# REPORT

# Mutations in Cytokine Receptor-Like Factor 1 (*CRLF1*) Account for Both Crisponi and Cold-Induced Sweating Syndromes

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Crisponi syndrome is a rare autosomal recessive disorder characterized by congenital muscular contractions of facial muscles, with trismus in response to stimuli, dysmorphic features, bilateral camptodactyly, major feeding and respiratory difficulties, and access of hyperthermia leading to death in the first months of life. The overlap with Stüve-Wiedemann syndrome (SWS) is striking, but the two conditions differ in that congenital lower limb bowing is absent in Crisponi syndrome, whereas it is a cardinal feature of SWS. We report here the exclusion of the leukemia inhibitory factor receptor gene in Crisponi syndrome and the identification of homozygote or compound heterozygote cytokine receptor-like factor 1 (*CRLF1*) mutations in four children from three unrelated families. The four mutations were located in the immunoglobulin-like and type III fibronectin domains, and three of them predicted premature termination of translation. Using real-time quantitative polymerase chain reaction, we found a significant decrease in *CRLF1* mRNA expression in patient fibroblasts, which is suggestive of a mutation-mediated decay of the abnormal transcript. CRLF1 forms a heterodimer complex with cardiotrophin-like cytokine factor 1, and this heterodimer competes with ciliary neurotrophic factor for binding to the ciliary neurotrophic factor receptor (CNTFR) complex. The identification of *CRLF1* mutations in Crisponi syndrome supports the key role of the CNTFR pathway in the function of the autonomic nervous system.

Crisponi syndrome (MIM 601378) is a rare autosomal recessive disorder characterized by congenital contractions of facial muscles, with trismus in response to stimuli or during crying; dysmorphic features, including round face, broad nose with anteverted nostrils, small mouth, and micrognathia; and bilateral camptodactyly. Major feeding and respiratory difficulties with access of hyperthermia occur in the course of the disease and usually lead to death in the first months of life. In the rare survivors, hyperexcitability disappears in the first years of life, but children develop scoliosis and psychomotor retardation.[1–3] So far, the disease has been reported in a total of 19 newborns from Sardinian, Italian, and Portuguese families.

The overlap of Crisponi syndrome with Stüve-Wiedemann syndrome (SWS, or Schwartz-Jampel syndrome type 2) is striking, since both conditions cause contractures at birth, pursed appearance of the mouth, camptodactyly, feeding and respiratory difficulties, hyperthermia, and progressive kyphoscoliosis.[4,5] However, congenital bowing of the lower limbs with internal cortical thickening and metaphyseal changes (which are cardinal features of SWS) have never been reported in newborn patients with Crisponi syndrome.

A total of four children belonging to three unrelated families were included in the study. The first patient was born to unrelated Sardinian parents (family 1), at 28 wk of gestation by cesarean section (birth weight 980 g). She presented at birth with facial muscle contractions, camptodactyly, and feeding difficulties, and she had major access of hyperthermia during her 1st year of life. Muscle biopsy and skeletal x-ray results were normal. Hyperexcitability and hyperthermia episodes disappeared after age 1 year. She then developed scoliosis, requiring surgery at age 12 years, and mild developmental delay with attention deficit disorder, requiring special schooling. She is now aged 13 years, and growth parameters are at $<-2$ SD.

The second patient was born to Yemenite parents who were first cousins (family 2). She was first given a diagnosis of neonatal Schwartz-Jampel syndrome type 2, since she presented suggestive dysmorphic features, pursed appearance of the mouth during crying, bilateral camptodactyly, poor suckling, and swallowing difficulties, but no bowing of the lower limbs. The family was then lost to follow up.

Patients 3 and 4 were first cousins born to related Gypsy parents (family 3). Patient 3 presented at birth with camptodactyly, overlapping toes, joint contractures of elbows, contractions of facial muscles with trismus, major feeding difficulties, and dysmorphic features (i.e., small nose with anteverted nostrils, small mouth, short neck, and low-set ears). He had transient increase of plasma creatine kinases but normal x-ray results. In the course of the disease, hyperexcitability disappeared and feeding difficulties im-

