndrome; NOMID, Neonatal-onset multisystem inflammatory disease; FMF, Familial Mediterranean fever; crFMF, colchicine refractory-familial Mediterranean fever; PAAND, Pyrin-associated autoinflammation with neutrophilic dermatosis; MKD, Mevalonate kinase deficiency; TRAPS, TNF receptor associated periodic syndrome.

stimulated with proinflammatory environmental triggers such as lipopolysaccharide (18). The variants associated with increased IL-1β production do not interfere with the production of regulatory natural antagonist protein IL-1Ra, which help limit the inflammatory episode within 2–3 days (18). Most of the FMF patients do not have constitutively enhanced autonomous production of IL-1β, and it is usually not possible to detect ongoing inflammation in between these "hyperinflammatory" episodes.

However, patients carrying dominantly inherited p.Ser242Arg mutation in exon 2 of the *MEFV* gene develop a different inflammatory phenotype, which is related to the constitutively active pyrin-inflammasome and continuously elevated IL-1β-driven acute phase response (19). The clinical picture associated with this *MEFV* variant has been named as pyrin-associated autoinflammation with neutrophilic dermatosis (PAAND); and it is characterized by fever lasting several weeks rather than days, neutrophilic dermatosis, arthralgia, myalgia, cardiomyopathy, anemia, pyogenic arthritis, and serositis (19). Phosphorylation of serine at position 242 of the pyrin protein is critical for the binding of its negative regulator 14-3-3 protein; and this regulatory mechanism is linked to the guard function of pyrin protein as a sensor of RhoA-mediated changes within the cytoplasm. Various bacterial toxins affecting the functions of Rho GTPases, such as TcdB toxin of *Clostridium difficile* and ADP-ribosylating C3 toxin of *Clostridium botulinum,* can induce pyrin-inflammasome through the decreased downstream activity of RhoA protein, which affects the binding of 14-3-3 to pyrin (19–21). Missense changes in the one of the phosphorylation sites of pyrin can mimic the intracellular changes induced by these bacterial toxins and result in autonomous activation of pyrin-inflammasome with more persistent inflammatory dynamics different from the characteristics of FMF.

Similarly, variants in the *NOD2 (CARD15)* gene are linked to both autonomous and hyperinflammatory disorders. Dominantly inherited gain-of-function mutations in the NACHT domain of the *NOD2* gene lead to the increased basal activity of NF-κB. This autonomous inflammatory response is associated with Blau syndrome, which is characterized by early-onset granulomatous uveitis, dermatitis, and arthritis with camptodactily deformities (22, 23). On the other hand, loss-of-function variants in the leucine-rich repeat (LRR) region of the *NOD2* gene are associated with the multifactorial Crohn disease; and these mutations are thought to be associated with dysregulated interaction between host and dysbiotic intestinal microbiota leading to hyperinflammatory responses (23–26).

To prevent the confusion associated with the "hyperinflammatory" state, it is necessary to note that a group of heterogeneous disorders have been grouped under the term of "hyperinflammatory syndromes," because of a common immunopathology associated with a cytokine storm or hypercytokinemia; which includes one of the hereditary autoinflammatory disorders, familial hemophagocytic lymphohistiocytosis (27). The hyperinflammatory response constitutes the shared pathogenic mechanism between hemophagocytic lymphohistiocytosis and macrophage activation syndromes, and the latter condition can develop in various autoinflammatory and autoimmune settings ranging from systemic onset juvenile idiopathic arthritis, Kawasaki disease to systemic lupus erythematosus (27). The hyperinflammatory syndromes associated with dysregulated cytokine production or cytotoxicity defects can also be seen in association with infections, malignancies, and immunodeficiency syndromes such as Chédiak Higashi, Griscelli 2, Hermansky Pudlak 2 syndromes (27). Infections are usually considered as the main triggers of hyperinflammation, which may be an example of maladaptive "trained immunity" response.

TRAINED IMMUNITY AND INNATE TOLERANCE

It has long been suggested that one of the critical differences between adaptive and innate immunity is that adaptive immune response can build immunological memory but innate immunity cannot (28). However, several recent studies have demonstrated that an innate version of immunological memory can be induced after infections or vaccinations, which results in a stronger inflammatory response with broader specificity following a secondary stimulation with different pathogens (**Figures 1i**) (28–30). This weeks or months-lasting memory is named as "trained immunity," and it is mainly associated with epigenetic re-programming of innate immune cells, especially of the cells of myeloid lineage (30). This stronger inflammatory response to various microbial triggers following the initial infections or vaccinations involves both histone modifications (i.e., H3K4me1, H3K4me3, H2K27Ac, H3K9me2), and metabolic changes (i.e., increased aerobic glycolysis through the mTOR pathway and increased production of mevalonate) in those cells (30, 31).

However, maladaptive conditions associated with inappropriate activation of trained immunity may result in immunodeficiency states or hyperinflammatory responses (28, 32). Inappropriate exposure of innate immune cells to bacterial endotoxins such as lipopolysaccharide (LPS) may result in a refractory state to subsequent challenges of LPS, which is known as "endotoxin tolerance," and it contributes to the immune paralysis observed in patients with sepsis (33, 34). Although several findings suggest changes in the polarization and cytokine production pattern of inflammatory cells, exact mechanism of the endotoxin tolerance has yet to be clarified (33, 35).

In the other end of the maladaptive conditions, hyperinflammatory responses due to induction of trained immunity may contribute to the pathogenesis and course of monogenic autoinflammatory disorders as well as several other inflammatory conditions (32), which may show variability in the expression and severity depending on the environmental factors (36, 37).

TRAINED IMMUNITY AND AUTOINFLAMMATORY DISORDERS

Non-specific hyperinflammatory response to a broad spectrum of triggers was first described as the "pathergy" reaction in 1933

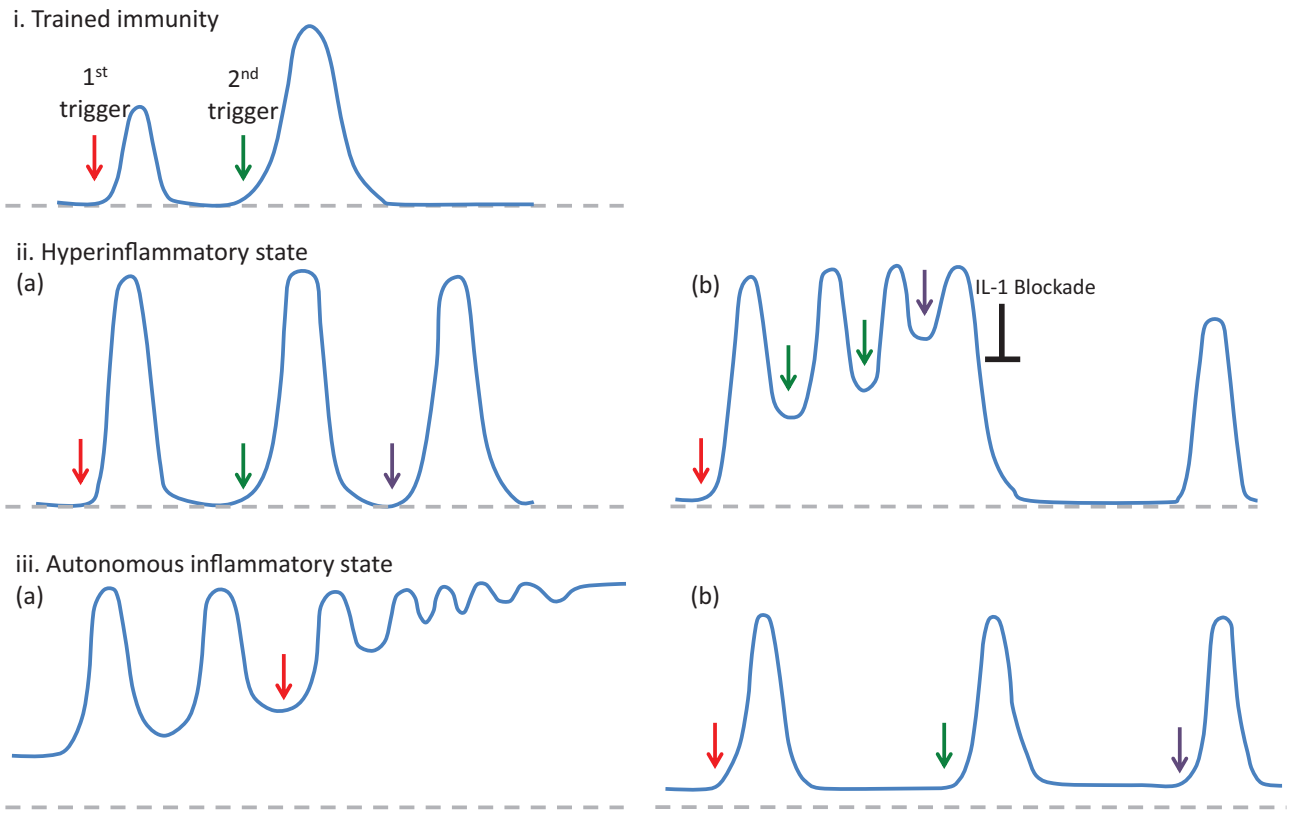

FIGURE 1 | The features of inflammatory responses developing in the trained immunity (i) and autoinflammatory disorders (ii and iii), in regard to the intensity and frequency of episodes and their resolution to the baseline. In trained immunity (i), after the resolution of the inflammatory response following an infection or vaccination (1st trigger), a following stimulation with different pathogens (2nd trigger) results in a stronger inflammatory response (28). In autoinflammatory disorders, triggering factors (arrows) may either induce a hyperinflammatory state (ii-a), defined as an enhanced inflammatory response developing after a stimulation, or an autonomous inflammatory state (iii-a) associated with gain of function mutations, which lead to the continuous production of IL-1β and ongoing inflammatory activity in between attacks. However, in autoinflammatory disorders associated with the hyperinflammatory dynamics, some patients may experience "autonomous" inflammatory states (ii-b), which require a therapeutic intervention to reset the inflammatory dynamics. On the other hand, in some autoinflammatory disorders associated with autonomous inflammatory characteristics, disease course may be very mild and obvious inflammatory findings could only be detected when the patients are exposed to triggering factors such as cold (iii-b).

by Rössle, and his definition corresponds well with the current understanding of the trained immunity associated with sustained changes in the expression of proinflammatory cytokines due to epigenetic modifications (38, 39). Therefore, long-standing observations on the pathergic response and trained immunity regarding the intensity of inflammatory response to various triggers may also have a potential to analyze the variability of clinical findings in the course of the autoinflammatory disorders, which cannot be explained by only genotype (32).

Investigation of pathogenic mechanisms associated with autosomal recessively inherited Mevalonate Kinase Deficiency (MKD) may provide the most direct clues for the role of trained immunity in autoinflammatory disorders. Recently, it has been shown that activation of the cholesterol synthesis pathway, but not the synthesis of cholesterol molecule itself, is involved in the stimulation of trained immunity, and mevalonate is the critical molecule of this pathway in the induction of epigenetic changes, such as the H3K4me3 change at the promoter regions of proinflammatory *TNFA* and *IL6* genes (31).

Retention of intracellular mevalonate in the monocytes of MKD patients due to decreased mevalonate kinase enzyme activity has also been shown to be associated with the same trained immunity phenotype, which leads to the autoinflammatory response (31). Prenylation defects associated mevalonate kinase enzyme defects has also been linked to the decreased RhoA-associated phosphorylation of pyrin protein and activation of the pyrin inflammasome (21). In addition to the mutation-specific conformational changes affecting the mevalonate kinase enzyme activity, the extent of epigenetic changes are expected to contribute to the variability in the clinical spectrum, ranging from the hyperinflammatory response due to temporary increases in the mevalonate concentration following stimuli such as vaccination in the mild end to the autonomous hyperinflammatory response resulting from constitutively increased production of mevalonate due to severely defective activity of the enzyme leading to the sustained changes (8, 40).

Similar to the *MVK* gene variants leading to MKD, the rare genetic variants responsible for other hereditary

autoinflammatory disorders can also be assumed to be associated with a much stronger and durable "trained immunity" response leading to maladaptive conditions (**Figure 1**). For example, country-dependent environmental factors affecting the risk of infections and infant mortality rate show an association with the risk of amyloidosis in FMF patients (37). In addition to the rare variants, even some common polymorphisms associated with multifactorial autoinflammatory disorders such as the NOD2 variants in Crohn disease may be involved in "hyperreactive" innate response and maladaptive trained immunity following an infection or associated with dysbiotic microbiota.

It may also be helpful to note that in the autoimmunity end of the inflammatory disease spectrum, more than half of the disease associated non-coding variants have been mapped to enhancer-like elements in immune cells, especially lymphocytes, possibly altering non-canonical regulatory sequences and causing context-dependent autoimmune responses (41).

IMPLICATIONS OF DEFINING HYPER-VERSUS AUTONOMOUS INFLAMMATORY STATES

Identification of molecular basis of several autoinflammatory disorders has led to development of targeted treatments with successful results such as IL-1 blocking agents in inflammasomopathies; and their classification according to underlying inflammatory pathways proved to be useful in explaining both the pathogenesis of clinical findings and variability in the treatment responses. On the other hand, addition of another dimension to the classification, by considering the dynamics of inflammatory response associated with hyperreactive or autonomously active innate immune cells may provide further benefits in the interpretation of clinical findings and developing better management strategies with the optimum use of available treatment options in individual patients (**Table 1**).

Bozkurt et al. developed a unifying mathematical model to understand the dynamics of recurrent nature of inflammation in FMF and CAPS, in the form of coupled nonlinear ordinary differential equations (42). Comprehensive bifurcation analyses of the model revealed that the concentration of active caspase 1 enzyme is the most critical parameter determining the healthy state as well as the inflammatory features of FMF and CAPS patients (42). In FMF patients, a self-limited inflammatory episode develops only when the system is triggered by an insult, compatible with the "hyperinflammatory" state (**Figures 1ii-a**). On the other hand, gain-of-function mutations in CAPS patients result in constitutively active caspase 1 leading to an autonomous periodicity with episodes developing even when there is no trigger (**Figures 1iii-a**) (42). In patients with low-penetrance variants periodicity of the attacks may decrease, but when the variants are penetrant and triggers are present, patients may develop a non-oscillatory, continuous inflammation representing the most severe end of the CAPS spectrum, NOMID (**Table 1**) (42).

In clinical practice, the inflammatory characteristics of a subgroup of FMF patients with inadequate response to colchicine treatment (also named as colchicine refractory-FMF patients) can be classified as an "autonomous" state due to genetic and/or environmental factors affecting the duration and sustainability of caspase 1 activity (**Table 1**). In some of the FMF patients, this autonomous inflammatory state may be temporary due to intervening stressful conditions resulting in a vicious circle characterized by continuous production of pro-inflammatory cytokines, which causes either unexpectedly long episodes or very frequently recurring attacks despite highest tolerable doses of colchicine along with an elevated acute phase response in between attacks (43). In this setting, blocking the activity of IL-1 by biologic agents may reset the autonomous production of IL-1β, and some of these colchicine refractory-FMF patients may experience a stable disease course with regained good response to colchicine (**Figures 1ii-b**) (44).

Within the same context, some patients with an autoinflammatory disorder characterized by gain-of-function mutations and associated with autonomous production of IL-1 may run a milder disease course with very rare inflammatory episodes (**Table 1**). In this situation, despite autonomous production of IL-1 at low level, inflammatory clinical findings can only be triggered following strong stimuli such as cold exposure, infections, or vaccinations, and they may not need continuous blockade of IL-1 to control inflammatory episodes (**Figures 1iii–b**).

Similarly, inhibition of IL-1 activity with potent drugs may reset the IL-1β-dependent vicious circle and cytokine-driven pathologies in patients with higher constitutive caspase 1 activity; and following a single high-dose administration of anti-IL-1β monoclonal antibody, some CAPS patients may not require additional treatment for months due to inhibition of IL-1β-dependent production of IL-1, which may be increased up-to-5-fold compared to healthy controls (10). Also, DNA methylation status of CAPS patients may become similar to that of healthy controls when they are using anti-IL-1 treatments, which suggests sustained improvements in the epigenetic programming (45).

CONCLUSIONS

In conclusion, adding the "hyper" or "autonomous" as well as the trained immunity dimensions to the concept of autoinflammation could provide further benefits for both understanding of the immunopathogenesis of the variable disease course in these conditions and developing better strategies for the management of inflammatory findings in regard to the timing, dosage, and administration intervals of IL-1 blocking agents.

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REVIEW
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Dermatologic and Dermatopathologic Features of Monogenic Autoinflammatory Diseases

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Autoinflammatory diseases include disorders with a monogenic cause and also complex conditions associated to polygenic or multifactorial factors. An increased number of both monogenic and polygenic autoinflammatory conditions have been identified during the last years. Although skin manifestations are often predominant in monogenic autoinflammatory diseases, clinical and histopathological information regarding their dermatological involvement is still scarce. Monogenic autoinflammatory diseases with cutaneous expression can be classified based on the predominant lesion: (1) maculopapular rashes or inflammatory plaques; (2) urticarial rashes; (3) pustular, pyogenic or neutrophilic dermatosis-like rashes; (4) panniculitis or subcutaneous nodules; (5) vasculitis or vasculopathy; (6) hyperkeratotic lesions; (7) hyperpigmented lesions; (8) bullous lesions; and (9) aphthous lesions. By using this classification, this review intends to provide clinical and histopathological knowledge about cutaneous involvement in monogenic autoinflammatory diseases.

Keywords: monogenic autoinflammatory diseases, autoinflammatory diseases, clinical dermatology, maculopapular rash, urticarial rash, dermatopathology, classification

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INTRODUCTION

The term "autoinflammatory diseases" was first used in 1999 to describe a group of rare diseases of the innate immunity presenting with recurrent episodes of uncontrolled systemic inflammation (1). Since then, the number of monogenic autoinflammatory conditions and other complex and polygenic disorders driven by autoinflammatory mechanisms have been in continuous expansion (2, 3). In addition, several autoimmune diseases and primary immunodeficiencies have been found to share pathogenic features with autoinflammatory diseases (4, 5).

The most frequent and well-known autoinflammatory mechanism is mediated by the inflammasomes, intracellular protein complexes acting as innate immune system receptors with an important role in the sensing of intracellular pathogen- and danger-associated molecular patterns. They are involved in the susceptibility to infection, autoinflammation, and tumorigenesis. Inflammasomes consist of a sensor part (the NOD-like-receptor), an adaptor protein (ASC), and caspase-1 as the downstream effector. Upon stimulation, inflammasome assembles and activates caspase-1 which cleaves pro-IL-1β and pro-IL-18 into IL-1β and IL-18. NRLP3 and pyrin inflammasomes are responsible for cryopyrin-associated

periodic syndromes (CAPS) and familial Mediterranean fever (FMF), respectively, and other inflammasomopathies (6, 7). Other relevant inflammasomes include NLRP1 and NLRP4 (8, 9).

Other pathogenic mechanisms causing autoinflammatory disorders include those related with the activation of NF-κB transcription factor and type I interferon (IFN) (6, 10). The transcription factor NF-κB is involved in processes related to inflammation, cellular differentiation, metabolism, cell survival, and acquired immune responses (6). In its inactive form, NF-κB is tied to inhibitors of kBs (IkBs). NF-κB can be activated by two mechanisms: the canonical pathway, induced by cytokines and toll-like receptors (TLR), and the non-canonical pathway, triggered by TNF-receptor family proteins. Both are controlled by the ubiquitin system. The canonical mechanism is regulated by K63 and linear Met1 ubiquitin chains. Both proteins are linked to their substrates (RIPK-1, which is one of the adaptor proteins on the TNF receptor 1, and IKKγ, part of the IKK complex) by LUBAC complex (composed by the proteins HOIP, HOIL-1, and SHARPIN), which increases NF-κB activity. Proteins A20 and OTULIN cleave K63 and Met1 from their substrates, which physiologically downregulate NF-κB signaling. Little is known about its exact role in the non-canonical pathway (10–12).

Type I interferons (IFNα and IFNβ) are the major effector cytokines against virus and intracellular pathogens. They induce the transcription of certain IFN stimulated genes with the subsequent viral clearance. Among the two IFN activating mechanisms, one is mediated by TLRs that detect viral nucleic acids within endosomes and induce proinflammatory cytokines and IFNα, and the other is mediated by cytosolic DNA and RNA sensors. DNA sensing is carried out by nucleotidyl transferase cyclic GMP-AMP synthase (cGAS), which produces cGAMP that binds to STING (stimulator of IFN genes) and induces transcription of IFNβ genes. RNA sensing is mediated by RIG-1-like helicase, RIG-1, and MDA-5 with the subsequently recruitment of MAVS (mitochondrial antiviral signal) and activation of NF-κB. IFN interacts with its surface receptor IFN-α and induces the STAT pathway, which induces the transcription of IFN genes and promotes antiviral activity. In addition, proteins regulating the synthesis or degradation of nucleic acids such as TREX1, SAMHDI, and RNase H2 play an important role in IFN genes activation. Immunoproteasomes are protein complexes that degrade ubiquitinated intracellular proteins and are implicated in cellular stress responses, as well as activating IFN (11, 13).

IL-1-mediated and IFN type I-mediated autoinflammatory diseases and their main genetic and pathogenic aspects are illustrated in **Figure 1**.

Over time, different classifications of monogenic autoinflammatory diseases have been proposed according to molecular and etiopathogenic mechanisms involved (11, 15), type of inheritance (16), genetic background and clinical presentation (17, 18). Apart from FMF and CAPS, other well-characterized monogenic inflammasomopathies comprise TNF receptor-associated periodic syndrome (TRAPS), hyper-IgD syndrome (HIDS), pediatric granulomatous arthritis (Blau syndrome and early onset sarcoidosis), pyogenic arthritis, pyoderma gangrenosum, and acne (PAPA), deficiency of IL-1 receptor antagonist (DIRA) and deficiency of interkeukin-36 receptor antagonist (DITRA). All the monogenic autoinflammatory diseases covered in this review classified according to the major pathogenic mechanism are listed in **Table 1**.

Polygenic or multifactorial autoinflammatory diseases are defined as complex systemic disorders sharing an autoinflammatory and sometimes autoimmune background, with an unknown genetic cause. The most prevalent polygenic conditions include Behçet disease, Schnitzler syndrome, periodic fever with aphthous stomatitis, pharyngitis, and cervical adenitis (PFAPA), systemic juvenile idiopathic arthritis (sJIA), adult onset Still disease (AOSD), Crohn disease and synovitis, acne, pustulosis, hyperostosis, and osteitis (SAPHO) (19).

Clinical features in autoinflammatory diseases are variable, heterogeneous and nonspecific, since most of the symptoms are often shared by different conditions. Common inflammatory manifestations include recurrent fever, musculoskeletal symptoms, abdominal and thoracic serositis, headache, ocular inflammation, and mucosal and skin lesions (11).

