Molecular mechanisms of phenotypic variability in monogenic autoinflammatory diseases

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Abstract | Monogenic autoinflammatory diseases are a group of rheumatologic disorders caused by dysregulation in the innate immune system. The molecular mechanisms of these disorders are linked to defects in inflammasome-mediated, NF-κB-mediated or interferon-mediated inflammatory signalling pathways, cytokine receptors, the actin cytoskeleton, proteasome complexes and various enzymes. As with other human disorders, disease-causing variants in a single gene can present with variable expressivity and incomplete penetrance. In some cases, pathogenic variants in the same gene can be inherited either in a recessive or dominant manner and can cause distinct and seemingly unrelated phenotypes, although they have a unifying biochemical mechanism. With an enhanced understanding of protein structure and functionality of protein domains, genotype–phenotype correlations are beginning to be unravelled. Many of the mutated proteins are primarily expressed in haematopoietic cells, and their malfunction leads to systemic inflammation. Disease presentation is also defined by a specific effect of the mutant protein in a particular cell type and, therefore, the resulting phenotype might be more deleterious in one tissue than in another. Many patients present with the expanded immunological disease continuum that includes autoinflammation, immunodeficiency, autoimmunity and atopy, which necessitate genetic testing.

Monogenic
A phenotype or disease that is caused by variation in a single gene and has well-defined inheritance pattern.

Phenotype
An organism's observable traits such as height, hair colour or blood type.

Null alleles
Genetic changes that cause a complete lack of protein expression or can notably alter protein function.

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Monogenic autoinflammatory disorders have been linked to germline and somatic pathogenic variants in over 40 genes[1,2]. As these disease-causing variants have a major effect on protein function, one might expect that phenotypes associated with each of these mutated genes have a high degree of clinical similarity, yet there are numerous examples of variability in disease severity and expression that are still poorly understood.

Various molecular mechanisms can lead to phenotypic variability in autoinflammatory conditions (Supplementary Table 1). For some of these disorders, the explanation is more straightforward than for others, as is generally the case with diseases linked to enzyme deficiencies. Null alleles tend to cause severe congenital syndromes, whereas hypomorphic or somatic variants lead to milder and late-onset forms of the disease, and this difference could translate into different clinical diagnoses[3]. Pathogenic variants that affect residues critical for protein function, such as phosphorylation, protease cleavage or glycosylation, will have more deleterious effects than other less-critical genetic alterations in the same gene. The magnitude of phenotypic effects of a pathogenic variant is also closely related to its location in distinct structural protein domains as the genetic change might interfere with intramolecular and intermolecular interactions. As more disease-associated variants are discovered and mapped onto protein domains, a better understanding of genotype–phenotype correlations is likely to emerge (BOXES 1,2).

In this Review, we discuss the mechanisms underlying the phenotypic variability of a subset of monogenic autoinflammatory disorders that present with remarkable phenotypic variability and, in some cases, different inheritance patterns in the same gene. The diseases covered include various inflammasome-mediated disorders, nucleotide-binding oligomerization domain 2 (NOD2)-associated diseases, caspase recruiting domain 14 (CARD14)-associated diseases, inflammatory actinopathies, interferonopathies and diseases caused by enzyme dysregulation.

Pyrin inflammasome-mediated diseases

Inflammasomes are multiprotein complexes that function as intracellular sensors for the recognition of pathogens (pathogen-associated molecular patterns) and endogenous danger-associated molecular patterns[4]. Inflammasomes are nucleated by proteins that belong to the family of NOD-like receptor (NLR)

Key points

- Mendelian-inherited pathogenic variants in a given gene can have different inheritance patterns and can cause distinct and sometimes opposing clinical phenotypes.
- Pathogenic variants in the same gene can have variable disease expressivity depending on their effect on protein function.
- Pathogenic variants in different genes can result in a similar phenotype by virtue of converging on the same signalling pathway (as exemplified by the spectrum of phenotypes denoted as familial cold autoinflammatory syndromes (FCAS).
- A growing number of autoinflammatory diseases can be explained by non-Mendelian inheritance of somatic variants.
- Although systemic inflammation and fevers are common features of autoinflammatory diseases, specific organ manifestations are determined by the tissue expression of mutated proteins.
- Other genetic alleles and risk factors, such as infections and stress, also contribute to the phenotypic variability of autoinflammatory diseases.

Somatic variants
Genetic alterations that occur post-zygotic in somatic cells (for example, leukocytes and keratinocytes) and are not passed on to children.

Genotype
An organism's complete set of genetic material; this term can be used to refer to the variants of a single gene or a set of variants in multiple genes.

Mendelian
The manner by which genes and traits are passed from parents to their offspring, described by Gregor Mendel.

Oligogenic
A phenotype or disease that is dependent on a few genes and is an intermediate between monogenic and polygenic inheritance.

proteins (also known as nucleotide-binding leucine-rich repeat receptors; discussed in a latter section on NLR-like receptor-associated diseases), the protein absent in melanoma 2 (AIM2) or pyrin (as discussed in this section). The aberrant activation of inflammasomes leads to the caspase 1-mediated production of pro-inflammatory cytokines of the IL-1 family. The activity of caspase 1 also induces a specific type of cell death known as pyroptosis, which results in the release of pro-inflammatory intracellular components and cytokines from dying cells[5,6]. Disease-causing variants have gain-of-function effects on the inflammasome pathway; however, these variants have different thresholds for the autoactivation of inflammasomes, which ultimately determines whether one or two pathogenic variants are necessary to trigger inflammation. Although high-penetrance variants lead to the constitutive activation of inflammasomes, by triggering ligand-independent protein oligomerization, milder variants might instead reduce the threshold for activation and enable inflammasome assembly (FIG. 1). The clinical presentation of inflammasomopathies is dependent not only on the protein domain affected by the pathogenic variant but also on the cell-specific expression of these proteins.

Box 1 | **Genotype–phenotype relationships**

Advances in high-throughput sequencing technologies have provided a vast amount of data on genetic variations in humans and thus have revolutionized the conceptual thinking from human monogenic disease towards a continuum of Mendelian–digenic–oligogenic inheritance and the occurrence of somatic variants. The assumption of one gene–one phenotype is overly simplified as heritability is more complex and might change over a lifetime with acquired genetic variations. Pathogenic variants in the same gene can have different inheritance patterns and can lead to distinct conditions and sometimes even contrasting phenotypes depending on whether the variants exert a gain-of-function or a loss-of-function effect on protein function. In the same gene, severe high-penetrance variants can cause the disease phenotype in a monoallelic state, whereas milder variants might need to be doubled in '*cis*' or '*trans*' to attain a deleterious effect. The effect of somatic pathogenic variants is influenced by the time of occurrence during development and the affected cell types. Genetic heterogeneity and digenic inheritance are other factors to consider for autoinflammatory disorders, exemplified by proteasome-associated diseases. Genetic testing has become instrumental in the diagnostics of most immunological diseases owing to an increasing number of patients who present with a continuum of features, including autoinflammation, autoimmunity and immunodeficiency.

Pyrin-associated autoinflammatory disease

Pyrin, encoded by *MEFV*, forms an inflammasome in response to bacterial toxin-induced inactivation of the host GTPase protein Ras homologue gene family member A (RhoA) and changes in the actin cytoskeleton[7]. GTPases function as 'molecular switches' by cycling between inactive (GDP-bound) and active (GTP-bound) conformations to regulate many effector proteins and a variety of signalling pathways[8]. Pathogens modulate RhoA GTPase activity to suppress host immune responses, and these changes are sensed by pyrin. The activation of RhoA results in pyrin inhibition through the phosphorylation of pyrin at residues Ser208 and Ser242 mediated by protein kinase N (PKN1 and PKN2)[9,10]. Pyrin is highly expressed in myeloid cells[11], and pathogenic variants activate pyrin in different ways but ultimately cause proteolytic cleavage of caspase 1 and the release of the pro-inflammatory cytokines IL-1β and IL-18 (FIG. 1). Activating variants result in a variety of phenotypes owing to alleles inherited in a dominant or recessive manner[12].

Familial Mediterranean fever. The most common pyrin-associated recessively inherited disease is familial Mediterranean fever (FMF). FMF is characterized by recurrent episodes of short-lasting fever accompanied by serositis, erysipelas-like erythematous rash and mono-articular arthritis, which can lead to potentially fatal amyloidosis[13]. Inflammatory attacks in FMF are often precipitated by stress[14]. Classical FMF is caused by biallelic missense variants that reside almost exclusively in exon 10, which encodes the C terminal B30.2/SPRY domain[15]. The function of the B30.2 domain is still unclear, and it remains to be seen whether this domain has a pro-inflammatory or autoinhibitory function. The most common pathogenic variants affect the amino acid residues Met680 and Met694[12] (FIG. 2a). Homozygosity for Met694Val is associated with the severe form of FMF and susceptibility to serum amyloid A (SAA) amyloidosis[16]. Although recessively inherited diseases typically result from loss-of-function variants, FMF-associated variants are considered gain-of-function and have a gene dosage effect[17]. Notably, approximately one-third of patients with clinical symptoms carry a single pathogenic variant in *MEFV*[18]. Additionally, asymptomatic individuals who are heterozygote carriers for FMF-associated variants have increased serum levels of acute phase reactants compared with individuals without an FMF-associated variant[19]. A study in primary human monocytes showed that FMF hypermorphic variants have an increased ability to sense bacterial toxin-mediated RhoA inhibition and that these variants lower the threshold for activation of the pyrin inflammasome[20]. Given the existence of an intermediate trait in heterozygote carriers, FMF might be considered a disease with an incomplete dominance inheritance pattern.

The carrier frequency of FMF-associated variants is as high as 10% in multiple Mediterranean populations[21], which has raised the question as to whether these variants might be under some form of positive evolutionary selection. Notably, one study has shown that FMF-associated mutations confer a heightened

Box 2 | **Effects of genotype on protein function**

Aside from enzyme deficiencies, there are common themes in the molecular mechanisms underlying autoinflammatory disorders. Disease-causing variants might follow a dominant or recessive inheritance pattern but fundamentally activate inflammatory pathways either by an upregulation of innate immune sensing pathways or by a lack of proteins that downregulate inflammatory responses. Other mechanisms that can cause unrestrained inflammatory responses are related to defects in protein degradation pathways that lead to upregulation in endoplasmic reticulum stress and the unfolded protein response. Nearly all disease-associated variants identified in 'immune sensors' (such as inflammasomes, nodosomes and signalosomes) are missense substitutions with a gain-of-function effect. High-impact pathogenic variants in highly conserved domains lead to the constitutive activation of proteins and result in severe early-onset phenotypes. Milder variants facilitate the autoactivation of inflammasomes or other immune sensors and, in some instances, necessitate additional factors such as stress, cold or heat to reach the threshold for autoactivation. The deficiency of proteins that negatively regulate inflammatory pathways, including NF-κB, type I interferon and IL-1 signalling pathways, leads to uncontrolled cytokine production manifesting with a variable degree of inflammation. With increasing knowledge about proteome function, regulation and interactions, we are beginning to elucidate the mechanisms of phenotypic variability in human traits and diseases. Cryogenic electron microscopy will ultimately help us better understand the functional consequences of various pathogenic variants and the molecular basis of diseases.

Dominant
The type of inheritance pattern referring to when a single copy of the altered gene is sufficient to cause disease or express the trait.

Recessive
A type of inheritance pattern referring to when both copies of a gene are required for the phenotype or disease expressivity.

Biallellic
A term used to refer to both alleles of a single gene or gene locus (both paternal and maternal).

Missense variants
Genetic changes in a single nucleotide that might or might not result in the substitution of one amino acid for another in the protein.

Hypermorphic variants
A type of genetic change (also known as a gain-of-function mutation) where the altered gene product has an increased level of activity or is expressed at higher levels. These mutations are typically dominantly inherited.

Monoallelic
A term used to refer to when only one of the two copies of a particular gene (alleles) is actively expressed and the other allele is silent.

Heterozygous
An individual who has two different alleles of a particular gene.

resistance to *Yersinia pestis*, the causative pathogen for plague pandemics[22]. Leukocytes from asymptomatic heterozygote carriers release heightened levels of IL-1β in response to *Y. pestis* compared with cells from non-carriers[22]. Thus, although monoallelic hypermorphic *MEFV* variants have been positively selected to confer heightened resistance to an endemic pathogen, biallelic hypermorphic variants cause a hyperinflammatory disease, that is, FMF.

Pyrin-associated dominant diseases. In addition to the recessively inherited FMF variants, other phenotypes exist that are associated with dominant pathogenic variants in *MEFV*. Booth et al. were the first to report patients with typical FMF and SAA amyloidosis carrying the single Met694del variant[23]. The clinical phenotype associated with this monoallelic pathogenic variant is similar to classical FMF but seems to occur at a later age of onset[24]. Another single amino acid deletion, Ile692del, has been linked to a dominantly inherited disease in some families. Interestingly, the Ile692del is also found in patients with the classical recessive FMF genotype, owing to the high carrier frequency of missense pathogenic mutations in affected populations[25]. The importance of this mutation hotspot is unclear and awaits the solving of the protein crystal structure.

