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"Molecular and genetic characterization, clinical evaluation and pilot study to assess the feasibility of a carrier screening for Crisponi syndrome in Sardinia"

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Genetic and molecular characterization, clinical evaluation and pilot study to assess the feasibility of a carrier screening for Crisponi Syndrome in Sardinia

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1 Introduction

Crisponi syndrome (CS; MIM# 601378) was initially described in 17 patients from 12 different families in central and southern Sardinia [Crisponi G., 1996]. 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. Hyperthermia as well as acute respiratory crises are 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. In 2007 *CRLF1* (MIM# 604237; *locus* 19p13.11) was identified as the gene involved in the pathogenesis of the syndrome [Crisponi et al., 2007; Dagoneau et al., 2007]. Mutations in this gene are also responsible for Cold induced sweating syndrome type 1 (CISS1; MIM# 272430) [Knappskog et al., 2003].

CISS1 was first described in two Israeli sisters [Sohar et al., 1978] and the similar clinical phenotype was reported later in two Norwegian brothers [Knappskog et al., 2003]. It involves paradoxical sweating at cold ambient temperatures on the upper part of the body, along with progressive scoliosis, dysmorphic features including a high arched palate, nasal voice and joint contractures.

Initially it was supposed that CS and CISS1 represented two allelic diseases [Crisponi et al., 2007] comprised in a new family of "CNTF receptor–related disorders," along with cold-induced sweating syndrome type 2 (CISS2; MIM#610313), caused by mutations in the *CLCF1* gene (MIM# 607672; *locus* 11q13.2), [Hahn et al., 2006; Rousseau et al., 2006], and Stüve–Wiedemann syndrome (SWS; MIM#601559), caused by mutations in the

LIFR gene (MIM# 151443; *locus* 5p13.1) [Dagoneau et al., 2004] (Fig. 1). Successively, both genotype/phenotype correlation and functional analysis on mutated CRLF1 proteins suggested that CS and CISS1 are manifestations of the same disease with different degrees of severity due to different ages of clinical evaluation and altered kinetics of secretion [Herholz, Meloni et al., 2011].

CRLF1 protein is a member of the ciliary neurotrophic factor receptor (CNTFR) pathway and interacts with CLCF1 to form a heterodimeric complex that binds to the CNTF receptor. This pathway is known to be important for the development and maintenance of the nervous system and muscles.

Locus heterogeneity for CS/CISS1 within the CNTF receptor–related disorders could be assumed just with CISS2, which shows the same phenotype, but it is due to mutations in the *CLCF1* gene. However, as there have been only three cases from two families described so far in literature, this assumption might be too early at the moment [Hahn et al., 2006; Rosseau et al., 2006; Hahn et al., 2010].

2 Clinical description of Crisponi syndrome

2.1 Presentation in Infancy

The phenotypic manifestations of the syndrome are already evident at birth. Typical findings include dysmorphic features, such as camptodactyly, foot anomalies, high arched palate and chubby cheeks. When crying or being handled, infants tend to startle excessively, and a state of massive muscular contraction occurs. This especially involves contraction of the mimic muscles of the face into an expression resembling that of a tetanic spasm. Furthermore the patients present contractions of the oropharyngeal muscles resulting in a excessive salivation, inability to suck or swallow. Cries appear as a

continuous weak lament, emitted in forced expiration, followed by short apneic spells with cyanosis. The muscles of the neck are contracted with hyperextension of the head and opisthotonus. Even the respiratory muscles are involved in the contraction phenomena, with dyspnea, cyanosis and apneic states during crying. Such episodes of contraction are of variable duration. When the baby is quiet and during sleep, they are not manifested. When crying or being handled, the muscle contractions are exacerbated as well as during fever episodes. Fever is the most sever manifestation of the syndrome. It appears in a temporal windows from a few days to a few weeks after birth. It presents as an intermittent character not linked to any infection with peaks over 42°C with rapid falls. Death usually occurs in concomitance with these episodes. [Crisponi,1996].

2.2 Presentation in Childhood and Adulthood

Once the difficulties of early childhood have been overcome, individuals with CS/CISS1 syndrome are, for the most part, able to lead a fairly normal and productive life, obtain a secondary education, and have children. Life expectancy is probably normal; although the neonatal mortality is very high, some patients survive (in Sardinia 8 out of 24) and to date only one individual has been followed to the eighth decade [Hahn et al., 2006]. Cold-induced sweating, the most disabling symptom in adulthood, is recognized during the first decade/puberty (age ≥3 years). At environmental temperatures of 22° C or less, affected individuals sweat profusely on their face and upper body, accompanied by intense shivering and dermal vasoconstriction, so that the fingers appear cold and cyanotic. Profuse sweating is also triggered by apprehension, nervousness, or by sweet gustatory stimuli, in particular by chocolate. In contrast, affected individuals sweat very little in heat and only in the lumbar region, the groin, and the anterior thigh. They become flushed and unpleasantly overheated in hot climates [Hahn et al., 2006, Hahn et al., 2010]. Although

the hyperhidrosis can be somehow treated [Hahn et al., 2006, Hahn et al., 2010, Herholz et al., 2010], heat intolerance is a lifelong problem.

Towards the end of the first decade, affected children develop a progressive thoracolumbar kyphoscoliosis that requires either bracing or spinal instrumentation.

The clinical manifestations of the syndrome in adult patients are still today object of study and continuous updating.

2.3 Differential diagnosis

A differential diagnosis is a critical step at birth; many common disorders can manifest the same features of CS/CISS1, such as hypertonia and contractures. These include neonatal tetanus, cerebral palsy due to sever perinatal asphyxia, but also the hyperekplexia or startle disease, Schwartz-Jampel syndrome, Isaacs-Mertens syndrome and the stiff-baby syndrome. Muscle contractions are common in several congenital muscular dystrophies. The frequent elevations in body temperature up to 42°C may be associated with malignant hyperthermia. But the syndromes showing the major clinical overlap and the more similar mode of inheritance are the Stuve-Wiedemann syndrome (SWS) and Cold Induced Sweating type 2 (CISS2) that, along with CS and CISS1 are identified as "CNTF receptor–related disorders" (Fig.1).

2.3.1 The Cold induced syndrome type 2 (CISS2)

Cold Induced Sweating Syndrome type 2 is caused by mutations in the *CLCF1* gene and shows characteristics similar to those described in CISS1. So far only three cases have been reported in literature [Rousseau et al., 2006; Hahn et al., 2010], an Australian and two Hungarian sisters. The symptoms are basically the same of CS/CISS1 with dysmorphic

features already present at birth, feeding difficulty, profuse sweating on the face, trunk and limbs during exposure to cold, inability to sweat properly in response to heat. Although patients suffering from CISS2 are only three, and it is still too early to arrive at a conclusion, we could hypothesize the presence of locus heterogeneity for CS/CISS1, with two loci involved, *CRLF1* and *CLCF1*, of which the first characterized by a wider number of mutations (approximately 95% in the *CRLF1* gene and 5% in the *CLCF1* gene).

2.3.2 The Stuve-Wiedemann syndrome (SWS)

Stuve-Wiedemann syndrome is caused by mutations in the *LIFR* gene [Dagoneau et al., 2004]. As in CS/CISS1 and CISS2, also this syndrome is characterized by different phenotypes at birth and during the developmental period. Many clinical features present in patients affected by SWS are the same described in patients suffering from CS/CISS1, including: camptodactyly, difficulty in sucking and swallowing, hyperthermia, respiratory distress, dysmorphic facial features, protrusion of the mouth, early death, progressive kyphoscoliosis, poor thermal regulation with severe heat intolerance and paradoxical sweating. However, some skeletal abnormalities such as bowing of the long bones and large metaphyses, associated with short stature, are a peculiar manifestation of SWS and never been described associated with CS/CISS1 or CISS2.

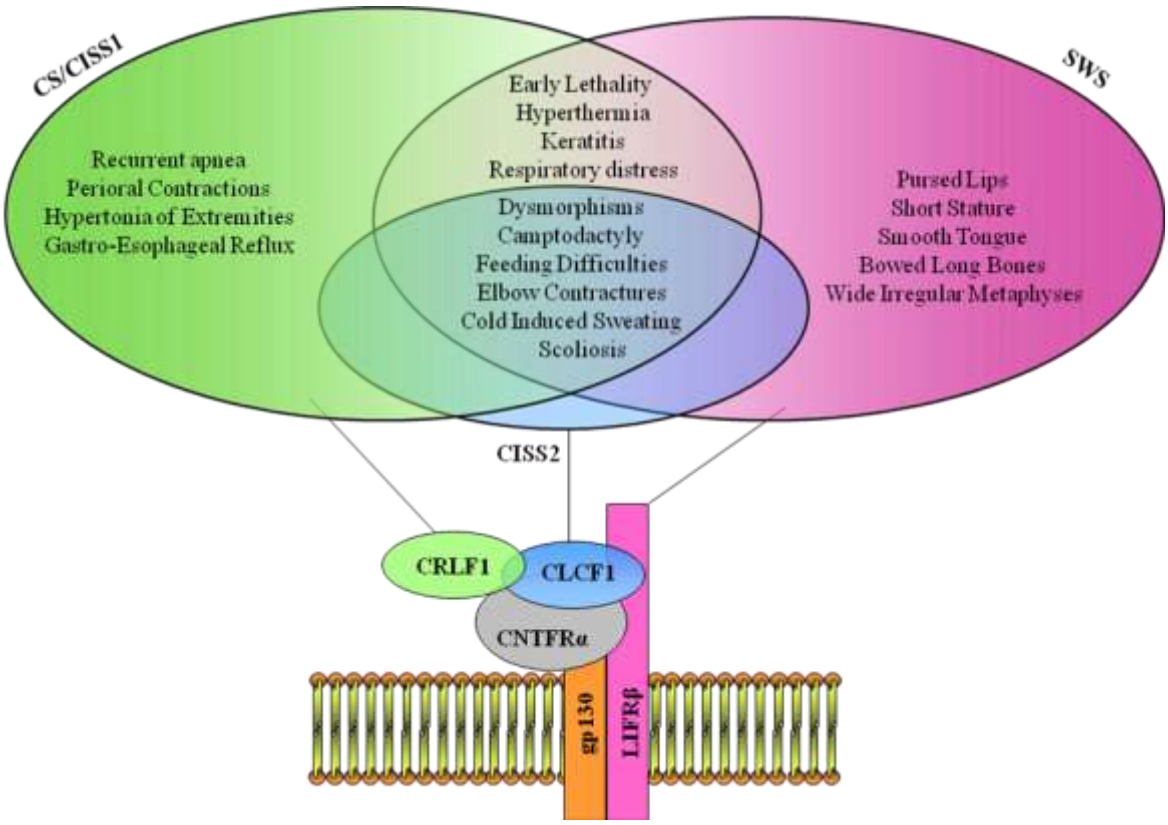

Fig.1 CS/CISS1 and other CNTF-receptor complex related disorders.

