Clinical and Experimental Rheumatology 2012; 30: 943-946.

Long-term clinical course of patients carrying the Q703K mutation in the *NLRP3* gene: a case series

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Received on December 22, 2011; accepted in revised form on March 16, 2012.

Key words: cryopyrin-associated periodic syndrome (CAPS), familial cold autoinflammatory syndrome, *NLRP3* gene, autoinflammatory disorders

Competing interests: none declared.

ABSTRACT

Background. *Cryopyrin-associated periodic syndromes (CAPS) comprise a spectrum of apparently distinct, rare, autosomal dominant autoinflammatory disorders of increasing severity caused by NLRP3 gene mutations. The Q703K allele is a variant of unknown pathogenetic significance, and has been considered to be both a clinically unremarkable polymorphism and a low-penetrance mutation.*

Objective. *To analyse the long-term clinical course in a cohort of patients presenting with periodic fever attacks and carrying the Q703K mutation in the NLRP3 gene.*

Methods. *Seven Caucasian patients (mean age 37.3±8.5 years, 2 males and 5 females) were identified as carriers of the Q703K mutation among 71 patients with CAPS-like symptoms.*

Results. *The mean age at disease onset was 25.58±16.08 years and the mean disease duration was 12.28±8.36. The mean number of febrile episodes was 7.56±6.48 and the mean duration of fever attacks was 6.66±4.71 days. Six out of 7 patients had a low grade fever, while 1 patient had no fever episodes. All patients were characterised by symptoms consistent with recurrent inflammatory syndrome. Six patients out of 7 presented skin lesions, 4/7 arthralgia, 4/7 myalgia, 4/7 conjunctivitis, 3/7 headache. All patients also complained of severe fatigue. In 4/7 patients symptoms were triggered or worsened by generalised cold exposure.*

Conclusion. *We suggest that patients carrying the low-penetrance Q703K mutation in the NLRP3 gene may present with FCAS-like symptoms. However, given the high frequency of healthy carriers, the role of additional, still unknown genetic and/or environmental modifiers is conceivable.*

Introduction

Cryopyrin-associated periodic syndrome (CAPS) comprises a spectrum of apparently distinct, rare, autosomal dominant inflammatory disorders of increasing severity, including familial cold autoinflammatory syndrome (FCAS), Muckle-Wells syndrome (MWS), and neonatal-onset multisystem inflammatory disorder (NOMID), also known as chronic infantile neurologic, cutaneous and articular syndrome (CINCA) (1). FCAS is characterised by early-onset urticarial rash with a typical neutrophilic infiltration (2), fever, arthralgia, myalgia, asthenia, headache and conjunctivitis, which can appear after generalised exposure to cold. In addition to the characteristics seen in FCAS, MWS is also characterised by sensorineural hearing loss. Chronic infantile neurological cutaneous and articular syndrome (CINCA), also called NOMID (neonatal onset multisystem inflammatory disease), is the most severe CAPS phenotype; diffuse erythema without pruritus may occur within a few days after birth. Chronic aseptic meningitis with neutrophils in the cerebrospinal fluid and mental retardation develop progressively during childhood. Sporadic manifestations include ocular involvement (conjunctivitis, uveitis, papillitis, and optic nerve atrophy potentially responsible for blindness), bilateral progressive sensorineural hearing loss, facial dysmorphia, lymphadenopathy, and hepatosplenomegaly (3). AA Amyloidosis leading to renal impairment is one of the most troublesome CAPS complications, occurring in about 25% of MWS and CINCA patients (4), but only infrequently in FCAS patients (2%) (5).

CAPS is associated with mutations in *NLRP3* (locus 1q44), the gene encoding cryopyrin, a component of the interleukin-1 inflammasome that regulates the production of interleukin-1β (6).

Advances in the understanding of the genetic basis and mechanisms of these disorders have led to the development of new treatments targeting the IL-1 pathway. These therapies are effective in the treatment of *NLRP3*-associated diseases, quickly controlling constitutional symptoms, urticaria-like rash and acute-phase reactant levels in all of these diseases (7).

