Expanding spectrum of neurologic manifestations in patients with *NLRP3* low-penetrance mutations

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ABSTRACT

Objective: To evaluate the frequency of the cryoporin/*NLRP3* low-penetrance mutations V198M and Q703K in patients who reported at least 2 symptoms compatible with cryopyrin-associated periodic syndromes (CAPS) and to characterize the phenotype in mutation-positive patients.

Methods: The frequency of the V198M and Q703K mutations was investigated in a selected cohort of 108 patients from our neuroimmunology department. We describe the clinical, neurologic, immunologic, and neuroradiologic features of the mutation carriers.

Results: Seventeen patients (16%) tested positive for either of the 2 mutations (V198M: n = 2; Q703K: n = 15). Eleven patients (65%) had severe headache syndromes. Six of these 11 patients were diagnosed with migraine. Nine patients (53%) had a concomitant diagnosis of multiple sclerosis (MS). In 3 patients, we identified additional family members with the respective mutation as well as the diagnosis of MS. Severe recurrent cranial nerve (CN) affection was the hallmark feature in 7 of the 8 (88%) non-MS mutation carriers. Brain MRI showed abnormalities in all but 2 patients (88%) and detected CN inflammation in 4 patients. Interleukin-6 was elevated in the CSF of 2 patients in the non-MS cohort during acute CAPS episodes with severe CNS inflammation. 5 of 9 treated patients (56%) responded to anti–interleukin-1 therapy.

Conclusion: CAPS constitute rare but treatable and commonly misdiagnosed autoinflammatory syndromes. Our data expand the spectrum of CAPS-associated neurologic manifestations. They also broaden our concept of autoimmunity and autoinflammation by linking CAPS and MS. ***Neurol Neuroimmunol Neuroinflamm* 2015;2:e109; doi: 10.1212/NXI.0000000000000109**

GLOSSARY

CAPS = cryopyrin-associated periodic syndromes; **CINCA** = chronic infantile neurological, cutaneous, and articular syndrome; **CN** = cranial nerve; **CRION** = chronic relapsing inflammation of the optic nerve; **DMT** = disease-modifying therapies; **EDSS** = Expanded Disability Status Scale; **FCAS** = familial cold-induced autoinflammatory syndrome; **GC** = glucocorticosteroid; **IL** = interleukin; **MS** = multiple sclerosis; **MTX** = methotrexate; **MWS** = Muckle-Wells syndrome; **OCB** = oligoclonal bands; **SAA** = serum amyloid A; **TNF-α** = tumor necrosis factor α; **TRAPS** = tumor necrosis factor receptor 1-associated periodic syndrome.

Cryopyrin-associated periodic syndromes (CAPS) are a group of hereditary systemic autoinflammatory diseases[1] encompassing 3 overlapping entities, benign familial cold-induced autoinflammatory syndrome (FCAS),[2] Muckle-Wells syndrome (MWS),[3] and chronic infantile neurological, cutaneous, and articular syndrome (CINCA),[4] which is the most severe variant. They are all due to gain-of-function mutations in exons 3, 4, or 6 of the *NLRP3* gene (formerly named *CIAS1*),[5–7] which encodes the protein cryopyrin, a component of the NLRP3 inflammasome. Its constitutive activation leads to an overproduction of the proinflammatory cytokine interleukin (IL)-1β and thus to inflammation.[8] The clinical picture is characterized by recurrent episodes of fever, abdominal pain, myalgia, arthralgia, and cutaneous inflammation as well as

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ocular and CNS involvement usually beginning in early childhood.[9] CNS manifestations are one of the prominent clinical features in children with CINCA, but they have been described only rarely in patients with FCAS and MWS. Recurrent chronic aseptic meningitis, increased intracranial pressure, headache, papilledema, and seizures as well as vision and hearing loss have been reported.[10]