Dermatologic involvement is common in monogenic autoinflammatory diseases and may represent the predominant and the initial event in some of them. Among all the cutaneous lesions present in monogenic autoinflammatory diseases, maculopapular, and urticarial rashes are by far the most prevalent manifestations. However, the identification of skin lesions as part of an autoinflammatory disease is often difficult

Abbreviations: AIKD, Autoinflammatory keratinization diseases; ANA, Antinuclear antibodies; ANCA, Antineutrophil cytoplasmic antibodies; AGS, Aicardi-Goutières syndrome; APLAID, Autoinflammation and PLCγ2-associated antibody deficiency and immune dysregulation; CAIN, C/EBPε-associated autoinflammation and immune impairment of neutrophils; CANDLE, Chronic atypical neutrophilic dermatitis with lipodystrophy and elevated temperature syndrome; CAPS, Cryopyrin-associated periodic syndrome; CINCA, Chronic infantile, neurologic, cutaneous and articular; CPR, C-reactive protein; CRMO, Chronic recurrent multifocal sterile osteomyelitis; DADA2, Deficiency of adenosine deaminase 2; DIRA, Deficiency of IL-1 receptor antagonist; DITRA, Deficiency of IL-36 receptor antagonist; EMA, European Medicines Agency; ESR, Erythrocyte sedimentation rate; FANF, Familial autoinflammatory necrotizing fasciitis; FCAS, Familial cold autoinflammatory syndrome; FDA, Food and Drug Administration; FMF, Familial Mediterranean fever; HA20, Haploinsufficiency of A20; HIDS, Hyper-IgD syndrome; Ig, Immunoglobulin; IL, Interleukin; IBD, Inflammatory bowel disease; IFN, Interferon; LUBAC, Linear ubiquitination chain assembly complex; MA, Mevalonic aciduria; MAS, Macrophage activation syndrome; MAVS, Mitochondrial antiviral signal; MKD, Mevalonate kinase deficiency; MVK, Mevalonate kinase; MWS, Muckle-Wells syndrome; NAIAD, NLRP-1 associated disease; NLRC4-AD, NLRC4-associated autoinflammatory diseases; NOMID, Neonatal-onset multisystem inflammatory disease; NSAID, Non-steroidal anti-inflammatory drugs; ORAS, OTULIN-related autoinflammatory syndrome; PAAND, Pyrin-associated autoinflammation with neutrophilic dermatosis; PAPA, Pyogenic sterile arthritis, pyoderma gangrenosum and acne; PFAPA, Periodic fever, aphthous stomatitis, pharyngitis and cervical adenitis; PFIT, Autoinflammatory periodic fever, immunodeficiency, and thrombocytopenia; PLAID, PLCγ2-associated antibody deficiency and immune dysregulation; SAA, Serum amyloid protein; SAPHO, Synovitis, acne, pustulosis, hyperostosis and osteitis; SAVI, STING-associated vasculopathy with onset in infancy; STING, Stimulator of IFN genes; SMS, Singleton–Merten syndrome; sJIA, Systemic juvenile idiopathic arthritis; SPENCDI, Spodyloenchondrodysplasia with immune dysregulation; TNF, Tumor necrosis factor; TACE, TNF-α converting enzyme;TRAPS, TNF receptor-associated periodic syndrome; TLR, toll-like receptors.

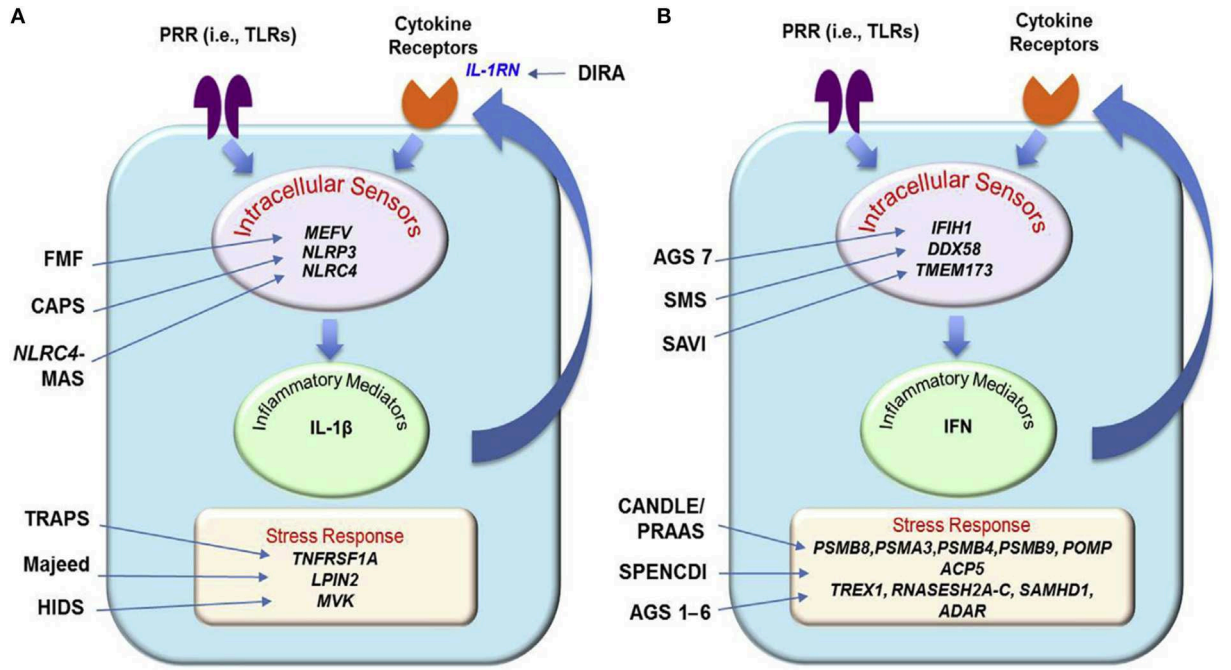

FIGURE 1 | Principal genetic and pathogenic mechanisms in IL-1 **(A)** and IFN type 1 **(B)** mediated autoinflammatory diseases [From Shwin et al. (14), with permission]. AGS, Aicardi–Goutières syndrome; CANDLE, chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperature; CAPS, cryopyrin-associated periodic syndrome (FCAS, familial cold autoinflammatory syndrome; MWS, Muckle–Wells syndrome; NOMID, neonatal-onset multisystem inflammatory disease); DIRA, deficiency of interleukin-1 receptor antagonist; FMF, familial Mediterranean fever; MKD/HIDS, mevalonate kinase deficiency/hyperimmunoglobulinemia D and periodic fever syndrome; NLRC4-MAS, NLRC4-associated macrophage activation syndrome; PRAAS, proteasome-associated autoinflammatory syndrome; PRR, Pattern recognition receptor; SAVI, STING-associated vasculopathy with onset in infancy; SMS, Singleton–Merten syndrome; SPENCDI, Spodyloenchondrodysplasia with immune dysregulation; TLRs, toll-like receptors; TRAPS, TNF receptor-associated periodic syndrome.

because of the potential wide spectrum of skin manifestations in these conditions, and also because the severity or extension of the cutaneous lesions may differ among patients with the same disease. In addition, some patients may exhibit overlapping skin manifestations. Consequently, differential diagnosis of dermatologic findings may be difficult, even for trained professionals. For instance, with regard to urticarial lesions, differential diagnosis should include all CAPS forms and other monogenic diseases in which urticariform features are the most characteristic cutaneous findings, but it must also comprise other monogenic autoinflammatory diseases presenting less frequently with urticarial rashes (e.g., TRAPS and HIDS), and several polygenic autoinflammatory diseases (e.g., Schnitzler syndrome, sJIA, and adult onset Still disease) (20). Moreover, clinical and histopathological data about dermatological involvement in monogenic autoinflammatory diseases are still scarce (14).

CLASSIFICATION OF MONOGENIC AUTOINFLAMMATORY DISEASES ACCORDING TO THE CUTANEOUS INVOLVEMENT

Several classifications based on clinical and histopathological features of cutaneous manifestations have been proposed for autoinflammatory diseases (6, 11, 15, 16, 21, 22). In 2017, Shwin et al. (14) divided monogenic autoinflammatory diseases into seven categories according to the predominant cutaneous lesion and the most clinically relevant aspect: (1) Nonspecific maculopapular rashes with recurrent episodic fever and abdominal pain; (2) Neutrophilic urticaria; (3) Pustular skin rashes and episodic fevers; (4) Vasculopathy and panniculitis/lipoatrophy syndromes; (5) Vasculopathy and/or vasculitis with livedo reticularis syndromes; (6) Autoinflammatory disorders with granulomatous skin diseases; and (7) Other autoinflammatory syndromes (14).

Because other cutaneous and mucosal lesions have been described to occur in monogenic autoinflammatory diseases, the current review propose a new classification that includes nine dermatologic categories:

1) Maculopapular rashes or inflammatory plaques;
2) Urticarial rashes;
3) Pustular, pyogenic, or neutrophilic dermatosis-like rashes;
4) Panniculitis or subcutaneous nodules;
5) Vasculitis or vasculopathy;
6) Hyperkeratotic lesions;
7) Hyperpigmented lesions;
8) Bullous lesions;
9) Aphthous lesions.

The main monogenic autoinflammatory diseases are divided in these nine groups and depicted in **Table 2**. By using this

TABLE 1 | Classification of autoinflammatory diseases based on the major pathogenic mechanism.

Groups based on pathogenic mechanism	Autoinflammatory disease	Gene/Locus	Inheritance pattern	Protein involved	GOF/LOF mutation
Inflammasomopathies	FMF	*MEFV*	AR	Pyrin	GOF
	TRAPS	*TNFRSF1A*	AD	TNF receptor 1	LOF
	HIDS/MKD	*MVK*	AR	Mevalonate kinase	LOF
	CAPS	*NLRP3*	AD	NLRP3/cryopirin	GOF
	NLRC4-AD (FCAS4)	*NLRC4*	AD	NLRC4	GOF
	PAPA	*PSTPIP1*	AD	CD2BP1	GOF
	DIRA	*IL1RN*	AR	IL-1 receptor antagonist	LOF
	Majeed syndrome	*LPIN2*	AR	Lipin-2	LOF
	PAAND	*MEFV*	AD	Pyrin	GOF
	NAIAD	*NLRC1*	AR/AD	NLRP1	LOF
	PFIT	*WDR1*	AR	WD40 repeat protein	LOF
	CAIN	*CEBPE*	AR	C/EBPε	GOF
NF-κB related diseases	Blau syndrome/Early-onset sarcoidosis	*NOD2/CARD15*	AD	NOD2	GOF
	NLRP12-AD (FCAS2)	*NLRP12*	AD	Monarch1	LOF
	Otulipenia/ORAS (Ubiquinopathy)	*OTULIN*	AR	Otulin	LOF
	HA20 (Ubiquinopathy)	*TNFAIP3*	AD	A20	LOF
	HOIL-1 deficiency (Ubiquinopathy)	*HOIL1*	AR	HOIL1	LOF
	CARD-14 psoriasis	*CARD14*	AD	CARD14	GOF
	NFKB1-AD	*NFKB1*	AD	p50/p105	LOF
	RELA haploinsufficiency	*RELA*	AD	RelA	LOF
	ADAM17 deficiency	*ADAM17*	AR	TACE	LOF
Interferonopathies	CANDLE/PRAAS syndrome	*PSMB8*	AR	β5i subunit of the proteasome	LOF
	SAVI	*TMEM173*	AD	STING	GOF
	Familial chilblain lupus	*TREX1* *SAMHD1* *TMEM173*	AD	3-prime repair exonuclease 1 enzyme dNTPs STING	LOF LOF GOF
	AGS	*TREX1, RNASEH2A, RNASEH2B, RNASEH2C and SAMHD1*	AR	Proteins involved in intracellular degradation or sensing of nucleic acids	LOF > GOF
		ADAR1 *IFIH1 and DDX58*	AD		
	SPENCDI	*ACP5*	AR	TRAP	LOF
	SMS	*IFIH1 and DDX58*	AD	MDA5 and RIG-1	GOF
Other	DADA2	*CECR1*	AR	ADA2	LOF
cytokine-signaling	DITRA	*IL36RN*	AR	IL-36 receptor antagonist	LOF
diseases	H syndrome	*SLC29A3*	AR	hENT3	LOF
	PLAID (FCAS3) / APLAID	*PLCγ2*	AD	PLCγ2	GOF
	Vibratory Urticaria	*ADGRE2*	AD	ADGRE2	LOF
	AP1S3 and autoinflammatory psoriasis	*AP1S3*	Not clear	AP1S3	LOF

(Continued)

TABLE 1 | Continued

Groups based on pathogenic mechanism	Autoinflammatory disease	Gene/Locus	Inheritance pattern	Protein involved	GOF/LOF mutation
	Monogenic forms of inflammatory bowel disease (IL-10 signaling defects)	*IL10RA, IL10RB and IL10*	AR	IL10 and IL10 receptor	LOF

AGS, Aicardi-Goutières syndrome; APLAID, autoinflammation and PLCγ2-associated antibody deficiency and immune dysregulation; CAIN, C/EBPε-associated autoinflammation and immune impairment of neutrophils; CANDLE, chronic atypical neutrophilic dermatitis with lipodystrophy and elevated temperature syndrome; CAPS, cryopyrin-associated periodic syndrome; DADA2 deficiency of adenosine deaminase 2; DIRA, deficiency of IL-1 receptor antagonist; DITRA, deficiency of the IL-36 receptor antagonist; dNTPs, deoxynucleoside triphosphate; FMF, familial Mediterranean fever; GOF, gain-of-function; HA20, haploinsufficiency of A20; HIDS/MKD, hyper-IgD syndrome/Mevalonate kinase deficiency; IL-10, interleukin 10; LOF, loss-of-function; MDA-5, melanoma differentiation-associated gene 5; NFKB1-AD, NFKB1-associated autoinflammatory diseases; NLRC4-AD, NLRC4-associated autoinflammatory diseases; NLRP12-AD= NLRP12-associated autoinflammatory disease; ORAS= OTULIN-related autoinflammatory syndrome; PAAND, pyrin-associated autoinflammation with neutrophilic dermatosis; PAPA syndrome, pyogenic sterile arthritis, pyoderma gangrenosum and acne syndrome; PFAPA, periodic fever, aphthous stomatitis, pharyngitis and cervical adenitis; PFIT, Autoinflammatory periodic fever, immunodeficiency, and thrombocytopenia; PLAID, PLCγ2-associated antibody deficiency and immune dysregulation; RIG-1, retinoic-acid-inducible gene; SAVI, STING-associated vasculopathy with onset in infancy; SMS= Singleton-Merten syndrome; SPENCDI, Spondyloenchondrodysplasia with immune dysregulation; TRAPS, TNF receptor-associated periodic syndrome.

dermatologic classification, this review intends to focus on dermatological and dermatopathologic aspects of monogenic autoinflammatory diseases.

Maculopapular Rashes or Inflammatory Plaques

Familial Mediterranean Fever (FMF)

FMF is the most frequent monogenic autoinflammatory disease caused by mutations in the *MEFV* gene, which encodes pyrin. Such mutations produce a constitutive activation of pyrin and lead to an uncontrolled release of IL-1β and IL-18 (23). FMF is classically inherited with an autosomal recessive fashion. However, an autosomal dominant pattern has also been described (24, 25). The most relevant pathogenic mutations, such as M694V, M694I, M680I, and V726A, are commonly placed in the exon 10 of *MEFV* gene (26).

FMF is clinically characterized by recurrent and self-limited inflammatory attacks lasting for 48–72 h with a variable periodicity (27). High fever (38–40°C) and serositis as abdominal and chest pain are constantly present. Large joints involvement and erysipeloid rash affecting the limbs are also quite common. Febrile protracted myalgia, pericarditis, scrotal pain, and lymphocytic meningitis may also occur. During attacks, acute phase reactants such as C-reactive protein (CPR), serum amyloid protein (SAA), erythrocyte sedimentation rate (ESR), and fibrinogen are significantly increased and tend to normalize during asymptomatic periods. Secondary amyloidosis, usually involving the kidneys, is the most common long-term complication, which is usually associated with a more severe disease or colchicine-resistant disease (14, 26).

Colchicine is the treatment of choice to control disease activity and to prevent the attacks. Colchicine also prevents the development of amyloidosis. In cases of proved intolerance or resistance to colchicine, anti-IL-1 agents have demonstrated efficacy in controlling disease activity and amyloidosis development. While canakinumab has been recently approved by the US Food and Drug Administration (FDA) and European Medicines Agency (EMA) (28), anakinra has also been proved to be useful, either with a continuous or on demand administration (29).

Dermatologic manifestations

Erysipeloid-like erythema is considered the pathognomonic lesion of FMF and consists of an uni- or bilateral well-defined, tender, erythematous, and edematous plaque, usually smaller than 15 centimeters, localized below the knee and on the dorsal aspect of the feet (**Figure 2**). Recurrences tend to occur in the same place, usually after long walking distances, and tend to subside within 24–48 h. It is common among Turks and Jews patients and those carrying the M694V mutation, with a variable frequency, ranging between 3 and 46% of FMF patients (30).

Other cutaneous lesions include diffuse palmoplantar erythema and purpuric papules involving the face, trunk, and extremities (31). FMF patients have an increased incidence of associated systemic vasculitis, such as IgA vasculitis (Henoch-Schönlein purpura), polyarteritis nodosa and Behçet disease (31, 32).

Cutaneous histopathology

Erysipeloid-like plaques are histologically characterized by slight edema of the superficial dermis and sparse perivascular infiltrates with lymphocytes, neutrophils, histiocytes, and nuclear dust. Blurring of the capillary walls is frequent. Direct immunofluorescence shows deposits of IgM, C3, and fibrinogen in the capillary walls of the papillary dermis (30). Slight changes of acanthosis and hyperkeratosis in the epidermis have also been described (33).

TNF Receptor-Associated Periodic Syndrome (TRAPS)

TRAPS is the most frequent autosomal dominant autoinflammatory disease. Mutations in the *TNFRSF1A* gene, encoding TNF receptor 1, induce an overproduction of IL-1β (11). T50M and cysteine mutations are associated with an earlier and more severe disease presentation and long-term development of complications, such as amyloidosis. Variants

TABLE 2 | Classification of monogenic autoinflammatory diseases based on the main cutaneous manifestation.

1	Maculopapular rashes or inflammatory plaques	Familial Mediterranean Fever (FMF)
		TNF receptor-associated periodic syndrome (TRAPS)
		Hyper-IgD syndrome/Mevalonate kinase deficiency (HIDS/MKD)
		Otulipenia/OTULIN-related autoinflammatory syndrome (ORAS)
		HOIL-1 deficiency
2	Urticarial rashes	Cryopyrin-associated periodic syndromes (CAPS)
		NLRP12-associated autoinflammatory disease (NLRP12-AD)
		PLCγ2-associated antibody deficiency and immune dysregulation (PLAID)
		NLRC4-associated autoinflammatory diseases (NRLC4-AD)
		Vibratory Urticaria
3	Pustular, pyogenic or neutrophilic dermatosis-like rashes	Pyogenic sterile arthritis, pyoderma gangrenosum and acne (PAPA)
		Syndromic forms of pyoderma gangrenosum
		Deficiency of IL-1 receptor antagonist (DIRA)
		Deficiency of IL-36 receptor antagonist (DITRA)
		CARD-14 mediated psoriasis (CAMPS)
		Majeed syndrome
		Pyrin-associated autoinflammation with neutrophilic dermatosis (PAAND)
		Singleton-Merten syndrome (SMS)
		ADAM17 deficiency
		AP1S3 and autoinflammatory psoriasis
		NFKB1-associated sterile familial autoinflammatory necrotizing fasciitis (FANF)
4	Panniculitis or subcutaneous nodules	Blau syndrome / Early-onset sarcoidosis
		Chronic atypical neutrophilic dermatitis with lipodystrophy and elevated temperature (CANDLE)
5	Vasculitis or vasculopathy	Deficiency of adenosine deaminase 2 (DADA2)
		STING-associated vasculopathy with onset in infancy (SAVI)
		Familial chilblain lupus
		Aicardi-Goutières syndrome (AGS) 1-7
		Spodyloenchondrodysplasia with immune dysregulation (SPENCDI)
6	Hyperkeratotic lesions	NLRP-1 associated disease (NAIAD)
7	Hyperpigmented lesions	H syndrome
8	Bullous lesions	Autoinflammation and PLCγ2-associated antibody deficiency and immune dysregulation (APLAID)
9	Aphthous lesions	Haploinsufficiency of A20 (HA20)
		Autoinflammatory periodic fever, immunodeficiency and thrombocytopenia (PFIT)
		C/EBPε-associated autoinflammation and immune impairment of neutrophils (CAIN)
		NFKB1-associated Behcet-like disease
		RELA haploinsufficiency
		Monogenic forms of inflammatory bowel disease (IL-10 signaling defects)

such as R92Q and P46L generally lead to a milder disease with a later onset (2).

TRAPS usually occurs in children as recurrent and irregular febrile episodes with generalized myalgia, arthralgia, abdominal pain, ocular lesions (conjunctivitis, uveitis, and periorbital edema) and skin involvement (16, 34). Attacks may be spontaneous or triggered by infections and other stress situations (35).

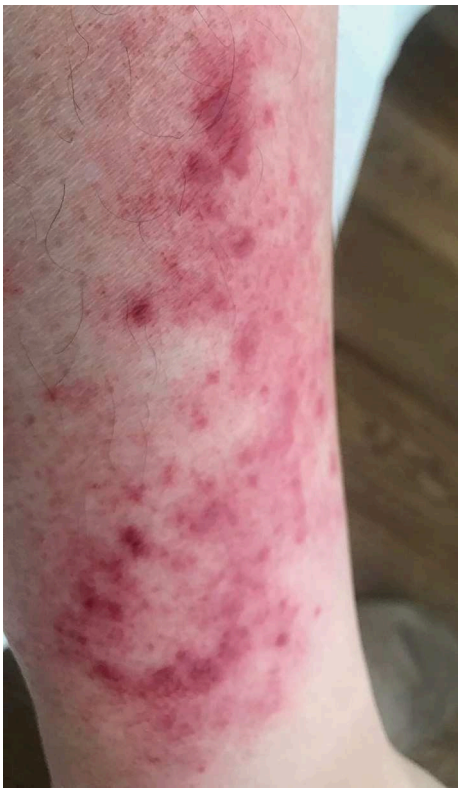

FIGURE 2 | Erysipeloid lesion in a leg of a patient with FMF. Written informed consent was obtained from the patient for the publication of this image.

Acute phase reactants, including CRP, ESR, and ferritin, are usually increased during attacks and subside after them. Secondary amyloidosis may occur in 25% of patients, mostly in those untreated (14, 34).

On demand use of non-steroidal anti-inflammatory drugs (NSAID) and glucocorticoids during attacks may improve symptoms in 40% of patients. With regard to anti-TNF agents, etanercept is the only proving efficacy in controlling attacks, since infliximab, and adalimumab have been associated with severe paradoxical reactions. IL-6 blockade with tocilizumab may also be of benefit in some cases. IL-1 inhibition seems to be the treatment of choice in TRAPS patients (36, 37). Anakinra is effective in most cases, administered either continuously or on demand (38), and canakinumab has been recently approved by the FDA and the EMA as first line therapy (28).

Dermatologic manifestations

About 80% of patients present with skin lesions. The most frequent is the painful erythema that consists of a migratory, centrifugal, erythematous, tender, non-purpuric, and well-demarcated plaque overlying migratory myalgia. The differential

diagnostic of these erythematous lesions comprises cellulitis plaque or panniculitis of the limbs (14–16). Other manifestations include (16) urticaria-like plaques, generalized serpiginous plaques, and small-sized vessel vasculitis (16, 32, 39, 40).

