Novel evidences of atypical manifestations in cryopyrin-associated periodic syndromes

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ABSTRACT

Objective. *Cryopyrin-associated periodic syndromes (CAPS) usually start during infancy as an urticarial-like rash and a marked acute phase response, with additional manifestations appearing during its evolution. The aim of this study was to expand the clinical diversity of CAPS by the description of novel atypical features.*

Methods. *Clinical data were collected from patients' medical charts. Sanger sequencing analysed NLRP3. Response to anti-IL-1 blockade was evaluated by clinical assessments and by measurements of laboratory parameters.*

Results. *Seventeen patients from two families (A and B), carrying the p.Ala439Thr and p.Arg260Trp NLRP3 mutations respectively, were enrolled. The disease was unexpectedly atypical in all members of Family A, with a 16-year-old asymptomatic carrier, and onset in adulthood associated with absence of skin lesions in four affected members. Surprisingly, one patient from each family suffered from severe haemorrhagic cystitis due to AA amyloidosis in the urinary bladder. Members of Family B displayed a classical phenotype, with two patients suffering from olfactive disorders.*

Conclusion. *Our evidence suggests that CAPS may occasionally be presented as a late-onset, recurrent inflammatory disease without urticarial-like rash. In some patients, AA amyloidosis in strange locations like urinary bladder may complicate the clinical course. The response to IL-1 blockade in these atypical CAPS was similar to that described in classical forms. Consequently, we suggest that CAPS should be included in the differential diagnosis of adult patients with unexplained, recurrent inflammatory diseases, and once confirmed, the early initiation of anti-IL-1 blockade will probably prevent the development of life-threatening complications.*

Introduction

Cryopyrin-associated periodic syndromes (CAPS) are dominantly inherited autoinflammatory diseases resulting from *gain-of-function NLRP3* mutations, which generate an overproduction of interleukin-1β (IL-1β). Three different phenotypes are included among CAPS: familial cold autoinflammatory syndrome, Muckle-Wells syndrome (MWS), and chronic infantile neurologic, cutaneous and articular syndrome (1). These phenotypes share some features, including an onset in infancy, and an urticarial-like and a marked acute phase response as the main symptoms at the disease onset. By contrast, as the disease progresses, additional manifestations may appear (*i.e.* deforming arthropathy, AA amyloidosis, deafness), which seem to be restricted to the severest phenotypes (1).

In recent years, atypical manifestations in patients carrying *NLRP3* mutations have been reported (2-3). Herein we provide additional evidences of the expanded clinical diversity of CAPS by the description of novel atypical features in patients with MWS, including asymptomatic carriers, late onset of the disease, absence of cutaneous lesions at the beginning of, or during, the disease evolution, severe haemorrhagic cystitis due to AA amyloidosis and anosmia. We also describe the excellent outcome of the IL-1 blockade in these patients, and emphasise the convenience to include CAPS in the differential diagnosis of adult-onset recurrent inflammatory diseases.

Material and methods

Patients

Patients' data were collected by direct interviews and by a review of their

medical histories. After obtaining written-informed consent from patients (or patients' parents), and approval given by the medical ethics committee of the Hospital Clinic, genetic studies were performed in accordance with the Declaration of Helsinki.

Analysis of NLRP3 gene

Genomic DNA from peripheral blood was isolated using QIAmp DNA Blood Mini Kit (QIAgen, Germany). *NLRP3* gene analysis was performed using polymerase chain reaction (PCR) and bidirectionally fluorescence Sanger sequencing as previously described (4).

