Concise report

Description of a new family with cryopyrin-associated periodic syndrome: risk of visual loss in patients bearing the R260W mutation

Nicolás Alejandre¹, Ana Ruiz-Palacios¹, Angel M. García-Aparicio², Fiona Blanco-Kelly³,4, Sandra Bermúdez⁵, Guillermo Fernández-Sanz¹,6, Fredeswinda I. Romero⁵, Juan I. Aróstegui⁷, Carmen Ayuso³,4, Ignacio Jiménez-Alfaro¹, Gabriel Herrero-Beaumont⁵ and Olga Sánchez-Pernaute⁵

Abstract

Objective. The aim of this study was to describe a family with cryopyrin-associated periodic syndrome (CAPS) in which the disease was unveiled after the ophthalmologic evaluation.

Methods. Family and personal histories from each of the patients were recorded. Each underwent a full ophthalmological examination along with the physical examination. The mutational analysis of the \textit{NLRP3} gene was performed by means of direct sequencing.

Results. The proband was admitted during an episode of unilateral anterior uveitis. She had a history of recurrent red eye and had been suffering episodes of skin rash and arthralgia induced by cold since childhood. At examination, she showed a reticulated corneal mid-stroma. Her mother and her younger sister also suffered from relapsing episodes of skin rash and fever triggered by cold as well as flares of red eye. They had developed premature hearing loss. In both cases, opacities in the corneal mid-stroma were evidenced with a slit lamp. The genetic analysis detected the heterozygous germline p.R260W mutation in the \textit{NLRP3} gene in the three women, confirming the diagnosis of CAPS. Treatment with anakinra resulted in complete remission of flares.

Conclusion. In this family, a structural \textit{NLRP3} mutation was associated with classic Muckle–Wells features of different degrees of severity. Interstitial keratitis with corneal opacification, usually ascribed to neonatal-onset multisystem inflammatory disease, was found. We underscore that ocular involvement in Muckle–Wells syndrome should be carefully assessed, since it can lead to visual impairment.

Key words: cryopyrin-associated periodic syndrome, interstitial keratitis, IL-1 blockage.

Introduction

Autoinflammatory diseases are characterized by unexplained flares of multisite inflammation without evidence of infection or an autoimmune response [1]. Cryopyrin-associated periodic syndromes (CAPS) are a subgroup of genetic autoinflammatory diseases inherited as autosomal dominant traits. They are caused by gain-of-function point mutations in the \textit{NLRP3} gene, which encodes cryopyrin [2]. The NLRP3 inflammasome is a multimolecular complex orchestrating innate responses in phagocytes, neutrophils and chondrocytes. Mutations causing CAPS lower the threshold for the activation of the NLRP3 inflammasome. This, in turn, results in the release of IL-1β.
and IL-18, both of which are largely responsible for the symptoms [3, 4].

Three types of CAPS have been described. The familial cold autoinflammatory syndrome (FCAS) has an early onset after birth and is characterized by recurrent episodes of urticarial skin rash triggered by exposure to cold. Low-grade fever, abdominal discomfort, conjunctivitis and arthromyalgia may appear during flares [5]. Patients with Muckle–Wells syndrome (MWS) usually present later in childhood with recurrent urticarial skin rash, fever, abdominal pain, arthromyalgia and arthritis [6, 7]. Compared with FCAS, flares in MWS have a more pronounced effect on the patient’s health. More serious manifestations such as chronic meningitis can occur and, in time, amyloidosis and/or premature deafness may develop [6]. Neonatal-onset multisystem inflammatory disease (NOMID) causes chronic urticaria, cartilage and joint swelling, recurrent fever and chronic meningitis starting shortly after birth [8, 9]. Symptoms in NOMID are frequently non-subsiding and result in profound alterations in growth, with joint deformities, mental retardation, sight impairment and hearing loss.

These distinct syndromes clearly represent three grades of severity in which FCAS and NOMID are the extremes in a single spectrum of clinical manifestations. However, there is considerable overlapping of features between entities as well as differences in expressivity between relatives bearing the same mutation [10]. This fact argues for the existence of either second genes or environmental factors acting as modifiers on expression of the disease. In this regard, the term cryopyrinopathy, encompassing all phenotypes, has been coined in order to acknowledge the continuum of the disease [11]. We describe a family with CAPS, as defined by characteristic clinical features and a frequently associated mutation, with three confirmed affected relatives and a family history suggesting the disease in another four members.

Patients and methods

Patients

The proband was recruited at the outpatient uveitis clinic at Jimenez Díaz Foundation University Hospital. Because of her characteristic symptoms, as well as positive family history, she was asked to undergo molecular sequencing of the NLRP3 gene. The other two cases were followed at Virgen de la Salud Hospital, Toledo. The family was Caucasian, of Spanish ancestry. Written consent to participate in the study was obtained from the three women according to the Declaration of Helsinki. The design of the study was approved by the Jimenez Díaz Foundation ethics committee. The women underwent an ophthalmological examination with slit lamp as well as the genetic study.

