

Report

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.

Chronic infantile neurological cutaneous and articular (CINCA) syndrome, also known as "neonatal onset multisystemic inflammatory disease," is a rare congenital inflammatory disorder characterized by a triad of (1) neonatal onset of cutaneous symptoms, (2) chronic meningitis, and (3) joint manifestations with recurrent fever and inflammation (Prieur and Griscelli 1981; Hassink and Goldsmith 1983; Prieur et al. 1987; Torbiak et al. 1989; Prieur 2001). Persistent and migratory skin rash associated with skin perivascular polymorphonuclear infiltrates is present in patients, starting at birth. A progressive neurological impairment results from chronic meningitis caused by polymorphonuclear cell (PMNC) infiltration. A progressive visual defect and perceptive deafness frequently occurs with increasing age. Joint symptoms manifest as recurrent joint flares with or without severe ra-

diologically evident modifications involving the growth cartilage or bone epiphysis (fig. 1). In addition, common morphological features (fig. 1), a shortening of distal limbs, and growth retardation give a sibling-like resemblance between unrelated patients. Intrafamilial recurrence, although much rarer than sporadic cases, is suggestive of an autosomal dominant inheritance pattern. However, the small number of familial cases have until now impeded a genomewide linkage approach. Recently, several genes involved in recurrent inflammatory syndromes, which also are associated with intermittent episodes of cutaneous rash, arthralgia, and fever, have been localized and identified. These include the *TNFRSF1A* gene on chromosome 12p13, associated with familial Hibernian fever (also called "TNF-receptor-associated periodic syndrome" [MIM 142680]) (McDermott et al. 1999); the *MEFV* gene on chromosome 16p13.3, associated with familial Mediterranean fever (FMF [MIM 249100]) (The French FMF Consortium 1997; The International FMF Consortium 1997); the *MVK* gene on chromosome 12q24, associated with hyper-IgD syndrome (MIM 260920) (Drenth et al. 1999; Houten et al. 1999); and the *CIAS1* gene on chromosome 1q44, associated

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Figure 1 Typical CINCA syndrome features. A, Facial appearance of patient 6 at age 12 years, with characteristic frontal bossing and protruding eyes. B, Radiological bone and joint modifications. Bilateral severe bone deformities of the knees of patient 7 at age 2.5 years, resulting in hard bony enlargement without any suggestion of synovial thickening on palpation. Arrows show the growth cartilage burst and increased irregular patellar opacity.

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 stud-

ied 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, was 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
CINCA Cases and Results of Mutations Analysis of *CIAS1*

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

^a 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.

^b Numbers represent the base location in the cDNA sequences, where base 1 is the first base of the second ATG codon.