Results

Family A

The propositus (Patient A1 in Fig. 1A) was a 71-year-old man presenting with severe haemorrhagic cystitis and nephrotic syndrome (proteinuria 3.9 g/24h), with no impaired renal function (serum creatinine level 1.08 mg/dl; normal: 0.85–1.3 mg/dl). A biopsy of urinary bladder revealed AA-type amyloid deposits at vascular and interstitial submucosa (See Fig. 1B). Similar deposits were detected in a rectal biopsy, supporting the diagnosis of systemic AA amyloidosis. Further research of the patient's medical history revealed no familial history of amyloidosis, haemorrhagic cystitis or any chronic infectious disease. His main prior incidences of health disorders were a heart infarction, an atrial fibrillation, an intermittent and self-limited monoarthritis, and a bilateral sensorineural hypoacusia starting at the third decade of life. Neither skin symptoms nor recurrent fever were detected. The arthritis started during the second decade and mainly affected large and medium joints. Despite its long evolution, no structural joint damage was detected. Laboratory analyses at admission revealed mild anaemia (Hb 11.4 g/dL; normal: 12.0–16.0 g/dL), normal counts of leukocytes and platelets, and normal or negative immunological analyses (serum and urine immunofixations, and circulating ANAs, ANCAs, anti-dsDNA and anti-CCP autoantibodies). By contrast, increased values of serum C-reactive protein (CRP) (3.7 mg/dL; normal <0.5 mg/dL), and

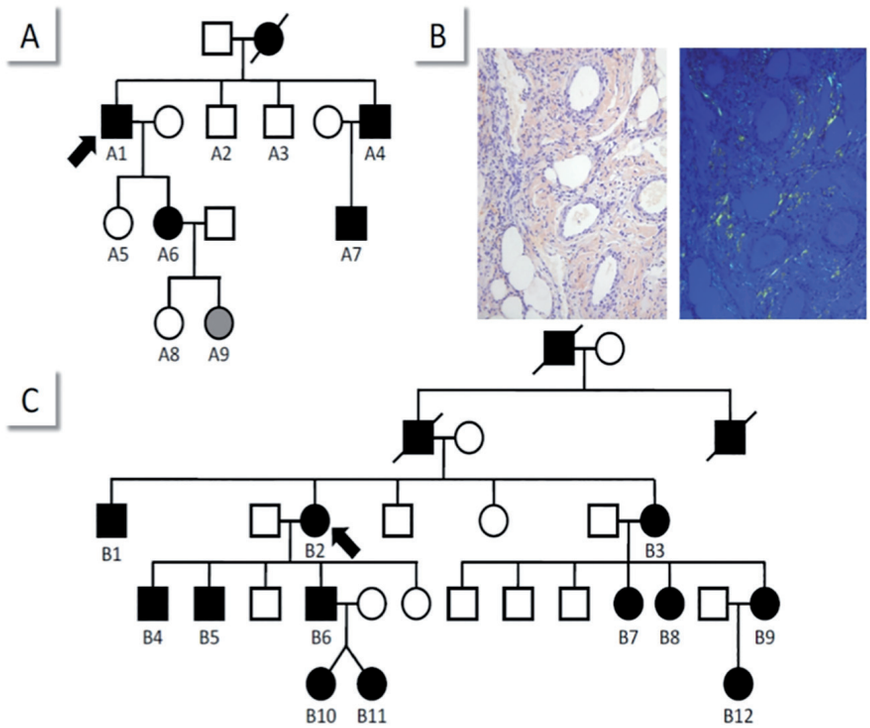

Fig. 1.