3 Genetic and molecular characterization

3.1 Genetic studies

By an homozygosity mapping approach, using high-density SNP arrays, in five Sardinian and three Turkish families with CS, Crisponi et al. in 2007 identified a critical region on chromosome 19p12-13.1. The most prominent candidate gene within this genomic interval was *CRLF1*, which was previously found to be involved in the pathogenesis of CISS1. The findings of mutations in this gene associated both to CISS1 and CS led to the hypothesis that they were allelic disorders.

3.2 Structure and expression of the *CRLF1* gene

The human *CRLF1* gene is localized on chromosome 19p13.11. It consists of 9 coding exons, spans for 14Kb and it is transcribed as a 1,824 bp linear mRNA (Ref. seq NM_004750.4). It encodes for a 422 amino acids protein (~ 46 KDa). This protein (NP_004741.1) has a domain structure that includes a signal sequence (positions 1–37) followed by an Ig-like C2-type N-terminal domain (positions 38–131), two consecutive fibronectin III–like domains (positions 134–229 and 234–334), and a C-terminal domain (positions 335–422). Each fibronectin type III repeat contains a highly conserved amino acid motif: the first has two cysteine doublets while the second has a WSXWS motif, at position 327. This motif is probably needed for correct folding and domain orientation of the protein [Bazan, 1990]. The C-terminus shows no homology to known functional domains [Elson et al., 1998].

The literature describes the *CRLF1* as a gene involved in regulating the immune system, in the development of the nervous system and with a key role in fetal development.

The highest levels of *CRLF1* mRNA were observed in lymph node, spleen, thymus, appendix, placenta, stomach, and fetal lung, with constitutive expression of *CRLF1* mRNA detected in a human kidney fibroblast cell line.

In the mouse embryo, expression of *Crlf1* mRNA is evident in different tissue and at different stages of development; in particular, at 11.5 days post-conception (dpc), *Crlf1* was detected in the mesonephric duct, limb buds, first branchial arch, nasal processes, and the dermatomyotome. At 14.5 dpc, *Crlf1* was detected in the lung, kidney, genital tubercle, precartilaginous condensations of the digital metacarpals, intervertebral discs, tongue, and facial mesenchyme. At 18.5 dpc, *Crlf1* expression was observed in the cortex and hippocampus regions of the brain [Alexander et al., 1999; Kass, 2011].

3.3 Mutational analysis of the *CRLF1* gene

The complete *CRLF1* coding sequence has been sequenced in all the patients analyzed, along with exon/intron junctions. All mutations are described according to the Human Variation Society (HGVS) nomenclature [den Dunnen and Antonarakis, 2000]. Mutation nomenclature has been then checked with the Mutalyzer program [Wildeman et al., 2008].

To date, overall 42 distinct *CRLF1* mutations have been found either as homozygous or compound heterozygous sequence changes in 63 patients with diagnosis of CS/CISS1 from 52 families apparently not related to each other; 13 Italians (in particular 9 from Sardinia), 18 Turkish, 8 Spanish and 24 from other different geographical areas (Table 1). Actually two of these 63 patients were found to be heterozygotes for one mutation (SC218 and SC247, see Table 3). In these cases we were not able to find a second mutation, probably due to the limits of the region analyzed and of the techniques used.

For these patients we hypothesized the presence of deletions/duplications in heterozygosity not readily detectable by sequence analysis of genomic DNA. To test their presence, a variety of methods including quantitative PCR, long-range PCR, multiplex ligation-dependent probe amplification (MLPA), CGH array or SNP array may be used.

In our cases, we decided to perform a long- range PCR using the following primers pairs: 1F-1Rintronic (3,8 Kb); 2Fintronic 2R (3,7 Kb); 2F-6R (3,5 Kb) and 5F-9R (3,8 Kb), but no additional bands were seen. So for one patient we decided to proceed by SNP array.

Case SC218 is a 6 months years old Spanish female. She was positive to *CRLF1* analysis, but we found only the maternally inherited c.713dupC mutation. The phenotype was clearly attributable to Crisponi syndrome; hyperthermia, contraction of facial muscle, trismus, swallowing, feeding difficulties, chubby cheeks and camptodactyly were evident. The analysis proceeded by Genome-Wide Human SNP array 6.0 (Affymetrix). The

reference sets used contained 57 samples. The results showed a loss of heterozygosity (LOH) of 120Kb on chromosome 19p12 (20,596,194-20,716,377 in reference hg19) (start marker CN_795771- end marker CN_165017), about 2 Mb 5' upstream of the *CRLF1* gene. We were not able to assess whether this variation was *de novo* or transmitted, since the parents could not be analyzed. In this region, are mainly present Zinc Finger Proteins (ZNFs). Although the analysis has not been exhaustive, these data deserve to be further evaluated.

It seems that all the 42 mutations found so far are inherited, although the inheritance could not be ascertained in some cases. Of these mutations, 16 (38%) are missense, 12 (28,6%) small indels, 6 (14,3%) splice site mutations, 4 (9,5%) nonsense and 4 (9,5%) large deletions. At least 20 (about 50%) of the total reported mutations are predicted to result in truncated proteins (Fig. 2). There is no apparent mutational hot spot in *CRLF1*, and there seems to be no correlation between the severity of the phenotype and the location/type of mutation.

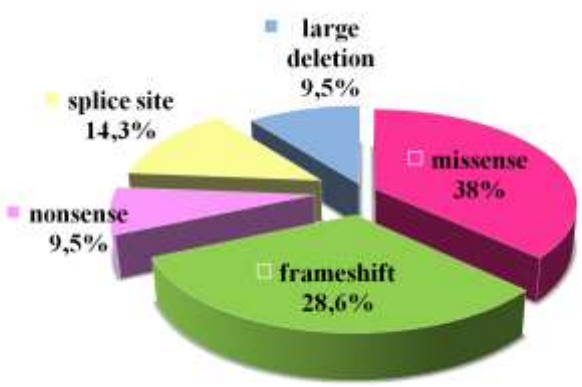

Fig.2 Different types of mutation found in the *CRLF1* gene .

Of these 42 mutations, 24 have already been reported in literature as associated to CS/CISS1, [Crisponi et al., 2007; Dagoneau et al., 2007; Herholz, Meloni et al., 2011; Hahn et al., 2006; Knappskog et al., 2003; Hahn et al., 2010; Okur et al., 2008; Thomas et

al., 2008; Yamazaki et al., 2010; Di Leo et al., 2010; Hahn et al., 2011; Tüysüz et al., 2012; Hakan et al., 2012; El-Assy et al., 2012] , 7 have been presented in a poster at the ESHG 2011 [Lebre et al., 2011] and 11 are novel and reported here for the first time (Table 1).

Of these 11 novel mutations found in our patient cohort, 5 are missense (c.433T>C, p.S145P; c.935G>C, p.R312P; c.[803T>C;1018C>T], p.[F268S; R340C]; c.221T>C, p.L74P and c.646C>T, p.R216C), 2 donor splice site defects (c.115+1G>A; c.527+5G>T), 2 large deletions (exon 3-exon 4 and exon 5_9), 1 small indels (c.721_737dup, p.G247Cfs*3) and 1 nonsense (c.776C>A; p.S259*). For these novel mutations, DNA sequences were compared to the reference sequence NM_004750.4. The sequence variants were confirmed by re-sequencing of PCR products obtained from a second amplification reaction. For the novel missense variants, 100 control chromosomes (of matched ethnicity where available) were screened by direct sequence. For c.433T>C, the novel identified variant lost a cleavage site for AvaII restriction enzyme, so we used enzymatic digestion for control screening**,** while for c.646C>T (Pakistan origin) we used the reference panel of 1000 Genome Project [The 1000 Genomes Consortium, Nature 2012].

The c.221T>C variant, was found in a Sardinian patient, in compound heterozygosity with the most frequent c.676_677dupA. To test the presence of the variant in control chromosomes, we took advantage of the SardiNIA Medical Sequencing Discovery Project which has been carried out by whole genome sequencing of 2100 individuals in the founder Sardinian Population, to map human genome variation that is rare or geographically restricted and unique to this specific population [Sidore et al., ASHG 2012].

The 2 donor splice site variants, c.115+1G>A and c.527+5G>T were detected in *trans* in the same patient. The mother was carrier of the new variant c.115+1G>A while the father was carrier of the other variant c.527+5G>T. Although this last variant affects the same

residue of another mutation described before in a CS patient from Yemen [Dagoneau et al., 2007], the substitution is different, G>T instead of G>A.

All these 11 mutations are not present in the SNP databases nor listed as non pathogenic variants in the literature. Prediction of splice sites was performed with NetGene2 (http://www.cbs.dtu.dk/services/NetGene2/), whereas for non synonymous SNPs functional prediction we employed dbNSFP (http://sites.google.com/site/jpopgen/dbNSFP) [Liu et al., 2011], an integrated database of functional predictions from four new and popular algorithms (SIFT, Polyphen2, LRT, and MutationTaster), along with a conservation score (PhyloP) multiple algorithms. All missense and splice site changes were predicted to be pathogenetic. Regarding the mutations *in cis* c.[803 T>C;1018C>T], the prediction by dbNSFP suggests that the causative one should be c.803T>C rather than c.1018C>T.

We also found 2 new large homozygous deletions (exon 3_4del and exon 5_9del). The first one was initially supposed by absence of PCR products for exons 3-4 in the patient. It was then confirmed by performing a long-PCR that covered the entire genomic region harboring exons 3-4 in both patient and parents (primer pairs 2F and 5R, Crisponi et al., 2007). It resulted in one shorter amplification product in the patient, and in two products in the parents, carriers of the deletion. The sequence analysis of this shorter product revealed that the deletion starts in intron 2 (position c.398-456) and ends in intron 4 (position c.697+747).

As for the first deletion, the second one (exon5_9) was also supposed by absence of PCR products for exons 5-9 in the patient. Unfortunately it could not be confirmed by performing a long PCR that covered the entire genomic region harboring exons 5 through 9, in both patient and parents (primer pairs 4F and 9R, Crisponi et al., 2007). We assumed that it spans over exon 9. We further confirmed such deletion by Real Time Quantitative

PCR analysis, which was performed on 7900HT Fast Real-Time PCR System (Applied Biosystem) using SYBR®Green assay. This is a sensitive and accurate method for the quantification of DNA in homogeneous solutions allowing to differentiate the presence of 0, 1, or 2 copies of the gene. This result confirmed the deletion previously seen with classic PCR assay (data not shown).

To date the most frequent mutations are found in the Sardinian (c.226T>G and c.676_677dupA), Turkish (c.708_709delCCinsT) and Spanish (c.713dupC) populations. The c.226T>G mutation results in a tryptophan-glycine substitution at position 76 of the Ig-like domain (p.W76G). Tryptophan 76 is likely buried within the molecule and a substitution by a glycine would be expected to result in a loss of tight internal side-chain arrangement and, thus, in a considerable decrease in stability. The W76 is strictly conserved within CRLF1 homologous proteins from different organisms [Crisponi et al., Dagoneau et al., 2007].

