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CLINICAL LETTER

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Hemophagocytic lymphohistiocytosis in an adolescent with *NLRP12*-related autoinflammatory disorder—A case report

To the Editor,

Systemic autoinflammatory syndromes are disorders generally characterized by recurrent episodes of fever accompanied by systemic inflammation without an identifiable infectious or autoimmune cause.[1]

The nucleotide-binding leucine-rich repeat-containing receptor 12 (*NLRP12*) gene is a member of the NLRP family. Through its activation by foreign microbial proteins and damaged intracellular components, *NLRP12* plays a role in steady-state regulation of innate immunity.[2] The nucleotide-binding leucine-rich repeat-containing receptor 12 heterozygous mutations are known to cause Familial Cold Autoinflammatory Syndrome 2 (FCAS2), inherited in an autosomal dominant fashion.[3] It is usually induced by cold, and in most cases, it has an early onset in childhood. The most common clinical presentation is fever associated with multisystemic symptoms such as polyarthralgia/arthritis, abdominal pain and diarrhea, rash, lymphadenopathy/splenomegaly, headache, neurosensory deafness, and aphthous stomatitis.[3] To date, approximately 33 cases of *NLRP12*-associated autoinflammatory syndromes in children have been reported and 21 different *NLRP12* disease causing variants have been associated with FCAS2, including 16 missense, three frameshift, and two nonsense.[3] In many cases of FCAS2, the *NLRP12* variant was present in nonaffected first-degree relatives, indicating incomplete penetrance of the disease and pointing the possibility of the contribution of other genetic and environmental factors for the clinical expression of the disease.[3]

With the aim of expanding the clinical spectrum of *NLRP12*-related autoinflammatory disorders, the authors describe the case of an adolescent with a novel *NLRP12* variant presenting with recurrent febrile episodes, cold urticaria, and hemophagocytic

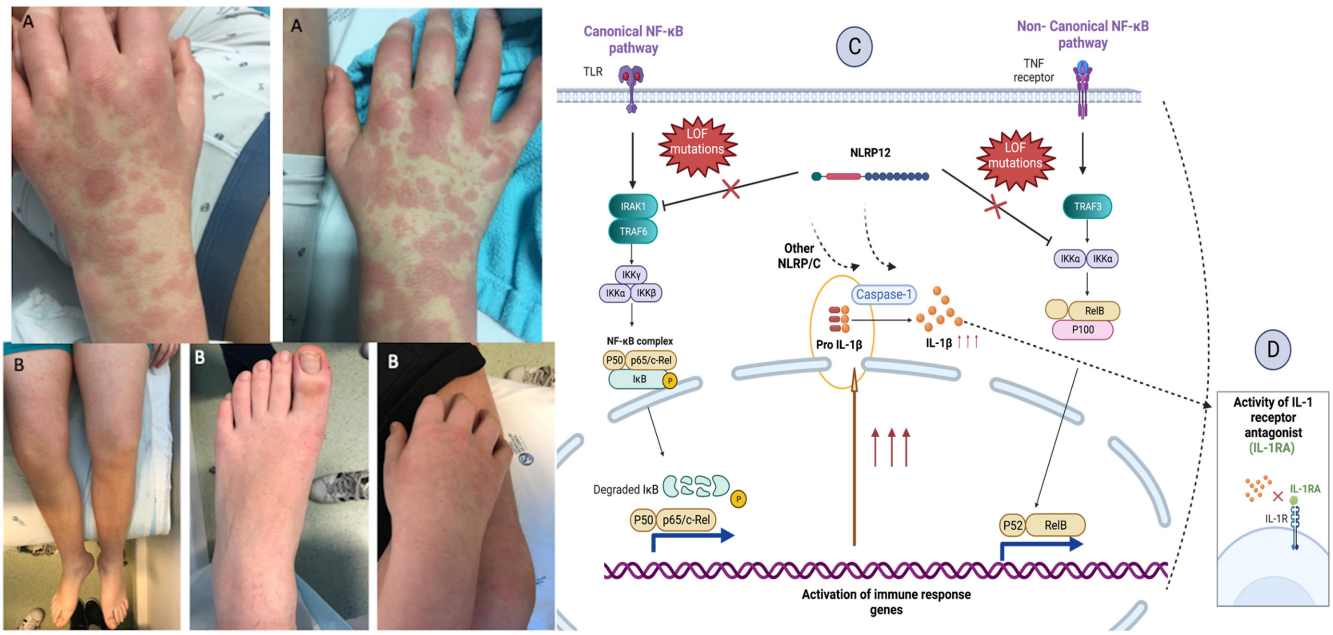

FIGURE 1 (Left—A) maculopapular rash with target lesions in upper limbs at initial clinical presentation; (B) maculopapular rash with target lesions in both upper and lower limbs during relapse. (Right—C) Overview of the *NLRP12* activity in the canonical and non-canonical NF-KB pathways, showing *NLRP12* role in inhibiting IRKA1 and IKK, leading to the control in the production of inflammatory cytokines, like IL-1β. Loss-of-function (LoF) mutations stop that inhibition leading to an increased transcription of pro-inflammatory cytokines, which promotes inflammation; (D) Role of IL-1 receptor antagonist (IL-1RA) as therapeutic drug that blocks the IL-1 receptor (IL-1R), inhibiting the action of IL-1 and controlling inflammation in these patients. For this manuscript, Figure right was created with BioRender.com and a publication license was obtained.

lymphohistiocytosis, never reported in patients harboring NLRP12 mutations.

