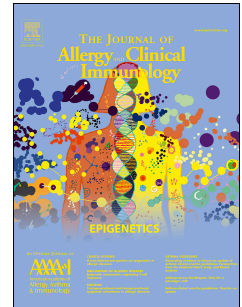


Accepted Manuscript

Severe autoinflammation in four patients with C-terminal variants in CDC42 successfully treated with IL-1 β inhibition

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Letters to the Editor

**Severe autoinflammation in four patients with C-terminal variants in CDC42
successfully treated with IL-1 β inhibition.**

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Capsule summary:

Four patients with novel variants in the C-terminal domain of CDC42, severe autoinflammation, constitutive elevation of serum interleukin 18, and predisposition to macrophage activation syndrome respond to treatment with interleukin-1 β signaling inhibition.

Key words: CDC42, autoinflammatory syndrome, IL-18 (interleukin), IL-1 β inhibition, inflammasome, Takenouchi-Kosaki syndrome

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Table: 1

Supplementary materials:

Figure: 1

Tables: 3

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All authors have disclosed all financial relationships for themselves and their immediate family/significant others.

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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 Interleukin(IL)-1 β and IL-18 (1). Here, we report four patients with severe autoinflammatory syndrome, presenting with cytopenias, rashes and significant increase in serum IL-18 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 3-5 amino acids of the *CDC42* C-terminus. The mutations predispose to the development of macrophage activation syndrome (MAS), and all patients significantly improved on therapy with IL-1 receptor inhibitors.

We report four patients from three different institutions. Clinical description and biological parameters are summarized in Table I and detailed in the supplementary materials. 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, 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

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depression (Figure 1B). The anemia (all patients) and thrombocytopenia (patients 3 and 4) were transfusion dependent. Skin biopsies of patients 2 and 3 revealed perivascular lymphocytic and neutrophilic infiltration, without vasculitis (not shown). Further laboratory evaluation revealed for all patients a dramatic increase in C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), and ferritin. In workup of their hyperferritinemic inflammation, all patients were found to have substantially elevated peripheral blood IL-18 levels (Figure 1D). Patients 2 and 3 met criteria for hemophagocytic lymphohistiocytosis (HLH)/ macrophage activation syndrome (MAS). None of the patients had a family history of consanguinity. Due to the complex nature of their disease, WES was performed in all patients and identified three 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 (see Figure 1C). 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 four patients with the rationale that local release of IL-1 β from monocytes and in the tissues promotes systemic inflammation and is associated with chronically elevated CRP and ESR (Figure 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 four patients started to meet developmental milestones. Anemia and thrombocytopenia resolved in three of the four patients, and CRP and ferritin significantly decreased in all four patients. Patient 3 continued with intermittent flares. Facial dysmorphisms persisted. In the

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one patient analyzed, pre-therapy abnormalities in monocytes and natural killer cells improved post-anakinra (Supplementary Figure E1). Remarkably, peripheral blood IL-18 levels showed little/no diminution even after months of treatment (Figure 1D and Table I). Whereas patient 1 has never experienced significant infections over one year of therapy, patients 2, 3 and 4 developed recurrent GI and respiratory infections (patient 2, over one year of therapy), staphylococcal lymphadenitis (patient 3 and 4, over 6 and 11 years of therapy, respectively), Group A streptococcal sepsis with purpura fulminans (patient 3), staphylococcus bacteremia and abscesses (patient 4). The infections in patient 3 occurred on canakinumab and in patient 4 on anakinra and prednisone at ~1mg/kg/day and during a short treatment period when he was switched from anakinra to canakinumab (Supplementary Materials).

Heterozygous mutations in the *CDC42* gene were recently reported to cause Takenouchi-Kosaki syndrome (TKS), manifesting with growth retardation, developmental delay, facial dysmorphism (diverse features depending on the type of *CDC42* mutation, Supplementary Materials, Table E1), and macro-thrombocytopenia (3). Like TKS, some of our patients have mild facial dysmorphic features (Supplemental Materials, Table E1). However, inflammatory disease manifestations including rashes, laboratory evidence of systemic inflammation and the development of MAS/HLH have not been reported in TKS.