The c.676_677dupA variant results in a threonine-asparagine change at position 226, followed by a frameshift, which leads to the deletion of a complete fibronectin domain as well as C-terminal domain (p.T226Nfs*104). This variant was found either in homozygous or compound heterozygous state [Crisponi et al., 2007].

These two mutations listed in Table 1, c.226T>G and c.676_677dupA, have been found so far only in Sardinian individuals, so deriving from a founder effect in this population (4 homozygous for c.676_677dupA, 3 compound heterozygous, and 1 homozygous for c.226T>G). In Sardinia we also found a third mutation c.221T>C, but only in one patient as compound heterozygote for c.676_677dupA.

In the Turkish families, the most frequent mutation, always found in homozigosity is c.708_709delCCinsT, which leads to a frameshift in the second fibronectin type III domain (p.P238Rfs*6). It was found in 6 patients from 5 families.

The c.713dupC variant is very common in the Spanish population. This mutation is located in the region encoding the second FNIII domain of the protein, and results in a premature termination of translation (p.P239Afs*91). It was found in 10 patients, either in homozygous or compound heterozygous state, from 6 families. Furthermore it was also found in a Turkish patient [Dagoneau et al., 2007] and in 3 French brothers [Lebre et al., ESHG 2011].

The c.708_709delCCinsT and the c.713dupC were frequently found in families of Gipsy origin, where often take place marriages between consanguineous.

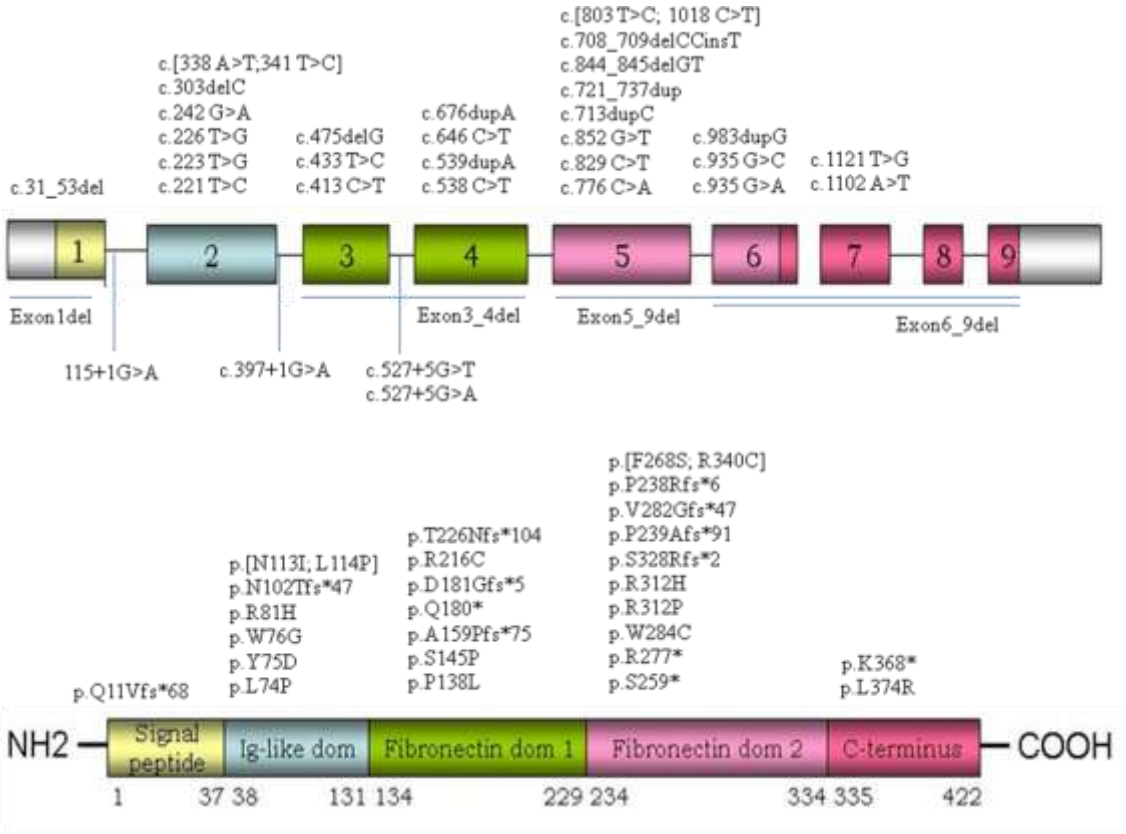

Fig.3 Distribution along the *CRLF1* mRNA and protein of 35 mutations found associated to CS/CISS1.

3.3.1 Geographical distribution of the molecular spectrum of *CRLF1* analysis

Up to date 63 patients affected by CS/CISS1 have been reported worldwide with 28,6% of the patients originating from Turkey (18), 20,6% from Italy (13, of which 9 from Sardinia), 12,7% from Spain (8) and the remaining 38% (24) from different geographical areas. In Italy the estimate reaches 40% if we consider also 15 Sardinian patients with a clinical diagnosis of CS [Crisponi, 1996] not confirmed by a molecular analysis for *CRLF1* since died before the discovery of the gene (Fig.4).

Considering this higher prevalence in Sardinia, Turkey and Spain, the most involved area is the one of the Mediterranean basin. In this area other cases have been described in Libya, Morocco and France (This report and Lebre et al., ESHG 2011). Other CS/CISS1 patients have been identified in Eastern countries, in particular in Israel [Knappskog et al., 2003], India [Thomas et al., 2008], Pakistan [This report], Yemen [Dagoneau et al., 2007], Saudi Arabia [El-Assy et al., 2012] and Japan [Yamazaki et al., 2010], while in the Western states one patient in Canada [Hahn et al., 2006], one in Australia [This report], and one in USA [Hahn et al., 2010].

Sardinia	24*
Turkey	18
Spain	8
France	5
Italy	4
Libia	3
Norway	3
Israel	3
Pakistan	2
Saudi Arabia	1
India	1
Yemen	1
Morocco	1
USA	1
Canada	1
Japan	1
Australia	1

***(9 *CRLF1* tested)**

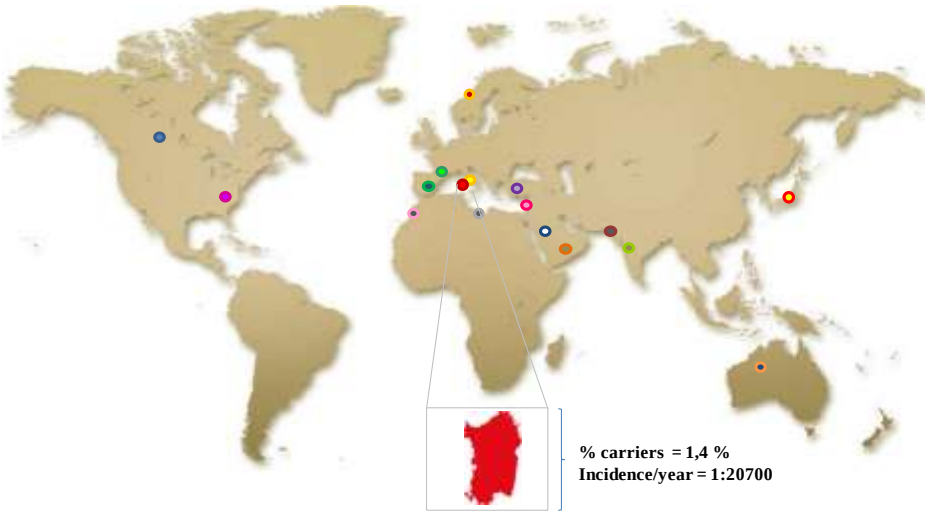

Fig.4 Geographical distribution of CS/CISS1. The left panel lists the numbers of patients identified in the different geographical areas.

3.3.2 Molecular analysis of *CLCF1* and *CNTFRa* genes

CRLF1 and CLCF1 proteins are involved in the ciliary neurotrophic factor (CNTF)-receptor pathway, important for embryonic development and maintenance of the nervous system [De Chiara, 1995]. This pathway supports the differentiation and survival of a wide range of neural cell types during development and in adulthood. To be efficient, the CLCF1 secretion requires the association with the soluble cytokine receptor CRLF1 [Rosseau et al., 2006; Herholz et al., 2011]. The stable heterodimeric complex of the CRLF1 and CLCF1 forms a ligand for CNTFRα, which, along with gp130 and LIFR, comprise the CNTF-receptor complex [Elson et al., 2000]. Binding of CRLF1/CLCF1 to CNTFRα leads to dimerization of gp130/LIFRβ, which in turn induces downstream signaling events, including activation of the Janus kinase 1/STAT3 pathway [Heinrich et al., 2003].

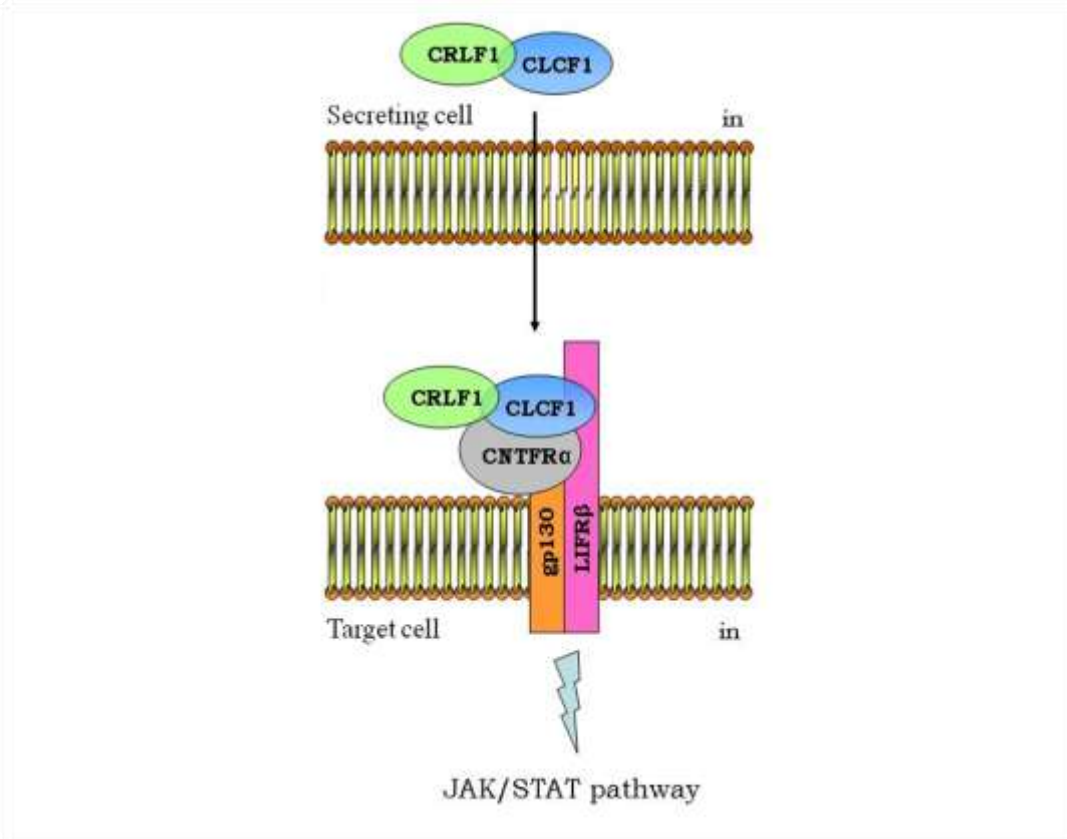

Fig.5 Schematic representation of the complex CRLF1/ CLCF1 with the CNTF receptor.

