

Clinical Characteristics of Patients Carrying the Q703K Variant of the *NLRP3* Gene: A 10-year Multicentric National Study

Aldo Naselli, Federica Penco, Luca Cantarini, Antonella Insalaco, Mariolina Alessio, Alberto Tommasini, Cristina Maggio, Laura Obici, Romina Gallizi, Marco Cimmino, Sara Signa, Orso Maria Lucherini, Sonia Carta, Francesco Caroli, Alberto Martini, Anna Rubartelli, Isabella Ceccherini, and Marco Gattorno

ABSTRACT. Objective. The aim of our study was to analyze the clinical and functional effect of the p.Q703K (p.Q705K, c. 2107C>A) variant of the *NLRP3* gene in a population of patients screened for suspected cryopyrin-associated periodic syndrome (CAPS).

Methods. Since 2002, 580 patients underwent molecular analysis for *NLRP3*. Data on clinical presentation, response to treatment, and longterm followup were collected using a uniform questionnaire. The pattern of cytokine secretion after lipopolysaccharide stimulation from isolated monocytes was analyzed in 3 patients carrying the p.Q703K variant and 1 patient with a chronic infantile neurologic, cutaneous, articular syndrome phenotype carrying both the p.M406I and p.Q703K, and compared with 7 patients with CAPS with sure pathogenic variants and 6 healthy controls.

Results. The p.Q703K variant was found in 57 screened patients with an overall allelic frequency of 5%. The frequency in normal controls was 5.5%. Clinical data at the moment of molecular analysis and at followup were available in 36 patients. Two patients displayed additional mutations of *NLRP3*. The mean followup was 2.5 years. Thirteen patients (39%) had a final diagnosis different from the original suspicion of CAPS. The remaining 21 patients displayed a mild phenotype mainly characterized by recurrent episodes of urticarial rash and arthralgia. Only 8 patients were treated with anti-interleukin (IL)-1 treatment, with a complete response in 5 patients. The pattern of secretion of IL-1 β and other cytokines (IL-6 and IL-1 receptor antagonist) in patients did not display the aberrancies observed in patients with CAPS and was similar to that observed in healthy controls.

Conclusion. The present study confirms the weak clinical and functional effect of the p.Q703K variant. (First Release April 1 2016; J Rheumatol 2016;43:1093–1100; doi:10.3899/jrheum.150962)

Key Indexing Terms:

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CRYOPYRIN

CRYOPYRIN-ASSOCIATED PERIODIC SYNDROME

INTERLEUKIN-1 β

From the Unità Operativa (UO) Pediatria 2, IRCCS G. Gaslini; Clinica Reumatologica, Dipartimento di Medicina Interna, Università di Genova; Laboratorio di Biologia Cellulare, IRCCS Azienda Ospedaliera Universitaria (AOU) San Martino Istituto Scientifico Tumori (IST); Unità Operativa Complessa (UOC) Genetica Medica, IRCCS G. Gaslini; Università degli Studi di Genova, Genoa; Dipartimento di Reumatologia, Policlinico Le Scotte, Università di Siena, UO Reumatologia, Siena; Ospedale Bambino Gesù, Rome; Dipartimento di Pediatria, Ospedale Federico II, Naples; Dipartimento di Pediatria, IRCCS Burlo Garofalo, Trieste; Dipartimento di Scienze per la Promozione della Salute e Materno Infantile “G. D’Alessandro”, Palermo; Centro per lo Studio e la Cura delle Amiloidosi Sistemiche Fondazione IRCCS Policlinico San Matteo, Pavia; Dipartimento Materno-Infantile, Ospedale Gaetano-Martino, Messina, Italy.

A. Naselli*, MD, UO Pediatria 2, IRCCS G. Gaslini; F. Penco*, MD, UO Pediatria 2, IRCCS G. Gaslini; L. Cantarini, MD, Dipartimento di Reumatologia, Policlinico Le Scotte, Università di Siena, UO Reumatologia; A. Insalaco, MD, Ospedale Bambino Gesù; M. Alessio, MD, Dipartimento di Pediatria, Ospedale Federico II; A. Tommasini, MD, Dipartimento di Pediatria, IRCCS Burlo Garofalo; C. Maggio, MD,

Dipartimento di Scienze per la Promozione della Salute e Materno Infantile “G. D’Alessandro”; L. Obici, MD, Centro per lo Studio e la Cura delle Amiloidosi Sistemiche Fondazione IRCCS Policlinico San Matteo; R. Gallizi, MD, Dipartimento Materno-Infantile, Ospedale Gaetano-Martino; M. Cimmino, MD, Clinica Reumatologica, Dipartimento di Medicina Interna, Università di Genova; S. Signa, MD, UO Pediatria 2, IRCCS G. Gaslini; O.M. Lucherini, MD, Dipartimento di Reumatologia, Policlinico Le Scotte, Università di Siena, UO Reumatologia; S. Carta, MD, Laboratorio di Biologia Cellulare, IRCCS AOU San Martino IST; F. Caroli, MD, UOC Genetica Medica, IRCCS G. Gaslini; A. Martini, MD, UO Pediatria 2, IRCCS G. Gaslini, and Università degli Studi di Genova; A. Rubartelli, MD, Laboratorio di Biologia Cellulare, IRCCS AOU San Martino IST; I. Ceccherini, MD, UOC Genetica Medica, IRCCS G. Gaslini; M. Gattorno, MD, UO Pediatria 2, IRCCS G. Gaslini. * These authors equally contributed to the paper.