The high serum IL-18 elevations and the predisposition to the development of MAS in our autoinflammatory cohort are laboratory and clinical features that overlap with patients with NLRC4-MAS and XIAP deficiency (4, 5). The significant clinical improvement with IL-1 blocking treatments supports a critical role for IL-1 driving the disease process, and together with the

chronically elevated IL-18 levels suggest inflammasome activation as 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 di-arginine motif implicated in membrane binding and lipidation of CDC42. Indeed, it has been reported that this di-arginine motif (Arg 186 and Arg 187) plays an essential role in the binding of CDC42 to liposomes containing phosphatidylinositol 4,5-bisphosphonate [PIP(2)] (7). The interaction of CDC42 and PIP(2) is critical in mediating PIP(2)-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 impact of actin polymerization defects, loss-of-function mutations in WDR1 encoding actin-interacting protein 1 (AIP-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 subject of ongoing studies.

Interestingly, three of the four patients with novel C-terminal CDC42 variants have mutations in additional genes that might modify the clinical phenotypes. These mutations include heterozygous *LYST* variants (patient 2 and 3), a rare *UNC13D* variant (patient 3), and a *WAS* mutation that has previously been associated with thrombocytopenia and a mild *WAS* phenotype (patient 4) (Supplementary Table 2). However, one of the four patients, patient 1,

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147 did not have any other mutations on WES (minor allele frequency <1%) that are expected to
148 confound the disease phenotype. Patient 1 also had the most complete response to anakinra
149 with reversal of clinical symptoms and rapid improvement of laboratory markers. Taken
150 together, these data suggest that the C-terminal CDC42 variants might be sufficient to trigger
151 an autoinflammatory syndrome that is responsive to IL-1 inhibition, although a modifying role
152 for the other rare variants cannot fully be ruled out without more patients and further
153 mechanistic studies.

154
155 In summary, these four patients' mutations, biomarker assessment, and response to IL-1
156 inhibition all support that C-terminal variants in CDC42 cause a novel, IL-1 inhibition-responsive
157 autoinflammatory syndrome. All patients had chronic and extreme elevation of IL-18, likely
158 predisposing to the development of MAS. The phenotypic overlap with patients with XIAP-
159 deficiency and activating *NLR4* mutations points to a shared pathogenesis (4, 5). Given the
160 remarkable treatment response to IL-1 blocking therapies, the recognition of this novel disease
161 will hopefully facilitate rapid diagnosis and early targeted therapies. Further studies are
162 necessary to elucidate the mechanistic basis and test the efficacy of other potential therapeutic
163 agents, including human recombinant IL-18 BP.

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Letters to the Editor

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ACCEPTED MANUSCRIPT

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Figure and Table

Figure I. (A) Picture and **(B)** Pedigree of four patients showing a *de novo* monoallelic mutation in *CDC42*. The affected individual(s): filled, circle (female), square (male), WT (wild type). **(C)** Crystal structure of CDC42 (orange). Highlighted are the WASp binding domain and the three reported C-terminal variants **(D)** Laboratory parameters: CRP, ESR, ferritin, hemoglobin, platelet, cytokines (IL-18, IL-18BP and CXCL9)

Table I: Clinical phenotype and biological parameters of the reported patients pre and post therapy with IL1-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 (day)	Prior to anakinra	1mg/kg	2.5mg/kg	Prior to anakinra	6mg/kg	5mg/kg	Prior to anakinra	3mg/kg	8.5mg/kg	Canakinu- mab 8mg/kg/ monthly	Prior to anakinra	3mg/kg	4.5mg/kg
Clinical presentation													
Facial dysmorphism	Mild frontal bossing, mild hypertelorism, depressed nasal bridge			Frontal bossing, macrocephaly, thin sparse hair, and depressed nasal bridge			None				Mild frontal bossing		
Weight (percentile)	<3 rd	<3 rd	5 th	<3 rd	<3 rd	5 th	3 rd	3 rd	3 rd	5 th	<3 rd	<3 rd	10 th
Appetite	Poor	Improve d	Normal	Poor	Improved	Improved	Poor	Improve d	Improved	Normal	Poor	Decrease d	Normal
Spleen size (cm) [patient age; SM or normal, UL for age] ~	11.4 [12mo; SM, UL 8.0]	8 [15mo; normal, UL 8.0]	8 [18 mo; normal, UL 8.0]	11.2 [neonatal; SM, UL 6.0]	NA	10.1 [12mo; SM, UL 8.0]	9.2 [neonatal; SM, UL 6.0]	9.9 [5mo; SM, UL 6.5]	10.8 [16mo; SM, UL 8.0]	11.9 [6 yo; SM, UL 10.0]	Hepatomegaly and SM	6.7 [15 months old; normal,	NA