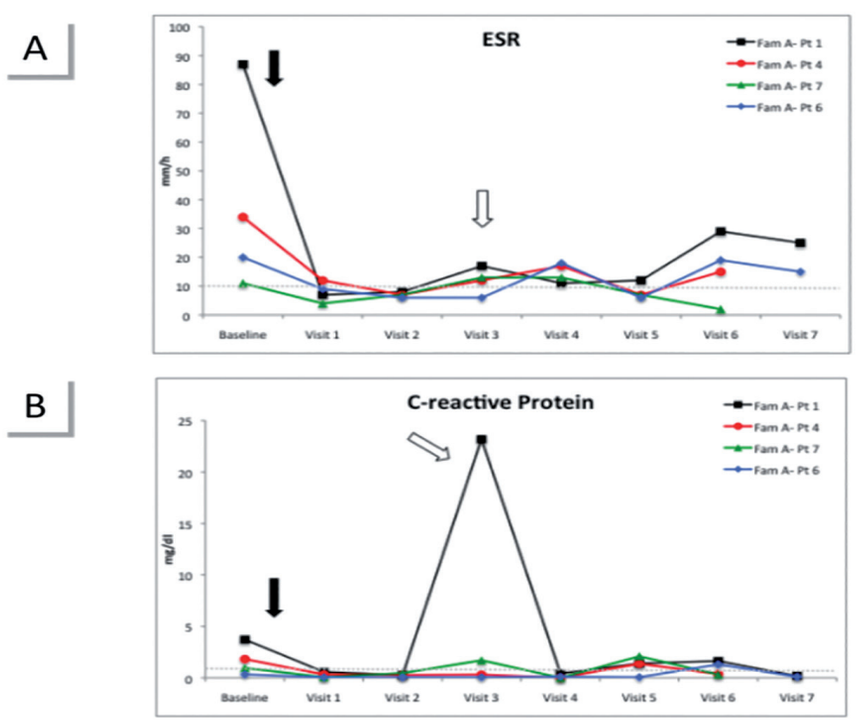

Fig. 2.

erytrocyte sedimentation rate (ESR) (87 mm/h; normal <10 mm/h) were detected. A suspicion of autoinflammatory disease complicated with AA amyloidosis was established. To evaluate this hypothesis, a complete genetic screening was performed. No mutations were detected in the *MEFV*, *TNFRSF1A* and *MVK* genes. By contrast, the heterozygous p.Ala439Thr *NLRP3* variant (NM_001243133.1) was detected. This variant was absent in different public

Table I. Summary of clinical data of patients.

Family - Patient	YOB	Fever	Skin rash	Arthritis (onset)	Pericarditis (onset)	CNS	Renal disease	Deafness (onset)	Others
A – 1	1940	-	-	Yes (≈20 yrs)	-	-	Haemorrhagic cystitis	Yes (≈35 yrs)	-
A – 4	1953	-	-	Yes (15 yrs)	Yes (43 yrs)	-	-	Yes (≈35 yrs)	-
A – 6	1966	-	-	-	-	-	-	Yes (20 yrs)	-
A – 7	1977	Yes	-	Yes (3 yrs)	Yes (17 yrs)	-	-	Yes (25 yrs)	-
A – 9	1998	-	-	-	-	-	-	-	-
B – 1	1933	-	Yes	Yes	-	Seizures, Anosmia	Kidney transplant	Yes (≈35 yrs)	Conjunctivitis
B – 2	1936	Yes	Yes	Yes	-	-	-	Yes (≈35 yrs)	Conjunctivitis
B – 3	1939	Yes	Yes	Yes	-	-	Haemorrhagic cystitis	-	Conjunctivitis
B – 4	1958	-	Yes	Yes	-	-	-	Yes (≈45 yrs)	-
B – 5	1961	Yes	Yes	Yes	-	Headache, Anosmia, Parosmia	Proteinuria	Yes (≈35 yrs)	-
B – 6	1968	-	Yes	Yes	-	Headache	Increased creatinine	Yes (≈30 yrs)	Conjunctivitis
B – 7	1968	-	Yes	Yes	-	Headache	-	Yes (≈35 yrs)	Conjunctivitis
B – 8	1971	-	Yes	Yes	-	-	-	-	Conjunctivitis
B – 9	1977	Yes	Yes	Yes	-	Headache	Increased creatinine	-	Conjunctivitis
B – 10	2002	-	-	Yes	-	-	-	-	-
B – 11	2002	-	Yes	Yes	-	-	-	-	-
B – 12	2007	-	Yes	Yes	-	-	-	-	Conjunctivitis

databases (NCBI dbSNP Build 137, NHBLI-ESP) and among healthy Spanish controls (n: 300), but it had been previously reported as the pathogenic mutation in several MWS patients (5). These data supported the definite diagnosis of CAPS in our patient. Anakinra (100 mg/24h s.c.) was then started (follow-up of 52 months), resulting in the disappearance of articular complaints, the decrease of proteinuria (currently 0.38 g/24h), and the normalisation of inflammatory markers (See Fig. 2). No significant progression of the neurosensorial hearing loss was detected. While he was receiving anakinra, a urinary infection associated with an inflammatory response was detected, revealing the patient's ability to fight against infections despite IL-1 blockade.