Cutaneous histopathology

Histological specimens of TRAPS are characterized by a mild to massive perivascular and interstitial lymphocytic and monocytic infiltrate (CD3+, CD4+, CD8+, CD68+, CD79a–, and CD20–) in edematous areas of the superficial and deep dermis, with no evidence of multinucleated macrophages nor granulomatous or leukocytoclastic vasculitis. Direct immunofluorescence reveals deposits of IgM and C3 at the dermal-epidermal junction or diffuse interstitial deposits of IgA, G, and C3. Perivascular C3 and C4 deposition in the dermis is also described (14, 16, 34, 39, 41).

Hyper-IgD Syndrome (HIDS)

HIDS and mevalonic aciduria (MA) represent parts of the spectrum of the mevalonate kinase (MVK) deficiency (MKD) (2, 42). Both diseases are inherited with an autosomal recessive pattern and caused by mutations in the *MVK* gene, which encodes MVK, an enzyme involved in the synthesis of non-steroidal isoprenoids and also in the caspase activation pathway (14, 43–45). The amount of residual enzymatic activity correlates inversely with phenotype severity. V377I and I268T are the most frequent pathogenic mutations. Most HIDS patients are heterozygous for two different variants. The presence of homozygous I268T mutations is associated with MA, the most severe phenotype (42).

MA has a neonatal onset with repeated attacks of fever accompanied with severe ocular and neurologic involvement, musculoskeletal abnormalities associated with growth retardation and dysmorphic features, hepatosplenomegaly, lymphadenopathy, and cutaneous lesions (46). HIDS is clinically characterized by an early onset of monthly or bimonthly recurrent febrile attacks lasting from 3 to 7 days. Other typical features include cervical or generalized lymphadenopathies, prominent oral aphthae, arthralgia or non-erosive arthritis of large joints, abdominal pain, and hepatosplenomegaly. Attacks of systemic and cutaneous symptoms are occasionally triggered by infections, vaccines, or trauma (47).

Acute phase reactants, IgD and IgA levels are usually elevated during attacks. An increase of urinary mevalonic acid levels during attacks is considered a specific marker for MKD. Secondary amyloidosis has been found in about 3% of patients (47).

Glucocorticoids at high doses are useful to control attacks in some patients, but most of them will require biologic therapy to avoid glucocorticoid adverse events. Among biologics, etanercept may improve symptoms in more than 50% of patients. However, IL-1 blockers are effective in the majority of cases (37). Anakinra has been proved to be useful in continuous or on demand administration (48) and canakinumab has been recently approved by the FDA and the EMA for HIDS treatment (28). Tocilizumab has been reported effective in some cases refractory to previous treatments (49).

Dermatologic manifestations

Skin involvement occurs in about 70% of MKD patients (47). Cutaneous lesions are heterogeneous and typically consist of non-specific maculopapular or morbilliform rashes. Small erythematous macules, papules, nodules, or cellulitis-like plaques are also frequent. Erythema nodosum and urticarial lesions have also been described, as well as petechiae or purpura resembling IgA vasculitis, erythema elevatum diutinum, and Sweet's syndrome (16, 31, 32, 50). Bipolar aphthae are present in almost 50% of patients (47).

Cutaneous histopathology

MKD cutaneous lesions are histologically variable. Endothelial swelling and perivascular inflammatory infiltrate are the main changes in a skin biopsy. In addition, signs of leukocytoclastic or necrotizing vasculitis, Sweet-like lesions, erythema elevatum diutinum, or erythema nodosum may also be observed. Direct immunofluorescence may show perivascular and linear deposits of IgD and C3 along the basal membrane (14, 51).

Otulipenia

Otulipenia, also known as OTULIN-related autoinflammatory syndrome (ORAS), is an autosomal recessive autoinflammatory disease due to mutations in the *FAM105B* gene, which encodes OTULIN, a Met-1 specific deubiquitinase that acts as a negative regulator of the NF-κB signaling pathway (10).

Clinically these patients present with an early-onset of prolonged recurrent episodes of fever, erythematous skin rash with nodules, arthralgia, abdominal pain, diarrhea, lymphadenopathy, and elevated acute phase reactants (10).

Treatment with TNF inhibitors is very effective in controlling disease activity (10).

Dermatologic manifestations

A painful erythematous rash with skin nodules is the most frequent cutaneous manifestation. Other features include pustular rash, lipoatrophy, and panniculitis (10, 52).

Cutaneous histopathology

Skin biopsies show different types of panniculitis and neutrophilic dermatosis. Small and medium-sized vessel vasculitis have also been reported (10, 52).

HOIL-1 Deficiency

HOIL-1 deficiency is an autosomal recessive disease caused by mutations in the *HOIL1* gene, which encodes HOIL1, a component of the linear ubiquitination chain assembly complex (LUBAC). These mutations result in destabilization of LUBAC complex with an impairment of the IL-1β dependent NF-κB activation in fibroblasts. However, myeloid cells, in particular monocytes, are hyperreactive to IL-1β. Therefore, the consequences of human HOIL-1 and LUBAC deficiencies for IL-1β responses differ between cell types (10).

HOIL-1 deficiency is clinically characterized by an early-onset of recurrent episodes of fever with gastrointestinal symptoms, such as abdominal pain, vomiting, and diarrhea with blood and mucus, and also lymphadenopathy, respiratory distress, failure to thrive, and muscular amylopectinosis (storage of abnormal

glycogen that leads to intracellular glycogen inclusions), which is complicated by myopathy and cardiomyopathy. Recurrent bacterial infections secondary to immunodeficiency features, including hyper-IgA syndrome and memory B-cell defects with antibody production deficiency and impaired response to vaccines have been reported. Inflammatory symptoms are accompanied by elevated acute phase reactants during flares (10, 53).

Dermatologic manifestations

Eczematous lesions, erythroderma, and exfoliative dermatitis occurred in different patients with HOIL-1 deficiency. Vaccination-induced subcutaneous inflammatory lesions have also been described (10, 53).

Cutaneous histopathology

No data regarding HOIL1 deficiency and cutaneous histology is available.

Urticarial Rashes

Cryopyrin-Associated Periodic Syndromes (CAPS)

CAPS or cryopirinopathies comprise three autosomal dominant conditions with different disease severity. The mildest form is familial cold autoinflammatory syndrome (FCAS), the intermediate phenotype is Muckle-Wells syndrome (MWS), and the most severe form is neonatal-onset multisystem inflammatory disease (NOMID), also known as chronic infantile, neurologic, cutaneous and articular (CINCA) (54). All CAPS are caused by mutations in the *NLRP3* gene, which encodes NLRP3 protein or cryopyrin and lead to constitutive activation of NLRP3 inflammasome and IL-1β overproduction. However, more than half of CINCA/NOMID cases are produced by *de novo* mutations. While the presence of pathogenic mutations predicts a more severe phenotype with neurologic complications and sensorineural hearing loss, low penetrance or uncertain significance variants are associated with milder disease phenotypes (11, 54).

Common clinical features to all CAPS forms include an early disease onset with fever or low-grade fever episodes, fatigue, urticarial rash, musculoskeletal symptoms, and ocular involvement as conjunctivitis and uveitis. During attacks, acute phase reactants tend to be elevated (14, 17). In FCAS, attacks are typically triggered by cold exposure and self-limited in <24 h. In MWS, attacks usually last 1–2 days and sensorineural hearing loss and amyloidosis are frequently developed, mostly in undiagnosed or untreated patients. CINCA/NOMID is characterized by a sustained systemic inflammatory response that included persistent fever, diffuse urticarial lesions and severe osteoarticular, ocular and neurologic involvement, usually leading to deforming and irreversible sequelae. Without a prompt directed treatment, CINCA/NOMID becomes a disabling and lethal disease.

Anti-IL-1 agents are considered the treatment of choice for CAPS (39, 55, 56) since anakinra and canakinumab are approved by the FDA and the EMA for CAPS treatment. While IL-1 blockade does not appear to influence established joint and bone damage, its early administration seems to reduce the risk of developing (or improve them when developed) amyloidosis, hearing loss, and neurologic complications (57).

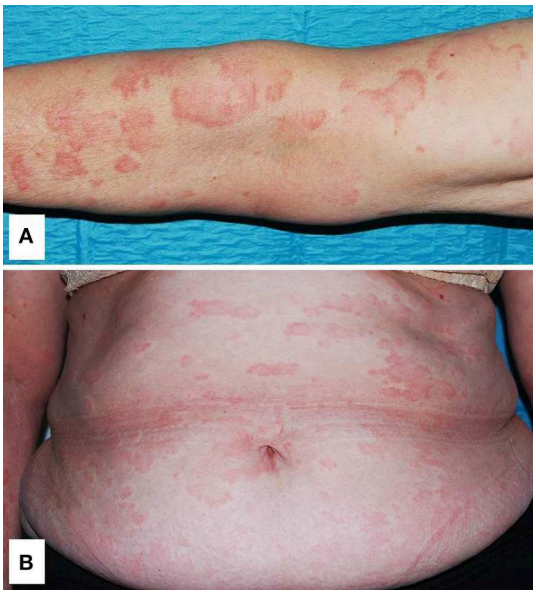

FIGURE 3 | Generalized urticarial rash with erythematous flat wheals without surrounding flare on the left arm **(A)** and trunk **(B)** in a patient with Muckle-Wells syndrome. Written informed consent was obtained from the patient for the publication of this image.

Dermatologic manifestations

A non-pruritic, somewhat symmetrical and evanescent urticarial rash involving the trunk and extremities, usually sparing the head, is the most frequent cutaneous event in CAPS (**Figure 3**) (20, 31, 58). As for CAPS, in other monogenic autoinflammatory diseases with urticarial lesions, hives are usually more flattened, painful or burning, and last longer than those of chronic spontaneous urticaria. In addition, they may also appear as erythematous patches or even solid lesions. Angioedema is not usually present. Although FCAS attacks are usually triggered by cold exposure, contact with cold objects does not cause a disease attack, and therefore, the ice cube test is negative (20).

Cutaneous histopathology

Neutrophilic urticarial dermatosis is the clinicopathological term used to describe dermatologic and histological findings in CAPS, which are different from those observed in ordinary neutrophilic urticaria. CAPS skin biopsies usually show no edema or mild dermal edema of the papillary dermis with a perivascular and neutrophilic infiltrates with limited leukocytoclasia (32) (**Figure 4**). The presence of neutrophilic epitheliotropism (neutrophils around or within eccrine glands or ducts, or inside the epidermis) is rather characteristic although it can be seen in other entities (**Figure 5**) (59). Interstitial neutrophilic infiltrates have also been described (14, 60, 61).

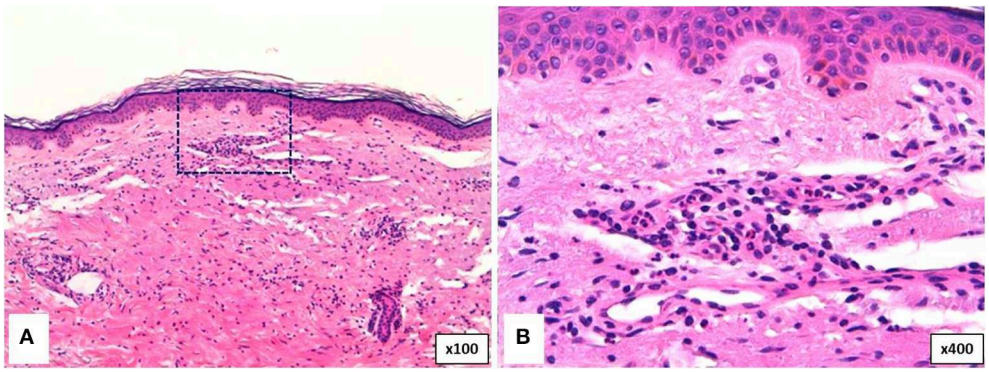

FIGURE 4 | Histopathology of a wheal from a patient with Muckle-Wells syndrome (MWS). **(A)** Dermal interstitial and perivascular infiltrates composed of lymphocytes and neutrophils consistent with neutrophilic urticaria. **(B)** Perivascular infiltrate in detail.

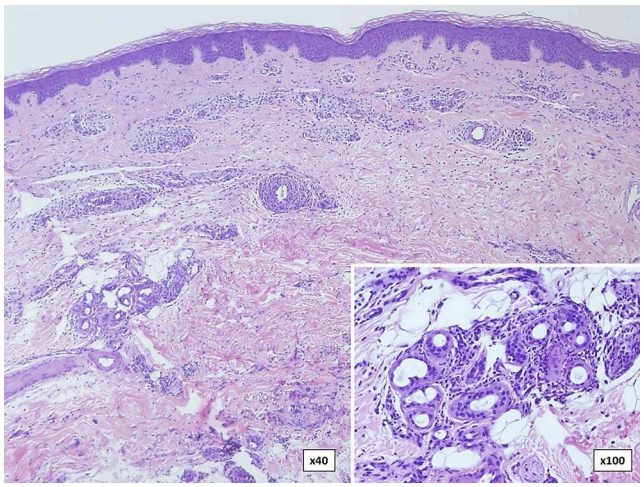

FIGURE 5 | Skin biopsy in a patient with familial cold autoinflammatory syndrome (FCAS) due to a somatic mutation in the *NLRP3* gene. There are dermal neutrophilic infiltrates between the collagen bundles and around blood vessels with presence (inset) of neutrophils around and within eccrine glands.

NLRP12-Associated Autoinflammatory Disease (NLRP12-AD)

NLRP12-AD, also known as FCAS2, is an autosomal dominant autoinflammatory disease caused by mutations in the *NLRP12* gene, which encodes Monarch-1 (31) and play a role in the activation of NF-κB and caspase 1 signaling pathways (62).

Similarly, to FCAS, patients with NLRP12-AD present with recurrent episodes of high fever triggered by cold exposure, lasting for 2–10 days, every 3–4 weeks. Fever is commonly accompanied by arthralgia, myalgia, abdominal pain, headache, lymphadenopathy, oral aphthae, and skin rash. Sensorineural hearing loss is the most common long-term complication. Acute phase reactants are elevated during attacks (63, 64).

Glucocorticoids, antihistamines, and NSAIDs may be useful in mild cases. Severe cases seem to respond to anakinra and also to anti-IL-6 and anti-TNFα agents (64, 65).

Dermatologic manifestations

Cold exposure usually induces the attack and cutaneous manifestations consisting of an evanescent urticarial rash involving the trunk, extremities and face. An erythematous malar rash (64) and cutis laxa (66) have also been described to occur. Contrary to FCAS, FCAS2 rashes tend to be itchy. The ice cube test is consistently negative (63, 64).

Cutaneous histopathology

No data about NLRP12-AD cutaneous histology is available. However, histopathological findings are expected to be similar to those described in CAPS.

PLCγ2-Associated Antibody Deficiency and Immune Dysregulation (PLAID)

PLAID, also known as FCAS3, is an autosomal dominant autoinflammatory disease due to mutations in the *PLCγ 2* gene, encoding phospholipase Cγ2 (PLCγ2), a transmembrane signaling enzyme with phospholipase activity. Cellular dysregulation is produced by a signaling reduction on pathways depending of PLCγ2, which are enhanced at low temperatures. B cells, NK cells, and mast cells are involved in the inflammatory dysregulation (67). *De novo* mutations have been also reported (68).

Clinical manifestations include an early onset of recurrent cutaneous lesions triggered by cold exposure and immunological abnormalities, such as the presence of antinuclear antibodies (ANA), immunoglobulin deficiencies (mostly IgM and IgA), elevated IgE levels, and decreased amounts of switched memory B-cells resembling a primary immunodeficiency, which leads to an increased susceptibility to infections (68).

Avoiding cold temperatures is the main preventive therapy. Depending on the history of repeated infections, intravenous immunoglobulins and prophylactic antibiotics can be used (69). Directed therapies with PLCγ2 inhibitors are not available yet (70).

Dermatologic manifestations

The main cutaneous manifestation of PLAID is a recurrent itchy cold-induced evaporative urticaria, since it appears in

cold-sensitive regions of the body after generalized exposure to cold air or evaporative cooling, but not after contact with cold objects. Lesions subside with an increase in temperature (69, 70). Other less common features include a neonatal ulceration of the nasal tip, which may show spontaneous regression or have a progressive and destructive course, and small papules and erosions on the fingers and toes that tend to resolve without sequelae. Granulomatous-like inflammatory lesions, usually presenting as red-brown, indurated and scaly plaques and nodules of the skin sparing warm regions, such as flexural surfaces and skinfolds (69), and infantile epidermolysis-bullosa-like eruption, initially generalized and later evolving to recurrent erythematous plaques and vesiculopustular photosensitive lesions (71) have also been reported.

Cutaneous histopathology

Urticarial lesions show an increased number of perivascular and interstitial mast cells, which appear degranulated after cold exposure (72). Biopsies of granulomatous lesions reveal well-delineated, non-necrotizing, non-caseating, or sarcoid-type granulomas, but also diffuse, poorly-defined granulomatous inflammation, particularly in the superficial dermis. Granulomatous infiltrates are composed by nodular foci of CD68+ epithelial histiocytes and multinucleated giant cells surrounded by a mild CD4/CD8+ lymphocytic infiltrate and scattered eosinophils. Perineural and lymph nodes granulomatous inflammation may also be observed (69).

NLRC4-Associated Autoinflammatory Diseases (NLRC4-AD)

NLRC4-associated macrophage activation syndrome (NLRC4-MAS) and familial cold autoinflammatory syndrome 4 (FCAS4) are part of NLRC4-AD (73). Both phenotypes are autosomal dominant diseases caused by mutations in the *NLRC4* gene, encoding NLRC4, which lead to a constitutive NLRC4 inflammasome activation resulting in an increased secretion of IL-1β and IL-18. IL-18 is found at extremely high levels in patients with NLRC4-MAS and may persist elevated, even in the absence of clinical activity (74, 75).

The most severe clinical phenotype (NLRC4-MAS) is dominated by a multisystemic inflammation starting in the first year of life with symptoms of chronic inflammatory bowel disease, MAS, or symptoms resembling CINCA/NOMID. Enterocolitis tends to subside over time (74). The mildest phenotype (FCAS4) usually starts at age of three with attacks after exposure to cold stimuli of urticaria, arthralgia, ocular inflammation, and fever in half of cases, in absence of visceral involvement. Although CRP levels are elevated, in severe cases, ESR values tend to decrease as the disease progresses.

Glucocorticoids and anakinra may be useful in most mild cases (76). IL-18 inhibitors and anti-interferon-gamma inhibitors have shown good response in severe cases (73, 75).

Dermatologic manifestations

Skin manifestations range from an unspecific rash to cold urticaria, evanescent urticarial, or linear erythematous lesions (74). While children commonly present with urticarial rash alone, in adult patients, urticarial lesions, and painful erythematous nodules on lower extremities are the most frequent signs (77, 78).

Cutaneous histopathology

NLRC4-AD histopathological findings are scarce. Nodular lesions show deep dermal and subcutaneous lymphohistiocytic infiltrates with septal and lobular panniculitis. Perivascular lymphocytic infiltrates without vasculitic changes have also been described. Direct immunofluorescence has not detected IL-1β staining (77).

Vibratory Urticaria

Vibratory urticaria is an autosomal dominant autoinflammatory disease caused by mutations in the *ADGRE2* gene, which encodes ADGRE2, a member of the epidermal growth factor seven transmembrane that acts as a cell surface receptor with two subunits, the extracellular α subunit and the transmembrane β subunit. It is predominantly expressed in leukocytes, especially in neutrophils and macrophages, but also in mast cells. The endogenous ligand of ADGRE2 is dermatan sulfate, which is the predominant glycosaminoglycan of the skin. The mutated ADGRE2 receptor undergoes autocatalytic cleavage, producing an extracellular subunit that non-covalently binds a transmembrane subunit with destabilization of the autoinhibitory subunit interaction and sensitization of mast cells to IgE-independent vibration-induced degranulation. Therefore, transitory high histamine serum levels seem to be responsible for the clinical manifestations in these patients (79).

Localized pruritic hives after repetitive vibratory or friction stimuli are the principal manifestations of the disease. Occasionally, cutaneous lesions may be accompanied by systemic symptoms (79).

Dermatologic manifestations

Skin lesions consist of localized pruritic hives, angioedema, erythema, and pruritus caused by repetitive physical stimulation. Cutaneous changes may appear from a few minutes to an hour after the vibratory stimulus. In prolonged or intense mechanical expositions, urticarial lesions may be associated with a more severe angioedema or systemic symptoms, such as headache, fatigue, facial flushing, and metallic taste. While dermographism is not present in patients with vibratory urticaria, urticarial rash can be provoked by stimulating the forearm with a laboratory vortex (79).

Cutaneous histopathology

Skin biopsies of vibration-induced lesions show a significant release of mast cell granular content in cases samples compared to controls (79).

Pustular, Pyogenic, or Neutrophilic Dermatosis-Like Rashes

Pyogenic Arthritis, Pyoderma Gangrenosum, and Acne Syndrome (PAPA)

PAPA is an autosomal dominant autoinflammatory disease caused by mutations in the *PSTPIP1* gene, encoding

TABLE 3 | Characteristics of the pyoderma gangrenosum-associated autoinflammatory syndromes.

	PASH syndrome (86–89)	PAPASH syndrome (83, 90)	PsAPASH syndrome (91)	PASS syndrome (92)
Complete name/Clinical manifestations	Pyoderma gangrenosum, acne, and hidradenitis suppurativa	Pyogenic/ psoriasis arthritis, pyoderma gangrenosum, acne, hidradenitis suppurativa	Psoriatic arthritis, pyoderma gangrenosum, acne, hidradenitis suppurativa	Pyoderma gangrenosum, acne, hidradenitis suppurativa, seropositive spondyloarthropathy
Year of description	2012	2013	2015	2012
Mutated genes	*NCSTN*, *PSMB8*, *NOD2*, *MEFV*, *IL1RN*, *NLRP3*, *PSTIP1*	*PSTPIP1* (E277D)	Unknown	Unknown
Treatment reported	Dapsone, cyclosporine, IL-1 blockers, infliximab, adalimumab	Glucocorticoids, cyclosporine, anakinra, adalimumab, infliximab, secukinumab	Glucocorticoids, cyclosporine, anakinra, adalimumab, infliximab	Infliximab

CD2BP1 or PSTPIP1 (80). Although the pathogenesis is not completely understood, PSTPIP1 seems to play a role in inflammasome activation and overproduction of IL-1β and IL-18 (81).

Clinical manifestations start at pediatric age with recurrent flares of erosive, sterile, and deforming arthritis of the elbows, ankles, and knees, leading to early joint destruction. Skin ulcers and severe acne occur during adolescence. Fever is rare and frequency of flares tends to decrease with age. Increased acute phase reactants and leukocytosis are observed during attacks (82).

Glucocorticoids, IL-1 blockers, and anti-TNF agents may be useful in treating arthritis and pyoderma gangrenosum (83).

After PAPA description in 1997 by Lindor et al. (84), other pyoderma gangrenosum-associated syndromes (85) with autoinflammatory background and a late-onset have been described. Those include PASH (86–89), PAPASH (83, 90), PsAPASH (91), and PASS (92). These PAPA-like syndromes are summarized in **Table 3**.