												UL 8.0]	
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	Lymph-adenitis	Lymph-adenitis	Lymph-adenitis, Streptococcal sepsis	None	Yes* Lymph-adenitis	Yes**
Biological parameters													
Platelet ($10^3/\mu\text{L}$)	72	155	141	182	267	276	13	22	148	202	54	381	307
Hemoglobin (g/dL)	Transfusion dependent Anemia (Hb<7g/dL)	13.8	13.3	Transfusion dependent Anemia (Hb<7g/dL)	11.2	11.7	8.1	7.9	10.1	12.3	7.4	13.3	13.5
RBC transfusion	Monthly	None	None	Q2-3month	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

LDH (ng/mL)	540	NA	547	584	269	271	817	998	1,115	1,633	NA	284	353
ESR (mm/hr)	>130	16	32	51	22	40	62	65	39	22	140	26	21
CRP (mg/L)	55	2	4	56	3.4	8.8	14.2	3.6	5.6	0.2	Increased	28.8	1.54
Ferritin (ng/mL) (<400)	2364	801	680	614	316	126	5191	3682	1329	557	NA	172	51
IL-6 pg/mL (<5)	18	NA	<5	NA	NA	NA	NA	NA	15.9	11.0	NA	NA	NA
IL-18 (pg/mL) ^ (100-500)	35,616	27,803	22,690	36,479 ^^	NA	83,268	NA	NA	28,407	26,402	NA	19,025	21,535
IL-18BP (pg/mL)^ (1000-6000)	14,418	8557	6264	NA	NA	14,653	NA	NA	11803	NA	NA	23,986	9626
CXCL9 (pg/mL)^ (500-3500)	8457	2978	4975	NA	NA	2064	NA	NA	3,942	NA	NA	11,473	4278

Table I. Clinical phenotype and biological parameters of the 4 reported patients pre and post IL-1 β inhibition. For patient 1 duplicate samples were run for all 3

timepoints of IL-18 and were 26,477, 31,134 and 25,431pg/ml respectively.

Abbreviations: SM: Splenomegaly, UL: upper limit of normal, CRP: C-reactive protein, ESR: erythrocyte sedimentation rate, IL: interleukin, LDH: lactate

dehydrogenase, NA: not applicable, NR: normal range, RBC: red blood cell, %: percentile, yo: years old, mo: months old

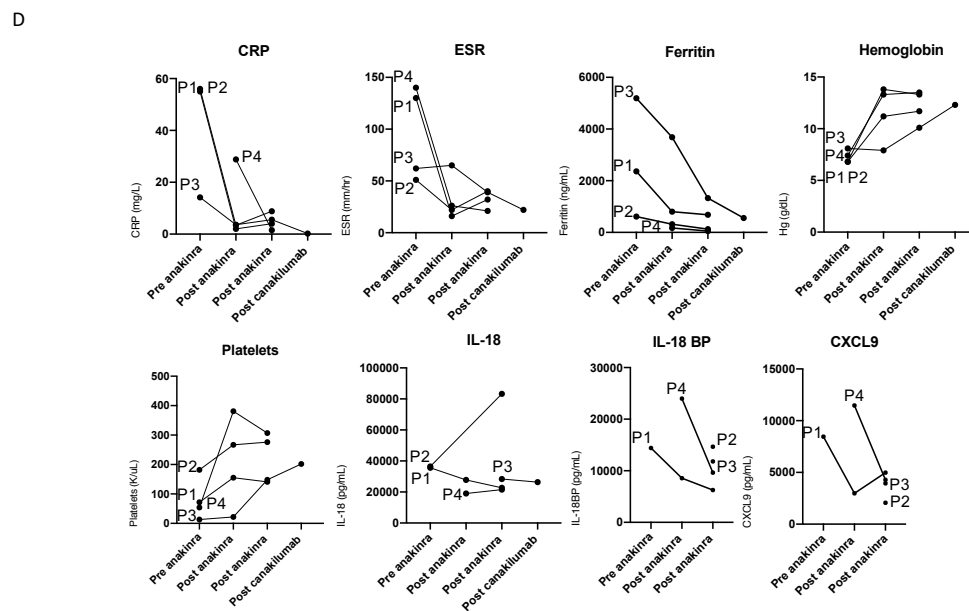
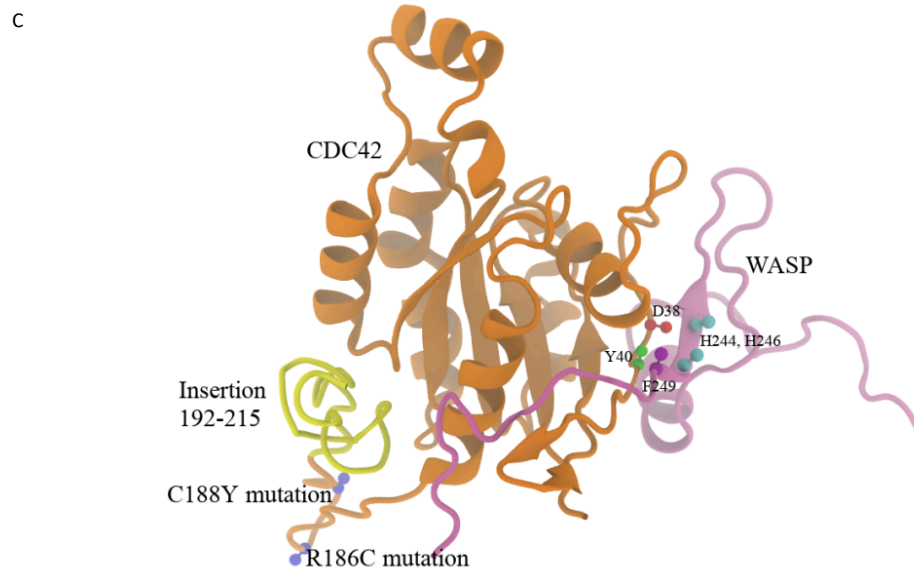
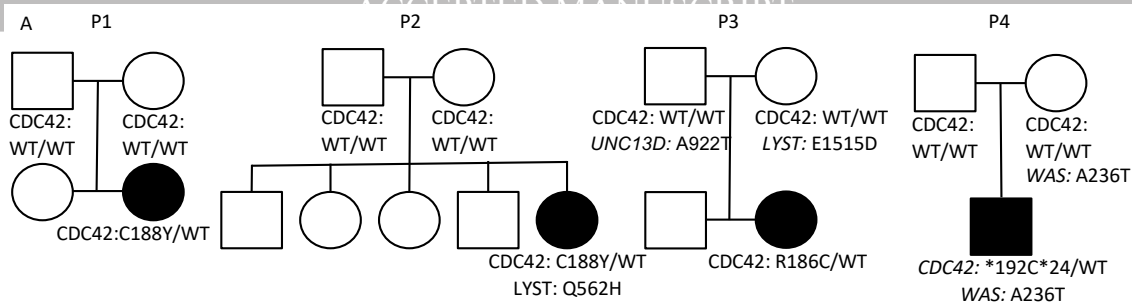
^ Performed by Dr. Scott Canna.

