Hemophagocytic lymphohistiocytosis in an adolescent with NLRP12 -related autoinflammatory disorder, a case report

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Clinical Letter to the Editor

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Main text:

To the editor:

Systemic autoinflammatory syndromes are rare monogenic 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). *NLRP12* 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 to 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 to FCAS2, including 16 missense, 3 frameshift and 2 nonsense (3). In many cases of FCAS2, the *NLRP12* variant was present in non-affected 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 lymphohistiocytosis, never reported in patients harbouring 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 maculo-papular 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^{o}C with 3

hours 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/ul, neutrophils 13440/ul, lymphocytes 630/ul). 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 (1gr/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 haemoglobin 7,7 g/dL, leucocytes 4300/ul, 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 (50252 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 five 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 five 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 (haemoglobin 10,5 g/dL, neutrophils 580/ul and mild thrombocytopenia 123000/ul), 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).

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 (6,7). *NLRP12*variants are associated with autosomal dominant autoinflammatory disorders with incomplete penetrance (6,7), 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.

NLRP12 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 both the canonical and non-canonical nuclear factor-kB (NF-kB) pathway (2,6,8). LoF variants compromise the regulation of the NF-kB pathway, leading to an increased and uncontrolled production of the pro-inflammatory cytokine IL-1ß (6) that can be targeted by interleukin-1 receptor antagonists (figure 2). 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 (9). 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 (10). This would support a role for *NLRP12* in the pathogenesis of HLH in our patient.

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.

Key words: familial cold autoinflammatory syndrome,*NLRP12* , adolescent, anakinra, canakinumab, autosomal dominant, incomplete penetrance, case report

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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.

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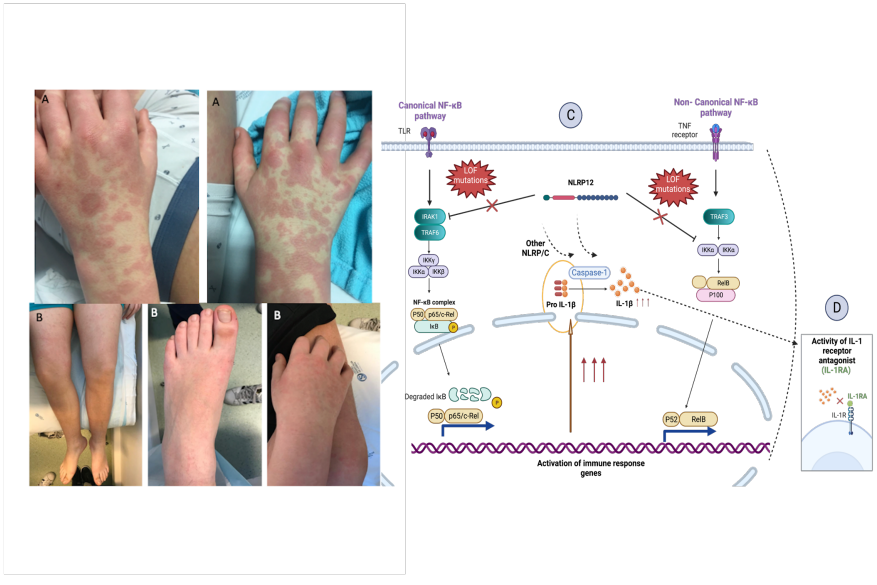
Table 1: Laboratory studies: immunological and infectious diseases screening during hospital admission



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