Journal of Clinical Immunology (2020) 40:567–570
https://doi.org/10.1007/s10875-020-00742-5

LETTER TO EDITOR

Systemic Inflammation and Myelofibrosis in a Patient with Takenouchi-Kosaki Syndrome due to *CDC42* Tyr64Cys Mutation

Giorgia Bucciol[1,2] · Bethany Pillay[3,4] · Jose Casas-Martin[1] · Selket Delafontaine[1,2] · Marijke Proesmans[5] · Natalie Lorent[6] · Johan Coolen[7] · Thomas Tousseyn[8] · Xavier Bossuyt[9,10] · Cindy S. Ma[3,11] · Rik Schrijvers[12,13] · Stuart G. Tangye[3,4] · Leen Moens[1] · Isabelle Meyts[1,2]

Received: 26 November 2019 / Accepted: 2 January 2020 / Published online: 18 January 2020
© Springer Science+Business Media, LLC, part of Springer Nature 2020

To the Editor:

Heterozygous mutations in *CDC42*, encoding the small GTP/GDP-binding protein cell division cycle 42 (CDC42) involved in eukaryotic actin cytoskeleton dynamics, cause Takenouchi-Kosaki syndrome, a rare developmental disorder. Hallmarks of Takenouchi-Kosaki syndrome are intellectual and growth delay, dysmorphisms, macrothrombocytopenia, camptodactyly, structural brain abnormalities with sensorineural deafness, hypothyroidism, and frequent infections [1]. The original patient identified with this condition carried a de novo p.Tyr64Cys (c.191A>G) mutation. Five patients with the Tyr64Cys variant and similar clinical phenotypes have now been described [1–5]. Fourteen additional patients with different developmental phenotypes due to autosomal dominant (AD) heterozygous mutations disrupting CDC42 function and downstream signaling were reported by Martinelli et al. [3]. Recently, Gernez et al. and Lam et al. described a syndrome of immune dysregulation caused by other *CDC42* mutations in eight additional patients [6, 7]. These patients presented with neonatal-onset severe auto-inflammation with increased serum inflammatory cytokines and chemokines (IL-6, IL-18, IL-18-binding protein (IL-18BP), CXCL9), as well as hepatosplenomegaly and hemophagocytic lymphohistiocytosis (HLH) [6, 7]. The unrelated patients carried C-terminal *CDC42* mutations (p.Arg186Cys in five; p.Cys188Tyr in two; p.*192Cys*24 in one) [6, 7]. The exact pathophysiology of inflammation remains elusive, yet Lam et al. did show that Arg186Cys causes aberrant subcellular localization of CDC42 with impaired cell polarity and migration. In contrast, mutations in the switch II domain of *CDC42* underlie Takenouchi-Kosaki syndrome, while mutations within or close to the nucleotide-binding pocket (NBP) cause

Leen Moens and Isabelle Meyts contributed equally to this work.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s10875-020-00742-5) contains supplementary material, which is available to authorized users.

✉ Isabelle Meyts
isabelle.meyts@uzleuven.be

[1] Inborn Errors of Immunity, Department of Microbiology, Immunology and Transplantation, KU Leuven, Leuven, Belgium

[2] Pediatric Immunodeficiency, Department of Pediatrics, University Hospitals Leuven, Leuven, Belgium

[3] Immunology Division, Garvan Institute of Medical Research, Darlinghurst, NSW, Australia

[4] St Vincent's Clinical School, UNSW Sydney Faculty of Medicine, Sydney, NSW, Australia

[5] Pediatric Pulmonology, Department of Pediatrics, University Hospitals Leuven, Leuven, Belgium

[6] Department of Pulmonology, University Hospitals Leuven, Leuven, Belgium

[7] Department of Radiology, University Hospitals Leuven, Leuven, Belgium

[8] Department of Pathology, University Hospitals Leuven, Leuven, Belgium

[9] Clinical and Diagnostic Immunology, KU Leuven, Leuven, Belgium

[10] Department of Laboratory Medicine, University Hospitals Leuven, Leuven, Belgium

[11] CIRCA (Clinical Immunogenomics Research Consortia Australia), Sydney, NSW, Australia

[12] Allergy and Clinical Immunology Research Group, Department of Microbiology, Immunology and Transplantation, KU Leuven, Leuven, Belgium

[13] Department of Internal Medicine, University Hospitals Leuven, Leuven, Belgium

growth deficiency, brain malformations, and striking facial dysmorphisms, and mutations affecting the association with CDC42/RAC-interacting binding (CRIB) motifs, especially p.Glu171Lys, underlie a phenotype resembling RASopathies [1–5]. The genetic and clinical characteristics of the reported patients with *CDC42* mutations are summarized in supplementary table S1, and the protein domains in supplementary fig. S1.