A very different phenotype is observed in patients with the heterozygous variants Ser242Arg or Glu244Lys, which affect the residues critical for pyrin inhibition. Variations at these residues prevent pyrin binding to inhibitory 14-3-3 proteins and cause constitutive activation of the pyrin inflammasome[26,27]. Individuals with these variants present with a specific condition, named pyrin-associated autoinflammation with neutrophilic dermatosis (PAAND), that presents with severe skin inflammation, myalgia, polyarthralgia, longer-lasting episodes of fever and, unlike in FMF, no evidence of serositis. Monocytes from patients with PAAND have a higher spontaneous production of IL-1β and IL-18 than monocytes from patients with FMF[26]. Although earlier reports have suggested that the Ser208 residue is also critical for pyrin inhibition[28], the inflammatory phenotype associated with variants at this location is present only in patients carrying biallelic pathogenic alleles, either Ser208Thr or Ser208Cys, at this residue. The disease is different from PAAND or FMF and manifests with fevers, oral ulcers, purpuric rash, lymphadenopathy and hypereosinophilia, without elevated secretion of IL-18 (REF.[29]).

Dominantly inherited pathogenic variants in a central domain of pyrin, which is composed of B-box and coiled-coil (CC) subdomains, have been linked to severe inflammatory phenotypes that often progress to SAA amyloidosis. Examples include the His478Tyr variant, found in a family presenting with colchicine-resistant long episodes of fever, peritonitis/pleuritis, severe polyarticular arthritis and renal SAA amyloidosis, and various heterozygous substitutions in residue Thr577 reported in several families[30,31]. Another dominantly inherited variant, Pro373Leu, has been reported in four generations of one family, and was associated with severe inflammation and a high incidence of SAA amyloidosis[32]. The molecular effect of these heterozygous variants on protein function is still unknown; however, they cause higher pyrin activation and enhanced cell death in in vitro conditions[33]. The CC domains play a role in protein oligomerization; thus, these monoallelic variants might trigger the assembly of the pyrin inflammasome.

The clinical relevance of low-penetrance variants, such as Glu148Gln (rs3743930) and Pro369Ser (rs11466023), is still debated, although recent functional studies have disputed their pathogenicity[33,34]. These variants have been linked to typical and atypical FMF as well as to many other polygenic, complex inflammatory diseases[35,36].

In summary, *MEFV*-associated disorders could be considered a continuous disease spectrum owing to the different activation levels of the pyrin inflammasome, which translates into variable responses to therapies. Most patients with FMF respond well to colchicine treatment, whereas patients with a severe phenotype and/or amyloidosis require targeted therapy with IL-1 inhibitors[37].

PSTPIP1-associated diseases

Proline serine threonine phosphatase-interacting protein 1 (PSTPIP1; also known as CD2-binding protein 1 (CD2BP1)) is a cytoskeleton-associated adaptor protein that regulates F-actin remodelling and cell migration and is predominantly expressed in leukocytes, including T cells[38]. PSTPIP1 interacting proteins include pyrin, the Wiskott–Aldrich syndrome protein (WASP), the protein-tyrosine phosphatase PTP-PEST and tyrosine-protein kinase ABL1. PSTPIP1 serves as a scaffold protein for guiding PTP-PEST for the dephosphorylation of WASP, a critical mediator of actin-cytoskeletal polymerization in haematopoietic cells (as discussed in more detail in the later section on actinopathies). WASP is of haematopoietic cells[39], and deficiency in WASP gives rise to an X-linked primary

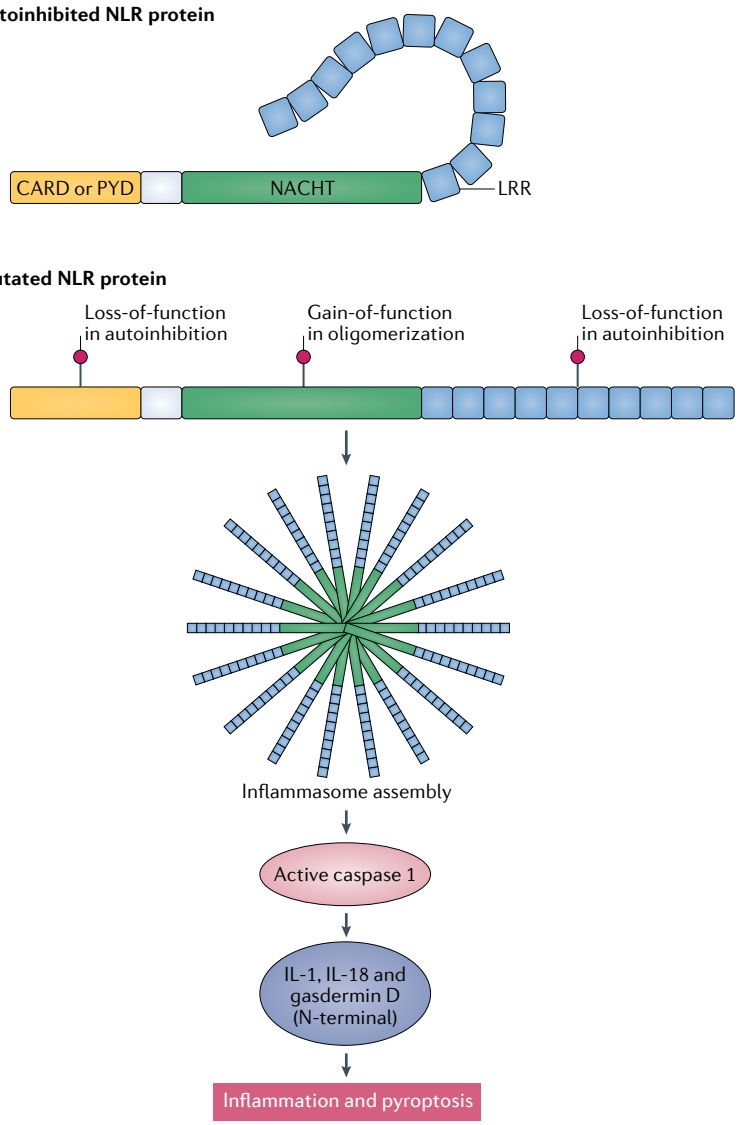

Fig. 1 | **Inflammasome-mediated autoinflammatory disorders. a** | Under physiological conditions, nucleotide-binding domain leucine-rich repeat (NLR) proteins are in a closed form owing to intramolecular domain interactions. **b** | Upon ligand binding (not shown) or in the presence of pathogenic variants, NLR proteins interact with apoptosis-associated speck-like protein containing a CARD (ASC) and caspase 1 to form canonical inflammasomes that process pro-IL-1β and pro-IL-18 into their active forms IL-1 and IL-18, resulting in inflammation and cell death by pyroptosis. The oligomerization of ASC is mediated through the pyrin domain (PYD) of NLRP1 and NLRP3 and the CARD of NLRC4. Gain-of-function variants in the NACHT domain cause inflammasome oligomerization and might result in constitutive activation. Loss-of-function variants in a putative autoinhibitory leucine-rich repeat (LRR) domain or PYD reduce the threshold for activation. The pyrin inflammasome has a different protein structure but is activated in a similar way (not shown).

immunodeficiency, Wiskott–Aldrich syndrome (WAS). Pyrin interacts with PSTPIP1 via its B-box–CC domain; however, the molecular mechanism of this interaction is unclear. The findings of one study suggested that the binding of PSTPIP1 activates pyrin inflammasome by releasing the effects of intramolecular autoinhibition[40].

PSTPIP1-associated arthritis, pyoderma gangrenosum and acne (PAPA) syndrome is a dominantly inherited disorder caused by heterozygous missense pathogenic variants in PSTPIP1 (FIG. 2b). Patients with PAPA present with a variable degree of skin inflammation from severe cystic acne to pyoderma gangrenosum and/or sterile joint inflammation[41]. The most common causal variants, Ala230Thr and Glu250Gln, are located in exons 10 and 11 of *PSTPIP*, which encodes the F-BAR domain[42]. Other pathogenic variants associated with PAPA syndrome are Asp246Asn and Glu256Gly[43,44]. Patients with the Glu250Lys pathogenic variant, which affects the same amino acid residue as in classical PAPA syndrome, or with the Glu257Lys variant present with a distinct phenotype named PSTPIP1-associated myeloid-related proteinemia inflammatory (PAMI) syndrome, also known as hyperzincaemia and hypercalprotectinaemia[45]. This phenotype is far more severe than PAPA syndrome and is characterized by very high serum levels of the pro-inflammatory alarmins S100A8 and S100A9 (REF.[46]). In addition to skin and joint inflammation, patients with PAMI have bone marrow abnormalities that manifest as recurrent infections, bleeding diathesis and autoimmunity similar to WAS[47,48].

Classical PAPA-associated variants are activating mutations that cause the increased production of various pro-inflammatory cytokines, including IL-1β[49]; however, the mechanism by which this effect occurs is not yet fully understood. The PAPA-associated form of PSTPIP1 is hyper-phosphorylated owing to a reduced interaction with PTP-PEST and has a stronger affinity for pyrin[50]. The PAMI-associated Glu250Lys variant has a stronger avidity to pyrin than the Glu250Gln variant, which is attributed to an altered electrostatic potential of PSTPIP1 rather than to an altered level of protein phosphorylation[45]. PSTPIP1 interacts with the B-Box–CC domains of pyrin via its CC–SRC homology domain 3 (SH3) domain, and both the F-BAR and CC domains are known to play a role in protein oligomerization. Fundamentally, all four pathogenic variants lead to the increased activity of the pyrin inflammasome through variable degrees of binding to pyrin; however, the strength of these interactions influences the activity of pyrin and the severity of the inflammatory phenotype.

The heterozygous variants Arg228Cys (rs781341816) and a novel Thr274Met mutation in PSTPIP1 have been identified in two patients with severe T cell deficiency but without signs of autoinflammation[51]. These variants cause an impairment in T cell differentiation by a reduction in F-actin polymerization that is essential for immune synapse formation. LPS-induced stimulation of peripheral blood mononuclear cells (PBMCs) from these patients did not result in increased levels of IL-1β compared with PAPA-associated variants, which might explain the lack of inflammation in these patients.

The C-terminal SH3 of PSTPIP1 is essential for the interaction with WASP and ABL1. The PSTPIP1 variant Arg405Cys (rs201253322) was identified in a boy with aggressive pyoderma gangrenosum and in his father, who had a history of severe acne[52]. This variant impairs PSTPIP1 binding to WASP and does not affect its interaction with PTP-PEST. Another missense variant in the SH3 domain (Gly403Arg) was identified in a patient with pyoderma gangrenosum, acne and ulcerative colitis[53]. Together, these data suggest that PSTPIP1,

Polygenic
A phenotype or disease that is influenced by several genes and often by environmental factors.

via its interaction with WASP, might negatively regulate macrophage migration and function. However, why some patients with pathogenic mutations in PSTPIP1 present only with systemic inflammation and others present with T cell dysfunction is unclear.

Pyoderma gangrenosum, acne and hidradenitis suppurativa (PASH) syndrome is a genetically heterogeneous disease linked to pathogenic variants in *PSTPIP1* and other genes, including *NCSTN*, *NOD2* and *MEFV*[54]. PASH syndrome can be distinguished from PAPA syndrome by the absence of pyogenic arthritis; however, there is a continuum of features common to both disease entities. An increased number of CCTG repeats in the 5′-untranslated region (UTR) of *PSTPIP* as well as Tyr345Cys and Arg405Cys variants in *PSTPIP1* have been identified in patients with PASH syndrome[55–57].