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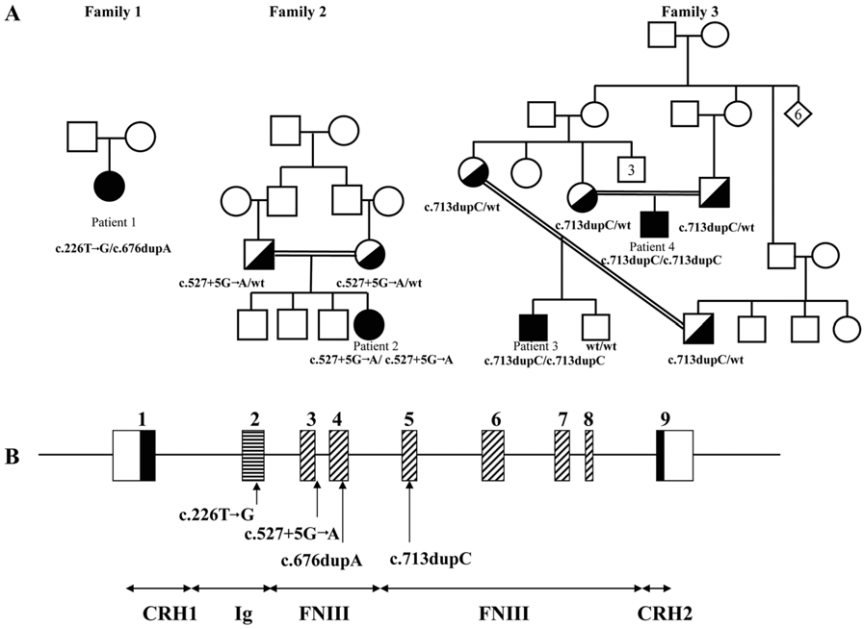


**Figure 1.** *A,* Pedigrees and segregation of the *CRLF1* mutations identified in the three families with Crisponi syndrome. *B,* Exon-intron structure of the *CRLF1* gene. Mutations are located in the region encoding the Ig and FNIII domains. wt = Wild type.

proved, but he presented iterative access of hyperthermia (body temperature >42°C) until age 3 years and then profuse sweating of the back. Kyphoscoliosis appeared at age 1 year. He is now aged 6 years (growth parameters at −2 SD) and has some speech delay. Results of brain magnetic resonance imaging, heart ultrasound, chromosome analysis, and skeletal muscle biopsy were normal. Patient 4 is the first cousin of patient 3 (fig. 1). He also presented at birth with facial muscle contractions, camptodactyly, elbow contractures, and feeding difficulties, and he developed seizures and temperature instability with access of hyperthermia and kyphoscoliosis.

Blood samples and skin fibroblasts (from patients 1, 3, and 4) were obtained with the written consent of the patients and relatives. For patient 1, samples from the parents were not available.

We first considered the leukemia inhibitory factor receptor gene (*LIFR,* or gp190 [GenBank accession number NM_002310]), the gene responsible for SWS, as a possible candidate gene. Binding of the leukemia inhibitory factor (LIF) to the LIFR complex (gp130-gp190 [GenBank accession number NP_002301]) is known to induce signaling through the Janus kinase (JAK)/signal transducers and activators of transcription (STAT) and mitogen-activated protein kinase pathways (fig. 2). The activation of STAT3 in response to LIF was therefore tested in patients with Crisponi syndrome (patients 1, 3, and 4) and in a patient with SWS carrying *LIFR* mutations. The cells were incubated in a serum-free medium for 15 min with 20 ng/ml LIF, as described elsewhere.[5] Western-blot analysis of fibroblast lysates with use of an antiphospho-STAT3 antibody showed that LIF normally triggered STAT3 phosphorylation in patients with Crisponi syndrome, whereas STAT3 failed to be phosphorylated in response to LIF in fibroblasts from a patient with SWS (data not shown). For patient 2, skin fibroblasts were not available. A series of 42 intronic primers were therefore used to amplify the 20 coding exons of *LIFR,* and results from the direct sequencing of *LIFR* were normal.

On the basis of the recent identification of cytokine receptor-like factor 1 (*CRLF1* [GenBank accession number NM_004750]) mutations in three families with cold-induced sweating syndrome (CISS [MIM #272430]),[6,7] we then considered *CRLF1* as a possible candidate gene in Crisponi syndrome. CRLF1 (GenBank accession number NP_004741) forms a heterodimer complex with cardiotrophin-like cytokine factor 1 (CLCF1 [GenBank accession number NP_037378]), and this heterodimer competes with ciliary neurotrophic factor (CNTF) for binding to the ciliary neurotrophic factor receptor (CNTFR [GenBank accession number NP_671693]) complex, which is composed of IL-6ST (gp130 [GenBank accession number NP_786943]), LIFR (gp190), and CNTFR (fig. 2).[8] Binding of the heterodimer to the receptor activates the JAK/STAT signaling pathway. The *CRLF1* gene is composed of nine exons and encodes a 422-aa protein comprising one immunoglobulin (Ig)–like domain and two fibronectin type III (FNIII) domains. A series of 18 primers was designed to amplify the nine coding exons of *CRLF1,* and a total of four distinct mutations were identified in the three families: c.226T→G and c.676dupA (p.W76G and p.Thr226AsnfsX104), c.527+5G→A/c.527+5G→A,

