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Differential secretion of the mutated protein is a major component affecting phenotypic severity in CRLF1-associated disorders

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ABSTRACT

Crisponi syndrome (CS) and cold-induced sweating syndrome type 1 (CISS1) are disorders caused by mutations in CRLF1. The two syndromes share clinical characteristics such as dysmorphic features, muscle contractions, scoliosis and cold-induced sweating, with CS patients showing a severe clinical course in infancy involving hyperthermia, associated with death in most cases in the first years of life.

To evaluate a potential genotype/phenotype correlation and whether CS and CISS1 represent two allelic diseases or manifestations at different ages of the same disorder, we performed a detailed clinical analysis of 19 patients carrying mutations in CRLF1. We studied the functional significance of the mutations found in CRLF1, providing evidence that phenotypic severity of the two disorders mainly depends on altered kinetics of secretion of the mutated CRLF1 protein. Based on these findings we believe that the two syndromes, CS and CISS1, represent manifestations of the same disorder, with different degrees of severity. We suggest to rename the two genetic entities CS and CISS1 with the broader term of Sohar-Crisponi syndrome.

KEYWORDS

Crisponi syndrome, cold-induced sweating, hyperthermia, CRLF1.
INTRODUCTION

Mutations in CRLF1 (cytokine receptor-like factor 1) account for both Crisponi syndrome (CS; MIM#601378) and cold-induced sweating syndrome type 1 (CISS1; MIM#272430).

Crisponi syndrome was initially described in 1996, in 17 patients from 12 different families in southern Sardinia.\textsuperscript{1} Further patients have been reported later.\textsuperscript{2-8} The syndrome usually manifests at birth, when patients present with hyperthermia and abnormal paroxysmal contractions of the facial and oropharyngeal muscles, as well as feeding and respiratory difficulties often requiring the use of nasogastric feeding. Physical dysmorphisms such as a large face, broad nose and camptodactyly have been described in most of the patients.\textsuperscript{3-5,7,8} Hyperthermia is frequently associated with death within the first months of life. Feeding difficulties and hyperthermia often resolve after infancy in the rare surviving patients, who then develop scoliosis and sometimes psychomotor retardation. In pre-adolescent patients, evidence of cold-induced sweating was reported.\textsuperscript{3}

Cold-induced sweating syndrome type 1 (CISS1) was first reported in two Israeli sisters in 1978.\textsuperscript{9} It involves paradoxical sweating at cold ambient temperatures on the upper part of the body, along with progressive scoliosis. In both sisters, dysmorphic features including a high arched palate, nasal voice and joint contractures have been observed. Two Norwegian brothers and a Canadian woman with a similar phenotype have been described more recently.\textsuperscript{10,11} CS and CISS1 belong to a group of genetic disorders with similar phenotypes associated with mutations of genes in the ciliary neurotrophic factor receptor (CNTFR) pathway, which is known to be important for development and maintenance of the nervous system and
This group includes cold-induced sweating syndrome type 2 (CISS2; MIM#610313), caused by mutations in \textit{CLCF1},\textsuperscript{10,13} and Stüve-Wiedemann syndrome (SWS; MIM#601559), caused by mutations in \textit{LIFR}.\textsuperscript{14} Mutations in \textit{CLCF1} lead to a phenotype similar to mutations in \textit{CLRF1}, with cold-induced sweating, scoliosis and cubitus valgus.\textsuperscript{10,13} Clinical features of SWS also include camptodactyly, feeding difficulties, scoliosis and temperature instability, also present in the other syndromes, but the characteristic bowing of the long bones is not present in CS, CISS1 and CISS2.\textsuperscript{14-19} Clinical similarities between Crisponi syndrome and cold-induced sweating syndrome type 1, along with the involvement of the same gene (\textit{CRLF1}), led to the question whether these two syndromes represent two allelic diseases or, in fact, manifestations of the same disorder, reported at different ages of affected patients.\textsuperscript{1,2}

In an effort to delineate the specific clinical features attributed to CS and CISS1, we investigated the clinical history, physical characteristics and experimental data of 19 patients with mutations in \textit{CRLF1}, 14 of them classified as CS (9 reported and 5 unreported cases) and 5 as CISS1 (all reported),\textsuperscript{9-11} by means of a standardized questionnaire.

Because a genotype/phenotype correlation has not been found in former studies\textsuperscript{2,3}, we attempted to carry out a detailed analysis of the clinical phenotype and compare it with the genetic and functional data from the investigation of the \textit{CRLF1} gene and protein for all the probands analyzed.