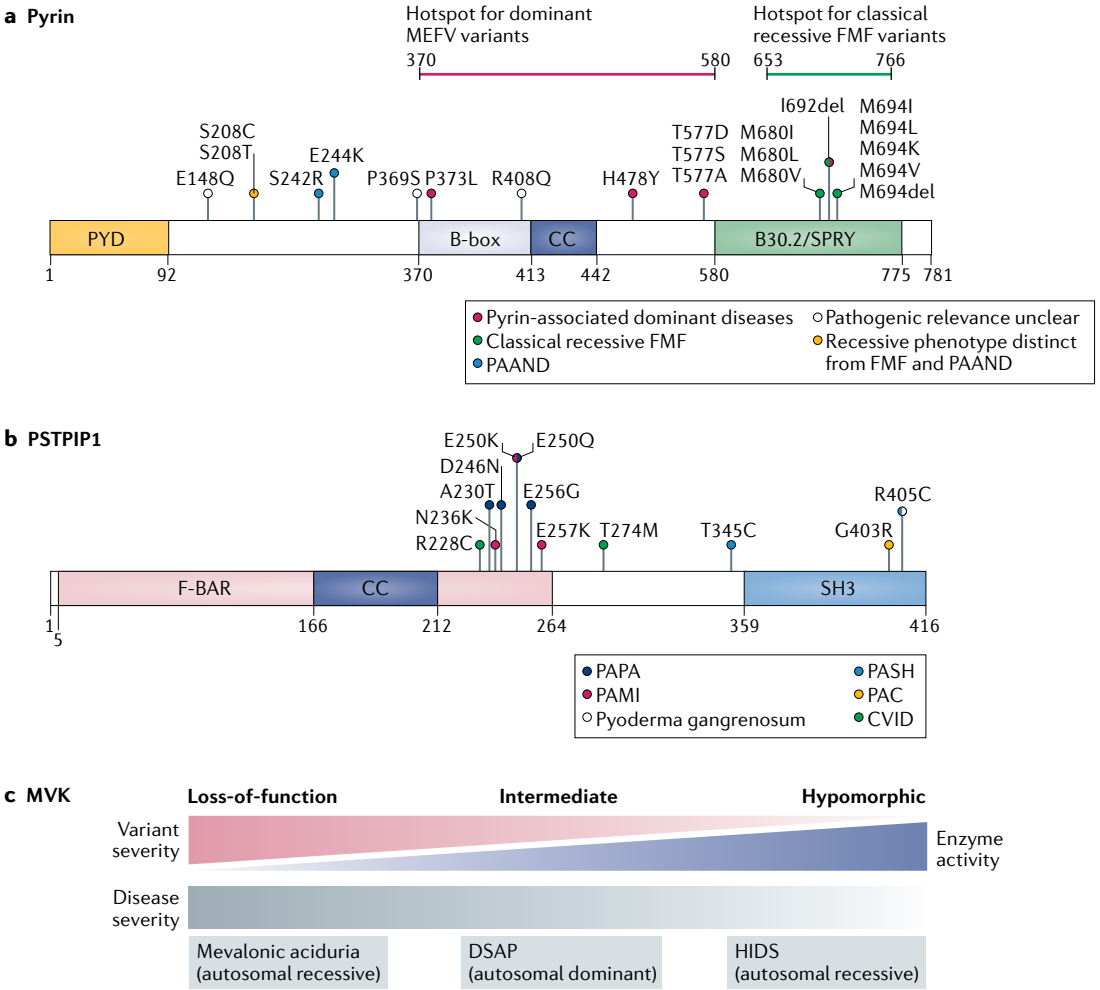

Fig. 2 | **Disease-causing variants of pyrin-associated autoinflammatory diseases.** Variants in pyrin, proline serine threonine phosphatase-interacting protein 1 (PSTPIP1) and mevalonate kinase (MVK) are associated with a spectrum of phenotypes, depending on its effect on protein function. The location and inheritance pattern of various disease-causing variants and their resulting clinical phenotypes are shown. Protein domains are annotated based on the UniProt database[253]. **a** | Dominantly inherited (or de novo) pathogenic variants can cause a severe inflammatory phenotype (shown in red) or the specific condition pyrin-associated autoinflammation with neutrophilic dermatosis (PAAND; shown in light blue), whereas other recessively inherited variants are either associated with classical familial Mediterranean fever (FMF; shown in green) or with a recessive phenotype distinct from FMF and PAAND (shown in yellow). Additional low-penetrance variants have been linked to typical and atypical FMF (shown in white) but their pathogenic relevance is unclear. **b** | Pathogenic variants in PSTPIP1 are either de novo or dominantly inherited and affect various protein interactions. Common variants associated with pyogenic arthritis with pyoderma gangrenosum and acne (PAPA) syndrome are shown in dark blue. The most severe pathogenic variants associated with PSTPIP1-associated myeloid-related proteinemia inflammatory (PAMI) syndrome are shown in red. Other disease-causing variants have been reported in single patients with variable clinical manifestations, including pyoderma gangrenosum (shown in white), pyoderma gangrenosum, acne and hidradenitis suppurativa (PASH) syndrome (shown in light blue), pyoderma gangrenosum, acne and ulcerative colitis (PAC; shown in yellow), or combined variable immunodeficiency (CVID; shown in green). **c** | Recessively inherited loss-of-function (null) variants in *MVK* cause the severe mevalonic aciduria phenotype. By contrast, recessively inherited hypomorphic variants exhibit some residual enzyme activity and are associated to the milder hyper IgD syndrome (HIDS) phenotype. Disseminated superficial actinic porokeratosis (DSAP) is within the MVK deficiency MKD disease spectrum and is caused by one dominantly inherited germline variant together with one skin-specific somatic variant in *MVK*.

Prenylation
A post-translational modification that involves covalent attachment of a lipid consisting of either three (farnesyl) or four (geranylgeranyl) isoprene units to a cysteine residue at or near the C terminus of a protein.

Hypomorphic variants
A type of genetic change (also known as loss-of-function mutation) where the altered gene product has decreased activity or expression. These mutations are typically recessively inherited.

Nonsense mutation
A genetic change that causes the translation of a protein to terminate earlier than would occur with the wild-type gene.

Tyr345 is critical for the dephosphorylation of PSTPIP1 by PTP-PEST[58]. The clinical relevance of the CCTG repeat remains elusive, although it might function as a gene expression modifier.

Thus, pathogenic variants in *PSTPIP1* are linked to a spectrum of diseases manifesting with neutrophilic dermatosis and variable degrees of immunodeficiency. Patients with PAPA require a high dose of anti-cytokine therapies to control inflammation[59], whereas patients with PAMI can be treated effectively with haematopoietic stem cell transplantation (HSCT)[60].

MVK-associated diseases

Mevalonate kinase (MVK) is an ubiquitously expressed enzyme with an important function in cholesterol and isoprenoid biosynthesis[61]. Defects in MVK activity lead to depleted levels of geranylgeranyl pyrophosphate, which is an important metabolic intermediate required for the prenylation of NACHT, LRR and PYD domains-containing protein 3 (NLRP3) and the small GTPases KRas and RhoA[10,62,63]. GTPases play a role in the regulation of the pyrin inflammasome by activating protein kinases (PKN1/PKN2) and phosphatidylinositol-3-OH kinase (PI3K), which keep pyrin in an inactive state. Thus, a dysregulation in protein prenylation in haematopoietic cells results in increased activity of the pyrin and NLRP3 inflammasomes.

The term MVK deficiency encompasses a continuum of mild to severe diseases. Patients with nearly absent enzymatic activity of MVK exhibit recurrent fevers and severe developmental disabilities, and this phenotype is named mevalonic aciduria. Biallelic hypomorphic variants in *MVK* are associated with a milder phenotype historically referred to as hyper IgD syndrome (HIDS) (FIG. 2c). In infancy, patients with HIDS present with episodes of high fever, erythematous rash, pharyngitis, swollen lymph nodes, abdominal pain, diarrhoea, aphthous ulcers, and arthralgia and/or arthritis and symptoms are often triggered by immunization[64]. Patients who carry one null variant and a second hypomorphic pathogenic variant have an intermediate phenotype, less severe than classical mevalonic aciduria and with chronic inflammation. Stimulated PBMCs from patients with MVK deficiency produce higher levels of IL-1β compared with PBMCs from healthy individuals[65].

Most variants causing mevalonic aciduria are nonsense mutations or create truncated proteins, whereas nearly all HIDS-associated variants are missense substitutions and are thought to impair protein stability in a temperature-sensitive manner[66]. Patients with biallelic HIDS-associated variants maintain residual enzyme activity in the range of 1–10%[66]. Two pathogenic variants (Ile268Thr and Val377Ile) account for more than 50% of patients with HIDS in multiple populations. The carrier frequency of Val377Ile in the founder Dutch population is high; however, the disease incidence is lower than predicted, which implies that Val377Ile has a milder effect on protein stability compared with other disease variants[67]. The severity of phenotype in HIDS correlates with the effect of the variant on the expression, folding or stability of MVK, whereas the catalytic properties of the enzyme are not affected[68].

A genome-wide association study identified novel heterozygous loss-of-function variants in *MVK* in Chinese patients with a distinct phenotype named disseminated superficial actinic porokeratosis (DSAP), which presents with potentially malignant keratotic skin lesions and without inflammatory features[69]. DSAP-associated variants were described as dominantly inherited with variable penetrance. The pathogenesis of DSAP was puzzling until a study in 2019 showed that DSAP was caused by a skin-specific deficiency of MVK[70]. Essentially, patients with DSAP carry one germline *MVK* pathogenic variant, which explains the familial inheritance of DSAP, and then acquire a second somatic *MVK* variant in their epidermis.

Overall, patients with nearly absent enzymatic activity have severe developmental disabilities, whereas a partial deficiency of MVK causes the HIDS phenotype. The inflammatory features in patients with HIDS are ameliorated with anti-IL-1 therapy whereas mevalonic aciduria can be effectively treated with HSCT[37,71].

NLR-associated diseases

NLRs are cytosolic innate immune sensors that regulate inflammatory responses and cell death by forming inflammasome complexes[72,73]. Their protein structure is highly conserved and includes a centrally located NACHT domain with ATPase activity, a C-terminal leucine-rich repeat (LRR) domain and a variable N-terminal domain that consists of either a CARD or a pyrin domain (PYD) (FIG. 1). The NACHT domain itself is composed of several subdomains: a nucleotide-binding domain (NBD), two helical domains (helical domain 1 (HD1) and HD2) and a winged-helix domain (WHD). Within the NACHT, several highly conserved motifs are essential for nucleotide-binding and/or hydrolysis, including a P-loop that is specific for ATPase and GTPase and the Mg^{2+}-binding site (known as Walker A and Walker B motifs, respectively)[74]. Nucleotide exchange of ATP for ADP is required for protein activation in response to ligand binding. The LRR domain is thought to serve as an autoinhibitory domain and to function as a ligand sensor. Through intramolecular interactions, the LRR domain keeps the protein in an autoinhibitory state. The ADP-bound 'closed' protein conformation is released upon the binding of specific ligands to the LRR domain and results in conformational changes and the exposure of the N-terminal effector domains (CARD or PYD). This change enables homotypic interactions of NLRs with other CARD-containing or PYD-containing proteins and subsequent inflammasome formation. Pathogenic variants in NLRs are activating variants and lead to the ligand-independent activation of the respective proteins that result in the overproduction of IL-1β and IL-18 (REF.[75]). Although NOD2 belongs to the family of NLR proteins, disease-associated variants in this protein lead to the ligand-independent activation of the NF-κB pathway.

IL-1-mediated or IL-18-mediated diseases

NLRP1-associated diseases. NLRP1 functions as a cytosolic sensor for bacterial toxins and viral dsRNA, including *Bacillus anthracis* lethal toxin[76], and regulates caspase

1-dependent cell death (pyroptosis)[77–79]. This protein is highly expressed in keratinocytes and haematopoietic cells[80]. NLRP1 is unique among inflammasomes in that its C-terminal end is composed of a 'function-to-find' domain (FIIND) and a CARD (FIG. 3a). The PYD and LRR domains of NLRP1 are assumed to suppress its activation. The autoproteolytic cleavage of FIIND leads to the release of a C-terminal CARD domain, which is sufficient for inflammasome activation[81]. The activity of the NLRP1 inflammasome is further regulated by the sequestration of its C terminus[82].

Activating variants in the NLRP1 inflammasome are linked to a spectrum of phenotypes, ranging from a hyperplastic skin disorder to autoinflammation. These pathogenic variants reside in different protein domains and can be inherited in a dominant or a recessive manner but eventually lead to increased NLRP1 self-oligomerization by disrupting autoinhibitory domains (FIG. 3a). For example, heterozygous missense variants in the PYD (Ala54Thr, Ala59Pro, Ala66Val and Met77Thr) are linked to multiple self-healing palmoplantar carcinoma (MSPC) and corneal dyskeratosis[83–85]. These patients have no features of systemic inflammation and instead present with epidermal hyperplasia and susceptibility to malignant squamous cell carcinoma. Biallelic loss-of-function variants in other domains of NLRP1 have been associated with an inflammatory skin phenotype denoted as familial keratosis lichenoides chronica (FKLC) or the phenotypically distinct disorder NLRP1-associated autoinflammation with arthritis and dyskeratosis (NAIAD)[84,86]. Two siblings with FKLC were found to be homozygous for the in-frame deletion Phe787–Arg843del in the first LRR domain of NLRP1. Heterozygous carriers for this variant have a mild phenotype, suggesting that two mutant alleles are necessary for a fully penetrant phenotype[84]. Patients with NAIAD carry the homozygous pathogenic variant Arg726Trp in the linker region between the NACHT and LRR domains and present with a spectrum of inflammatory features, which is in contrast to patients with MSPC or FKLC[86]. Another homozygous variant in the linker domain (Thr775Asn) is reported to cause a severe phenotype called juvenile-onset recurrent respiratory papillomatosis (JRRP), a rare disease that manifests with keratotic skin lesions and recurrent respiratory papillomas that can cause potentially life-threatening airway obstructions[87]. Finally, one patient has been found to carry a de novo heterozygous variant (Pro1214Arg) close to the cleavage site in the FIIND domain, which is notable as NLRP1 activity is dependent on autolytic cleavage within this domain. This patient presented with a more severe inflammatory phenotype and much higher serum levels of IL-1β and IL-18 than two patients with NAIAD[86].

