

A novel mutation in the CIAS1/NLRP3 gene associated with an unexpected phenotype of cryopyrin-associated periodic syndromes

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ABSTRACT

Background. Cryopyrin-associated periodic syndromes (CAPS) comprise a spectrum of distinct, rare, autosomal dominant autoinflammatory disorders of increasing severity caused by NLRP3 gene mutations

Methods. We describe a 13-year-old female who presented, in the initial phase of the disease, recurrent episodes of high fever, pericarditis, arthralgia, arthritis of the knees, abdominal pain and marked increase in inflammatory markers.

In the subsequent months she developed recurrent episodes of chest pain, skin rash and swelling of the subcutaneous tissue, without fever, and with spontaneous resolution.

Results. Molecular analysis of the CIAS1 gene revealed the presence of the Q703K variant and also a c.1105C>A mutation in the heterozygous state, that predicts a L369M amino acid substitution. The latter variant has never been reported. The L369M mutation was predicted to significantly affect protein structure (scoring as "dangerous" and "deleterious") by the Variant Effect Predictor tool. Therapy with anakinra was started with rapid disappearance of clinical symptoms and normalization of CRP levels in 24 hours

Conclusion. The rapid response to IL-1 inhibition suggests that the disease of this patient is driven by IL-1 and supports the conclusion that this novel mutation is pathogenic and may be associated with a new CAPS phenotype. The role played by the concomitant presence of the mutation Q703K remains to be clarified

Introduction

The term CAPS (cryopyrin-associated periodic syndromes) indicates a spectrum of autoinflammatory diseases caused by heterozygous mutations of the CIAS1/NLRP3 gene. It has been clearly established that cryopyrin mutations in CAPS are gain of function mutations leading to caspase-1 activation and increased cleavage and secretion of the mature form of IL-1 β .

Affected individuals may present three different phenotypes: familial cold autoinflammatory syndrome (FCAS),

Muckle-Wells syndrome (MWS), and neonatal onset multisystem inflammatory disease (NOMID), also known as chronic, infantile, neurological, cutaneous, and articular (CINCA) syndrome, the most severe form of the clinical disease spectrum. FCAS is the mildest form of CAPS: it usually starts in the first year of life and is characterised by rash, fever, arthralgia and conjunctivitis induced by the exposure to cold temperature. Muckle-Wells syndrome is the intermediate-severity disease, characterized by fever, chronic evanescent recurrent urticaria-like rash, arthralgia, conjunctivitis and headache with attacks lasting usually 1–3 days: sensorineural hearing loss is frequent, while in 25% of patients are at risk of renal amyloidosis.

NOMID is the most severe form of CAPS; NLRP3 mutations are found only in 60% of the patients. It begins during the neonatal period, affecting skin, central nervous system and joints. Skin rash, present at birth or even *in utero*, is the usual initial presentation of NOMID, while the degree of neurological and articular involvement is highly variable. Severe cases suffer from sensorineural deafness, eye involvement, meningitis, joint contractures and secondary amyloidosis.

The systemic inflammatory disease manifestations in all CAPS patients include episodes of fever, non-pruritic urticarial rash, joint pain and elevations in acute phase reactants. The current standard of care to treat CAPS patients is lifelong treatment with IL-1 blocking agents, which are promptly effective (3-5).

Case report

We describe a 13-year-old female, the first child of healthy unrelated parents, who presented, since 12 years of age, with recurrent monthly episodes of high fever, non-infectious pericarditis, arthralgia, arthritis of the knees, abdominal pain and marked increase in inflammatory markers and in serum amyloid A levels (mean CRP value 6.76 mg/dl). The duration of febrile episodes was variable, they were not triggered by the exposure to cold temperature, nor were characterised by a circadian pattern.

Competing interests: none declared.

She presented 5 episodes in the first 5 months of disease. The arthritis of the knees was transient, non associated with *calor* and *rubor* and resolved at the end of febrile episode. Abdominal ultrasonography, performed during all febrile episodes because of the presence of marked abdominal pain, did not show signs of peritonitis or abdominal effusion, except in one of these episodes that was characterised also by pleural and abdominal effusions. Pericarditis was diagnosed by performing an echocardiogram that showed pericardial effusion in each episode. Pericarditis is an unusual manifestation in CAPS. Pericarditis recurred together with febrile episodes in five instances and was invariably characterised by pericardial effusion of variable magnitude. One episode occurred in another hospital was associated with remarkable effusion, with initial myocardial dysfunction. High dose steroid lead to prompt improvement

In order to prevent recurrence we started treatment with colchicine and indomethacin without benefit; therefore glucocorticoid treatment was initiated with resolution of clinical manifestations. Any attempt to stop glucocorticoids was followed by recurrence of symptoms.