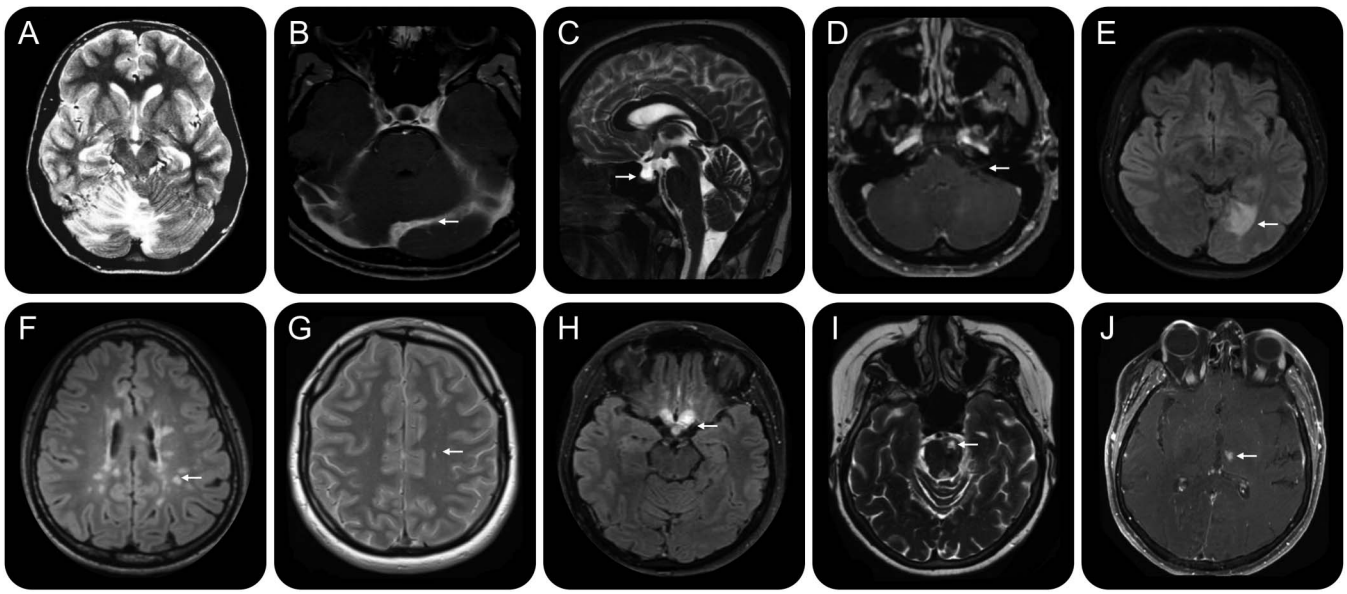
The clinical significance of the V198M and Q703K variants is still under debate. In one study, human monocytic cells transduced with the Q703K variant expressed increased IL-1β and IL-18 levels compared with wild-type cells, demonstrating an overactive inflammasome.[11] V198M and Q703K mutation carriers usually have a milder phenotype with heterogeneous symptoms such as fatigue and malaise, late disease onset, and lack of CNS manifestations.[12,13]

Prompted by the severe neurologic manifestations in 1 patient (patient 1, index case) carrying the cryopyrin/*NLRP3* Q703K (+/−) variant, we aimed to investigate the frequency of the V198M and Q703K mutations encoded by exon 3 of the *NLRP3* gene in a larger cohort and to characterize the clinical phenotype in mutation-positive patients.

METHODS

Index patient. In 2011, a 26-year-old Caucasian man was referred to our neuroimmunologic outpatient clinic for evaluation of presumed neurosarcoidosis (patient 1 in table 1). Symptoms started in 1999 at the age of 13 with aseptic meningitis and cerebellitis. Cerebral MRI showed signs of cerebellar and tentorial inflammation with granulomatosis-like enhancement of the meninges and intracerebral edema (figure 1A). His condition improved after polypragmatic therapy with antibiotics, antiviral drugs, and glucocorticosteroids (GCs). In 2000, he experienced sensorineural hearing loss and tinnitus with dizziness followed by short recurrent episodes of cranial nerve (CN) affection (V, VI, and XII) and accompanied by neck stiffness and myalgias. In 2005, he developed papilledema and vision loss with increased intracranial pressure up to 29 mm Hg. A diagnosis of idiopathic

Figure 1 Neuroradiologic characteristics in patients with CAPS



Brain MRI scans of the index patient are shown in A–E. MRI scans during adolescence, in 1999, demonstrated signs of pronounced cerebellar as well as tentorial inflammation with granulomatosis-like enhancement and intracerebral swelling; an axial T2-weighted image in 1999 demonstrates pronounced edema with signal hyperintensities in the right cerebellar hemisphere, vermis, and, to a lesser extent, the left cerebellar hemisphere (A). In April 2011, marked tentorial thickening with pronounced enhancement is shown in an axial T1-weighted, fat-saturated image after the IV administration of a gadolinium-based contrast medium (B); a sagittal T2-weighted image at the same time point demonstrates signs of chronic intracranial hypertension with enlarged nerve sheaths around the optic nerves (not shown) and empty sella (arrow; C); an axial T1-weighted, fat-saturated image after the IV administration of a gadolinium-based contrast medium at this time shows mild cranial nerve inflammation with slightly increased enhancement of the vestibulocochlear nerve (D). In December 2011, a new meningoencephalitic lesion was identified on MRI during therapy with anakinra (axial fluid-attenuated inversion recovery [FLAIR] image; E). The lower panel demonstrates the neuroradiologic spectrum in other patients from our cryopyrin-associated periodic syndromes (CAPS) cohort. (F) Axial FLAIR image (year 2013) in patient 14 with a diagnosis of multiple sclerosis (MS) demonstrating multiple typical hyperintense white matter lesions in the periventricular region and corona radiata. (G) In contrast, axial T2-weighted image (year 2013) in patient 3 with migraine shows unspecific white matter lesions without evidence for dissemination in time and space, thus not fulfilling the diagnostic criteria for MS. (H) Axial FLAIR sequence (year 2014) illustrates a pronounced swelling of the chiasma and pituitary stalk in patient 5. (I) T2-weighted axial sequence (year 2014) shows a hyperintense lesion in the left anterior pontomesencephalic junction. (J) Axial T1-weighted, fat-saturated image (year 2014) after the IV administration of a gadolinium-based contrast medium demonstrates an enhancing lesion in the left anterior thalamus (same patient as panel I).