Molecular analysis

Genomic DNA was isolated from peripheral blood leucocytes with the BioRobot EZ1 DNA extractor (Qiagen, Hilden, Germany). Exon 3 of the NLRP3 gene was directly amplified by PCR from genomic DNA using primers previously described [7, 12]. The PCR amplicons were purified with the Illustra ExoStar 1-step kit (GE Healthcare, Albany, NY, USA) and sequenced using the ABI BigDye terminator cycle sequencing kit (Applied Biosystems, Fosters City, CA, USA). The sequencing products were purified using Sephadex G-501 columns (Princeton Separations, Adelphia, NJ, USA), subjected to electrophoresis and analysed using the ABI Prism 3730XL (Applied Biosystems).

Results

Family description

Case V:2 (proband)

At 24 years of age, she attended the emergency eye clinic of our institution during an episode of unilateral anterior uveitis. She had a history of recurrent red eye since she was 10 years old and was diagnosed with anterior uveitis and keratitis. She also had one to two yearly episodes of exanthematous rash and arthralgia, frequently induced by cold. The episodes were mild and of short duration, rarely associated with fever. She complained of large joint swelling during flares and occasionally had abdominal pain and oral ulcers. In our unit, slit lamp examination showed reticulated mid-stromal changes in the cornea without significant opacification. Visual acuity and eye pressure were normal. Fundoscopy showed no abnormalities. Blood tests were normal except for a slight increase in acute phase reactants (ESR 27 mm/h (normal <25 mm/h) and CRP 9.1 mg/l (normal <5.0 mg/l)). During follow-up she had additional episodes of anterior uveitis with keratitis and received local corticosteroids.

Case V:3

Two years younger than the proband, she suffered a first episode of urticarial rash and red eye shortly after birth. From then on she had continuous episodes of widespread urticaria, large joint arthralgia, fever and malaise. She reported an average of two episodes per week, worsening with exposure to cold weather and also triggered by emotional stress. She had relapsing flares of red eye, which occurred independently. She frequently complained of headache accompanying eye flares. At the age of 9, sensorineural hearing loss in her left ear was detected. Her blood tests showed a persistent increase in acute phase reactants. Her renal function was preserved. An echocardiogram showed no abnormalities. Previous descriptions of eye involvement included uveitis, episcleritis and keratitis. She also developed corneal ulcers and synchiae. Slit lamp examination showed a central circular corneal opacification in both eyes. The mid- and posterior stroma showed a calcification band-like image. No endothelial damage or corneal oedema was found. Visual acuity was 0.9.

Case IV:2

The mother of the two siblings was 54 years old at the time of evaluation in our clinic. She had a history of
Relapsing fever, arthralgia and red eye from her youth. The episodes were triggered by cold weather and emotional stress and were associated with malaise and increased ESR. She had five to six monthly episodes in winter months and one to two during warmer months. Skin involvement consisting of urticarial rash had an independent course, with a short interval of 1–3 days between attacks. From the age of 20 she needed hearing devices because of advanced sensorineural bilateral deafness. She had cardiac enlargement in X-ray studies, but a normal echocardiogram. Her renal function was normal. Previous red-eye episodes had been diagnosed with conjunctivitis and anterior uveitis. Episcleritis and keratitis had also been observed. She had developed corneal ulcers. The slit lamp examination performed in our unit revealed bilateral, round, central corneal opacity involving the mid- and posterior stroma. Visual acuity was 0.7. Table 1 summarizes the clinical findings in the three subjects.

### Additional family information
Case IV:2 reported a positive family history of hearing loss. Her father suffered from adult-onset sensorineural deafness and three of her father’s cousins—a female and two males who were siblings—needed hearing devices in their youth.

### Genetic study
A genetic study of the proband was performed to assess possible CAPS-associated mutations at the NLRP3 gene. The study revealed the heterozygous c.778C>T transition, located at exon 3 of the gene. This genetic variant provokes the arginine-to-tryptophan amino acid exchange at position 260 (p.R260W). The same missense mutation was detected in cases V:3 and IV:2, while none of the analysed asymptomatic relatives (cases III:6, IV:1 and V:1) carried the mutation. The family lineage is shown in Fig. 1.

### Treatment and outcome
At referral, the women were treated with steroids and MTX, with poor results. Case IV:2 also received adalimumab for a brief period, before the treatment was discontinued due to a pulmonary infection. Once definitive CAPS diagnosis was achieved, patients V:3 and IV:2 started treatment with anakinra, which resulted in complete remission of the inflammatory flares. In parallel with the clinical improvement, ESR and CRP dropped to normal levels.

### Discussion
The missense p.R260W mutation detected in this family was described in 2002 in a French cohort of patients with CAPS [13] and later was found in several families from different countries [14, 15]. It is considered a frequent or unambiguous CAPS-linked mutation [16]. It has been shown in vitro that this single amino acid substitution provokes spontaneous activation of the NLRP3 inflammasome [3].