We found that phenotypic severity of \textit{CRLF1}-associated disorders depends on altered kinetics in the secretion of the mutated CRLF1 protein, suggesting that CS and CISS1 are manifestations of the same disease.
MATERIALS AND METHODS

Patients and clinical data

Inclusion in the study was based on the diagnosis of CS or CISS1 with proven mutations on both alleles of the CRLF1 gene. Diagnosis of either syndrome was established based on compilation of symptoms: 14 patients diagnosed with CS and 5 patients diagnosed with CISS1 were included in the study. The 14 patients classified as CS (9 females, 5 males) originated from 11 families from Italy, Turkey, Spain and Libya (Table 1). The 5 patients (3 females, 2 males) classified as CISS1 originated from 3 families from Norway, Canada and Israel and showed a heterogenic phenotype (Table 1). The study protocol was approved by the Münster University Hospital Ethical Committee in Germany and all subjects involved in this study gave informed written consent.

Clinical data and patient history were gathered through a standardized questionnaire, which was sent to the responsible clinicians and through review of the clinical data presented in the literature. Data on facial and skull height, as well as nasal and philtrum length were obtained from a subset of probands, measurements were plotted according to the Handbook of Normal Physical Measurements, 1989.20

DNA extraction and mutation analysis

Genomic DNA was isolated from peripheral blood by a salting-out protocol. All the nine coding exons and surrounding intronic regions of CRLF1 (GenBank accession number NM_004750U) were amplified by PCR using specific primers3, and the amplicons subsequently analyzed by direct sequencing (ABI3130XL, Applied Biosystems).
DNA cloning

The clone pCMV6-XL5CRLF1 (Origene cat.TC126412 NM_004750.2) was used to subclone the cDNA encoding the human CRLF1 in the PEF5HA vector (provided by Doctor Nunzio Bottini, La Jolla Institute for Allergy and Immunology, CA) using EcoRI and XbaI restriction enzymes. The clone pCMV6-XL5CLCF1 (Origene cat.TC122789 NM_013246.2) was used to be co-transfected with PEF5HA-CRLF1, wild type and mutated, in COS-7 cells.

CRLF1 Site-directed Mutagenesis

The PEF5HA-CRLF1 clone was subjected to site-directed mutagenesis using the QuickChange™ site-directed mutagenesis kit (Stratagene) according to the manufacturer’s instructions. Primer sequences are available on request.

Cell culture

COS-7 cells were maintained in Dulbecco’s Modified Eagle’s Medium (DMEM; Gibco-Invitrogen CA, USA) supplemented with 10% fetal bovine serum, 100U/ml penicillin, 100µg/ml streptomycin and 2mM glutamine (Gibco Invitrogen). IMR32 neuroblastoma cell lines (DSMZ ACC165) were grown in RPMI 1640 (Gibco Invitrogen) supplemented with 10% fetal bovine serum, 100U/ml penicillin, 100µg/ml streptomycin and 2mM glutamine, 1% essential amino acids, 1% sodium pyruvate (Gibco Invitrogen).
**DNA transfection and cell lysis**

COS-7 cells were transfected using Lipofectamine 2000 reagent (Invitrogen) according to the manufacturer’s instructions and after 24 h, 36 h, 48 h and 72 h were lysed with Lysis Buffer, (150mM NaCl, 50mM TrisHCl pH 7.5, 1mM EDTA, 1% Triton X-100, 0.1% Sodium deoxycholate, 4mM pefablock ) containing Complete Protease Inhibitor Cocktail (Roche).

**Western blotting**

Protein concentrations were determined by spectrophotometer, using Bio-Rad Protein Assay (BIO-RAD). Then 50µg of lysates and 30µl of supernatants were submitted to SDS-PAGE with 9% polyacrylamide gels and transferred to PVDF membranes Hybond-P (Amersham) by electroblotting. The membranes were blocked in PBS with 1% Tween 20 (PBS-T) and with 5% of milk for 1 h at room temperature and then incubated with the anti-CRLF1 (mouse monoclonal Abcam ab26125) or anti-CLCF1 (rabbit polyclonal Abcam ab56500) antibodies overnight at 4°C, with a dilution 1:500 and 1:1000 respectively. The membranes were washed 3 times for 10 minutes in PBS-T and incubated in the appropriate secondary antibody for 1 h at room temperature. After washing, the blots were developed by chemiluminescence using ECL plus reagent (Amersham Biosciences), according to the manufacturer’s instructions. To perform densitometric analysis to evaluate CRLF1 secretion level, we used Image J software (http://rsbweb.nih.gov/ij/). We defined a rectangular shaped area surrounding each band, and keeping this area constant we measured the corresponding mean of intensities. We then subtracted the values of
background area and calculated the percentage rate related to the mean of
the intensities between supernatants and lysates. The level of wild type
CRLF1 secretion in the supernatants was considered as 100%. The result is
representative of at least three independent experiments.