Address correspondence to Dr. M. Gattorno, UO Pediatria 2, IRCCS Giannina Gaslini Institute and University of Genoa, Largo G. Gaslini 5, 16147 Genoa, Italy. E-mail: marcogattorno@ospedale-gaslini.ge.it
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Cryopyrin-associated periodic syndromes (CAPS) are a group of autoinflammatory diseases associated to dominantly inherited or *de novo* mutations of the *NLRP3* gene¹. Representing the clinical spectrum of CAPS are familial cold autoinflammatory syndrome (FCAS), Muckle-Wells syndrome (MWS), and neonatal-onset multisystem inflammatory disease [NOMID; also known as chronic infantile neurological, cutaneous, articular syndrome (CINCA syndrome)]^{2,3,4,5,6}.

To date, more than 170 different variants of *NLRP3* have been identified (fmf.igh.cnrs.fr/InfEVERS), the large majority of which is localized in the exon 3 that encodes the oligomerization (NLR-binding) domain of cryopyrin⁷. Somatic mosaicism has been reported in patients with a severe CAPS phenotype negative to germ-line mutations of *NLRP3*^{8,9}. Pathogenic variants of *NLRP3* result in substantial upregulation of caspase 1-mediated cleavage of pro-interleukin (IL)-1 β and secretion of active IL-1 β ^{10,11}. For this reason, IL-1 blockade is extremely effective in these patients^{12,13,14}. Even if a strict genotype-phenotype correlation cannot be ascertained, it is now clear that some variants found with high penetrance in large families are usually associated to a milder phenotype, whereas rare and *de novo* mutations are usually associated to the more severe CINCA phenotype^{3,4,15}. On the other hand, other variants, such as V198M and I313V, are characterized by a low penetrance and their clinical effect is debated^{7,16}. The p.Q703K variant (p.Q705K, c. 2107C>A) has been considered for many years as a clinically unremarkable polymorphism because of its presence in 5–11% of the general population (fmf.igh.cnrs.fr)^{7,17}. However, 2 subsequent studies from Sweden have shown that the p.Q703K variant displays a higher secretion of IL-1 β , thus suggesting a possible pathogenic involvement of this variant^{18,19}. This hypothesis was supported by a preliminary clinical study performed in 7 adult patients²⁰.

The aims of our study were (1) to analyze the prevalence of p.Q703K mutation in pediatric and adult patients screened for *NLRP3* in the context of a diagnostic workup for a suspected CAPS and to compare it to its prevalence in the normal population, (2) to describe the clinical findings at presentation, the disease outcome, and response to treatment in patients carrying the p.Q703K *NLRP3* variant, and (3) to analyze the pattern of IL-1 β secretion in this subgroup of patients.

MATERIALS AND METHODS

Patients' selection. From March 2002 to January 2013, molecular analysis of the *NLRP3* was performed in 580 patients for a clinical suspicion of CAPS and other periodic fevers by 2 national referral centers (1 pediatric, 1 adult) collecting samples from Italian centers (local hospitals, secondary and tertiary referral centers) distributed in all regions of the country. Subjects who displayed features possibly associated to autoinflammatory diseases other than CAPS or presenting an unspecific phenotype underwent additional screening for the following genes associated to monogenic periodic fevers: *MEFV* [associated with familial Mediterranean fever (FMF)], *TNFRSF1A*

[associated with tumor necrosis factor (TNF) receptor-associated periodic syndrome], *MVK* (associated with mevalonate kinase deficiency), and *NLRP12* (FCAS 2). All patients carrying the p.Q703K (p. Q705K, c. 2107C>A) *NLRP3* variant were enrolled in our study. Detailed information concerning the family history, personal history, and clinical manifestations at disease presentation were collected at the time of genetic analysis with a uniform questionnaire.

To collect information on the followup of the patients, a second questionnaire was sent to all participating centers on March 2013. Data were collected on modification of the clinical picture during the followup, including the possible appearance of complications such as hearing loss, eye manifestations, or amyloidosis. Data were also evaluated on the association between the clinical manifestations and the elevation of acute-phase reactants and on response to different therapeutic strategies. Centers were also asked to indicate whether a possible alternative diagnosis was made during the followup period subsequent to the genetic analysis. The study was approved by the ethics board of the G. Gaslini Institute, Genoa, Italy. Local ethical boards also approved the study in each center.

