J Clin Immunol (2008) 28:134–138
DOI 10.1007/s10875-007-9150-7

Phenotype–Genotype Analysis of Cryopyrin-Associated Periodic Syndromes (CAPS): Description of a Rare Non-Exon 3 and a Novel *CIAS1* Missense Mutation

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Received: 4 October 2007 / Accepted: 24 October 2007 / Published online: 15 December 2007

Abstract We describe in this paper the phenotype–genotype analysis of a Brazilian cohort of patients with cryopyrin-associated periodic syndromes (CAPS). Patient 1 presented with an urticarial rash and recurrent fever exacerbated by cold weather, arthritis, and anterior uveitis, thus, receiving a clinical diagnosis of familial cold autoinflammatory syndrome. *CIAS1* sequencing identified the T436I mutation, previously associated to a clinical phenotype of chronic infantile neurological cutaneous and articular/neonatal onset multisystem inflammatory disease. Patient 2 developed a papular exanthema with daily fever shortly after birth, frontal bossing, patellae enlargement, and cognitive and motor impairments. Sequencing identified the exceedingly rare G755R *CIAS1* mutation in exon 4. Patient 3 developed skin rash and articular symptoms 6 h after birth, followed by aseptic meningitis. He was found to have the novel C148Y missense mutation in *CIAS1*. This report expands the spectrum of *CIAS1* mutations associated to clinical disease, suggests that the same mutation can be associated with different clinical syndromes, and supports the evidence that CAPS patients should always be screened for mutations outside exon 3.

Keywords Cryopyrin · periodic fever · NOMID · CINCA · CIAS1

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Introduction

The systemic autoinflammatory diseases are a group of rare disorders characterized by inflammatory episodes that include autosomal recessive diseases (familial Mediterranean fever and hyperimmunoglobulinemia D with periodic fever syndrome) and autosomal dominant diseases (tumor necrosis factor receptor-associated periodic syndrome and cryopyrin-associated periodic syndromes, CAPS) [1, 2]. CAPS comprise three clinical entities linked to mutations in *CIAS1* (cold induced autoinflammatory syndrome gene): familial cold autoinflammatory syndrome (FCAS; OMIM 120100), Muckle–Wells syndrome (MWS; OMIM 191900), and chronic infantile neurological cutaneous and articular syndrome

(CINCA; OMIM 607115), also known as neonatal onset multisystem inflammatory disease (NOMID) [3].

CINCA/NOMID is the most severe syndrome and is characterized by symptoms of almost continuous inflammation [1–3]. The initial manifestations usually include urticaria-like cutaneous rash that develops during the neonatal period or early infancy. Later, patients develop symptoms of severe central nervous system inflammation, including chronic aseptic meningitis and cognitive and motor deficits. Some patients develop a characteristic bony overgrowth, predominantly involving the knees. Other common manifestations include short stature and forehead bossing. Amyloidosis develops in a minority of patients reaching adulthood [1–3].

MWS is often associated with sensorineural hearing loss, conjunctivitis, episcleritis, optic disc edema, and musculoskeletal manifestations. The urticarial rash develops up to the second decade of life and the incidence of amyloidosis is about 25% [1–3]. FCAS is the mildest cryopyrinopathy and is characterized by cold-induced urticaria-like rash. Patients develop urticarial rash, fever, and arthralgia that begin within 1 to 2 h after relatively mild cold exposure and usually persist for 12 to 24 h. Neurological abnormalities, severe articular involvement, and amyloidosis are very uncommon in FCAS [1–3].

In this report, we describe the genetics and clinical features of three Brazilian patients with CAPS. Our studies reveal a novel disease-associated *CIAS1* mutation and a rare *CIAS1* mutation outside exon 3.

Methods

Genomic DNA samples were isolated from patients and healthy individuals (wild-type control) using PureLink (Invitrogen, Carlsbad, CA, USA). Exonic regions of *CIAS1*, including splice sites, were amplified by polymerase chain reaction (PCR) as described [4]. The PCR products were directly sequenced by using ABI Prism BigDye (version 1.1) terminators and analyzed on an ABI 3100 sequencer (Applied Biosystems, Foster City, CA, USA). Primer sequences and annealing temperatures are available on request. The sequencing data for the patient and wild-type control was compared with Ensembl (http://www.ensembl.org) data (version 35, Nov. 2005) for each locus. All identified mutations/single nucleotide polymorphisms (SNPs) were confirmed by sequencing a second PCR product. A formal consent for publishing photographs was obtained from the patients' parents.