^^ IL-18 measured after 1 dose of Anakinra

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

* While on 1mg/kg/day of prednisone and anakinra, he developed respiratory syncytial virus (RSV) pneumonia, septic arthritis and cervical lymphadenitis, which resolved when steroids were discontinued.

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



Supplemental Materials

Figures: 1

Tables: 3

Patient 1 is an 18-month-old female who initially presented with hydrops fetalis and mild facial dysmorphism (mild frontal bossing, hypertelorism and depressed nasal bridge), and later developed failure-to-thrive, hepatosplenomegaly with transaminitis, chronic cytopenias, arthralgias, nodular rashes, and recurrent febrile episodes. Her anemia was transfusion dependent, her baseline platelet count was approximately $30 \times 10^3/\text{ul}$. Both cytopenias were non-autoimmune in nature, but secondary to hypersplenism. Her parents were not related. Further laboratory evaluation revealed a massive increase in CRP (55 mg/L), ESR (>130 mm/hr), and ferritin (2364 ng/mL). She had normal levels of plasma sIL-2R (sCD25). Serum interleukin-6 (IL), IL-18 as well as IL-18 BP levels were significantly elevated (Table I). Immunophenotypic changes in monocytes and natural killer cells (Supplemental Materials, Figure 1 A, B) were also noted. Due to the complex nature of the disease, WES was performed and identified a heterozygous, possibly pathogenic *de novo* variant in *CDC42* c.563G>A (p.C188Y) (Figure I). Therapy with the IL-1 receptor antagonist anakinra was initiated and within 48 hours the patient experienced an increase in appetite and improved mobility. Over the course of the following weeks, fever, anemia, thrombocytopenia and rash entirely resolved, the spleen massively decreased in size and the patient started to meet developmental milestones. CRP, ESR normalized over a period of 6 months, while ferritin and free IL-18 continue to trend down

(Table I, Figure 1D). Elevations in monocyte phosphoproteins also normalized after 4 months of treatment (Supplemental Materials, Figure 1 D)