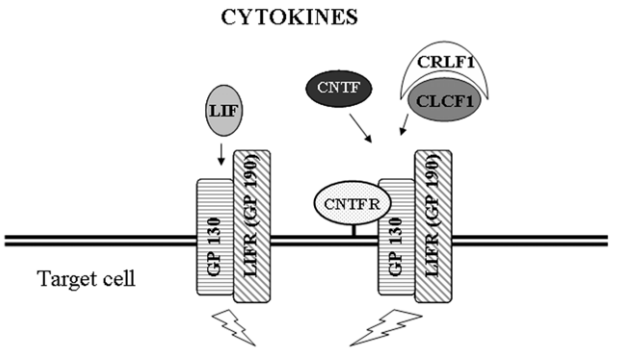


**Figure 2.** Schematic representation of the LIFR and CNTFR pathways (from the work of Plun-Favreau et al.[8]). Binding of LIF to the LIFR complex (gp130-gp190) and binding of CNTF or CRLF1-CLCF1 to the CNTFR complex (CNTFR-gp130-gp190) activate the JAK/STAT pathway.

and c.713dupC/c.713dupC (p.Asp240AlafsX91/p.Asp240AlafsX91) (fig. 1). The mutations were located in the regions encoding the Ig-like domain and the two FNIII domains of the protein, and three of them predicted premature termination of translation. The base changes were not identified in 210 control chromosomes.

We then analyzed the pattern of expression of *CRLF1* in tissues presumably involved in Crisponi syndrome. Total RNA was extracted from human tissues or primary cultured cells (i.e., muscle, chondrocytes, fibroblasts, and adult brain) with use of the RNeasy Mini Kit (Qiagen). RT-PCR analysis of *CRLF1* RNA transcripts detected a single amplification product in muscle, chondrocytes, fibroblasts, and brain from controls. By contrast, RT-PCR analysis of the fibroblast RNA transcripts detected only a weak signal in patients 1, 3, and 4 (fig. 3). We finally performed a real-time quantitative PCR, using LightCycler technology (Roche Manheim). RT-PCR was performed using the following primers: forward 5′-TACCAGATCCGCTACCGAGTG-3′ and reverse 5′-GCTCCACTCACTCCAGATCC-3′ (product length 180 bp). The experiments were performed two times. In each experiment, samples were run in triplicate. The amounts of *CRLF1* mRNA were normalized to the amount of $\beta$-actin mRNA measured in each sample. The expression of *CRLF1* mRNA appeared significantly lower in the three patients, with 13%–21% of control values (fig. 4). These findings were highly suggestive of a mutation-mediated decay of the abnormal transcript.

Mutations of both *CRLF1* and *CLCF1* (GenBank accession number NM_013246) have been recently identified in CISS, which is characterized by profuse sweating induced by cool temperatures, nasal voice, depressed nasal bridge, inability to fully extend elbows, and kyphoscoliosis.[6,7,9] This entity is distinct from Crisponi syndrome, in that it has a later onset, a less severe outcome, no facial muscle contractions or trismus, and no hyperthermia (body temperature >42°C) in the neonatal period. The reported patients with CISS ranged in age from 13 years to 46 years, whereas, among the 22 patients with Crisponi syndrome, only 6 are still alive. The neonatal features of Crisponi syndrome (i.e., hyperthermia, facial contractures, trismus, and feeding difficulties) disappear after the first years of life, and the clinical course becomes quite similar to that of CISS (i.e., flexion deformities of the fingers, progressive kyphoscoliosis, and cold-induced sweating). This last feature was observed in only one of four patients from our series; however, another patient of the four was lost to follow up, and the development of paradoxical sweating in the course of the disease cannot be excluded.

Whether Crisponi syndrome and CISS are allelic disorders or parts of a single entity described at different stages is questionable. Among the four mutations identified in Crisponi syndrome, three are splice-site and frameshift mutations, whereas, among the five mutations identified in CISS, three are missense mutations. Moreover, the two brothers with CISS reported with homozygous frameshift deletions have an early-onset form of CISS with neonatal manifestations (i.e., feeding difficulties and difficulty opening mouth).[6] These findings may suggest that nonsense mutations are associated with the severe neonatal manifestations of Crisponi syndrome, whereas missense mutations are associated with late-onset CISS.