To date, more than 90 different mutations of *NLRP3* have been identified (online at http:// fmf.igh.cnrs.fr/infevers/), the majority of which are localised in the exon 3 that encodes the oligomerisation (NLR binding) domain of cryopyrin. Some degree of genotype-phenotype correlation has been

observed in the cryopyrinopathies; in particular some mutations are associated with more severe phenotypes than others associated with milder phenotypes. The lack of a clear genotype/phenotype correlation for some mutations has been also reported (8-10).
The aim of our study was to analyse the long-term clinical course of disease in a cohort of patients with periodic fever carrying the Q703K mutation in the *NLRP3* gene, to date considered a variant of unknown pathogenetic significance.

Patients and methods
A cohort of 7 Caucasian patients (mean age 37.3±8.5 years, 2 males and 5 females) were retrospectively identified as carriers of the Q703K mutation on the *NLRP3* gene, among 71 consecutive patients with CAPS-like symptoms received at our Institution from October 2007 to October 2011. Detailed information regarding family history, personal history and clinical manifestations was collected; medical records were accurately reviewed for demographic characteristics and past clinical history regarding the duration and periodicity of febrile episodes, associated symptoms and signs, laboratory findings, disease course and outcome following treatments.
All patients underwent detailed investigations including neurological and ophthalmologic examinations, brain magnetic resonance imaging (MRI), and audiometry exam tests. Laboratory analyses included white blood cell count, kidney function evaluation, platelet count, blood haemoglobin concentration, erythrocyte sedimentation rate (ESR), C-reactive Protein (CRP), serum-amyloid A (SAA), serum IL-1β, IL-6 and IL-18 levels. After providing written consent for genetic testing, in accordance with the Declaration of Helsinki and local Ethics Committee regulations, all subjects were tested for mutations in the *MEFV* gene (from exon 1 through 10), responsible for familial Mediterranean fever (FMF), the *TNFRSF1A* gene (from exon 1 through 6), responsible for tumour necrosis factor receptor-associated periodic syndrome (TRAPS) and *MVK* (from exon 1 through 12), responsible for hyperimmunoglobulinaemia D (HIDS). Typing of HLA-B51 alleles was performed using polymerase chain reaction-sequencing based typing (PCR-SBT).