Here, we present a patient with the Tyr64Cys *CDC42* mutation who manifested immune dysregulation, including hepatosplenomegaly, myelofibrosis, systemic inflammation, and pulmonary hemorrhage. The patient was born at term from non-consanguineous Belgian parents. She was small for gestational age (weight − 2 SD), microcephalic (head circumference − 2.5 SD), hypotonic, and mildly dysmorphic. She manifested psychomotor developmental delay, feeding difficulties, and growth retardation. A brain MRI scan revealed a Dandy-Walker variant with a posterior fossa arachnoid cyst, requiring cysto-peritoneal drainage at age 18 months. She had microretrognathia, hypertelorism with incomplete eyelid closure, depressed nasal bridge, strabismus, severe astigmatism, arachnodactyly, and sensorineural hearing loss. She developed scoliosis, pes planus, camptodactyly, fingernail exostosis, hepatosplenomegaly with multiple spleen and kidney hyperechogenic lesions (which were not biopsied), liver hemangiomas, and a thoracic-abdominal aortic aneurysm with a maximal diameter of 4.5 cm (supplementary fig. 2A). She suffered recurrent lower respiratory tract infections since childhood with a diagnosis of bronchiectasis at age 15 years despite antibiotic treatment and physiotherapy. Laboratory evaluations at that time showed profound lymphopenia, especially of B cells, neutropenia, anemia, and thrombocytopenia. A bone marrow biopsy at age 16 showed dysmegakaryopoiesis but otherwise normocellular marrow with normal differentiation. She had hypogammaglobulinemia in the first year of life with IgA and IgM deficiency, but only IgA deficiency persisted later in life. Laboratory results are summarized in supplementary table S2.

At age 25 years, she experienced weight loss (> 10%), increased coughing, fever, and elevated inflammatory markers. C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) increased to 45 mg/L and 45 mm/h, respectively. Fibrinogen was 4 g/L with D-dimers > 7500 μg/L (normal < 500), but ferritin was normal (highest value 31 μg/L). Approximately 50% of T cells expressed HLA-DR. HLH could not be confirmed. The patient's state did not improve despite broad antifungal and antibiotic treatment, but responded to steroids. Bone marrow biopsy showed myeloid hypercellularity, erythroid hypoplasia, and fibrosis grade 2/3 according to the WHO classification of myeloid neoplasm [8], suggestive of primary myelofibrosis (supplementary fig. S3). As part of the hematology workup, a NGS panel (Illumina platform) on peripheral blood was performed to screen for driver variants in *JAK2*, *CALR*, and *MPL*, as well as for non-driver variants in a large set of genes (see the list in supplementary material). No somatic or germline variants could be identified. At age 26 years, she was admitted with cachexia, dyspnea, and hypoxemia (O_2 saturation 78%, arterial pO_2 34 mmHg). A chest CT scan showed bilateral ground-glass opacities, honeycombing, and crazy paving pattern with cyst formation (supplementary fig. S2B–C). Bronchus aspirate revealed MRSA and HSV-1 for which she was treated accordingly. Maximal CRP and ESR were 179 mg/L and 50 mm/h, respectively. Ventilatory support was escalated to extracorporeal membrane oxygenation (ECMO) from D+7. Suspecting an inflammatory component, she was started on 80 mg methylprednisolone per day on D+11, after which she could be weaned from ECMO. Upon tapering of steroids, lung infiltrates flared—a pattern seen twice, after which glucocorticoid therapy was maintained from D+41. During this course, she had several episodes of pulmonary hemorrhages. CT angiography showed several hypertrophic bronchial arteriae which were embolized. At D+41 after admission, *Stenotrophomonas maltophilia* was cultured from the sputum. Ultimately the patient succumbed to shock associated with *Stenotrophomonas maltophilia* bacteremia at D+68 after admission.