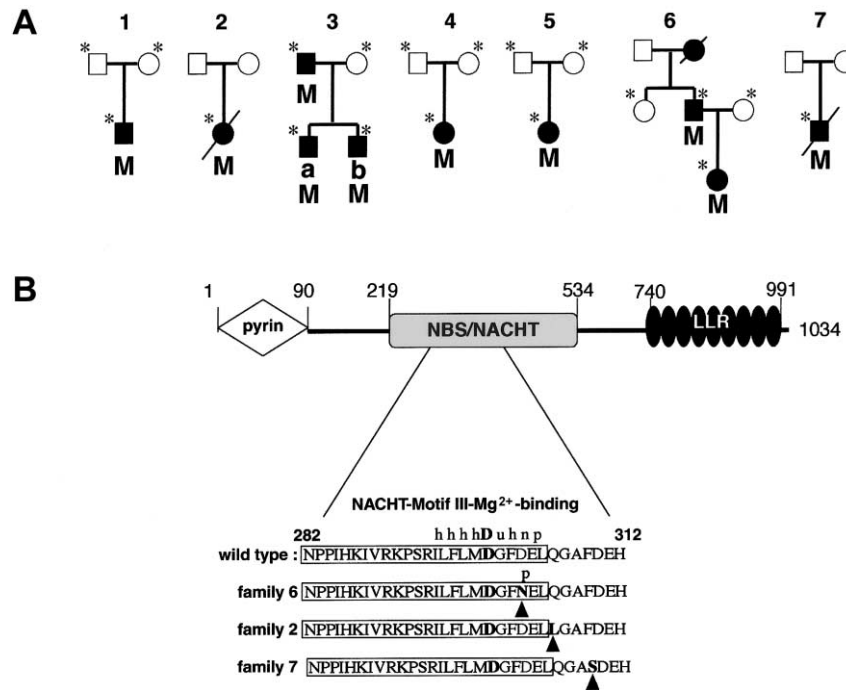


Figure 2 A, Mutation segregation in pedigrees of families with CINCA. Square symbols denote males; circles denote females; affected individuals are denoted by blackened symbols, and segregating *CIAS1* mutations are denoted by the capital letter M. In each pedigree, family members from whom DNA samples 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 Mg²⁺-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.

mon polymorphisms. No mutation could be identified in an additional patient with a diagnosis of CINCA syndrome who presented with neonatal onset of skin lesions, joint inflammation, and radiologically severe arthropathy but without detectable chronic meningitis at the time of the study. Although this result does not rule out the possibility that there is a mutation elsewhere in the sequence that affects mRNA translation, this may alternatively suggest either genetic heterogeneity of this disease or the occurrence of an acquired form in this sporadic case.

CIAS1 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 Mg²⁺-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- κ 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 *CIAS1* (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- κ B (Manji et al. 2002), which suggests a function of *CIAS1* related to inflammation.

All of the observed mutations in patients with CINCA fall within exon 3 of *CIAS1*; five of them modify the region encoding the NBS/NACHT domain, with a cluster of three mutations very close to the Mg²⁺-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

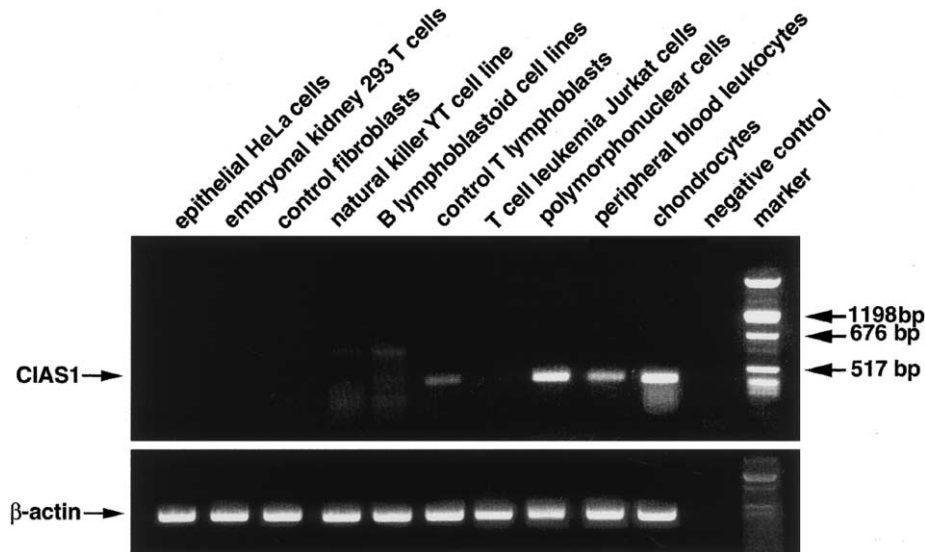


Figure 3 Semiquantitative RT-PCR of *CIAS1* mRNA expression in various tissues with β -actin levels shown below. RNAs (50ng) from each tissue was reverse transcribed and PCR amplified by a one-step RT-PCR using the following oligonucleotides: 5'-TTGAAGAGGAGTGGATGGGT-3' (sense on exon 2) and 5'-GATGGGTGGGTTGGGTC-3' (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.

regulators, mutations in this region may affect the control of NF- κ B 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- κ B 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- κ B, 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- κ B activation pathway in a way that leads to a de-

fective switching of NF- κ B between these two functions. This hypothesis would be in agreement with the in vivo model of rat carageenin-induced acute inflammation, in which inhibition of NF- κ B 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-