Low-penetrance common single nucleotide polymorphism (SNP) variants in the promoter and non-coding regions of *NLRP1* are associated with susceptibility to vitiligo and other autoimmune and autoinflammatory diseases[88–90]. These variants might augment the transcription or translation of NLRP1 (REF.[89]). The inflammatory phenotype in both monogenic and polygenic NLRP1-associated disorders is most prominent in the skin; however, the disease expression is variable[91].

Overall, irrespective of the mode of inheritance, NLRP1 pathogenic variants activate the pro-inflammatory milieu in the skin, especially IL-1 and IL-18, leading to epidermal hyperplasia.

***NLRP3*-associated diseases.** The NLRP3 inflammasome functions as a cytosolic sensor for a number of pathogen-associated molecular patterns and danger-associated molecular patterns and is critical for host defences[92,93]. NLRP3 is highly expressed in haematopoietic cells, primarily in myeloid cells[94], and its regulation involves various multiple positive and negative regulators, including reactive oxygen species, calcium signalling, ubiquitylation, prenylation, SUMOylation and microRNAs. NLRP3 is further regulated through phosphorylation at residue Tyr861 in the LRR domain and through its interaction with the Ser/Thr protein kinase NEK7 (REF.[95]).

Heterozygous gain-of-function variants in *NLRP3* cause a continuum of phenotypes, denoted as cryopyrin-associated periodic syndrome (CAPS), that ranges from familial cold autoinflammatory syndrome (FCAS) to Muckle–Wells syndrome (MWS) to the most severe phenotype denoted as neonatal-onset multisystem inflammatory disease (NOMID; also known as chronic infantile neurological, cutaneous and articular syndrome (CINCA))[96] (FIG. 3b). Clinical features span from recurrent fevers, urticaria-like rash and arthralgia in patients with FCAS, to early-onset severe systemic inflammation, epiphyseal bone overgrowth, sensorineural hearing loss, vision loss, aseptic meningitis and cognitive disability in patients with NOMID. NOMID is associated with systemic inflammation that is chronic and persistent, whereas flares in FCAS are triggered by cold temperature and humidity. Patients with severe chronic inflammation in the MWS–NOMID spectrum can develop SAA amyloidosis[96].

Most CAPS-associated variants are missense substitutions that reside in the NACHT domain (FIG. 3b). However, the severity of the clinical presentation depends on the position and biochemical properties of these substitutions[93]. Disease-causing variants located around the ATP-binding pocket are usually inherited de novo and cause constitutive inflammasome activation[95]. Other 'milder' variants probably destabilize the interdomain interaction and facilitate the active state of NLRP3.

The only nonsense pathogenic variant in *NLRP3*, Arg554Ter (also known as Arg556Ter), has been described in a patient with FCAS and causes a complete loss of the autoinhibitory LRR domain[97]. Several missense pathogenic variants in the LRR domains have been reported in patients with late-onset symptoms, hearing loss and atypical presentation[98–101] (FIG. 3b). The hearing loss is not always accompanied by clinical evidence of inflammation, indicating a milder impact of these variants.

Thus far, only two pathogenic variants have been identified in the PYD of NLRP3. A novel substitution, Asp31Val, was found in a patient with MWS, and this mutation increases the interaction of NLRP3 with apoptosis-associated speck-like protein containing a CARD (ASC)[102]. A second rare variant, Asp21His

Homozygous
An individual who has inherited the identical alleles of a particular gene from both parents.

De novo
A genetic change that arises in a germ cell or fertilized egg and is not inherited from the parents.

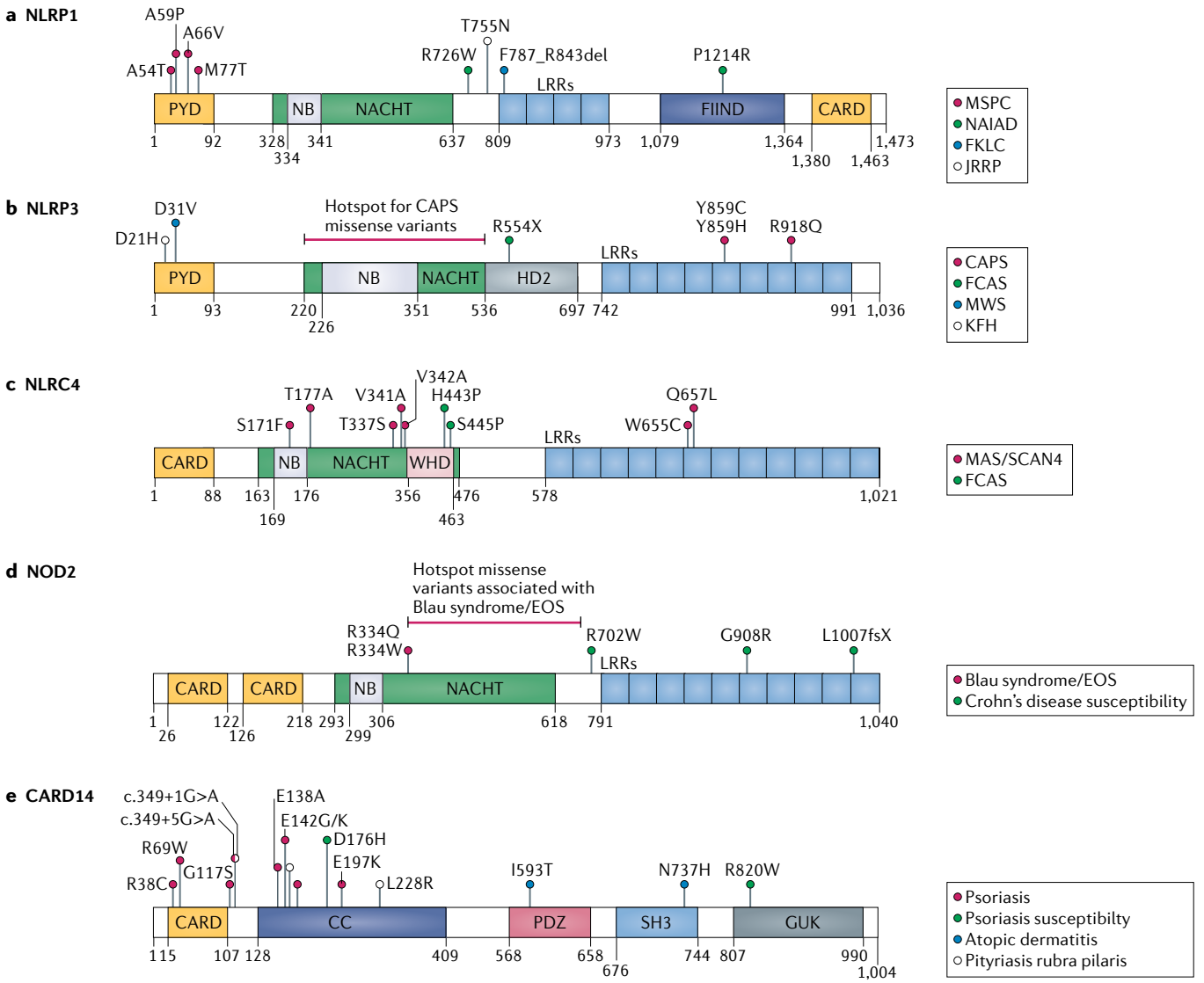

Fig. 3 | **Disease-causing variants of NOD-like receptor-associated diseases.** Numerous variants in the proteins NACHT, LRR and PYD domains-containing protein 1 (NLRP1), NLRP3, NLR family caspase activation and recruitment domain (CARD) domain-containing protein 4 (NLRC4), NACHT domain of nucleotide-binding oligomerization domain-containing protein 2 (NOD2) and CARD14 can cause a variety of autoinflammatory diseases. The inheritance pattern of NOD-like receptor-associated autoinflammatory diseases can be autosomal dominant (or occur de novo) or autosomal recessive. Pathogenic variants are shown in various colours to depict different associated phenotypes. Protein domains are annotated based on the UniProt database[253]. **a** | Pathogenic variants in the pyrin domain (PYD) of NLRP1 that are linked to autosomal dominant multiple self-healing palmoplantar carcinoma (MSPC) are highlighted in red . Recessively inherited variants (or dominantly inherited variants in the case of P1214R) in other domains that cause either juvenile-onset recurrent respiratory papillomatosis (JRRP), NLRP1-associated autoinflammation with arthritis and dyskeratosis (NAIAD) or familial keratosis lichenoides chronica (FKLC) are shown in white, green or blue, respectively. **b** | The NACHT domain of NLRP3 can contain various missense gain-of-function variants (shown across as a red line) that are associated with severe cryopyrin-associated periodic syndrome (CAPS) phenotypes (including Muckle–Wells syndrome (MWS) and neonatal-onset multisystem inflammatory disease (NOMID)), as well as other missense variants associated with the milder familial cold autoinflammatory syndrome (FCAS) phenotype. The only described nonsense variant in *NLRP3* that can cause FCAS is shown in green, whereas the D21H variant (shown in white) is associated with the eye disease keratoendotheliitis fugax hereditaria (KFH). A pathogenic variant D31V (shown in blue) in the pyrin domain has also been reported in patients with MWS. Pathogenic variants in the leucine-rich repeat (LRR) domain are milder but can be associated with neurological phenotypes, for example, sensorineural deafness. **c** | Heterozygous gain-of-function variants in NLRC4 that cause the severe life-threatening condition syndrome of enterocolitis and autoinflammation associated with mutation in NLRC4 (SCAN4) or macrophage-activation syndrome (MAS) are shown in red and milder variants, associated with FCAS, are highlighted in green. **d** | Missense high-penetrance variants in the NOD2 that are associated with Blau syndrome are depicted in red (only the two most common variants are shown). Most common low-penetrance single nucleotide polymorphisms that are associated with susceptibility to Crohn's disease are shown in green. **e** | CARD14 can contain various dominantly inherited novel or rare pathogenic variants that are associated with psoriasis (shown in red), pityriasis rubra pilaris (shown in white) or atopic dermatitis (shown in blue) as well as low-penetrance common variants linked to susceptibility to psoriasis (shown in green). This diagram only shows variants for which there is a sufficient functional evidence that they cause an increase in NF-κB activity. EOS, early-onset sarcoidosis; FIIND, 'function-to-find' domain; GUK, guanylate kinase domain; NB, nucleotide binding core residues; PDZ, PDZ domain; SH3, SRC homology domain 3; WHD, winged-helix domain.

Disease expressivity
The extent to which a genotype shows its phenotypic expression in different people with the same genetic disease.

(rs200154873), has been identified in seven Finnish families diagnosed with keratoendotheliitis fugax hereditaria; however, the effect of the variant on inflammasome activation is unclear[103]. NLRP3 is expressed in the human cornea and eye inflammation is a known feature of CAPS. Indeed, patients with these variants present with conjunctival injection, corneal opacities, pain and photophobia albeit with no evidence of systemic inflammation.

In addition to germline inheritance, somatic missense variants have been reported in about 30% of patients with classical and late-onset CAPS[104–108]. The mutant alleles are found predominantly in myeloid cells with a variable allele frequency as low as 5%[108,109]. These somatic variants often arise at residues critical for protein function, which suggests that germline mutations at these residues are likely incompatible with life[110]. Specifically, two highly conserved subdomains, NBD and HD2, harbour many CAPS-associated somatic mutations. A somatic in-frame deletion of three amino acids (Gly309, Ala310 and Phe311) in close proximity to the Walker B motif was identified in a patient with adult-onset urticaria and systemic inflammation[109].

A low-penetrance variant, Val198Met (rs121908147; also known as Val200Met), has been linked to Schnitzler syndrome, a late-onset disease manifesting with neutrophilic urticaria and monoclonal gammopathy[111]. This variant was germline inherited in two patients with classical (IgM) Schnitzler and was found as a somatic variant in two patients with variant (IgG) Schnitzler[112,113]. The same Val198Met and the rare variant Lys488Arg (rs145268073; also known as Lys490Arg) have been reported in patients with mild non-specific inflammatory phenotypes[114]. Whether these variants influence inflammasome function is still debated.

In summary, pathogenic variants in *NLRP3* enhance inflammasome activity; however, although some variants maintain the protein in the constitutively active state, milder variants might require environmental factors such as cold or stress to trigger inflammasome activation. Mutant cells spontaneously secrete high levels of IL-1β and treatment with IL-1 inhibitors is highly effective[37,115,116].