Patient 2 is a 22-month-old female who presented in the neonatal period with dysmorphic features (frontal bossing, macrocephaly, thin sparse hair, and depressed nasal bridge), respiratory distress, and cholestasis with transaminitis requiring intensive care. Subsequently, she had recurrent febrile episodes, associated with respiratory symptoms and macular skin lesions. In addition to failure-to-thrive, her physical exam showed generalized lymphadenopathy and hepatosplenomegaly. Infectious history was positive for two episodes of otitis media with perforation of the tympanic membrane and one episode of severe bronchiolitis with enterovirus. The latter required invasive ventilation. Laboratory evaluation revealed thrombocytopenia with a baseline platelet count of $35 \times 10^3/\text{ul}$ and a transfusion dependent anemia. There was no history of consanguinity. Further investigations demonstrated an elevated CRP (56 mg/L), ferritin (875ug/L), and LDH (540ng/L). She had an elevated sIL-2R (sCD25) (8657 U/ml [nl range 278-1,580 U/mL]) (Table 1). With concern for MAS, therapy with anakinra was initiated and titrated to a dose of 6mg/kg. This led to a dramatic improvement in the inflammatory markers and resolution of fevers. WES was performed and identified a monoallelic, possibly pathogenic *de novo* mutation in *CDC42* c.563G>A (p.C188Y). Twelve months post initiation of treatment with anakinra, the patient had significantly improved growth parameters and started to meet developmental milestones. Her cytopenias and rashes resolved, transaminitis and splenomegaly improved (Figure 1D and Table 1). She continues with subclinical inflammation and mildly elevated CRP and serum ferritin. Through her disease course, she developed 2 episodes of suspected viral

gastroenteritis. Her clinical course was also complicated by multiple respiratory infections with negative viral studies, except for one which had a positive respiratory culture to human metapneumovirus.

Patient 3 is a now 6.5-year-old Caucasian girl who presented at birth with purpuric rash, grade II brain hemorrhage, hepatosplenomegaly, thrombocytopenia ($\sim 25 \times 10^3/\text{ul}$) and leukocytosis. She also rapidly developed macular rashes, frequent febrile episodes and chronic cytopenias (anemia, thrombocytopenia and neutropenia). Her anemia and thrombocytopenia were transfusion dependent. Her parents were not related. Further laboratory evaluation revealed an increased CRP 142mg/L, ESR 62mm/h, hyperferritinemia (5191ug/L) and elevated LDH (817ng/L) (see Table I). She poorly responded to prednisone, immunoglobulin replacement therapy and rituximab. At the age of 5 months, she was started on anakinra (3mg/kg) with improvement of the rash and fever. Anakinra dose was progressively increased to 8.5mg/kg resulting in an improvement but not complete resolution of anemia, thrombocytopenia, hyperferritinemia and systemic inflammation (Figure 1D and Table 1). WES was performed and identified a monoallelic, possibly pathogenic *de novo* mutation in *CDC42* c.556C>T (p.R186C). She also had rare variants in *LYST* and *UNC13D* (Figure 1A and Table SII). Anakinra was stopped and Canakinumab doses were increased to eventually 8.0 mg/kg once monthly. This resulted in further improvement of the anemia, thrombocytopenia and normalization of the WBC. The need for transfusions was dramatically decreased. She has had recurrent episodes of anterior cervical lymphadenitis (some with abscess formation) and one episode of beta-hemolytic strep sepsis with purpura fulminans. She has chronic hepatosplenomegaly.

Growth is at 5th percentile for height and weight. Social and intellectual development is normal.

Patient 4 is a now 12-year-old Hispanic male, without any family history of consanguinity, who presented in the neonatal period with fever, papular erythematous rash, hepatosplenomegaly, transaminitis and leukocytosis. Cerebrospinal fluid collected had 60 WBC with 60% neutrophils and increased protein. He also developed anemia and thrombocytopenia. At 7 weeks of age, he presented with seizures caused by a small intracranial hemorrhage/hematoma secondary to severe thrombocytopenia. Between 2 and 8 months of life, his anemia (~7.4g/dL) and thrombocytopenia (~56x10³/ul) were transfusion depended, he still had hepatosplenomegaly, recurrent fever, rash, increased CRP (44mg/L on 2.5mg/kg of anakinra), ESR (140mm/h) and LDH (322 U/L, normal range: <226) on 2.5mg/kg of anakinra. At 8 months, he was found to have retinal detachment, phthisis of his right eye and papilledema of the left eye, which were both presumed to be secondary to his inflammatory disease and increased intracranial pressure. Due to the patient's phenotypic similarities to patients with NOMID, he was started on prednisone and anakinra at the age of 8 months with resolution of systemic inflammation and cytopenias. Before 18 months of age, while on 1mg/kg/day of prednisone and anakinra, he developed respiratory syncytial virus (RSV) pneumonia, septic arthritis and cervical lymphadenitis. The infections resolved when steroids were discontinued. His psychomotor development was mildly delayed. At age 9 years, when anakinra was switched to canakinumab, he developed Staphylococcus aureus bacteremia and septic myositis with multiple abscesses and was switched back to anakinra with ongoing control of the systemic inflammation while completing antibiotic therapy. The patient has not presented with chronic hyperferritinemia and the

