REVIEW

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# When less is more: primary immunodeficiency with an autoinflammatory kick

*Angeliki Giannelou, Qing Zhou, and Daniel L. Kastner*

**Purpose of review**
Next-generation sequencing is revolutionizing the molecular taxonomy of human disease. Recent studies of patients with unexplained autoinflammatory disorders reveal germline genetic mutations that target important regulators of innate immunity.

**Recent findings**
Whole-exome analyses of previously undiagnosed patients have catalyzed the recognition of two new disease genes. First, a phenotypic spectrum, including livedo racemosa, fever with early-onset stroke, polyarteritis nodosa, and Sneddon syndrome, is caused by loss-of-function mutations in cat eye syndrome chromosome region, candidate 1 (*CECR1*), encoding adenosine deaminase 2. Adenosine deaminase 2 is a secreted protein expressed primarily in myeloid cells, and a regulator of macrophage differentiation and endothelial development. Disease-associated mutations impair anti-inflammatory M2 macrophage differentiation. Second, patients presenting with cold-induced urticaria, granulomatous rash, autoantibodies, and common variable immunodeficiency, or with blistering skin lesions, bronchiolitis, enterocolitis, ocular inflammation, and mild immunodeficiency harbor distinct mutations in phospholipase $C\gamma_2$, encoding a signaling molecule expressed in natural killer cells, mast cells, and B lymphocytes. These mutations inhibit the function of a phospholipase $C\gamma_2$ autoinhibitory domain, causing increased or constitutive signaling.

**Summary**
These findings underscore the power of next-generation sequencing, demonstrating how the primary deficiency of key molecular regulators or even regulatory motifs may lead to autoinflammation, and suggesting a possible role for cat eye syndrome chromosome region, candidate 1 and phospholipase $C\gamma_2$ in common diseases.

**Keywords**
autoinflammation and $PLC\gamma_2$-associated antibody deficiency and immune dysregulation, deficiency of adenosine deaminase 2, $PLC\gamma_2$-associated antibody deficiency and immune dysregulation, vasculitis, vasculopathy

## INTRODUCTION

The concept of autoinflammation was introduced 15 years ago to define a group of clinical disorders characterized by seemingly unprovoked episodes of inflammation in the absence of high-titer autoantibodies or antigen-specific T cells, distinguishing them from the classical autoimmune diseases. This idea was stimulated by the discovery of the genes underlying two of the prototypic hereditary fever syndromes, familial Mediterranean fever (FMF) and the tumor necrosis factor receptor-associated periodic syndrome [1–3]. In 1997, the gene that, when mutated, causes FMF was identified by positional cloning [1,2]; and 2 years later, the discovery of mutations in the extracellular domain of TNF receptor 1 provided the explanation for a syndrome with an FMF-like picture but dominant inheritance and a longer duration of attacks [3].

A growing body of evidence has implicated the innate immune system in the pathogenesis of the autoinflammatory diseases. A breakthrough discovery was that gain-of-function mutations in the

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## KEY POINTS

- Whole-exome sequencing analysis has revolutionized the discovery of the genetic causes of heretofore molecularly uncharacterized diseases.
- The discovery of pathogenic mutations associated with autoinflammatory diseases has provided significant insights into molecules that are important for the regulation of inflammatory pathways.
- The discovery of ADA2 mutations in patients with inflammation and vasculopathy/vasculitis identified an extracellular adenosine deaminase-related growth factor as a key player for the maintenance of vascular development and integrity.
- Mutations altering the function of $PLC\gamma_2$ are associated with a spectrum of immune phenotypes ranging from allergy and immunodeficiency to autoimmunity and autoinflammation.

NLRP3 inflammasome cause the cryopyrinopathies, a group of autoinflammatory diseases that include familial cold autoinflammatory syndrome [4], Muckle–Wells syndrome [4], and neonatal-onset multisystem inflammatory disease (also known as chronic infantile neurologic cutaneous and arthropathy syndrome) [5,6]. Inflammasomes are a group of intracellular protein complexes that respond to a broad set of pathogen-associated molecular patterns and danger-associated molecular patterns with the production of the proinflammatory cytokines, IL-1β and IL-18 [7]. In addition to providing insight into the function of the human innate immune system, the discovery of the prominent role of IL-1β has provided the scientific basis for targeted therapies that have already had an enormous impact on the natural history and prognosis of several of these otherwise devastating disorders [8▪▪].