NLRC4-associated diseases. The NLR family CARD domain-containing protein 4 (NLRC4, also known as IPAF) inflammasome functions as a cytosolic sensor in innate immune and intestinal epithelial cells[117–119]. Through its interaction with the NLR family apoptosis inhibitory protein (NAIP), NLRC4 senses and restricts the intraepithelial replication of Gram-negative bacteria. NLRC4 is a unique NLR protein in that NLRC4 does not bind to bacterial ligands but rather this protein co-assembles with the NAIP receptor to form a functional inflammasome. In contrast to other inflammasomes, the NLRC4 protein contains an N-terminal CARD, instead of a PYD, and can activate caspase 1 independent of ASC[120]. As with other inflammasomes, NLRC4 autoinhibition relies on complex interdomain interactions that stabilize a closed and inactive conformation[121]. Phosphorylation at residue Ser533 is critical for protein function[122]. The NLRC4 inflammasome uses two functions to eliminate bacteria from epithelial cells: the release of pro-inflammatory cytokines (IL-1β and IL-18) and pyroptosis.

Heterozygous gain-of-function missense variants in *NLRC4* result in a spectrum of autoinflammatory phenotypes ranging from milder FCAS to severe life-threatening enterocolitis or macrophage-activation syndrome (MAS)[123,124]. In patients who survive the early-onset severe disease, intestinal inflammation can subside[123], suggesting that host–microbiome interactions probably regulate the disease expressivity.

Various severe MAS-associated variants (Thr337Ser, Val341Ala and Val342Ala) reside in a highly conserved subdomain of NACHT, the HD1, and cause constitutive protein activation (FIG. 3c). A second cluster of pathogenic variants includes the germline variant Ser171Phe and the somatic variant Thr177Ala[125,126]. The Ser171Phe substitution was identified in an infant with congenital anaemia, systemic inflammation, ascites, hepatosplenomegaly and haemophagocytosis, who died at the age of 2 months[125]. The somatic variant Thr177Ala was identified at a high variant frequency in induced pluripotent stem cell clones from a child with NOMID-like features, including severe central nervous system inflammation[126]. These pathogenic variants reside in the vicinity of the ATP-binding pocket and result in a highly active protein. Two other MAS-associated variants, Trp655Cys and Gln657Leu, are thought to increase protein activation by creating an LRR–LRR interface important for NLRC4 oligomerization[127,128].

FCAS-associated *NLRC4* variants are inherited in an autosomal-dominant manner and reside in the WHD of NACHT. The His443Pro pathogenic variant was identified in a three-generation family with cold-induced rash[129]. This variant could trigger constitutive caspase 8-mediated Fas-associated protein with death domain (FAAD)-dependent cell death, independent of Ser533 phosphorylation[130]. A second family with 13 affected members was found to carry the Ser445Pro variant[131]. In this family, all patients presented with cold-induced and/or stress-induced skin lesions, arthralgia and conjunctivitis, and only 2 of the 13 patients had enterocolitis[131]. Thus, similar to CAPS, pathogenic variants in NLRC4 lead to enhanced inflammasome activity; however, although variants affecting the highly conserved motifs spontaneously activate the protein, milder variants increase the propensity for inflammasome activation. Cultured myeloid cells of patients with NLRC4-MAS spontaneously secrete IL-18 and patients respond well to blockade with IL-18 binding protein[132].

NF-κB mediated diseases

NOD2-associated granulomatous disease. NOD2 (also known as CARD15) is a cytosolic sensor for bacterial muramyl dipeptides derived from the cell wall of bacteria[133]. NOD2 is expressed in myeloid and lymphoid cells as well as in intestinal epithelial cells[134]. Ligand binding to the LRR domain triggers conformational changes that lead to self-oligomerization (to form a signalling complex known as the nodosome[135]), recruitment of receptor-interacting serine/threonine-protein kinase 2 (RIPK2), and activation of the NF-κB and mitogen-activated protein kinase (MAPK) signalling pathways.

NOD2 was first linked to human disease as one of the strongest Crohn's disease susceptibility genes. Common low-penetrance SNP variants have been linked to Crohn's disease in multiple, predominantly Caucasian populations (FIG. 3d). Although Crohn's disease-associated SNPs are found throughout the gene, the most common risk alleles are Arg702Trp, Gly908Arg and Leu1007fsX, and it is estimated that up to 30% of patients with Crohn's disease carry one or two copies of these SNPs. All three variants are located in the LRR domain and are thought to affect the sensing activity of LRRs or its function as a putative autoinhibitory domain. The detailed mechanism of intestinal inflammation in Crohn's disease is still unclear; however, Crohn's disease susceptibility alleles are considered functionally hypomorphic as they impair mucosal barrier function and bacterial clearance. Other possible contributing mechanisms include alterations in the immunomodulatory function of NOD2 in regulating TLR responses and autophagy[136,137].

By contrast, high-penetrance novel or rare pathogenic variants in the NACHT domain are associated with a severe dominantly inherited disorder, Blau syndrome (also known as early-onset sarcoidosis), which is characterized by granulomatous arthritis, skin lesions and uveitis[138,139]. In addition to germline-inherited pathogenic variants, somatic de novo and gonosomal variants have been reported in some patients with variable frequencies of a mutant allele in different cells[140,141]. The Blau syndrome-associated missense gain-of-function variants facilitate the formation of the constitutively active NOD2 nodosome and result in increased NF-κB basal activity and IFNγ-mediated inflammatory response[142–144].

A common missense variant in the non-coding region of *NOD2*, c.2798 +158C>T (rs5743289), has been linked to a non-specific inflammatory phenotype termed NOD2-associated autoinflammatory disease[145]. Patients with NOD2-associated autoinflammatory disease typically present in their mid-30s with fevers, arthralgia and erythematous patches or plaques on the trunk. This variant is associated with increased mRNA levels of NOD2 and increased basal MAPK pathway activity in PBMCs but to a much lesser extent than that seen with Blau syndrome-associated mutations[146]. Additional risk factors, such as age-dependent changes in commensal microbiota, might contribute to the disease expression.

In general, high-impact gain-of-function mutations associated with Blau syndrome result in constitutive activation of the NF-κB pathway, whereas common hypomorphic variants in the LRR domain are associated with a susceptibility to Crohn's disease. In contrast to the other NLR-associated diseases, Crohn's disease and Blau syndrome are not mediated by increased IL-1β secretion[147], and anti-TNF therapy has been highly efficacious in these patients[148].

CARD14-associated psoriasis. CARD14 is a scaffold protein, highly expressed in keratinocytes and endothelial cells, that functions as an epidermal regulator of NF-κB signalling[149]. CARD14 exists in a closed autoinhibited form; upon stimulation, CARD14 is phosphorylated by protein kinase C and binds to MALT1/BCL10 to activate the NF-κB signalling pathway[150].

Pathogenic variants in CARD14 have been linked to a spectrum of skin inflammatory phenotypes, including psoriasis vulgaris, familial pityriasis rubra pilaris and pustular psoriasis, with variable disease expressivity and penetrance. Fever and other inflammatory manifestations are not generally present in these patients. Collectively, these diseases are described as CARD14-mediated pustular psoriasis (CAMPS) (also denoted as psoriasis susceptibility 2 locus; PSOR2).

Pathogenic CAMPS-associated variants in *CARD14* are dominant gain-of-function missense substitutions that lead to a range of amplified NF-κB activities[151]. These variants are found in all protein domains, although there is enrichment of pathogenic mutations in exon 4 that encodes the CC domain, which suggests that the CC domain is important for the regulation of CARD14 activity[152–156]. The CC domain is known to mediate protein oligomerization upon its activation. Three mutations, Gly117Ser, and intronic variants, c.349+5G>A and c.349+1G>A, create a cryptic splice site that results in a 22 amino acid insertion, disrupting the CARD domain (FIG. 3e). The most severe phenotype has been observed in a patient with early-onset general pustular psoriasis who had a de novo variant, Glu138Ala[153]. The mutant protein induced the highest level of NF-κB activity relative to the other CAMPS-associated rare mutations and CAMPS-associated SNPs as shown by in vitro experiments. This missense substitution disrupts the autoinhibitory linker domain of CARD14 and causes constitutive activation of the protein. The mutant protein facilitates the formation of the B cell lymphoma/leukaemia-10 (BCL-10) and mucosa-associated lymphoid tissue lymphoma translocation protein 1 (MALT1) signalosome and promotes pro-inflammatory gene expression in keratinocytes[151]. Most other CAMPS-associated variants result in moderately increased NF-κB activity in overexpression experiments[157].

Low-penetrance rare and common variants in *CARD14*, including Arg820Trp (rs11652075) and Asp176His (rs144475004), have been associated with the susceptibility to psoriasis and general pustular psoriasis in large cohort studies[152,149,158]. These missense variants probably destabilize the inactive form of the protein but require additional risk factors for disease expressivity. Interestingly, dominant-negative variants that impair downstream NF-κB signalling in keratinocytes have been associated with a severe form of atopic dermatitis[159].

Thus, on the whole, activating variants in *CARD14* increase the transcription of pro-inflammatory chemokines and cytokines in the skin[152,153]. A range of phenotypes has been observed even in patients with the same pathogenic variants, suggesting a role for environmental and genetic modifiers. Patients with CARD14-associated diseases have a favourable response to treatment with ustekinumab[160].

Inflammatory actinopathies

The actin cytoskeleton is critical for the regulation of many cellular functions, including inflammatory and immune responses[161,162]. The maintenance of cytoskeleton dynamics, including its assembly and

depolymerization, requires the highly coordinated action of many effector and regulatory proteins. In immune cells, the actin cytoskeleton is required for functions such as immune synapse stability, signalling, migration and cytokine release. Pathogenic variants in proteins involved in cytoskeleton organization have been mainly reported in patients with early-onset primary immunodeficiencies and thrombocytopenia[162]. However, variable degrees of autoinflammatory features, including a predisposition to haemophagocytic lymphohistiocytosis (HLH), have been described in all these diseases.

The actin-related protein 2/3 (ARP2/3) complex is pivotal for assembling branched actin filaments by bringing actin monomers into the complex to initiate actin polymerization (FIG. 4). The small GTPases cell division control protein 42 (CDC42) and RAC1 are essential for the activation of the ARP2/3 complex via WASP or the WASP-family verprolin-homologous protein (WAVE) regulatory complex (WRC), respectively[163]. The Nck-associated protein 1-like (NCKAP1L) functions as a haematopoietic lineage-specific regulator of the actin cytoskeleton in response to the engagement of immune receptors in lymphoid and myeloid cells. NCKAP1L is required for the RAC1-mediated stabilization and activation of the WRC[164]. The function of the WRC complex is similar to WASP in terms of enabling the ARP2/3 complex to initiate and coordinate