As CAPS are dominantly inherited disorders, a complete evaluation of the patient's relatives was performed. Genetic analyses revealed four additional living relatives carrying the heterozygous p.Ala439Thr *NLRP3* mutation (See Fig. 1A). Interestingly, one of them (Patient A9) is a 16-year-old asymptomatic girl. The remaining individuals showed variable clinical features, with male patients (Patient A4 and Patient A7) suffering from late-onset recurrent pericarditis, arthritis and bilateral sensorineural deafness, whereas the female patient (Patient A6) referred exclusively bilateral sensorineural deafness. The familial history revealed no information regarding incidences of recurrent fever, urticaria-like rash, CNS involvement, abdominal pain, renal disease or AA amyloidosis. At the time of clinical evaluation, mild-to-moderate increases of ESR and CRP were detected in all symptomatic individuals (See Fig. 2). All patients started treatment with anakinra (100 mg/24h s.c.), which provoked the disappearance of the pericarditis and articular complaints in Patient A4 and Patient A7, and the normalisation of inflammatory parameters in all of them (See Fig. 2). No significant hearing changes were detected.

Family B

This is a Spanish family comprising five generations afflicted by a dominantly inherited disorder with 15 affected individuals (See Fig. 1C). The patients' clinical data include urticaria-like rash presenting in infancy, recurrent fever, recurrent arthritis, headache, recurrent conjunctivitis and late-onset bilateral sensorineural deafness (See Table for a complete summary of clinical data). The *NLRP3* analysis revealed the heterozygous p.Arg260Trp variant in all analysed patients, which has been reported as one of the most common disease-causing *NLRP3* mutations (6-8). All these data supported the MWS diagnosis for this family. Interestingly, during the study of this family, atypical clinical features were detected in certain patients, some of which are shared with patients from Family A. At the age of 73 years, Patient B2 developed a severe haemorrhagic cystitis with a progressive descrease of haemoglogin (range 12.4–8.9 g/dL; normal 12.0–16.0 g/dL) and haematocrit (range 36.1–27.1%; normal 37-47%), with haemodynamic instability that required an urgent arteriography and embolisation. A biopsy of the urinary bladder revealed AA-amyloid deposits at vascular, perivascular and interstitial submucosa, with inflammatory cells infiltrates, supporting the diagnosis of AA amyloidosis complicating the course of the MWS. Additionaly, two patients (Patient B1 and Patient B5) referred olfactive disorders: anosmia in both patients, and parosmia only in Patient B5.

Discussion

Like most monogenic autoinflammatory diseases, the diagnosis of CAPS is definitely achieved through a combination of clinical observations and genetic analyses. Until recently, the clinical features that suggested these syndromes included a dominant inheritance pattern,

an onset of the disease during infancy or early childhood, and a generalised urticarial-like rash associated with a marked acute phase response as the first symptoms (1). In recent years, the clinical diversity of CAPS expanded by means of the description of atypical presentations. Thus, Verma and colleagues reported a Swedish family with the novel and private p.Met299Val *NLRP3* mutation in which the patients referred a late onset (fifth decade) inflammatory disease mainly characterised by low-grade fever, red eyes, fatigue, headache, pleural and pericardial effusions, and the absence of urticaria-like rash (2). One year later, Murphy and colleagues reported four Irish MWS patients carrying the already known p.Glu311Lys *NLRP3* mutation that referred the near absence of urticaria (3). Finally, *de novo* somatic *NLRP3* mutations have been detected in patients with late-onset of otherwise typically MWS (9). We herein describe two MWS families carrying the structural, germline p.Ala439Thr and p.Arg260Trp *NLRP3* mutations, respectively. Both mutations have been previously identified in different CAPS families, and are currently considered as two of the most common disease-causing *NLRP3* mutations (5-8). However, the clinical phenotypes of some of these patients were notable for their atypical presentations. In the case of Family A, the atypical features were present in all individuals and provoked that CAPS had not been initially considered in the differential diagnosis. These atypical features included the existence of an asymptomatic teenage carrier; the late onset of the disease; the absence of urticarial-like rash at the onset or during the course of the disease, the presence of recurrent pericarditis, and marked gender differences with regard to the disease severity. The occurrence of haemorrhagic cystitis as the first symptom of AA amyloidosis was identified as an atypical feature in one patient from each family, and olfactive disorders were detected as atypical symptoms in two patients of Family B.

