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Erythematous nodes, urticarial rash and arthralgias in a large pedigree with NLRC4-related autoinflammatory disease, expansion of the phenotype

Running title: Skin lesions and arthralgias in NLRC4-related autoinflammatory disease

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Bulleled statements

What is already known about this topic?

- Gain of function mutations in *NLRC4* have recently been found to be associated with autoinflammatory disease, presenting predominantly with recurrent febrile episodes, urticarial skin rash and enterocolitis.

What does this study add?

- We report a novel *NLRC4* variant that is associated with autoinflammatory disease, and provide a detailed description of the phenotype in a 6-generation pedigree.
- It is the first report of cutaneous histopathological findings in patients with gain of function mutations in *NLRC4*. Skin biopsies showed a dermal lymphocytic-histiocytic infiltrate, a feature that has not been described before in autoinflammatory skin disease.

Dear Editor,

Autoinflammatory disorders (AID) are a heterogeneous group of diseases, characterized by an unprovoked innate immune response, resulting in recurrent or ongoing systemic inflammation and fever¹⁻³. Inflammasomes are protein complexes with an essential role in pyroptosis and the caspase-1-mediated activation of the proinflammatory cytokines IL-1 β , IL-17 and IL-18 (reviewed in^{4,5}). Excessive activation of inflammasomes results in systemic autoinflammatory disease. Various inflammasomes have been identified, of which the NLRP3 inflammasome is most widely studied. Gain of function mutations in the *NLRP3* gene are associated with cryopyrin-associated periodic syndromes (CAPS), with urticarial skin rash as one of the hallmarks^{6,7}. Recently, gain of function mutations in *NLRC4* (*IPAF/CARD12*) were found to associate with a clinically heterogeneous AID, characterized by recurrent episodes of fever, periodic urticarial rash, enterocolitis, splenomegaly, and macrophage activation syndrome (MAS)⁸⁻¹⁰. Five different mutations in *NLRC4* have been reported⁸⁻¹⁰ (reviewed in¹¹). Gastrointestinal symptoms and hemophagocytosis distinguish the *NLRC4*-associated phenotype from CAPS⁸.

In this study, we describe the phenotype and skin histology of a large pedigree with 13 affected family members due to a novel mutation in *NLRC4* (pedigree in suppl. Fig. 1).

Disease onset occurred in infancy in all patients. Symptoms could be triggered by changes in weather conditions, emotional stress, and infection. Disease episodes were characterized by skin lesions, conjunctivitis, arthralgias, and - in two patients - colitis. Skin lesions, the predominant feature, varied in severity and localization. In contrast to previous reports, we observed different skin

phenotypes between the pediatric and adult patients⁸⁻¹⁰. The adult patients had painful erythematous nodes on the lower legs and feet, either isolated or in combination with urticarial patches on arms, legs, trunk and face. In two patients, soles were involved, which had not been reported before in AIDs. Remarkably, the pediatric cases in our cohort suffered from urticarial rash only (Fig. 1a).

One patient had enterocolitis with histological signs of inflammatory bowel disease, a feature that was previously described⁹. Histological evaluation of colon biopsies revealed active inflammation, with moderate infiltration of neutrophilic granulocytes and a submucosal mononuclear infiltrate. In contrast to the other reports where neonatal-onset enterocolitis spontaneously resolved after the age of 1 year, the onset of enterocolitis in our patient was in adulthood, with a chronic course⁸. The low number of patients with gastrointestinal symptoms in this family is remarkable, as enterocolitis was thought to distinguish the NLRC4-associated AID from CAPS⁸. Hemophagocytosis was not observed. Response to treatment with an IL-1 receptor antagonist (anakinra) varied (Table 1).

WES revealed the novel heterozygous c.1333T>C p.(Ser445Pro) variant in *NLRC4* in all affected family members. This variant is absent in the dbSNP or ExAC databases. Prediction software programs (Sift score 0.01, Polyphen-2 score 0.997) indicate that this mutation is probably damaging. The mutation is located next to the recently described pathogenic c.1328A>C p.(His443Pro) mutation and segregated with the disease phenotype (LOD-score 3.58).