According to previous descriptions, the p.R260W mutation is associated with exanthematous rash, joint symptoms and red eye, while fever is unusual. Patients usually fit into the diagnosis of MWS, as did cases IV:2 and V:3 from our family. The mutation can also render mild phenotypes, which are closer to the description of FCAS, like our proband [13]. Both women with an MWS-like phenotype had developed sensorineural deafness, a trait that is

### Table 1: Description of disease features in the three patients

<table>
<thead>
<tr>
<th>Case</th>
<th>V:2</th>
<th>V:3</th>
<th>IV:2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>F</td>
<td>F</td>
<td>F</td>
</tr>
<tr>
<td>Age at onset</td>
<td>10 years</td>
<td>At birth</td>
<td>Teens</td>
</tr>
<tr>
<td>Age at the time of the study, years</td>
<td>24</td>
<td>22</td>
<td>54</td>
</tr>
<tr>
<td>Frequency of flares</td>
<td>2 yearly</td>
<td>2-3 weekly</td>
<td>1-2 monthly</td>
</tr>
<tr>
<td>Duration of flares</td>
<td>Up to 48 h</td>
<td>24 h</td>
<td>24 h</td>
</tr>
<tr>
<td>Fever</td>
<td>Absent</td>
<td>38-39°C</td>
<td>38-39°C</td>
</tr>
<tr>
<td>Arthralgia</td>
<td>Large joints</td>
<td>Large joints</td>
<td>Large joints</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>Present</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>Malaise</td>
<td>Mild</td>
<td>Severe</td>
<td>Moderate</td>
</tr>
<tr>
<td>Headache</td>
<td>Absent</td>
<td>Present</td>
<td>Absent</td>
</tr>
<tr>
<td>Skin involvement</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rash occurrence</td>
<td>During flares</td>
<td>During flares</td>
<td>Independent of flares</td>
</tr>
<tr>
<td>Rash severity</td>
<td>Mild</td>
<td>Moderate</td>
<td>Severe</td>
</tr>
<tr>
<td>Eye manifestations</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Severity</td>
<td>Moderate</td>
<td>Severe</td>
<td>Moderate</td>
</tr>
<tr>
<td>Conjunctivitis</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>Uveitis</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>Keratitis</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>Hearing loss</td>
<td>Absent</td>
<td>Moderate</td>
<td>Severe</td>
</tr>
<tr>
<td>Acute phase response</td>
<td>Slightly increased</td>
<td>Increased</td>
<td>Increased</td>
</tr>
<tr>
<td>Amyloidosis</td>
<td>Not studied</td>
<td>Not studied</td>
<td>Not studied</td>
</tr>
</tbody>
</table>
frequently observed in carriers of the p.R260W mutation [13, 17]. Interestingly, the father of case IV:2 (case III:5) and three of his cousins also suffered from premature hearing loss. This fact could point to a second genetic trait in the family. However, case III:5 most probably carried the mutation, since the genetic study of his wife (case III:6) confirmed that she was unaffected. This suggests that cases with deafness in this family represent CAPS with low expressivity.

In addition, our family shows that the p.R260W mutation can lead to sight impairment. Almost all patients with FCAS or with MWS have recurrent flares of red eye, but in most cases they are diagnosed with conjunctivitis. Although there are also descriptions of episcleritis, uveitis and keratitis, eye involvement in these conditions is regarded as mild [18]. The appearance of severe ophthalmic features is more often found in patients with NOMID. In this disease, chronic meningitis results in increased intracranial pressure and papilledema, eventually leading to visual loss. The anterior pole can also be affected in NOMID, and chronic interstitial keratitis with corneal clouding was found in some patients included in two large cohorts [19, 20]. The lesions that were observed in these patients are similar to the ones that we found in cases V:3 and IV:2. Interestingly, a recent report has described stromal keratitis in another patient with MWS-like features [21].

In the recently proposed autoinflammatory diseases activity index, conjunctivitis was chosen as the eye variable scoring activity in CAPS [22]. However, we show here that a careful evaluation of CAPS-associated red eye might unmask more serious involvement, such as uveitis or interstitial keratitis. It should be borne in mind that interstitial keratitis is not restricted to patients with NOMID, but may appear in other phenotypes. Moreover, patients with interstitial keratitis should be considered for treatment with IL-1-blocking agents, since the condition can result in opacification and loss of vision. In this regard, corneal inflammatory infiltrates disappeared after treatment with anakinra in several patients [23, 24]. On the other hand, the ability of therapies to improve established corneal clouding is more questionable. A histopathological examination of the cornea in one patient with CAPS showed that clouding was due to calcium deposits in the inner tissue [21, 23], which probably represents a non-reversible lesion. In this regard, it is important to emphasize early intervention.

**Rheumatology key messages**
- Interstitial keratitis can be observed in patients with otherwise mild forms of cryopyrinopathy.
- A slit lamp examination is warranted to identify cryopyrinopathy patients at risk of visual loss.
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References