The interaction between genes of the CNTF receptor complex, the functional link between CRLF1 and CLCF1 and in particular the clinical overlap between CS/CISS1 and CISS2 created the basis for undertaking a mutational analysis for the *CLCF1* and the *CNTFRα* (MIM# 118946; *locus* 9p13) genes, respectively in 35 and 20 patients negative for *CRLF1* analysis. To date, only 4 causative mutations in the *CLCF1* gene have been described in CISS2 patients [Rousseau et al., 2006; Hahn et al., 2010], while disease-causing mutations have never been reported for the *CNTFRα* gene. The results of our analyses were negative since we did not find causative mutations in these genes, but only polymorphisms previously described as benign variants. In details, we found only 2 non synonymous mutations in heterozygosity in the *CLCF1* gene, which by different prediction softwares such as SIFT, Polyphen and ESEfinder, were predicted not to be disease-causing mutations. We didn't perform analysis of the *LIFR* gene, since mutations in this gene cause

SWS, which, although having a phenotype very similar to CS/CISS1 and CISS2 (Fig.1) presents in addition to short stature, the typical curvature of the long bones, absent in the 35 patients analyzed.

4 Clinical evaluation

The CS/CISS1 is a rare disease, of recent identification and characterization, with a quite complex phenotype and with different levels of severity, so it is complicated to reach a correct diagnosis. The clinical diagnosis of CS/CISS1 is based on the critical analysis of medical data and family history. This is then confirmed at a genetic level by positivity to the molecular analysis of the causative *CRLF1* gene.

Before requesting a molecular analysis for the *CRLF*1 gene, neonatologists and pediatricians as well as geneticists are required to complete a detailed clinical questionnaire, to acquire all the information useful to support the suspect of CS/CISS1 diagnosis and to standardize clinical data for all patients. Furthermore these information are also useful to allow forthcoming studies on genotype/phenotype correlation, or to extend the analysis to other genes implicated in similar disorders.

4.1 Genotype/phenotype correlation

With the exception of SWS with the characteristic bowing of the long bones, caused by *LIFR* mutations, manifestations of the CNTF receptor–related disorders are very similar both when caused by mutations in *CRLF1*, as in CS/CISS1 and by mutations in *CLCF1*, as in the case of CISS2. In particular CISS1 and CISS2 are clinically indistinguishable.

In table 3 are shown the clinical phenotypes of all CS/CISS1 patients reported so far and mutated for the *CRLF1* gene. There is no evident correlation between the phenotype and

the type/localization of mutations found. A functional study on the mutated forms of CRLF1, [Herholz, Meloni et al 2011], showed that CS and CISS1 are actually the same disease and that the phenotypic severity depends on altered kinetics in the secretion of the mutated CLRF1 proteins. So a defective secretion is a major component affecting phenotypic severity of CLRF1- associated disorder [Herholz, Meloni et al., 2011].

4.2 Management and therapy

At the moment there is no treatment available for this syndrome. At birth the patients require close monitoring in case of episodes of laryngospasm with respiratory distress, crisis of acute hyperthermia, or like-epileptic crisis, in particular when contractions of the oropharyngeal and respiratory muscles occur, that may lead to sudden death. In all these cases it is important to prepare appropriate countermeasures, such as supplemental oxygen, cooling blankets, anti-epileptic drugs. It is recommended a monitor for sleep apnea. A serial and continuous EEG monitoring may be required during the first few weeks of life. To overcome the sucking and feeding problems, the infants require prolonged use of a nasogastric tube. Bracing, occupational therapy or plastic surgery may be necessary to correct congenital finger and hand deformities. Surgical intervention or prolonged bracing may be required to treat the progressive thoracolumbar kyphoscoliosis. Sweating triggered by cold or apprehension can be effectively treated with clonidine/amitriptyline or moxonidine [Herholz et al., 2010; Hahn et al., 2010]. Heat exposure and prolonged physical activity in hot climate are to avoid.

Keratopathy is a constant of these patients, and the use of artificial tears or lubricating gel since birth could prevent the onset of surface erosion or more severe corneal damage.

Furthermore, it was seen that some patients have decreased pain perception, so the quantitative sensory testing (QSART), nerve biopsies with morphometric analyses and skin

biopsy with quantitative analysis of sensory innervations, could be performed to further explore the possibility of impaired development of sensory neurons.

This disease is still poorly understood and often not diagnosed correctly because the phenotype is relatively new and extremely complex, with marked clinical overlaps to other diseases. The identification of mutations in the *CRLF1* gene provides a definite diagnosis in patients with suspected diagnosis of CS/CISS1. Furthermore the genetic test available can be carried out for determining the carrier status in at-risk relatives in families with history of disease, and also for prenatal-testing if the disease-causing mutations in the family have been identified.

5 Pilot study to assess the feasibility of a carrier screening for CS in Sardinia

5.1 Requirements to perform a population screening

In 1975, genetic screening has been defined as the search in a population, for individuals possessing certain genotypes with or predisposing to certain diseases. To date, genetic screening can be defined as a type of test performed for the early detection or exclusion of a hereditary disease, for knowing the predisposition toward a disease or to determine whether a person is a carrier of a disease that can be inherited to the offspring [Godard et al., 2003]. In particular, population-based carrier screening for autosomal recessive disorders tends to define at-risk couples in which both members are heterozygotes and therefore at risk of having affected children in 25% of cases. Some considerations for this kind of screening have been discussed in 2008 at a meeting sponsored by the National Human Genome Research Institute (http://www.genome.gov/27026048):

- the disorder impairs health in the homozygous affected offspring,
- there is a high frequency of carriers in the screened population,

- technically and clinically valid screening methods are available and cost effective to all,
- IVF, prenatal diagnosis, and termination are reproductive options,
- consent (informed and voluntary participation) is obtained,
- potential benefits and risks of carrier testing are communicated before and after the test,
- privacy is protected,
- stigmatization of the carrier by the community is minimized,
- experienced professional resources are available.

5.2 Sardinia as a model of "Founder Population"

In a founder population, the geographical isolation, lack of immigration and/or high levels of endogamy and consanguinity preserve the genetic features of the original founders over time. Their genetic makeup can change over the centuries under the effect of several evolutionary mechanisms, such as bottleneck and genetic drift. These processes alter allele frequencies, and while common variants are barely lost, rare variants may be either lost or drifted to higher frequencies than in the original population [Peltonen et al., 2000]. Sardinia is the second largest island in the Mediterranean sea. Its modern population is of approximately 1.65 million inhabitants and constitutes a genetically isolated founder population, which has already aided in the identification of genes involved in several Mendelian disorders (β- and α-thalassemia, APECED and Wilson's disease) wherein the detection of gene mutations has confirmed the existence of a strong "founder" effect.

In addition, its relatively large size offers adequate statistical power for the genetic analysis of many diseases common in the island. Furthermore, due to its organization into long-established settlements, it simplifies analysis of micro-isolates. Mutations that arose in

ancient times and were transmitted to offspring, can be found today in apparently unrelated families, with a frequency higher than in other populations, and this is manifested by an increased incidence of rare autosomal recessive diseases. So in Sardinia a population screening is technically feasible and justifiable for many genetic disorders.

5.3 Pilot study to assess the frequency of founder mutations

The mutational analysis of the *CRLF1* gene in Sardinian patients affected by CS/CISS1, showed the presence of allelic heterogeneity, with three mutations found. Among these, c.676_677dupA and c.226 T>G are the most frequent, whereas c.221 C>T is found in only one patient.

Sardinia is one of the geographical regions with the highest number of patients. Despite this, the complexity of the phenotype and the early lethality complicate diagnosis, and this could lead to an underestimation of the number of cases. Before implementing a population-based screening program, studies should be carried out to establish a reliable prevalence of the disease as well as to assess the feasibility of routine screening. For these reasons we performed a pilot study to assess the frequency of the two most frequent mutations found associated with CS/CISS1 in Sardinia thus far. This allowed us to assess the carrier frequency and to determine the incidence of the syndrome in Sardinia. The two mutations c.226 T>G and c.676_677dupA were analyzed on 1194 anonymous DNA, selected from a cohort of about 3000 healthy donors with Sardinian origin by at least two generations, available in our laboratory and originated from four provinces, Cagliari (CA/VS), Ogliastra (OG), Sassari (SS) and Oristano (OR). The assay used was the Custom TaqMan ® SNP Genotyping Assays provided by Applied Biosystem, which allows to design oligonucleotide probes containing the desired mutations. The allelic discrimination was conducted by 7900HT Fast Real-Time PCR System (Applied Biosystem) and it was

possible to evaluate the heterozygous/homozygous state for both mutations investigated. In heterozygote controls, the result was confirmed by Sanger sequencing using the 3130 Genetic Analyzer instrument.

The third variant c.221T>C identified in Sardinia, was not considered in this pilot study since it was found very recently and only in one individual. Furthermore it was not present in any Sardinian healthy controls from the SardiNIA Medical Sequencing Discovery Project. This could be explained in two ways: either the origin of the carrier parent is not completely Sardinian, or its frequency is very rare.

We found 5 carriers for c.226T>G and 12 for c.676_677dupA, and these data allowed us to estimate a percentage of carriers of 1,4% with an incidence of about 1 affected per 20,700 newborns, i.e. 0,005 %, calculated on 15,000 live newborns/year in Sardinia. The data for c.226T>G were confirmed successively by the search of this variant within the SardiNIA Medical Sequencing Discovery Project [Sidore et al., ASHG 2012]. A more detailed analysis taking into account the different provinces of origin, showed that the most involved area is the Ogliastra, with an incidence of 1:10,200 and a percentage of carriers of 1.9% (Table 4). These findings approximately confirm the epidemiological data collected in 40 years, during which 24 CS/CISS1 patients were identified. In fact, according to our results, we would have expected about 28 affected individuals.

The data obtained so far on carrier frequency does not justify the extension of carrier screening to all couples of childbearing age, but only to at-risk relatives in family with a clear history of the disease.

6 Discussion

6.1 Biological relevance

A murine *Crlf1* knock-out (KO) model was developed in 1999 by Alexander and colleagues [Alexander et al., 1999]. The loss of *Crlf1* doesn't compromise embryonic survival but is lethal during the first day of life. Neonatal KO mice for *Crlf1* fail to suckle and die of starvation within 24 hours of birth, with their stomachs devoid of milk, suggesting that *Crlf1* is necessary for the recognition or processing of pheromonal signals or for the mechanics of suckling itself. In addition, *Crlf1* KO mice show motor neuron deficits in the facial nucleus and ventral horn of the lumbar spinal cord [Zou et al., 2009].