Serum analysis of IL-1 β , IL-6, IL-10, IL-18, TNF α , and IFN γ showed extremely elevated IL-18 concentrations in 8 studied patients (median 4,324 pg/ml, range 3,097-13,984 pg/ml, normally 0-34 pg/ml). Concentrations of the other cytokines were in the normal range.

Skin biopsies were taken from nodes on the shins and/or calf of three adult patients. Histopathological examination in patient V5 showed a deep dermal and subcutaneous lymphocytic-histiocytic infiltrate with (para)septal and lobular panniculitis. In patient VI5, a perivascular infiltrate of lymphocytes and perivascular edema was observed. Patient VI1 showed a perivascular and interstitial infiltrate of lymphocytes, and in the deep dermis an infiltrate of lymphocytes, histiocytes and a few mast cells (Fig. 1b). Vasculitis was absent in all samples. Skin biopsies of clinically uninvolved skin (V5 and VI1) showed no abnormalities (not shown).

Skin biopsies in CAPS and in the related Schnitzler's syndrome mostly show a neutrophilic dermal infiltrate without vasculitis¹². The absence of a neutrophilic infiltrate in the skin biopsies of our patients is remarkable. Immunofluorescence studies did not detect IL-1 β in the skin biopsies, whereas it was found in mast cells in a skin biopsy from a Schnitzler's syndrome patient (data not shown). NLRC4 could not be detected in affected or unaffected skin (not shown), whereas it was present in the positive control (spleen).

In this pedigree, the response to anakinra varied widely, from no response to complete remission. The partial response to anakinra may be explained by the upregulated IL-18 production in response to NLRC4 inflammasome assembly. Additional deletion of the IL-18 receptor in mice with *Nlrp3* mutations, CAPS-mice, abolished the skin and visceral phenotype in young mice, and normalized the concentrations of most inflammatory markers in serum. However, with aging, a profound systemic inflammatory response occurred, even though the skin phenotype remained absent¹³. Possibly, IL-18 plays a role in various stages of the disease and may explain the partial anakinra-resistance.

CAPS-mice lacking both IL-1 and IL-18 receptors still showed clinical features associated with autoinflammation, indicating a role for additional players in the development of the autoinflammatory

phenotype as well¹³. As caspase-1 deficiency provided protection from autoinflammation in CAPS-mice, it can be hypothesized that caspase-1 exerts additional effects in NLRC4-mediated autoinflammatory disease. This is supported by the caspase-1 dependent, but IL-1 β and IL-18 independent, 'eicosanoid storm', which was recently described upon NLRC4 inflammasome activation in mice¹⁴.

In conclusion, we describe a novel mutation in *NLRC4* causing an NLRC4-associated, partially anakinra-responsive AID, dominated by cutaneous erythematous nodes and urticarial rash, arthralgias, and late-onset enterocolitis. We present the first histopathological study on the skin lesions, showing a lymphocytic-histiocytic infiltrate and septal/lobular panniculitis. The absence of both a neutrophilic dermal infiltrate and IL-1 β -positive mast cells distinguishes the urticarial rash observed in NLRC4-mediated AID from that observed in CAPS and Schnitzler's syndrome.

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Figure legends

Fig 1. Skin features and histology in patients with the c.1333T>C p.Ser445Pro variant in NLRC4

Urticarial skin rash in patient VII2, photographed at age 2 and 5 years old (at age 5 skin lesions appeared during temporary anakinra withdrawal): the face, trunk, extremities and soles are affected. Erythematous nodes on the lower legs of patient VI5; and on the lower legs (taken at the end of attack) and feet (during attack and at the end of attack) of patient VI2.