Table 1 Clinical characteristics of the 17 *NLRP3* mutation-positive patients

Patient no./ age, y/sex/ origin	Age at onset of CAPS, y	Prior diagnosis	Systemic CAPS symptoms	Neurologic manifestation of CAPS	MS	MRI	Elevation of inflammatory markers	CSF	Therapy for CAPS	Mutation	Family history
1/26/M/C	13	Neurosarcoidosis, encephalitis of unknown origin	Arthralgias/arthritis, myalgias	Recurrent aseptic meningoencephalitis; headache; CN affection: VIII with bilateral SNLH and tinnitus, VI, and V; papillitis and papilledema	No	Leptomeningeal enhancement, recurrent meningoencephalitic lesions, intermittent CN enhancement, extended nerve sheaths around the optic nerves and empty sella	SAA, IgM	Mild pleocytosis, mild elevation of intracranial pressure, IL-6 elevation, OCB	GCs, anakinra, canakinumab	Q703K (+/−)	Mother Q703K (+/−), no relevant symptoms
2/53/F/C	30	Fibromyalgia	Arthralgias, myalgias, fatigue, abdominal pain	Migraine; CN affection: VIII with unilateral SNLH	No	Unspecific WML	Normal	Normal	Anakinra discontinued, NSAD	Q703K (+/−)	Unremarkable
3/40/F/C	28	Fibromyalgia	Arthralgias, tendinitis, fatigue, conjunctivitis, urticarial rash	Migraine	No	Unspecific WML	Leukocytosis	Normal	Anakinra, NSAD	Q703K (+/−)	Unremarkable
4/52/F/C	50	Somatoform disorder, generalized pain syndrome, neurosarcoidosis	Multiple exostosis, recurrent maculopapular rash, severe arthralgias, myalgias, severe chronic pain syndrome	CN affection: VII and VI palsy	No	Cranial and spinal MRI normal	SAA	Mild pleocytosis, OCB negative	Azathioprine, anakinra discontinued, GCs, NSAD	Q703K (+/−)	Son exostosis Q703K (−/−)
5/44/F/C	42	Neurosarcoidosis, CRION	Tendinitis	Headache; CN affection: severe bilateral optic nerve inflammation with vision loss of one eye; asymptomatic CN III inflammation	No	Bilateral optic nerve inflammation with Gd enhancement and optic nerve atrophy, inflammation of pituitary gland and CN III	SAA	OCB, mild pleocytosis, IL-6 elevation	Azathioprine, anakinra, and canakinumab discontinued; methotrexate and GCs	Q703K (+/+)	Mother with MS Q703K (+/−)
6/45/F/C	41	Somatoform disorder	Arthralgias, myalgias, fever flares, intermittent rash, severe fatigue	CN affection: VIII with unilateral SNLH and V with hypesthesias and pain	No	Cranial and spinal MRI normal	SAA	Normal	Anakinra discontinued, canakinumab, NSAD	Q703K (+/−) R92Q (+/−)	Mother with arthralgias Q703K (−/−)
7/49/F/C	23	CRION	None	CN affection: severe bilateral optic nerve inflammation with vision impairment; episode with aseptic meningitis	No	Bilateral atrophy of optic nerves	Normal	Normal	None	Q703K (+/−)	Unremarkable
8/63/F/C	NK	Cerebral vasculitis with recurrent strokes	Arthalgias, myalgias, urticarial rash	CN affection: IV palsy and VIII with unilateral SNLH; cerebral vasculitis with strokelike episodes; myelitis	No	Right pontine and thalamic vasculitic infarction, optic nerve inflammation with Gd enhancement, myelitis and spinal leptomeningeal enhancement	SAA, CRP	OCB, mild pleocytosis	GCs, anakinra	Q703K (+/−)	Unremarkable
9/62/F/C	29	Fibromyalgia	Arthralgias, myalgias, uveitis, abdominal pain, aphthous ulcera	Migraine	Yes	MS pathology, subcortical atrophy	SAA, IgM	OCB, mild pleocytosis	Anakinra, GCs, NSAD	V198M (+/−)	Unremarkable
10/42/M/C	39	NA	Recurrent urticarial rash, bursitis, tendinitis	None	Yes	MS pathology	SAA, CRP	OCB, mild pleocytosis	None	V198M (+/−)	Daughter with recurrent urticarial rash
11/49/F/C	36	Fibromyalgia	Recurrent urticarial rash, arthralgias, myalgias, conjunctivitis	None	Yes	MS pathology	None	OCB, mild pleocytosis	Intermittent low-dose GCs and NSAD	Q703K (+/−)	Unremarkable
12/35/F/C	33	NA	Arthralgias, uveitis	CN affection: VIII with tinnitus and hypacusis	Yes	MS pathology	SAA	OCB, normal cell count	NSAD	Q703K (+/−)	Aunt, grandmother with arthralgias; mother with MS; all Q703K (+/−)

