Chronic Infantile Neurological Cutaneous and Articular Syndrome Is Caused by Mutations in CIAS1, a Gene Highly Expressed in Polymorphonuclear Cells and Chondrocytes

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Chronic infantile neurological cutaneous and articular (CINCA) syndrome is a severe chronic inflammatory disease of early onset, characterized by cutaneous symptoms, central-nervous-system involvement, and arthropathy. In the present study, we report, in seven unrelated patients with CINCA syndrome, distinct missense mutations within the nucleotide-binding site of CIAS1, a gene encoding cryopyrin and previously shown to cause Muckle-Wells syndrome and familial cold urticaria. Because of the severe cartilage overgrowth observed in some patients with CINCA syndrome and the implications of polymorphonuclear cell infiltration in the cutaneous and neurological manifestations of this syndrome, the tissue-specific expression of CIAS1 was evaluated. A high level of expression of CIAS1 was found to be restricted to polymorphonuclear cells and chondrocytes. These findings demonstrate that CIAS1 missense mutations can result in distinct phenotypes with only a few overlapping symptoms and suggest that this gene may function as a potential inducer of apoptosis.

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with the Muckle-Wells syndrome (MWS [MIM 191900]) and familial cold autoinflammatory syndrome (FCAS [MIM 120100]) (Hoffman et al. 2001).

In the familial cases of CINCA syndrome that we have investigated, disease segregation was compatible with linkage to the CIAS1-containing genetic region on chromosome 1 (data not shown) (Cuisset et al. 1999), a finding that prompted us to search for mutations of CIAS1 in patients with CINCA. We have therefore studied seven unrelated white families with classic features of CINCA (table 1) and have sequenced the CIAS1 coding sequence (GenBank accession number AF427617), to search for the presence of mutations. Both strands of the CIAS1 coding sequence, as well as each exon/intron flanking sequence, were screened by direct sequencing of PCR fragments, through use of combinations of primer pairs described elsewhere (Hoffman et al. 2001). In these families, mutation analysis identified distinct missense mutations, all located in CIAS1 exon 3 (table 1 and fig. 2). In family 1, a T1718C de novo transition appeared in the proband, changing a phenylalanine into a serine codon (F573S). A C916A transition (leading to Q306L) was identified in the proband from family 2. In family 3, both patients and their affected father display a C1307A transition, leading to a threonine-to-asparagine codon change (T436N). A G907A transition changes an aspartic acid (a negative acidic residue highly conserved at this position among members of the NACHT subfamily [Koonin and Aravind 2000]) into an asparagine codon (D303N; fig. 2B) in patient 6 and in her affected father. A nucleotide transition was also found in patient 4 (A1073G, leading to H358R), in patient 5 (T1985C, leading to M662T), and in patient 7 (T926C, leading to F309S); these appeared as de novo mutations in these three probands. These mutations were not present in any of the parents of patients with sporadic cases, in any unaffected individual in these families (fig. 2A), or in >70 ethnically related healthy control individuals, which excludes the possibility that these mutations are com-

Table 1

<table>
<thead>
<tr>
<th>Proband in Family</th>
<th>Clinical Characteristics</th>
<th>DNA Base Change</th>
<th>Amino Acid Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Neonatal onset, skin lesion, chronic meningitis, joint inflammation, sensory organ impairment, dysmorphy</td>
<td>T1718C</td>
<td>F573S</td>
</tr>
<tr>
<td>2</td>
<td>Neonatal onset, skin lesion, chronic meningitis, joint inflammation, sensory organ impairment, dysmorphy</td>
<td>C916A</td>
<td>Q306L</td>
</tr>
<tr>
<td>3a</td>
<td>Onset at age 6 mo, skin lesion, chronic meningitis, joint inflammation, sensory organ impairment, dysmorphy</td>
<td>C1307A</td>
<td>T436N</td>
</tr>
<tr>
<td>3b</td>
<td>Onset at age 3 years, joint inflammation, sensory organ impairment, dysmorphy</td>
<td>C1307A</td>
<td>T436N</td>
</tr>
<tr>
<td>4</td>
<td>Neonatal onset, skin lesion, joint inflammation, sensory organ impairment, dysmorphy</td>
<td>A1073G</td>
<td>H358R</td>
</tr>
<tr>
<td>5</td>
<td>Onset during the first year of life, skin lesion, chronic meningitis, joint inflammation, sensory organ impairment, dysmorphy</td>
<td>T1985C</td>
<td>M662T</td>
</tr>
<tr>
<td>6</td>
<td>Neonatal onset, skin lesion, chronic meningitis, joint inflammation, sensory organ impairment, dysmorphy</td>
<td>G907A</td>
<td>D303N</td>
</tr>
<tr>
<td>7</td>
<td>Neonatal onset, skin lesion, chronic meningitis, joint inflammation, radiological severe arthropathy, sensory organ impairment, dysmorphy</td>
<td>T926C</td>
<td>F309S</td>
</tr>
</tbody>
</table>