With regard to the onset of the disease, it is highly remarkable that the first manifestations in all patients from Family A started during the second decade or beyond. The only previous reports describing a late onset CAPS are those above-mentioned, which were detected in patients carrying private or somatic *NLRP3* mutations (2, 3, 9). By contrast, our observations could be considered as different because all patients from Family A carried a well-known, germline *NLRP3* mutation, which was first reported in MWS patients in whom the disease started during early childhood (5). In this Family A, marked differences in the disease severity depending on the patient's gender were detected. Thus, the first symptom, and the most relevant during the course of the disease, in all female patients was the bilateral sensorineural deafness. By contrast, the disease seemed more diverse and severe in all male patients both at the disease onset and during its course. The data collected in this particular family are at variance with a previous report that described female gender and the deafness as the two features that define a group of high risk for severe MWS (10). It is also extremely unusual the case of Patient A9, a 16-year-old asymptomatic girl who carried the p.Ala439Thr *NLRP3* mutation. In previous reports of CAPS, the phenomenon of asymptomatic carriers has been exclusively described in individuals harbouring the p.Val198Met, p.Arg488Lys and p.Gln703Lys *NLRP3* variants, which are currently considered as variants of uncertain significance (4, 6-8, 11). Though we can not exclude the possibility of the low-penetrance phenomenon, on the basis of the type of mutation and the evolution of the disease in her relatives, we believe as the most probable option that we are actually challenging with a presymptomatic individual. In agreement with that consideration, this girl is not being currently treated with anti-IL-1 drugs, but it is being periodically visited and her inflammatory parameters and hearing function evaluated.

Systemic AA amyloidosis is a late complication of MWS (6-8, 12, 13). AA amyloid deposits can occur in any organ or tissue, but the renal dysfunction dominates its clinical course (13). We herein describe two unrelated, aged patients, one from each family, with severe haemorrhagic cystitis as the first symptom of AA amyloidosis. The presence of amyloid deposits in the urinary bladder is markedly unusual, with only few cases described to date (14, 15). The fact that two unrelated MWS patients showed AA amyloid deposits at this strange location deserves consideration concerning the possibility that other local factors can play a role to favour the amyloid deposit at the bladder, and support to include CAPS syndromes in the differential diagnosis of non-malignant haemorrhagic cystitis. Finally, two patients from Family B referred persistent isolated olfactive disorders (anosmia and parosmia), not associated with ageusia. The main causes of these olfactive disorders among the general population are exposure to tobacco, chronic rhinosinusitis, nasal polyps, trauma, aging, and tumours, which have been reasonably ruled out in both patients. Despite the fact that these observations may be merely incidental, we can not totally exclude that they could be rare manifestations of CAPS resulting from chronic inflammation affecting the nose area and/or the olfactive nerve.

In conclusion, we describe atypical manifestations in MWS patients harbouring well-known *NLRP3* mutations that expand the clinical diversity of CAPS. Our data suggests the inclusion of these syndromes in the differential diagnosis of adult patients with unexplained, recurrent inflammatory diseases, even in the absence of early-onset or cutaneous manifestations. Patients with atypical CAPS exhibited a similar response to IL-1 blockade than patients with the typical forms. Consequently, the initiation of anti-IL-1 therapy as early as possible might avoid the appearance of life-threatening complications in these patients.

Competing interests

S. Bujan-Rivas has received honoraria with an amount less than 1000 € from Novartis Farmaceutica S.A. as scientific speaker. F. Martinez-Valle has received a grant from Aspire for research in Attr. Hereditary Amyloidosis. J.I. Arostegui has received honoraria from Novartis and Sobi, and a grant support from Novartis. All the other authors have declared no competing interests.

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