Molecular analysis of *NLRP3*. The exon 3 (1.7Kb) of the gene was amplified by PCR and the 5 overlapping amplification products were sequenced by using the BigDye Terminator Cycle Sequencing Ready reaction Kit 3.0 and loading the reactions into an automated DNA sequencer²¹.

Cell preparation and culture. These functional experiments could be performed only in freshly isolated monocytes and have been done in a limited number of patients followed by the Gaslini Institute. Fresh monocytes were enriched and activated with lipopolysaccharide (LPS) as described^{11,22} in 3 patients carrying the p.Q703K variant, 1 patient with a CINCA phenotype carrying both the p.M406I and p.Q703K, and 7 additional patients with CAPS (Supplementary Table 1, available online at jrheum.org). Six age-matched healthy individuals were used as controls. Cytokines in supernatants were quantified by ELISA assay (R&D Systems). The Student t test (significance level defined as p values < 0.05) was used for statistical analysis.

RESULTS

Prevalence of the p.Q703K variant in patients with suspected CAPS and in the normal population. From March 2002 to January 2013, the p.Q703K (p. Q705K, c. 2107C>A) variant was detected in 57/580 white patients (41 children and 16 adults). Fifty-five were heterozygous and 2 were homozygous. The overall allelic frequency was 5%. Twenty-seven patients were exclusively screened for *NLRP3*, whereas additional genes associated to periodic fevers were analyzed in the remaining 30 patients (52%; Supplementary Figure 1, available online at jrheum.org).

The clinical features at the moment of genetic analysis were available for 46 patients and are summarized in Supplementary Table 2 (available online at jrheum.org). No positive family history of urticarial skin rash, unexplained fever, or other inflammatory manifestations possibly related to a CAPS-like phenotype was reported in any of the patients. Molecular analysis for Q703K was performed in 1 or 2 parents for 15 patients. In 12 patients, 1 asymptomatic parent carrying the same variant was identified. In the remaining 3 patients, 1 parent only was analyzed.

To estimate the prevalence of the p.Q703K mutation in the general population, 90 healthy individuals were also analyzed. Ten individuals displayed a p.Q703K heterozygous mutation with an overall allelic frequency of 5.5%. Such a

Table 1. Demographic features of the patients at the time of molecular analysis.

| Patient | Sex | Age at Onset, yrs | Age at Molecular Analysis, yrs | Mutations in <i>NLRP3</i> or in Other Genes |
|---------|-----|-------------------|--------------------------------|---|
| S1 | F | 30 | 41 | Q703K |
| S2 | F | 45 | 60 | Q703K |
| S3 | F | 22 | 26 | Q703K |
| S4 | F | 7 | 11 | Q703K |
| S5 | F | 4 | 21 | Q703K |
| S6 | M | 17 | 25 | Q703K |
| S7 | F | 15 | 19 | Q703K |
| S8 | F | 57 | 58 | Q703K |
| S9 | F | 28 | 28 | Q703K |
| S10 | F | 57 | 64 | Q703K |
| S11 | M | 33 | 43 | Q703K |
| S12 | F | 10 | 23 | Q703K |
| S13 | F | 33 | 38 | Q703K |
| S14 | M | 15 | 45 | Q703K |
| S15 | M | 6 | 41 | Q703K |
| S16 | M | 8 | 8 | Q703K |
| S17 | F | 1 | 6 | Q703K |
| S18 | F | 1 | 8 | Q703K |
| S19 | F | 1 | 7 | Q703K |
| S20 | M | 1 | 9 | Q703K |
| S21 | F | 3 | 16 | Q703K |
| S22 | F | 4 | 7 | Q703K |
| S23 | M | 3 | 3 | Q703K |
| S24 | M | 1 | 1 | Q703K |
| S25 | M | 1 | 1 | Q703K |
| S26 | M | 2 | 4 | Q703K-M406I |
| S27 | F | 29 | 37 | Q703K |
| S28 | F | 14 | 16 | Q703K |
| S29 | M | 3 | 11 | Q703K |
| S30 | M | 1 | 1 | Q703K |
| S31 | M | 21 | 23 | Q703K |
| S32 | F | 1 | 1 | Q703K |
| S33 | M | 5 | 6 | Q703K/V726A (<i>MEFV</i>) |
| S34 | M | 1 | 5 | Q703K |
| S35 | F | 1 | 2 | Q703K |
| S36 | F | 1 | 1 | Q703K-D303N + V198M |

control frequency estimate has been compared with data from the 1000 Genomes project, HapMap, and Exome Aggregation Consortium (ExAC), available in dbSNP (www.ncbi.nlm.nih.gov/SNP) or Ensembl (www.ensembl.org), finding that the “A” allele, associated with the variant protein, was reported with frequencies of 5.1%, 5.8%, and 4.1%, respectively. The former 2 frequencies were referred to the European/white population while the latter one was calculated on the basis of a mixed group of 60,706 subjects.