The finding of *CRLF1* mutations in Crisponi syndrome further illustrates the key role of the CNTFR pathway in the function of the autonomous nervous system. Indeed, Crisponi syndrome is mainly characterized by dysautonomia symptoms—that is, disturbance in temperature regulation; neonatal feeding, swallowing, and respiratory difficulties; trismus and contractions of facial muscles; camptodactyly; and paradoxical sweating. All these symptoms are also observed in SWS. By contrast, Crisponi

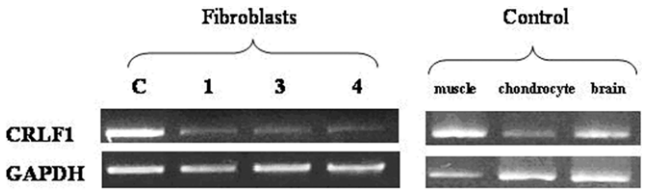


**Figure 3.** RT-PCR detection of *CRLF1* transcripts. RT-PCR analyses were done using RNA isolated from control muscle, chondrocytes, and brain from fibroblasts of controls (C) and patients (1, 3, and 4). Thirty PCR cycles were performed at an annealing temperature of 60°C to amplify a 381-bp fragment specific to the *CRLF1* gene (forward primer 5′-AAGAACATGAAGGACTTGACCTG-3′; reverse primer 5′-GTATTTGGCTTGAAAGAGGAAATC-3′). Sense and antisense primers used for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) amplification were 5′-AGACAGCCGCATCTTCTTGT-3′ and 5′-CCACAGTCTTCTGAGTGGCA-3′ (product length 587 bp). The specific RNA transcript was found in control tissues but was barely detectable in Crisponi syndrome fibroblasts.

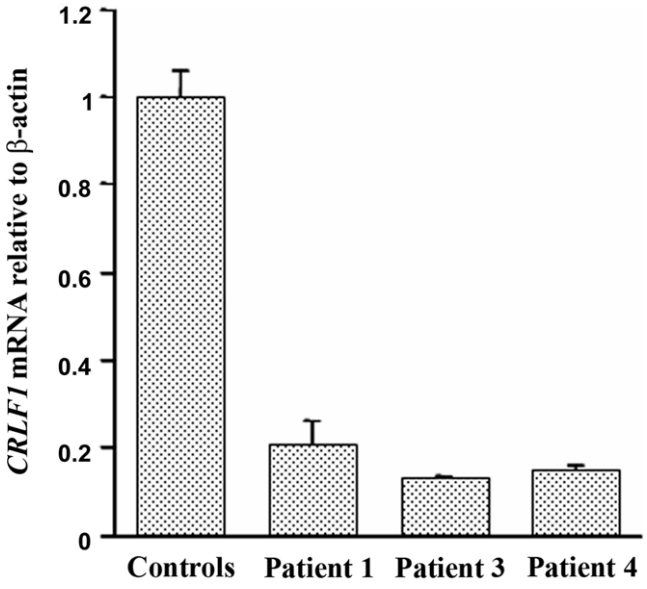


**Figure 4.** Quantitative analysis of *CRLF1* gene expression in fibroblasts from patients 1, 3, and 4. The relative amounts of *CRLF1* mRNA levels compared with $\beta$-actin mRNA levels were determined for controls and for the three patients. The results shown are means $\pm$ SDs of two independent experiments. In each experiment, samples were run in triplicate.

syndrome is not characterized by congenital bowing of the lower limbs, a characteristic feature of SWS. Hence, dysautonomia symptoms observed in SWS would seem to result from the impairment of the CNTFR pathway, whereas the bone and cartilage manifestations characteristic of SWS (and not observed in Crisponi syndrome) would seem to be due to the specific impairment of the LIFR pathway.

CRLF1 is a soluble cytokine receptor that associates with CLCF1 to form a soluble functional heteromeric ligand and competes with CNTF for the binding to CNTFR.[10] CNTF has an ability to maintain the survival of parasympathetic neurons of chick ciliary ganglions, but mice lacking CNTF appear remarkably normal, and CNTF homozygous mutations have been identified in healthy humans.[11] CRLF1-deficient mice die shortly after birth because of a lack of suckling and also exhibit a significant reduction in facial and lumbar motor neurons.[12] Similarly, mice lacking CNTFR exhibit profound motor neuron deficits at birth and die perinatally.[13] The identification of *CRLF1* mutations in a severe congenital syndrome mainly characterized by dysautonomic symptoms supports the key role of the CNTFR pathway in the autonomic nervous system function. We conclude that the proper binding of CLCF1-CRLF1 to CNTFR is a major event for the autonomic function and that impairment of this pathway is associated with both Crisponi syndrome and CISS in humans.

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## Web Resources

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

GenBank, http://www.ncbi.nlm.nih.gov/Genbank/ (for *LIFR* [accession number NM_002310], LIFR [accession number NP_002301], *CRLF1* [accession number NM_004750], CRLF1 [accession number NP_004741], CLCF1 [accession number NP_037378], CNTFR [accession number NP_671693], IL-6ST [accession number NP_786943], and *CLCF1* [accession number NM_013246])

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for Crisponi syndrome and CISS)

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