Continued

Table 1 Continued

Patient no./ age, y/sex/ origin	Age at onset of CAPS, y	Prior diagnosis	Systemic CAPS symptoms	Neurologic manifestation of CAPS	MS	MRI	Elevation of inflammatory markers	CSF	Therapy for CAPS	Mutation	Family history
13/34/M/C	16	NA	Fever, arthralgias, myalgias	Migraine with aura	Yes	MS pathology	None	OCB, mild pleocytosis	None	Q703K (+/−)	Mother with MS Q703K (+/−)
14/21/M/C	13	NA	Adolescent arthritis, uveitis	Tension-type headache, tinnitus left ear	Yes	MS pathology	None	OCB, mild pleocytosis	NSAD, GCs	Q703K (+/−)	Mother: arthralgias, fever; brother: deafness Q703K (−/−)
15/50/F/C	43	Somatoform disorder	Recurrent maculopapular rash, arthralgias, recurrent lymphadenitis, abdominal pain and diarrhea, fever flares, aphthous ulcera	CN affection: V with neuralgia, VIII with unilateral SNLH; tension-type headache	Yes	WML	Leukocytosis	Intrathecal antibodies against measles, rubella, and zoster; mild pleocytosis	Anakinra, NSAD	Q703K (+/−)	Son: Crohn disease Q703K (−/−)
16/48/F/C	29	NA	Arthralgias, uveitis	Migraine with aura	Yes	MS pathology	SAA, IgM	OCB, mild pleocytosis	None	Q703K (+/−)	Daughter with MS
17/39/F/C	NK	NA	Raynaud phenomenon	Migraine	Yes	MS pathology	Leukocytosis	OCB, mild pleocytosis	None	Q703K (+/−)	Unremarkable

Abbreviations: (+/−) = heterozygous; (+/+) = homozygous; C = Caucasian; CAPS = cryopyrin-associated periodic syndromes; CN = cranial nerve; CRION = chronic relapsing inflammation of the optic nerve; CRP = C-reactive protein; IL = interleukin; GC = glucocorticosteroid; Gd = gadolinium; MS = multiple sclerosis; NA = not applicable; NK = not known; NSAD = nonsteroidal antiphlogistic drug; OCB = oligoclonal bands; SAA = serum amyloid A; SNLH = sensorineural hearing loss; WML = white matter lesions.

intracranial hypertension syndrome was made, and therapy with acetazolamide was initiated. All symptoms and signs responded promptly to repeated short courses of IV or oral GC therapy.

On neurologic examination in March 2011, he had a mild CN XII palsy on the right side. Generalized myoclonic jerks were also noted. Laboratory examinations revealed increased levels of IgM but otherwise no evidence of acute infection, rheumatologic disease, or sarcoidosis. CSF analysis showed a normal cell count (5 cells/μL, 4% neutrophils), mildly elevated protein (58 mg/dL), and 2 CSF-specific oligoclonal bands (OCB), with an increased opening pressure up to 36.7 mm Hg. Audiometry revealed sensorineural hearing loss in the right ear.

The clinical phenotype pointed to an autoinflammatory condition. Genetic testing revealed a heterozygous Q703K substitution encoded by exon 3 of the *NLRP3* gene; no other mutation was detected in the *NLRP3*, *TNFRSF1A*, and *MEFV* genes. His mother reported intermittent back pain and also tested positive for the mutation.

Repeat cerebral MRI demonstrated several features of chronic CNS inflammation and increased intracranial pressure (figure 1, A–E). The patient's clinical course correlated with intermittent mild elevations of serum amyloid A (SAA). Stimulation of nonclassical monocytes (CD14++CD16++) with lipopolysaccharides[14] (for methods see appendix e-1 at Neurology.org/nn) showed increased IL-1β secretion, which was abolished after therapy with IL-1 blockers (see figure e-1).

Therapy with the IL-1 receptor antagonist anakinra was started in August 2011. He was subsequently switched to the IL-1 antibody canakinumab after worsening of the CNS inflammation with a new-onset meningoencephalitic lesion in December 2011 (figure 1E), resulting in an improvement of the CAPS-associated symptoms.

Investigated cohort. Patients consecutively seen at our neuroimmunologic outpatient clinic between 2011 and 2013 were screened for the V198M and Q703K mutations (for methods see appendix e-1) if they reported episodes with at least 2 symptoms compatible with CAPS, such as myalgias, arthralgias/arthritis, fever episodes, migraine, rash, ocular inflammation, hearing loss, papilledema, or aseptic meningitis. A detailed medical history and clinical data, including cerebral MRI, audiogram, and blood and CSF samples, were collected from all mutation-positive patients. Classic rheumatologic diseases were excluded by consultation with the rheumatology unit. In patients with multiple sclerosis (MS), the Expanded Disability Status Scale (EDSS) and the MS Severity Scale[15] scores were also determined. Family members were invited for interview and gene analysis. Mutation carriers were screened for additional mutations in exons 4 and 6 of the *NLRP3* gene as well as in exons 2, 3, and 10 of the *MEFV* gene and exons 2, 3, 4, and 6 of the *TNFRSF1A* gene. In a subset of mutation-positive patients, serum and CSF cytokine concentrations of IL-1β, tumor necrosis factor α (TNF-α), IL-17a, and IL-6 were measured (for methods see appendix e-1). All patients were followed for at least 1 year. All mutation-positive individuals were seen on a regular basis every 3–6 months. Therapy and treatment responses were assessed using a standardized questionnaire.

Standard protocol approvals, registrations, and patient consents. All participants gave their written informed consent, and the study was approved by the local ethics committee (project 159-03).

RESULTS **Genotypes of the analyzed patients.** A total of 108 patients (87 women, 21 men; mean age 41 ± 12 years; 53 patients with the diagnosis of MS[16]) were investigated for mutations in the *NLRP3* gene. In 17 patients (16%) (13 women, 4 men; mean age 44 ± 11 years, range 28–64 years; 9 with the diagnosis of MS), an alteration in exon 3 of the *NLRP3* gene (V198M: n = 2; Q703K: n = 15) was identified. One woman (patient 5) was homozygous for the Q703K substitution and another woman (patient 6) was additionally found to carry a R92Q low-penetrance mutation encoded by exon 4 of the *TNFRSF1A* gene (table 1).