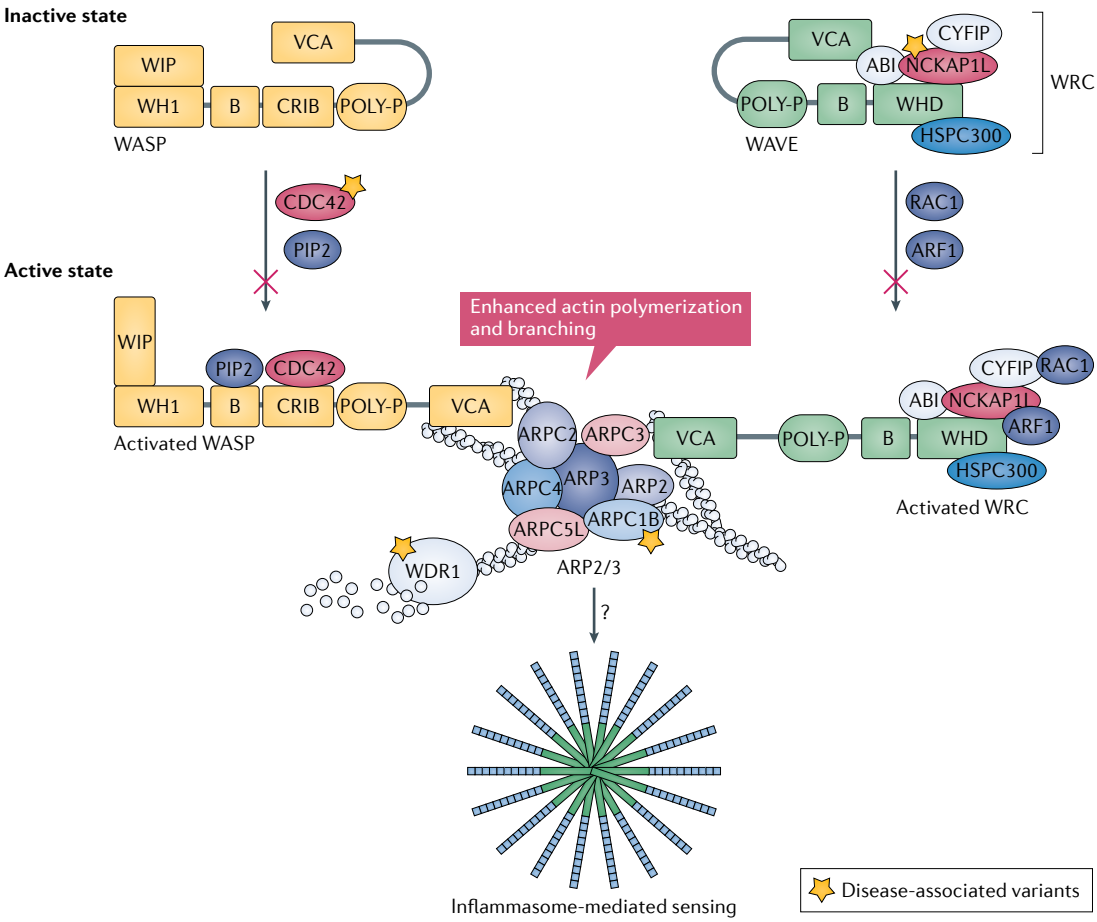

Fig. 4 | **Pathogenesis of inflammatory actinopathies.** Wiskott–Aldrich syndrome protein (WASP, shown on the left) and the WASP-family verprolin-homologous protein (WAVE) regulatory complex (WRC, shown on the right) can both promote actin polymerization via the actin-related proteins 2/3 (ARP2/3) complex, which is composed of seven subunits (shown in the centre). In the inactive state, WASP is inhibited by an interaction between the CDC42/RAC interactive binding motif (CRIB) domain and the verprolin homology/central/acidic (VCA) region. Cooperative binding of active CDC42 to the CRIB domain and phosphatidylinositol 4,5-bisphosphate (PIP2) to the B domain exposes the VCA domain and results in the activation of the ARP2/3 complex. Disease-associated forms of CDC42 are unable to interact with various effectors and regulators and fail to activate WASP. In its inactive state, WRC contains WAVE, ABI (Abl interactor protein), CYFIP (cytoplasmic FMR1-interacting protein), HSPC3000 (haematopoietic stem/progenitor cell protein 300) and NCK associated protein 1-like (NCKAP1L). The binding of active RAC1 to CYFIP, and ARF1 to NCKAP1L, shifts the WRC from an inactive to an active state and enables ARP2/3 complex activation. Disease-associated forms of NCKAP1L impair ARF1 binding and cause the destabilization of the WRC. Activated ARP2/3 triggers actin polymerization and branching. Pathogenic variants in the ARP2/3 subunit ARPC1B cause impaired actin polymerization, whereas biallelic loss-of-function variants in WDR1 lead to defective actin depolymerization. Changes in the cytoskeleton dynamics might be sensed by the inflammasome or perhaps by other cytosolic sensors and can result in an inflammatory phenotype. POLY-P, poly-proline; WH1, WASP homology region 1; WHD, winged-helix domain; WIP, WAS/WASL-interacting protein family member 1.

actin polymerization. Conversely, WD-repeat protein 1 (WDR1, also known as AIP1) and actin-depolymerizing factor 1 (ADF1) are critical for actin depolymerization and thus for the regulation of the dynamics of the actin cytoskeleton. Together, all these proteins play a critical role in the maintenance and turnover of the actin filament network. Studies of the pyrin inflammasome have provided insights on how cells might sense changes in the cytoskeleton dynamics. In this section, we discuss the phenotypes associated with deficiencies in CDC42, NCKAP1L, ARP2/3 complex subunit 1B (ARPC1B; a component of the ARP2/3 complex) and WDR1.

CDC42 deficiency

Dysregulation of the function of CDC42 has been linked to various immune and non-immune phenotypes, depending on the effect of the mutation on cell-specific transcript isoforms. Heterozygous dominant loss-of-function variants in the brain-restricted isoform of CDC42 (isoform 2) cause diverse neurodevelopmental phenotypes that include growth failure, facial dysmorphism, brain malformations, intellectual disability and cardiac defects[165]. These variants disrupt the switch between the active and inactive state of the protein and its interaction with various effector proteins and consequently affect multiple cellular functions. Heterozygous loss-of-function de novo variants in the ubiquitously expressed transcript of CDC42 (isoform 1) have been identified in patients with a severe and potentially fatal haematological disease named neonatal onset of pancytopenia, autoinflammation, rash and haemophagocytosis (NOCARH)[166,167]; some of these patients have mild dysmorphic features. The recurrent NOCARH-associated Arg186Cys variant affects a highly conserved di-arginine motif (Arg186 and Arg187) critical for the binding of CDC42 to the interacting proteins such as WASP and Ras GTPase-activating-like protein IQGAP1. This genetic alteration results in protein mislocalization, disruption of the actin architecture and defects in cell differentiation, polarity and migration[166]. Other pathogenic variants in the C-terminal domain reside in close spatial proximity to the di-arginine motif[167]. Fundamentally, all the NOCARH-associated variants affect the last five amino acids of the protein that are required for post-translational processing and its proteolytic cleavage[168]. Bone marrow-derived mononuclear cells from these patients spontaneously release IL-1 and IL-18 and, during HLH episodes, the plasma levels of CXCL9 and IFNγ are very high[166]. Most patients improve considerably with IL-1 inhibitor therapy[167], and the targeting of IL-18 or IFNγ and HSCT are other promising therapeutic options[166]. How exactly defects in actin assembly induce the high production of IL-1 and IL-18 and predispose to HLH remains to be investigated.

NCKAP1L deficiency

NCKAP1L deficiency in humans leads to profound immune dysregulation with features of immunodeficiency, lymphoproliferation, atopy and severe inflammation[169]. Pathogenic biallelic loss-of-function variants in NCKAP1L (also known as HEM1) dysregulate the activation of the WRC, which leads to defective F-actin polymerization, abnormal immune cell activation, differentiation and migration[170]. Two patients with NCKAP1L deficiency had flares of disease consistent with HLH that are thought to result from prolonged immune stimulations owing to impaired T cell activation and pathogen control[169]. The respective pathogenic variants include a missense substitution (Val141Phe) and a splice site variant (c.2862+1G>A) that leads to skipping of exon 26. Data from structural modelling predict that these variants affect the binding of NCKAP1L to the Abl interactor 2 (ABI2; a regulator of actin cytoskeleton dynamics). Additional missense variants (Arg258Leu, Pro359Leu, Met371Val and Val519Leu) have been reported in five patients with a complex immune dysregulation but without features of HLH. These pathogenic variants affect the stability of WRC or the binding of NCKAP1L to ARF1 (REF.[170]), which is notable as ARF1 is critical for the activation of the WAVE complex[171]. T cells and neutrophils containing this variant exhibit abnormal F-actin formation, loss of lamellipodia extensions and migratory dysfunction. In addition, independently of its function as a WAVE regulator, mutated forms of the NCKAP1L protein can abrogate the mTORC2-mediated activation of protein kinase B (AKT) signalling, which might explain the abnormalities in T cell activation and function[170].

ARPC1B deficiency

ARPC1B is one of the seven subunits of the human ARP2/3 protein complex, and its expression is restricted to blood cells[172]. Given the central role that ARPC1B plays in the development and function of haematopoietic cells, patients with recessive loss-of-function variants in *ARPC1B* present with a broad spectrum of immune dysregulations[172–174]. These symptoms include combined immunodeficiency, bleeding disorder, eczema and autoimmunity similar to that seen in patients with WAS. A deficiency of ARPC1B leads to microthrombocytopenia and defective platelet function, whereas persistent thrombocytopenia is observed in patients with null mutations in *ARPC1B*. Immunological features in patients with ARPC1B deficiency include impaired TCR-mediated proliferation, T cell lymphopenia, loss of cytotoxic T lymphocyte function, eosinophilia and elevated IgE antibody levels[175], whereas inflammatory manifestations can include cutaneous vasculitis and inflammatory bowel disease.

WDR1 deficiency

Recessive loss-of-function variants in the *WDR1* gene are linked to the disease named periodic fevers, immunodeficiency and intermittent thrombocytopenia[176,177]. WDR1 deficiency leads to a defect in actin depolymerization, which affects neutrophil morphology and function. WDR1 also plays a crucial role in lymphocyte development and activation, particularly in the B cell department. Consequently, neutropenia, thrombocytopenia and B cell lymphopenia are features of this phenotype. Affected individuals suffer from severe recurrent respiratory infections, impaired wound healing and autoinflammatory features, including severe stomatitis, perianal ulceration and periodic fever. LPS-stimulated myeloid cells from these patients have increased

caspase 1 activity and produce very high levels of IL-18, although IL-1 secretion is not upregulated. In transfected HEK293T cells, overexpressed mutated forms of WDR1 colocalize with pyrin, suggesting that the pyrin inflammasome activation probably contributes to these patients' inflammatory manifestations.

In summary, pathogenic variants in proteins involved in cytoskeleton organization affect the function of all haematopoietic cells, leading to defects in innate and adaptive immune responses. Dysregulation in actin polymerization also results in a spectrum of inflammatory manifestations. Further studies are necessary to elucidate the molecular abnormalities causing the overproduction of IL-18 in these diseases.

Interferonopathies

The interferon pathway is critical for the recognition of pathogen-generated nucleic acids and the generation of antiviral responses. Monogenic diseases caused by constitutive upregulation in type I interferon signalling are known as primary interferonopathies. The inheritance pattern of pathogenic variants correlates with the function of the mutated proteins. Interferonopathies can arise from a heterozygous gain-of-function variant in a sensor protein, for example, in melanoma differentiation-associated protein 5 (MDA5), stimulator of interferon signalling (STING) or retinoic acid inducible gene 1 (RIGI), or from biallelic loss-of-function variants in a protein with nuclease activity, for example, in three-prime repair exonuclease 1 (TREX1) or deoxynucleoside triphosphate triphosphohydrolase (SAMHD1), or from biallelic loss-of-function variants in a protein that downregulates type I interferon responses, for example, in ubiquitin-like protein ISG1 (ISG15) or in Ubl carboxyl-terminal hydrolase 18 (USP18)[178,179]. The binding of type I interferons to heterodimeric IFNAR1–IFNAR2 receptors induces the activation of Janus tyrosine kinases (JAK) and the dimerization of activator of transcription 1 (STAT1) and STAT2, which then bind to interferon regulatory factor 9 (IRF9) to form the phosphorylated transcription factor complex interferon-stimulated gene factor 3 (ISGF3). The activated transcription complex translocates to the nucleus and upregulates the gene expression of interferon-stimulated genes (ISGs). The clinical presentation of patients with primary interferonopathies is in the autoinflammatory–autoimmune disease spectrum. In this section, we focus on two diseases that have complex inheritance patterns and present with notable disease variability: STING-associated vasculopathy with onset in infancy (SAVI) and proteasome-associated autoinflammatory syndromes (PRAAS).

STING-associated diseases

STING functions as a cytosolic DNA-sensing adaptor protein. STING is bound and activated by cyclic GMP-AMP (cGAMP), which is produced by the cyclic GMP-AMP synthase (cGAS) in response to pathogen-derived and mitochondrial DNA[180] and induces both interferon type I gene expression and NF-κB-mediated cytokine production[181,182]. cGAMP binding causes the release of the STING inhibitory C-terminal tail and its polymerization[183]. Residue 263 on STING is essential for ligand binding, whereas phosphorylation at residue 366 is necessary for TBK1–IRF3-mediated interferon type I production and antiviral activity[184]. STING is encoded by *TMEM173* and is highly expressed in myeloid cells, natural killer (NK) cells, T cells, vascular endothelial cells, alveolar pneumocytes and the bronchial epithelium[185].

Heterozygous gain-of-function variants in *TMEM173* are linked to a systemic inflammatory disease named SAVI[186] (FIG. 5a). SAVI manifests with fevers, erythematous rash, acrocyanosis, telangiectasia, small vessel vasculitis, peripheral amputations, interstitial lung disease and failure to thrive[187]. The activity of the mutated form of STING in SAVI is particularly high in dermal vascular endothelial cells, which explains the severity of cutaneous vasculitis in patients with this disease. The first reported disease-causing variants (Val147Leu/Met, Phe153Val, Asn154Ser and Val155Met) were identified as de novo variants in patients with severe early-onset vasculitis[186]. In addition to these germline variants, Val147Leu has also been found to be a somatic mutation in haematopoietic cells, dermal fibroblasts and other cell types[186]. Collectively, these pathogenic variants are known as class 1 mutations and they reside in a highly conserved domain, the connector helix loop, which controls the ligand-induced rotation of the dimers required for the activation of STING. These hyperactive variants reside in close proximity to the cysteine residue 148, which is critical for the stabilization of STING dimers[183]. Essentially, class 1 mutations cause ligand-independent activation of STING. The Val155Met variant is associated with a variable disease expressivity, and patients who carry this variant present with a spectrum of phenotypes, including chilblain lupus, severe vasculopathy or severe pulmonary fibrosis, that could be the first manifestation of the disease[188,189]. In addition to these class 1 mutations, a Gly166Glu variant has been identified in four generations of one family with chilblain lupus and without fever episodes or lung disease[190]. This variant is postulated to increase interactions at the dimer interface by inducing stronger hydrogen bonds between monomers. Furthermore, variants that reside in the polymerization interface (Cys206Tyr, Gly207Glu, Arg281Gln and Arg284Gly/Ser) cause a constitutive activation of STING either by making the polymerization interface available or by preventing the binding of a putative inhibitor[191–193]. Specifically, Arg281Gln and Arg284Gly/Ser are proposed to affect the binding of a C-terminal tail that keeps STING in an inactive state[183]. Patients who carry these mutations present with early-onset symptoms, failure to thrive, and variable degrees of skin and lung disease.

