Severe autoinflammation in 4 patients with C-terminal variants in cell division control protein 42 homolog *(CDC42)* successfully treated with IL-1β inhibition

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To the Editor:

Autoinflammatory syndromes are caused by dysregulation of the innate immune system, often affecting the inflammasome and other pathogen recognition pathways that lead to overproduction of IL-1b and IL-18.[1] Here we report 4 patients with severe autoinflammatory syndrome, presenting with cytopenias, rashes, and a significant increase in serum IL-18 levels secondary to novel specific mutations in cell division control protein 42 homolog *(CDC42). CDC42* encodes a small Rho family GTPase that regulates multiple signaling pathways controlling cell polarity and migration, actin polarization, cytoskeletal architecture, endocytosis, and cell-cycle progression.[2] All mutations affect the last 3 to 5 amino acids of the *CDC42* C-terminus. Mutations predispose to the development of macrophage activation syndrome (MAS), and all patients improved significantly on therapy with IL-1 receptor inhibitors.

We report 4 patients from 3 different institutions. Clinical descriptions and biological parameters are summarized in Table 1 and detailed in the Methods section in this article's Online Repository at www.jacionline.org. In brief, all patients presented at birth or in the neonatal period with growth restriction, hepatosplenomegaly, transaminitis, recurrent febrile episodes, urticaria-like rashes, and significant cytopenias. Patient 4 had aseptic meningitis, papilledema, and posterior retinitis and was thought to have clinical features consistent with the IL-1–mediated autoinflammatory disease neonatal-onset multisystem inflammatory disease (NOMID).[1] Most patients displayed some facial dysmorphisms, such as mild frontal bossing, mild hypertelorism, and nasal bridge depression (Fig 1, B). Anemia (all patients) and thrombocytopenia (patients 3 and 4) were transfusion dependent. Skin biopsy specimens of patients 2 and 3 revealed perivascular lymphocytic and neutrophilic infiltration without vasculitis (data not shown). Further laboratory evaluation revealed for all patients a dramatic increase in C-reactive protein (CRP) levels, erythrocyte sedimentation rates (ESRs), and ferritin levels. In workup of their hyperferritinemic inflammation, all patients were found to have substantially increased peripheral blood IL-18 levels (Fig 1, D). Patients 2 and 3 met the criteria for hemophagocytic lymphohistiocytosis/MAS. None of the patients had a family history of consanguinity.

Because of the complex nature of their disease, whole-exome sequencing (WES) was performed in all patients and identified 3 distinct monoallelic, heterozygous, possibly pathogenic *de novo* variants in *CDC42.* Patients 1 and 2 carried *CDC42* c.563G>A (p.C188Y), patient 3 carried *CDC42* c.556C>T (p.R186C), and patient 4 carried *CDC42* c.576A>C (p.*192C*24), which causes read-through of the stop codon and adds 24 amino acids to the C-terminus of the protein (Fig 1, C).

Given their inflammation, MAS-like features, and concern for NOMID (in patient 4), therapy with the IL-1 receptor antagonist anakinra was initiated in all 4 patients, with the rationale that local release of IL-1β from monocytes and in tissues promotes systemic inflammation and is associated with chronically increased CRP levels and ESR (Fig 1, D). On anakinra, fever and rashes entirely resolved, and the spleen and liver size of all patients significantly decreased. In all patients failure to thrive significantly improved with respect to both longitudinal growth and weight gain. Three of the 4 patients started to meet

developmental milestones. Anemia and thrombocytopenia resolved in 3 of the 4 patients, and CRP and ferritin levels decreased significantly in all 4 patients. Patient 3 continued with intermittent flares. Facial dysmorphisms persisted. In the 1 patient analyzed, pretherapy abnormalities in monocytes and natural killer cells improved after anakinra (see Fig E1 in this article's Online Repository at www.jacionline.org). Remarkably, peripheral blood IL-18 levels showed little or no diminution, even after months of treatment (Fig 1, D, and Table 1). Although patient 1 has never experienced significant infections over 1 year of therapy, patients 2, 3 and 4 had recurrent gastrointestinal and respiratory tract infections (patient 2, over 1 year of therapy), staphylococcal lymphadenitis (patients 3 and 4, over 6 and 11 years of therapy, respectively), group A streptococcal sepsis with purpura fulminans (patient 3), and *Staphylococcus* bacteremia and abscesses (patient 4). Infections in patient 3 occurred during canakinumab treatment and in patient 4 during anakinra and prednisone treatment at approximately 1 mg/kg/d and during a short treatment period when he was switched from anakinra to canakinumab (see the Methods section in this article's Online Repository).