Longterm followup. Complete clinical data at the moment of the molecular analysis and at the last followup were available in 36 patients (20 children and 16 adults). The remaining 21 patients were lost to followup by the centers (Supplementary Figure 1, available online at jrheum.org).

The main clinical findings observed in the 36 patients carrying the p.Q703K (p. Q705K, c. 2107C>A) substitution

of *NLRP3* with complete followup data are reported in Table 1 and Table 2. The mean age at presentation was 3 years (range 1–14) for pediatric patients and 26.3 years (range 4–57) for adult patients. The mean followup after molecular analysis was 2.5 years (range 0.6–8).

Additional *NLRP3* mutations were found in 2 patients: M406I in S26 and D303N + V198M in S36 (Table 1). Additional genes were searched in 18 patients. One patient displayed a monoallelic V726A variant of the *MEFV* gene (Table 1). The 2 patients who carried additional *NLRP3* mutations displayed a clinical phenotype consistent with a diagnosis of CAPS: patient S36 displayed a clinical phenotype characterized by recurrent episodes of fever, urticarial rash, arthralgia, and arthritis associated to elevation of acute-phase reactants consistent with an MWS phenotype, and patient S26, already described¹², displayed a clear CINCA/NOMID phenotype characterized by persistent inflammation, headache, hearing loss, mental retardation, and typical facies (frontal bossing and midface hypoplasia) without a typical urticarial rash (Table 2).

At the last followup, 13 out of the 34 remaining patients (39%) were classified by the centers with a diagnosis different from the original suspicion of CAPS. Three patients (S20, S28, and S30) displayed a clinical phenotype characterized by intermittent fever, arthralgia/arthritis, and recurrent urticarial skin that, according to the physicians in charge, was consistent with a systemic-onset juvenile idiopathic arthritis (SoJIA). Five patients (S5, S14, S18, S19, and S23) screened for *NLRP3* for recurrent urticarial rash, despite the lack of clear signs of systemic inflammation, were classified as idiopathic urticaria. Four patients (S9, S17, S25, and S33) displayed recurrent fever episodes or chronic inflammation without signs of urticarial rash, receiving a final classification of undifferentiated periodic fevers (Table 2). One patient (S34) was screened because of the presence of an isolated overgrowth of distal phalanges, in the absence of a clear history of persistent or recurrent inflammation and urticarial rash. A final diagnosis of primary hypertrophic osteoarthropathy was pointed out (Table 2).

According to the centers, at the last followup, 21 patients presented an inflammatory phenotype consistent with the original suspicion of a CAPS-like disorder. Nine patients presented a history of chronic or subchronic inflammation with elevation of acute-phase reactants associated with persistent or recurrent urticarial rash and muscle-skeletal manifestations. Twelve patients displayed a clear recurrent disease course with episodes of rash and arthralgia associated with systemic inflammation of variable duration alternating with periods of complete well-being. Six patients (50%) with a recurrent disease course presented 1 or 2 episodes/year only. None of the 21 patients displayed complications possibly associated to a severe CAPS such as hearing loss, mental retardation, eye manifestations (e.g., papilledema), or amyloidosis (Table 2). One patient (S24) displayed some