Phenotype of patients with cryopyrin/*NLRP3* V198M or Q703K mutations. ***Systemic CAPS symptoms.*** The patients' clinical characteristics are summarized in table 1. Median age at onset of CAPS symptoms was 31 years (±11 years). Five patients (patients 2, 3, 4, 9, and 11; 29%) had a chronic course with daily symptoms, whereas 12 patients (71%) had an episodic course with recurrent attacks. Systemic CAPS symptoms mainly consisted of arthralgias, myalgias, recurrent uveitis/conjunctivitis, urticarial or maculopapular

Table 2 Clinical characteristics of 9 MS patients with a cryopyrin/*NLRP3* mutation

Patient no./age, y/sex	Disease course	Age at clinical onset of MS, y	EDSS	MSSS	Current DMT	Treatment of CAPS-associated symptoms
9/62/F	SP-MS	50	6	5.23	None	Anakinra, GCs, NSAD
10/42/M	RR-MS	40	2.5	2.91	Interferon β-1	None
11/49/F	RR-MS	43	4	3.37	Fingolimod	Intermittent low-dose GCs and NSAD
12/35/M	RR-MS	34	3	1.5	Azathioprine	NSAD
13/34/F	RR-MS	33	4.0	8.14	Glatiramer acetate	None
14/21/M	RR-MS	18	5.5	3.79	Natalizumab	NSAD, GCs
15/50/F	SP-MS	43	6	7.41	Intermittent high-dose GCs	Anakinra, NSAD
16/48/F	RR-MS	29	1	1.0	None	None
17/39/F	RR-MS	36	2	5.41	Dimethylfumarate	None

Abbreviations: CAPS = cryopyrin-associated periodic syndromes; DMT = disease-modifying therapies; EDSS = Expanded Disability Status Scale; GCs = glucocorticosteroid; MS = multiple sclerosis; MSSS = MS Severity Scale; NSAD = nonsteroidal antiphlogistic drugs; RR-MS = relapsing-remitting MS; SP-MS = secondary progressive MS.

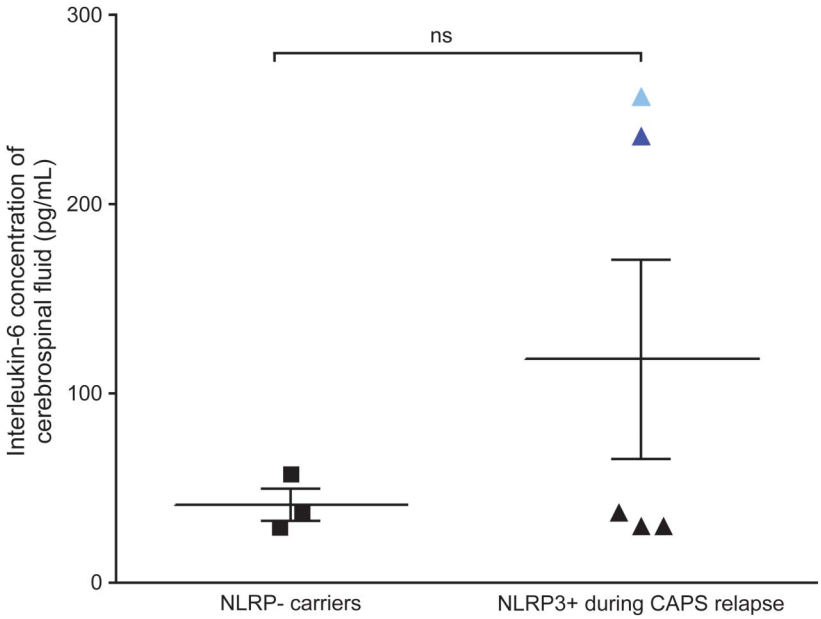
Table 3 Clinical characteristics of mutation-positive patients compared with mutation-negative patients

Clinical characteristics	*NLRP3*+ (n = 17)	*NLRP3*− (n = 91)	Significance, uncorrected *p* value	Significance, corrected *p* value
CN affection (n = 108)	10/17 (59%);	12/91 (13%);	0.00014	0.00111
	7/8 non-MS patients (88%)	10/47 non-MS patients (21%)	0.00062	0.00501
Headache (n = 108)	11/17 (65%)	29/91 (32%)	0.01400	0.11199
Uveitis/conjunctivitis (n = 108)	6/17 (35%)	10/91 (11%)	0.01907	0.15255
Urticarial rash (n = 108)	7/17 (41%)	10/91 (11%)	0.00531	0.04248
Arthralgias (n = 108)	13/17 (76%)	18/91 (20%)	0.00001	0.00009
Myalgias (n = 108)	8/17 (47%)	3/91 (3%)	0.00001	0.00007
MS (n = 108)	9/17 (53%)	44/91 (48%)	0.79541	6.36327

Abbreviations: CN = cranial nerve; MS = multiple sclerosis.
p Value (uncorrected and corrected with Bonferroni) by Fisher exact test; *NLRP*+ (mutation-positive patients) vs *NLRP*− (mutation-negative patients). Logistic regression analysis with stepwise optimization of factors revealed myalgia ($\beta_1 = 2.57$), arthralgia ($\beta_2 = 2.22$), and CN affection ($\beta_3 = 2.50$) as predictive parameters for an *NLRP3* mutation that correctly classified 93% of patients in this training dataset ($\beta_0 = -3.97$, $p < 0.01$ for all coefficients): $P = 1/1+e^{-(\beta_0+\beta_1 \text{ myalgia}+\beta_2 \text{ arthralgia}+\beta_3 \text{ CN affection})}$.