**Tyrosine Phosphorylation Analysis**

After a 24 h serum starvation, IMR32 cells were stimulated for 10 min with
the medium recovered from COS-7 cells co-transfected with wild type or
mutant CRLF1 and CLCF1, with serum free medium as negative control
and with 100pM human recombinant Ciliary Neurotrophic Factor, expressed
in E. coli (C3710 #086k1965-SIGMA) in serum free medium as positive
control. The cells were then lysed with Lysis Buffer, (150mM NaCl, 50mM
TrisHCl pH 7.5, 1mM EDTA, 1% Triton X-100, 0.1% Sodium
deoxycholate, 4mM pefablock) containing Complete Protease Inhibitor
Cocktail (Roche) and HaltPhosphataseInhibitor Cocktail (PIERCE). Then
50 µg of lysates were subjected to SDS PAGE analysis with 9%
polyacrylamide gel and transferred to PVDF membrane Hybond-P
(Amersham) by electroblotting. The filter was immunoblotted with an
antibody specific for all of the phospho-STAT3 isoforms (Cell Signaling--
#9131s dil. 1:1000). The membrane was then stripped in 0.1N NaOH, and
reblotted with an antibody specific for STAT3 (Cell Signaling-#9132 dil.
1:1000).
RESULTS

Clinical features of patients classified with Crisponi syndrome and CISS1 syndrome

In our study, patients classified as CS, presented with typical symptoms in infancy, including severe hyperthermia, contraction of the facial muscles, feeding difficulties with hypersalivation, which often required nasogastric feeding, and dysmorphic features, e.g., camptodactyly, foot anomalies, high arched palate and chubby cheeks (Figure 1, Table 1 and Supplementary Table 1). The severe manifestations led to death in most of the historically described cases.

However, these classical symptoms of CS could be observed also in some patients with the established diagnosis of CISS1 (Figure 2, Table 1 and Supplementary Table 1). The Norwegian brothers described initially by Knappskog et al.11 feature a phenotype that shows considerable resemblance to Crisponi patients. Feeding difficulties and the need of nasogastric tube during infancy were described in the paper, while in one of the two brothers episodes of hyperthermia in the first period of life was reported only later during a reconsideration of the cases for this study. Also the eldest brother showed contractions of the facial muscles in infancy (Figure 2b). Feeding difficulties in infancy were also described in the Canadian woman.10

Physical measurements performed in 14 patients with CRLF1 mutations did not reveal a significant difference of the facial attributes in comparison to the general population (data not shown). These observations show, in contrast to previous studies1, that the attribute of a long and large face, long philtrum and broad nose cannot be included in the obligatory features of patients with CRLF1 mutations.
All surviving patients older than 2 years of our study cohort developed scoliosis and those older than 6 years developed paradoxical sweating in response to low ambient temperatures. Some patients had been treated with Clonidine to reduce the sweating. Patients 15 and 18 showed long lasting positive effects, whereas patient 16 showed no significant improvement and preferred physical training as a method against the sweating. Patients 17 and 19 showed positive but decreasing effects over time. Also, patients 7 and 8, originally classified as CS patients, responded to alpha-adrenergic agents with regard to paradoxical sweating.

Diagnostic studies such as electroencephalogram, cranial computed tomography, muscle biopsy and nerve conduction velocity were performed in several cases mostly showing normal results (Supplementary Table 2). Most of the initial symptoms resolved after the first 1 or 2 years of life. Some patients still showed hypersalivation or hyperthermia in adulthood (Table 1 and Supplementary Table 1).

In our cohort, there is a subgroup of patients with a mild phenotype in early childhood presenting no hyperthermia or feeding difficulties after birth (patients 17-19, Table 1). In pre-adolescence, these patients developed scoliosis and cold-induced sweating (Table 1).