As well as for *Crlf1*, mice KO for Cntf receptor α (*Cntf-Rα*) and *Clcf1* mirror the same phenotype, with perinatal death, decreased facial motility, inability to suckle and significant reductions in motor neuron number, while mice and humans deficient of CNTF, the primary ligand to CNTFR, were healthy [Takahashi et al., 1994, De Chiara et al., 1995; Zou et al., 2009]. The same phenotype was also seen in the *gp130* and *Lifr* null mice. [Li et al., 1995; Nakashima et al., 1999].

The findings are analogous to those of infants with CS/CISS1 and CISS2 who suffer from severe oral-facial weakness and impaired suckling. The observations illustrate the importance of the CNTFR/gp130/LIFR tripartite receptor and its ligand CLCF1/CRLF1 for development and maintenance of the nervous system in particular for the embryonic development of facial motor neurons.

It is known that the IL6 cytokines acting through gp130 receptors are required for the cholinergic differentiation of sympathetic neurons innervating sweat glands [Stanke et al., 2006]. CRLF1 and CLCF1 are cytokines expressed in sweat-gland tissue, and currently, this complex is one of the most likely candidates to mediate the switch from noradrenergic

to cholinergic phenotype of sympathetic neurons via gp130/LIFR pathway [Stanke et al., 2006].

Cholinergic sympathetic neurons innervate, as additional target tissues, the skeletal muscle vasculature and the periosteum, the connective tissue covering the bone [Francis et al., 1999]. Skin biopsies from a CS/CISS1 patient, derived from areas of hyperhidrosis showed that the sweat glands lacked cholinergic innervation while adrenergic supply was amply maintained [Di Leo et al., 2010]. If confirmed, these results would indirectly support a role for CLCF1/CRLF1 in mediating the switch from noradrenergic to cholinergic properties of sympathetic neurons that innervate sweat glands and periosteum during development.

Although the defects observed in mice and humans suggest vitally important functions of *CRLF1* expression in developmental pathways, new evidences suggest that changes in *CRLF1* expression may also be associated with several post-natal disease processes [Kass, 2011]. A paper published in 2009 suggests that the CRLF1/CLC complex disrupts cartilage homeostasis and promotes the progress of Osteoarthritis (OA) by enhancing the proliferation of chondrocytes and suppressing the expression level of cartilage structural proteins [Tsuritani et al., 2009]. Furthermore it has also been recently supported a potentially important antifibrotic role for CRLF1 in Idiopathic Pulmonary Fibrosis, suggesting that its expression in the lung could be a potentially reparative response to fibrotic lung injury [Kass et al., 2012]. Both these studies show that the CRLF1 is involved in other more common diseases. This could further explain the complexity of the CS/CISS1 phenotype.

Recently Crabe et al., in 2009 found that similar to CLCF1, the p28 subunit of IL-27 could associate with CRLF1 to form a new complex that can bind IL-6R, a tripartite receptor of IL-6Ra, WSX-1, and gp130. Activation of this receptor leads to downstream signaling events via the JAK/STAT pathway (particularly STAT3), MEK/ERK, and PI3K/AKT.

This recent discovery suggests that CRLF1 can stimulate cell populations that may not express CNTFR, so up to now the full range of cells that are potentially responsive to CRLF1 stimulation is unknown, as well as the biological activity of CRLF1 on these cells. These data highlights how CRLF1 function and is still little known, as well as its involvement in other pathways. These new interactions could further elucidate the complexity of the CS/CISS1 phenotype and could explain a definitive relationship with the mutations found.

6.2 Clinical and Diagnostic relevance

Before 2007 it was thought that CS and CISS1 were different disorders, with CS reported for its neonatal phenotype and CISS1 for its evolutive phenotype. In 2007, the identification of mutations in the *CRLF1* gene led to the conclusion that they were allelic forms of the same disease. Functional studies on mutated forms of CRLF1, gave the hint that the two syndromes, CS and CISS1, represent manifestations of one single disorder, with different degrees of severity. The rare cases of CISS1 most likely correspond to CS survivors. This led to the old matter about ‘lumpers' and ‘splitters' and thus to the critical question of how to classify and name these genetic entities. In 2011 it was suggested to rename the two genetic entities CS and CISS1 with the broader term of Sohar–Crisponi syndrome [Herholz, Meloni et al., 2011]. However the discussion about what term is more appropriate to define the syndrome is still open. Since 2007, a molecular genetic testing for *CRLF1* mutations is available, allowing for a reliable genetic counseling. It comprises the sequence analysis of all 9 exons and exon-intron boundaries. If a variant is found in a patient, several types of analyses are performed to determine its pathogenicity. The variant is checked in mutation databases and published data, any novel non synonymous variant is checked in 100 alleles from control samples (with matched ethnicity where possible). The

variant is also checked on the dbSNFP software database, which runs several prediction programs (SIFT, Polyphen, Mutation taster, Mutation assessor, LRT, GERP and PhyloP) that predict the likely effect of the missense mutation on the CRLF1 protein. Variants predicted to affect splicing are also checked using Netgene2. The presence of the variant is also evaluated in the parents and other family members. The results of our study have shown that mutations in the *CRLF1* gene are responsible for CS/CISS1, representing a single genetic entity with variable degrees of severity. Functional studies have shown that altered kinetics of protein secretion associated with mutated CRLF1 proteins is associated with various degrees of severity in CS/CISS1. However, there is currently no clear genotype/phenotype correlation for both type and location of mutations in *CRLF1*. The distinctions are further complicated by the combination of different mutations (compound heterozigosity) in some patients. Further functional studies on mutated CRLF1 proteins will be needed to better define their role in the pathogenesis of CS/CISS1.

7 Future prospects

The functions of CRLF1 need to be further explored. Little is known about other interacting proteins and receptors involved. Future research will be directed toward a better understanding of the molecular disease mechanisms, of the genotype–phenotype correlations and of potentially modifiers of the phenotype, making use of recombinant systems, proteomics approaches or mouse models. In particular, since the *Crlf1* null mouse dies on postnatal day 1, a conditional model using the cre-lox system may be effective in dissecting the organ-specific effects of *Crlf1* deficiency. A more deep understanding of CRLF1 signalling pathways would be critical to the development of novel therapeutic strategies for CS/CISS1 as well as other diseases.

Furthermore a short-time goal will be the clinical and genetic delineation of CS/CISS1-like phenotypes, which are not caused by *CRLF1/CLCF1* mutations. In such cases, the identification of new disease-causing genes, after exclusion of rearrangements by SNP/CGH arrays would be achievable by whole-exome sequencing, and will help in better dissecting pathways and networks where CRLF1 is involved and function.

8 Materials and methods

8.1 Clinical Questionnaire and Consent Form for Genetic Analysis

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. Neonatologists, pediatricians or geneticist who request the molecular analysis for *CRLF1* are invited to fill in and complete a detailed clinical questionnaire, necessary for a critical evaluation of the phenotype and for a future genotype/phenotype correlation. The written consent and clinical questionnaire were attached in the supplementary materials.

8.2 DNA extraction

DNA used for PCR-based diagnostic analysis has originated from white cells fractionated from whole blood in EDTA. The method used was saline extraction (*salting-out*) based on osmotic lysis of red blood cells and then white cells isolations. So the cells were lisated with SDS 10% and Proteinase K that degrades the proteins permitting the nucleic acids extraction. The last step is the precipitation with isopropanol.

The DNA quality and its concentration was determined using both the spectrophotometric reading (Nanodrop 2000c Spectrophotometers-Thermo Scientific) and by loading an aliquot of DNA on agarose gel electrophoresis (0,8%)

8.3 PCR (Polymerase Chain Reaction) and Sequencing

The PCR was performed in 25 µL of final volume; the protocol is the following:
50 nanograms DNA, 1XBuffer, 1.5millimoles of MgCl2, 200µM of dNTP and 25 picomoles of primer pairs. 1U of TaQ Polymerase was added to solution.
We used Buffer GC-rich (1X final) and DMSO (1X final) in place of normal Buffer for amplification of the GC rich regions. See below the tables of the primers (5'-3') used for the analysis :

CRLF1

Exon	Forward	Reverse	bp
1	ttagcgccttgtcaattcggc	tgttccccggccgtccagg	394
2	gacaatcattaacagcgtc	agtgtgcccacagctcatcc	507
3	ggagatcgagtcaccagcctc	ggcagcctcagggtgcagac	441
4	cttgaccaacgcggaccct	acttacctaccttccctctg	456
5	acagaggcaggttccatc	caggaggtctggttgctcac	250
6	ctaccgagtggaggacagtg	tatgcgacagaatgaggccg	421
7	tcggtccttgagaaacggg	ttggagcagtacgcgtgc	252
8	agctcaagcagttcctgg	gggtgtgaacaagacctgc	377
9	ggacaggacacgaatgaagc	cattaagacgcctcacattccc	518
2Fint.*	gacacactataggtaccctg	1Rint* tctcgttccacacagagtgg	-

*The primer pairs 2Fint and 1Rint were used only for Long Range PCR.

CLCF1

Exon	Forward	Reverse	bp
1	tcctgggagtcctcagacg	aggacgggaaccggatctc	484
2	cctctctttctctcccgtctt	actggtgggagccaaagagc	473
3	tcacctgcatacaaatgatatcc	actccctcgagcatgacttc	931
3'UTR	aggtctaccgagctggggag	ggcaagagtctgatgagcacc	847

CNTFRα

Exon	Forward	Reverse	bp
1	ggagagtgagtgtgaagga	tcgtgaactttcctgtcg	845
2	gtgctgggaggggtctgtatta	gcatgaaaagcctcagccag	455
3	cctactccgtgtcagtcggg	ctccatgtccctctgggtgg	388
4	ggagctttgaacactcttcatc	ggctcagaggccagaagag	562
5/6	ccacaactttggcatcaatg	ggcatgtacatgccatgtatac	700
7	ggggatatcagacttggaac	gcagagagcctgatgcatc	479
8	ccttctcctcagggaagtcc	ggcaggagttggacagacag	462
9/10	ggaaacaggatctgcctgatg	ccctcacgtcccccaagg	700
10	ccaagctggccctccttcc	caccttccccaaccacaatttc	559

The PCR products were purified with ExoSAP-IT® (Invitrogen) and sequenced using the relative primers and BigDye Terminator v3.1 Cycle Sequencing kits (Applied Biosystem). by the 3130xl Genetic Analyzer (Applied Biosystem).

8.4 Quantitative PCR with SYBR®Green assay

The quantitative PCR was performed by SYBR®Green assay (Applied Biosystem life Technologies) using the 7900HT Fast Real Time PCR system instrument (Applied Biosystem). Quantification was performed using the comparative C_t method also referred to as the $2^{-\Delta\Delta C}{}_T$ method [Schmittgen & Livak, Nature 2008]. The copy numbers of the target gene were normalized against a calibrator DNA sample with disomic copy number of all exons (normal human DNA). The results were normalized and we estimated a copy number value of about 0,5 for the parents, (one allele), carriers of the deletion; of 1,00 for the reference sample (both alleles), and of 0 (absence of final product), in the patient [Barrois et al., 2004, Rose-Zerilli et al., 2009].