Since the initial discovery of the FMF gene, the field of autoinflammation has expanded with a burgeoning number of monogenic diseases and their underlying genes (Table 1) [1–7,8▪▪,9▪▪,10–13, 14▪,15▪,16–32,33▪,34▪▪–36▪▪,37–40]. There are also a number of genetically complex disorders that are frequently placed under the autoinflammatory rubric, such as the syndrome of periodic fever with aphthous stomatitis, pharyngitis, and adenopathy, systemic-onset juvenile idiopathic arthritis, adult-onset Still disease, and Behçet disease [41]. In addition, disorders such as gout and atherosclerosis are sometimes considered autoinflammatory because of evidence implicating IL-1 in their pathogenesis [42,43].

The advent of next-generation sequencing has permitted the molecular analysis of small families and isolated cases that were previously intractable to genetic study, leading to the recent identification of heretofore unrecognized clinical disorders and their underlying molecular mechanisms. The purpose of the present review is to focus on two newly established disease genes and their associated clinical syndromes. These examples highlight how the inherited deficiency of a key immune regulatory element may sometimes lead to autoinflammatory or autoimmune disease.

## A SPECTRUM OF VASCULOPATHY ASSOCIATED WITH THE DEFICIENCY OF ADENOSINE DEAMINASE 2

In 2014, two independent groups utilized whole-exome sequencing to discover recessively inherited loss-of-function mutations in the cat eye syndrome chromosome region, candidate 1 (*CECR1*) gene, encoding the adenosine deaminase 2 (ADA2) protein, associated with a syndrome that includes recurrent fever, early-onset vasculopathy, inflammation, and mild immunodeficiency in a total of 33 patients described in back-to-back studies in the *New England Journal of Medicine* [35▪▪,36▪▪] (Table 2). Subsequent reports prompted by these studies have added five more patients with this syndrome to the literature and, by whole-exome sequencing, extended the phenotype to five members of a family with Sneddon syndrome, a later-onset disease also characterized by fever and vasculopathy [44▪–47▪]. The term deficiency of adenosine deaminase 2 (*DADA2*) has been proposed to subsume the spectrum of clinical phenotypes caused by loss-of-function *CECR1* mutations [35▪▪].

Zhou and her colleagues at the National Institutes of Health (NIH) identified six patients of mixed European ancestry presenting before the age of 5 with intermittent fevers, systemic inflammation, lacunar strokes, hepatosplenomegaly, and hypogammaglobulinemia M. The strokes were primarily ischemic strokes of the deep-brain nuclei and the brain stem; hemorrhagic strokes were noted less frequently, and in some cases might have been attributable to anticoagulant therapy. The study included an additional three Turkish patients with polyarteritis nodosa (PAN) or small-vessel vasculitis, two of whom also had ischemic strokes. Navon Elkan *et al.* [36▪▪] at the Shaare Zedek Medical Center in Israel studied a cohort of patients of Georgian Jewish ancestry with familial PAN, most with a childhood onset and some with strokes, and extended their genetic analyses to PAN patients from Turkey and Germany.

PAN is a systemic necrotizing vasculitis involving the medium and small vessels, leading to



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**Table 1.** Molecular basis of selected monogenic autoinflammatory diseases

| Disease | Gene | Protein | Proposed mechanism | References |
|---|---|---|---|---|
| Hereditary periodic fever syndromes | | | | |
| Familial Mediterranean fever (FMF) | *MEFV* | Pyrin | Pyrin senses modifications of Rho GTPases induced by bacterial toxins. In FMF, increased IL-1β production plays a major role in mediating inflammation | [1,2,9▪▪,10] |
| TNF receptor-associated periodic syndrome (TRAPS) | *TNFRSF1A* | p55 TNF receptor | Protein misfolding causes retention of mutant TNFR1 in the endoplasmic reticulum, increased mitogen-activated protein (MAP) kinase activation,increased mitochondrial reactive oxygen species (ROS) production, and increased proinflammatory cytokine production | [3,11] |
| Hyper IgD syndrome (HIDS) | *MVK* | Mevalonate kinase enzyme | Mutations decrease geranylgeranylation of RhoA GTPase, leading to increased Rac1 activity and increased IL-1β production | [12,13,14▪,15▪] |
| Cryopyrinopathies | | | | |
| Familial cold autoinflammatory syndome (FCAS); Muckle–Wells syndrome (MWS); neonatal-onset multisystem inflammatory disease (NOMID)/chronic infantile neurologic cutaneous and articular syndrome (CINCA) | *NLRP3* | Cryopyrin | Mutations cause constitutive activation of the NLRP3 inflammasome, leading to excessive IL-1β production; mutant NLRP3 has diminished binding affinity for the inhibitory effects of cAMP | [4–6,16] |
| Other inflammasomopathies | | | | |
| Deficiency of IL-1 receptor antagonist (DIRA) | *IL1RN* | IL-1 receptor antagonist | Impaired inhibition of the IL-1β signaling pathway | [17,18] |
| Deficiency of IL-36 receptor antagonist (DITRA) | *IL36RN* | IL-36 receptor antagonist | Impaired inhibition of the IL-36 signaling pathway | [19,20] |
| Pyogenic arthritis, pyoderma gangrenosum, and acne syndrome (PAPA) | *PSTPIP1* | PSTPIP1/CD2BP1 | Increased binding of PSTPIP1 to pyrin, increased production of IL-1β | [21–23] |
| NF-κB disorders | | | | |
| Blau syndrome | *NOD2* | NOD2 (CARD15) | Constitutive activation of NF-κB pathways | [24,25] |
| Early-onset enterocolitis | *IL10RA, IL10RB, IL10* | IL-10R1, IL-10R2, IL-10 | Impaired signaling through the anti-inflammatory IL-10 pathway | [26,27] |
| Familial psoriasis (PSORS2) | *CARD14* | CARD14 | Activation of NF-κB and upregulation of psoriasis-related genes in keratinocytes | [28] |
| Interferonopathies | | | | |
| Chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperature (CANDLE) | *PSMB8* | PSMB8 | Impaired proteasome catalytic activity or assembly, impaired removal of damaged proteins. Increased production of proinflammatory cytokines, interferon-signaling signature on gene-expression profiling | [29–32,33▪] |