Dermatologic manifestations

Skin involvement includes pyoderma gangrenosum and severe cystic acne, which gets worse with puberty. Pyoderma gangrenosum may occur spontaneously or be triggered by trauma (pathergy) and starts as a violaceous tender papule, nodule or a sterile pustule that rapidly expands with necrosis of the surrounding tissue, and finally results in a poor-healing and painful ulcer with undermined borders (**Figure 6**). Granulation tissue, necrosis or purulent discharge is common in the middle of the ulcer. Cribriform scarring is a hallmark of the disease and may help with the diagnostic (93). Psoriasiform lesions and rosacea-like eruptions have also been reported (94).

Cutaneous histopathology

The typical histological feature consists of central sterile neutrophilic infiltrates in the dermis that becomes with mixed cellularity in the peripheral areas (**Figure 7**) (14, 93).

Deficiency of IL-1 Receptor Antagonist (DIRA)

DIRA is an autosomal recessive autoinflammatory disease caused by mutations in the *IL1RN* gene, encoding IL-1 receptor antagonist (IL-1RA) (95). This mutations lead to the absence of IL-1RA and produce an overactivity of IL-1 (57).

DIRA is clinically characterized by a neonatal-onset of chronic-recurrent flares with cutaneous pustulosis, joint swelling, and bone pain due to painful multifocal aseptic osteomyelitis, long bone periostitis, epiphyseal overgrowth, and secondary skeletal malformations. Interstitial lung disease, vasculitis of the central nervous system, thrombosis, and respiratory distress are much less frequent manifestations (96, 97). Although fever is usually absent, acute phase reactants are constantly elevated during attacks. If untreated, the disease tends to evolve to multiorgan failure with a high mortality rate (57, 96).

Anakinra at doses of 1–5 mg/kg/day remains the treatment of choice for DIRA since it produces a fast and complete clinical and biological resolution in the majority of patients (98).

Dermatologic manifestations

Newborn children present with localized or generalized erythematous plaques and overlying sterile pustules sparing palms and soles. These plaques may evolve to diffuse desquamation resembling ichthyosiform lesions. Nail changes with pitting and onychomadesis, similar to those experiencing in psoriasis, are frequent. Oral lesions such as ulcers and vesicular stomatitis may also occur (97).

Cutaneous histopathology

Histological findings resemble those of pustular psoriasis and skin biopsies show acanthosis and hyperkeratosis of the epidermis with extensive epidermal and dermal neutrophilic infiltrates developing pustules around hair shafts. Vasculitis in subcutaneous tissue adjacent to the bone have also been described (96, 99).

Deficiency of IL-36 Receptor Antagonist (DITRA)

DITRA is an autosomal recessive autoinflammatory disease caused by mutations in the *IL36RN* gene, which encodes IL-36

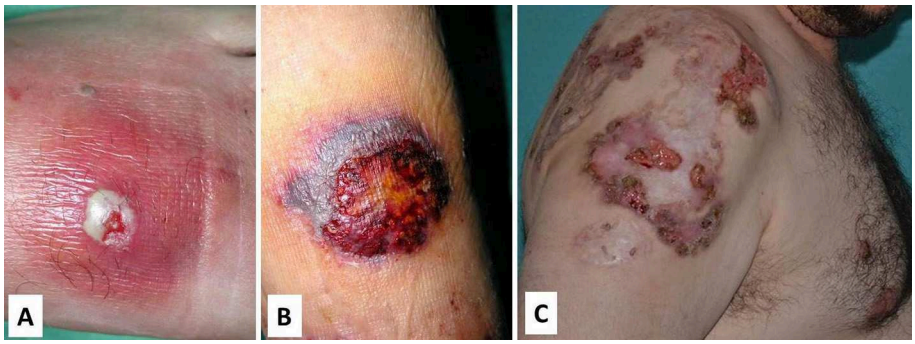

FIGURE 6 | Different stages of pyoderma gangrenosum in PAPA. **(A)** Initial lesion with an erythematous and tender plaque with a central sterile pustule; **(B)** Ulceration with necrotic borders; and **(C)** Poor-healing and painful ulcer with undermined borders and cribriform scarring. Written informed consent was obtained from the patients for the publication of these images.

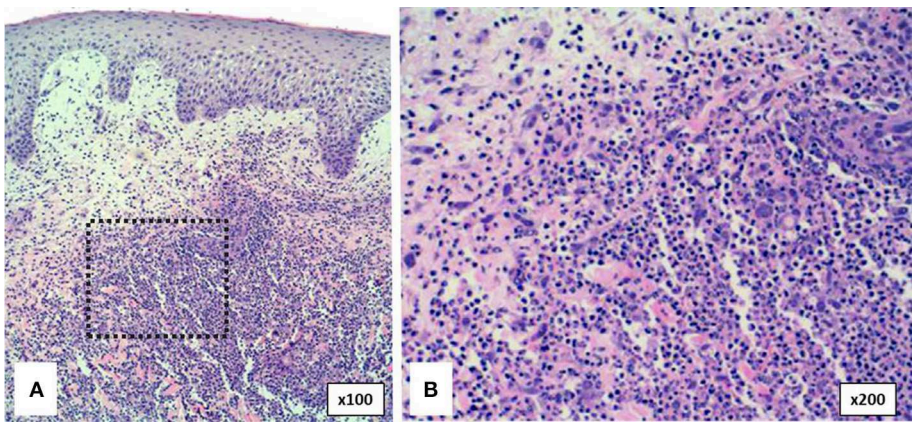

FIGURE 7 | Histopathology of pyoderma gangrenosum in a PAPA patient. **(A)** Dense neutrophilic infiltrate with upper dermis edema. **(B)** Neutrophilic infiltrate in detail.

receptor antagonist (IL36Ra) (100). These mutations are involved in NF-κB activation and overproduction of proinflammatory cytokines such as IL-36 and IL-8 (100, 101). In recent years, late-onset cases have been described in patients carrying heterozygous mutations (101).

DITRA is clinically included in generalized pustular psoriasis. These patients may have a pediatric and adult onset consisting of irregular episodes of high-grade fever, generalized pustulosis, and asthenia, with elevated acute phase reactants and leukocytosis. Attacks have been reported to be triggered by infections, pregnancy, and menstruation (100). Several authors have suggested that patients with early-onset generalized pustular psoriasis without concomitant psoriasis vulgaris are often diagnosed with DITRA (102).

DITRA is currently included in the group of autoinflammatory keratinization diseases (AIKD), a term first used in 2017 to cluster those disorders characterized by keratinized lesions caused by an autoinflammatory mechanism (103).

Conventional topical and systemic therapies used for psoriasis may also be useful in DITRA patients (16). Anakinra (anti-IL-1), adalimumab, infliximab (anti-TNFα), ustekinumab (anti-IL-12/23), and secukinumab (anti-IL-17) have shown efficacy in isolated cases (104–106). Recently, a phase 1 clinical trial in patients with generalized pustular psoriasis treated with a single intravenous dose of a monoclonal antibody against the IL-36 receptor has shown promising results by reducing the severity of the disease over a 20-week period, regardless of the presence of the *IL36RN* mutation (107).

Dermatologic manifestations

Cutaneous lesions resemble those of generalized pustular psoriasis and consist of a diffuse erythematous skin eruption that tends to be rapidly covered by pustules with subsequent desquamation (**Figure 8**). Skin eruptions may mimic all forms of psoriasis ranging from psoriasis vulgaris to acrodermatitis continua (100, 108).

Cutaneous histopathology

Histological features are indistinguishable from classical pustular psoriasis and include epidermal hyperplasia with acanthosis, irregular papillomatosis, subcorneal spongiform pustules, compact orthokeratosis, or parakeratosis and neutrophilic infiltration (**Figure 9**). Immunohistochemistry of the dermis

shows superficial perivascular infiltrates of CD8 and CD3 T cells, macrophages and neutrophils (100, 108).

CARD-14 Mediated Psoriasis (CAMPS)

CAMPS is an autosomal dominant inherited disease due to mutations in the *CARD14* gene encoding CARD14. Such mutations produce an overactivation of NF-κB pathway (109). Keratinocytes show high levels of CARD14 (110). CAMPS is currently classified as AIKD (103).

Disease presentation may vary among monogenic psoriasis, pustular psoriasis, psoriatic arthritis, or pityriasis rubra pilaris. Features of systemic inflammation are usually absent. Therapeutic options are mainly the same as those used for treating psoriasis and DITRA (111–113).

Dermatologic manifestations

Clinical manifestations are mostly cutaneous presenting as a plaque and pustular psoriasis. Other diseases, such as pityriasis rubra pilaris or acute generalized exanthematous pustulosis, have also been associated with CARD14 mutations. Disease extension may vary from localized to generalized, as well as severity, which may range from mild to severe (111, 113–115).

Cutaneous histopathology

Skin biopsies show histopathological features of psoriasis or pityriasis rubra pilaris (116).

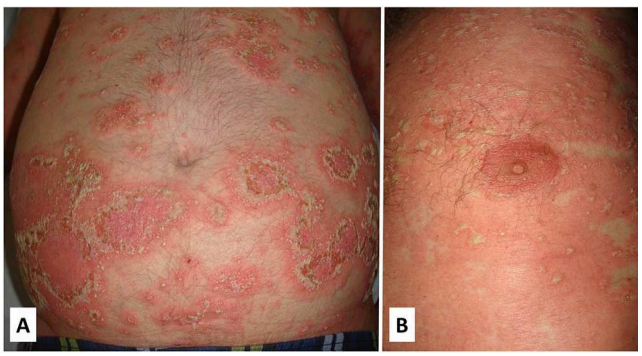

FIGURE 8 | Clinical features of a patient with DITRA with a heterozygous mutation in the *IL36RN* gene. The clinical picture started in adulthood with flares of diffuse erythematous plaques covered with pustules that often involved the whole body **(A,B)**. The episodes were often triggered by bacterial infections. Written informed consent was obtained from the patient for the publication of this image.

Majeed Syndrome

Majeed syndrome is an autosomal recessive autoinflammatory disease caused by mutations in the *LPIN2* gene, which encodes phosphatase lpin2 (117). Mutated LPIN-2 induces NLRP3 activation with the consequent IL-1β overproduction (118).

The clinical triad is characterized by the early onset of chronic recurrent multifocal sterile osteomyelitis (CRMO), congenital dyserythropoietic anemia and neutrophilic skin lesions (119). Other manifestations during attacks include fever and swelling of large joints. Growth retardation and permanent flexion contractures are long-term complications in untreated patients (120). Abnormal laboratory tests include raised acute phase reactants levels, anemia, and variable leukocytosis.

Treatment with NSAIDs and glucocorticoids may be useful in controlling CRMO-related pain. Anti-TNF agents, bisphosphonates, and interferon gamma show variable success rates. IL-1 blockers have been useful in controlling inflammatory manifestations (121–123).

Dermatologic manifestations

Inflammatory dermatoses are the most frequent cutaneous symptoms, and may occur as neutrophilic dermatoses and erythematous and scaly plaques. The prototypic findings are Sweet syndrome-like lesions, seen as erythematous plaques, pseudovesiculous or target lesions (120, 124). CRMO has also been associated with generalized pustulosis, palmoplantar psoriasis, pyoderma gangrenosum, acne, recurrent subcutaneous abscesses, and SAPHO syndrome (31, 120, 125–128).

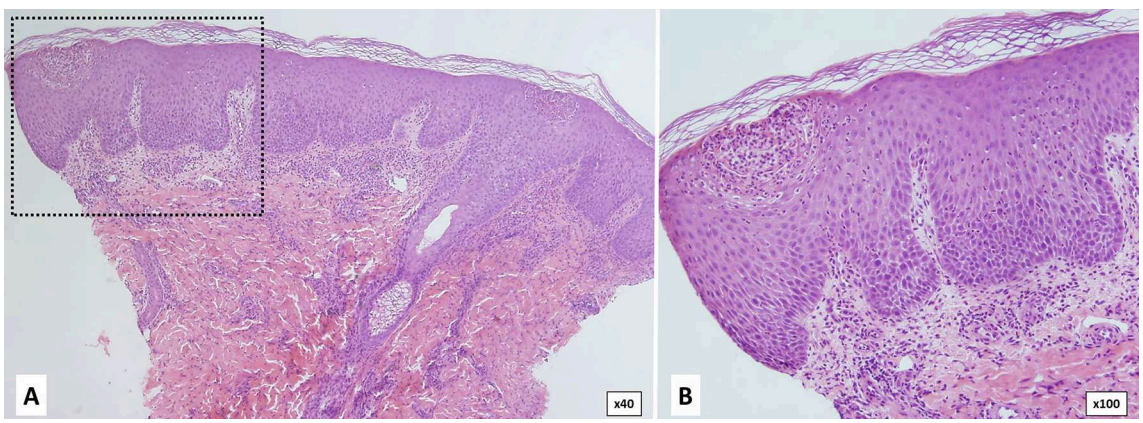

FIGURE 9 | Histology of DITRA shows epidermal acantosis with edema and dilated blood vessels in the papillary dermis. There is epidermal spongiosis with the presence of neutrophils migrating through the epidermis **(A,B)**. In the most superficial part of the epidermis (inset-**B**) there is a subcorneal pustule that is formed through the aggregation of neutrophilic spongiform pustules.

Cutaneous histopathology

Skin histopathology displays edema of the papillary dermis with dense dermic neutrophilic infiltrates. A bone biopsy usually shows subacute and chronic inflammatory changes (129).

Pyrin-Associated Autoinflammation With Neutrophilic Dermatosis (PAAND)

PAAND is an autoinflammatory disease caused by mutations in the *MEFV* gene, the same gene responsible for FMF. However, contrarily to the autosomal recessive but inconstant pattern observed in FMF patients, PAAND has an autosomal dominant inheritance with complete penetrance. PAAND mutations (S242R and E244K) are associated with pyrin inflammasome activation (130).

PAAND has a childhood-onset characterized by recurrent febrile episodes lasting for several weeks accompanied with arthralgia, myalgia and cutaneous inflammatory lesions. During attacks, acute phase reactants and circulating proinflammatory cytokines (IL-1β, IL-6, TNF-α, and IL-1Ra) levels are normally increased (130).

Treatment with IL-1 blockers has shown a rapid control of clinical and laboratory abnormalities. Infliximab and adalimumab have been used with success in anakinra-resistant patients (130).

Dermatologic manifestations

Severe neutrophilic dermatoses in PAAND have a wide spectrum of presentation, including pustular acne, pyoderma gangrenosum, sterile skin abscesses, neutrophilic small vessel vasculitis, severe hidradenitis suppurativa, and neutrophilic panniculitis (32, 130, 131).

Cutaneous histopathology

Histopathology reveals an spared epidermis and dense dermal neutrophilic infiltrates both interstitial and perivascular (131).

Other Novel Psoriasiform Monogenic Autoinflammatory Diseases

Singleton-merten syndrome (SMS)

SMS is an autosomal dominant transmitted disease caused by mutations in *IFIH1* or *DDX58* genes. The resulting proteins (melanoma differentiation associated protein 5 [MDA5] and retinoic-acid-inducible gene I [RIG-I], respectively) are involved in type I interferon induction pathways (132).

Clinical manifestations occur after childbirth and are characterized by dental dysplasia, tendon rupture, osteoporosis, arthropathy, neurologic abnormalities, aortic calcification, and glaucoma. Cutaneous involvement as localized or generalized psoriasis is present in the majority of patients (132).

As in other type I interferonopathies, the use of a Janus kinase (JAK) inhibitor has been useful in a SMS patient (132).

ADAM17 deficiency

ADAM17 deficiency is considered an autoinflammatory disease (6) caused by autosomal recessive mutations in the *ADAM17* gene, encoding TNF-α converting enzyme (TACE), which is necessary for the cleavage and secretion of TNF-α, epidermal growth factor, transforming growth factor alpha (TGF-α), and some desmogleins (6, 133).

Clinical features were described in two consanguineous siblings with neonatal-onset of pustular psoriasis followed by chronic bloody diarrhea and cardiomyopathy. Skin lesions were characterized by perioral and perianal erythema with fissuring and a generalized pustular rash that evolved to psoriasiform erythroderma, with flares of erythema, scaling, and widespread pustules. Cutaneous infections were frequent. Other dermatologic manifestations included hair abnormalities (short or fragile hair and wiry eyelashes and eyebrows), and thickened nails with frequent episodes of paronychia. Dermatopathology revealed infiltrates of T cells in the epidermis. CD3+ T cells were located around the skin follicles and in the epithelium, CD4+ T cells in the perifollicular region and CD8+ T cells in the epithelium at the neck of the follicle. B cells (CD20+), natural killer cells (CD56+), or neutrophils were scarce within the infiltrates (133).

Treatment with acitretin, ciclosporin, methotrexate, and adalimumab has not been useful in patients with ADAM17 deficiency. However, anti-IL1 and anti-IL6 therapy may be potential agents since peripheral-blood mononuclear cells from patients overproduced IL-1β and IL-6 after lipopolysaccharide stimulation (133).

AP1S3 and autoinflammatory psoriasis

Pustular psoriasis may be caused by mutations in the *AP1S3* gene encoding AP1S3, a protein implicated in autophagosome formation, which is elevated in keratinocytes. Its deficiency disrupts keratinocyte autophagy and causes abnormal accumulation of p62, an adaptor protein mediating NF-kB activation, with subsequent up-regulation of IL-1 signaling and overexpression of IL-36. The inheritance pattern is not clear since patients with *de novo* mutations and with a mutated allele from an unaffected parent have been reported. Treatment with IL-36 blockade has demonstrated to reverse skin lesions (134).

Although inflammatory symptoms such as arthritis may be present, pustular psoriasis is the most prominent clinical feature. This may be localized to the palms and soles (palmar plantar pustulosis) or to the toes and fingertips (acrodermatitis continua of Hallopeau), but it may also be generalized (134).

Panniculitis or Subcutaneous Nodules

Blau Syndrome and Early-Onset Sarcoidosis

Blau syndrome and early-onset sarcoidosis are the two forms of pediatric granulomatous arthritis caused by mutations in the *NOD2*/*CARD15* gene encoding NOD2. While Blau syndrome is inherited in an autosomal dominant manner, early-onset sarcoidosis is the spontaneous form, caused by the novo mutations (135).

In both disorders, symptoms onset occurs during the first decade of life with the sequential, but not constant, triad of maculopapular rash, non-erosive arthritis of wrists, hands, elbows and ankles, and uveitis. Other less frequent manifestations include fever, large and small vessel vasculitis, interstitial lung disease, cranial neuropathies, and granulomatous involvement of salivary glands, kidneys, spleen, and liver

(136, 137). Laboratory studies are typically normal, although elevated ESR and angiotensin-converting enzyme levels and hypergammaglobulinemia have been reported (137).

With regard to treatment, high-dose glucocorticoids may be useful for inflammatory symptoms. Limited reports have shown effectiveness with thalidomide, methotrexate, cyclosporine and other conventional immunosuppressants, together with anti-IL-1 and anti-IL-6 agents. However, anti-TNF blockers (infliximab and adalimumab) seem to be the drugs associated with better responses (15, 135–137).

Dermatologic manifestations

Skin involvement is the most prominent and the earliest expression of the disease, which is manifested as an erythematous maculopapular fine scaly rash on the trunk and extremities, resembling an ichthyosiform exanthema. Progressively it becomes tan-colored with lichenoid characteristics and dirty scaly appearance. This later stage tends to last longer (22, 138). Erythema nodosum-like lesions, pityriasis lichenoides, leg ulcers, and leukocytoclastic vasculitis have also been observed (136, 139).

Cutaneous histopathology

Histopathology of the cutaneous lesions shows non-caseating, sarcoid-type granulomas in the subpapilar dermis with a variable number of lymphocytes and eosinophils (138). Biopsies from purpuric lesions display vasculitis, and leg ulcers can show both, granulomatous infiltrates and chronic granulation with mononuclear infiltration in the fat tissue (139).

Chronic Atypical Neutrophilic Dermatitis With Lipodystrophy and Elevated Temperature Syndrome (CANDLE)

CANDLE syndrome, also called proteasome associated autoinflammatory syndrome (PRAAS), is an autosomal recessive autoinflammatory disease caused by mutations in the *PSMB8* gene, which encodes the β5i subunit of the immunoproteasome (5, 11). *PSMB9*, *PSMA3*, *PSMB4,* and *POMP* are other proteasome genes recently identified as also causing CANDLE/PRAAS (140). This condition is considered an interferonopathy since mutant genes cause defective proteasome/immunoproteasome assembly and accumulation of ubiquitinated proteins that induce intracellular stress and increased IFN-1 production through JAK signaling pathway (141, 142).

Classical manifestations include neonatal onset of recurrent or persistent high-fever, cutaneous lesions, and facial and generalized lipodystrophy. Arthralgia, muscle atrophy, hepatosplenomegaly, lymphadenopathy, and inflammatory involvement of other territories, such as ocular, meningeal, epididymis and parotids, are also common (143). Raised acute phase reactants are constant and muscle and hepatic enzymes are frequently elevated. Positive ANA and antineutrophil cytoplasmic antibodies (ANCA) may be present without pathogenic significance (144).

Glucocorticoids, conventional immunosuppressive drugs, and biologic agents, such as anti-TNF, anti-IL-1, or anti-IL-6 have been used without complete response (143, 144). Baricitinib, a JAK inhibitor that prevents the expression of IFN-induced genes and the autoinflammatory loop, has shown efficacy in CANDLE/PRAAS patients (145).

Dermatologic manifestations

Perinatal-onset fever attacks are accompanied by annular erythemato-violaceous edematous plaques on trunk and extremities, and stable violaceous erythemas on the perioral and periorbital areas. Most of these lesions resolve within few days or weeks leaving purpuric pigmentation, but recurrences are common. Other less frequent manifestations include violaceous nodules, hirsutism, and acanthosis nigricans. The development of progressive lipoatrophy of the face, extremities, and trunk occurs in the late phase of the disease (22, 143, 144).

Cutaneous histopathology

Histopathology of cutaneous lesions is characterized by dense interstitial and perivascular atypical-looking (because of the presence of mitotic figures) mononuclear infiltrates with karyorrhexis in the deep dermis and fat tissue. Neutrophils and eosinophils may also be observed within the infiltrates. Immunohistochemistry shows strong and diffuse positivity for myeloperoxidase, lysozyme, CD68, and CD45, which confirms the myeloid lineage of the infiltrate by revealing the presence of macrophages and histiocytes. T cells and B cells, identified by positivity for CD3, CD45RO, and CD20, are also present to a lesser extent (143, 144).

Vasculitis or Vasculopathy

Deficiency of Adenosine Deaminase 2 (DADA2)

DADA2 is an autosomal recessive autoinflammatory disease caused by mutations in the *CECR1* gene, encoding ADA2 (146, 147). ADA2 acts as a growth factor in the myeloid lineage promoting differentiation into anti-inflammatory macrophages, and has also a role in the development and maintenance of endothelial cells. Mutant ADA2 promotes vascular damage by affecting endothelial cells and inducing neutrophil-driven cell damage (146, 147).

DADA2 patients commonly exhibit persistent or recurrent fever, skin lesions (mostly livedo reticularis or racemosa and subcutaneous nodules), peripheral neuropathy and vascular lesions secondary to distal ischemia or hemorrhage of the affected territories, especially involving the brain. Disease phenotype is frequently indistinguishable from polyarteritis nodosa. Oral aphthae, arthralgia and hepatosplenomegaly are also frequent. Acute phase reactants are increased during attacks and the presence of variable peripheral blood cytopenias and low immunoglobulin levels contribute to develop a certain degree of immunodeficiency. Although disease typically occurs in early childhood, later-onset cases have also been described (146, 147).