The specific primers have been designed using the software Primer Express v2.0 (Applied Biosystem). An intergenic region of chromosome 3,was used as reference gene. (Forward tgttcacagccacaaaccagat; Reverse cctaccacagtctccacacctgta)

The protocol in 10 µL final volume is the following: 10 nanograms DNA; 1X SYBR®Green solution and 2,5 µM primer pairs.

8.5 Allelic discrimination

The allelic discrimination was performed with Custom TaqMan®SNP Genotyping Assays (Applied Biosystem) designed specifically for the desired mutations (c.226 T>G and c.676_677dupA). The instrument is 7900HT Fast Real-Time PCR system (Applied Biosystem). In a final volume of 5 µL the protocol is the following: 10 nanograms DNA; 1X TaqMan®Genotyping Master Mix; 1X Assay Mix

9 List of software and databases used

For the functional prediction of the new mutations found we used the following software:

- dbNSFP (http://sites.google.com/site/jpopgen/dbNSFP);
- FastNP (http://fastsnp.ibms.sinica.edu.tw/pages/input_CandidateGeneSearch.jsp);
- PolyPhen (http://genetics.bwh.harvard.edu/pph/);
- SIFT (http://sift.jcvi.org/);
- ESEfinder (http://rulai.cshl.edu/cgi-bin/tools/ESE3/esefinder.cgi?process=home);
- NetGene2 (http://www.cbs.dtu.dk/services/NetGene2/).

For the analysis of the variants we used the following databases:

- dbSNP (http://www.ncbi.nlm.nih.gov/projects/SNP/)
- Exome Variant Server (http://evs.gs.washington.edu/EVS/)
- 1000GenomeProject (http://www.1000genomes.org/).

For the mutation nomenclature we used the following software and databases:

- Mutalyzer (https://mutalyzer.nl/)
- HGVS (http://www.hgvs.org/)
- NCBI (http://www.ncbi.nlm.nih.gov/)

10 Tables

						Prediction with dbNSP										
Origin/Ethnicity	**Dna variant**	**Number**	**Effect**	**Domain**	**Mutation type**	**SIFT**	**Polyphen2_HDIV**	**Polyphen2_HVAR**	**LRT**	**MutationTaster**	**Mutation assessor**	**GERP++_NR**	**GERP++_RS**	**phyloP**	**NetGene2**	**Previous_pubblications**
Sardinia	c.226 T>G	M1	p.W76G	Ig-like	Missense	D (0.00)	D (997)	D (986)	D (0.00)	D (0.99)	L (975)	5.23	5.23	1.98		Crisponi et al., 2007; Dagoneau et al., 2007
Sardina	c.676dupA	M2	p.T226Nfs*104	FNIII 1	Insertion											Crisponi et al., 2007; Dagoneau et al., 2007
Turkey	c.708_709delCCinsT	M3	p.P238Rfs*6	FNIII 2	Del/ins											Crisponi et al., 2007
Turkey	c.1102 A>T	M4	p.K368*	C-terminus	Nonsense											Crisponi et al., 2007
Spain	c.223 T>G	M5	p.Y75D	Ig-like	Missense	T (> 0.11)	D (994)	P (832)	D (0.00)	D (0.96)	L (805)	5.23	5.23	1.98		Heroltz et al., 2011
Spain/Turkey/France	c.713dupC	M6	p.P239Afs*91	FNIII 2	Insertion											Dagoneau et al., 2007; Herolz et al., 2011; Lebre 2011*
Libya	c.539dupA	M7	p.D181Gfs*5	FNIII 1	Insertion											Heroltz et al., 2011
Italy	c.[338 A>T;341 T>C]	M8	p.[N113I;L114P]	Ig-like	Missense	[D (0.04);D (0.00)]	[D (999);D(999)]	[D (994);D (997)]	[D (0,00);D (0,00)]	[D (0,99); D (0,99)]	[L (975) L (975)]	[5,23; 5,23]	[5,23;5,23]	[1.98; 1.98]		Heroltz et al., 2011
Canada	c.538C>T	M9	p.Q180*	FNIII 1	Nonsense											Hahn et al., 2006
Canada	c.852G>T	M10	p.W284C	FNIII 2	Missense	D (0.00)	D (1.0)	D (998)	D (0.00)	D (0.99)	M (3.21)	4.78	4.78	2.38		Hahn et al., 2006
Norway	c.844_845delGT	M11	p.V282Gfs*47	FNIII 2	Deletion											Knappskog et al., 2003; Hahn et al., 2010
Israel	c.242G>A	M12	p.R81H	Ig-like	Missense	T (0.17)	B (4)	B (3)	N (0.0013)	D (0.50)	N (695)	5.23	4.18	1.20		Knappskog et al., 2003
Israel	c.1121T>G	M13	p.L374R	C-terminus	Missense	D (0.01)	P (838)	B (276)	D (0.00)	D (0.71)	L (895)	3.91	3.91	1.64		Knappskog et al., 2003
Turkey	c.829 C>T	M14	p.R277*	FNIII 2	Nonsense											Okur et al., 2008 ; Benoit 2012**
India	c.1-?_c.115+?del	M15	p.0?		Deletion											Thomas et al., 2008
Pakistan	c.433 T>C	M16	p.S145P	FNIII 1	Missense	T (0,11)	D (999)	D (996)	D (0.00)	D (0.99)	M (2.72)	5.3	5.3	2.01		This report
Australia	c.721_737dup	M17	p.G247Cfs*3	FNIII 2	Insertion											This report
Australia	c.935 G>C	M18	p.R312P	FNIII 2	Missense	D (0,00)	D (999)	D (998)	D (0.00)	D (0.99)	M (3165)	4.98	4.98	2.50		This report
Spain	c.115+1 G>A	M19	p.0?		Splice site										loss of donor splice site	This report
Yemen	c.527+5 G>A	M20	p.0?		Splice site										loss of donor splice site	Dagoneau et al., 2007
Turkey	c.475delG	M21	p.A159Pfs*75	FNIII 1	Deletion											This report; Hahan et al., 2012
Turkey	c.398-456_c.697+747del	M22	p.0?		Deletion											This report
Japan/USA/Israel	c.31_53del	M23	p.Q11Vfs*68	Signal peptide	Deletion											Yamazaki et al., 2010; Hahn et al., 2010
USA	c.303delC	M24	N102Tfs*47	Ig-like	Deletion											Hahn et al., 2010
Spain	c.[803 T>C;1018 C>T]	M25	p.[F268S; R340C]	FNIII 2/C-term.	Missense	[D (0,03); T (0,11)]	[P (948);D (998)]	[P (765);P (585)]	[D (0,00);N (0,24)]	[D (0,99); N (0,29)]	[M (2005); N (345)]	[4,7; 4,98]	[4,7;3,87]	[1.90;2.50]		This report
Italy	c.935 G>A	M26	p.R312H	FNIII 2	Missense	D (0,01)	D (1.0)	D (997)	D (0.00)	D (0.99)	M (2615)	4.98	4.98	2.50		Di Leo et al., 2010
Italy	c.856-?_c.1269+?del	M27	p.0?		Deletion											Di Leo et al., 2010
Arabia Saudita	c.983dupG	M28	p.S328Rfs*2	FNIII 2	Insertion											This report; El Assy et al., 2012
Turkey	c.698-?_c.1269+?del	M29	p.0?		Deletion											This report
Morocco	n.a	M30	n.a	-	Missense											Lebre et al., 2011*
Italy	n.a	M31	n.a	-	Missense											Lebre et al., 2011*
Italy	n.a	M32	n.a	-	Deletion											Lebre et al., 2011*
France	n.a	M33	n.a		Splice site											Lebre et al., 2011*
France	n.a	M34	n.a	-	Missense											Lebre et al., 2011*
Turkey	n.a	M35	n.a	-	Splice site										-	Lebre et al., 2011*
Turkey	n.a	M36	n.a	-	Insertion											Lebre et al., 2011*
Turkey	c.413 C>T	M37	p.P138L	FNIII 1	Missense	D (0,00)	D (998)	D (966)	D (0.00)	D (0.99)	M (2.85)	5.3	5.3	2.49		Tuysuz et al., 2013
-	c.397+1G>A	M38	p.0?		Splice site										loss of donor splice site	Hahn and Boman, 2011
Turkey	c.776 C>A	M39	p. S259*	FNIII2	Nonsense											This report
Sardinia	c.221 T>C	M40	p.L74P	Ig-like	Missense	D (0,00)	D (999)	D (997)	D (0.00)	D (0.99)	L (975)	5.23	5.23	1.98		This report
Spain	c.527+5 G>T	M41	p.0?		Splice site										loss of donor splice site	This report
Pakistan	c.646 C>T	M42	p.R216C	FNIII 1	Missense	D (0,01)	D (999)	P (894)	D (0.00)	D (0.70)	L (895)	4.81	2.59	0.52		This report

Table 1 Summary of the 42 known *CRLF1* mutations found so far in CS/CISS1 patients. For SIFT, Polyphen and LRT and Mutation Taster predictions: D indicates Damaging, P indicates Probably Damaging, T indicates Tolerated and B indicates Benign. For Mutation Assessor prediction L indicates Low; M, Medium and N, neutral.

				Fast-SNP			
Number	**ex/int**	**DNA variant**	**Effect**	**SNP**	**Variant type**	**Effect**	**Transcription regulatory**
1	exon 1	c.73_75delCTG	p.Leu25del	rs137853925	In frame deletion	Untested	
2	exon 2	c.237 C>T	p.N79N	rs2238647	Synonymous	Sense/Synonymous with very low risk	
3	intron 4	c.698-19 T>G	-	rs7247346	Intronic	-	
4	exon 2	c.266 G>A	p.R89H	rs143326783	Missense	-	
5	intron 2	c.398 - 57 C>T	-	rs8108207	Intronic	Intronic with no Known function	-
6	intron 4	c.697 + 67 G>A	-	rs35521276	Intronic	Intronic ehancer: lower risk (very low), upper risk (low)	transcription factor binding site*
7	intron 4	c. 698 - 19 T>G	-	rs7247346	Intronic	Intronic with no Known function	-
8	intron 2	c.398-50 C>T		rs28579583	Intronic	Intronic with no known function	
9	intron 6	c.1025-65 C>A		rs79743774	Intronic	-	

(*GATA-2; *NF-kappaB1)

Table 2 Summary of the non causative variants found in our patient cohort.