Whole exome sequencing (WES) was performed on whole blood DNA from the patient and her parents, after informed consent from the parents (research protocol S58466 approved by the ethical committee of the University Hospitals Leuven). WES revealed a de novo heterozygous *CDC42* mutation in the patient, c.191A>G (p.Tyr64Cys), associated with Takenouchi-Kosaki syndrome (supplementary methods and supplementary fig. S4) [1–5]. In-depth immune phenotyping at age 25 years showed reduced frequencies of B cells and NK cells with increased proportions of T cells in the patient compared with healthy controls (supplementary methods and supplementary fig. S5A). Proportions of total CD4+ and CD8+ T cells were preserved. Differentiation of CD4+ and CD8+ T cells was skewed to effector memory and effector memory expressing CD45RA (TEMRA) subsets, respectively, at the expense of naïve cells. No exhaustion signature was present in either CD4+ or CD8+ T cells from the patient (supplementary fig. S5F–G). Normal proportions of NKT and mucosal-associated invariant T (MAIT) cells were observed (supplementary fig. S5E). Transitional and memory B cells were decreased and naïve B cells were increased (supplementary fig. S5B–D). Following in vitro activation, *CDC42* mutant CD8+ T cells exhibited poor upregulation of the activation markers CD25, CD69, and CD95 when tested after 5 days. Expression of CD107a was not affected (supplementary fig. S5H). Patient memory CD4+ T cells exhibited normal production of IL-2 as well as cytokine characteristic of T helper (Th)1 (interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α)), Th2

(IL-4, IL-9), Th17 (IL-17A, IL-17F), and T follicular helper (Tfh) (IL-21) cells, although the production of IL-13 was reduced (supplementary fig. S5I). Inspired by recent reports [6, 7], pro-inflammatory cytokines (IL-6, IL-18, IL-18BPa, CXCL9) were measured in serum samples collected between the age of 25 and 26 years (see supplementary methods). All cytokines tested as well as CRP and ESR were elevated, compared with those of healthy controls (Fig. 1). Taken together, these data suggest that auto-inflammation is also a feature of Takenouchi-Kosaki syndrome caused by Tyr64Cys in the switch domain of CDC42.

The patient manifested the typical traits of Takenouchi-Kosaki syndrome [1–5]. The immunophenotype is characterized by lymphopenia, particularly B lymphopenia with excess naïve B cells. The vascular malformations (liver hemangiomas) and the aneurysm are comparable with those described in DOCK8 deficiency, Wiskott-Aldrich syndrome, and/or actin-cytoskeleton disorders in general [9, 10]. We also show evidence of auto-inflammation in this patient with a *CDC42* mutation outside of the C-terminal domain. Patients with Takenouchi-Kosaki syndrome due to Tyr64Cys have not been reported to suffer from auto-inflammation [1–5], although a single patient with the switch II domain Arg68Gln variant suffered splenomegaly, fever, intermittent skin rash, and serositis [3]. It is possible that Tyr64Cys is associated with milder auto-inflammation, as reflected by the lower values of inflammatory cytokines in our patient compared with those reported previously with full-blown HLH [6, 7]. This demonstrates the potential of using serum cytokine pattern analysis in patients with an ill-defined disease course, as it would aid in therapeutic decision-making. In hindsight, this patient may have benefited from IL-1 inhibition or anti-IFN-γ antibody emapalumab to control inflammation. On the other hand, given the myelofibrosis and the likely necessity to continue anti-inflammatory treatment lifelong, hematopoietic stem cell transplantation may be a valuable alternative for these patients, as shown in many other recently described severe auto-inflammatory manifestations/primary immunodeficiencies. In general, heterozygous *CDC42* mutations result in a spectrum of immune deficiency, developmental features, and auto-inflammation, much resembling phenotypes caused by mutations in other genes governing the complex actin cytoskeleton dynamics [9].

Finally, the patient had grade 2 myelofibrosis with a bone marrow phenotype comparable with a primary form of