Results
The mean age at disease onset was 25.58±16.08 years and the mean number of febrile episodes at disease onset was 8.33±5.12 per year, while the mean number of febrile episodes at the time of observation was 7.56±6.48. The mean duration of fever attacks was 6.66±4.71 days, and the mean disease duration was 12.28±8.36 years. Six out of 7 patients had a low grade fever, ranging from 37.5°C to 38.2°C , whereas one patient had no fever episodes. All seven patients were characterised by symptoms consistent with recurrent inflammatory syndrome. None of the patients had a family history relevant for recurrent fever episodes; however, asymptomatic relatives were screened for mutations in the *NLRP3* gene, and in 5 out 7 cases, at least one relative carried the same mutation as the proband. In 2/7 patients the mutation was likely to have occurred *de novo*. Clinical manifestations observed in our cohort were: asthenia in 7/7, skin lesions in 6/7, headache in 3/7, arthralgia in 4/7, conjunctivitis in 4/7 and myalgia in 4/7. In 4/7 patients the clinical manifestations were triggered or worsened by generalised cold exposure. Four out of 7 showed a chronic disease course (symptoms persisting during fever-free intervals), whereas 3/7 had no symptoms during fever-free episodes. Three out of 4 patients with a chronic disease course tended to have lower temperatures (~37.5°C) and longer fever episodes.
Skin lesions were an urticarial-like rash in 4/6 and a CAPS-like rash in 2/6. Kidney function was normal in all patients, and none showed proteinuria. Audiometry exam tests and brain MRI were normal in all subjects. Laboratory investigations performed during febrile episodes showed neutrophilic leukocytosis (9.036±4.840/mm^3), ESR (41±26; n.v. <35 mm/h), CRP (2.88±1.32 mg/dl; n.v. <0.5 mg/dl) and SAA (92.75±53.74 mg/l; n.v. <10 mg/l). Characterisation of the cytokine pattern revealed elevated interleukin (IL)-1ß (7.27±1.22; n.v 0-2 pg/ml), elevated IL-6 (33.25±19.89 pg/ml; n.v 0.45-10 pg/ml) and normal IL-18 (32.65±10.84 ng/ml; n.v 0-100 ng/ml) serum levels. IL-1β and IL-6 serum levels were within normal values during fever-free intervals, whereas patients showing a chronic disease course had a persistent mild elevation of SAA. Kidney function was normal in all patients and none showed proteinuria. None of the patients carried mutations in the *MEFV*, *MVK* or *TNFRSF1A* genes. All subjects were negative for the HLA-B51 antigen.
Febrile episodes showed a poor response to non-steroidal anti-inflammatory agents (NSAIDs) and colchicine (1–2 mg/daily). Short courses of oral corticosteroids, administered on demand at the beginning of fever attacks (0.5–1 mg/kg/daily prednisone), were given in 4/7 patients (3/3 patients with remittent disease course and 1/4 with chronic disease course). Corticosteroid administration led to a complete clinical remission within a few days of the onset of fever in patients showing a remittent disease course, whereas the patient characterised by a chronic disease course required a more prolonged prednisone administration. Lower corticosteroid doses resulted in only a partial amelioration of symptoms. Skin lesions did not respond to antihistamines. None of the patients was prescribed a second-line treatment such as IL-1β inhibitors due to steroid resistance or steroid dependency.

Discussion
The Q703K mutation is a variant of unknown pathogenetic significance of the *NLRP3* gene. It has been considered to be both a clinically unremarkable polymorphism and a low-penetrance mutation found at an allele frequency of 3% in the Caucasian population (11). The rate of prevalence of *NLRP3* mutations has recently been assessed in a population of 806 randomly selected Swedish individuals. The Q703K variant was found to be a common polymorphism, present at an allele frequency of 6.5% (12). However, Verma *et al*. also showed that the Q703K mutation may cause excessive IL-1β production, and

Table I. A summary of the main clinical and demographic characteristics of patients.

Patient	1	2	3	4	5	6	7
Gender	female	male	male	female	female	female	female
Age at disease onset (years)	10	36	17	42	22	45	4
Disease duration (years)	2	26	8	14	4	15	17
Fever	+	+	+	+	+	+	–
Fever duration (days)	4-5	10-14	5-8	2-3	9-11	2-4	2-3
Fever episodes/year	8-10	10-12	8-11	10-14	1-4	3-4	9-13
Disease course	remittent	chronic	chronic	remittent	chronic	chronic	remittent
Skin rash	+	–	+	+	+	+	+
Type of skin rash	CAPS-like	–	CAPS-like	Urticarial	Urticarial	Urticarial	Urticarial
Headache	–	+	+	–	–	–	+
Arthralgia	+	–	–	+	+	+	–
Conjunctivitis	+	+	+	–	–	–	+
Myalgia	+	+	–	–	+	–	+
Fatigue	+	+	+	+	+	+	+
Cold induced symptoms	–	–	+	+	–	+	+
Positive family history	–	–	–	–	–	–	–

that patients' symptoms can be eliminated by IL-1β inhibitors such as anakinra. The authors suggested that the Q703K polymorphism in *NLRP3* could represent a gain-of-function alteration leading to excessive Interleukin-1β and interleukin-18 production (12).

The Q703K mutation has been also identified in more than 200 normal European (Spanish) controls (http://fmf.igh.cnrs.fr/infevers/) (13). To the best of our knowledge, the carrier frequency of the Q703K mutation in the Italian population is currently unknown.