Pathogenic variants in STING can have additive effects. Two de novo variants, Ser102Pro and Phe279Leu, were inherited on the same chromosome (in *cis*) in a patient with systemic inflammation, telangiectatic skin lesions, brain infarctions, pulmonary dysfunction and recurrent infections[194]. This finding suggests that mutations with a weaker effect on STING function might not be sufficient as a single variant or in a monoallelic state to induce protein activation. In support of this hypothesis, six patients with a severe, potentially lethal

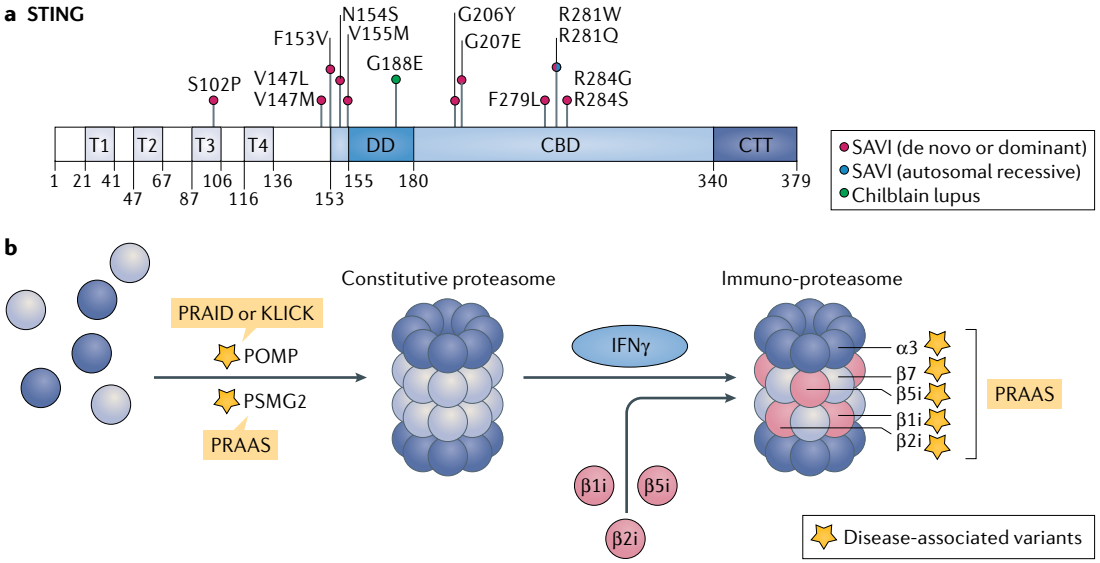

Fig. 5 | **Interferonopathies and disease-causing variants.** Various pathogenic variants in stimulator of interferon signalling (STING) and proteasome or immunoproteasome components can cause type I interferon-mediated diseases. **a** | A number of de novo or dominantly inherited gain-of-function variants in STING can cause STING-associated vasculopathy with onset in infancy (SAVI; shown in red), whereas another variant Arg281Gln (shown in blue) causes autosomal recessive SAVI. The variant shown in green is associated with chilblain lupus. **b** | The constitutive proteasome complex is expressed in all cell types and is composed of four stacked rings, each consisting of either 7α or 7β subunits. The immunoproteasome differs from the proteasome in that three of the proteasome subunits are replaced by different subunits, β1i, β2i and β5i, which are encoded by *PSMB9*, *PSBM10* and *PS*MB8, respectively. IFNγ can also induce immunoproteasome assembly. Heterozygous de novo variants in the proteasome maturation protein (POMP), which serves as a chaperone for proteasome assembly, function as dominant-negative variants and cause POMP-related autoinflammation and immune dysregulation disease (PRAID). The recessively inherited single nucleotide deletion in the 5′ untranslated region of POMP (rs112368783) is associated with the distinct syndrome keratosis linearis with ichthyosis congenita and sclerosing keratoderma (KLICK). Biallelic compound heterozygous variants in the chaperone protein PSMG2 cause a proteasome-associated autoinflammatory syndrome (PRAAS)-like phenotype with autoimmune haemolytic anaemia. Recessively inherited loss-of-function variants in α3 (encoded by *PSMA3*), β7 (encoded by *PSMB4*), β5i, β1i and/or β2i are associated with PRAAS. In cells that contain mutated components of the proteasome or immunoproteasome, ubiquitylated proteins accumulate and might trigger cellular stress and the upregulation of the type I interferon response (not shown). CBD, cGAMP binding domain; CTT, C-terminal tail; DD, dimerization domain.

SAVI phenotype were found to carry the homozygous Arg281Trp variant[195]. A missense variant at the same amino acid residue, Arg281Gln, was reported in another patient to be dominantly inherited[196]. The nature of the amino acid change from a positively charged arginine (Arg) to an uncharged glutamine (Gln) probably explains the high impact of this mutation and the difference in the inheritance pattern compared with the homozygous Arg281Trp variant. Common nucleotide variants (SNPs) in *TMEM173* might further contribute to the varying phenotype spectrum in SAVI[193].

Hence, in general, pathogenic STING variants are activating mutations that have a variable effect on protein activation and different inheritance patterns. The cells of patients with STING have a highly elevated ISG expression signature and produce high levels of interferon-induced cytokines[186]. Treatment with JAK inhibitors is beneficial but does not entirely ameliorate pulmonary involvement and skin disease[197].

PRAAS

Proteasomes are multiprotein complexes responsible for the K48 ubiquitin-dependent degradation of proteins and the maintenance of cellular homeostasis[198]. The constitutive proteasome is ubiquitously expressed, whereas the immunoproteasome is highly expressed in immune cells (FIG. 5b). A deficiency in proteasome activity leads to a build-up of undegraded proteins in cells, resulting in endoplasmic reticulum (ER) stress, the unfolded protein response and activation of type I interferon signalling[199].

Biallelic pathogenic variants in the immunoproteasome catalytic subunits or in the constitutive proteasome subunits are associated with a spectrum of autoinflammatory diseases collectively described as PRAAS (also known as chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperature)[200–203]. Patients with PRAAS present with an early-onset recurrent fever, skin lesions, lipodystrophy, progressive joint contractures, multi-organ failure and neurological impairments[204]. PRAAS-associated variants are typically recessively inherited loss-of-function variants, and they affect proteasome assembly, maturation and, ultimately, proteasome activity. However, the inheritance pattern of PRAAS can be monogenic or digenic. Most patients are homozygous for the Thr75Met variant in *PSMB8*, which encodes the catalytic subunit β5i of the immunoproteasome[204]. Biallelic pathogenic variants have

Digenic
A phenotype or disorder that is expressed only when two non-allelic controlling genes interact.

also been identified in the immunoproteasome-specific gene *PSMB10* and in the *PSMB4* gene, which encodes the constitutive proteasome β7 subunit[205,206]. A subset of patients carry pathogenic variants at two separate genetic loci (*PSMB8* and *PSMA3*, *PSMB8* and *PSMB4*, or *PSMB9* and *PSMB4*)[205]. These patients tend to present with a severe phenotype, presumably because they have a defect in a constitutively expressed proteasome subunit. Recently, a de novo monoallelic mutation Gly156Asp in *PSMB9*, which suppresses protein expression, was reported in a patient who was subsequently successfully treated with HSCT[207].

Further expanding on what is known on the phenotype and inheritance pattern of PRAAS, three unrelated patients have been reported to carry de novo heterozygous variants in the proteasome maturation protein (POMP) that serves as a chaperone for proteasome assembly[208]. Two of these pathogenic variants are frameshift deletions in the penultimate exon 15 of *POMP* and produce truncated proteins with a dominant-negative effect. This distinct phenotype, termed POMP-related autoinflammation and immune dysregulation disease (PRAID), is characterized by severe neonatal-onset neutrophilic dermatosis, susceptibility to infections and autoimmunity features[208]. Interestingly, a homozygous 1bp-deletion in the 5′-UTR of another POMP transcript isoform causes a very different phenotype: keratosis linearis with ichthyosis congenita and sclerosing keratoderma syndrome (KLICK)[209], which is hypothesized to arise from a deficiency of a skin-specific isoform of POMP. The genetic heterogeneity of PRAAS is further emphasized by a report of compound heterozygous pathogenic variants in another chaperone protein (PSMG2) in a patient with features of PRAAS and autoimmune haemolytic anaemia[210].

Therefore, patients with PRAAS have complex inheritance patterns that include genetic heterogeneity, digenic inheritance, and a dominant or recessive inheritance pattern. Nonetheless, all PRAAS-associated variants are loss-of-function mutations that cause a defect in the proteasome function. Mutant cells have a strong type I interferon gene expression signature and JAK inhibitors ameliorate disease activity[197].

Diseases caused by enzyme dysregulation

PLCγ$_2$-associated diseases

Phosphoinositide-specific phospholipase Cγ2 (PLCγ2) is an enzyme that catalyses the hydrolysis of phosphatidylinositol 4,5-bisphosphate to the secondary messengers inositol triphosphate (IP3) and diacylglycerol (DAG) and is an important component of the B cell receptor signalosome[211]. In activated immune cells, IP3 induces the release of Ca^{2+} from the ER. High intracellular levels of Ca^{2+} activate various signalling pathways, including the protein kinase C (PKC) and the extracellular signal-regulated kinase (ERK)–MAPK pathway[212].

Monogenic *PLCG2*-associated autoinflammatory diseases include PLCγ2-associated antibody deficiency and immune dysregulation (PLAID)[213] and autoinflammation and PLAID (APLAID)[214] (FIG. 6a). Patients with PLAID or APLAID present with distinct skin and organ-specific disease manifestations. Features common to both diseases include low serum titres of IgA and IgM antibodies and decreased numbers of class-switched memory B cells, circulating $CD19^+$ B cells and NK cells. PLAID is characterized by cold-induced urticarial rash, skin granuloma, soft tissue destruction, and variable degrees of immunodeficiency and autoimmunity[213], whereas patients with APLAID present early in life with blistering skin lesions on exposure to heat or the sun, cutis laxa, arthralgia, ulcerative colitis, central nervous system inflammation, interstitial lung disease, and recurrent skin and sinopulmonary infections[214–217]. Both conditions result from activating, dominantly inherited pathogenic variants in PLCγ2 but differ in the nature of the causal variants. Patients with PLAID carry heterozygous in-frame genomic deletions of exon 19 or exons 20–22 spanning the C-terminal SRC homology 2 (SH2) autoinhibitory domain of PLCγ2, whereas APLAID is linked to the de novo missense variants Ser707Tyr, Ala708Pro, Leu848Pro and Leu845_Leu848del (FIG. 6a).

The pathophysiology of PLAID and APLAID is caused by a combination of gain-of-function and loss-of-function cell-specific effects on the PLCγ2 signalling pathway[218]. PLAID-associated deletions render the protein constitutively active; however, B cells and NK cells from patients with PLAID have an anergic phenotype at physiological temperatures owing to feedback inhibition secondary to chronic activation. T cell function is not affected, whereas mast cells spontaneously activate following exposure to temperatures lower than 37 °C, which explains the occurrence of cold urticaria in this disease. The APLAID missense variants Ser707Tyr and Ala708Pro reside in the same SH2 domain and have a gain-of-function effect that occurs in a temperature-independent manner but requires upstream activation by receptor tyrosine kinases. Two other APLAID causal mutations, Leu848Pro and Leu845_Leu848del, are located in the split pleckstrin homology (spPH) autoinhibitory domain. The most severe phenotype has been observed in a patient with the Leu845_Leu848del variant who presented at birth with a complete absence of B cells[217]. APLAID-associated missense substitutions enhance the basal activity of PLCγ2 and are hypersensitive to stimulation by GTPase Rac2 (REF.[219]). The net effect of these missense substitutions is an elevated production of IP3 and DAG and the calcium-dependent activation of the ERK–MAPK pathway.

In addition to PLAID and APLAID, rare missense variants in PLCγ2 have also been reported in patients with FCAS[220]. Finally, somatic variants that arise at the same residues as APLAID causal mutations (Ser707Tyr and Leu845Phe) give rise to Bruton tyrosine kinase inhibitor (ibrutinib)-resistant chronic lymphocytic leukaemia[221].

Overall, pathogenic variants in PLCγ2 activate the protein by altering the protein structure, resulting in the upregulation of various downstream signalling cascades and inflammatory features that are non-responsive to targeted cytokine therapies. As PLCγ2 is an essential regulator for B cell differentiation and proliferation, hypogammaglobulinemia is a prominent feature of the disease.