Heterozygous mutations in the *CDC42* gene were recently reported to cause Takenouchi-Kosaki syndrome (TKS), which manifests with growth retardation, developmental delay, facial dysmorphism (diverse features depending on the type of *CDC42* mutation, see Table E1 and the Methods section in this article's Online Repository at www.jacionline.org), and macrothrombocytopenia.[3] Like TKS, some of our patients have mild facial dysmorphic features (see Table E1 and the Methods section in this article's Online Repository). However, inflammatory disease manifestations, including rashes, laboratory evidence of systemic inflammation, and development of MAS/hemophagocytic lymphohistiocytosis have not been reported in patients with TKS.

The high serum IL-18 level increase and the predisposition to development of MAS in our autoinflammatory cohort are laboratory and clinical features that overlap with patients with NLRC4-MAS and X-linked inhibitor of apoptosis deficiency.[4,5] The significant clinical improvement with IL-1–blocking treatment supports a critical role for IL-1 driving the disease process and together with chronically increased IL-18 levels suggests inflammasome activation as a pathomechanism.[4,6]

This points to the need for further investigations to mechanistically connect the C-terminal *CDC42* mutations with overproduction of IL-18 and IL-1 activation. The novel C-terminal variants in *CDC42*, which are associated with severe autoinflammation, all occur within or in close spatial proximity to a C-terminal diarginine motif implicated in membrane binding and lipidation of *CDC42*. Indeed, it has been reported that this diarginine motif (Arg186 and Arg187) plays an essential role in binding of *CDC42* to liposomes containing phosphatidylinositol 4,5-bisphosphonate (PIP_2).[7] The interaction of *CDC42* and PIP_2 is critical in mediating the PIP2-induced actin assembly.[8]

Emerging evidence highlights the importance of the actin cytoskeleton in modulating inflammatory responses. A growing number of pathogenic mutations cause aberrant actin depolymerization, activate the inflammasome, and modulate other innate immune functions.[6,9] Illustrating the functional effect of actin polymerization defects, loss-of-function mutations in *WDR1*, encoding actin-interacting protein 1, cause defective

neutrophil mobilization, opportunistic infections, and activation of monocyte/macrophages, leading to autoinflammation.[6] How exactly defects in actin depolarization predispose to exaggerated inflammasome activation or propagation remains the subject of ongoing studies.

Interestingly, 3 of the 4 patients with novel C-terminal *CDC42* variants have mutations in additional genes that might modify the clinical phenotypes. These mutations include heterozygous lysosomal trafficking regulator *(LYST)* variants (patients 2 and 3), a rare *UNC13D* variant (patient 3), and a Wiskott-Aldrich syndrome *(WAS)* mutation that has previously been associated with thrombocytopenia and a mild Wiskott-Aldrich syndrome phenotype (patient 4, see Table E2 in this article's Online Repository at www.jacionline.org). However, one of the 4 patients, patient 1, did not have any other mutations on WES (minor allele frequency, <1%) that are expected to confound the disease phenotype. Patient 1 also had the most complete response to anakinra, with reversal of clinical symptoms and rapid improvement of laboratory markers. Taken together, these data suggest that the C-terminal *CDC42* variants might be sufficient to trigger an autoinflammatory syndrome that is responsive to IL-1 inhibition, although a modifying role for the other rare variants cannot fully be ruled out without more patients and further mechanistic studies.