rash, aphthous ulcera, and gastrointestinal symptoms as well as constitutional symptoms such as severe fatigue and malaise. Mild fever episodes up to 38°C were observed in only 3 patients (patients 6, 13, and 15). Thirteen patients (76%) showed a recurrent elevation of inflammatory markers, including SAA in 9 patients (53%) and C-reactive protein in 4 patients (24%). SAA correlated with clinical disease activity in 5 patients (patients 1, 5, 6, 8, and 12).

Figure 2 Interleukin-6 concentrations in the CSF



In 2 mutation carriers (patient 1 in dark blue triangle and patient 5 in light blue triangle carrying the Q703K variant in heterozygous [+/−] and homozygous [+/+] form), interleukin (IL)-6 levels in the CSF were elevated during an acute exacerbation of cryopyrin-associated periodic syndromes (CAPS)-related CNS symptoms, whereas other mutation carriers (represented by black triangles) lacked increased IL-6 levels in CSF during acute CAPS exacerbation compared to controls without mutations in the *NLRP3* gene (black squares; n = 3). *p* Value did not differ significantly (ns = not significant) as assessed by Mann-Whitney *U* test.

Neurologic symptoms. Nine of the 17 mutation-positive patients (53%) fulfilled the revised diagnostic criteria for MS.[16] The mean EDSS, disease duration, and age at onset of MS did not differ between MS patients with mutation and MS patients without mutation. Clinical characteristics of patients with MS are summarized in table 2. Onset of CAPS-associated symptoms occurred 3.2 years (mean ± 3.4) prior to the diagnosis of MS.

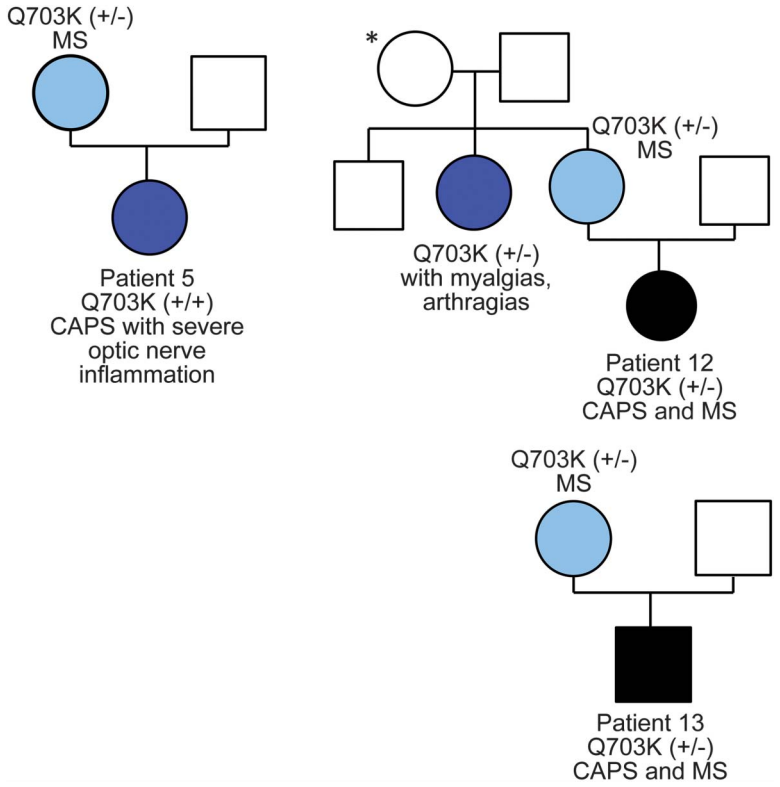
Eleven patients (65%) had severe headache syndromes. Six of them were diagnosed with migraine. Seven of 8 mutation carriers without a diagnosis of MS (88%) had severe recurrent episodes of CN involvement of the identical CN(s), including, II, III, IV, V, VI, VII, and VIII. Two of them (patients 5 and 7) showed recurrent severe inflammation of the optic nerve with consecutive optic nerve atrophy and had the prior diagnosis of chronic relapsing inflammation of the optic nerve (CRION).

Two patients had a history of aseptic meningitis (patients 1 and 7). Recurrent stroke due to small vessel vasculitis was observed in patient 8. Five patients showed sensorineural hearing loss on audiometry, and 2 patients reported tinnitus.

Clinical features such as arthralgias, myalgias, headache syndromes, urticarial rash, uveitis/conjunctivitis, and CN affection as well as the frequency of MS were compared between mutation carriers and nonmutation carriers and are shown in table 3. A logistic regression model[17] identified myalgia, arthralgia, and CN affection as relevant predictors in this dataset (for methods see appendix e-1).

Neuroimaging. Cerebral MRI before and after IV administration of gadolinium-based contrast agents was available for analysis in all mutation-positive patients. All but 2 patients (patients 4 and 6) showed abnormalities on cerebral MRI (figure 1, A–J).