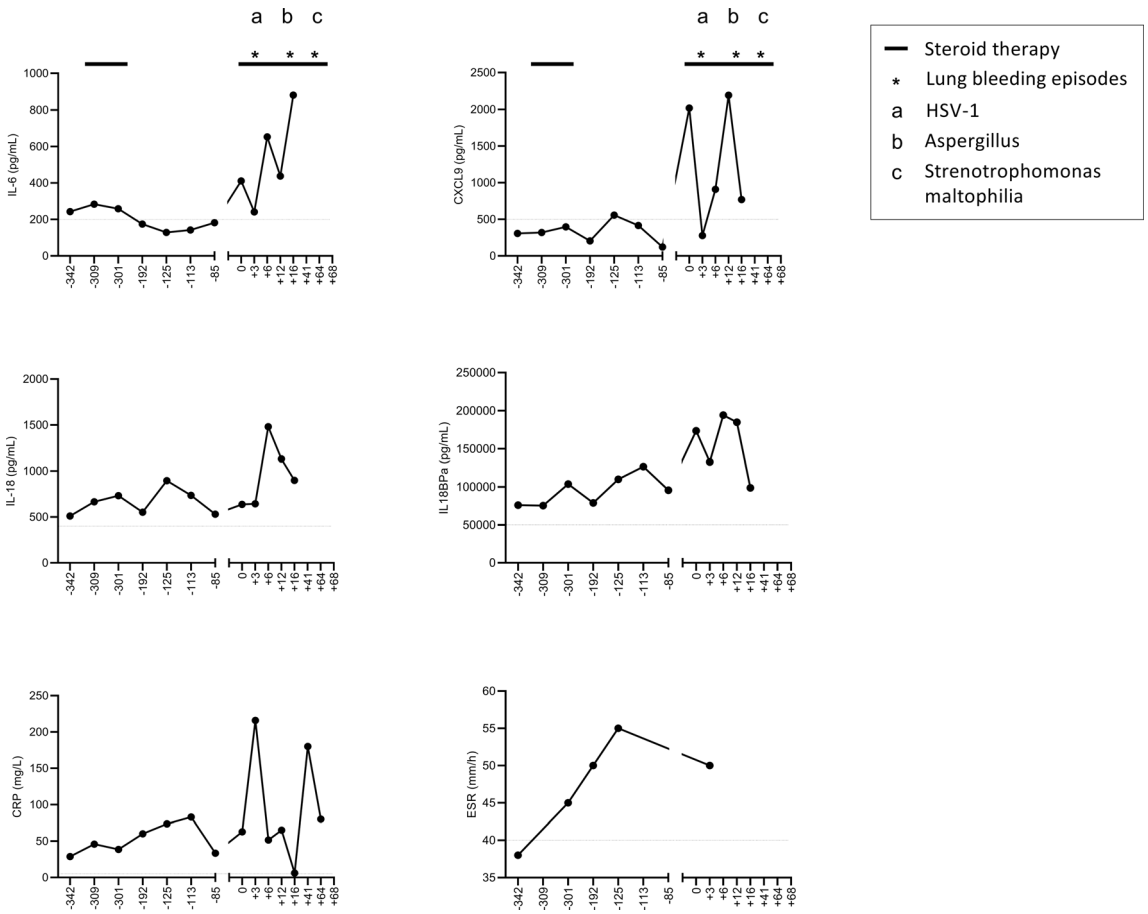

Fig. 1 Serum levels of IL-6, CXCL9, IL-18, IL-18BPa, CRP, and ESR were measured in the patient. Time points are given in days from the last hospital admission (day 0). Dashed lines represent normal intervals (based on in-house healthy controls for the cytokines measured by ELISA ($n = 13$) and standard ranges for CRP and ESR). Relevant clinical manifestations and treatment are indicated in the legend

disease, even though genetic causes of primary myelofibrosis were excluded by gene sequencing and WES. Chronic inflammation is a cause of secondary myelofibrosis, yet this was deemed unlikely given the patient's young age and the absence of amyloid deposits in the bone marrow. Interestingly, downregulation of CDC42 has been identified as a key driver of myeloproliferative disease and erythroid hypoplasia in mice [11]. Moreover, CDC42 is significantly downregulated in primary myelofibrosis and its Rho GTPase activity was also shown to play an important role in the development of myelofibrosis by a study that compared differential microRNA and gene expression in the bone marrow of 42 myelofibrosis and 16 healthy individuals [12, 13]. The Tyr64Cys mutation carried by our patient results in almost completely abolished GTPase activity in response to guanine nucleotide exchange factor (GEF) and a profoundly decreased GTPase activity in response to GTPase-activating protein (GAP) [3]. This suggests a direct role for the disturbed Rho GTPase activity of the Tyr64Cys mutant in the development of myelofibrosis.

In conclusion, we here demonstrate that auto-inflammation is a feature not only of defects of the C-terminal domain, but also of other *CDC42* mutations, at least of the switch II domain. Moreover, this case expands the clinical phenotype of *CDC42* mutations to include myelofibrosis and aortic aneurysm.