highest documented ferritin was 470ng/ml (normal range: <336) during the episode of septic myositis. His WES identified a monoallelic, possibly pathogenic *de novo* variant in *CDC42* c.576A>C (stop lost) (p.*192C*24). He is currently 12 years old and on treatment with anakinra. His systemic inflammation presenting with fever, rash, anemia and thrombocytopenia has resolved and the liver and spleen size has normalized (Figure 1D and Table 1). His growth parameters have improved, he grows along the 10th percentile for height and weight.

Methods:

Serum cytokine measurement:

Total IL-18, IL-18BP, and CXCL9 were measured as in Weiss *et al* (1).

Mass cytometry

Whole blood and antibody staining

Blood was drawn in EDTA vacutainer tubes. The samples used for experiments were fixed within one hour of blood draw using Smart Tube Proteomic Stabilizer (Smart Tube, Inc. San Carlos, CA) and stored at -80°C. The samples were thawed in a cold room and red cells lysed using 1X Smart Tube Thaw-Lyse Buffer (Smart Tube, Inc.). Each sample was labeled and acquired at a CyTOF machine individually. Samples were stained with an Fc receptor blocker (Human TruStain FcX™, Biolegend, San Diego, CA), followed by incubation with a cocktail of pre-titrated metal-conjugated antibodies (23 antibodies, see Supplementary Table I) for surface staining for 30

minutes at room temperature. Samples were then washed, fixed with 1.6% formaldehyde (Thermo Scientific, Rockford, IL), and permeabilized with 90% ice-cold methanol overnight. Next day samples were washed and stained with a cocktail of antibodies for intracellular protein and phosphoprotein (7 antibodies, See Supplementary Table I) for 30 minutes at room temperature. After washing, the samples were incubated with 0.125 nM iridium (191Ir and 193Ir) Cell-ID™ Intercalator-Ir, Fluidigm) to enable cell identification based on DNA content and acquired on the same day.

Data acquisition and analysis.

For acquisition, the samples were resuspended at a concentration of approximately 1 million cells/mL in a solution of 1:10 Calibration Beads (Calibration Beads, EQ™ Four ELEMENT, Fluidigm). Samples were acquired on a Helios mass cytometer (Fluidigm) using a SuperSampler (VictorianAirships, Alamo, CA) fluidics system. For quality control, the acquisition event rate was maintained at lower than 300 events/s. The resulting FCS files were normalized using the Calibration Beads and cell subpopulations were determined using FlowJo (FlowJo, LLC). Cells events were identified as Ir191/193 double positive events, and doublets were excluded on the basis of higher DNA content (Ir191) and longer event length. Analysis were performed using FlowJo and Cytobank (Cytobank, Inc, Santa Clara, CA).