In summary, these 4 patients' mutations, biomarker assessment, and response to IL-1 inhibition all support that C-terminal variants in *CDC42* cause a novel IL-1 inhibition–responsive autoinflammatory syndrome. All patients had chronic and extreme increases in IL-18 levels, likely predisposing to development of MAS. The phenotypic overlap with patients with X-linked inhibitor of apoptosis deficiency and activating *NLRC4* mutations points to a shared pathogenesis.[4,5] Given the remarkable treatment response to IL-1-blocking therapies, the recognition of this novel disease will hopefully facilitate rapid diagnosis and early targeted therapies. Further studies are necessary to elucidate the mechanistic basis and test the efficacy of other potential therapeutic agents, including human recombinant IL-18BP.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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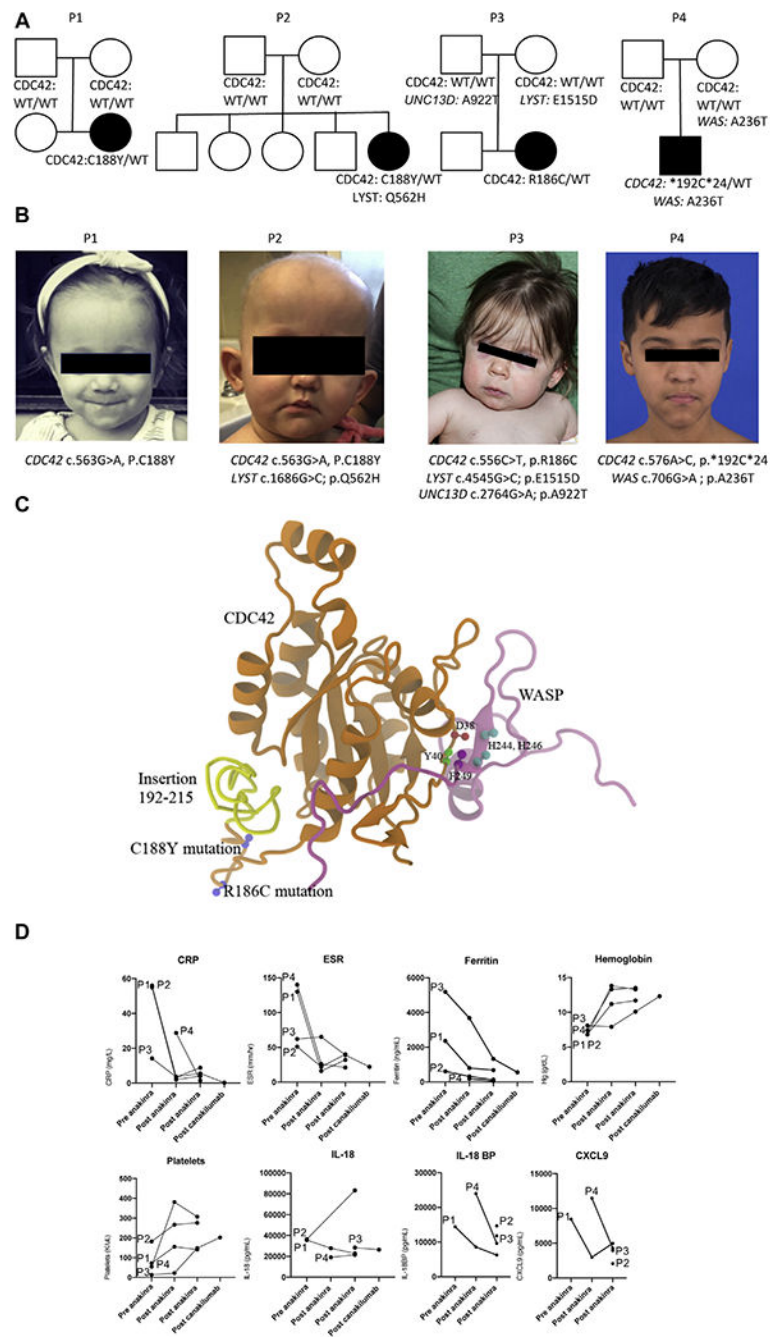
FIG 1.
A and **B,** Picture (Fig 1, A) and pedigree (Fig 1, B) of 4 patients showing a *de novo* monoallelic mutation in *CDC42*. The affected subjects are indicated as follows: *circles*, female subjects; *squares*, male subjects. *WT*, Wild-type. **C** and **D,** Crystal structure of *CDC42* (orange). Highlighted are the Wiskott-Aldrich syndrome protein *(WASP)* binding domain (Fig 1, C) and the 3 reported C-terminal variants (Fig 1, D). Laboratory parameters

measured were CRP, ESR, ferritin, hemoglobin, platelet, and cytokines (IL-18, IL-18BP, and CXCL9).

TABLE I.

Clinical phenotype and biological parameters of the reported patients before and after therapy with IL-1 inhibitors