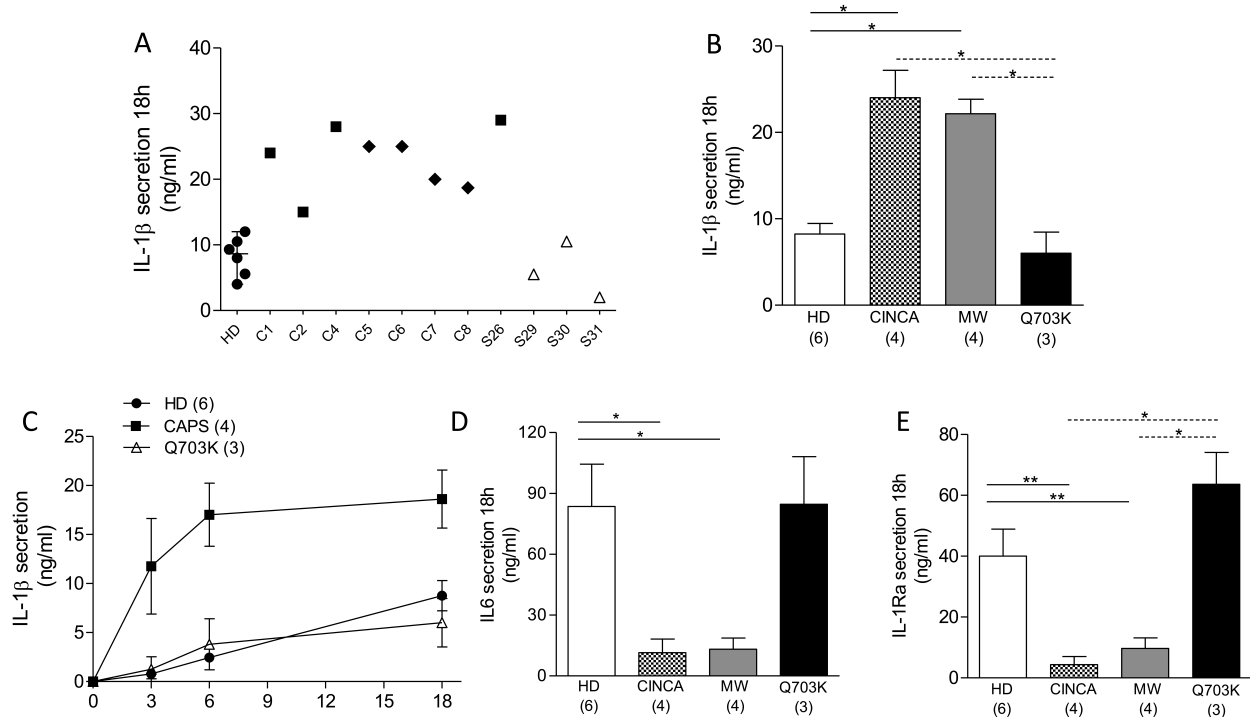


Figure 1. Pattern of cytokine secretion in patients carrying the Q703K variant compared with healthy controls and patients with CAPS. **A.** Secretion of IL-1 β after 18 h of stimulation with LPS in patients carrying the Q703K variant only (white triangles), Muckle-Wells patients (black rhombus), patients with CINCA (black squares), and age-matched healthy controls (black circles). Patient S26 displayed a CINCA phenotype and was a carrier of either the M406I or Q703K variants. **B.** Comparison of secretion of IL-1 β in the 4 subgroups of patients (* $p < 0.05$ at nonparametric test). S26 is included in the CINCA group. **C.** Kinetics of IL-1 β secretion in 4 patients with CAPS (C1, C2, C6, C7), 6 healthy controls (HD), and 3 patients carrying the Q703K variant only. In panels D and E, comparison of secretion of IL-6 and IL-1Ra, respectively, in the 4 subgroups of patients (* $p < 0.05$ at nonparametric tests, ** $p < 0.01$). S26 is included in the CINCA group. CAPS: cryopyrin-associated periodic syndrome; IL-1: interleukin 1; LPS: lipopolysaccharide; CINCA: chronic infantile neurologic, cutaneous, articular syndrome; IL-1Ra: IL-1 receptor antagonist; MW: Muckle-Wells.

dysmorphic features (gross face and skeletal dysplasia) and slight mental retardation, which according to the expert physician in charge, were not compatible with a CINCA/NOMID phenotype.

The therapeutic strategy used in the 21 patients carrying the Q703K *NLRP3* mutation with CAPS-like manifestations is reported in Supplementary Figure 2 (available online at jrheum.org). Steroids were used in 16/21 patients (12 on-demand vs 4 continuous treatment), often in association with nonsteroidal antiinflammatory drugs. In 7 out of 16 patients (44%), this strategy led to a complete control of clinical manifestations, especially in those patients presenting a recurrent or episodic disease course. Anti-IL-1 treatment (anakinra) was attempted in 8 patients (38%) displaying a chronic or subchronic disease course, requiring continuous steroid treatment or a frequent use of steroid on demand. A complete normalization of the clinical manifestations and laboratory variables was observed in 5/8 patients (62%, complete responders), whereas 3/8 patients experienced a partial or absent response, with withdrawal of the treatment after some weeks or months (nonresponders). None of the 5 complete responder patients was to date investigated for a

possible somatic mosaicism of *NLRP3* or for mutations in other exons of the gene. Other treatments were used with incomplete or no response, such as anti-TNF (3 patients), colchicine (2 patients), and various combinations of disease-modifying antirheumatic drugs (3 patients; Table 2; Supplementary Figure 2, available online at jrheum.org).

Pattern of IL-1 β secretion in patients with Q703K compared with healthy controls and patients with CAPS. Fresh monocytes from 3 patients carrying the p.Q703K (p.Q705K, c. 2107C>A) variant (S29, S30, S31) and 1 patient with a CINCA phenotype carrying both the p.M406I and p.Q703K variants (S26) were analyzed for the IL-1 secretion, and compared with monocytes from 7 patients with CAPS (3 CINCA and 4 MWS) carrying variants surely pathogenic and from 6 age-matched healthy controls, as reported^{11,22}.

The genotype/phenotype correlation, ongoing disease activity, and treatment at the moment of the study of the patients analyzed are reported in Supplementary Table 1 (available online at jrheum.org).