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- κ B, we speculate that mutations in *CIAS1* may disturb the physiological balance between the proinflammatory and proapoptotic function of NF- κ B, 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:

GenBank, <http://www.ncbi.nlm.nih.gov/Genbank/> (for *CIAS1* [accession number AF427617])

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])

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Errata

In the February 2002 issue of the *Journal*, in the article “Genomic Screening of Fibroblast Growth-Factor Receptor 2 Reveals a Wide Spectrum of Mutations in Patients with Syndromic Craniosynostosis,” by Kan et al. (70:472–486), the following errors occurred: (1) on the

10th line in the left hand column on page 476, “mM” should read “ μ M,” in both cases; and (2) in table 4, the exon 15 polymorphism “1673-47G→A” should be “1673-49G→A,” and “1673-10C→T” should be “1673-12C→T.” The authors regret these errors.

In the July 2002 issue of the *Journal*, in the article “Chronic Infantile Neurological Cutaneous and Articular Syndrome Is Caused by Mutations in *CIAS1*, a Gene Highly Expressed in Polymorphonuclear Cells and

Chondrocytes,” by Feldmann et al. (71:198–203), in table 1, the amino acid change in the second row (family 2) should be Q306K. The authors regret this error.

In the October 2002 issue, the *Journal* published a letter entitled “Increased Rate of Twins among Affected Sib Pairs,” by Peter Visscher (71:995–996), and a response to this letter by Hodge et al. (71:996). The Hodge et al. response discussed, among other things, an error in a

formula in Dr. Visscher’s letter. This error was corrected in Dr. Visscher’s letter before publication, but, because of an oversight by the *Journal*, Hodge et al. were not given an opportunity to alter their response accordingly. The *Journal* regrets this error.

In the June 2002 issue of the *Journal*, in the article “A Comprehensive Mutation Analysis of *RP2* and *RPGR* in a North American Cohort of Families with X-Linked Retinitis Pigmentosa,” by Breuer et al. (70:1545–1554), the authors have identified errors in the numbering of *RPGR-ORF15* mutations, in table 4, that were due to the use of prepublication sequence from Vervoort and colleagues (Vervoort R, Lennon A, Bird AC, Tulloch B, Axton R, Miano MG, Meindl A, Meitinger T, Ciccodicola A, Wright AF [2000] Mutational hot spot within a new *RPGR* exon in X-linked retinitis pigmentosa. *Nat Genet* 25:462–466). These errors have now been identified and corrected, and a revised version of table 4 is presented below. (We thank Dr. Dror Sharon for bringing this to our attention.) The authors regret the errors.

Table 4

Mutations in *RPGR-ORF15* in Affected Hemizygotes with XLRP

Mutation ^a	Protein Change ^b	No. of Patients Affected
Deletion:		
432delG	Glu144fsTer230	1
483-4delGA	Glu161fsTer182	9
481-4delGAGA	Arg160fsTer229	1
499-502delAGGA	Lys166fsTer229	1
503-6delGGGA	Gly168fsTer231	1
570-1delAG	Arg190fsTer248	1
631delA	Glu210fsTer229	1
652-3delAG	Glu217fsTer248	7
673-4delAG	Glu224fsTer248	2
689-92delAGAG	Glu230fsTer233	3
1184-5delGG	Glu395fsTer493	1
1244-5delGG	Glu415fsTer493	1
1339-40delAG	Glu446fsTer493	1
Insertion:		
185ins, 5bp	Ala61fsTer111	1
352-460dup		1
Nonsense:		
369G→T	Glu123Ter	1
393G→T	Glu131Ter	1
423G→A	Glu141Ter	3
507G→T	Glu169Ter	3

^a Nucleotide positions and nomenclature are based on the work of Vervoort et al. (2000).

^b Frameshift mutations are designated according to the following example: Glu144fsTer230 refers to a frameshift mutation in which Glu144 is the first amino acid altered, with termination of the ORF at residue 230.