	Patient 1			Patient 2			Patient 3				Patient 4		
CDC42 variant	c.563G>A (p.C188Y)			c.563G>A (p.C188Y)			c.556C>T (p.R186C)				c.576A>C (stop lost [p.*192C*24])		
Dose of anakinra (d)	Before anakinra	1 mg/kg	2.5 mg/kg	Before anakinra	6 mg/kg	5 mg/kg	Before anakinra	3 mg/kg	8.5 mg/kg	Canakinumab, 8 mg/kg/mo	Before anakinra	3 mg/kg	4.5 mg/kg
Clinical presentation													
Facial dysmorphism	Mild frontal bossing, mild hypertelorism, depresse nasal bridge			Frontal bossing, macrocephaly, thin sparse hair, depressed nasal bridge			None				Mild frontal bossing		
Weight (percentile)	<3rd	<3rd	5th	<3rd	<3rd	5th	3rd	3rd	3rd	5th	<3rd	<3rd	10th
Appetite	Poor	Improved	Normal	Poor	Improved	Improved	Poor	Improved	Improved	Normal	Poor	Decreased	Normal
Spleen size* (cm [patient age; SM or normal, UL for age])	11.4 (12 mo; SM, UL 8.0)	8 (15 mo; normal, UL 8.0)	8 (18 mo; normal, UL 8.0)	11.2 (neonatal; NA SM, UL 6.0)	NA	10.1 (12 mo; SM, UL 8.0)	9.2 (neonatal; SM, UL 6.0)	9.9 (5 mo; SM, UL 6.5)	10.8 (16 mo; SM, UL 8.0)	11.9 (6 y; SM, UL 10.0)	Hepatomegaly and SM	6.7 (15 mo old; normal, UL 8.0)	NA
Painful rash	Daily	None	None	Bimonthly	Monthly	None	Daily	Resolved	Resolved	Resolved	Daily	Recurrent	None
Ibuprofen intake	Daily	None	None	None	None	None	None	None	None	None	None	None	None
Severe infections	None	None	None	None	None	None	None	Lymphadenitis	Lymphadenitis	Lymphadenitis, streptococcal sepsis	None	Yes† Lymphadenitis	Yes†
Biological parameters													
Platelet (10³/mL)	72	155	141	182	267	276	13	22	148	202	54	381	307
Hemoglobin (g/dL)	Transfusion-dependent anemia (Hb, <7 g/dL)	13.8	13.3	Transfusion-dependent anemia (Hb, <7 g/dL)	11.2	11.7	8.1	7.9	10.1	12.3	7.4	13.3	13.5
RBC transfusion	Monthly	None	None	Q2-3 mo	None	None	Weekly or biweekly	Sporadic	Rare	Rare	Multiple	None	None
Platelet transfusion	None	None	None	None	None	None	Multiple	Sporadic	Rare	Rare	Multiple	None	None

	Patient 1				Patient 2				Patient 3			Patient 4	
LDH (ng/mL)	540	NA	547	584	269	271	817	NA	998	1,115	1,633	284	353
ESR (mm/h)	>130	16	32	51	22	40	62	140	65	39	22	26	21
CRP (mg/L)	55	2	4	56	3.4	8.8	14.2	Increased	3.6	5.6	0.2	28.8	1.54
Ferritin (ng/mL [<400])	2,364	801	680	614	316	126	5,191	NA	3,682	1,329	557	172	51
IL-6 (pg/mL [<5])	18	NA	<5	NA	NA	NA	NA	NA	NA	15.9	11.0	NA	NA
IL-18§ (pg/mL [100-500])	35,616	27,803	22,690	36,479//	NA	83,268	NA	NA	NA	28,407	26,402	19,025	21,535
IL-18BP§ (pg/mL [1,000-6,000])	14,418	8,557	6,264	NA	NA	14,653	NA	NA	NA	11,803	NA	23,986	9,626
CXCL9§ (pg/mL [500-3,500])	8,457	2,978	4,975	NA	NA	2,064	NA	NA	NA	3,942	NA	11,473	4,278

For patient 1, duplicate samples were run for all 3 time points of IL-18 and were 26,477, 31,134, and 25,431 pg/mL, respectively.

Hb, Hemoglobin; LDH, lactate dehydrogenase; NA, not applicable; SM, splenomegaly; UL, upper limit of normal.

* Suggested upper limit splenic length in 230 infants and children (PMID: 2048509): 0 to 3 months, 6.0 cm; 3 to 6 months, 6.5 cm; 6 to 12 months, 7.0 cm; 1 to 2 years, 8.0 cm; 2 to 4 years, 9.0 cm; 4 to 6 years, 9.5 cm; 6 to 8 years, 10.0 cm; 8 to 10 years, 11.0 cm; and 10 to 12 years, 11.5 cm.

† While receiving 1 mg/kg/d prednisone and anakinra, he had respiratory syncytial virus pneumonia, septic arthritis, and cervical lymphadenitis, which resolved when steroids were discontinued.

‡ At age 9 years, when anakinra was switched to canakinumab, he had Staphylococcus aureus bacteremia and septic myositis with multiple abscesses and was switched back to anakinra with significant improvement.

§ Performed by Dr Scott Canna.

// IL-18 levels were measured after 1 dose of anakinra.