Rare *NLRP12* variants associated with the NLRP12-autoinflammatory disorder phenotype: an Italian case series

Sirs,

The NLRP12-autoinflammatory disorder (NLRP12-AD) is a rare autosomal dominant disease, also known as familial cold autoinflammatory syndrome 2 (or "FCAS 2"), caused by mutations in the *NLRP12* gene (1), which shares common clinical features with familial cold autoinflammatory syndrome (FCAS), the mildest phenotype among cryopyrin-associated periodic syndromes (CAPS) (2), associated with mutations in the *NLRP3* gene, directly involved in the interleukin-1 (IL-1) processing and secretion. NLRP12-AD main features are recurrent bouts of fever, severe fatigue, and musculo-skeletal symptoms, which are typically activated or worsened by cold exposure (3). The onset usually occurs in the neonatal period or in early infancy, and skin manifestations can be encountered in about one half of cases (4, 5). The knowledge of NLRP12-AD, its pathogenetic mechanisms and the genotype-phenotype correlations are still limited by both rarity of the disease and its recent identification.

In the present study, we report 6 Italian Caucasian patients with symptoms suggestive of NLRP12-AD, carrying *NLRP12* variants never before correlated with the NLRP12-AD phenotype. Table I reports the main clinical features of these patients: all underwent a full screening for infectious diseases, autoimmunity, thyroid and blood disorders, which were completely normal.

The *NLRP12* gene encodes for monarch 1 (also known as NLRP12), a regulator of NF-κB activation, which acts as a negative controller of inflammation, suppressing both canonical and noncanonical NF-κB activation and subsequent production of pro-inflammatory cytokines and chemokines, such as IL-1 (6-8), with protean effects. Williams *et al*. found that reduced *NLRP12* expression in myeloid/monocytic cells led to the activation of NF-κB and increased proinflammatory cytokine expression. To date, R284X, D294E, R352C, c.2072+3insT and c.1352G>A represent the main *NLRP12* sequence variations linked to the NLRP12-AD phenotype (1, 4, 5). To the best of our knowledge, there are no data available on F402L and G448A variants. Mutations affecting the NBD domain of *NLRP12* (*e.g*. F402L and G448A) could impair the monarch 1 anti-inflammatory function, leading to subverted pattern of inflammation. Despite the F402L mutation showed high frequency (up to 5%) in databases that report sequence variations from the general population, the presence of this mutation was not detected in our cohort of 72 origin-matched healthy individuals. Multiple alignment of monarch 1 (NP_001230062.1) from several species (chimpanzee, XP_524387.2; mouse, NP_001028603.1; rat, XP_001066862.1; dog, Ensemble accession no. ENSCAFP00000003989) demonstrated that p.Phe402 and p.Gly448 are conserved across different species. By virtue of our findings, we suggest that the present case series is important in showing that subjects carrying these variants might present with NLRP12-AD symptoms. Nevertheless, since asymptomatic carriers can

Table I. General and clinical features of patients with the NLRP12-autoinflammatory disorder phenotype.

	1	2	3	4	5	6
Age	26	17	39	54	3	39
Gender	F	F	F	F	F	M
Age at onset of symptoms	22	7	18	20 (disappeared around 45)	2	36
NLRP12 mutation	F402L	F402L	F402L	F402L	F402L	G448A
Mutated relatives	+ (mother)	–	+	+ (daughter)	+ (mother, two sisters)	–
Affected relatives	–	–	mother	mother, daughter, cousin	–	–
Fever	+	+	+	+	+	–
Fever ≥38°C	–	–	–	–	+	–
Fever duration (days)	Daily fever	5-10	Up to 3 weeks	Daily fever	3	NK
Fever episodes/year		12	12		NK	
Disease course	Chronic	Recurrent episodes	Recurrent episodes	Chronic	Recurrent episodes	Chronic
Cold-induced symptoms	+	+	+	+	+	+
Skin rash (type)	–	+ (maculopapular and urticarial)	+ (urticarial, pseudofolliculitis)	–	+ (urticarial, swelling of the dorsum of hands and feet)	+ (urticarial)
Lymphadenopathy	–	–	+	–	+	–
Headache	–	+	+	+	–	–
Arthralgia	+	+	–	–	+	+
Arthritis	–	+	+	–	+	–
Myalgia	+	+	+	–	+	+
Fatigue	+	+	+	+	+	+
Dyspnea	–	+	–	–	–	–
Conjunctivitis	–	–	–	–	+	–
Sensorineural deafness	–	+	–	–	–	–
Abdominal pain	–	–	+	–	–	–
Thoracic pain	–	+	–	–	–	–
Diarrhoea	–	–	+	–	–	–
Oral aphthosis	–	–	–	+	–	–
Increased CRP	–	+	+	NK	–	–
Increased ESR	–	+	+	NK	+	–
Increased SAA	+	+	+	NK	+	–
Response to NSAIDs	NR	PR	NR	NK	PR	NK
Response to colchicine	NR	NK	NK	NK	NK	NK
Response to antihistamines	NK	NK	NK	NK	NK	CR
Response to corticosteroids	PR (prednisone: 7.5-25 mg/day)	CR (prednisone: up to 50 mg/daily)	CR (methylprednisolone: 0.3 mg/kg/day)	NK	CR (prednisone: dose unknown)	CR (prednisone: 5 mg/day)
Response to anakinra (100 mg/daily)	NR	CR	NK	NK	NK	NK

CR: complete response; PR: partial response; NR: no response; NK: not known.

be observed (as the mother of Case 1), we also suggest that F402L and G448A variants might represent low-penetrance mutations rather than genetic polymorphisms, as described for other mutations studied in other autoinflammatory syndromes, as the Q703K in the *NLRP3* gene and the R92Q in the *TNFRSF1A* gene (9, 10). Currently, treatment of NLRP12-AD is mostly empirical: antihistamines and corticosteroids administered during winter can bring about the resolution of clinical symptoms and even the prevention of different disabling manifestations. As regards biologic agents, although a recent study reveals failure of anakinra, anti-IL-1 treatment may be a therapeutic choice in severe cases (10). Two out of our 6 patients underwent anakinra administration, but only one showed a prompt and sustained improvement. We could hypothesise that this heterogeneous therapeutic response might be related to a wide alteration in the cytokine network regulated by NF-κB cascade, and that the main cytokines involved in NLRP12-AD patients can vary in each patient. In support of our hypothesis, Jerù *et al*. found that anakinra led to an initial clinical improvement with rapid near-normalisation of IL-1β secretion in two NLRP12-AD patients, but that a progressive clinical relapse occurred secondarily, associated with increased TNF-α secretion, persistent elevated levels of IL-1 receptor antagonist and interleukin-6 (IL-6), with final reactivation of IL-1β secretion (5). In addition, serum IL-6 levels seem to be considerably increased in patients with NLRP12-AD in comparison with healthy controls (5). For these reasons, it would be interesting to evaluate the clinical response to anti-TNF agents, but more interestingly to IL-6 antagonists.

In conclusion, we suggest that F402L and G448A *NLRP12* variants may be associated with the peculiar symptoms of NLRP12-AD. However, given the possibility of healthy carriers, the role of additional and still unknown genetic and/or environmental modifiers is conceivable. For this reason we suggest that caution should be used in the interpretation of positive findings for this mutation in order to avoid false positive diagnoses and overtreatment. Lastly, we suggest anti-TNF or anti-IL-6 agents as potential therapeutic approaches in this category of patients.

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