As shown in Figure 1A, the level of secretion after 18 h of stimulation with LPS in monocytes from the 3 patients carrying uniquely the p.Q703K variant (S29, S30, S31) was

Table 2. Clinical features of patients who are carriers of the Q703K variant.

| Patient | Fever | Elevation of Acute-phase Reactants | Urticarial Rash | Arthralgia/arthralgia | Visceromegaly/abdominal Pain | Conjunctivitis | Headache | Hearing Loss | Papilledema | Typical Facies | Bone Overgrowth | Mental Retardation | Treatment | Final Diagnosis |
|---------|-------|------------------------------------|-----------------|-----------------------|------------------------------|----------------|----------|--------------|-------------|----------------|-----------------|--------------------|---|---------------------------------------|
| S1 | + | + | + | +/- | -/- | - | - | - | - | - | - | - | Steroids/NSAID | Alternative diagnosis |
| S2 | + | + | + | +/- | -/- | - | - | - | - | - | - | - | Steroids/NSAID | Alternative diagnosis |
| S3 | + | + | + | +/- | -/- | - | - | - | - | - | - | - | Steroids/NSAID | Alternative diagnosis |
| S4 | + | + | + | +/- | -/- | + | + | - | - | - | - | - | Steroids/NSAID/anti-TNF/anti-IL-1 | Alternative diagnosis |
| S5 | - | - | + | -/- | -/- | + | + | - | - | - | - | - | Steroids/NSAID | Idiopathic urticaria |
| S6 | + | + | + | +/- | -/- | + | + | - | - | - | - | - | NSAID/anti-TNF/anti-IL-1 | Alternative diagnosis |
| S7 | + | + | + | +/- | -/- | + | + | - | - | - | - | - | NSAID/anti-TNF | Alternative diagnosis |
| S8 | + | + | + | +/- | -/- | + | - | - | - | - | - | - | NSAID/anti-IL-1 | Alternative diagnosis |
| S9 | + | + | - | -/- | -/- | + | + | - | - | - | - | - | NSAID/anti-IL-1 | Undifferentiated periodic fevers |
| S10 | + | + | + | +/- | -/- | + | + | - | - | - | - | - | Steroids/NSAID/colchicine | Alternative diagnosis |
| S11 | + | + | + | +/- | -/- | + | + | - | - | - | - | - | Steroids/NSAID | Alternative diagnosis |
| S12 | + | + | + | +/- | -/- | - | + | - | - | - | - | - | Steroids/NSAID | Alternative diagnosis |
| S13 | + | + | + | +/- | -/- | - | + | - | - | - | - | - | Steroids/NSAID | Alternative diagnosis |
| S14 | - | - | + | +/- | -/- | - | - | - | - | - | - | - | Steroids/NSAID | Idiopathic urticaria |
| S15 | - | + | + | +/- | -/- | - | - | - | - | - | - | - | Steroids/NSAID | Alternative diagnosis |
| S16 | - | + | + | -/- | -/- | - | - | - | - | - | - | - | Steroids/NSAID | Alternative diagnosis |
| S17 | + | + | - | -/- | +/+ | - | - | - | - | - | - | - | Steroids/colchicine | Undifferentiated periodic fevers |
| S18 | - | - | + | -/- | -/- | - | - | - | - | - | - | - | - | Idiopathic urticaria |
| S19 | - | + | + | -/- | -/- | - | - | - | - | - | - | - | Steroids/NSAID | Idiopathic urticaria |
| S20 | + | + | + | +/+ | -/- | - | - | - | - | - | - | - | Anti-IL-1 | SoJIA |
| S21 | + | + | + | +/+ | -/- | - | + | - | - | - | - | - | - | Alternative diagnosis |
| S22 | + | + | + | +/+ | -/- | - | - | - | - | - | - | - | Anti-IL-1 | Alternative diagnosis |
| S23 | - | - | + | -/- | -/- | - | - | - | - | - | - | - | - | Idiopathic urticaria |
| S24 | + | + | + | +/- | -/- | + | + | - | - | + | + | - | Steroids/NSAID | Alternative diagnosis |
| S25 | + | + | - | +/- | -/- | - | + | - | - | - | - | - | Steroids/NSAID | Undifferentiated periodic fevers |
| S26 | + | + | + | +/+ | -/- | - | + | + | - | + | + | - | Anti-IL-1 | CINCA |
| S27 | + | + | + | +/+ | -/- | + | - | - | - | - | - | - | Steroids/NSAID/CSA/MTX/AZA/MMF/anti-IL-1 | Alternative diagnosis |
| S28 | + | + | + | +/+ | -/- | - | - | - | - | - | - | - | Steroids/NSAID/CSA/MTX/Anti-TNF/anti-IL-1 | SoJIA |
| S29 | + | + | + | +/+ | -/- | + | - | - | - | - | - | - | Steroids/anti-IL-1 | Alternative diagnosis |
| S30 | + | + | + | +/+ | -/- | - | - | - | - | - | - | - | Steroids/NSAID/MTX/anti-TNF/anti-IL-1 | SoJIA |
| S31 | - | + | + | +/+ | -/- | - | - | - | - | - | - | - | Steroids/CSA/colchicine/anti-IL-1 | Alternative diagnosis |
| S32 | + | + | + | +/+ | -/- | - | - | - | - | - | - | - | Steroids/NSAID/CSA | Alternative diagnosis |
| S33 | + | + | - | -/- | +/+ | - | - | - | - | - | - | - | Steroids | Undifferentiated periodic fevers |
| S34 | - | - | - | +/- | -/- | - | - | - | - | - | + | - | Steroids/NSAID | Primary hypertrophic osteoarthropathy |
| S35 | + | + | + | +/+ | -/- | - | - | - | - | - | - | - | Steroids/NSAID/anti-IL-1 | Alternative diagnosis |
| S36 | + | + | + | +/+ | -/- | - | - | - | - | - | - | - | Anti-IL-1 | MWS |