* Precise clinical information was not available for the two patients from family 3 during the neonatal period or for the affected fathers in families 3 and 6.

* Numbers represent the base location in the cDNA sequences, where base 1 is the first base of the second ATG codon.
Figure 2  A, Mutation segregation in pedigrees of families with CINCA. Square symbols denote males; circles denote females; affected individuals are denoted by blackened symbols, and segregating CIAS1 mutations are denoted by the capital letter M. In each pedigree, family members from whom DNA samples were available for analysis are marked by an asterisk. Autosomal dominant inheritance of CINCA is suggested by the segregation observed in families 3 and 6. B, Mutation locations on CIAS1 in three patients and domain structure of CIAS1. Residues 1–90 form the N-terminal pyrin domain, residues 219–534 form the NBS/NACHT domain, and residues 740–991 form the C-terminal leucine rich repeats. Shown at the bottom is the sequence of the NBS domain around the consensus motif-III Mg$^{2+}$-binding site (boxed) found in the NACHT subfamily of NTPases (following Koonin and Aravind 2000) with the mutations (arrowheads) found in three families with CINCA. h = hydrophobic residues; u = tiny residues; n = negatively charged residues; p = polar residues.

CIA$S1$ encodes cryopyrin, a protein composed of two protein-protein interaction domains, an amino-terminal pyrin-like domain, and a carboxy-terminal domain containing leucine-rich repeats (“LLR” in fig. 2B) (Hoffman et al. 2001). In addition, cryopyrin includes a central nucleotide-binding site (NBS) domain that belongs to the NACHT subfamily of NTPases, which is characterized by the presence of seven distinct characteristic conserved motifs, including the ATP/GTPase-specific P loop and the Mg$^{2+}$-binding site (Koonin and Aravind 2000). Several other proteins from the CED4/Apaf1 family of proteins (Zou et al. 1997), including members of the caspase activation and recruitment domain family, share the same domain architecture (Hlaing et al. 2001; Martinon et al. 2001; Miceli-Richard et al. 2001). This family of proteins coordinates the assembly of signaling complexes that regulate the activation of NF-$\kappa$B, cytokine processing, and apoptosis (Martin 2001). They are thought to occupy a crucial position in stress-associated signaling pathways that culminate in cell death or inflammatory responses. Accordingly, the pyrin domain of CIA$S1$ (also known as “pyrin-containing Apaf1-like protein 1” [PYPAF1]) was recently shown to interact selectively with the pyrin domain of the apoptosis protein ASC, leading to the activation of NF-$\kappa$B (Manji et al. 2002), which suggests a function of CIA$S1$ related to inflammation.

All of the observed mutations in patients with CINCA fall within exon 3 of CIA$S1$; five of them modify the region encoding the NBS/NACHT domain, with a cluster of three mutations very close to the Mg$^{2+}$-coordinating aspartate (D300) (fig. 2B) (Koonin and Aravind 2000; Falcon-Perez et al. 2001) and thus potentially disrupting nucleotide binding-site function. Since this region is thought to contain binding sites for upstream
regulators, mutations in this region may affect the control of NF-κB activation.