Figure 3 Pedigrees of 3 multiplex families with *NLRP3* mutations and MS



The patient numbers refer to the patients listed in table 1. The respective cryopyrin/*NLRP3* mutations are indicated. Patients with clinically proven cryopyrin-associated periodic syndromes (CAPS) and multiple sclerosis (MS) are represented by filled black symbols; patients with only CAPS are shown by dark blue symbols; patients with MS without CAPS symptoms are represented by light blue symbols. * = positive history for symptoms compatible with CAPS; +/+ = homozygous, +/− = heterozygous.

MRI characteristics of the index patient are given in the case description and are shown in figure 1, A–E. Patients with the diagnosis of MS demonstrated typical multiple white matter lesions on cerebral MRI. Eight of them fulfilled the MRI criteria according to Barkhof et al.[18] (figure 1F, patient 14). In 4 patients, CN inflammation could be demonstrated on MRI. Detailed neuroradiologic analyses are presented in figure 1.

CSF findings and immunologic parameters. OCB were present in 11 patients. Eight of them were diagnosed with MS. In 5 non-MS patients (patients 1, 4, 5, 6, and 8), the CSF was investigated during acute CAPS episodes. Four of them (patients 1, 4, 5, and 8) had a mild pleocytosis as well as an elevated protein concentration up to 104 mg/dL. In 4 patients, intracranial pressure was measured during lumbar puncture; it was elevated in only the index patient. IL-6 was elevated in the CSF in patients 1 and 5 during acute severe attacks of CAPS-related CNS inflammation, but all other tested cytokine concentrations (including IL-1β, TNF-α, and IL-17a) were unremarkable in serum and CSF (figure 2).

Treatment. ***Patients with MS.*** Most patients with MS were treated with disease-modifying therapies (DMT) and therefore did not receive additional anti–IL-1 therapy for CAPS-related symptoms. Two patients with MS (patients 9 and 15) without DMT were treated with the IL-1 receptor antagonist anakinra and showed improvement in systemic CAPS symptoms and headache. So far, their MS has remained stable (clinically and radiologically) during anti–IL-1 therapy for ≥1 year. Five of 9 patients with MS occasionally received oral GC therapy and/or nonsteroidal antiphlogistic drugs for CAPS-associated symptoms and responded partially.

Non-MS patients. CAPS-associated neurologic manifestations during acute attacks (e.g., cranial nerve palsy, aseptic meningitis, optic nerve inflammation, cerebral vasculitis) showed a favorable response to GC therapy. In 7 of 8 non-MS patients with CAPS, treatment with anakinra was started because of recurrent severe CNS inflammation and/or systemic CAPS symptoms. Five patients showed a response, but 3 of them (patients 1, 5, and 6) were switched to canakinumab because they had only a partial response. MRI changes improved during canakinumab therapy in the index patient; the treatment is currently suspended after stabilization of his disease activity. In patient 6, who carried the combined tumor necrosis factor receptor 1-associated periodic syndrome (TRAPS) and CAPS mutations, canakinumab therapy led to an improvement of systemic as well as neurologic CAPS symptoms. Patient 5 with severe bilateral optic nerve inflammation did not have a response to anakinra or canakinumab and finally stabilized with a combination of low-dose GC and methotrexate (MTX). Patients 2 and 4 discontinued anakinra therapy after several weeks due to lack of efficacy.

Family history. We were able to test additional family members of 4 patients for the respective mutations and identified 6 mutation-positive individuals. Four of them also reported symptoms compatible with CAPS. In 3 family members, the mutation cosegregated with MS (figure 3).

DISCUSSION

Previously, we reported that the autoinflammatory syndromes TRAPS and familial Mediterranean fever may be associated with MS.[19,20] The *TNFRSF1A* R92Q mutation, which is frequently observed in patients with MS, was identified as an independent MS risk factor.[21] In contrast, a clinical association between CAPS and MS has not been reported so far. Nine of our 17 mutation-positive patients had a diagnosis of MS. Possibly coincident MS and CAPS has only been described in very few cases and it has been questioned whether MS-like lesions on brain MRI represent CAPS-related CNS

manifestations or subclinical MS in CAPS.[22,23] Our patients clearly fulfilled the diagnostic criteria for MS and additionally had systemic CAPS-associated symptoms, strongly suggesting concomitant MS and CAPS. However, it can be difficult to distinguish MS and coexisting CAPS from CAPS with CNS manifestations, as both conditions show episodic exacerbations and promptly respond to GC therapy, particularly in patients with optic nerve involvement or cerebral vasculitis, as shown in 4 of our patients. CAPS in patients with MS should be considered in individuals with additional unexplained recurrent symptoms such as myalgia/arthralgias, urticarial rash, uveitis/conjunctivitis, and severe headache syndromes. Careful evaluation of the medical history and family history is also essential in order to identify such patients.