(*Continued*)



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**Table 1** (*Continued*)

| Disease | Gene | Protein | Proposed mechanism | References |
|---|---|---|---|---|
| STING-associated vasculopathy with onset in infancy (SAVI) | *TMEM173* | STING | Constitutive activation of STING and the interferon-β pathway | [34▪▪] |
| Other | | | | |
| Deficiency of adenosine deaminase 2 (DADA2) | *CECR1* | ADA2 | Impaired endothelial development and M2 macrophage differentiation | [35▪▪,36▪▪] |
| PLCγ2-associated antibody deficiency and immune dysregulation (PLAID) | *PLCG2* | PLCγ2 | Constitutive activation of PLCγ2 | [37] |
| Autoinflammation and PLCγ2-associated antibody deficiency and immune dysregulation (APLAID) | *PLCG2* | PLCγ2 | Decreased threshold for triggering PLCγ2, without constitutive activation | [38] |
| HOIL-1 deficiency | *RBCK1* | RBCK1 | Increased production of inflammatory cytokines by monocytes in response to IL-1β stimulation | [39,40] |

vascular stenosis and/or aneurysms and tissue ischemia. Although uncommon, PAN can cause significant morbidity and, if untreated, it is usually fatal, most frequently affecting the vasculature of the kidneys, gastrointestinal tract, central and peripheral nervous system, and skin. In some cases, PAN is caused by chronic hepatitis B infection, but in the majority of patients, no underlying cause is identified, and prior to the identification of *CECR1* mutations, there were no known predisposing genes.

Both groups used whole-exome sequencing in a small number of patients and their parents to discover the underlying gene, and then conventional candidate-gene sequencing in additional patients. Variant filtering assumed an autosomal recessive mode of inheritance. *CECR1* is located in the cat-eye syndrome critical region on chromosome 22. All disease-associated variants were either novel or extremely rare in all available sequence databases, occurred at sites that are evolutionarily highly conserved, and are predicted by computer modeling to have a deleterious effect on protein function. The functional consequences of these mutations were confirmed by documenting marked reductions in ADA2 enzymatic activity in patients' blood, by demonstrating reduced levels of ADA2 in the supernatants of cultured macrophages and transfected cell lines, and by biophysical analysis of mutant protein [35▪▪,36▪▪].

To date, a total of 15 missense mutations broadly distributed throughout the protein sequence and a single 28 kb genomic deletion have been reported [35▪▪,36▪▪,44▪–47▪]; a regularly updated listing of mutations can be found online at http://fmf.igh.cnrs.fr/ISSAID/infevers/. Although *CECR1* mutations are found at a very low frequency in multiple ethnic groups, the p.Gly47Arg mutation has an estimated carrier frequency of 10% in the Georgian Jewish population [36▪▪]. Most likely, this is due to a founder effect, and the data suggest that there may be as yet unrecognized patients with milder phenotypes in this population. It is also noteworthy that residue 47 appears to be a mutational 'hot spot', with three different mutations documented to date. Another mutation, p.Tyr453Cys, was observed in the heterozygous state in two brothers with late-onset lacunar strokes, raising the intriguing possibility that variants in this gene may be associated with more common vascular phenotypes in the general population [35▪▪].