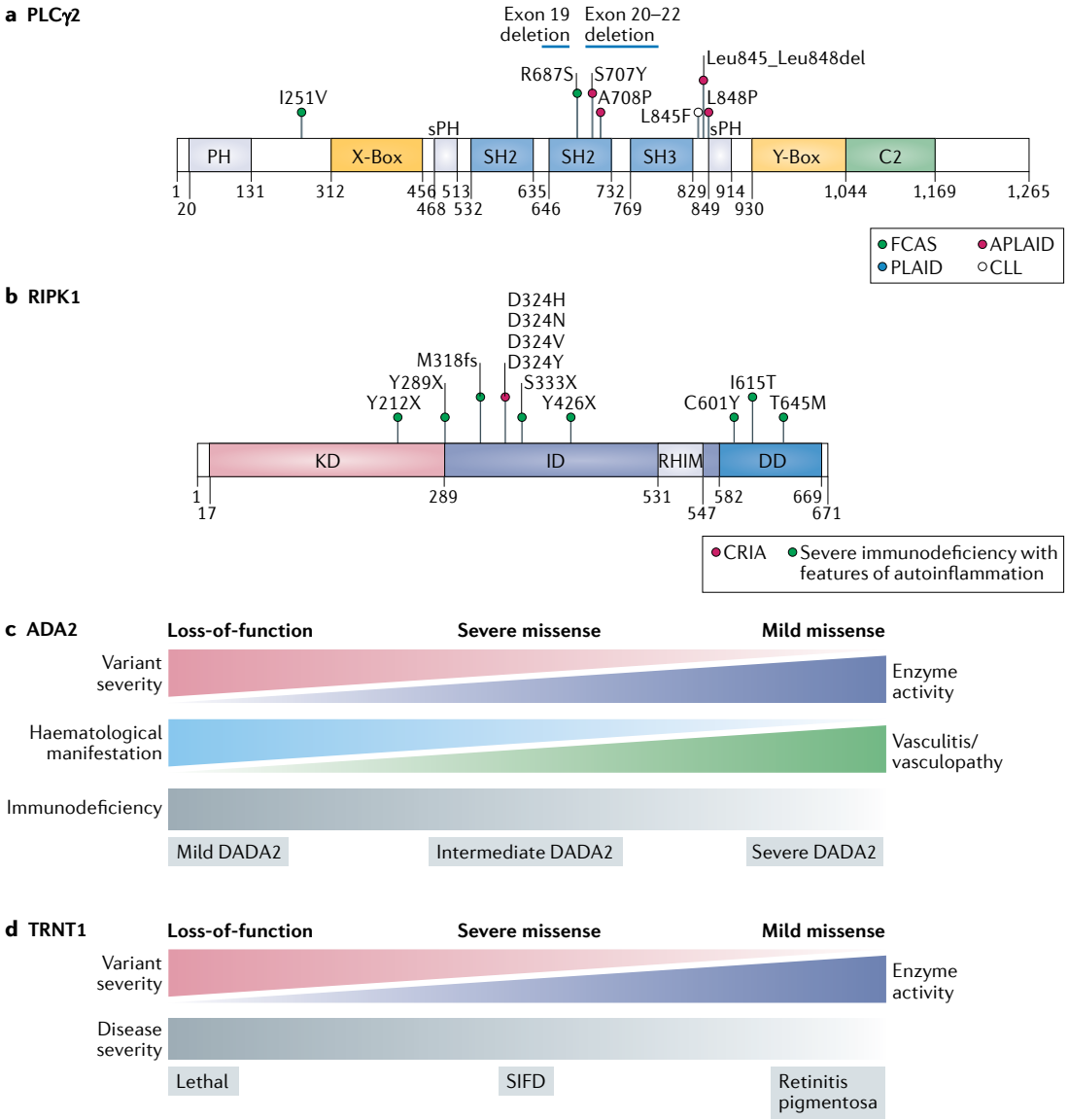

Fig. 6 | **Genotype–phenotype correlations in autoinflammatory diseases associated with enzyme deficiencies.** The dysregulation of various enzymes (ranging from missense to loss-of-function mutations) can cause a variety of autoinflammatory phenotypes, depending on the location and severity of the mutation. Pathogenic variants are colour-coded according to their associated phenotype. **a** | Rare variants in phosphoinositide-specific phospholipase Cγ2 (PLCγ2) have been identified in patients presenting with familial cold autoinflammatory syndrome (FCAS; shown in green). Other variants include in-frame genomic deletions that are associated with PLCγ2-associated antibody deficiency and immune dysregulation (PLAID; shown in blue); variants that are associated with autoinflammation, PLCγ2-associated antibody deficiency and immune dysregulation (APLAID, shown in red); and somatic variants that can cause chronic lymphocytic leukaemia (CLL; shown in white). **b** | Various recessively inherited loss-of-function pathogenic variants in receptor-interacting serine/threonine-protein kinase 1 (RIPK1) can cause severe immunodeficiency with features of autoinflammation (shown in green). Other dominant variants that affect the cleavage site of RIPK1 (residue 324) are associated with cleavage-resistant RIPK1-induced autoinflammatory (CRIA) syndrome (shown in red). **c** | Variants in adenosine deaminase 2 (ADA2) can lead to a spectrum of disease referred to as deficiency of adenosine deaminase 2 (DADA2). Variants leading to complete loss of ADA2 activity are associated with a higher incidence of severe haematological manifestations. By comparison, milder missense variants that exhibit some residual enzyme activity (>3%) are more often observed in patients with vasculitis or vasculopathy. Immunodeficiency can occur in patients carrying pathogenic variants across the whole mutational spectrum. **d** | The enzyme function of tRNA nucleotidyltransferase, CCA-adding 1 (TRNT1) correlates with disease severity. Complete TRNT1 enzyme deficiency is lethal early in development, whereas severe missense variants that exhibit some residual enzyme activity are associated with early-onset congenital sideroblastic anaemia, immunodeficiency, fevers and developmental delay (SIFD). Milder missense variants often lead to non-syndromic retinitis pigmentosa. DD, death domain; ID, intermediate domain; KD, kinase domain; PH, pleckstrin homology domain; RHIM, RIP homotypic interaction motif; SH, SRC homology domains.

RIPK1-associated diseases

Receptor-interacting serine/threonine-protein kinase 1 (RIPK1) is an important regulator of TNF-induced activation of the canonical NF-κB signalling pathway and cell death[222], and this kinase is expressed in many tissues. The function of RIPK1 is regulated by changes in post-translational modifications, including phosphorylation, ubiquitylation and caspase 8-mediated cleavage[223]. The disruption of TNF-mediated ubiquitylation of RIPK1 induces the activation of cell-death pathways (apoptosis or necroptosis).

Biallelic loss-of-function variants in *RIPK1* are linked to a disease consisting of primary immunodeficiency, early-onset inflammatory bowel disease and polyarthritis[224,225] (FIG. 6b). Cells from patients with these variants have reduced levels of NF-κB and MAPK signalling and defective B cell and T cell differentiation, which explains the susceptibility of these patients to infections. The inflammatory component in this disease might result from increased inflammasome activity or the dysregulated response of intestinal epithelial cells to TNF-induced apoptosis[225].

In addition to RIPK1 deficiency, pathogenic variants in this gene can lead to a dominantly inherited inflammatory phenotype named cleavage-resistant RIPK1-induced autoinflammatory (CRIA) syndrome. Patients with CRIA syndrome carry heterozygous missense variants at residue Asp342 that function as gain-of-function variants by inhibiting the cleavage and inactivation of RIPK1. The associated disease manifests with early-onset recurrent periodic fevers and severe intermittent lymphadenopathy[226,227]. Studies in primary cells have shown that the activating *RIPK1* variants sensitize PBMCs but not fibroblasts to TNF-induced cell death by apoptosis, necroptosis and ferroptosis[226].

In summary, pathogenic variants in RIPK1 are either recessively inherited hypomorphic variants or dominantly inherited activating variants and they cause opposing clinical phenotypes. Patients with CRIA syndrome respond to therapy with a cytokine inhibitor[227], whereas HSCT might be a curative option for patients with RIPK1 deficiency[224].

ADA2-associated diseases

The deficiency of adenosine deaminase 2 (DADA2) is caused by biallelic hypomorphic variants in the gene encoding adenosine deaminase 2 (ADA2)[228,229]. ADA2 is a secreted enzyme that regulates purine metabolism by breaking down adenosine and 2′-deoxyadenosine at sites of inflammation and has growth factor-like properties[230]. ADA2 is highly expressed in myeloid cells and is produced by activated monocytes, macrophages and dendritic cells[231].

Patients with DADA2 present with early-onset recurrent fever, hepatosplenomegaly and variable degrees of vasculopathy ranging from livedo racemosa and reticularis to polyarteritis nodosa. Severe disease manifestations consist of recurrent life-threatening ischaemic and/or haemorrhagic strokes and haematological manifestations, including pure red cell aplasia, immune thrombocytopenia and neutropenia, bone marrow failure, combined variable immunodeficiency and lymphoproliferation[232,233].

DADA2-associated variants are located over the entire gene, with no predilection to cluster in specific protein domains. Ultimately, pathogenic variants affect various protein functions of ADA2 such as its catalytic activity, dimerization, glycosylation and interactions with other proteins[234]. Disease severity correlates with the effect of the variants on ADA2 enzyme activity. Low or absent ADA2 activity is associated with severe haematological manifestations, whereas a higher residual activity is linked to vascular phenotypes[235] (FIG. 6c).

ADA2-deficient myeloid cells are prone to activation and produce high amounts of TNF, which causes tissue inflammation and damage to endothelial cells[229]. The underlying mechanism of bone marrow dysregulation is unclear; it can result either from a lack of ADA2 growth factor activity or from the infiltration of bone marrow with activated immune cells. The establishment of genotype–phenotype correlations in DADA2 has important implications for the therapy of these patients. Treatment with TNF inhibitors reduces inflammation and restores vascular integrity[236]. Bone marrow transplantation is an option for patients presenting with severe haematological disease and not responding to TNF inhibitors[237].

TRNT1 deficiency

TRNT1 encodes the ubiquitously expressed transfer RNA nucleotidyltransferase 1 (TRNT1), an enzyme that is responsible for transferring CCA trinucleotides to the 3′ end of all precursor transfer tRNAs. This post-transcriptional modification is essential for the accurate attachment of amino acids and proper protein translation[238]. TRNT1 also plays a critical role in maintaining the homeostasis of cellular tRNAs by selectively marking structurally defective or unstable tRNAs for degradation[239].

Biallelic loss-of-function variants in *TRNT1* were first reported in patients with early-onset congenital sideroblastic anaemia, immunodeficiency, fevers and developmental delay (SIFD)[240,241] (FIG. 6d). Disease-linked variants affect the protein stability and catalytic activity[242]. Patients at the severe end of the disease spectrum present with neonatal-onset anaemia and prominent extramedullary erythropoiesis, profound immunodeficiency, and metabolic and neurological abnormalities[243]. Disease mortality is high in these patients owing to multi-organ or cardiac failure. Immunodeficiency can be the first disease manifestation and is caused by defects in B cell differentiation[244]. SIFD-associated mutations include missense, nonsense, frameshift and splice site pathogenic variants, whereas biallelic nonsense or truncating variants are non-viable[245–248]. Unprocessed and misfolded proteins accumulate in TRNT1-deficient cells, which can exhaust the protein degradation machinery and the autophagy pathway and induce ER stress and cell death[246]. At the milder end of the TRNT1 deficiency spectrum are patients with a very different phenotype that includes non-syndromic retinitis pigmentosa and subtle haematological features[249,250]. Retinitis pigmentosa is characterized by the progressive degeneration of photoreceptors leading to low night vision and visual field defects. Although vision loss is observed in patients with severe

SIFD, the haematological features of these patients often require immediate medical interventions.

Hence, the severity of TRNT1-associated diseases correlates with the levels of residual enzyme activity. Treatment modalities include blood transfusions, intravenous immunoglobulin and anti-TNF therapy to alleviate inflammatory symptoms[246]. HSCT performed early in life can effectively treat the haematological phenotype[240].

Conclusion

The classic concept of one gene–one phenotype is overly simplified as different disease-causing variants within a gene might affect various aspects of protein function and lead to clinically distinct conditions. High-impact, mostly de novo, pathogenic variants that cluster in specific protein domains often result in a similar phenotype. By comparison, milder variants tend to present with variable disease expressivity and are more influenced by other genetic and non-genetic modifying factors. Furthermore, pathogenic variants in the same gene can have different inheritance patterns that translate into different effects on protein function. Adding to the complexity of genetics in autoinflammatory diseases, various reports have emerged of several diseases caused by somatic variants primarily in myeloid cells. These patients typically present with late-onset symptoms, and their disease severity might be related to the type and proportion of cells carrying a mutant allele[251]. By contrast, somatic variants that occur in pluripotent cells during early embryogenesis might result in a clinical phenotype similar to those observed in patients with germline pathogenic variants[252]. Understanding the factors contributing to the variable disease penetrance and expressivity of monogenic autoinflammatory and other human diseases will ultimately require large-scale genomic, epigenomic, transcriptomic and proteomic studies. Understanding these aspects will also help to understand variabilities in treatment response and enable patient-tailored therapy as we approach the era of precision medicine.

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