In 3 patients with mutation-proven CAPS (2 with MS), additional family members carrying the same mutation were diagnosed with MS, pointing to a possible cosegregation of CAPS with MS. So far, single nucleotide polymorphisms in genes of the NLRP3 inflammasome have been associated with other autoimmune disorders, such as inflammatory bowel diseases, but not with MS.[24,25] Nevertheless, increased levels of caspase-1 and IL-1β have been reported in the CSF and in cerebral lesions of patients with MS.[26,27] Furthermore, a role of the NLRP3 inflammasome complex in the development of experimental autoimmune encephalomyelitis, an animal model of MS, has been demonstrated.[28,29]

Consistent with a previous report,[10] headache syndromes were present in more than half of our mutation-positive patients. Particularly, in 2 of them, unspecific white matter lesions were observed, suggesting CNS inflammation. In our non-MS cohort, CN affection was a distinct feature. It was the hallmark of CAPS-associated neurologic manifestations, with a frequency of more than 80%. Optic nerve involvement was a prominent feature in 3 patients and had led to a prior diagnosis of CRION in 2 of them. Optic nerve inflammation and atrophy have been described in patients with mutations in the *NLRP3* gene but so far not with distinct MRI characteristics, as observed in our cohort.[10,30] Whether *NLRP3* mutations are more frequent in CRION in general is unknown and needs to be determined in larger cohorts. So far neurologic involvement in patients with low-penetrance mutations has rarely been reported and was not described in a recent study by the Eurofever registry.[13,22,23] Usually, adult patients with autoinflammatory syndromes are seen by rheumatologists. Neurologic symptoms might have been overlooked, as neurologic examinations as well as MRI and CSF analysis are not routinely performed in such settings. Conversely, neurologists are usually not familiar with CAPS, so molecular testing for *NLRP3* mutations is not performed.

Systemic CAPS symptoms were mild to moderate in our cohort, in agreement with recent observations.[12,13] However, uveitis was not observed in patients with low-penetrance mutations in the recent series of patients from the Eurofever registry.[13]

Overall, the frequency of the V198M and Q703K mutations (16%) in our selected group of patients was higher than previously reported, reflecting the estimated number of unreported cases among Europeans.[12,31] In the Caucasian population, the V198M mutation is observed with a frequency of 1% and classified as a low-penetrance mutation, whereas the Q703K variant with an allele frequency of up to 5% is usually regarded as a variant of unknown significance.[31,32] The high allele frequency together with the heterogeneous phenotype and variable penetrance suggests that these mutations may exert proinflammatory effects, leading to inflammation potentially in combination with other environmental or genetic factors.

Inflammatory markers in serum were associated with the clinical disease course in the majority of patients. In 2 patients, elevations of IL-6 in the CSF correlated with severe CNS inflammation that has not been described in patients with the Q703K mutation. The proinflammatory cytokine IL-6 is classically considered to act downstream of IL-1β.[33] Therefore, the use of IL-6 blockers could theoretically be of benefit for patients with CAPS. Despite simultaneously increased acute-phase reactants, corresponding IL-6 levels in serum remained normal, suggesting a predominantly local inflammation of the CNS. In contrast to our observation and those of others,[34] a patient with mutation-proven CAPS who presented with severe arthropathy and CNS inflammation had increased serum levels of IL-6.[6]

A number of patients with atypical CAPS symptoms benefit from anti–IL-1 blockade, whereas others do not.[35] Ongoing CNS inflammation despite anti–IL-1 therapy has been observed in patients with CINCA in long-term follow-up studies.[36] In addition, bioavailability of canakinumab and anakinra in the CNS after systemic administration may be low and thus may not reach effective levels to alleviate severe CNS inflammation.[36] In patients with MS, anti–IL-1 therapy is usually not an option since the effects of such therapies on MS and interactions with DMT are not known. In 2 patients with MS without DMT who were treated with anti–IL-1 for CAPS-associated symptoms, the MS course has remained stable so far. In our non-MS cohort, response to anti–IL-1 treatment was heterogeneous, whereas anti–IL-1 therapy clearly failed in the patient with severe bilateral optic nerve affection who was

homozygous for the Q703K substitution and finally responded to combined low-dose GC and MTX. A response to MTX was reported in a single patient with a novel *NLRP3* mutation accompanied by predominant bilateral optic nerve atrophy and elevated IL-6 concentrations in the CSF, similar to our patient 5.[34]

Our observations expand the spectrum of neurologic manifestations in patients carrying the low-penetrance cryopyrin/*NLRP3* mutations V198M and Q703K. Undoubtedly, prompt CAPS diagnosis and treatment is of great importance. However, this is a challenging task in patients who show CAPS-like symptoms but lack classic manifestations of FCAS, MWS, or CINCA and may have concomitant MS. The association of CAPS and MS indicates that the *NLRP3* gene may be another immunologically relevant gene locus linking autoimmunity with autoinflammation. In future studies we will delineate the immunologic phenotype of *NLRP3* low-penetrance mutations and their potential contribution to MS.

AUTHOR CONTRIBUTIONS

E.S.: acquisition, analysis and interpretation of data, patient care and evaluation, manuscript writing and editing. P.L.: genetic testing, reviewing and editing of manuscript. B.E.-W.: acquisition and interpretation of MRI data, reviewing and editing of manuscript. M.W.: patient care and contribution to clinical data. M.K.: statistical analysis and interpretation of data, manuscript editing. M.F.: acquisition, analysis and interpretation of experimental data. L.-A.G.: patient care and evaluation, reviewing and editing of manuscript. R.H.: supervision of patient care, reviewing and editing of manuscript. T.K.: development of study concept, study supervision, patient care and evaluation, analysis and interpretation of data, writing, reviewing and editing of manuscript. All authors discussed the results and commented on the manuscript.

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Expanding spectrum of neurologic manifestations in patients with *NLRP3* low-penetrance mutations

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