There are two human proteins with adenosine deaminase activity, ADA1 and ADA2, both of which catalyze the conversion of adenosine and 2′-deoxyadenosine to inosine and 2′-deoxyinosine, respectively. ADA1 is ubiquitously expressed and is the primary intracellular adenosine deaminase. It



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**Table 2.** Salient features of ADA2 deficiency and phospholipase Cγ2-associated diseases

| Disease | DADA2 | PLAID | APLAID |
|---|---|---|---|
| Gene | *CECR1* | *PLCG2* | *PLCG2* |
| Protein | Adenosine deaminase 2 | Phospholipase $C\gamma_2$ | Phospholipase $C\gamma_2$ |
| Type of mutation | Missense mutations and 28-kb deletion | In-frame genomic deletions | Missense mutation |
| | | | p.Ser707Tyr |
| Effect on the protein | Loss-of-function | Gain-of-function | Gain-of-function |
| Mode of inheritance | Autosomal recessive | Autosomal dominant | Autosomal dominant |
| Clinical characteristics[a] | Fever | Cold-induced urticaria | Neutrophilic dermatosis |
| | Livedo racemosa | Granulomatous disease | Blistering skin rash |
| | Lacunar strokes | Allergy | Nonspecific interstitial pneumonitis with respiratory bronchiolitis |
| | Polyarteritis nodosa (PAN) | Autoimmune disease | Arthralgia |
| | Hepatosplenomegaly with portal hypertension | | Ocular inflammation |
| | Sneddon syndrome | | Enterocolitis |
| | | | Cellulitis |
| Immunodeficiency | B cell immunodeficiency, hypogammaglobulinemia M | Hypogammaglobulinemia | Decreased serum IgM and IgA |
| | | Recurrent infections | Recurrent sinopulmonary infections |
| | | Common variable immunodeficiency disease | |

[a]Variable percentages of affected patients.

prevents the accumulation of intracellular toxic derivatives of adenosine and 2′-deoxyadenosine, which have a profound inhibitory effect on lymphocyte development. Recessive ADA1 deficiency is the second most common cause of severe combined immunodeficiency disease [48].

*CECR1* is highly expressed in monocytes and other cells of the myeloid lineage. In contrast to ADA1, which acts intracellularly and is monomeric, ADA2 bears a signal peptide sequence that permits protein secretion and is a highly glycosylated homodimer in the extracellular space. Human ADA2 has a lower adenosine deaminase activity than ADA1 (owing to ADA2's 100-fold higher $K_m$) and has optimal activity in an acidic environment, suggesting a specialized role in an inflammatory milieu. The catalytic sites of ADA1 and ADA2 are structurally similar, but not identical. ADA2 has additional domains that mediate its dimerization and may permit binding to cell surface receptors. ADA2 is secreted by monocytes undergoing differentiation into macrophages and dendritic cells, and acts on receptors on monocytes, B cells, and T cells [49]. Based both on phylogenetic analysis and its function in promoting human macrophage and T cell proliferation and differentiation, ADA2 is considered a member of the family of adenosine deaminase-related growth factors [49,50].

Although ADA1 deficiency causes severe combined immunodeficiency disease and ADA2 is a known growth factor for T cells and macrophages, patients exhibited relatively mild immunodeficiency. Four of five patients seen at the NIH had varying degrees of lymphopenia, and all five had consistently low IgM levels, but only two had recurrent bacterial and viral infections prior to the initiation of immunosuppressive therapy [35▪▪]. Levels of cytokines in the serum or secreted by stimulated peripheral blood mononuclear cells (PBMCs) were normal. Despite an extensive evaluation, T cell numbers and function were largely unremarkable, with normal numbers of recent thymic emigrants and naïve T cells, and normal short-term T cell activation and normal proliferative responses to anti-CD3 antibodies. In contrast, B cell abnormalities were observed in the NIH cohort. This included a decreased number of memory B cells in the peripheral blood, lower expression of CD27 and IgG on B cells after a number of in-vitro stimuli, and decreased terminal differentiation of B cells and plasma cells after T cell stimulation. In addition, higher rates of spontaneous B cell death were observed when patients' PBMCs were cultured without stimulation, relative to controls. The underlying cause of the immunological abnormalities in the B cell line is not yet known [35▪▪].

In order to explore the mechanism by which ADA2 deficiency causes stroke, the NIH group sought to establish an animal model. Although there is no clear ortholog of *CECR1* in the mouse,



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there are two paralogs in the zebrafish: *cecr1a* and *cecr1b*. On the basis of existing random mutagenesis data that a *cecr1a* hypomorphic zebrafish line had no overt phenotype, Zhou *et al.* [35▪▪] set out to use knockdown morpholinos to inhibit *cecr1b* expression transiently in zebrafish embryos. Forty-eight hours after injection of the inhibitory antisense constructs, dramatic intracranial bleeds were observed in the developing embryos, as well as evidence for ischemia, phenotypes that could be rescued by expression of the nonmutated human *CECR1*, but not by disease-associated mutants. These data strongly suggest a role for the zebrafish ADA2 homolog in vascular development. Moreover, consistently with the known role of ADA2 in leukocyte development, *cecr1b* morpholinos exhibited marked neutropenia, which could be blocked by expression of the wild type but not the mutant human *CECR1*.