Results

Patient 1

Patient 1 is now an 8-year-old girl that was noted to have a disseminated erythematous and macular rash at 20 days of age. At 3 months of age, she developed recurrent fever lasting from a few days to 3 weeks and ranging between 38 and 39.5°C (100.4–103.1°F). Fever was exacerbated by cold weather and was associated to pallor, asthenia, and worsening of cutaneous rash. Family history was unremarkable.

Physical examination revealed a generalized macular and papular skin rash, weight and height between percentiles 10 and 25, and normal musculoskeletal and neurological evaluation (Figs. 1a and b). Cervical and inguinal lymphadenopathies were present. Laboratory results are shown in Table I. A skin biopsy demonstrated chronic superficial perivascular dermatitis (not shown). Lymphnode biopsy showed nonspecific lymphoid tissue hyperplasia (not shown).

During the initial 2-year follow-up, the patient developed recurrent acute arthritis of large joints without articular limitation or bone erosions. She was treated with nonsteroidal anti-inflammatory drugs (NSAIDs) and colchicine, with a decrease in the frequency of fever episodes but maintenance of skin rash and elevated acute phase reactants. Follow-up was lost for almost 2 years, when she returned with a unilateral anterior uveitis. Currently, she persists with weekly fever for 2 to 3 days but with normal development, neurological examination, and brain magnetic resonance imaging.

Mutational analysis for familial Mediterranean fever excluded five most common *MEFV* mutations (M680I,

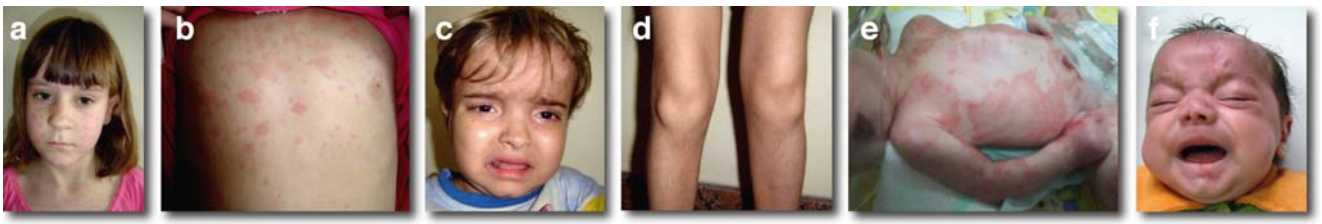

Fig. 1 Salient clinical features of three CAPS patients. Note the erythematous and macular rash affecting the face and trunk of patient 1 (**a** and **b**), and forehead bossing (**c**) and patellar enlargement (**d**) of patient 2; severe urticarial rash 6 h after birth on patient 3 (**e**), followed by scar formation on the face (**f**).

Table I Laboratory Values at Hospital Admission

	Patient 1	Patient 2	Patient 3[a]
Hemoglobin, g/l	10.2	8.7	15.4
Leukocyte count, 10^9/l	15.2	16.4	10.0
Neutrophil count, 10^9/l	8.8	9.1	7.2
Platelet count, 10^9/l	622	375	199
C-reactive protein, mg/dl	15.2	149	10.9
ESR, mm/1 h	30	57	40

ESR Erythrocyte sedimentation rate
[a] Values at 3 days of age

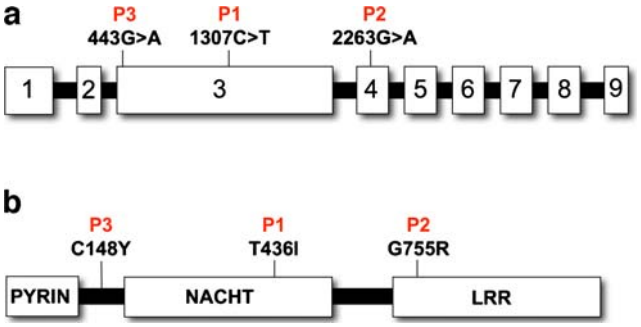

Fig. 3 Schematic representation of the mutations at the DNA (**a**) and protein (**b**) levels. Numbering inside boxes representing *CIAS1* exons. *P1* Patient 1, *P2* patient 2, *P3* patient 3.

M694V, M694I, V726A, and E148Q). Sequencing of *CIAS1* identified the p.T436I (c.1307C>T) mutation in exon 3 (Figs. 2a and 3).