A previous study has shown that expression of CIAS1 is restricted to peripheral blood leukocytes (Hoffman et al. 2001). Tissue expression of CIAS1 was examined, and CIAS1 was found to be strongly expressed in the PMNC, whereas it was undetectable or expressed at a lower magnitude in B and T lymphoblasts, respectively (fig. 3). Interestingly, high-level expression was also detected in chondrocytes (fig. 3)—a provocative finding, given the phenotype of patients with CINCA syndrome, since most of the clinical features result from the deleterious effect of PMNC infiltration of various organs and from manifestations such as growth retardation, cartilage burst, epiphyseal anomalies, late closure of the fontanel, and morphological anomalies (frontal bossing, saddle-back nose), which suggest a cartilage target. These data suggest that CIAS1 may function as an inducer of apoptosis in these cells. Thus, in addition to its role in NF-κB activation, CIAS1 may also be connected to the apoptosis pathway. Of great interest are the recent studies that highlight a potential dual role of NF-κB, as an inducer both of proinflammatory mediators during the onset of inflammation and of anti-inflammatory mediators and apoptosis during the resolution of inflammation (Lawrence et al. 2001; Karin and Lin 2002). An attractive hypothesis would be that mutations in the regulatory nucleotide-binding domain of CIAS1 affect the NF-κB activation pathway in a way that leads to a defective switching of NF-κB between these two functions. This hypothesis would be in agreement with the in vivo model of rat carrageenin-induced acute inflammation, in which inhibition of NK-κB during the resolution of inflammation protracts inflammatory responses and prevents apoptosis (Lawrence et al. 2001).

The association of chronic inflammation with recurrent fever, cutaneous rash, and joint symptoms is a common characteristic of severe disorders within the group of autoinflammatory diseases. However, CINCA syndrome is an original entity defined by neonatal onset and a unique cluster of manifestations, including chronic PMNC meningitis, as well as the severe and unique joint manifestations, characterized by symmetrical epiphyseal and metaphyseal cartilage anomalies. These features differentiate CINCA from FCAS (Zip et al. 1993) and MWS (Muckle 1979), recently shown to result from missense mutations in the same exon of CIAS1 (Hoffman et al. 2001). None of the mutations identified in the patients with CINCA reported in the present study have so far been associated with FCAS or MWS. The mutations occur de novo in five of the seven patients with CINCA syndrome, a higher proportion than that observed in the families with FCAS and MWS that have previously been reported (2/4). Although these data are not statistically significant, the early onset and severity of CINCA syndrome, which can be fatal in some patients, in comparison with the delayed and milder phenotype occurring in FCU/FCAS, may favor the occur-

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**Figure 3**  
Semiquantitative RT-PCR of CIAS1 mRNA expression in various tissues with β-actin levels shown below. RNAs (50ng) from each tissue was reverse transcribed and PCR amplified by a one-step RT-PCR using the following oligonucleotides: 5′-TTGAAGAGGAGTGGAGGTT-3′ (sense on exon 2) and 5′-GATGGGTGGGTTTGGGTC-3′ (antisense on exon 3), which predict a 507-bp product of CIAS1. β-actin oligonucleotides, 5′- TACCACTGGCATCGTGATGGACT-3′ (sense on exon 2) and 5′-TCCTTCTGCATCCTGTCGGCAAT-3′ (antisense on exon 3), were used in the same conditions as a control for RNA integrity and RT efficiency. A negative control containing no input RNA was also included.
rence of de novo mutations. The analysis of a greater number of patients with each condition is required to validate this preliminary observation and to determine whether disease expression strictly depends on the nature and localization of the CIAS1 mutation, or whether it is also influenced by still-undefined environmental or genetic factors.

In conclusion, we show that CIAS1 mutations can lead to very distinct phenotypes among the autoinflammatory diseases, including CINCA syndrome. Given the peculiar phenotype observed in patients with CINCA, in terms of PMNC activation and cartilage overgrowth, the high level of expression of CIAS1 that we show to be restricted to these two types of cells strongly supports the hypothesis of a role of CIAS1 in apoptosis. Since CIAS1 was recently shown in vitro to activate NF-κB, we speculate that mutations in CIAS1 may disturb the physiological balance between the proinflammatory and proapoptotic function of NK-κB, by favoring the former. Further studies will be necessary to better identify this anti-inflammatory pathway. Its elucidation may provide a potential new target for the treatment of inflammatory disease.

Acknowledgments

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Electronic-Database Information

Accession numbers and URLs for data presented herein are as follows:


Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for FMF [MIM 249100], TNF-receptor-associated periodic syndrome [MIM 142680], HID [MIM 260920], MWS [MIM 191900], and FCAS [MIM 120100])

References