* Absence of typical daily urticarial rash, few episodes of diffuse erythematous rash at limbs. NSAID: nonsteroidal antiinflammatory drugs; anti-TNF: antitumor necrosis factor; anti-IL-1: anti-interleukin 1; CSA: cyclosporine A; MTX: methotrexate; AZA: azathioprine; MMF: mycophenolate mofetil; SoJIA: systemic-onset juvenile idiopathic arthritis; CINCA: chronic infantile neurologic, cutaneous, articular syndrome; MWS: Muckle-Wells syndrome.

comparable to that observed in healthy controls and much less than that found in monocytes from patients with MWS and CINCA. Conversely, patient S26, carrying an additional *NLRP3* mutation and displaying a severe CINCA phenotype, displayed the typical IL-1 β over-secretion observed in patients with CAPS (Figure 1A and Figure 1B). In previous studies, we have shown that monocytes from patients with CAPS displayed peculiar kinetics of IL-1 secretion that reached the peak at 3–6 h after LPS stimulation, well before monocytes from healthy subjects²³. Conversely, patients carrying the p.Q703K variant show a kinetics comparable to those observed in healthy controls (Figure 1C). Monocytes from patients with CAPS displayed impaired secretion of second-wave cytokines such as IL-6 and IL-1 receptor antagonist (IL-1Ra) independently of disease activity and ongoing treatment²². This is due to the insurgence of oxidative stress in CAPS monocytes following Toll-like receptor stimulation. In fact, a common cell response to oxidative stress is the block of protein synthesis, which occurs in stressed CAPS monocytes with consequent impaired translation of IL-1Ra and IL-6 mRNA^{22,24}. Unlike monocytes from patients with CAPS, monocytes from patients carrying uniquely the p.Q703K variant displayed a normal secretion of these 2 cytokines (Figure 1D and Figure 1E, respectively).

DISCUSSION

In our current study, we analyze the clinical phenotype and longterm followup of a selected population of patients screened for *NLRP3* for a suspected CAPS in 2 national referral centers during the last 10 years and found to be positive for the p.Q703K (p. Q705K, c. 2107C>A) variant. Despite the evident bias of selection toward patients with a possible CAPS-like phenotype, the results of our study add further evidence to clarify the poor clinical significance of this variant. These conclusions are driven by the high proportion of patients receiving a final alternative diagnosis and by the observation of the same prevalence of the p.Q703K variant in this selected group of patients when compared with the healthy population. Moreover, most of the patients carrying this variant displayed a very mild phenotype with the absence of the most typical CAPS-associated manifestations (hearing loss, neurological involvement, bone dysplasia). In contrast with “classical” CAPS, a low percentage of patients carrying the Q703K variant required an anti-IL-1 treatment, with a relatively high proportion of poor responders. The p.Q703K variant of the *NLRP3* gene has been considered for a long time an unremarkable, likely neutral polymorphism^{4,17}. This policy was challenged by the description of a patient carrying the p.Q703K showing a clinical phenotype consistent with CAPS with a good response to anti-IL-1 treatment¹⁸. The patient’s monocytes showed an overactivation of caspase 1 and higher amounts of secreted active IL-1 β after LPS stimulation compared with 5 healthy controls¹⁸. The same group performed an *in vitro*

study transfecting the p.Q703K into the human monocytic cell line THP-1¹⁹. After stimulation, THP-1 cells transduced with *NLRP3*-Q703K secreted higher levels of IL-1 β compared with *NLRP3*-WT, but much less compared with the *NLRP3*-R260W variant, a proven pathogenic *NLRP3* variant usually associated to a mild form of CAPS (FCAS and MWS)¹⁹.

After this report¹⁹, a note added to the guidelines for the genetic diagnosis of hereditary recurrent fevers pointed out the possibility that this variant could be considered in the group of variants of uncertain significance and therefore recommended that it be reported to the clinicians¹⁷.