Family	A	B	C	D	E	F	G	G	H	I	I	I	J	K	L	L	L	M	N	N	O
Reference	Crisponi 2007	Crisponi 2007	Crisponi 2007	Crisp.2007/Dag.2007	Crisponi 2007	Crisponi 2007	Crisponi 2007		Crisponi 2007	Herolz 2011			Herolz 2011	Herolz 2011	Knappskog2003		Hahn 2010	Hahn 2006	Sohar 1978/Knappskog2003		This report
Patient code	SC03	SC07	SC10	SC14	SC17	SC37	SC41	SC42	SC21	SC84	SC85	SC86	SC78	SC48							SC175
Patient	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Sex	F	M	F	F	F	M	M	F	F	F	F	M	M	F	M	M	M	F	F	F	F
Age at Publication	28	9	12	14	3	30 mo.	17	16	5	16	14	4	20	3	33	28	19	24	21	20	
Age at Diagnosis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3
Origin	Sardinia	Sardinia	Sardinia	Sardinia	Sardinia	Turkey	Turkey	Turkey	Turkey	Libya	Libya	Libya	Spain	Italy	Norway	Norway	Norway	Canada	Israel	Israel	Sardinia
Original Classification	CS	CS	CS	CS	CS	CS	CS	CS	CS	CS	CS	CS	CS	CS	CISS1	CISS1	CISS1	CISS1	CISS	CISS	CS
Mutation	M1/M2	M2	M2	M1/M2	M1	M3	M3	M3	M4	M7	M7	M7	M5/M6	M8	M11	M11	M11	M9/M10	M12/M13	M12/M13	M1/M2
Type of mutation	Mis/Ins	Ins	Ins	Mis/Ins	Mis	Delins	Delins	Delins	Nons	Ins	Ins	Ins	Ms/Ins	Mis	Del	Del	Del	Nons/Mis	Mis/Mis	Mis/Mis	Mis/Ins
Phenotypic severity	Severe	Severe	Severe	Severe	Severe	Severe	Severe	Severe	Severe	Severe	Severe	Severe	Severe	Severe	Severe	Interm	severe	Interm	Mild	Mild	
Hyperthermia	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	n.a	n.a	n.a	+
Contraction of oropharyngeal muscles	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	n.a	n.a	n.a	+
Dehydration	-	-	-	-	+	+	-	-	n.a	-	+	-	-	-	+	-	n.a	n.a	n.a	n.a	-
Cyanosis	+	+	+	+	+	-	-	-	+	+	+	+	-	-	-	-	n.a	n.a	n.a	n.a	+
Swallowing	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+
Nasogastric feeding	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	n.a	-	-	
Camptodactyly	+	+	+	+	+	+	+	+	+	-	-	-	+	+	+	+	+	+	n.a	n.a	+
Chubby cheeks	+	+	+	+	+	+	-	-	+	-	-	-	-	-	+	-		n.a	n.a	n.a	+
Foot anomalies	-	-	+	-	-	+	+	+	n.a	-	-	+	-	+	-	+	+	+	+	+	-
High arcade palate	+	n.a	-	-	-	+	+	-	n.a	+	+	+	+	+	+	+	+	+	+	+	n.a
Depressed nasal bridge	+	+	+	-	-	n.a	+	+	n.a	-	-	-	-	-	-	-	+	n.a	+	+	+
Cold induced sweating	+	+	+	+			+	+	n.a	+	+	+	+		+	+	+	+	+	+	n.a
Scoliosis	+	+	+	+	+		+	+	n.a	+	+	+	+	+	+	+	+	+	+	+	n.a
Joint contractures	+	+	n.a	n.a	-	+	+	+	n.a	-	-	+	+	-	+	+	+	+	+	+	n.a
Psychomotor retardation	+	+	+	+	-	+	+	+	n.a	-	-	-	-	-	-	-	?	-	-	-	n.a

Table 3 (I): Genetic and clinical data for CS/CISS1 patients (from case 1 to 21) reported so far and positive to mutations in the *CRLF1* gene

Family	P	Q	R	S	S	T	T	U	V	W	X	Y	Z	AA	AB	AC	AD	AE	AF	AG	AH
Reference	This report	This report	This report	This report		Dagoneau 2007		Okur 2008	Thomas 2008	Dagoneau 2007	Yamazakhi 2010	Hahn 2010	Di Leo 2010	This report	This report	This report	Hakan 2012	This report	This report	This report	This report
Patient code	SC115	SC185	SC154	SC104	SC107	3	4	SC54	SC61					SC119	SC148	SC161	SC178	SC157	SC189	SC212	SC226
Patient	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42
Sex	F	F	M	F	F	M	M	M	M	F	F	F	M	M	M	M	F	M	M	M	F
Age at Publication						6	?	9 mo	10 mo	?	30	24	16								
Age at Diagnosis	36 days	2 mo	2 mo	11 mo	22 mo	-	-	-	-	-	-	-	-	1 mo	3	14 mo	13 mo.	2 mo	10	-	4 mo
Origin	Sardinia	Turkey	Turkey	Spain	Spain	Spain	Spain	Turkey	India	Yemen	Japain	USA	Italy	Pakistan	Australia	Spain	Turkey	Turkey	Spain	Turkey	Turkey
Original Classification	CS	CS	CS	CS	CS	CS	CS	CS	CS	SWS	CISS1	CISS1	CISS1	CS	CS	CS	CS	CS	CS	CS	CS
Mutation	M2	M3	M6	M6	M6	M6	M6	M14	M15	M20	M23	M23/M24	M26/M27	M16	M17/M18	M19/M41	M21	M22	M25	M3	M29
Type of mutation	Ins	Delins	Ins	Ins	Ins	Ins	Ins	Nons	Del	Ss	Del	Del/Del	Mis/Del	Mis	Ins	Ss/Ss	Del	Del	Mis	Delins	Del
Phenotypic severity						severe			severe		severe	severe								no QC	severe
Hyperthermia	+	+	+	+	+	+	+	+	+		+	+		+	+	+	+	+	-		+
Contraction of oropharyngeal muscles	+	+	+	+	+	+	+	+	+	+	-	+		+	+	+	+	+	-		-
Dehydration	-	+	-	n.a	n.a	n.a	n.a					n.a		n.a	-	-	n.a	+	-		
Cyanosis	-	+	+	n.a	n.a	n.a	n.a		+		+	n.a		n.a	-	+		-	-		+
Swallowing	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-		+
Nasogastric feeding		-	+			?		+	+	?	+	+		+		+			-		
Camptodactyly	+	+	-	+	+	+	+	+	+	+	+	+		+	+	+	n.a	+	+		+
Chubby cheeks	+	+	-	+	+	n.a		-	+		+	-		+	+	+	+	+	+		+
Foot anomalies	+	+	-	-	-	+					+	n.a		+	-	-	n.a	+	+		-
High arcade palate	-	+	+	-	-			+	+		+	+	+	-	-	+	n.a	+	+		
Depressed nasal bridge	-	+	+	-	-	?			+		+	+		-	-	-	n.a	+	-		+
Cold induced sweating	n.a	n.a	n.a	n.a	n.a	+					+	+	+	n.a	-	+	n.a	n.a	+		+
Scoliosis	n.a	-	n.a	n.a	n.a	+	+				+	+	+	n.a	+	+	n.a	n.a	+		-
Joint contractures	-	+	-	-	-	+	+		+		+	+		+	n.a	?	n.a	+	+		-
Psychomotor retardation	n.a	+	-	n.a	-	+			+			-		n.a	-	+	n.a	+	+		+

Table 3 (II): Genetic and clinical data for CS/CISS1 patients (from case 22 to 42) reported so far and positive to mutations in the *CRLF1* gene

Family	AI	AJ	AK	AL	AL	AM	AM	AM	AN	AO	AP	AQ	AR	AS	AT	AU	AV	AW	AX	AY	AZ
Reference	El-Assy 2012	Cosar 2011	This report	Tuysuz 2012		Lebre 2011			Lebre 2011							This report	This report	This report	Benoit 2012	This report	This report
Patient code	SC231	Cosar 2011	SC246			ESHG 2011	ESHG 2011	ESHG 2011	ESHG 2011	ESHG 2011	ESHG 2011	ESHG 2011	ESHG 2011	ESHG 2011	ESHG 2011	SC255	SC264	SC278	ASHG 2012	SC218	SC247
Patient	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63
Sex	F	M	F	M	M											F	M	M	?	F	M
Age at Publication	5.5 mo	6 mo		22	13														?		
Age at Diagnosis	-	-	12 mo	-	-	-	-	-	-	-	-	-	-	-	-	6 mo	7 mo	4 mo	-	6 mo	?
Origin	Saudi Arabia	Turkey	Sardinia	Turkey	Turkey	France	France	France	Morocco	Italy	Italy	France	France	Turkey	Turkey	Turkey	Israel	Sardinia	Turkey	Spain	Pakistan
Original Classification	CS	CS	CS	CISS1	CISS1	CS	CS	CS	CS	CS	CS	CS	CS	CS	CS	CS	CS	CS	CS	CS	CS
Mutation	M28	M3	M2	M37	M37	M6	M6	M6	M30	M31	M32	M33	M34	M35	M36	M39	M23	M2/M40	M14	M6/?	M42/?
Type of mutation	Ins	delins	Ins	Mis	Mis	Ins	Ins	Ins	Mis	Mis	Del	Ss	Mis	Ss	Ins	Nons	Del	Ins/Mis	Nons	Ins/?	Mis/?
Phenotypic severity	severe	severe	severe	Mild	Mild												severe	Mild			
Hyperthermia	+	+	-	+	+	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	+	+	-	n.a	+	?
Contraction of oropharyngeal muscles	+	+	+	-	-	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	+	+	+	n.a	+	?
Dehydration	-		-			n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	-	-	-	n.a	-	?
Cyanosis	+		-			n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	+	-	-	n.a	-	?
Swallowing	+		+	+	+	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	-	+	-	n.a	+	?
Nasogastric feeding	+	+	+			n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	+	-	-	n.a	+	?
Camptodactyly	+	+	+	+	+	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	+	+	-	n.a	+	?
Chubby cheeks	+	+	+	+	+	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	+	+	+	n.a	+	?
Foot anomalies	-		-			n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	-	+	-	n.a	-	?
High arcade palate	+	+	+	-	+	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	+	n.a	+	n.a	-	?
Depressed nasal bridge	+	+	+	+	+	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	+	+	-	n.a	-	?
Cold induced sweating	n.a		-	+		n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	-	n.a	+	?
Scoliosis	-		-	+	+	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	-	-	-	n.a	-	?
Joint contractures	-	+	-			n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	+	+	-	n.a	+	?
Psychomotor retardation	n.a		-	-	+	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	+	-	-	n.a	-	?

Table 3 (III): Genetic and clinical data for CS/CISS1 patients (from case 43 to 63) reported so far and positive to mutations in the *CRLF1* gene

Provincies	Controls	Carriers		Allele p* frequency	Allele q* frequency	2pq* frequency	Incidence
		c.226T>G	c.676_677dupA				
CA/VS	340	2	3	0,993	0,007	0,014	1: 19050
OG	450	2	7	0,99	0,01	0,019	1: 10200
SS	287	0	1	0,998	0,002	0,004	1: 250000
OR	117	1	1	0,992	0,008	0,016	1: 15600
Sardinia	**1194**	**5**	**12**	**0,993**	**0,007**	**0,0139**	**1: 20700**

Table 4. Estimate of the allele frequency and incidence of CS/CISS1 in the Sardinian population**.** For the Hardy-Weinberg equilibrium $(p+q)^2=1$: **(**p* = frequency of the dominant allele; q* = frequency of the recessive allele; 2pq* = frequency of heterozygous **).**
The incidence in the four different provinces has been calculated on 15,000 new birth/ year in Sardinia (http://www.sardegnastatistiche.it).