Although high-dose glucocorticoids can be effective in some patients, low-dose glucocorticoids, conventional immunosuppressive drugs, anti-CD20 therapy and anti-IL-1 and anti-IL6 blockers do not seem to provide a clear benefit. However, anti-TNF agents, in particular etanercept, have demonstrated to control systemic inflammatory manifestations and progression

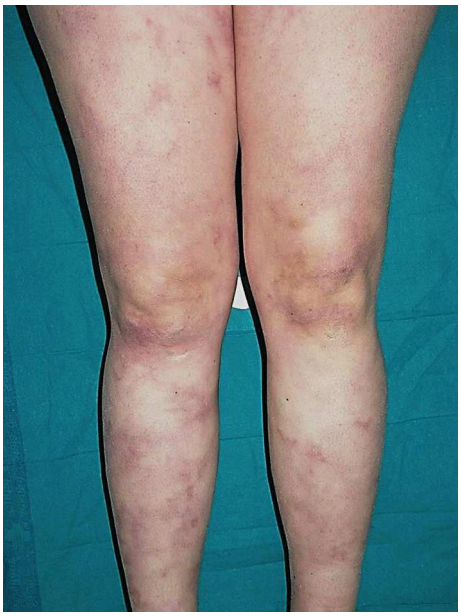

FIGURE 10 | Livedo racemosa on the lower limbs in a patient with DADA2. Written informed consent was obtained from the patient for the publication of this image.

of vascular disease, in the absence of normalization of ADA2 enzyme activity. To date, allogeneic hematopoietic stem cell transplantation is the only therapy that has demonstrated to cure the disease (146–148).

Dermatologic manifestations

The most frequent cutaneous lesions are livedo reticularis or racemosa (**Figure 10**) and subcutaneous nodules. Pediatric and adult presentations may have a phenotype resembling cutaneous arteritis or polyarteritis nodosa refractory to conventional immunosuppressive therapy. Raynaud syndrome, digital necrosis, ulcers, and erythema nodosum may also occur (146, 147, 149).

Cutaneous histopathology

Skin biopsies are characterized by dermal interstitial neutrophilic infiltrates which stain positive for myeloperoxidase and CD68 confirming the existence of macrophages and a perivascular lymphocytic infiltrate. Livedo and nodular lesions may display non-granulomatous necrotizing medium-sized vessels vasculitis. However, leukocytoclastic vasculitis or panniculitis have also been reported (32, 146, 147, 149).

STING-Associated Vasculopathy With Onset in Infancy (SAVI)

SAVI is an autosomal dominant autoinflammatory disease caused by mutations in the *TMEM173* gene encoding STING, an indirect sensor of cytosolic DNA that activates IRF3 and induces transcription of IFN-1 related genes. Mutant STING results in overactivation of IRF3 and transcription of IFNβ (150).

SAVI is clinically characterized by a neonatal-onset of recurrent febrile attacks with cutaneous rash, small-vessel vasculitis, and interstitial lung disease. During flares, acute phase reactants are elevated and low-titer autoantibodies, such as ANA, ANCA, and antiphospholipid antibodies, are frequent (150, 151).

As in other interferonopathies, IFN-1 pathway blockade with JAK inhibitors, in particular baricitinib, seems to be effective in SAVI, since glucocorticoids, conventional immunosuppressive, and anti-cytokines agents have not demonstrated efficacy (145).

Dermatologic manifestations

Skin is the initial territory involved in SAVI. Lesions are caused by vasculitic changes with subsequent tissue damage and are manifested as violaceous, scaly and atrophic plaques affecting hands, cold-induced ulcerative distal lesions and erythemato-violaceous nodules on the cheeks, ears and nose, nail dystrophy, distal digital gangrene, and nasal septum perforation. Other cutaneous lesions such as telangiectasia, pustules, blisters, erythematous plaques may also occur, mostly on acral sites (14).

Cutaneous histopathology

Dermatopathology shows medium and small-vessel vasculitis with dense neutrophilic infiltrates and karyorrhexis in the vessel wall, as well as fibrin endovascular microthrombi (32, 151). Biopsies of telangiectatic plaques show perivascular infiltration by lymphocytes and neutrophils with leukocytoclasia, without involvement of the vessel walls (14).

Familial Chilblain Lupus

Familial chilblain lupus or TREX1-associated systemic lupus erythematosus is an autosomal dominant autoinflammatory disease caused by either loss-of-function mutations in *TREX1* and *SAMHD1* genes or gain-of-function mutations in the *TMEM173* gene, both leading to type I IFN overproduction (152–154).

Clinical manifestations consist of early-onset of mucocutaneous lesions and arthralgia, with occasional periodic fever and infrequent increased inflammatory markers. Low-titer autoantibodies, including ANA and anti-C1q-autoantibodies are usually present. Successful treatment with JAK inhibitors has been described (153, 155).

Dermatologic manifestations

Patients present with cold-induced chilblain lesions at acral locations (fingers, toes, nose, and ears) with subsequent ischemia and ulceration of these regions. Nails can show dystrophy or onychomadesis. Nailfold capillaroscopy may appear with irregular capillary loops and tortuous appearance. Livedo reticularis, malar rash, photosensitivity, and oral and nasal ulcers have also been described (152–154).

Cutaneous histopathology

Histological examination of skin reveals perivascular lymphohistiocytic infiltrates along with expression of the type I IFN-induced myxovirus resistance protein A within the endothelial cells (153).

Aicardi-Goutières Syndrome (AGS)

AGS comprise a group of seven monogenic autoinflammatory diseases, most of which are inherited with an autosomal recessive pattern, caused by mutations in several genes encoding proteins involved in intracellular degradation or sensing of nucleic acids. *TREX1*, *RNASEH2A*, *RNASEH2B*, *RNASEH2C*, *SAMHD1*, *ADAR1*, *IFIH1,* and *DDX58* are the genes involved in AGS. Mutations in these genes induce high levels of IFNα, both in blood and cerebrospinal fluid, which are thought to be responsible for systemic and cerebral tissue damage (156). In addition, dyschromatosis symmetrica hereditaria is an autosomal dominant skin disease caused by mutations in the *ADAR1* gene consisting of hyper- and hypo-pigmented macules on the dorsal aspects of the extremities. Patients with homozygous or compound heterozygous *ADAR1* mutations may present with a combination of AGS6 and dyschromatosis symmetrica hereditaria (157).

All forms of AGS share several features in common, such as a neonatal-onset encephalopathy consisting in basal ganglia calcifications, spasticity, dystonia, progressive cerebral atrophy, and microcephaly, as well as fever and hepatosplenomegaly (14). Abnormal laboratory results include lymphocytosis and elevated IFNα levels in cerebrospinal fluid. Patients may develop some autoimmunity features resembling systemic lupus erythematosus, such as arthritis, lymphopenia, thrombocytopenia, and ANA positivity (156).

No traditional treatment options, including glucocorticoids and conventional immunosuppressants and anti-cytokines agents, are useful. As for previous interferonopathies, JAK inhibitors seem also to control AGS activity (145, 156, 158, 159).

Dermatologic manifestations

Skin involvement comprises chilblain lesions on the feet, hands, and ears, digital vasculitis, generalized skin mottling, lipoatrophy, panniculitis, and acral necrotic lesions (14, 156, 160).

Cutaneous histopathology

No data regarding cutaneous histopathology is available in AGS.

Spondyloenchondrodysplasia With Immune Dysregulation (SPENCDI)

SPENCDI is an autosomal recessive autoinflammatory disease caused mutations in the *ACP5* gene encoding tartrate-resistant phosphatase. The lack of activity of this enzyme leads to a constitutive gain-of-function of osteopontin, a multifunctional protein involved in bone remodeling and immune regulation causing autoimmunity through a type I interferon expression signature (161).

SPENCDI is clinically characterized by bone dysplasia with subsequent growth retardation, and neurologic manifestations, such as cerebral atrophy, intracranial calcifications, seizures, and spastic paraparesis. Systemic and organ-specific autoimmune diseases are commonly present. These include systemic lupus erythematosus, antiphospholipid syndrome, Sjögren syndrome, Raynaud's disease, inflammatory myositis, arthritis, vitiligo, hypothyroidism, hemolytic anemia, and thrombocytopenia. Consequently, autoimmune markers are also frequently present, including positive ANA, anti-DNA antibodies, and hypocomplementemia (161).

Glucocorticoids, chloroquine, and other additional immunosuppressive agents, such as cyclophosphamide, azathioprine, mycophenolate mofetil, and rituximab have been used with good results (161).

Dermatologic manifestations

Cutaneous manifestations include severe eczema, Raynaud's phenomenon, distal sclerodermatous/acrocyanotic changes, and leukocytoclastic vasculitis presenting with purpuric lesions. Livedo reticularis and occlusive vasculitis leading to digital auto-amputation have also been described. Capillaroscopy may reveal edema and sludging or disappearance of parallel loops of some dilated capillaries (161).

Cutaneous histopathology

The skin biopsy from a patient with SPENCDI confirmed a non-specific leukocytoclastic vasculitis with perivascular neutrophilic infiltrate, without deposition of complement or immunoglobulin at direct immunofluorescence (161).

Hyperkeratotic Lesions

NLRP-1 Associated Disease (NAIAD)

NAIAD is an autoinflammatory disease inherited with a recessive or dominant pattern due to mutations in the *NLRP1* gene, which encodes NLRP1 protein. NLRP1 is the central inflammasome in the skin. Mutations in PYRIN or LRR domains lead to constitutive NLRP1 inflammasome activation and IL-18 production (138). NAIAD is currently categorized as AIKD (103).

Patients present with infantile-onset attacks of recurrent fever lasting 3–4 days, accompanied by hyperkeratotic lesions, polyarticular arthritis and chronic relapsing infections. Blood tests show high CRP levels during flares, low-titer of ANA, vitamin A deficiency, and raised transitional B cells (162). Treatment with vitamin A and acitretin has been associated with clinical improvement.

Dermatologic manifestations

Most patients show disseminated erythematous follicular hyperkeratosis. Cases of familial keratosis lichenoides chronica (also considered an AIKD), associated with multiple self-healing palmoplantar carcinoma, as well as larynx involvement resembling human papillomavirus infection have been reported in NAIAD patients (162, 163).

Cutaneous histopathology

Skin biopsy shows orthokeratotic hyperkeratosis with papillomatosis, acanthosis and hypergranulosis. Numerous dyskeratotic cells sparse throughout the epidermis, without involving the basal layer, have been observed (162).

Hyperpigmented Lesions

H Syndrome

H syndrome is an autosomal recessive autoinflammatory disease caused by mutations in the *SLC29A3* gene encoding ENT3 (164).

This syndrome is referred to as "H syndrome" to describe some of the disease hallmarks: hyperpigmentation, hypertrichosis, hepatosplenomegaly, heart anomalies, hearing loss, and hypogonadism. Therefore, the disease is clinically characterized by progressive sclerotic skin lesions, cardiac anomalies, short stature, and childhood-onset sensorineural hearing loss. Other manifestations include scrotal masses, azoospermia, hepatosplenomegaly, micropenis, dilated scleral vessels, exophthalmos, facial telangiectasia, and camptodactyly (164, 165). Laboratory tests reveal high ESR values and growth hormone deficiency (164).

Most treatments, including glucocorticoids, colchicine, cytotoxic immunosuppressants, IFNα, anakinra, canakinumab, adalimumab, and radiotherapy, are associated with an inadequate response (164).

Dermatologic manifestations

Cutaneous involvement starts with progressive sclerotic, hyperpigmented plaques on the lower limbs with hypertrichosis, and less frequently with ichthyotic desquamation. These lesions usually appear on the inner aspect of the thighs and progress to the abdomen, genitalia, ankles and feet, with sparing of the knees and buttocks. The presence of plaques in axillae and trunk is infrequent (164, 165).

Cutaneous histopathology

Dermatopathology is characterized by an increase of melanocytes with acanthosis in the basal layer and sclerodermiform changes with interstitial macrophagic infiltrates in the dermis and fat tissue. Perivascular infiltrates of lymphocytes, mast cells and plasma cells are also noted. Emperipolesis (cell engulfment phenomena) may be occasionally observed (22, 165).

Bullous Lesions

Autoinflammation and PLCγ2-Associated Antibody Deficiency and Immune Dysregulation (APLAID)

APLAID is an autosomal dominant autoinflammatory disease caused by two missense mutations (S707Y and L848P) in the *PLCγ2* gene. Despite being produced by the same gene that PLAID, mutants in APLAID result in hyperactivation of PLCγ2 signaling pathway and are associated with a different disease phenotype (71, 166).

APLAID patients present with early-onset recurrent attacks of blistering skin lesions, eye inflammation with ocular hypertension, inflammatory bowel disease, arthralgia, and sinopulmonary infections caused by a mild humoral immunodeficiency. Acute phase reactants tend to be normal and switched memory B-cells are almost absent. Contrary to PLAID, APLAID is not characterized by cold-induced urticaria and the presence of circulating autoantibodies (71).

While TNF-blockers have not shown efficacy, high-dose glucocorticoids, and anakinra have been reported to control inflammatory symptoms (71).

Dermatologic manifestations

Early-onset recurrent blistering lesions resembling epidermolysis-bullosa is the most common picture. Later in life, these lesions tend to evolve to recurrent erythematous plaques and vesiculopustular lesions that get worse with sun and heat exposure. Cellulitis-like plaques and granulomata, as well as cutis laxa, have also been described (70, 71, 166). More recently, three cases with different presentation have been reported: a newborn patient with generalized erythematous papules evolving to vesicles, pustules, and crusts involving face, gluteal region, and extremities (167) and two patients of 6 and 14 years-old with a papulovesicular skin rash with granuloma formation and cutis laxa (168).

Cutaneous histopathology

A biopsy from a plaque lesion showed a dense dermal interstitial and perivascular mixed inflammatory infiltrate composed of lymphocytes, histiocytes, eosinophils, and karyorrhectic nuclear debris (71).

Aphthous Lesions

Haploinsufficiency of A20 (HA20)

HA20, also known as monogenic Behçet-like disease, is an autosomal dominant autoinflammatory disease caused by mutations in the *TNFAIP3* gene encoding protein A20. Mutated A20 results in increased NF-κB signaling and NLRP3 hyperactivity (11, 169).

The clinical picture of HA20 is characterized by the triad of orogenital ulcers, ocular inflammation and non-deforming polyarthritis (170). Abdominal pain, pharyngitis, pericarditis, retinal vasculitis, and central nervous system vasculitis are also frequent manifestations (32, 171). Several organ-specific and systemic autoimmune diseases have been associated with HA20. Some of them include Hashimoto thyroiditis, type 1 diabetes mellitus, neutrophilic dermatosis, erythema nodosum, pseudofolliculitis, central nervous system vasculitis, Kawasaki disease, IgA vasculitis, nephrotic syndrome, idiopathic thrombocytopenic purpura, or interstitial lung disease. Acute phase reactants are increased during flares and low-titer autoantibodies may be present in cases with autoimmune diseases (171).

Treatment with colchicine, glucocorticoids, methotrexate and thalidomide may be useful. Refractory cases to previous drugs have been reported to respond to anti-TNFα, anti-IL-1, and anti-IL-6 agents (170, 171).

Dermatologic manifestations

Oral, genital, and gastrointestinal non-scarring ulcers are the most frequent manifestations (**Figure 11**). Skin involvement is characterized by pustular or vesicular rashes, acne, dermal abscesses, mild desquamation, and hyperkeratosis. Pathergy test can be positive in some patients (170, 171).

Cutaneous histopathology

Histological data of the skin is limited. The presence of an epidermal infiltrate of lymphocytes and neutrophils with extensive intradermal mucin accumulation and scarce inflammatory infiltrates has been reported (171).

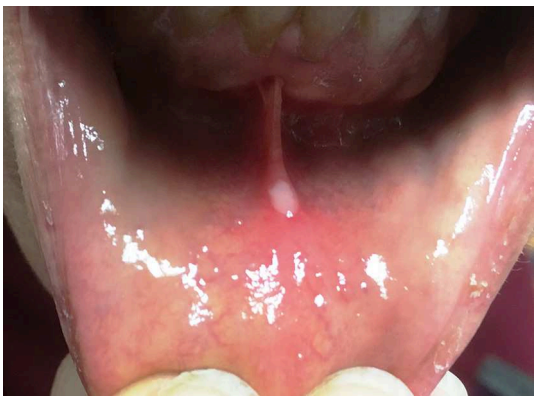

FIGURE 11 | Oral aphtous lesion in a patient with HA20. Written informed consent was obtained from the patient for the publication of this image.

Autoinflammatory Periodic Fever, Immunodeficiency, and Thrombocytopenia (PFIT)

PFIT is an autoinflammatory disease caused by a homozygous missense mutation in the actin regulatory gene *WDR1*, which encodes WDR1. Mutant WDR1 is thought to facilitate assembly of pyrin inflammasome, leading to excessive IL-18 production (172).

PFIT clinical features include recurrent fever attacks, lasting from 3 to 7 days and with 6–12 weeks periodicity. Fever is accompanied by oral ulcers, intermittent thrombocytopenia and cellular immunodeficiency, increasing the rate of infections. Raised acute phase reactants, leukocytosis, hyperferritinemia, and thrombocytopenia are observed during attacks (172).

Glucocorticoids, colchicine, conventional immunosuppressive drugs, and anakinra have been associated with poor responses. A case treated with an allogeneic hematopoietic stem cell transplantation has been reported with success (172).

Dermatologic manifestations

The most critical skin manifestation is the presence of severe oral ulcers and inflammation that cause scarring and microstomia (172).

Cutaneous histopathology

There are no reports regarding histopathological features in PFIT skin lesions.

C/EBPε-Associated Autoinflammation and Immune Impairment of Neutrophils (CAIN)

CAIN is an autosomal dominant autoinflammatory disease caused by mutations in the *CEBPE* gene encoding the transcription factor C/EBPε, which regulates both the inflammasome and the interferome.

CAIN is characterized by a combination of autoinflammation, immunodeficiency and neutrophil dysfunction. Disease onset has been reported during adolescence and tends to subside after menopause. The clinical presentation consists of periodic attacks of abdominal pain and high fever during 4–5 days, every 2–4 weeks. Other manifestations during attacks include oral ulcers, cutaneous abscesses, pyoderma gangrenosum, intra-abdominal granulomas, and upper respiratory tract infections. Mild bleeding diathesis with frequent nosebleeds and hematomas after needle sticks and surgical procedures have also been described. ESR elevation is frequent.

In CAIN patients, blockade of IL-1β and anti–IL-18 are candidate therapies, still untested (173).

Dermatologic manifestations

Crater-like buccal ulcers are the most frequent mucocutaneous features. Severe recurrent tongue, gluteal, submandibular abscesses, purulent paronychia, pyoderma gangrenosum, and wounds with delayed healing have also been described.

Cutaneous histopathology

No information about dermatopathologic features in CAIN lesions has been reported.

NFKB1-Associated Autoinflammatory Diseases (NFKB1-AD)

NFKB1-AD comprise a group of three different autosomal dominant diseases due to mutations in the *NFKB1* gene. These mutations affect the NK-κB subunits p50 and p105, resulting in an increased expression of IL-1β and TNF in some cases (174).

Initial descriptions of patients with *NFKB1* gene mutations were associated with an immunodeficiency phenotype consisting of recurrent respiratory tract infections leading to chronic lung disease with bronchiectasis, diarrhea, lymphadenopathy, splenomegaly, recurrent autoimmune phenomena (hemolytic anemia, thrombocytopenia, and leukopenia), hypogammaglobulinemia, deficient production of specific antibodies, and decreased class-switched and memory B cells (175, 176).

Subsequently, two additional autoinflammatory phenotypes associated to different mutations in the *NFKB1* gene have been described in two families (177). The first autoinflammatory phenotype is NFKB1-associated Behçet-like disease, which has been associated with the non-truncating mutation H67R in the *NFKB1* gene. It was described in six individuals within the same family presenting with clinical manifestations similar to those observed in Behçet disease (mucosal ulcers, arthritis, and abdominal pain) (177). Notably, mutations in *NFKB1* affect the same pathway as in HA20. However, Behçet-like disease associated with NFKB1 mutations was also associated with IgG-hypogammaglobulinemia, depletion of switched memory B cells and increased susceptibility to respiratory tract infections, thus overlapping somewhat with the immunodeficiency and autoimmunity phenotype described first for NFKB1-associated disease (175, 176). Behçet-like phenotype seems not to cause canonical inflammasome overactivation *in vitro*, thus targeting IL-1β and TNF might not be useful (177).

The second autoinflammatory phenotype is NFKB1-associated sterile familial autoinflammatory necrotizing fasciitis (FANF), which is caused by the truncating mutation R157X in the *NFKB1* gene. It was described in two brothers who presented with recurrent, sterile, isolated necrotizing inflammation

after tissue trauma caused by minor surgery, and rapidly extending into muscle fasciae, thus corresponding to necrotizing fasciitis. Patients had no other organ or systemic involvement nor any obvious manifestations of immunodeficiency (177). This mutation caused increased inflammasome activation *in vitro*, suggesting that agents targeting IL-1β or TNF might be useful in such autoinflammatory necrotizing fasciitis patients (174).

Dermatologic manifestations

The most common cutaneous lesions in patients with NFKB1-associated Behçet-like disease are mucosal aphthae affecting oral mucosa, esophagus, and genitalia. Lesions consisting of postoperative deep necrotizing fasciitis have been described in two patients of the same family with FANF (177). Of note, NFKB1-associated FANF lesions have been included in the section of pustular, pyogenic or neutrophilic dermatosis-like rashes in **Table 2**.

Cutaneous histopathology

Information about genital aphthae biopsy displayed a small vessel vasculitis, similar to that seen in Behçet's patients (177).

RELA Haploinsufficiency

RELA haploinsufficiency is an autosomal dominant autoinflammatory disease caused by mutations in the *RELA* gene, which encodes RelA, a subunit of NF-κB. The heterodimer RelA/NF-κB1 constitutes the predominant form of NF-κB, critical for cell survival. A biallelic requirement for RelA in order to maintain the normal cell function in stromal and epithelial cells, which is essential for mucosal integrity, has been reported. However, lymphocyte function is preserved in mice with RELA haploinsufficiency. This would explain why these patients with an impaired NF-κB signaling and an increased sensitivity to TNF have mucosal abnormalities without immunodeficiency (178).

Clinically these patients present with mucosal ulcers and gastrointestinal symptoms, such as abdominal pain, vomiting, and diarrhea, which can resemble an inflammatory bowel disease. Fever and elevated acute phase reactants are also present.

Treatment with glucocorticoids and TNF-α inhibitors have shown efficacy (178).

Dermatologic manifestations

Oral and/or genital ulcers are the most common mucocutaneous feature described in patients with RELA haploinsufficiency (178).

Cutaneous histopathology

No information regarding cutaneous histopathological features in RELA haploinsufficiency has been reported yet.

Monogenic Forms of Inflammatory Bowel Disease

Crohn's disease and ulcerative colitis are considered inflammatory bowel diseases (IBD), diseases with a polygenic or multifactorial etiology, in which complex interactions between genetic and environmental factors play an important role. Although over 150 genetic loci are associated with IBD, the genetic contribution toward heritability of the majority of those loci is low. However, recent studies have reported an increasing spectrum of human monogenic diseases that can present with IBD-like intestinal inflammation. Most of patients with those genetic defects present with very early onset IBD (during early childhood). However, as occur in polygenic IBD, in the monogenic forms, a variable penetrance of the clinical phenotype has been similarly described, also suggesting a role for modifier genes and/or gene–environmental interactions (179).