A 14-year-old boy, born of nonconsanguineous parents, presented to the emergency department with a three-week history of a pruritic, generalized maculopapular rash with centrifugal distribution (Figure 1). In the 2 days previous to his first hospital observation, he had sore throat and high fever (maximum 39.5°C with 3-h intervals). On the day of observation, he additionally had bilateral conjunctival hyperemia, headache, and myalgia of the upper thighs. He had a previous medical history of growth hormone deficiency treated with growth hormone substitution therapy: myositis of unknown origin at 8 years of age, an episode of cold urticaria at 10 years of age treated with methylprednisolone, and three previous episodes of fever of unknown origin. Regarding his family history, his father had a previous diagnosis of cold urticaria and migraine.

At hospital admission, laboratory investigation revealed an elevated C-reactive protein (CRP) of 113.4 mg/L, D-Dimer of 4986 ng/mL, lactate dehydrogenase (LDH) of 432 UI/mL, together with neutrophilia, and lymphopenia (leucocytes 14.410/μL, neutrophils 13.440/μL, lymphocytes 630/μL). He was admitted and started intravenous ceftriaxone. On the following day, he developed dyspnea and hypoxemia. The chest X-ray showed a diffuse "cotton-like" infiltrate, confirmed by thoracic CT-scan that also revealed a bilateral pleural effusion. Invasive streptococcal disease was suspected, and he was started on intravenous penicillin, clindamycin, and two consecutive administrations of intravenous immunoglobulin (1g/kg/day). Although all the laboratory investigations for infectious diseases were negative (Table 1), he had a progressive clinical improvement after this treatment was started, with his fever settling, partial resolution of the skin rash, and a significant and progressive improvement of the inflammatory parameters. On Day 15 of admission, he presented again with high fever, confluent skin rash, and hepatosplenomegaly. Laboratory evaluation showed hemoglobin 7.7 g/dL, leucocytes 4300/μL, increased liver enzymes (AST 465 UI/L, ALT 136 UI/L, GGT 232 UI/L), elevated inflammatory parameters (CRP 220 mg/L; serum amyloid A 1200 mg/L), elevated ferritin (50,252 ng/mL), soluble CD25 (5227 pg/mL), and fasting triglycerides (423 mg/dL), which together with splenomegaly fulfilled 5/8 criteria for hemophagocytic lymphohistiocytosis (HLH).[4] NK cytotoxicity and degranulation assays were both normal. He was started on daily methylprednisolone pulses (30 mg/kg) for 5 days, followed by oral prednisolone (2 mg/kg/day for 2 weeks), with resolution of the fever episodes and a progressive improvement of the inflammatory markers. When clinically stable, he was discharged with a tapering steroid scheme. Four weeks after stopping steroids, following exposure to cold water, he again developed fever, elevated inflammatory markers, and a maculopapular rash with target lesions (Figure 1). The symptoms resolved with prednisolone but relapsed 5 days after its interruption. He was then started on long-term treatment with the recombinant interleukin-1 receptor antagonist, anakinra, 100 mg (2 mg/kg) daily, with transient complete resolution of the episodes. After 1 year on therapy with anakinra, he had a new relapse with fever, skin rash and splenomegaly, pancytopenia (hemoglobin 10.5 g/dL, neutrophils 580/μL and mild thrombocytopenia 123,000/μL), mild elevation of ferritin (600.5 ng/mL), and increased liver enzymes (AST 281 UI/L, ALT 328 UI/L). A quick response to 2 mg/kg/day of prednisolone was observed. Anakinra was then substituted by canakinumab leading to sustained remission. The analysis of a whole-exome sequencing (WES) based custom-designed virtual panel with 93 genes for auto-inflammatory syndromes found a novel nonsense heterozygous variant in the *NLRP12* gene [c.1952C > A; p.(Ser651*)], inherited from his father. This variant falls between the NACHT and LRR domains of the protein, a location that was previously shown to be critical for protein function.[5]

TABLE 1 Laboratory studies: immunological and infectious diseases screening during hospital admission.