**CRLF1 mutational analysis**

The *CRLF1* gene (GenBank accession number NM_004750) is localized on human chromosome 19p13.11 and is composed of 9 coding exons (Figure 3a). The encoded protein (GenBank accession number NP_004741) comprises 422 amino acids. By direct sequencing of all the 9 coding exons
as well as exonic/intronic boundaries of the *CRLF1* gene, different types of mutations have been identified in all the patients included in this study (Figure 3, Table 1, Table 2). Six patients (Table 1) were compound heterozygotes for different mutations, the remaining were homozygotes for one type of mutation. No obvious correlation between the type/localization of mutation and the severity of the clinical course was detected (Table 1).

**CRLF1 functional analysis**

CRLF1 protein is known to be a member of the ciliary neurotrophic factor receptor (CNTFR) pathway by forming a complex with CLCF1. The stable secreted complex of CRLF1 and CLCF1 forms a ligand for CNTFRα, which, along with gp130 and LIFR, compose the CNTF-receptor complex. CRLF1/CLCF1 binding to CNTFRα leads to dimerization of gp130/LIFR, which then induces downstream signalling events, including activation of the JAK1/STAT3 pathway. To investigate the biological significance and to assess the secretion capacities of mutated CRLF1, mutations found in the 19 patients (Table 1) were introduced into the wild-type *CRLF1* cDNA and co-transfected with wild type *CLCF1* cDNA in COS-7 cells. Non-transfected COS-7 cells were used as control.

After a 48 h culture period, strong expression of wild type CRLF1 and mutants was detected in cell lysates, whereas only wild type CRLF1, mutants K368X (M4), W284C (M10), R81H (M12) and L374R (M13) were strongly detected in the supernatant of transfected cells. CRLF1 mutants W76G (M1), T226NfsX104 (M2), P238RfsX6 (M3), and V282GfsX47 (M11) were only partially secreted into the medium, while Y75D (M5), P239AfsX92 (M6), D181GfsX5 (M7), [N113I;L114P] (M8) and Q180X
Previous studies reported that CLCF1 must interact with CRLF1 in the ‘producer’ cell to be released. To evaluate the ability of mutated CRLF1 to stimulate CLCF1 secretion, we co-transfected wild type and mutant forms of CRLF1 together with wild type CLCF1. After a 48 h culture period, we found CLCF1 in culture supernatants when transfected alone, as well as when co-transfected with the wild type and mutants CRLF1, although with different signal intensities (Supplementary Figure 1b). To better understand these findings, we performed a time course experiment with different culture periods (24 h, 36 h, 48 h and 72 h) for both wild type CRLF1 and CLCF1 transfected alone or together in COS-7 cells. Non-transfected COS-7 cells were used as control (Supplementary Figure 2a, b). The results indicate that in the cell lysate, CLCF1 alone is always expressed from 24 h to 72 h, whereas in the supernatants it is detected at 48 h and 72 h. When co-transfected with CRLF1, CLCF1 was only faintly detected in the supernatants at 24 h, while it was strongly detected at 36 h, 48 h and 72 h. Thus, it seems that co-expression with CRLF1 is not necessary for CLCF1 secretion, but is required to accelerate the kinetics of secretion into the extracellular medium.

Analysis of induction of STAT3 tyrosine phosphorylation by the CRLF1/CLCF1 complex

It has been previously reported that the secreted heterodimer CRLF1/CLCF1 activates the CNTF receptor with the induction of downstream signaling events including activation of the JAK1/STAT3
We therefore assessed the ability of CRLF1 mutants to induce STAT3 signal transduction in the IMR32 neuroblastoma cell line, expressing the CNTF receptor complex (gp130, LIFRβ and CNTFRα). The supernatants of COS-7 cells transfected with wild type CLCF1 or wild type CRLF1 alone, and with wild type CLCF1 and wild type or mutant forms of CRLF1, were added to an IMR32 cell line after 24 h serum starvation. CNTF was used as a control at a concentration of 100pM. After a 10 min incubation, cells were lysed and analyzed by western blotting with anti STAT3 and anti phosphoSTAT3 antibodies. We found that CLCF1 alone can induce STAT3 phosphorylation in an IMR32 cell line, but CRLF1 alone cannot (data not shown). However, the complexes of wild type CLCF1 and wild type or mutated forms of CRLF1 were able to elicit STAT3 signaling.