Oral aphthae may occur in polygenic and monogenic forms of IBD. With regard to monogenic forms, IL-10 signaling defects associated with very early onset IBD is an autosomal recessive monogenic autoinflammatory disease caused by mutations in genes encoding IL-10 and IL-10-receptor. Clinical manifestations start within the first 3 months of life and include bloody diarrhea, abscesses, perianal fistula, folliculitis, oral aphthous lesions and arthritis. The intermittent course of colitis with deep ulcerations is also indistinguishable from that of Crohn disease (179).

CONCLUSIONS

Cutaneous inflammatory lesions are commonly present in most of monogenic autoinflammatory diseases. Among them, non-specific maculopapular rashes and urticarial lesions are the most frequent manifestations, which have some differential traits regarding similar lesions without an autoinflammatory cause. While the evidence of systemic involvement will draw the attention toward an autoinflammatory origin, a genetic test showing pathogenic mutations in causal genes will confirm the diagnosis of a monogenic autoinflammatory disease.

Because the information regarding skin manifestations is still scarce, this review analyzes the most relevant histopathological and clinical features of cutaneous involvement in monogenic autoinflammatory diseases and groups the diseases based on the predominant cutaneous lesions, which were divided in: (1) maculopapular rashes or inflammatory plaques; (2) urticarial rashes; (3) pustular, pyogenic, or neutrophilic dermatosis-like rashes; (4) panniculitis or subcutaneous nodules; (5) vasculitis or vasculopathy; (6) hyperkeratotic lesions; (7) hyperpigmented lesions; (8) bullous lesions; and (9) aphthous lesions.

Therefore, the classification based on the predominant skin lesion in patients in whom a monogenic autoinflammatory disease is suspected may be a supporting tool to guide final diagnosis.

AUTHOR CONTRIBUTIONS

IF-N wrote and reviewed the article and provided most of the images for the article. JM collaborated with the writing, reviewed the manuscript, and provided some images. XS reviewed the article and contributed with useful corrections and some images. JH-R assisted with the writing, extensively reviewed the manuscript, and provided some images.

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REVIEW
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Current Therapeutic Options for the Main Monogenic Autoinflammatory Diseases and PFAPA Syndrome: Evidence-Based Approach and Proposal of a Practical Guide

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Monogenic autoinflammatory diseases are rare conditions caused by genetic abnormalities affecting the innate immunity. Previous therapeutic strategies had been mainly based on results from retrospective studies and physicians' experience. However, during the last years, the significant improvement in their genetic and pathogenic knowledge has been accompanied by a remarkable progress in their management. The relatively recent identification of the inflammasome as the crucial pathogenic mechanism causing an aberrant production of interleukin 1β (IL-1β) in the most frequent monogenic autoinflammatory diseases led to the introduction of anti–IL-1 agents and other biologic drugs as part of the previously limited therapeutic armamentarium available. Advances in the treatment of autoinflammatory diseases have been favored by the use of new biologic agents and the performance of a notable number of randomized clinical trials exploring the efficacy and safety of these agents. Clinical trials have contributed to increase the level of evidence and provided more robust therapeutic recommendations. This review analyzes the treatment of the most frequent monogenic autoinflammatory diseases, namely, familial Mediterranean fever, tumor necrosis factor receptor–associated periodic fever syndrome, hyperimmunoglobulin D syndrome/mevalonate kinase deficiency, and cryopyrin-associated periodic syndromes, together with periodic fever with aphthous stomatitis, pharyngitis, and cervical adenitis syndrome, which is the most common polygenic autoinflammatory disease in children, also occurring in adult patients. Finally, based on the available expert consensus recommendations and the highest level of evidence of the published studies, a practical evidence-based guideline for the treatment of these autoinflammatory diseases is proposed.

Keywords: monogenic autoinflammatory diseases, PFAPA syndrome, colchicine, anakinra, canakinumab, anti-TNF agents, tocilizumab, Janus kinase (JAK) inhibitors

INTRODUCTION

Over the past 20 years, pathogenic insights into the mechanisms of autoinflammation completely changed treatment and prognosis of many inherited autoinflammatory diseases, also known as "hereditary periodic fever syndromes," a group of rare diseases characterized by difficult diagnostic and therapeutic approaches. The most extensively studied and better pathogenically defined monogenic autoinflammatory conditions have been familial Mediterranean fever (FMF), tumor necrosis factor (TNF) receptor–associated periodic fever syndrome (TRAPS), hyperimmunoglobulin D syndrome/mevalonate kinase deficiency (HIDS/MKD), and cryopyrin-associated periodic syndromes (CAPS), which comprise three disorders with the same genetic background, and different phenotypes and outcomes. The CAPS spectrum includes familial cold autoinflammatory syndrome (FCAS), the mildest form; Muckle-Wells syndrome (MWS), the intermediate presentation; and chronic infantile neurological, cutaneous, and articular syndrome (CINCA) or neonatal-onset multisystem inflammatory disorder (NOMID), the most severe disease (1).

Until the late 1990s, traditional drugs, such as colchicine and glucocorticoids, had been used to treat autoinflammatory diseases. The pathogenic and therapeutic revolution started when NLRP3, one of the NOD-like receptors (NLRs) and part of the NLRP3 inflammasome, was discovered as the main actor in the activation of caspase 1 and the subsequent production of active interleukin 1β (IL-1β) (2). Mutations of genes involved in the inflammasome function or its related pathways were then identified as responsible for most of the monogenic autoinflammatory disorders recognized so far, also known as inflammasomopathies (3).

The discovery of the aberrant production of IL-1β as the final cause of all inflammasomopathies led to the introduction of anti–IL-1 agents and other biologic drugs to the very limited therapeutic armamentarium available for such diseases until then (4). In addition, the more recent knowledge of other autoinflammatory mechanisms, such as the activation of nuclear factor κB (NF-κB) and type I interferon (IFN) pathways, also provided new therapeutic options, such as anti-TNF and Janus kinase (JAK) inhibitors agents, directed to the specific blockade of cytokines and molecules involved in these novel inflammatory mechanisms (5–9).

Because monogenic autoinflammatory diseases are rare conditions, therapeutic strategies had been mainly based on results from retrospective studies and expert physicians' experience. Nevertheless, during the last years, the vast improvement in their genetic and pathogenic knowledge has been accompanied by a great effort in improving their management. In this sense, a remarkable number of randomized clinical trials exploring the efficacy and safety of new biologic agents in autoinflammatory diseases have been conducted, and therefore, therapeutic recommendations can be based now on higher evidence levels.

OBJECTIVES AND METHODOLOGY OF THE REVIEW

This review intends to analyze the use of conventional and biologic agents in the most prevalent monogenic autoinflammatory diseases (FMF, TRAPS, HIDS/MKD, and CAPS) and periodic fever syndrome, aphthous stomatitis, pharyngitis, and cervical adenitis (PFAPA) syndrome. Although PFAPA syndrome is a polygenic or multifactorial disease with an unidentified genetic background, its treatment is reviewed because PFAPA syndrome is the most frequent autoinflammatory condition in childhood and has to be frequently considered part of the differential diagnosis of some monogenic disorders in children and adults (10).

Evidence-based recommendations for the management of FMF, TRAPS, HIDS/MKD, and CAPS have been provided from previous consensus studies using the European League Against Rheumatism (EULAR) standard operating procedures for developing best practice (**Table 1**) (11, 12), such as the EULAR recommendations for the management of FMF (13), and the European SHARE (Single Hub and Access point for pediatric Rheumatology in Europe) recommendations for the management of CAPS, TRAPS, and MKD (14). For all the autoinflammatory diseases, results of new clinical trials, international multicenter collaborative studies and registries, and retrospective data preferably from referral centers, have also been used to generate levels of evidence. The most relevant international multicenter initiatives and registries include the PRINTO Registry (a secured web-based registry hosted at the Pediatric Rheumatology International Trial Organization website - PRINTO, http://www.printo.it) and EUROFEVER Registry (an international registry for autoinflammatory diseases, https://www.printo.it/eurofever/) (15).

Because, by definition, the strength of recommendation has to be based on a combination of data extracted from systematic literature review (providing levels of evidence) and consensus expert opinion (providing homogeneous and measurable information to generate the recommendations), in those conditions or situations for which any new therapeutic information has been reported but no opinion of the experts has been issued yet, only the level of evidence is mentioned (11, 12).

TRADITIONAL DRUGS

Colchicine

Colchicine is an alkaloid extracted from two plants of the Lily family: *Colchicum autumnale* and *Gloriosa superba*. Historically, the therapeutic role of colchicine was for the first time raised up for the treatment of gout in the sixth century (16). More recently, colchicine use has been extended to other diseases such as primary biliary cirrhosis, Behçet disease, recurrent pericarditis, alcohol induced hepatitis, psoriasis, scleroderma, Sweet syndrome, amyloidosis, sarcoidosis and, in lesser extent, to inflammatory disorders prone to fibrosis (17–19).

In 1972, the efficacy of colchicine was first described in five FMF patients who experienced a dramatic reduction of

TABLE 1 | Categorization of the level of clinical evidence and strength of recommendation based on the EULAR standardized operating procedures for EULAR-endorsed recommendations (11, 12).

Category	Evidence from
LEVELS OF EVIDENCE	
1A	Meta-analysis of randomized controlled trials
1B	At least one randomized controlled trial
2A	At least one controlled study without randomization
2B	At least one type of quasi-experimental study
3	Descriptive studies, such as comparative studies, correlation studies, or case–control studies
4	Expert committee reports or opinions and/or clinical experience of respected authorities
Grade	**Directly based on**
STRENGTH OF RECOMMENDATION	
A	Category 1 evidence
B	Category 2 evidence or extrapolated recommendation from category 2 evidence
C	Category 3 evidence or extrapolated recommendation from category 1 or 2 evidence
D	Category 4 evidence or extrapolated recommendation from category 2 or 3 evidence

the number of attacks (20). Since then, colchicine has become the basis of FMF treatment, and later on, several clinical trials definitively established its efficacy in preventing attacks and developing amyloidosis (21–23).

Colchicine was officially approved in 2009 by the US Food and Drug Administration (FDA) for the acute flares of gout and FMF, as a single-ingredient oral formulation (Colcrys®) (24). Nowadays, according to 2016 EULAR recommendations for the management of FMF, colchicine represents the first-line treatment in FMF, with a level of evidence 1B and grade of recommendation A (13).

Among other biological functions, colchicine has anti-inflammatory properties based on the inhibition of leukocyte chemotaxis, which is caused by its interaction with tubulin and the resulting dysfunction of microtubules. Microtubules, composed by α- and β-tubulin heterodimers, are involved in cell division, signal transduction, migration, secretion, and regulation of gene expression (25). Colchicine has the ability to bind in a poorly reversible manner to soluble non-polymerized tubulin in the cells, with the formation of a tubulin–colchicine complex (26, 27), and the subsequent movement inhibition of intracellular granules (28).

The predominant effect of colchicine on leukocytes, and more specific on granulocytes, has been correlated with the high concentrations of the drug in neutrophils compared to lymphocytes and monocytes. In this regard, defects in the efflux pumps with low activity of the P-glycoprotein 1 (PGY-1) efflux pump of granulocytes might explain the accumulation of colchicine in their cytoplasm (29, 30).

Other anti-inflammatory effects of colchicine include the reduction of TNF-α production by macrophages and TNF-α receptors on endothelial cells and the abrogation of neutrophil binding to adhesion molecules on vascular endothelium. Colchicine also decreases phospholipase A_2 activity, release of lysosomal enzymes, and phagocytosis (31–33).

After the discovery of the *MEFV* gene as the gene encoding for pyrin and responsible for FMF, *MEFV* was found fully expressed in neutrophils, and some authors evidenced a colchicine-related cytosolic modulation of pyrin expression (34–36). Finally, colchicine has been shown to suppress the activation of caspase 1, the enzymatic component of NLRP3 inflammasome, with the subsequent inability to convert pro–IL-1β to active IL-1β (37).

Pharmacokinetic properties of colchicine include a narrow therapeutic range because the half-life after oral ingestion ranges between 7 and 9 h. The drug is absorbed in the jejunum and ileum and is metabolized in the liver by the cytochrome P (CYP) 450 3A4 and PGY-1. It is finally excreted mainly by the biliary, intestinal, and renal systems. Colchicine use in pregnant or nursing patients is considered relatively safe as long as hepatic and renal functions are intact. In this regard, because colchicine is metabolized via CYP3A4, its concomitant administration with agents that inhibit this isoenzyme (e.g., macrolide antibiotics, azole antifungals, statins) may produce elevated colchicine plasma concentrations, resulting in severe, and sometimes fatal complications. Colchicine intoxication may be also induced in patients with renal and/or liver disorders (38, 39).

Colchicine in Autoinflammatory Diseases

Familial Mediterranean Fever

Therapeutic doses of colchicine to treat FMF should be adjusted to the body weight, with a mean optimal dose of 0.03 ± 0.02 mg/kg per day. Total doses usually range from 0.5 to 2 mg/d in children and from 1 to 3 mg/d in adults (40, 41). Colchicine usually reduces severity, duration, and frequency of the attacks in the majority of FMF patients. Among them, ∼30–40% of individuals experience a partial response (42). After ruling out any colchicine-associated gastrointestinal intolerance, other adverse effect, or poor patient compliance, only 5–10% of FMF cases can be finally considered colchicine-resistant FMF (crFMF) patients (15, 43).

Intravenous colchicine has shown efficacy in some patients unresponsive or with partial response to the oral formulation (43–45). An adjunctive weekly infusion of (1 mg) colchicine in FMF patients with frequent attacks despite a maximum tolerated oral dosage of 2–3 mg/d was associated with 50% reduction in frequency of attacks after 6 months (45). However, because of the potential risk of toxicity, intravenous colchicine is currently not recommended in FMF patients unresponsive to the oral formulation.

Other Autoinflammatory Diseases

Colchicine has not demonstrated effectiveness in HIDS/MKD and CAPS (14, 15). However, it has shown to be somehow effective in TRAPS and PFAPA syndrome. In the Eurofever Registry, colchicine was used in 39 TRAPS patients, with complete and partial response in 3 (8%) and 18 (46%) individuals, respectively (15). In a recent study of 24 patients with TRAPS,

colchicine resulted to be of some help in 71% of cases, with a complete response in 12.5% of them. No differences in colchicine response were observed with regard to age at disease onset (pediatric vs. adult), type of variant (low vs. high penetrance), and colchine daily doses (1 mg vs. higher doses) (46). In 303 PFAPA patients from a tertiary Turkish center, colchicine was used as regular prophylactic treatment with high rate of response in terms of reduction of the frequency of episodes. Interestingly, heterozygous mutations in the *MEFV* gene were found in 25% of PFAPA subjects, who obtained even better results in terms of reduction of attacks. The potential modifier role of *MEFV* mutations in PFAPA patients seems associated with attenuated disease severity and higher response rate to colchicine compared to non-carriers of *MEFV* variants (47–49).

Non-steroidal Anti-inflammatory Drugs

The main therapeutic effects of non-steroidal anti-inflammatory drugs (NSAIDs) are determined by the inhibition of cyclooxygenases, enzymes that convert arachidonic acid in prostaglandins, and thromboxanes. Some of these eicosanoids participate in different pathways involved in the inflammatory response. However, because NSAIDs do not inhibit the biosynthetic cascade originating arachidonic acid, these drugs do not usually influence the underlying cause of the disease.

NSAIDs in Autoinflammatory Diseases

NSAIDs have been used in monogenic autoinflammatory diseases as symptomatic treatment, alone or in addition to the baseline therapy. Although a complete response has been reported in a minority of patients with autoinflammatory conditions in the Eurofever Registry (8% of FMF and TRAPS, 13% of HIDS/MKD, 6% of CAPS, and 4% of PFAPA patients), NSAIDs, alone or in combination with glucocorticoids, appear to be of some help in 70–80% of all cases (15).

In some FMF patients with exertional leg pain and protracted febrile myalgia, similarly to glucocorticoids, NSAIDs seem to be also effective (50). Protracted febrile myalgia is a condition characterized by prolonged episodes of muscle pain affecting limbs with marked systemic inflammatory response in the absence of rhabdomyolysis. The EULAR recommendations for treating this complication with NSAIDs have a level of evidence 2B and grade of recommendation C (13).

Patients with TRAPS associated with R92Q mutations have been reported to respond better to the combination of colchicine and NSAIDs than those carrying clearly pathogenic *TNFRSF1A* mutations (15, 51). However, in TRAPS, HIDS/MKD, and CAPS patients, the use of NSAIDs as pain relievers during inflammatory attacks is recommended with a level of evidence 3 and strength of recommendation D (for TRAPS) and C (for HIDS/MKD, CAPS, and PFAPA syndrome) (14, 15).

Glucocorticoids

Glucocorticoids suppress the production and effects of several proinflammatory mediators, induce the inhibition of leukocytes migration to the inflammatory foci, and interfere with the function of fibroblasts and endothelial cells, thereby exerting a powerful anti-inflammatory action, both on acute manifestations (i.e., pain, edema) and on late inflammatory stages (including reparative processes, such as cell proliferation and fibrosis). Glucocorticoids bind to a specific intracytoplasmic receptor, a ligand-activated transcription factor with both positive and negative effects on the regulation of gene expression. Consequently, the activated glucocorticoid receptor–glucocorticoid complex up-regulates the expression of anti-inflammatory proteins and suppresses the transcription of proinflammatory cytokines and chemokines by blocking the NF-κB signaling pathway (52).

Glucocorticoids in Autoinflammatory Diseases

Familial Mediterranean Fever

In FMF patients, when colchicine becomes insufficient to control the disease activity, glucocorticoids (always in combination with colchicine) have shown positive effects with variable response in 83% of the 47 cases recruited from the Eurofever Registry (15). The use of intravenous methylprednisolone (40 mg) on demand has also demonstrated efficacy in decreasing fever and abdominal and pleuritic pain during attacks in a study of 31 FMF patients (53).

FMF patients with protracted febrile myalgia may be controlled with intravenous and/or oral administration of high-dose glucocorticoids (54). In fact, glucocorticoids have been recommended for treating this complication with a level of evidence 2B and grade of recommendation C (13). A recent study on eight FMF patients with protracted febrile myalgia has shown good response with the use of intravenous methylprednisolone pulses, at a dose of 10 mg/kg per day, for 3 days, followed by oral glucocorticoids, at initial doses of 1–2 mg/kg and gradual tapering down over 6 weeks (55). A small comparative study on 15 FMF patients with protracted febrile myalgia suggested similar efficacy of oral glucocorticoids and NSAIDs (50).

Tumor Necrosis Factor Receptor–Associated Periodic Fever Syndrome

In TRAPS patients, prednisone (or equivalent) may be useful on demand, administered during the attacks at a dose of 1 mg/kg per day, usually with rapid tapering and cessation in the following days (51, 56, 57). In the Eurofever Registry, short-term glucocorticoids, with or without concomitant NSAIDs, were considered effective for controlling inflammatory attacks in 91% of TRAPS patients, with complete response in 41% of them (15). Glucocorticoids do not seem to have any significant effect in preventing amyloidosis, and its intermittent use does not reduce either the frequency of attacks or subclinical inflammation between them. Glucocorticoids in TRAPS are recommended either as short-term courses or as maintenance treatment with a level of evidence 3 and strength of recommendation C (14).

As in other monogenic autoinflammatory disorders, some TRAPS patients may require long-term glucocorticoid therapy. In these glucocorticoid-dependent situations, a biological agent is often warranted in order to avoid steroid side effects. Indeed, almost 80% of TRAPS patients initially treated with glucocorticoids may finally receive biologic agents as maintenance therapy to control symptoms (14, 58).

Hyperimmunoglobulin D Syndrome/Mevalonate Kinase Deficiency

Similarly to TRAPS patients, in HIDS/MKD, high-dose glucocorticoids may be useful during the attacks with some benefit in 73% of cases. However, complete improvement is achieved by only 9% of patients, and glucocorticoids do not decrease neither the intensity nor the frequency of the acute episodes (15). Short-term glucocorticoids, with or without NSAIDs, may be effective for alleviating inflammatory attacks and are recommended with a level of evidence 3 and strength of recommendation C (14).

Cryopyrin-Associated Periodic Syndromes

In CAPS patients, glucocorticoids, mostly used on demand, resulted in some benefit in 80% of patients included in the Eurofever Registry. However, glucocorticoids do not control the underlying inflammatory process or decrease the frequency of the attacks (15, 59). Anyway, for symptomatic adjunctive therapy, short courses of NSAIDs and glucocorticoids may be used, with a level of evidence 3 and strength of recommendation C (14). However, they should not be used for primary maintenance therapy (level of evidence 4 and grade of recommendation D) (14).

PFAPA Syndrome

Glucocorticoids are the treatment of choice in PFAPA syndrome, because their prompt administration is able to discontinue the attacks rapidly and completely in the majority of patients. Such dramatic response is usually considered a peculiar diagnostic feature in pediatric and adult patients (15, 60–63). The conventional dosage from 0.5 to 2 mg/kg of prednisone (or equivalent) in a single dose at the time of fever onset has been proved useful in a randomized clinical trial (64), and therefore, glucocorticoids can be recommended in PFAPA patients with a level of evidence 2B (15). However, additional doses of glucocorticoids may be occasionally necessary. Their use does not prevent further attacks and may even be associated with an increased frequency of the attacks in 25–50% of cases (15, 60).

Of note, the evidence for the effectiveness of tonsillectomy in children with PFAPA syndrome is based on a systematic review that included two small randomized controlled trials studying the effects of tonsillectomy compared to no surgery. Tonsillectomy was associated with immediate and complete clinical resolution and significant reduction in the frequency and severity of symptoms (65). Although these results have to be interpreted considering the clinical severity, the previous response to a single dose of prednisone, and the surgical risk in every individual situation, tonsillectomy as a therapy for PFAPA syndrome can be recommended with a level of evidence 1A (15).

Other Conventional Agents

Thalidomide

Thalidomide exerts its immunomodulatory and anti-inflammatory actions through inhibiting TNF-α and IFN-γ synthesis, leukocyte chemotaxis, and angiogenesis. Teratogenicity and polyneuropathy are known as the most feared thalidomide-related serious adverse events. Thalidomide is an accepted treatment for dermatological diseases (such as leprous erythema nodosum, severe mucosal ulcers, cutaneous lupus erythematosus, and chronic graft-vs.-host disease), refractory multiple myeloma, and other systemic autoimmune or inflammatory conditions. Evidence on the efficacy of thalidomide in autoinflammatory diseases is limited to sporadic case reports and series. In three crFMF patients treated with thalidomide in addition to colchicine, the combination did not show clear benefit (66). Similarly, thalidomide failed to demonstrate any biological or clinical effect in six patients with HIDS/MKD (67).

Dapsone

Dapsone is an antibacterial sulfonamide drug with anti-inflammatory properties due to the inhibition of function and chemotaxis of neutrophils and blockade of the inflammatory effects of multiple prostaglandins and leukotrienes. Dapsone is currently used for the treatment of different infectious and immune-mediated systemic and dermatologic conditions (68). In monogenic autoinflammatory diseases, dapsone has been used in a case series of 10 crFMF patients with control of attacks in half of them. The authors postulated that dapsone might be considered as an alternative therapy in selected FMF cases not responding to colchicine (68).