With the animal model data in hand, the NIH group then turned their attention back to the human condition. Although *CECR1* transcripts are not expressed and the ADA2 protein is not detectable in cultured human endothelial cells, skin and brain biopsy specimens from patients with DADA2 exhibited endothelial damage and activation of endothelial cells [35▪▪]. There was also increased perivascular staining for IL-1β, TNF-α, and inducible nitric oxide synthase, most likely because of mononuclear and T cell infiltration. On the basis of the known effects of ADA2 on macrophage development, the NIH group examined the differentiation of cultured patient or control monocytes into macrophages, and observed a skewing toward the proinflammatory M1 macrophage subset in patients, and a relative deficiency in the differentiation of the M2 subset that is typically implicated in tissue repair and healing (Fig. 1). Moreover, when patient or control monocytes were cocultured with monolayers of primary dermal microvascular endothelial cells, the patient monocytes induced disruption of the endothelial layers, relative to controls.

Taken together, the zebrafish and human data suggest a model in which ADA2 is a growth factor both for endothelial cells and for certain macrophage subsets. Deficiency of ADA2 may lead to vasculopathy both because of a direct effect on endothelial cells and by skewing monocyte and macrophage subsets, thus establishing a positive feedback loop driven by proinflammatory M1

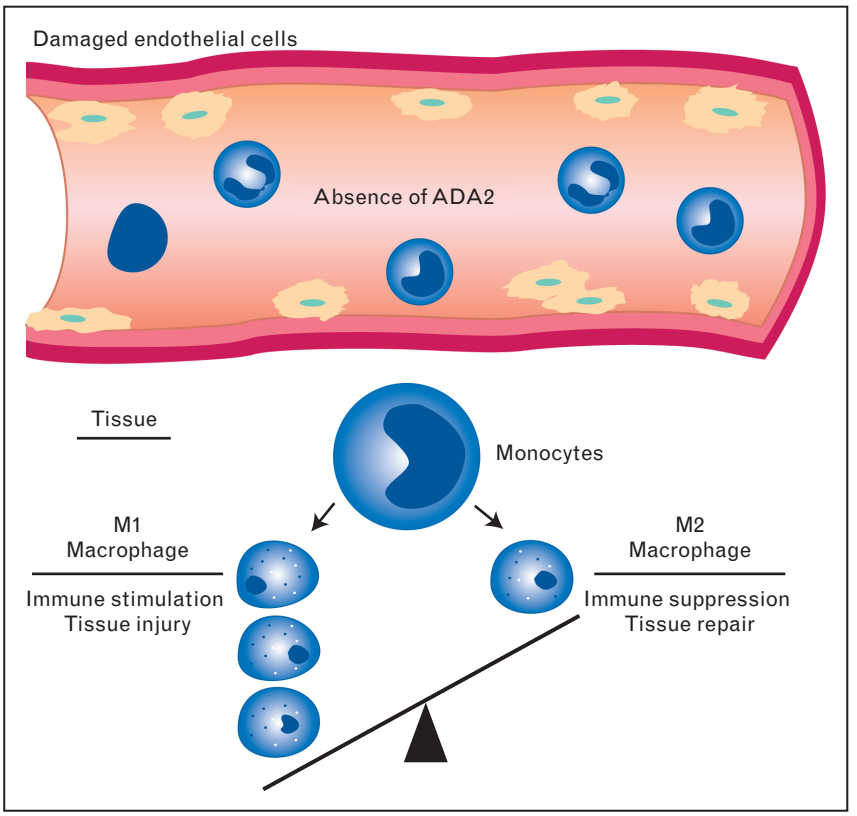


**FIGURE 1.** Effect of adenosine deaminase 2 deficiency on endothelial and inflammatory cells. In the absence of ADA2, endothelial cells appear damaged and express activation markers, such as E-selectin, with an overall loss of endothelial integrity. In the tissue, skewing toward proinflammatory M1 macrophages leads to accumulation of proinflammatory cytokines and tissue injury. ADA2, adenosine deaminase 2.



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macrophages and cytokines and by the lack of M2 macrophages that would mediate repair (Fig. 1).