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FIGURE E1

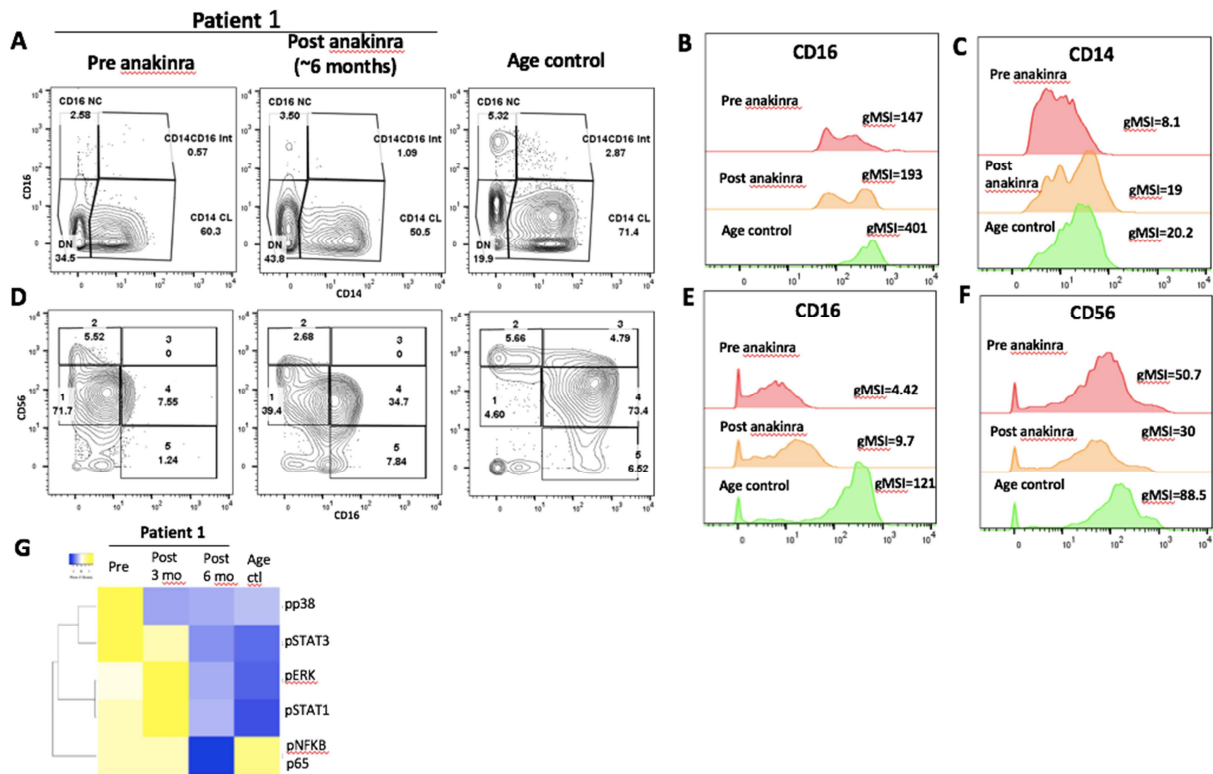


Figure E1. CyTOF analysis of distribution of selected subsets (monocytes and natural killer (NK) cells) in peripheral blood mononuclear cells of patient 1. A: Low percentage of CD16⁺ monocytes. Two parameter plots (CD14 x CD16) of monocytes (CD3⁻CD19⁻CD7⁻). DN, double negative (for CD14 and CD16); NC, Nonclassical; Int, Intermediate; CL, Classical. Numbers in quadrants show percentages. B, C: Low CD16 expression on monocytes. Geometric mean signal intensity (gMSI) of CD16 in nonclassical monocytes and of CD14 in classical monocytes. D: Decreased percentage of CD16⁺CD56⁺ NK cells, with partial recovery after treatment. Two parameters plots (CD16 x CD56) of NK (CD7⁺) cells. Gates based on Poli *et al* (2): 1, CD56⁺CD16⁻; 2, CD56^{bright}CD16⁻; 3, CD56^{bright}CD16⁺; 4, CD56⁺CD16⁺; 5, CD56⁻CD16⁺; this subset is observed at

low percentage in healthy adults (<5%, Milush *et al*) (3). 2nd line numbers in quadrants shows percentage. E, F: Low expression of CD16 in total NK cells of Patient 1. gMSI of CD16 (E) and CD56 (F) in total NK cells. G: Decreasing levels of several phosphorylated signaling proteins in CD14 classical monocytes following treatment. Signaling heat map from serial samples, pre- and post-anakinra, based on intracellular levels of phosphorylated proteins. Age ctl: gender-matched, pediatric control.

Table E1. Comparison of the clinical phenotypes and biological parameters of patients with Takenouchi-Kosaki syndrome and of our cohort of 4 patients with a de novo heterozygous C-terminal* mutation in CDC42 (*affecting the last 3-5 amino acids of CDC42).

	Takenouchi-Kosaki syndrome			C-terminal* mutations in CDC42
	Group I	Group II	Group III	
Clinical presentation				
Failure to thrive, postnatal weight Weight <2SD	3/5	2/4	3/4	4/4
Intellectual disability	5/5	4/4	2/6	1/4
Facial dysmorphism including: Frontal bossing, large forehead	2/5	2/4	1/6	3/4
Cardiac abnormality	3/5	2/4	2/5	0/4
Rash	0/5	0/4	0/5	4/4
Recurrent severe infections	4/5	3/4	1/6	3/4 (2/3 were on immunosuppression)
Biological parameters				
Thrombocytopenia	4/4	1/3	0/5	4/4
Anemia	NA	NA	NA	4/4

Systemic inflammatory syndrome Increased CRP, ESR, ferritin)	NA	NA	NA	4/4
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501 **Legend:** group I: switch mutations in CDC42, group II: pocket mutations of CDC42 and group III CRIB502 mutation as per Martinelli *et al* (4). NA for not applicable.