In our present study, the unique criterion for the analysis of the *NLRP3* gene was the request from the referral center. In more than 50% of patients, a gene other than *NLRP3* was requested for screening, likely because of the presence of an undefined phenotype. In addition to patients identified by experienced physicians, there were a number of patients directly referred by local hospitals with limited experience in autoinflammatory diseases. This explains the variability of the clinical phenotypes reported at the time of molecular analysis and also the rather relevant number of patients presenting features not consistent with a possible CAPS. The absent elevation of acute-phase reactants should be considered a criterion of exclusion for the request of the molecular analysis for *NLRP3*.

Therefore, we decided to focus the analysis of the actual clinical effect of the p.Q703K variant only on those patients for whom followup data were available. According to the evaluations by the centers, in 38% of the patients the final diagnosis at the last followup was not consistent with the original suspicion of a CAPS-like phenotype. After followup, 21 patients presented some clinical features still consistent with the original suspicion of a CAPS-like disorder, generally with a very mild clinical course. Most of the patients presented a recurrent disease course with few episodes during the year. None of these patients developed clinical features classically associated to a severe CAPS phenotype (skeletal and neurological involvement, hearing loss, papilledema). These data are in contrast with a recent report showing a possible correlation between the presence of *NLRP3* low penetrance mutations and a variable spectrum of neurological manifestations²⁵. Moreover, in contrast with the common finding usually observed in FCAS and MWS, no clear positive family history was observed. These findings reduce the actual pathogenic significance of the p.Q703K *NLRP3* variant and can be explained by the relatively low specificity of the clinical manifestations presented by the patients at the moment of molecular analysis, essentially consisting of systemic inflammation associated with some urticarial rash of variable duration and severity. It should be noted that the good response to anti-IL-1 treatment is not strictly limited to CAPS but is also observed in a number of other genetic and multifactorial disorders, including patients with a CAPS-like

phenotype but negative for mutations of the *NLRP3* gene^{26,27}. On the other hand, we cannot exclude, in the few patients with a more severe CAPS-like phenotype presenting a complete response to anti-IL-1 blockade, the presence of either *NLRP3* germ line mutations in unscreened gene regions or *NLRP3* somatic mutations, these latter having been detected in CAPS and recognized as causative of even severe disease forms⁸.

The limited pathogenic effect of the p.Q703K variant is further supported by our experimental approach on patients' primary cells. In the last few years, we characterized in detail the peculiar pattern of activation and secretion of IL-1 β in patients with CAPS carrying variants of certain pathogenic effect with different degrees of disease severity^{11,22,23}. Because of a severe alteration in the redox balance, NLRP3-mutated monocytes display a fast activation and secretion of IL-1 β , followed by an exhaustion of their capacity to further produce other proteins and cytokines, such as IL-6 and IL-1Ra^{22,23}. This immunological phenotype is specific for CAPS and is not observed in inflammatory conditions, such as SoJIA²³ and FMF²⁴. In our study, we were not able to replicate the results obtained by Verma, *et al* in a single patient and in their *in vitro* study^{18,19}. Independently from their disease activity and final diagnosis, the pattern of cytokine secretion of patients carrying the p.Q703K variant was similar to those observed in healthy individuals and not comparable to those of patients with CAPS carrying sure pathogenic variants. This observation is in line with a recent study by Rieber, *et al* showing a clear difference in the production of IL-1 β , IL-18, and caspase 1 in patients with low penetrance mutations compared to patients carrying pathogenic variants²⁸.

The relatively small number of patients and healthy controls analyzed in our present study does not allow us to definitively establish or refute a causal relationship from the genetic point of view. Nonetheless, because similar Q703K variant frequencies (range 4.1–5.8%) were reported in a very large number of subjects in the 1000 Genomes project, HapMap, and ExAC, we can reasonably conclude that there is no difference between patients and healthy controls regarding the presence of the variant “A” allele. In any case, the evidence we report suggests that the *NLRP3* p.Q703K variant should be considered a polymorphism without an evident functional and clinical effect. This is new evidence against the need to report this variant after genetic analysis, as originally suggested by the guidelines proposed by Shinar, *et al*¹⁷.

ONLINE SUPPLEMENT

Supplementary data for this article are available online at jrheum.org.

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Correction

Clinical Characteristics of Patients Carrying the Q703K Variant of the *NLRP3* Gene: A 10-year Multicentric National Study

Aldo Naselli, Federica Penco, Luca Cantarini, Antonella Insalaco, Mariolina Alessio, Alberto Tommasini, Cristina Maggio, Laura Obici, Romina Gallizzi, Marco Cimmino, Sara Signa, Orso Maria Lucherini, Sonia Carta, Francesco Caroli, Alberto Martini, Anna Rubartelli, Isabella Ceccherini, and Marco Gattorno

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The correct last name of the coauthor R. Gallizi is Romina Gallizzi.

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