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LETTER TO EDITOR

Autoinflammation Masquerading as Autoimmunity in an Adult with Heterozygous p.E250K *PSTPIP1* Mutation

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To the Editor,

PSTPIP-1-associated myeloid-related proteinemia inflammatory syndrome (PAMI) is a rare autoinflammatory disease that typically presents in childhood, characterized by high serum levels of zinc and the alarmin calprotectin. It is associated with the p.E250K (c.748G>A) or p.E257K (c.769G>A) charge-reversal mutations in the *PSTPIP1* gene, which encodes Proline-Serine-Threonine Phosphatase Interacting Protein 1 (PSTPIP1) [1]. PAMI is distinct from Pyogenic Arthritis, Pyoderma gangrenosum, and Acne (PAPA) syndrome that arises from different mutations in the same gene. The clinical manifestations of this disease, which include neutropenia, arthritis, and hepatosplenomegaly, often develop in the absence of typical febrile episodes, and therefore pose significant diagnostic challenges, particularly in adults. We describe the 38-year diagnostic odyssey of a patient who was diagnosed with multisystem autoimmunity and proteinuria, a novel manifestation of PAMI associated with glomerular calprotectin deposition.

A 56-year-old female with diseases in multiple systems was referred for evaluation of a possible unifying diagnosis (see Table 1). She had a seronegative symmetrical deforming non-erosive polyarthritis since the age of 18. Her rheumatologist had unsuccessfully treated her with corticosteroids, sulfasalazine, and methotrexate. Notably, she reported joint swelling following minimal trauma and on one occasion had pus drained from her elbow, which was culture-negative. She was referred to a hepatologist at the age of 43 when she was diagnosed with macronodular cirrhosis, mild portal hypertension (hepatic vein pressure gradient 6 cm), and splenomegaly. Extensive investigations including transjugular liver biopsy did not reveal the underlying cause. She was found to be pancytopenic, with marked neutropenia, and hypogammaglobulinemia. Bone marrow biopsy showed reactive changes and excluded a hematological malignancy. Infusion of intravenous immunoglobulin and subcutaneous injection of G-CSF both resulted in serum sickness. Interestingly, she reported recurrent childhood chest infections which resolved after she developed polyarthritis. Breast reduction surgery at age 48 resulted in postoperative hematoma, poor wound healing, and wound dehiscence. She was found to also have von Willebrand's

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Table 1 Clinical and laboratory findings in patient before and after treatment with colchicine. Blue indicates present or elevated; red indicates decreased; green indicates return to normal; black indicates within range. *ND* not done

	Reference range:	Before colchicine	After colchicine
Arthritis		**Yes**	**Yes - improvement**
Hepatic cirrhosis		**Yes**	**Yes**
Portal hypertension		**Yes**	**Yes**
Splenomegaly		**Yes**	**Yes**
Lymphadenopathy		No	No
Cutaneous inflammation		No	No
Serum calprotectin	500 μg/l*	**>5 million μg/l**	**550,000μg/l**
Serum zinc	10.1-16.8 μmol/l	**98.2**	**59.4**
IgG	6.7-15.1 g/l	**2.7**	**2.8**
IgA	0.50-4.60 g/l	0.71	0.78
IgM	0.30-2.20 g/l	0.89	0.64
IgE	0-180 KU/l	6	ND
CRP	<5.0 mg/ml	**21.2**	**4.0**
ESR	2-25 mm/h	**38**	**23**
Serum amyloid A	0.0-6.4 mg/ml	**26.3**	**<4.0**
Hemoglobin	115-165 g/l	**81**	**112**
White cell count	4.0-11.0 x 10^9/l	**1.4**	**1.4**
Neutrophil count	2.0-7.5 x 10^9/l	**0.4**	**0.5**
Platelet count	150-400 x 10^9/l	204	165
24-hour urinary protein	0.00-0.15 g/day	**2.78**	**2.05**

*Normal healthy control

disease. At the age of 51, she developed ankle swelling and proteinuria. Renal biopsy showed podocyte effacement and dense deposits on electron microscopy; there was no amyloid or immune complex deposition.

Over the course of her disease, the patient had attended numerous alternative health practitioners who repeatedly tested her for heavy metal poisoning. These all showed persistent elevation of serum zinc levels to 98.2 μmol/l (reference range 10–16 μmol/l). This, and her history of "pathergy" and poor wound healing, prompted us to measure serum calprotectin levels by a chemiluminescent assay (Diasorin Liaison XL), which was markedly elevated at > 5 million μg/l (normal control 500 μg/l). A diagnostic Sanger sequence of her *PSTPIP1* gene revealed the heterozygous p.E250K (c.748G>A) mutation that had been reported in 13 of 14 patients with PAMI [1].