503

500 **Table E2.** WES analysis of all 4 reported patients

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Patient 1	CDC42	c.563G>A	p.C188Y	De novo	Possibly pathogenic 0.337*
Patient 2	CDC42	c.563G>A	p.C188Y	De novo	Possibly Pathogenic 0.337*
	LYST	c.1686G>C	p.Q562H		Possibly pathogenic 0.035
	LPIN2	c.446C>T	p.P149L		Possibly pathogenic 0.049
	MEFV	c.442G>C	p.E148Q		Likely Benign 0.024
Patient 3	CDC42	c.556C>T	p.R186C	De novo	Possibly pathogenic 0.053*

	LYST	c.4545G>C	p.E1515D	Het from father	Likely benign 0.003
	UNC13D	c.2764G>A	p.A922T	Het from mother	Possibly Pathogenic 0.047
Patient 4	CDC42	c.576A>C (stop lost)	p.*192C*24	De novo	Possibly pathogenic 0.053*
	WAS	c.706G>A	p.A236T	X-linked from mother	Possibly pathogenic 0.964

Legend: (*) M-CAP pathogenicity scores were used to interpret the pathogenicity of nonsynonymous CDC42 variants (5). The pLI score measures the likelihood that *CDC42* is intolerant to loss of function variants; pLI score for *CDC42* for patient 1, 2 and 3 was 0.79 (6). As further support for the claims of pathogenicity, these variants are observed in 0 individuals from the gnomAD control population consisting of over 120,000 presumably healthy individuals [https://www.biorxiv.org/content/10.1101/531210v2].

500 **Table E3.** CyTOF antibody panel

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Number	Label	Target	Clone	ul per sample	Target location	Source
1	89Y	CD45	HI30	0.75	Surface	Fluidigm
2	141Pr	CD235ab (Glycophorin)	HIR2	0.1	Surface	Fluidigm
3	142Nd	CD19	HIB19	0.9	Surface	Fluidigm
4	144Nd	CD11b (Mac-1)	ICRF44	0.9	Surface	Fluidigm
5	146Nd	CD64	10.1	0.9	Surface	Fluidigm
6	147Sm	CD11c	Bu15	0.9	Surface	Fluidigm
7	149Sm	CD66a/CEACAM1	CD66a-B1.1	0.9	Surface	Fluidigm
8	150Nd	CD192 (CCR2)	K036C2	0.9	Surface	BioLegend ; In house labelling ¹
9	151Eu	CD123 (IL-3R)	6H6	0.7	Surface	Fluidigm
10	152Sm	CD36	5271	0.9	Surface	Fluidigm
11	153Eu	CD7	CD7-6B7	0.9	Surface	Fluidigm
12	154Sm	CD163	GHI/61	1	Surface	Fluidigm
13	155Gd	CD45RA	HI100	0.5	Surface	Fluidigm
14	156Gd	p-p38 [T180/Y182]	D3F9	1	Intracellular	Fluidigm

					r (phospho)	
15	158Gd	CD33	WM53	0.9	Surface	Fluidigm
16	161Dy	pSTAT1 [S727]	A15158B	1	Intracellular (phospho)	BioLegend ; In house labelling ¹
17	162Dy	CD80 (B7-1)	2D10.4	1	Surface	Fluidigm
18	163Dy	pStat3 [Y705]	4/P-Stat3	1	Intracellular (phospho)	BioLegend ; In house labelling ¹
19	164Dy	IκBα	L35A5	1	Intracellular (total protein)	Fluidigm
20	165Ho	CD61	VI-PL2	0.4	Surface	Fluidigm
21	166Er	pNF-κB p65 [S529]	K10- 895.12.50	0.5	Intracellular (phospho)	Fluidigm Fluidigm
22	168Er	CD206 (MMR)	15-2	1	Surface	Fluidigm
23	170Er	CD3	UCHT1	0.9	Surface	Fluidigm
24	171Yb	pERK 1/2 [T202/Y204]	D13.14.4E	1	Intracellular	BioLegend ; In house

					(phospho)	labelling ¹
25	172Yb	CX3CR1	2A9-1	1	Surface	Fluidigm
26	173Yb	Iba1	ab5076	1	Intracellular (total protein)	Abcam; In house labelling*
27	174Yb	HLA-DR	L243	0.9	Surface	Fluidigm
28	175Lu	CD14	M5E2	1	Surface	Fluidigm
29	176Yb	CD56 (NCAM)	NCAM16.2	0.9	Surface	Fluidigm
30	209Bi	CD16	3G8	0.9	Surface	Fluidigm

Legend: * labeled using Maxpar® X8 Antibody Labeling Kit.