Finally, the discovery of DADA2 raises a number of new questions. For patients with this condition, there are a number of new therapeutic possibilities, including recombinant ADA2 protein, administration of fresh-frozen plasma (which contains ADA2), and hematopoietic stem cell transplantation, which was effective in two recent reports [44▪,45▪]. Navon Elkan *et al.* [36▪▪] have presented preliminary evidence that treatment with anti-TNF agents may be effective in this disorder. It will also be of paramount importance to further delineate the role of ADA2 in normal immune and vascular development, and to explore further the role of *CECR1* variants in other forms of vasculitis and stroke, as well as conditions such as Sneddon syndrome and HHV-8-negative Castleman disease [47▪,51▪].

## PHOSPHOLIPASE C$\gamma_2$-ASSOCIATED DISEASES

Shortly before the discovery of the vasculopathies associated with recessively inherited mutations in ADA2, two recent studies used next-generation sequencing techniques to delineate clinical phenotypes associated with dominantly inherited mutations in *PLCG2*, encoding the phospholipase C$\gamma_2$ (PLC$\gamma_2$) signaling molecule. The patients described in these reports presented with features of both immunodeficiency and autoimmunity or autoinflammation.

In the first of these manuscripts, Ombrello *et al.* [37] described three families with in-frame genomic deletions in *PLCG2* and a dominantly inherited form of cold-induced urticaria, and proposed the term PLC$\gamma_2$-associated antibody deficiency and immune dysregulation (PLAID) to denote this condition (Table 2). These patients also manifested a spectrum of immune abnormalities that included granulomatous rash, sinopulmonary infections, hypogammaglobulinemia, B cell and natural killer (NK) cell defects, autoantibodies, and, in some cases, symptomatic autoimmune disease (Table 2). All 27 affected patients exhibited an urticarial rash that had its onset in childhood, and typically was triggered by exposure to cold wind rather than by contact with cold objects. The rash could be induced by an evaporative cooling challenge, but not by ice cube challenge or cold-water immersion. More than half of the patients (17/27) had a history of recurrent infections, most frequently of the upper and lower respiratory tract, and three were diagnosed with common variable immunodeficiency disease. Seven patients from two of the families had granulomatous disease. Three of these patients had transient cutaneous granulomatous lesions of the fingers and nose during infancy, and the rest had persistent skin disease, including soft palate and laryngeal involvement in one patient. Fifty-six percent of the patients had demonstrable autoantibodies or symptomatic autoimmune disease, and a similar percentage had a history of allergy, including asthma, eczema, allergic rhinitis, allergic conjunctivitis, and drug or food allergies.

On routine laboratory testing, patients had low serum immunoglobulin levels (15/20 who were tested), with IgM and IgA the most frequently affected [37]. There were also decreased numbers of $CD19^+$ B cells in the peripheral blood, decreased numbers of $IgA^+$ and $IgG^+$ class-switched memory B cells, and decreased numbers of NK cells. No abnormalities were detected in the numbers of neutrophils, monocytes, basophils, eosinophils, or numbers of naïve or memory T cells. IgE levels were often elevated. In addition, patients manifested decreased IgG and IgA class switching upon stimulation, and impaired termination of secondary recombination, as assessed by $J_\kappa$ region usage.

The causative gene was identified by an integrated approach that included linkage and haplotype analysis, targeted genomic and cDNA sequencing of the *PLCG2* candidate gene, and confirmatory analyses of whole-genome sequence from one of the patients [37]. In two of the families, there were distinct genomic deletions resulting in the in-frame loss of exon 19 of *PLCG2*, whereas the third family exhibited a genomic deletion that resulted in the in-frame loss of exons 20–22. Five of the six deletion breakpoints observed in these three families occurred within repetitive elements known to facilitate aberrant recombination.

PLC$\gamma_2$ is a member of the phosphoinositide-specific phospholipase C family, a key family of enzymes involved in trans-membrane signaling. A variety of extracellular stimuli, including hormones, antigens, and growth factors initiate intracellular signaling cascades through tyrosine phosphorylation and activation of phospholipase C enzyme isoforms. Phosphoinositide-specific phospholipase C isoenzymes hydrolyze phosphatidyl-inositol 4,5-diphosphate ($PIP_2$) to form inositol 1,4,5-triphosphate ($IP_3$) and diacylglycerol. $IP_3$ mediates the release of calcium from the endoplasmic reticulum, an intermediate step in cellular activation (Fig. 2a). There are two PLC$\gamma$ isoenzymes: PLC$\gamma_1$ that is widely expressed and PLC$\gamma_2$ that is critical for signaling in B lymphocytes, NK cells, and mast cells [52].