The patient was given a therapeutic trial of colchicine 1 mg twice daily. This resulted in normalization, for the first time, of her C-reactive protein, erythrocyte sedimentation rate, and serum amyloid A protein after 3 months. Her serum zinc level decreased and notably her serum calprotectin declined by 10-fold (see Table 1). Clinically, her joints symptoms improved and her use of non-steroidal antiinflammatory drugs decreased. Concomitantly, we also noted a decline in her

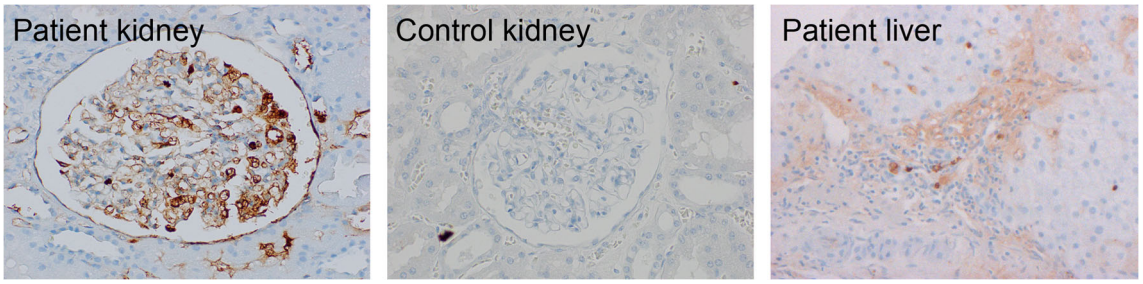

Fig. 1 Deposition of calprotectin in the kidney. Immunohistochemistry showing extracellular glomerular deposition of calprotectin (brown) in the patient kidney (left) but not control kidney (middle) or patient liver (left)

proteinuria from 2.78 to 2.05 g/day (see Table 1). This prompted a re-examination of her renal biopsy, and immunohistochemistry showed that the dense deposits were indeed calprotectin (see Fig. 1). This is the first description of dense extracellullar deposition of calprotectin in the glomerular basement membrane, and is distinct from the detection of intracellular calprotectin located inside the cytoplasm of infiltrating macrophages and neutrophils that have been described in the kidneys of patient vasculitis and glomerulonephritis [2]. There was no deposition of calprotectin in the liver (see Fig. 1). Thus, the extreme elevation of serum calprotectin may have resulted in dense deposits in her kidney and proteinuria, and this report expands the phenotypic spectrum of PAMI. Interestingly, a 6-year-old boy with PAMI and minimal change glomerulonephritis has previously been reported [1]; it will be interesting to see if this patient also had dense deposits of calprotectin in his kidney.

Autoinflammatory diseases are disorders of innate immunity [3]. The prototypical monogenic autoinflammatory disease, Familial Mediterrannean Fever, due to mutations in pyrin encoded by the *MEFV* gene, is associated with recurrent fever and serositis. However, autoinflammatory diseases may also present with autoimmunity and immunodeficiency, often in the absence of stereotypical febrile episodes. PAMI is one such autoinflammatory disease in which patients, such as in this report, may present with pancytopenia, hepatosplenomegaly, and hypogammaglobulinemia in the absence of fever to alert the physician to the diagnosis.

PSTPIP1 is a tyrosine-phosphorylated cytoskeletal protein that interacts with pyrin [4]. It is perplexing how the specific p.E250K mutation gives rise to such a distinct phenotype from other mutations in the gene, including the p.E250Q (c.748G>C) mutation in which the same base is mutated to a different nucleotide to give rise to PAPA syndrome [1, 5]. Nevertheless, the hallmark of PAMI is marked elevation of serum calprotectin, often to levels much higher than seen in PAPA syndrome and other autoinflammatory diseases [1].

Calprotectin is a dimer of S100A8 (MRP8) and S100A9 (MRP14) with high affinity for divalent cations including zinc. It constitutes 60% of the neutrophil cytosol [6] and its detection in neutrophil extracellular traps (NETs) suggests it is involved in extracellular control of pathogens such as *Candida albicans* [7]. Accordingly, fecal calprotectin is used as a surrogate measure of neutrophil infiltration and active colonic inflammation in patients with inflammatory bowel disease [8]. While antineutrophil antibodies have been detected in PAMI [1], it is conceivable that the p.E250K mutation constitutively induces inflammasome activation and neutrophil necroptosis, leading to the extracellular release of calprotectin and secondary elevation of zinc sequestered by calprotectin. In our case, colchicine was effective in reversing her hypercalprotectinemia, inflammatory markers, and proteinuria, possibly by inhibition of microtubule-mediated assembly of the inflammasome and calprotectin release from necroptotic neutrophils.

In summary, PAMI is an autoinflammatory disease that may masquerade as a multisystem autoimmune disease without overt symptoms or signs of systemic inflammation. Patients may also have proteinuria, and disease control with colchicine may be associated with a decrease in serum calprotectin and urinary calprotectin.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Abbreviations PSTPIP1, Proline-Serine-Threonine Phosphatase Interacting Protein 1; PAMI, PSTPIP-1-associated myeloid-related proteinemia inflammatory syndrome; MEFV, Mediterranean Fever; G-CSF, granulocyte colony stimulating factor

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