Histopathological examination of lesional skin in patient V5 showed a deep dermal and subcutaneous lymphocytic-histiocytic infiltrate with (para)septal and lobular panniculitis; in patient VI5 a perivascular infiltrate of lymphocytes and perivascular edema without signs of vasculitis or urticaria; and in patient VI1 a perivascular and interstitial infiltrate of lymphocytes, and in the deep dermis a granulomatous infiltrate of lymphocytes, histiocytes and a few mast cells. Panel (a) shows 100x magnification; panel (b) 400x magnification of the cellular infiltrate. Bar: 100nm.

Table 1

Patient no.	Current age (yrs)	Age at disease onset (yrs)	Gender	Episodes	Organs involved					Responsiveness to Anakinra
					Eyes	Skin	Joints/Muscle	Gastro-intestinal system	Other	
IV1	88	5	F	Intermittent, 6x/yr	Dry, conjunctivitis	Itching skin rash, erythematous nodes	Swelling, arthralgia, myalgia	Colitis ulcerosa (age 75)	Fatigue, elevated ESR, chronic renal insufficiency	Not tried
V1	63	<4	F	Intermittent, frequency unknown	Dry, conjunctivitis, episcleritis, cataract insipiens	Urticarial rash, erythematous nodes, itch, edema of lower legs	Swelling, arthrosis (Heberden noduli), rheumatic complaints	-	Elevated ESR, no response to medication	Not tried
V3	61	<4	F	Intermittent, 0-2x/yr	-	Painful skin rash, non-itching.	Pain, swelling, redness of joints. Myalgia.	-	-	Not tried
V5	58	Birth	F	Intermittent 12-60x/yr	Dry, conjunctivitis	Painful erythematous nodes, itch.	Swelling, erosive polyarthrits, tendinitis, osteoarthritis	-	-	Partial response: good effect on skin phenotype. Worsening of erosive arthritis despite Anakinra
V7	Deceased	<7	F	Intermittent, 24x/yr	Conjunctivitis	Painful skin rash	Swelling	-	-	Not tried
V8	70	<24	M	unknown	Dermatitis eyelids, conjunctivitis, episcleritis	Urticarial rash, dermatitis neck, itch, psoriasis	Painful joints, myalgia	-	Fever, sensorineural hearing loss	Not tried
V9	68	Child-hood	F	unknown	Conjunctivitis	Urticarial rash	Pain	-	Fever	Not tried
VI1	33	6 months	F	Never symptom-free, frequency of flares varies	Conjunctivitis, recurrent uveitis, keratitis, episcleritis	Urticarial rash (age 12 yrs), erythema nodosum, secondary	-	-	Fatigue, lymphedema, temporary IgA-deficiency,	Not tried

VI5	32	6	F	Intermittent, 2x/yr. Before Anakinra continuously subfebrile temperature	Dry, conjunctivitis	lymphedema Urticarial rash, painful erythematous nodes, itch, eczema	Pain, swelling, stiffness	Inflammatory bowel disease, developed during treatment with Canakinumab	- elevated CRP	Good effect on skin phenotype and subfebrile temperature
VI7	39	unknown	M	unknown	Dry	Urticarial rash	+	-	-	Not tried
VII1	7	9 months	F	Continuous rash; intermittent flares, frequency varies	Conjunctivitis	Urticarial rash; non-itching	Red, painful, swelling	-	Microcytic anemia, elevated CRP	Not tried
VII2	6	Birth	F	Intermittent, 12x/yr. Continuous complaints without Anakinra	Conjunctivitis	Urticarial rash	Painful, swelling	-	Fever (infrequent)/ subfebrile temperature, microcytic anemia, elevated CRP	yes, immediate flare up once treatment with Anakinra is interrupted
VII3	8	2 months	F	Continuous	-	Urticarial rash	+	-	-	Not tried

Table 1: Patient characteristics: Legends: All patients carry the c.1333T>C (p.Ser445Pro) mutation in *NLRC4*. Patient numbers are indicated similar to numbering in pedigree. Gender: F, female; M, male.