PLAID-associated mutations were found in the C-terminal Src-homology domain 2 (cSH2) of PLC$\gamma_2$, which is part of a larger autoinhibitory domain (the X-Y linker) that prevents constitutive



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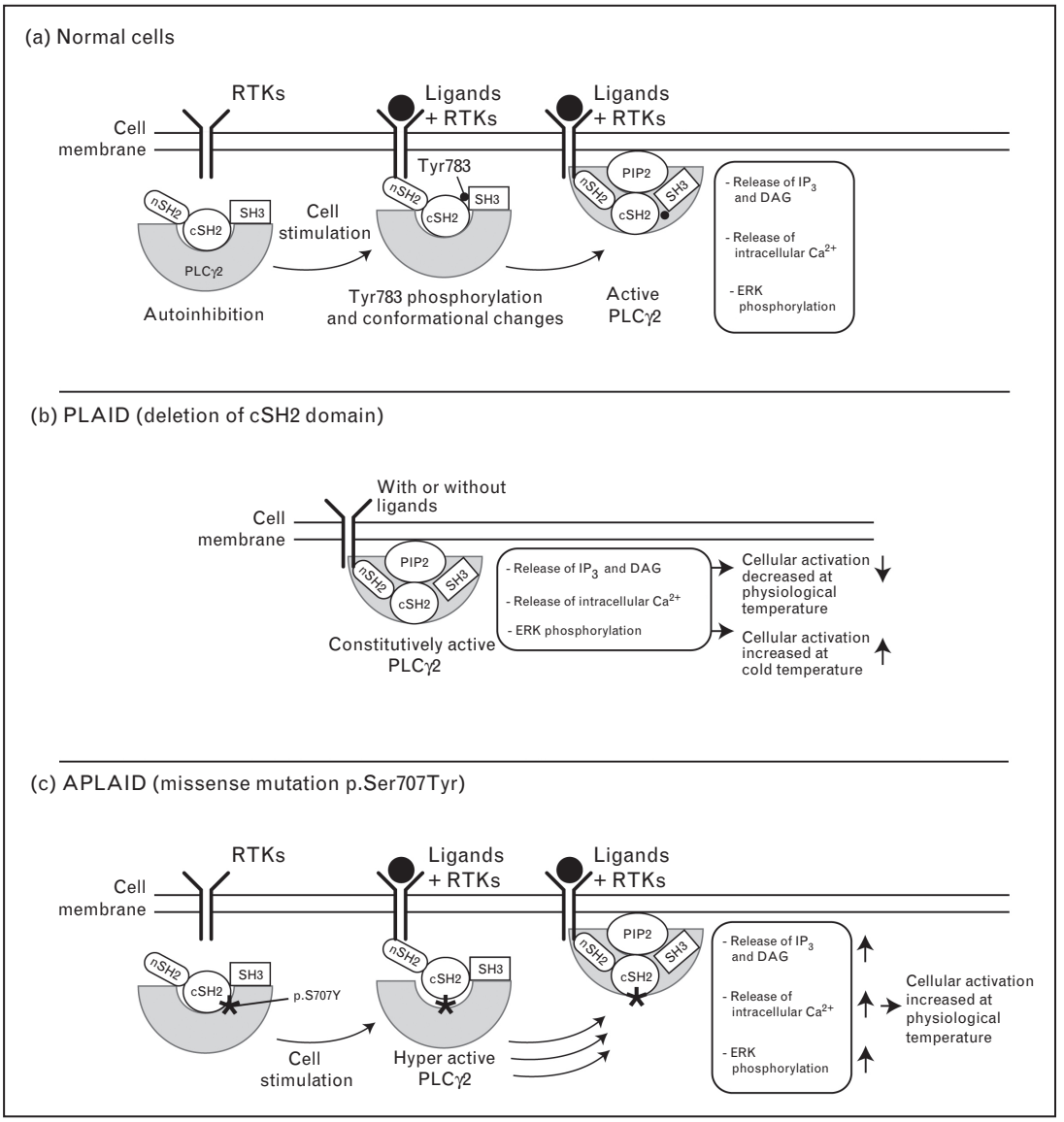


**FIGURE 2.** Schematic diagrams of the function of the phospholipase $C\gamma_2$ enzyme under normal conditions, in PLAID and in APLAID. (a) In normal unstimulated cells, $PLC\gamma_2$ is in a state of autoinhibition, with the cSH2 domain blocking the catalytic site. Upon stimulation of receptor tyrosine kinases (RTKs) by various ligands, $PLC\gamma_2$ is recruited to the cell membrane. Through a chain of interactions $PLC\gamma_2$ becomes phosphorylated at tyrosine residue 783, causing conformational changes that lead to exposure of the catalytic site to its substrate, phosphatidylinositol 4,5-bisphosphate (PIP2). The active $PLC\gamma_2$ enzyme then catalyzes the formation of $IP_3$ and diacylglycerol (DAG) from PIP2, which leads to the downstream effects of increased intracellular calcium ($Ca^{2+}$) and extracellular signal-regulated kinase (ERK) phosphorylation. (b) In PLAID, genomic deletions affecting the autoinhibitory cSH2 domain cause constitutive activation of $PLC\gamma_2$ even in the absence of RTK ligands, leading either to substrate depletion or to activation of inhibitory pathways. Cellular activation is therefore not observed under physiologic conditions but only upon exposure to cold. (c) In APLAID, the missense mutation p.Ser707Tyr may either create an additional tyrosine residue available for phosphorylation or may disrupt the interaction of the catalytic domain with the autoinhibitory cSH2 domain. In either case, the net effect is an increase in the $PLC\gamma_2$ enzymatic activity and an increase in inducible cellular activation at physiologic temperatures.