Interferon α

The use of IFN-α in patients with FMF resistant to colchicine has been reported with controversial results. While in several crFMF cases IFN-α seemed to induce a marked decrease in both severity and frequency of the attacks, alone or in combination with colchicine (69–72), a double-blind randomized clinical trial of 22 FMF patients comparing IFN-α with placebo (without concomitant colchicine) did not demonstrate clear efficacy of IFN-α in reducing severity of attacks or inflammatory markers (73).

Other Drugs

Other anti-inflammatory, immune-modulatory, or immunosuppressive agents, such as azathioprine, methotrexate, cyclosporine, leflunomide, mycophenolate mofetil, sulfasalazine, statins, cimetidine, and antihistamines, have been historically used in monogenic autoinflammatory diseases mostly with unclear or unsatisfactory results (15, 51).

BIOLOGIC AGENTS

Interleukin 1 Blockers

It is well-known that IL-1 production has strong impact on initiation and maintenance of inflammatory process. Both IL-1α and IL-1β bind to the IL-1 receptor type, which is expressed by nearly every human cell and is responsible for triggering the cascade of inflammatory processes (74). IL-1 inhibitory molecule was first described over 30 years ago in the urine from patients with fever and monocytic leukemia (75, 76). This molecule was later cloned and identified as IL-1 receptor antagonist (IL-1Ra) (77), and subsequently, it was hypothesized that the inhibition of IL-1 with IL-1Ra could be a potential therapeutic option for treating inflammatory diseases (78).

TABLE 2 | Anti-IL-1 agents, types of studies supporting the maximum evidence level for their use, and common pediatric and adult doses given in the main monogenic autoinflammatory diseases (crFMF, TRAPS, HIDS/MKD, and CAPS) and PFAPA syndrome.

Anti-IL-1 agent	Disease	Type of study	EMA/FDA approval	Doses (children)	Doses (adults)	References
Anakinra	crFMF	RCT, RCS	No/No	50–300 mg/day	50–100 mg/day	(80, 81)
	TRAPS	OLS, CS	No/No	1.5 mg/kg/day	100 mg/day	(82, 83)
	HIDS/MKD	OLS	No / No	1 mg/kg/day*	100 mg/day*	(84)
	CAPS	OLS, RCS, CS	Yes/Yes**	1–1.5 mg/kg/day, 1–2 mg/kg/day, 1.5–8 mg/kg/day	100 mg/day, 1–2 mg/kg/day, 1.5–8 mg/kg/day	(85–92)
	PFAPA	CS, CR	No/No	1 mg/kg/day*	100 mg/day*	(93, 94)
Canakinumab	crFMF	RCT, OLS	Yes/Yes	2 mg/Kg q4w or q8w	150–300 mg q4w or q8w	(95–97)
	TRAPS	RCT, OLS	Yes/Yes	2 mg/Kg q4w or q8w	150–300 mg q4w or q8w	(95, 98)
	HIDS/MKD	RCT, OLS	Yes/Yes	2 mg/Kg q4w or q8w	150–300 mg q4w or q8w	(95, 99)
	CAPS	RCT, OLS	Yes/Yes	2–10 mg/Kg q4w or q8w	150–300 mg q4w or q8w	(100–104)
	PFAPA	CR	No/No	2 mg/kg q8w	150 mg q8w	(105, 106)
Rilonacept	crFMF	RCT	No/No	2.2 mg/Kg qw	160 mg qw	(107)
	CAPS	RCT, OLS	No/No	4.4 mg/Kg followed by 2.2 mg/Kg qw	320 mg followed by 160 mg qw	(108, 109)

**In some cases, on demand administration at the beginning of the febrile episode may also be used.*

***Only for CINCA/NOMID cases.*

CAPS, cryopyrin-associated periodic syndromes; CINCA/NOMID, chronic infantile neurological, cutaneous and articular syndrome/neonatal onset multisystem inflammatory disorder; CR, case report; CS, case series; crFMF, colchicine-resistant familial Mediterranean fever; EMA, European Medicines Agency; FDA, Food and Drug Administration; HIDS/MKD, hyperimmunoglobulin D and periodic fever syndrome/mevalonate kinase deficiency; OLS, open-label study; PFAPA, periodic fever with aphthous stomatitis, pharyngitis and cervical adenitis; RCS, retrospective cohort study; RCT, randomized controlled trial; TRAPS, TNF-receptor associated periodic fever syndrome.

With regard to inflammasome-mediated autoinflammatory diseases, such as FMF, TRAPS, HIDS/MKD, and CAPS, IL-1 blockade has become the most specific and useful treatment, as first-line therapy or when previous conventional anti-inflammatory or immunosuppressive agents are not useful. Although several IL-1 blockers are currently under development (79), the three biologic agents targeting IL-1 with special interest in autoinflammatory diseases, commercially available nowadays, are anakinra, canakinumab, and rilonacept.

Table 2 illustrates the most relevant studies supporting the maximum evidence level for the use of IL-1 blockers in the main monogenic autoinflammatory diseases (crFMF, TRAPS, HIDS/MKD, and CAPS) and PFAPA syndrome and includes the doses used in pediatric and adult patients.

Anakinra

Anakinra is a recombinant non-glycosylated form of the human IL-1 receptor antagonist (rhIL-1Ra) that binds to IL-1 receptor type I (IL-1RI) and acts as competitive inhibitor with IL-1α and IL-1β, in a way that mimics the activity of endogenous IL-1Ra (110). Anakinra has been approved by the FDA and European Medicines Agency (EMA) for rheumatoid arthritis and by the EMA for the treatment of Still disease, including systemic juvenile idiopathic arthritis and adult-onset Still disease. In 2012, anakinra was approved for the treatment of CINCA/NOMID in the United States. In the European Union, anakinra was approved by the EMA for all types of CAPS in 2013.

The recommended initial dose of anakinra is 100 mg/d subcutaneously in adults and 0.5–2 mg/kg per day in children, who may require increasing dosage up to 5–8 mg/kg per day to maintain remission. The need of higher requirements of anakinra in pediatric patients might be related to pharmacokinetics of the drug whose effective steady-state concentration in young children seems to be higher than in adults (14, 111). Anakinra terminal half-life ranges from 4 to 6 h, and ∼80% of the drug is excreted by renal clearance (112). Consequently, the mean plasma clearance of the drug strongly depends on normal kidney function, and anakinra removal from plasma is minimally feasible through dialysis (113).

An animal study showed that up to two-thirds of serum IL-1Ra are able to cross blood–brain barrier. On clinical grounds, anakinra administration has determined improvement of memory and cognitive functions in CAPS patients (85, 114).

Safety data on anakinra comes from rheumatoid arthritis randomized controlled trials and long-term observational studies, in which good safety profiles have been observed with no increase of opportunistic infections or malignancies (141, 142). Data from the British Society of Rheumatology Biologic Register reported higher rates of serious infections of the skin and respiratory tract in subjects with more severe disease and higher exposure to glucocorticoids (143). The increased susceptibility to respiratory infections in patients treated with anti–IL-1 might be explained by the fact that IL-1β seems to play a role in the resistance to *Streptococcus pneumoniae* infection (144). Although the most frequent adverse event by far of anakinra is the injection site skin reaction, which is observed in up to 70% of patients, this local reaction tends to decline over time without the need of treatment discontinuation (145). Anakinra use during

pregnancy has not been associated with an increased rate of miscarriages or congenital malformations, neither in a registry of 40 patients (146) nor in small series of FMF and CAPS patients (147, 148).

Canakinumab

Canakinumab is a fully humanized IgG1 monoclonal antibody that acts specifically against IL-1β. In 2009, canakinumab received the FDA approval for CAPS treatment, specifically for FCAS and MWS, and EMA approval for children older than 2 years and adults with all forms of CAPS. More recently, in 2016, the FDA and EMA approved canakinumab for the treatment of crFMF, TRAPS, and MKD, based on the results of the phase 3 Canakinumab Pivotal Umbrella Study in Three Hereditary Periodic Fevers (CLUSTER) trial (95). Additional indications of canakinumab include systemic juvenile idiopathic arthritis (by the FDA) and Still disease (systemic juvenile idiopathic arthritis and adult-onset Still disease) and gouty arthritis (by the EMA).

Given its long half-life of 26 days, canakinumab administration is recommended subcutaneously at 2–4 mg/kg in children and at a minimal dose of 150 mg in adults, every 4–8 weeks in both age groups. Patients with more severe phenotypes may require dose escalation. For instance, patients with CINCA/NOMID (the most severe CAPS phenotype) may require up to 8 mg/kg dosage every 4 weeks to control symptoms (100, 101). Similarly, in crFMF, TRAPS, and MKD patients, the recommended starting dose is usually 150 mg or 2 mg/kg (in children with body weight between 7.5 and 49 kg) every 4 weeks, with the possibility to increase up to 300 mg every 4 weeks (or 4 mg/kg in children), which is more often required by MKD patients (95).

Its penetrance of the blood–brain barrier seems incomplete because canakinumab does not normalize intrathecal inflammatory markers, as demonstrated in two case series of CINCA/NOMID patients (149, 150). Renal function does not influence the pharmacokinetics of canakinumab (151). Data on pregnancy and breastfeeding are still scarce. However, seven of eight pregnancies of patients on canakinumab were reported uneventful, and no developmental abnormalities were reported in four breastfed infants while mothers were on canakinumab treatment (152).

Mild infections, involving mostly the urinary tract and upper airway, represent the most frequent canakinumab-associated adverse events. However, serious infections have been reported in 5.4/100 patients-years in 285 patients included in the long-term CAPS registry (153) and in 7.4/100 patients-years of FMF, TRAPS, and MKD patients treated with higher doses of canakinumab in the CLUSTER trial (95).

Additional data on safety can be extrapolated from the randomized, double-blind, placebo controlled trial of canakinumab in patients with atherosclerotic disease, with more than 10,000 individuals and ∼30% of them receiving placebo (154). Neutropenia and thrombocytopenia were observed in the treatment group, with more deaths attributed to infections or sepsis, however, with no statistically significant differences in the overall rates of adverse events compared with placebo (154).

Rilonacept

Rilonacept is a dimeric fusion glycoprotein consisting of the Fc portion of human IgG1 and the human IL-1 receptor extracellular domains that binds IL-1α and IL-1β. In 2008, rilonacept was the first agent approved by the FDA for the treatment of FCAS and MWS in patients older than 12 years, based on two sequential phase III clinical studies, which demonstrated rilonacept safety and effectiveness in adult patients with CAPS (108). Rilonacept obtained a marketing authorization only in the United States.

Initial dose in adults is recommended at 320 mg subcutaneously, followed by a weekly dose of 160 mg. In children older than 11 years and adolescents, the dose has to be adjusted at 4.4 mg/kg (maximum of 320 mg) and then 2.2 mg/kg (maximum of 160 mg) once weekly. The circulating half-life may vary from 6 to 9 days (155, 156). Being a large molecule, it is expected not to cross the blood–brain barrier and to be cleared primarily by the reticuloendothelial system rather than by the kidney. Therefore, a dose adjustment in patients with renal disease is not required (157).

Injection site reactions, headache, and upper respiratory tract and urinary infections have been described as the most common adverse events in CAPS patients treated with rilonacept (109). Data from studies and trials on gout and gouty arthritis suggest also that several laboratory changes may occur, including transient neutropenia and a small increase in liver transaminases, triglycerides, and creatine phosphokinase (158).

IL-1 Blockers in Autoinflammatory Diseases

Familial Mediterranean Fever

IL-1 blockade with anakinra is currently recommended for FMF in case of true colchicine resistance, with a level of evidence 2B and strength of recommendation B, and as an optional treatment for protracted febrile myalgia, with a level of agreement 2B and grade of recommendation C (13).

Although in 2011 the Eurofever Registry included only three FMF patients treated with anakinra with complete response (15), a 2013 literature review identified 30 FMF cases resistant or intolerant to colchicine treated with anakinra, and four with canakinumab, with good clinical and laboratory outcomes (110). A 2015 systematic review reported 64 patients treated with anakinra and 40 with canakinumab. A complete response without attacks occurred in 76.5 and 67.5% of patients on anakinra and canakinumab, respectively. In patients with established type AA amyloidosis, both anti–IL-1 agents demonstrated to reverse proteinuria (159). Anakinra has been administered on demand with efficacy to some selected crFMF patients, mainly those with prominent prodromal manifestations or recognizable triggers of the attacks (160).

Ben-Zvi et al. (80) enrolled 25 adult patients with crFMF in the first double-blind randomized placebo-controlled trial, aiming to assess efficacy and safety of anakinra at a dose of 100 mg/d during 4 months of treatment. Adult FMF patients experiencing at least one attack per month despite the maximum tolerated dose of colchicine (up to 3 mg/d) were enrolled. Patients treated with anakinra had an attack rate of 1.7 per month, whereas those receiving placebo suffered 3.5 attacks per month.

In addition, anakinra was associated with better quality of life, and no differences in the development of adverse effects were found. Interestingly, the best results with anakinra were observed in terms of amelioration of joint manifestations (80). In a Turkish multicenter retrospective study of 172 crFMF patients, in whom 151 were treated with anakinra and 21 with canakinumab, both drugs reduced the yearly attack frequency from 16.8 to 2.4 ($p < 0.001$). Forty-two percent of crFMF patients were attack-free, and proteinuria was significantly reduced in those patients with amyloidosis (81).

Canakinumab has proved effective in several retrospective FMF reviews (161–163) and two open-label phase II studies with nine and seven crFMF patients (96, 97). Both phase II trials established the efficacy of canakinumab in reducing the frequency of FMF attacks and maintaining low levels of acute phase reactants, with no unexpected adverse events (96, 97). The recent FDA and EMA approval of canakinumab for patients with FMF resistant or intolerant to colchicine has been based on data from the CLUSTER trial (95).

A total of 63 patients with crFMF were randomized to receive canakinumab 150 mg or placebo every 4 weeks. At week 16, canakinumab compared with placebo produced a significantly higher response rate by day 15 (61 vs. 6%), higher rates of physician global assessment of disease activity (minimal/none) (65 vs. 9%), and higher C-reactive protein (CRP) levels ≤10 mg/L (68 vs. 6%) and serum amyloid A (SAA) levels ≤10 mg/L (65 vs. 9%) (95). Canakinumab also demonstrated a rapid and sustained disease control assessed with the Autoinflammatory Disease Activity Index (AIDAI) over 16 weeks, and approximately half of crFMF patients had inactive disease after the same period (164). In the open-label phase from weeks 16–40, all FMF patients with a previous complete response to canakinumab 150 mg every 4 weeks maintained the absence of flares with canakinumab 150 mg every 8 weeks. After that period, the same dose interval of every 8 weeks was sufficient to maintain disease control in 46% of patients. An increase in the dose to 300 mg every 4 weeks was required by 10% of crFMF patients (95).

Adverse events and serious adverse events were more frequent in canakinumab-treated patients than in those receiving placebo. Overall, the most frequently reported adverse events were infections (mostly those affecting the respiratory tract), headache, abdominal pain, and injection-site reactions. Rates of infections and serious infections were of 173.3 and 6.6 per 100 patient-years of treatment, respectively (95).

With regard to rilonacept, this agent was administered to 14 FMF patients with one or more attacks per month in a randomized double-blind, single-participant alternating treatment study, with a significant reduction in the number of FMF attacks. Injection site reactions were the only adverse events associated to rilonacept administration (107).

Tumor Necrosis Factor Receptor–Associated Periodic Fever Syndrome

Anti–IL-1 agents have demonstrated efficacy in the majority of TRAPS patients. Anakinra seems to be superior to etanercept in retrospective TRAPS studies (15). In the Eurofever Registry, anakinra provided some benefit in ∼90% of TRAPS patients, with a complete remission in 67% of them (15). Anakinra has shown to be effective, both in continuous and on-demand administration (14, 15, 57, 82, 83, 165), and is recommended in TRAPS patients with a level of evidence 2B and strength of recommendation B (14).

Canakinumab induced a complete response in 19 of 20 TRAPS patients in an open-label, phase II study (98), but it has been recently labeled by the FDA and EMA for TRAPS patients based on data from the CLUSTER trial (95). In such trial, among the 46 TRAPS patients randomized to receive canakinumab 150 mg or placebo every 4 weeks, at week 16, canakinumab, compared to placebo, was associated with a higher response rate by day 15 (45 vs. 8%), higher rates of (minimal/none) physician global assessment of disease activity (45 vs. 4%), and higher CRP levels ≤10 mg/L (36 vs. 8%) and SAA levels ≤10 mg/L (27 vs. 0%) at week 16. Canakinumab also produced a rapid and sustained disease control assessed with AIDAI over 16 weeks, and about half of TRAPS patients had inactive disease after the same period (164). In the open-label phase from week 16–40, 83% of TRAPS patients with a previous complete response to canakinumab 150 mg every 4 weeks maintained the absence of flares with canakinumab 150 mg every 8 weeks. After that period, the same dose interval of every 8 weeks was sufficient to maintain disease control in 53% of patients. An increase in the dose to 300 mg every 4 weeks was required by 8% of TRAPS patients (95).

Similarly to FMF patients of the CLUSTER study, TRAPS patients treated with canakinumab developed more frequently adverse events and serious adverse events than patients receiving placebo. The rate of infections was of 148 per 100 patient-years of treatment, and no serious infections were observed (95).

Hyperimmunoglobulin D Syndrome/Mevalonate Kinase Deficiency

In HIDS/MKD, anakinra, and canakinumab may control or attenuate the intensity of attacks in most of patients. In the Eurofever Registry, among the 62 HIDS/MKD patients treated with anakinra, 84% obtained a positive response, which was complete in only 29% of them (15). On-demand administration of anakinra in HIDS/MKD patients decreases the duration and severity of symptoms attacks when started within 24 h after the onset of symptoms. However, on-demand regimen does not influence the frequency of attacks (84). Anakinra is recommended for treating HIDS/MKD patients with a level of evidence 2B and strength of recommendation C (14).

Efficacy and safety of canakinumab have been recently investigated in an open-label phase II study in nine patients (six pediatric and three adults) with HIDS, using a predefined dosage of 300 mg or 4 mg/kg for patients ≤40 kg (higher than the usual dose of 150 mg used in CAPS), with an interval administration of every 6 weeks (99). A significant reduction of attacks frequency and complete clinical response with normalization of inflammatory markers within the first month of treatment were noted in all patients (99).

As for crFMF and TRAPS, canakinumab has also been recently approved by the FDA and EMA for HIDS/MKD treatment, based on the data derived from the CLUSTER trial (95). Among the 72 HIDS/MKD patients included in the study (treated with

canakinumab 150 mg or placebo every 4 weeks), those receiving canakinumab experienced better response rate by day 15 (35 vs. 6%), higher rates of (minimal/none) physician global assessment of disease activity (46 vs. 6%), and higher CRP levels ≤10 mg/L (41 vs. 6%) and SAA levels ≤10 mg/L (41 vs. 6%) at week 16. Canakinumab was also associated with a rapid and sustained disease control assessed with AIDAI over 16 weeks, and 40% of HIDS/MKD patients had inactive disease after the same period (164). In the open-label phase from week 16–40, 82% of HIDS/MKD patients with a previous complete response to canakinumab 150 mg every 4 weeks maintained the absence of flares with canakinumab 150 mg every 8 weeks (95). After that period, the same dose interval of every 8 weeks maintained the disease controlled in only 23% of patients. An increase in the dose to 300 mg every 4 weeks was required by 29% of patients (95).

Similarly to FMF and TRAPS patients, in HIDS/MKD patients of the CLUSTER study, adverse events and serious adverse events were more frequent in patients treated with canakinumab than with placebo. Rates of infections and serious infections in HIDS/MKD were of 313.5 and 13.7 per 100 patient-years of treatment, which were higher than for FMF and TRAPS patients (95).

Cryopyrin-Associated Periodic Syndromes

Anakinra, canakinumab, and rilonacept are approved by the FDA and EMA for CAPS treatment. All the three anti–IL-1 agents are currently recommended as first-line therapy in CAPS patients of any age, with a level of evidence 1B for canakinumab and rilonacept, and 2A for anakinra, and a strength of recommendation A and B, respectively (14).

Anakinra has demonstrated to control clinical and biological activity in CAPS. FDA approval of anakinra was based on a long-term, open-label, and uncontrolled study of 18 CINCA/NOMID patients, in whom symptoms and inflammatory markers improved in all of them (86). This study provided relevant information about dosage variability of anakinra to control CAPS activity. Patients were treated with an initial dose of 1–2.4 mg/kg body weight and an average maintenance dose of 3–4 mg/kg per day (with a maximum dose administered of 7.6 mg/kg per day). Although most of individuals received a single daily dose, some of them achieved better control of disease by splitting the dose into two daily administrations. Upon withdrawal of treatment, disease flare occurred after a median time of 5 days (86).

Several open-label and prospective studies (85–90) and retrospective series (91, 92) supported EMA approval of anakinra in adults and pediatric patients 8 months or older with a body weight of 10 kg or greater, diagnosed with any type of CAPS [CINCA/NOMID (85, 86, 91, 92), MWS (85, 87, 88), and FCAS (88–90)].

Although anakinra does not seem to control the progression of osteoarticular deformities in CINCA/NOMID patients (166, 167), it improves leptomeningeal and cochlear involvement, due to its ability to penetrate the blood–brain barrier (15, 168, 169). Therefore, anakinra appears to be more effective than canakinumab in the intrathecal compartment (149).

Anakinra efficacy and safety were analyzed in a prospective, open-label, single-center clinical cohort study, including 43 severe CAPS patients followed during a mean of 5 years (170). Anakinra was safe and well-tolerated both in pediatric and adult patients, with most adverse events emerging during the first months after treatment initiation. The most frequent adverse events included headache, arthralgia, and injection site reactions. Infections, such as pneumonia and gastroenteritis, occurred in ∼25% of patients, but they did not require permanent discontinuation of treatment. Interestingly, an increase of anakinra dose was required in two cases during an infectious event with a concomitant disease flare (170).

Canakinumab induced clinical and biological remission in 75–90% of CAPS patients in the Eurofever Registry (15). The approval of canakinumab for CAPS was based on a 48-week, double-blind, placebo-controlled trial of 35 patients with CAPS (mainly MWS patients) treated with 150 mg every 8 weeks, in which 97% of patients had a complete response to canakinumab during the study period (102). In addition, another randomized controlled trial (103) and two observational studies on CAPS patients including all disease phenotypes similarly showed good results (100, 104).

A prospective study comparing the efficacy and safety of canakinumab and anakinra in 26 MWS patients concluded that both agents equally controlled disease activity and inflammatory markers and that canakinumab may be effective in some patients not responding to anakinra (104). Occasionally, patients receiving anakinra and those treated with canakinumab (mainly CINCA/NOMID patients) may require an increase of dose or frequency of administration of the drug (101, 104).

Rilonacept showed efficacy in 47 adult patients with CAPS (44 with FCAS and 3 with MWS) in a randomized controlled trial and in an extended open-label study of up to 96 weeks including 101 patients with favorable safety and tolerability profile in adult and pediatric patients (108, 109).