**DISCUSSION**

**Clinical and diagnostic considerations**

In this study, we collected and analyzed in detail clinical data from 19 patients diagnosed with CS or CISS1. Patients with mutations in the CRLF1 gene until now have been classified as CS or CISS1 based on the severity of their clinical symptoms and the age at diagnosis. However, a detailed analysis of the clinical phenotypes in this study showed that there is a clinical course of varying severity in both groups of patients. The Israeli sisters described in 1978 by Sohar et al., exhibited a less severe phenotype and have formed the entity of CISS1. Further patients like the Norwegian brothers, have been put in this group. However, our retrospective study showed that they presented symptoms in early childhood including feeding...
difficulties and hyperthermia, which have been reported only for CS. On the other hand, CS patients who have survived the critical first months of life, develop typical symptoms of CISS1 as they grew older. This makes it very likely that the initial CS and CISS1 patients reported in the paper by Crisponi, 1996\textsuperscript{1} and by Sohar et al., 1978\textsuperscript{9} have been considered different entities only because the patients were reported at different ages. This is in line with a most recent study reporting a CISS1 patient with neonatal features of Crisponi syndrome supporting the notion that CISS1 and CS may be a single clinical entity.\textsuperscript{25}

The overlapping phenotypes (Table 1 and Supplementary Table 1), make it difficult today to classify new patients. In order to elucidate a potential genotype/phenotype correlation, we performed a detailed analysis in relation to the mutations found in the \textit{CRLF1} gene.

**Classification according to the type of mutation**

The different types of mutations were analyzed for all patients included into this study. Missense, nonsense, insertions, and frameshift mutations were found. Strikingly, patients who have suffered a severe clinical course with hyperthermia and feeding difficulties during childhood display all different kinds of mutations. The deceased patients do not differ from other patients with regard to the type of mutation. Also, the same class of mutation is responsible for different clinical courses, as exemplified by patient 5, who displays an extremely severe phenotype until now, and patients 18 and 19, who are known to have shown a very mild clinical course. As missense mutations were detected in all 3 of them, a strict correlation between the type of mutation and the phenotype seems very unlikely. The variety of
mutations leading to the same phenotype and the variety of phenotypes caused by the same type of mutation lead to the conclusion that there is no strict correlation between phenotype and genotype based on the type/localization of CRLF1 mutation.

Classification according to the biochemical characteristics of CRLF1

We also performed a functional analysis of the mutated forms, showing that different mutations can be grouped according to their biochemical quality. In the first group, absent or weak secretion (less than 40% compared to wild type) of mutant CRLF1 occurred; in the second group, the mutated protein was strongly secreted (more than 40% compared to wild type, Table 1, Table 2).

Patients 1-15 are carriers of mutations belonging to the first group. The clinical phenotype was severe for all patients in this group (Table 1). All these patients showed hyperthermia, contraction of the facial muscles, hypersalivation and feeding difficulties in early childhood and required nasogastric feeding. All of these patients older than two years developed a scoliosis, those older than six years also cold-induced sweating.

Patients 18 and 19 carry mutations associated with a strong CRLF1 secretion. These patients described by Sohar et al. exhibit a mild phenotype without severe manifestations in infancy such as hyperthermia, but with cold-induced sweating, scoliosis and some dysmorphic features. Based on these observations, we conclude that phenotypic severity of CRLF1 associated disorders mainly depends on altered secretion of the CRLF1 protein. Accordingly, patient 17, who is a compound heterozygous carrier of two mutations, associated with absent and strong CRLF1 secretion
respectively, presents an “intermediate” phenotype with feeding difficulties in infancy, scoliosis and cold-induced sweating in adolescence.

However, it is likely that in some cases, the severity of the phenotype could also be affected by other factors. For example, in patient 16, genetic factors may account for the milder phenotype compared to his brother (patient 15), who carries the same mutation.

Our analysis supports the conclusion that absent or weak CRLF1 secretion is associated with a severe clinical phenotype, whereas strong secretion is associated with a milder clinical phenotype. Based on the fact that our retrospective clinical study was limited either by the small number of patients in each CRLF1-secretion-based group and by clinical information not exhaustive, we suggest that defective secretion is a major component affecting phenotypic severity of CRLF1-associated disorders.

**Analysis of the CRLF1/CLCF1 pathway**

CRLF1 protein is a member of the ciliary neurotrophic factor -receptor (CNTFR) pathway by forming a complex with CLCF1, which binds to the CNTF-receptor. In contrast to the observations previously reported, where CLCF1 can be actively secreted from cells by forming a complex with CRLF1, we found that CLCF1 was detected in culture supernatants when transfected alone, as well as when co-transfected with the wild type and mutant CRLF1, although with different signal intensities (Supplementary Figure 1b).