signaling [53▪]. It was therefore not surprising that overexpression studies documented increased basal and Rac-activated enzymatic activity of the altered $PLC\gamma_2$ [37]. However, it was surprising that in studies of patients' B lymphocytes and NK cells, the downstream effects of $PLC\gamma_2$ measured by intracellular calcium levels and extracellular signal-related kinase (ERK) phosphorylation were paradoxically decreased at physiologic temperatures and elevated only by exposure to cold temperature



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(perhaps accounting for the cold urticaria that patients experience) (Fig. 2b). Possible explanations for these paradoxical cellular effects of PLC$\gamma_2$ mutations at physiologic temperatures include depletion of the $PIP_2$ substrate or the activation of negative regulatory feedback molecules. Signaling defects in PLAID B cells would predictably result in the observed antibody deficiencies and impaired class switching, as well as abnormalities in receptor editing that could lead to autoimmunity.

In the second of the two manuscripts, Zhou *et al.* [38] utilized whole-exome sequencing to study a family with recurrent blistering skin lesions since childhood, cellulitis, recurrent sinopulmonary infections, nonspecific interstitial pneumonitis with respiratory bronchiolitis (NSIP), enterocolitis, ocular inflammation, arthralgia, and mild immunodeficiency (Table 2). A young woman and her father were affected, but neither of the paternal grandparents manifested symptoms. Remarkably, when whole-exome data from the daughter, father, and unaffected mother were filtered on the assumption of a de novo dominant mutation in the father, only a single variant met the tests of having a significant effect on the encoded protein, evolutionary conservation, and familial cosegregation: the p.Ser707Tyr mutation in *PLCG2,* which resides in the same autoinhibitory cSH2 domain that harbors mutations in PLAID. This variant was not found in 1488 genotyped control chromosomes.

The two patients had low levels of serum IgM and IgA and markedly diminished numbers of class-switched memory B cells in the periphery [38]. Numbers of circulating T cells and NK cells were normal, although numbers of NK T cells were reduced in both affected individuals. Despite multiple studies, autoantibodies were not detected in either affected individual.

In order to assess the functional consequences of the p.Ser707Tyr mutation, transient transfection studies were performed, which documented increased ERK phosphorylation, intracellular $IP_3$ levels, and intracellular calcium upon stimulation in cells transfected with mutant constructs, relative to wild type [38]. Similar results were observed when patients' PBMCs were compared with PBMCs from healthy controls. It is noteworthy that, whereas the cellular phenotype of PLAID is decreased signaling, the cellular phenotype of the p.Ser707Tyr mutation is increased signaling (Fig. 2c). Most likely, this is because PLAID mutations cause constitutive signaling and substrate depletion or negative feedback, while p.Ser707Tyr causes more easily inducible although not constitutive signaling, perhaps by introducing an extra tyrosine phosphorylation site in the autoinhibitory cSH2 domain. In light of the difference in cellular phenotype between p.Ser707Tyr and PLAID, as well as the fact that patients with p.Ser707Tyr do not have autoantibodies, the term autoinflammation and PLC$\gamma_2$-associated antibody deficiency and immune dysregulation (APLAID) has been proposed [38].

## CONCLUSION

The monogenic autoinflammatory diseases are inborn errors of the innate immune system that have provided important insights into human immune function and disease. As illustrated in this article, next-generation sequencing techniques have catalyzed the genetic analysis of phenotypes that would have been considered intractable even 5 years ago. Although the discovery of genes, such as *CECR1* and *PLCG2*, has opened new vistas, it has also blurred the boundaries between autoinflammation, autoimmunity, and immunodeficiency. These advances underscore the fact that autoinflammation, immunodeficiency, and autoimmunity represent axes of a multidimensional phenotypic space created by human genetic variation.

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### Conflicts of interest

*There are no conflicts of interest.*

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Papers of particular interest, published within the annual period of review, have been highlighted as:
■ of special interest
■■ of outstanding interest

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