PFAPA Syndrome

Although genetic and pathogenic mechanisms of PFAPA syndrome are unknown, in a case report and a small cohort of five PFAPA patients resistant to glucocorticoids, the administration of anakinra has been effective in suppressing disease flares during the long-term follow-up (93, 94). Similarly, a pediatric case and an adult case of PFAPA syndrome have been reported to respond to canakinumab at a dose of 2 mg/kg and 150 mg every 8 weeks, respectively (105, 106).

TNF Blockers

Anti-TNF agents, mainly etanercept (a dimeric human TNF receptor p75-Fc fusion protein), infliximab (a chimeric monoclonal antibody against TNF-α), and adalimumab (a fully human monoclonal antibody against TNF-α), have been used in different monogenic autoinflammatory inflammasomopathies, generally with poorer efficacy than IL-1 blockers. These biologic agents have been approved for several autoimmune/inflammatory conditions. Etanercept has FDA and EMA indications for rheumatoid arthritis, juvenile

idiopathic arthritis, ankylosing spondylitis, psoriatic arthritis, and plaque psoriasis; infliximab was approved by the FDA and EMA for rheumatoid arthritis, ankylosing spondylitis, psoriatic arthritis, plaque psoriasis, Crohn disease, and ulcerative colitis, and adalimumab is indicated by the FDA and EMA for all the previous diseases (approved for etanercept and infliximab) plus non-infectious uveitis. In addition, the FDA approved adalimumab as therapy for hidradenitis suppurativa (acne inversa). None of the anti-TNF drugs have been approved by either the FDA or EMA for any of the monogenic autoinflammatory diseases.

TNF Blockers in Autoinflammatory Diseases

Familial Mediterranean Fever

In FMF, the experience with anti-TNF agents has been overall scarce and unclear. Two small series of 10 and 14 patients with crFMF and concomitant inflammatory conditions (chronic arthritis, ankylosing spondylitis, juvenile idiopathic arthritis, psoriasis, or Crohn disease) treated with all the three TNF blockers resulted in good control of FMF and the associated disease (115, 116). Retrospective data included in the Eurofever Registry showed complete and partial response with etanercept (in seven and nine cases, respectively), infliximab (seven with complete and eight with partial response), and adalimumab (three with complete and two with partial response) (15). Similarly to the previous series, in this registry, anti-TNF agents seemed also to exert more benefit in cases with predominant arthritis (15). In a multicenter international retrospective study including 27 FMF patients treated with biologic agents, two patients received adalimumab and two etanercept as first agent. While one patient experienced a complete response, the remaining three had to discontinue the drug due to lack of efficacy after a mean duration of therapy of 9.3 months (117). The level of evidence of the efficacy of anti-TNF agents in crFMF, especially in those with articular involvement, is 3, but no grade of recommendation has been provided (13).

Tumor Necrosis Factor Receptor–Associated Periodic Fever Syndrome

In TRAPS, etanercept is the anti-TNF agent with the best reported results, because it has shown to prevent or reduce the intensity of attacks and the dose of glucocorticoids previously controlling disease activity (15, 117). However, it is relatively common that etanercept has to be discontinued because of lack of efficacy (15, 117), which has been reported after a period of 3.3 years (118). Although SHARE consensus recommends etanercept in some patients with a level of evidence 2B and grade of recommendation C, the experts also inform that the effect might decline over time (14).

In the Eurofever Registry, among 121 TRAPS patients treated with etanercept, 88% experienced a satisfactory response, which was complete in 26% of them (15). A multicenter international retrospective study including 47 TRAPS patients, 41 with pathogenic mutations and 6 with the R92Q variant, analyzed 20 and 4 patients treated with etanercept in each group, respectively. Among TRAPS caused by pathogenic mutations, ∼50% of those receiving etanercept could achieve a complete clinical response. In addition, a total of 13 of 20 (65%) TRAPS patients discontinued anti-TNF, mostly due to a lack of efficacy. Compared to patients receiving anakinra, those treated with etanercept experienced less frequently a complete response and higher drug discontinuation rates (117). With regard to the four R92Q-TRAPS patients receiving etanercept as first-line therapy, only one (25%) had a complete response (117). Etanercept has also been reported effective in isolated TRAPS patients carrying the R92Q variant (57).

The use of infliximab and adalimumab has been associated with severe paradoxical reactions, and therefore, they are currently not recommended in TRAPS. While etanercept is a receptor fusion protein, infliximab, and adalimumab are TNF monoclonal antibodies. One of the proposed mechanisms by which infliximab is involved in a hyperinflammatory response is related to the failure in shedding infliximab-bound receptor from the cell surface, with the subsequent activation of antiapoptotic mechanisms and a widespread inflammatory response (171).

Hyperimmunoglobulin D Syndrome/Mevalonate Kinase Deficiency

In HIDS/MKD, anti-TNF therapy can improve frequency and intensity of attacks. However, because anti–IL-1 agents can be considered the first-line therapy for HIDS/MKD patients (14, 95), TNF blockers are recommended as a second option (together with IL-6 blockade) in case of IL-1 blockers are ineffective or not tolerated, with a level of evidence 4 and grade of recommendation D (14).

In the Eurofever Registry, etanercept was the most frequently used anti-TNF drug, providing any improvement in 26 of 44 (59%) of HIDS/MKD patients, which was complete in seven (16%) of them (15). A multicenter retrospective study of eight HIDS/MKD patients treated with etanercept showed higher complete response rates (88%). However, etanercept was discontinued in four patients (50%) due to lack of efficacy (117).

Cryopyrin-Associated Periodic Syndromes

No evidence of efficacy of biological therapy (including anti-TNF agents) other than IL-1 blockade in CAPS patients is available (119).

PFAPA Syndrome

To date, no PFAPA cases treated with anti-TNF agents have been reported.

Anti–IL-6 Agents: Tocilizumab

Tocilizumab is a humanized monoclonal anti–IL-6 receptor antibody currently labeled by the FDA and EMA for rheumatoid arthritis, systemic and polyarticular juvenile idiopathic arthritis, giant cell arteritis and for the treatment of chimeric antigen receptor T cell–induced severe or life-threatening cytokine release syndrome. Tocilizumab has not been approved for any of the monogenic autoinflammatory diseases because the experience of its use, usually in patients unresponsive to other biologic agents, is still occasional.

TABLE 3 | Biological agents (other than IL-1 blockers), types of studies supporting the maximum evidence level, and response to treatment of the main monogenic autoinflammatory diseases (crFMF, TRAPS, HIDS/MKD, and CAPS)*.

Drug	Disease	Type of study	Response	References
Anti-TNF *Etanercept Infliximab Adalimumab*	crFMF	RCS	Partial or complete response in few patients. Better efficacy in patients with articular involvement	(15, 115, 116)
	TRAPS**	RCS	Good response in 88% of patients, complete in 25–50% of them	(15, 117, 118)
	HIDS/MKD	RCS	Good response in 59–88% of patients, complete in 16% of them Lack of efficacy over time	(15, 117)
	CAPS	CR	No response	(119)
Anti- IL-6 *Tocilizumab*	crFMF	RCS, CS, CR	Good response in patients resistant to colchicine, anti-IL-1, and anti-TNF agents	(120–128)
	TRAPS	CR	Good response in patients refractory to anti-TNF and anti-IL-1 agents	(129–131)
	HIDS/MKD	CR	Good response in patients refractory to anti-TNF and anti-IL-1 agents	(132–136)
	CAPS	CR	No response	(119, 137)
JAK-inhibitors *Tofacitinib*	crFMF	CR, CS	Good response in patients refractory to anti-TNF, anti-IL-1, and anti-IL-6 agents	(138–140)

*No evidence about the use of these drugs in PFAPA syndrome.

**Infliximab and adalimumab are not recommended in TRAPS since their use has been associated with severe paradoxical reactions.

CAPS, cryopyrin-associated periodic syndromes; CR, case report; CS, case series; crFMF, colchicine-resistant familial Mediterranean fever; HIDS/MKD, hyperimmunoglobulin D and periodic fever syndrome/mevalonate kinase deficiency; IL, interleukin, JAK, Janus kinase; PFAPA, periodic fever with aphthous stomatitis, pharyngitis and cervical adenitis; RCS, retrospective cohort study; TNF, tumor necrosis factor; TRAPS, TNF-receptor associated periodic fever syndrome.

Tocilizumab in Autoinflammatory Diseases

Familial Mediterranean Fever

Tocilizumab has been successful in controlling disease activity in the majority of patients with crFMF reported as single cases, with good control of secondary amyloidosis in some of them (120–125). Tocilizumab effects over proteinuria in FMF-associated amyloidosis has been analyzed in two series of 11 and 12 patients resistant to colchicine, anti–IL-1, or anti-TNF agents, in whom the previous colchicine therapy was maintained (126, 127). Proteinuria improvement in any degree was achieved by 7 of 11 patients (64%) and 9 of 12 patients (75%), respectively (126, 127). In two of the responder patients, tocilizumab was discontinued, and proteinuria returned, with good control after restarting IL-6 blockade (128).

Other Autoinflammatory Diseases

Tocilizumab has been successfully used in three patients with TRAPS (129–131) and in seven HIDS/MKD cases (132–136) in whom anti-TNF and/or anti–IL-1 agents had previously failed. Negative results have been reported in two CAPS (CINCA/NOMID) patients treated with tocilizumab (119, 137). No evidence on the use of tocilizumab in PFAPA syndrome is currently reported.

JAK Inhibitors

JAK inhibition suppresses the constitutive phosphorylation of the transcription factor STAT-1, which blocks the induction of IFN-stimulated genes and subsequently leads to the regulation of the uncontrolled IFN production, causing inflammatory manifestations. Janus kinase inhibitors, such as tofacitinib, baricitinib, and ruxolitinib, are currently approved by the FDA and EMA for rheumatoid arthritis, psoriatic arthritis, and ulcerative colitis (tofacitinib), rheumatoid arthritis (baricitinib), and myelofibrosis and polycythemia vera (ruxolitinib, also approved for steroid-refractory acute graft-vs.-host disease by the FDA).

JAK inhibitors have demonstrated positive effects in several monogenic autoinflammatory diseases mediated by type I IFN (also named interferonopathies), including chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperature/proteasome-associated autoinflammatory syndrome, STING-associated vasculopathy with onset in infancy, familial chilblain lupus, and Aicardi–Goutières syndrome (172–175).

Because the pathogenic mechanism of the diseases included in this review consists mainly in the abnormal function of the inflammasome, as expected, the use of JAK inhibitors in monogenic inflammasomopathies remains anecdotal and limited to six crFMF patients previously failing to IL-1, TNF, and IL-6 blockers. However, interestingly, these cases were successfully treated with tofacitinib (138–140).

Table 3 summarizes all the biological agents (other than IL-1 blockers) used in the main monogenic autoinflammatory diseases and PFAPA syndrome, and types of studies supporting the maximum evidence level for their use.

PROPOSAL OF A PRACTICAL GUIDE FOR TREATING THE MAIN MONOGENIC AUTOINFLAMMATORY DISEASES AND PFAPA SYNDROME

Based on the maximum level of evidence and grade of recommendation for FMF, CAPS, TRAPS, HIDS/MKD, and PFAPA syndrome, a proposed practical scheme for the treatment of these conditions is illustrated in **Figure 1**. Evidence-based recommendations for the treatment of the main monogenic autoinflammatory diseases have been

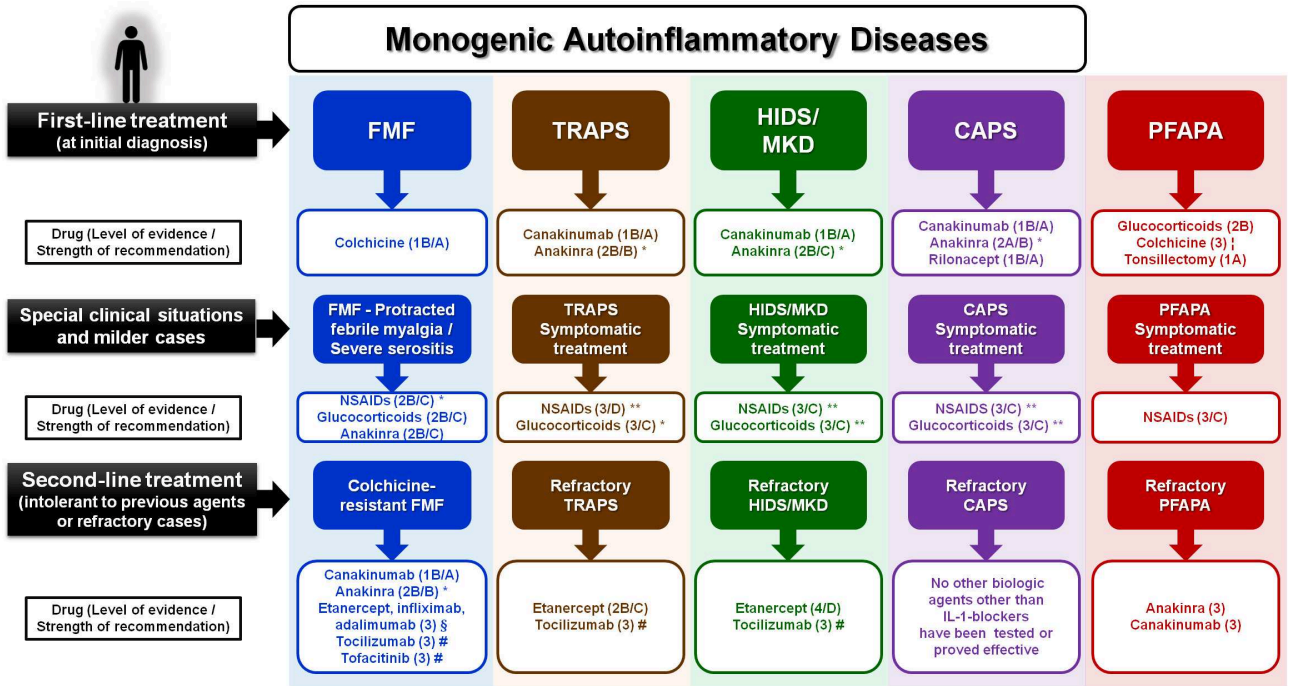

FIGURE 1 | Proposed approach for the treatment of the main monogenic autoinflammatory diseases and PFAPA syndrome, based on the maximum level of evidence and grade of recommendation (when available) for each drug and disease.
No strength of recommendation is provided when expert opinion consensus still does not exist.
Thalidomide and dapsone have also been reported of benefit in some colchicine-resistant FMF patients (data within the text).
*This drug has been proved effective with continuous and on demand administration.
**This drug is recommended mostly in short courses (on demand) during attacks.
¦Colchicine in PFAPA patients has been reported more effective in carriers of heterozygous variants in the *MEFV* gene.
§Anti-TNF agents (etanercept, infliximab, and adalimumab) have been shown more useful in FMF patients with prominent articular manifestations.
#Tocilizumab has been used with efficacy in few patients with FMF, TRAPS, and HIDS/MKD who failed to anti–IL-1 and/or anti-TNF agents.
#Tofacitinib has shown good response in few patients with colchicine-resistant FMF who also failed to IL-1, TNF, and IL-6 blockers.
CAPS, cryopyrin-associated periodic syndrome; FMF, familial Mediterranean fever; HIDS/MKD, hyperimmunoglobulin D and periodic fever syndrome/mevalonate kinase deficiency; PFAPA, periodic fever with aphthous stomatitis, pharyngitis and cervical adenitis; TRAPS, TNF receptor–associated periodic syndrome.

extracted from previous consensus studies, such as the EULAR recommendations for the management of FMF (13) and the European SHARE recommendations for the management of TRAPS, HIDS/MKD, and CAPS (14). Additional information used to generate levels of evidence has been incorporated mostly from results of new clinical trials, international multicenter registries, and retrospective data mainly from referral centers. According to the EULAR standard operating procedures for developing best practice to endorse recommendations, only the level of evidence is provided in those diseases with new therapeutic information but without recommendations issued by consensus expert opinion (11, 12).

The proposed therapeutic approach includes three different levels or situations: (a) first-line treatment (at the time of disease diagnosis or when symptomatic treatment used for milder situations is not effective); (b) milder cases or special clinical situations (including severe serositis or protracted febrile myalgia in case of FMF, or symptomatic treatment in milder presentations for the remaining conditions); and (c) second-line treatment (in case of patients not responding to first-line drugs or other biologic agents).

STILL UNSOLVED AND OPEN QUESTIONS

Despite the great efforts and progresses achieved during the last years in the treatment of monogenic autoinflammatory diseases, several questions remain still pending to be answered:

- how to define resistance or failure to any biologic drug?
- how to demonstrate the effect of biologic agents in preventing secondary amyloidosis?
- which is the right therapeutic window to initiate or switch to a biologic agent?
- which biological agent should be used as first?
- when it is time to switch to another biologic agent, and which biologic?
- how to identify the right moment to start on-demand treatment and when changing from continuous to on demand administration is appropriate?

- how to establish, if possible, reduction and discontinuation of any treatment used?

NEW TREATMENT STRATEGIES IN AUTOINFLAMMATORY DISEASES

It is of crucial importance to maintain and boost the international collaboration with patients' registries for all the autoinflammatory diseases and also to promote initiatives that allow the generation of genetic, clinical, and therapeutic research. The latter should include drug development in preclinical (phases I and II) trials, whereas phase III trials (evaluating safety) should be now focused on testing new drugs and also on performing head-to-head comparisons between the current biologic agents, respectively.

With regard to preclinical trials, because the abnormal activation of the NLRP3 inflammasome seems to be implicated in conditions other than autoinflammatory diseases, such as diabetes, atherosclerosis, and cardiovascular and neurodegenerative diseases, several *in vitro* and *in vivo* studies are exploring the potential role of different pharmacological inhibitors of the NLRP3 inflammasome in NLRP3-associated diseases (176). While some of these new agents are small molecule inhibitors directly acting on the NLRP3 protein, others are targeting different components and products of the inflammasome. These new agents include glyburide, 16673-34-0, JC124, FC11A-2, parthenolide, VX-740, VX-765, Bay 11-7082, BHB, MNS, CY-09, OLT1177, oridonin, MCC950, and tranilast (176). Among them, tranilast and other small molecules, such as IZD174 and IZD334 (related to MCC950), are being used in patients with CAPS, in phases I and II clinical trials.

Currently, the efficacy and safety of alternative biologic drugs and small molecules are being studied or pending of results in several ongoing clinical trials, including:

1) Tocilizumab for the treatment of FMF—a randomized, double-blind, phase II proof-of-concept study (ClinicalTrials.gov identifier: NCT03446209). This trial will analyze the efficacy of tocilizumab in adult patients with crFMF, also failing to other biologic agents.
2) A randomized, double-blind, parallel-group comparison trial of tocilizumab for colchicine-resistant FMF (UMIN-CTR Clinical Trial ID: UMIN000028010). This (phase III) trial will analyze the efficacy of tocilizumab in patients from 12 to 75 years with crFMF (177).
3) An open-label continuation trial of tocilizumab for FMF with colchicine ineffective or intolerance (UMIN-CTR Clinical Trial ID: UMIN000032557). This trial is expected to obtain evidence regarding the long-term safety of tocilizumab in crFMF patients (178).
4) Safety and tolerability, pharmacokinetic, and pharmacodynamic study with IZD334 (ClinicalTrials.gov identifier: NCT04086602). This is the first-in-human, single-center, double-blind, randomized, crossover (phase I) study of IZD334 (a small molecule inhibitor of the NLRP3 inflammasome) conducted in healthy adults as well as an open-label cohort in adult patients with CAPS.
5) Safety and tolerability, pharmacokinetic, and pharmacodynamic study with inzomelid (ClinicalTrials.gov identifier: NCT04015076). A phase I, randomized, double-blind, placebo-controlled, single- and multiple-ascending-dose study to determine the safety, tolerability, pharmacokinetics, pharmacodynamics, and food effect of inzomelid (or IZD174, a small molecule inhibitor of the NLRP3 inflammasome) in healthy adult participants, as well as an open-label cohort to confirm the safety, pharmacokinetics, and pharmacodynamics of inzomelid in adult patients with CAPS.
6) A clinical study of tranilast in the treatment of CAPS (ClinicalTrials.gov identifier: NCT03923140). A single-arm prospective cohort (phase II) study designed to observe the efficacy and safety of tranilast (a small molecule inhibitor of the NLRP3 inflammasome) in CAPS patients.
7) Ilaris® (canakinumab) in PFAPA patients (ClinicalTrials.gov identifier: NCT02775994). This single-arm open-label pilot study will analyze the efficacy of canakinumab during the first 2 months of treatment in 10 children with PFAPA syndrome experiencing frequent flares.

CONCLUDING REMARKS

- Colchicine, used at therapeutic doses, is the criterion-standard treatment for FMF and of some help in PFAPA patients, mostly in those carrying heterozygous *MEFV* gene mutations.
- NSAIDs improve symptoms in most patients with any autoinflammatory disease, but they are usually insufficient to control symptoms and do not influence the underlying cause of the disease in any of them.
- Glucocorticoids, generally used at medium or high doses, may be effective when administered on demand (in short courses) in most of the monogenic conditions. In some TRAPS patients, continuous administration of glucocorticoids may be also useful. Anyway, because most patients will require a biologic agent to control the disease activity, glucocorticoids may be reserved as initial treatment to prove response or may be also administered in a continuous or on-demand modality for mild/non-severe cases. Glucocorticoids have demonstrated special utility in FMF patients experiencing protracted febrile myalgia and severe serositis (in a short course at high doses) and in PFAPA patients (in a single high-dose administration), representing the treatment of choice in PFAPA syndrome.
- Among biologic agents, IL-1 blockers are the most relevant and useful drugs in the treatment of the main monogenic autoinflammatory diseases, especially in those considered inflammasomopathies.
- Anakinra and canakinumab have FDA and EMA approval as first-line treatment for CAPS patients, whereas rilonacept has been approved only by the FDA. Both anakinra and canakinumab are of clear utility in crFMF, TRAPS, and HIDS/MKD patients, and canakinumab has been recently approved by the FDA and EMA for these three conditions. Anakinra has been used with success on demand in patients

with all monogenic diseases and PFAPA syndrome and therefore may be reserved for selected patients, mainly for those presenting with prominent manifestations or recognizable triggers preceding the attacks.
- Information regarding other alternative biologic agents is scarce and extracted from small case series and case reports including patients who did not respond to other biologics.
- Anti-TNF agents, and in particular, etanercept, seem to exert some benefit in FMF patients with prominent articular manifestations and also in TRAPS and HIDS/MKD patients.
- Tocilizumab (anti–IL-6 agent) has been successful in controlling disease activity and improving proteinuria in patients with FMF-associated amyloidosis and also in few TRAPS and HIDS/MKD patients resistant to anti-TNF and/or anti–IL-1 agents.
- The use of JAK inhibitors in monogenic inflammasomopathies is limited to the use of tofacitinib with good response in few crFMF patients previously failing to IL-1, TNF, and IL-6 blockers.
- The knowledge of genetic and pathogenesis of these autoinflammatory diseases has to be encouraged in order to discover new potential molecular targets leading to new specific drugs.
- Further randomized clinical trials proving the efficacy and safety of the currently promising biologic drugs and the new potential agents and small molecules are still needed.

AUTHOR CONTRIBUTIONS

AS and JH-R designed, wrote and reviewed the article, and created the original figure. MS, GEs, RM, GEm, and LC collaborated with the writing and the revision of the manuscript.

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