An autoinflammatory syndrome was suspected and molecular analysis of the MEFV, TNFR1 and MVK genes was performed without evidence of pathogenic mutations.

In the subsequent months a change in the clinical picture was observed. She developed recurrent (up to daily) episodes of chest pain not related to pericardial effusion, non pruriginous skin rash and pomfoid-like swelling of the subcutaneous tissue of limbs, trunk (Fig. 1), joints and lips in the absence of fever, with spontaneous resolution. These episodes were associated with a elevation of acute phase reactants. Given the recurrent appearance of swelling of the lips, a deficiency of the complement was suspected; however, levels of C1q inhibitor were normal.

Molecular analysis of the CIAS1 gene revealed in exon 3 heterozygosity for a c.1105C>A base change, that predicts a p.L369M amino acid substi-

Fig. 1. Pomfoid-like swelling of the subcutaneous tissue of trunk.



tution. To the best of our knowledge this variant has not been reported before in association with CAPS or other autoinflammatory disorders; it is not reported in the Single Nucleotide Polymorphism DataBase (dbSNP). One hundred chromosomes were examined and the variant was not found. Prediction of the effect of this mutation on the protein function has been performed, in silico, by subjecting the p.L369M substitution to the Variant Effect Predictor tool (http://www.ensembl.org/Homo_sapiens/UserData/UploadVariations?db=core), including the PolyPhen (<http://genetics.bwh.harvard.edu/pph2/>) and SIFT (<http://sift.jcvi.org/>) analysis, and the SeattleSeq Annotation tool (<http://snpgs.washington.edu/SeattleSeqAnnotation131/index.jsp>). According to the in silico data obtained, the mutation was predicted to “significantly affect protein structure (scoring as “dangerous” and “deleterious” according to the above mentioned tools); indeed, the aminoacid at position 369 (Leu) has a very high conservation score, suggest-

ing that its substitution cannot occur without phenotypic consequences.

In the same exon, a heterozygous c.2107C>A nucleotide substitution, leading to the known p.Q703K amino acid substitution has also been detected. The p.Q703K mutation is a variant of unknown pathogenic significance of the *NLRP3* gene. It has been considered as both a common polymorphism or a low-penetrance mutation. The prevalence of this variant in the general population ranges from 5–11% (6). Some authors suggested that the Q703K polymorphism could represent a gain-of-function alteration leading to excessive Interleukin-1 β and interleukin-18 production (7). In a recent paper Vitale *et al* showed that subjects carrying the Q703K mutation may present with FCAS-like clinical manifestations (8). DNA from the parents was not made available for genetic analysis. Therefore we cannot reconstruct the phase of the two nucleotide variants found in our patient nor assess the possible *de novo* or inherited nature of the two mutations. We then evaluated the potential

relevance of this mutation on the de-regulation of IL-1 β production. Plasma IL-1 β levels (measured by ELISA, HS h IL-1 β , R & D System) were elevated (10.6 pg/ml) compared to healthy controls (n=30; 0.24 $\pm$ 0.15 pg/ml). Moreover, *in vitro* IL-1 β production by peripheral blood mononuclear cells, was increased: stimulation with LPS alone for 18 hours (100 ng/ml) induced higher IL-1 β release in our patient (1643 pg/ml) compared to age-matched healthy controls (n=3, 655.6 $\pm$ 54.45 pg/ml). This is in agreement with observations in CAPS patients (9). In order to verify the potential *in vivo* role of the observed p.L369M amino acid substitution daily therapy with anakinra (2 mg/kg/day) was started. This was associated with the rapid disappearance of clinical symptoms in 24 hours and normalisation of CRP levels. The rapid and complete response to IL-1 inhibition suggests that the clinical and laboratory features of the disease of this patient are driven by IL-1. Our data *in silico*, *in vitro* and *in vivo* support the

conclusion that the p.L369M mutation is pathogenic and may be associated with a new CAPS phenotype; this may differ from the known phenotypes for the characteristics of the rash and for the presence of pericarditis and abdominal pain. The role played by the concomitant presence of the Q703K variant remains to be clarified, though an effect in modifying the disease features cannot be excluded.

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