	During admission	Reference values
$CD3^+$ T cells (cells/μL)	753	750–3477
$CD4^+$ T cells (cells/μL)	453	361–1900
$CD45RA^+CD27^+CD4^+$ T cells (naïve) (%)	53.9	31%–97%
$CD8^+$ (cells/μL)	271	160–1189
$CD45RA^+CD27^+CD8^+$ T cells (naïve) (%)	66.4	20–95
$CD16^+/56^+$ NK cells (cells/μL)	48.7	81–1348
$CD19^+$ B cells (cells/μL)	64.9	59.5–1194
$CD19^+CD27^-IgD^+IgM^+$ pregerminal center B cells (%)	93.99	59.0–87.4
$CD19^+CD27^+$ postgerminal center B cells (%)	5.86	7.0%–29.0%
$CD19^+CD27^+IgD-$ switched postgerminal center B cells (%)	5.55	0.9–21.2
IgG (g/L)	11.8	8.4–20.1
IgM (g/L)	1.59	0.57–3.0
IgA (g/L)	1.2	0.4–2.30
Serum soluble interleukin-2 receptor (sIL2Rα-CD25) pg/mL (maximum value during admission)	10,700	458–1997
CH50	54%	>66
C3 (g/L)	0.6	0.9–1.8
C4 (g/L)	0.1	0.1–0.4
Anti-Dnase B (U/mL)	<71	0–200
ANA	Negative	
Antiglobulin test (Coombs)	Weak positive	
IGRA T-Spot	Negative	
Blood, urine, and fecal cultures	Negative	
Tuberculous nAAT test in bronchoalveolar lavage	Negative	
Mycological culture in bronchoalveolar lavage	Negative	
Epstein–Barr Virus serology	Negative	
Cytomegalovirus serology	Negative	
PCR of enterovirus in feces	Negative	

This variant is present in a very low frequency (0.0016%) in genome aggregation database (GnomAD) and has a CADD Score of 34. A few other loss-of-function (LoF) variants have already been reported in *NLRP12*, associated with FCAS, suggesting its sensitivity to LoF.[6] Studies have suspected that *NLRP12*-related conditions have a paternal origin, with phenotypic variability.[7,8] The nucleotide-binding leucine-rich repeat-containing receptor 12 variants are associated with autosomal dominant autoinflammatory disorders with incomplete penetrance,[7,8] which is consistent with the milder phenotype in the father of this case, who only had cold urticaria, and in which we found the same nonsense heterozygous variant in the *NLRP12* gene.

The nucleotide-binding leucine-rich repeat-containing receptor 12 is a pleiotropic protein, which is activated by microbial proteins and intracellular components after cell damage. It works as an immune regulator of the innate immune response, inhibiting the both canonical and non-canonical nuclear factor-kB (NF-kB) pathway.[2,7,9] Loss-of-function variants compromise the regulation of the NF-kB pathway, leading to an increased and uncontrolled production of the pro-inflammatory cytokines IL-1β and IL-6.[6,7] On the contrary, FCAS2 is also known to interact with apoptosis-related dot-like protein (ASC) and pro-caspase-1, forming inflammasome, and mutations in NLRP12 can also be related to an hyperactive inflammasome activity, leading to uncontrolled IL-1 production that can be targeted by interleukin-1 receptor antagonists (Figure 1). Three drugs that target IL-1 have been used to treat FCAS: anakinra, a short-acting recombinant IL-1 receptor antagonist; and rilonacept and canakinumab, two long-acting IL-1-blockers.[10] Due to its rarity, the prognosis of FCAS2 is unknown; however, good results have been reported with prolonged and sustained remission using IL-1 receptor antagonists.[3]

Very interestingly, it has been shown that single allelic variants in *NLRP12* were associated with HLH in a group of patients with HLH but without biallelic mutations in the recognized familial HLH genes.[11] This would support a role for *NLRP12* in the pathogenesis of HLH in our patient, as individuals harboring pathogenic variants in the NLRP12 lead to dysregulated immune activation that can trigger HLH in the absence of abnormal cytotoxicity.

In conclusion, we describe a novel *NLRP12* variant, identified in an adolescent with HLH and recurrent episodes of cold-induced rash and fever. This report expands the range of disease causing variants in the *NLRP12* gene and further illustrates the complexity of phenotypes associated with this gene dysfunction.

AUTHOR CONTRIBUTIONS

Inês Hormigo: Conceptualization; methodology; writing – original draft; investigation; resources. **Marta Valente Pinto:** Software; methodology; writing – review and editing; supervision; investigation; project administration; validation. **Ana Isabel Cordeiro:** Writing – review and editing; visualization. **Cristina Henriques:** Visualization; writing – review and editing; data curation; investigation. **Catarina Martins:** Visualization; writing – review and editing. **João Parente Freixo:** Visualization; writing – review and editing. **Marta Conde:** Visualization; writing – review and editing. **Catarina Gouveia:** Visualization; writing – review and editing. **João Farela Neves:** Visualization; writing – review and editing; investigation; project administration; supervision; validation.

CONFLICT OF INTEREST STATEMENT

No potential conflict of interest is declared for any of the authors.

PEER REVIEW

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