Aksentijevich *et al*. screened 125 unrelated patients with clinically uncharacterised autoinflammatory disease for mutations in exon 3 of *NLRP3*. Ten patients, including 1 with NOMID/CINCA syndrome, were found to carry the Q703K mutation (allele frequency 0.04). They also evaluated whether the Q703K mutation could be a disease-associated mutation with variable expressivity, and found 1 homozygous subject and 34 carriers in a panel of 374 control Caucasian DNA samples (allele frequency 0.05) (10). Based on the similar allele frequency of Q703K in patients and controls, they concluded that Q703K was unlikely to be pathogenic.

Ting *et al*. described the cases of three children with varying clinical phenotypes, all of whom had in early childhood periodic symptoms, neurologic manifestations, elevated inflammatory markers, and expression of the *NLRP3* Q703K mutation (14). Although these patients did not fit the classic diagnosis of any of the cryopyrin-associated periodic syndromes, they manifested elements of each (14).

Our data show that although asymptomatic carriers are commonly observed, subjects carrying the Q703K mutation may present with FCAS-like clinical manifestations, thus suggesting that this allele may be a low-penetrance mutation rather than a polymorphic variant. In addition, as in FCAS, generalised cold exposure typically triggered the onset (or worsening) of symptoms in most of our patients. Nonetheless, some differences can be identified: i) late disease onset ii) the eosinophilic skin infiltrate noted in one patient, as opposed to the more typical neutrophilic FCAS infiltrate. Although use of an IL-1β inhibition has also been suggested in milder CAPS phenotypes in order to improve patients' quality of life (15), none of the patients in the present study was prescribed a second-line treatment, as they were responsive to short courses of oral corticosteroids administered on demand during febrile episodes. However, despite the mild phenotype, most patients showed no reduction in the number of fever episodes over time, as had been previously described for other low-penetrance mutations (16).

Even though the presence of other genetic and environmental factors may initiate or modulate CAPS manifestations (16), specific *NLRP3* mutations seem likely to affect disease expression, as demonstrated by some degree of genotype/phenotype correlation observed within this spectrum of phenotypic expression (8). Low-penetrance mutations such as V198M and R488K can lead to a mild CAPS phenotype, but asymptomatic carriers have also been evaluated (10, 17), thus supporting the concept of mutations with reduced penetrance that require additional genetic factors in order to produce a disease phenotype (10). The presence of healthy donors and asymptomatic familial cases carrying this mutation and the heterogeneous phenotype observed in the Q703K patients has raised the question of whether this variant is a low-penetrance disease-associated mutation or an asymptomatic polymorphism. These observations suggest the hypothesis of a possible pro-inflammatory effect of the Q703K mutation which may contribute to an inflammatory phenotype in concomitance with other eventual environmental and genetic factors. It can also be speculated that the Q703K could act as a possible susceptibility factor in other autoinflammatory disorders, but for the moment this remains mere hypothesis, and further studies are still needed. For this purpose, *in*

vitro functional tests may be useful for understanding its pathogenicity. Study limitations: we have studied only seven patients with a specific genetic status, therefore the results cannot be generalised to all patients. In addition, since 5 of the patients also have asymptomatic relatives carrying the same mutation, it would have been interesting to examine laboratory data from the healthy carriers in order to determine whether they have evidence of subclinical inflammation. Furthermore, due to the small number of patients in the study, we did not search for correlations between clinical manifestations, disease duration and laboratory data. However, to the best of our knowledge this is the largest report to date of extensively phenotyped Q703K patients. In conclusion, we suggest that the present results are important in showing that subjects carrying the Q703K mutation in the *NLRP3* gene, a variant of unknown pathogenetic significance, may present with FCAS-like symptoms. Given the high frequency of healthy carriers, the role of additional, still unknown genetic and/or environmental modifiers is conceivable. For this reason we suggest that caution be used in the interpretation of positive findings for this mutation in order to avoid false positive diagnoses and overtreatment.

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