Acknowledgments We thank the parents for their trust and the patient for teaching us joy and gratitude. We thank the entire team of clinicians, physiotherapists, and other care providers. Special thanks are given to Kris De Boeck and Myriam Vreys.

Funding Information GB is supported by the Research Foundation - Flanders (project G0C8517N). RS is supported by a senior clinical investigator fund of the Research Foundation - Flanders. Research performed in the Tangye/Ma lab is supported by the National Health and Medical Research Council of Australia, and the Office of Health and Medical Research of the New South Wales Government. IM is supported by the Jeffrey Modell Foundation, by the Research Foundation - Flanders (project G0C8517N), and by the CSL Behring Chair in Primary Immunodeficiency in Children. IM and RS are supported by the VIB Grand Challenge Project Primary Immunodeficiencies.

Compliance with Ethical Standards

Conflict of Interest IM is supported by the CSL Behring Chair in Primary Immunodeficiency in Children, paid to institution. The other authors have no conflict of interest to declare.

References

1. Takenouchi T, Kosaki R, Niizuma T, Hata K, Kosaki K. Macrothrombocytopenia and developmental delay with a de novo CDC42 mutation: yet another locus for thrombocytopenia and developmental delay. Am J Med Genet A. 2015;167(11):2822–5.
2. Takenouchi T, Okamoto N, Ida S, Uehara T, Kosaki K. Further evidence of a mutation in CDC42 as a cause of a recognizable syndromic form of thrombocytopenia. Am J Med Genet A. 2016;170(4):852–5.
3. Martinelli S, Krumbach OHF, Pantaleoni F, Coppola S, Amin E, Pannone L, et al. Functional dysregulation of CDC42 causes diverse developmental phenotypes. Am J Hum Genet. 2018 Feb 1;102(2):309–20.
4. Motokawa M, Watanabe S, Nakatomi A, Kondoh T, Matsumoto T, Morifuji K, et al. A hot-spot mutation in CDC42 (p.Tyr64Cys) and novel phenotypes in the third patient with Takenouchi-Kosaki syndrome. J Hum Genet. 2018 Mar;63(3):387–90.
5. Uehara T, Suzuki H, Okamoto N, Kondoh T, Ahmad A, O'Connor BC, et al. Pathogenetic basis of Takenouchi-Kosaki syndrome: electron microscopy study using platelets in patients and functional studies in a Caenorhabditis elegans model. Sci Rep. 2019;9(1):1–9.
6. Gernez Y, de Jesus AA, Alsaleem H, Macaubas C, Roy A, Lovell D, et al. Severe autoinflammation in 4 patients with C-terminal variants in cell division control protein 42 homolog (CDC42) successfully treated with IL-1β inhibition. J Allergy Clin Immunol. 2019;144(4):1122–1125.e6.
7. Lam MT, Coppola S, Krumbach OHF, Prencipe G, Insalaco A, Cifaldi C, et al. A novel disorder involving dyshematopoiesis, inflammation, and HLH due to aberrant CDC42 function. J Exp Med. 2019:jem.20190147.
8. Arber DA, Orazi A, Hasserjian R, Thiele J, Borowitz MJ, Le Beau MM, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood. 2016;127(20):2391–405.
9. Tangye SG, Bucciol G, Casas-Martin J, Pillay B, Ma CS, Moens L, et al. Human inborn errors of the actin cytoskeleton affecting immunity: way beyond WAS and WIP. Immunol Cell Biol. 2019;97(4):389–402.
10. Chu EY, Freeman AF, Jing H, Cowen EW, Davis J, Su HC, et al. Cutaneous manifestations of DOCK8 deficiency syndrome. Arch Dermatol. 2012;148(1):79–84.
11. Yang L, Wang L, Kalfa TA, Cancelas JA, Shang X, Pushkaran S, et al. Cdc42 critically regulates the balance between myelopoiesis and erythropoiesis. Blood. 2007;110(12):3853–61.
12. Calura E, Pizzini S, Bisognin A, Coppe A, Sales G, Gaffo E, et al. A data-driven network model of primary myelofibrosis: transcriptional and post-transcriptional alterations in CD34+ cells. Blood Cancer J. 2016;6(6):e439–9.
13. Zhang L, Sankaran VG, Lodish HF. MicroRNAs in erythroid and megakaryocytic differentiation and megakaryocyte–erythroid progenitor lineage commitment. Leukemia. 2012;26(11):2310–6.

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.