Patient 2

Patient 2 is now a 7-year-old boy that developed an erythematous and papular exanthema involving the chest, abdomen, and extremities 3 h after birth. A skin biopsy suggested urticaria. Since the age of 4 months, he presented with two daily fever episodes and temperatures ranging from 38 to 38.5°C (100.4–101.3°F). He was born to healthy parents, and family history was unremarkable, except for an older brother with vitiligo.

The patient was admitted at our service at the age of four. Physical examination revealed weight and height bellow percentile 2.5, generalized eythematous and papular exanthema, forehead bossing (Fig. 1c), and marked swelling of knees with patellae enlargement (Fig. 1d). A delayed neurological development was noted, with cognitive and motor deficits. Ophthalmological evaluation demonstrated a chronic papilledema, suggesting chronic increase in intracranial pressure. Lab results are shown on Table I.

Since the age of five, he has presented acute recurrent arthritis of hips, knees, and ankles, and persistent daily fever episodes. Abdominal ultrasound demonstrated bilateral parenchymal nephropathy, suggesting secondary amyloidosis.

Because of the strong clinical suspicion for CINCA/NOMID, *CIAS1* was sequenced, and the c.2263G>A (p.G755R) mutation was found. This mutation is located in exon 4, affecting the leucine-rich repeats (LRR) domain of cryopyrin (Figs. 2b and 3).

Patient 3

Patient 3 is a preterm newborn who developed urticarial skin lesions and joint limitations in the elbows and knees 6 h after birth (Fig. 1e). Atypically, perioral, nasal, and frontal skin lesions evolved to necrosis with scarring (Fig. 1f). Initial investigation showed elevated inflammatory activity and negative antinuclear antibody (ANA), anti-Sjogren syndrome antigen B (autoantigen La) (anti-SSB/La), anti-SSA/Ro, and Venereal Disease Research Laboratory (VDRL; Table I). Therapy with corticosteroid was

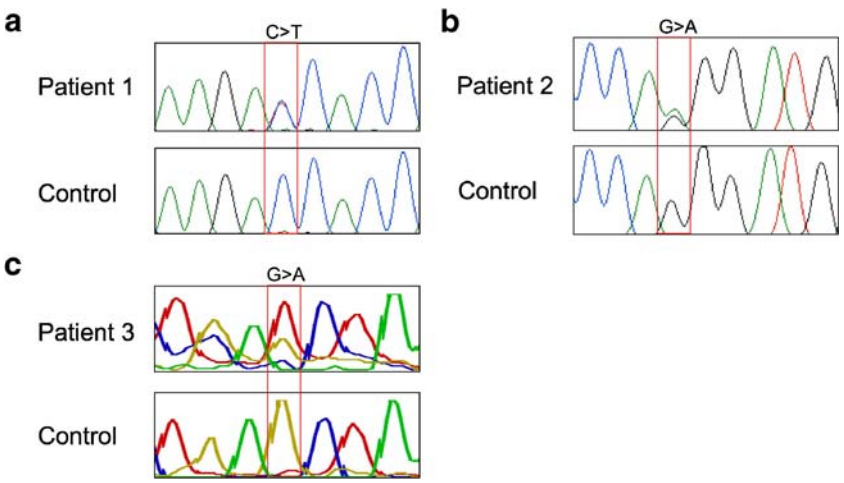

Fig. 2 Electropherograms demonstrating the C > T change at position 1307 of CIAS1 in patient 1 (**a**), G > A change at position 2263 in patient 2 (**b**), and G > A change at position 443 in patient 3 (**c**), as compared to a normal control.

started, with clinical improvement and a decrease in inflammatory markers. However, 2 weeks later, the medication was discontinued. After hospital discharge, the patient presented with otitis media at 1 month of age, pneumonia at 2, and aseptic meningitis at 3 months of age. He was referred to our service at the age of 4 months because of failure to thrive, post-feeding vomits, and irritability. Lab tests showed an elevated C reactive protein (CRP) and low levels of C3 and C4, and he was started on prophylactic sulphametoxazole-trimethoprim and prednisolone 0.25 mg/kg per day. After the introduction of these medications, the patient showed clinical improvement, and normalization of complement and CRP levels. The patient remains well, with normal development and without major complications at the age of 1 year and 5 months.

Genomic DNA sequencing demonstrated the novel p. C148Y (c.443 G>A) mutation in *CIAS1*, at the beginning of exon 3. This mutation was not found on 40 normal controls from the same population, dbSNP, Ensembl, or Infevers databases. The mutation affects a protein region of unknown function between the PYRIN and NACHT domains (Figs. 2c and 3).