It is noteworthy that an alteration in the kinetics of CLCF1 secretion is associated with the co-expression with some mutant forms of CRLF1, which
may act to inhibit its secretion into the medium. Further studies aiming to identify the CRLF1 interaction sites with CLCF1 will help to better understand the heterodimer complex formation as well as the role of the CRLF1 mutations analyzed.

It has been previously reported that the secreted heterodimer CRLF1/CLCF1 activates the CNTF receptor with the induction of downstream signaling events including activation of the JAK1/STAT3 pathway. Dagoneau et al.\textsuperscript{2}, already reported that STAT3 phosphorylation was normally triggered in fibroblasts of patients with Crisponi syndrome by LIF. We found the same result in our experiments (data not shown). Although CRLF1 is not needed to activate the CNTF receptor, early secretion of the full CRLF1 protein seems to be necessary for adequate CLCF1 secretion and proper activation of the CNTF receptor pathway.

However, the results from the cell culture experiments cannot be directly transferred to the mechanisms in humans, so that it cannot be fully estimated how a delay of 48 hours in a cell culture would affect function in organ systems. Alternatively, another receptor may be involved, and the alteration of its pathway may cause the phenotype. This hypothesis gains some evidence from the recent detection of a previously unknown receptor for CRLF1.\textsuperscript{26}

Our findings lead to the old matter about “lumpers” and “splitters”\textsuperscript{27} and thus to the critical question how to classify and name these genetic entities. In the presence of bowing of the long bones, the pediatric manifestation would lead to Stüve Wiedemann syndrome.\textsuperscript{28} In the absence of bowing of the long bones, based on our findings, we believe that the two syndromes, CS and CISS1, represent manifestations of one disorder, with different
degrees of severity. The rare cases of CISS1 most likely correspond to CS survivors. Genetic heterogeneity could be assumed with CISS2, which shows the same phenotype as CISS1, but is due to mutations in CLCF1. However, since there has been only one case described in literature, this assumption might be too early at the moment.

Use of one facet of the syndrome, such as cold-induced sweating as a name for the whole, has some limitations since this feature is not the only one, may occur as an isolated anomaly or may be a feature of other syndromes. Therefore, we suggest to rename the two genetic entities CS and CISS1 with the broader term of Sohar-Crisponi syndrome. Furthermore, we conclude that defective secretion of the CRLF1 protein is a major factor contributing to the phenotypic severity of this syndrome.

**CONFLICT OF INTEREST**

The authors declare no conflict of interest.

**ACKNOWLEDGEMENTS**

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SUPPLEMENTARY INFORMATION

Supplementary information is available at EJHG’s website

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Abbreviations: Mis, missense; Nons, nonsense; Fs, frameshift; n.a., not available. *CRLF1 secretion level of mutant forms compared to wild type.
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<th>Mutation number</th>
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<th>CRLF1 exon</th>
<th>DNA sequence variant</th>
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<th>Mutation type</th>
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FIGURE LEGENDS

Figure 1. Clinical characteristics of patients classified as Crisponi syndrome.  a-d: Patient 5, at 7 months of age, showing contractions of the facial muscles, chubby cheeks and hypersalivation.  e,f: Patient 5 at 1 year of age; camptodactyly of both hands.  g,h: Patient 5 at 1 year of age with contractions of the facial muscles (h).  i,j: Patient 5 at 5 4/12 years of age, showing hyperkyphosis (i) and scoliosis (j).  k,l: Patient 5 at 5 4/12 years of age; camptodactyly of both hands.  m: Typical fever course of a patient with Crisponi Syndrome (Patient 2) after birth. Graphs show the core temperature during 66 days of measurement with peaks up to 41°C.

Figure 2. Clinical characteristics of two Norwegian patients classified as CISS1.  a: Patient 15, in infancy, presenting contractures in elbows and knees.  b: Patient 15, showing ocular and oropharyngeal contractions, and chubby cheeks.  c: Patient 15, hand with typical camptodactyly.  d: Patient 16, at the age of 18 years; camptodactyly of both hands.  e: Patient 16, at the age of 18 years, presenting micrognathia, kyphosis, scoliosis and flexion contractures of the elbows.

Figure 3. Structure of the human CRLF1 gene and protein.  a: Relative positions of the mutations found in both CS and CISS1.  b. Alignment and amino acid conservation for both Crisponi and CISS syndrome missense mutations analyzed. SIFT (Sorting Intolerant From Tolerant http://sift.jcvi.org/) predicted that mutations Y75D, W76G, [N113I;L114P], W284C and L374R are not tolerated, while R81H is tolerated. Pink: CS mutations, green: CISS1 mutations.