Discussion

Cryopyrinopathies represent a spectrum of diseases caused by mutations in the *CIAS1* gene, and in which severity of symptoms is likely influenced by other genetic and environmental factors [4, 5]. *CIAS1* is a nine-exon gene that encodes the protein known as cryopyrin (NALP3, PYPAF1), which consists of three major domains: PYRIN, NACHT/NOD, and LRR [6]. Nearly all of the more than 50 known disease-associated mutations in cryopyrin are missense substitutions in the NACHT/NOD domain, encoded by the exon 3 of *CIAS1* (Infevers database, http://www.fmf.igh.cnrs.fr/ISSAID/infevers, accessed 2007–09–27) [4]. Recently, the fist mutations outside exon 3 were described (G755R, G755A, Y859C) [4]. We describe in this paper three patients with peculiar findings and diverse *CIAS1* mutations.

Patient 1 had a less severe phenotype, without clinical or radiological evidence of neurological disease for an extensive follow-up period and a skin rash worsened by cold. This clinical course suggests a clinical diagnosis of FCAS. However, cerebral spinal fluid was not evaluated, and we cannot rule out subclinical central nervous system inflammation. This patient harbored a T436I exon 3 mutation. The only other case described in the literature with the same mutation was diagnosed with CINCA/NOMID, thus, indicating a role for other genetic and possibly nongenetic modifiers in severity of disease in CAPS patients [7].

Patient 2 had the salient features of CINCA/NOMID syndrome and was found to have a mutation in the region between the first and second LRR domains of cryopyrin, encoded by the exon 4 of *CIAS1*. Mutations outside the NACHT/NOD domain are extremely rare, with only three such cases reported thus far [8]. Our finding confirms glycine 755 as the first mutational hotspot outside exon 3 and supports the notion that patients with clinical disease compatible with CAPS should have the entire coding region of *CIAS1* sequenced.

Patient 3 had very early onset NOMID associated with a novel missense mutation in the exon 3 of *CIAS1*. This mutation caused a cysteine-to-tyrosine amino acid change at position 148, in the NACHT-associated domain, which lies in between the PYRIN and NACHT domains. This finding expands the spectrum of CAPS-associated *CIAS1* mutations. Although this patient had typical NOMID clinical findings, such as forehead bossing, bony overgrowth, and aseptic meningitis, atypical features in this case include a normal neurological development, cutaneous lesions with necrosis and scars, and decreased complement levels that recovered after introduction of corticosteroids and prophylactic antibiotics.

But how do *CIAS1* mutations cause excessive inflammation? Cryopyrin/NALP3 interacts with adaptor proteins ASC and cardinal in a multiprotein complex known as the cryopyrin inflammasome [9]. This inflammasome regulates IL-1β release by bringing together and activating two pro-caspase-1 molecules, which subsequently cleave pro-IL1β to active IL-1β. In accordance to this model, monocytes from patients with mutations in the NACHT/NOD domain of cryopyrin show increased activation of caspase-1 and increased release of IL-1β [10]. Current evidence suggests that the wild-type cryopyrin is kept in an inactive state through auto-inhibition likely mediated by the interaction between the LRR domains with the NACHT/NOD domain of the protein. Mutations in the NACHT/NOD domain or some regions of the LRR domain might, thus, disrupt this auto-inhibitory mechanism, resulting in increased inflammasome activation and subsequent IL-1β release.

The key role of IL-1β in the cryopyrinopathies is confirmed by success of treatment with IL-1 blockers in all three clinical syndromes. In a series of studies, anakinra, a human recombinant IL-1 receptor antagonist, effectively prevented clinical symptoms or acute-phase elevations in patients with FCAS [11], induced a complete remission in MWS and resulted in the resolution of uveitis, rash, fever, and significant decline in cerebrospinal fluid pressure in NOMID patients [12, 13]. The possibility of reducing clinical symptoms of inflammation and of improving the quality of life and prognosis of CAPS patients makes molecular diagnosis of these specific hereditary periodic fever patients extremely important. Unfortunately, anakinra is not commercialized in Brazil.

Conclusions

This report expands the spectrum of disease-associated *CIAS1* mutations, suggests that the same mutation can be associated with different clinical syndromes, and supports the evidence that CAPS patients should always be screened for mutations outside exon 3.

Acknowledgment We thank the patients and their families for their kind collaboration with the study.

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