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Study on Crisponi Syndrome and Cold Induced Sweating Syndrome

Study Coordinator: PD Dr. Frank Rutsch, University Children's Hospital, Albert-Schweitzer-Strasse 33, D-48149 Münster, Germany

Consent Form for Genetic Analysis and for Participation in the Patient's Registry

Patient Name: ……

Date of birth: …… / …../ …….

Today, I was informed by Dr. _____ , that a sample of me (my child) is planned to be taken for molecular genetic studies.

It is confirmed, that the sample will not be used for commercial purposes.

It is confirmed, that the sample will only be used for genetic analysis of the disorder I am (my child) is presumably suffering from, _____ (please specify). The results of the genetic studies will only be told to the physician involved. Protection of privacy is ascertained.

□ (please check) I agree, that the sample indicated above can be taken from me (my child).

□ (please check) I agree, that the clinical data of me (my child) will be used in a registry for the disease in an pseudonymous fashion. This means that my name (my child's name) will be substituted by a code.

□ (please check) I agree that in the course of the research study my data (my child's data) concerning the health status and the clinical history, age, gender, weigth and height and ethnic origin will be gathered and saved in a pseudonymous fashion.

□ (please check) I agree that these data will be given to the study coordinator and to the official supervising committee of the research study, who will check if the study is run according to standards.

□ (please check) I agree that an authorized person of the study coordinator or an authorized person of the supervising committee can gain insight into these data, if this is necessary to check the correct guidance of the study.

I know, that I can repeal this consent at any time and without giving reasons.
Place: …… Date: ….. / …../ ……..

…… / …… ……
Signatures of the parents/ patient Signature of the physician

Name: …… / …….. Name: ……

CRISPONI SYNDROME
AND
COLD INDUCED SWEATING SYNDROME

- clinical data sheet –

Patient Initials:
Date of birth: ____.____._____ (dd.mm.yyyy)
Sex: m □ f □

Physician:

Hospital / Address:

Email:

Date: ____.____._____ (dd.mm.yyyy)

Case History

Past Medical History

Twin: yes □ no □

Delivery: spontaneous □ C-Section □ Other □ (please specify: _____)

Gestional Age: _____

Birth Weight: _____ g

Birth Length: _____ cm

Birth Head Circumference: _____ cm

Apgar Score: ____ at 1min. ____at 5 min. ____ at 10 min

Umbilical Cord pH: _____

Family History (please attach pedigree-tree)

Nationality / Ethnic Origin: _____

Consanguinity of Parents: yes □ no □ unknown □

Abortions: yes □ no □ unknown □
If yes, how many: _____

Still born Children: yes □ no □ unknown □
If yes, how many: _____

Siblings with Diagnosis Crisponi Syndrome: yes □ no □ unknown □ deceased □
If yes, how many: _____
(Please attach an additional form for each sibling)

Siblings with Diagnosis CISS: yes □ no □ unknown □ deceased □
If yes, how many: _____
(Please attach an additional form for each sibling)

Deceased Siblings: yes □ no □ unknown □
If yes, how many: _____

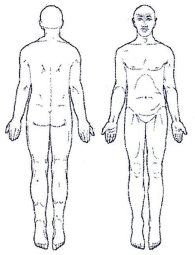
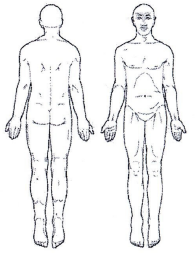
Healthy Siblings: yes □ no □ unknown □
If yes, how many: _____

Clinical Findings from Infancy until now

Please mark observed symptoms: ☒

	Symptom	Onset (Month, Year)	Dates of further incidence (Month, Year)	Cessation (Month, Year)	Please specify special findings
☐	**Hyperthermia:**				Please specify body temperature:
☐	**Contraction of facial muscles:**				Please specify regions:
☐	**Trismus:**				
☐	**Hypersalivation:**				
☐	**Swallowing / Feeding Difficulties:**				
☐	**Hypertonia of neck muscles:**				
☐	**Cyanosis:**				
☐	**Dehydration:**				
☐	**Gastrooesophageal Reflux:**				Proven by:
☐	**Hyporeflexia:**				
☐	**Apnea:**				
☐	**Reduced Appetite:**				

Please mark observed symptoms: ☒

	Symptom	Onset (Month, Year)	Dates of further incidence (Month, Year)	Cessation (Month, Year)	Please specify special findings
☐	**Cold Induced Sweating:**				 Please specify affected areas ⇩ Inducing Temperature: from _____ to _____ °C
☐	**Sweet Induced Sweating:**				 Please specify affected areas ⇩
☐	**Restricted Jaw Movements:**				
☐	**Reduced Sensitivity to Heat:**				
☐	**Reduced Sensitivity to Pain:**				
☐	**Psychomotor Retardation:**				
☐	**Muscle Dystrophia:**				
☐	**Other, especially autonomic dysfunction:**				

Physical Features

Date of measuring: ____.____._____ (dd.mm.yyyy)

Body Height: _____cm
Body Weight: _____kg

Photo: if possible, please attach a recent photo of the patient.

Head

Chubby Cheeks: yes ☐ no ☐

Micrognathia: yes ☐ no ☐

Anteverted Nostrils: yes ☐ no ☐

High Arched Palate: yes ☐ no ☐

Low Set Ears: yes ☐ no ☐

Rotated Ears: yes ☐ no ☐ direction: _____

Depressed Nasal Bridge: yes ☐ no ☐

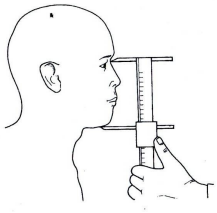
Expressionless Face: yes ☐ no ☐

Nasal Voice: yes ☐ no ☐

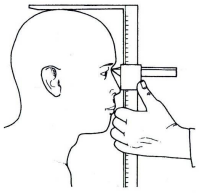
Other important findings: yes ☐ no ☐
(please specify) _____
_____

Head Circumference:
_____ mm

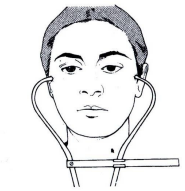
Facial Height
(distance from the root of the nose to the inferior border of the mandible):
_____ mm



Skull Height
(distance from the root of the nose to the highest point of the vertex):
_____ mm

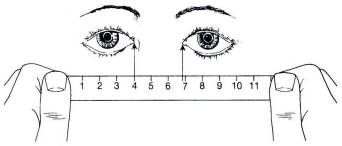


Bizygomatic Distance
(distance between the most lateral points of the zugomatic arches):



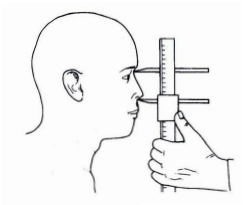
_____ mm

Inner Canthal Distance
(distance between the inner canthi of the two eyes):



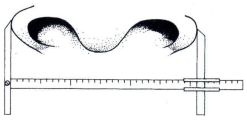
_____ mm

Nasal Length
(distance from the nasal root to the nasal base):



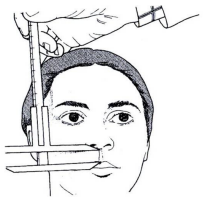
_____ mm

Interalar Distance (Nasal Width)
(distance between the most lateral aspects if the alae nasi):



_____ mm

Philtrum Length
(distance between the base of the nose and the border od the upper lip, in the midline):



_____ mm

Body

Scoliosis: yes □ no □
(please specify) _____
Surgery: yes □ no □ if yes, please specify age:_____(Month, Year)

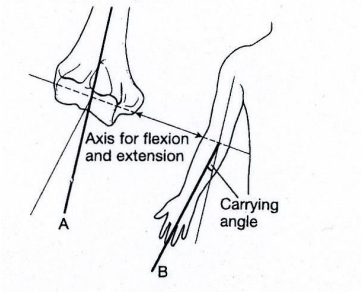
Camptodactyly: bilateral □ unilateral □ no □
Surgery: yes □ no □ if yes, please specify age:_____(Month, Year)

Feet anomalies: yes □ no □
Over-Riding Toes: yes □ no □
Rocker-bottom Feet: yes □ no □
Clinodactyly: yes □ no □
Syndactyly: yes □ no □

Torticollis: yes □ no □

Joint contractures: yes □ no □
(please specify region) _____

Carrying Angle
(angle subtended by the forearm on the humerus): _____°



Cubitus Valgus: yes □ no □

Hypospadias: yes □ no □

Other important findings: yes □ no □
(please specify) _____
_____

Diagnostic Studies

EEG: yes □ no □ date: ____.____._____ (dd.mm.yyyy)
(please specify findings) _____
_____
_____

CT: yes □ no □ date: ____.____._____ (dd.mm.yyyy)
(please specify findings) _____
_____
_____

MRT: yes □ no □ date: ____.____._____ (dd.mm.yyyy)
(please specify findings) _____
_____
_____

EMG: yes □ no □ date: ____.____._____ (dd.mm.yyyy)
(please specify findings) _____
_____
_____

Nerve Conduction Velocity: _____ m/s date: ____.____._____ (dd.mm.yyyy)

Sweat Test: yes □ no □ date: ____.____._____ (dd.mm.yyyy)
(please specify findings or attach report)_____
_____

Sleep Studies: yes □ no □ date: ____.____._____ (dd.mm.yyyy)
(please specify findings or attach report)_____
_____

Muscle Biopsy: yes □ no □ date: ____.____._____ (dd.mm.yyyy)
(please specify findings) _____

Laboratory Studies

	Date: (dd.mm.yyyy)	Date: (dd.mm.yyyy)	Date: (dd.mm.yyyy)
Plasma			
Noradrenaline (please select correct unit)			
Adrenaline (please select correct unit)			
Renin (please select correct unit)			
Vasopressin (please select correct unit)			
Dopamine (please select correct unit)			
Liquor			
GABA (please select correct unit)			
Aspartate (please select correct unit)			
Lactate (please specify correct unit)			
Glucose (please select correct unit)	mmol/L mg/dl	mmol/L mg/dl	mmol/L mg/dl
Dopamine (please select correct unit)			

Genetic Studies

Mutations in CRLF1 (Chromosome 19p12): yes □ no □
(Please specify mutations or attach report) _____
_____

Other Mutations: yes □ no □
(if yes, please specify) _____
_____

Diagnosis

Please mark correct diagnosis:
Crisponi Syndrome □ Date : ____.____._____ (dd.mm.yyyy)
Cold Induced Sweating Syndrome □ Date : ____.____._____ (dd.mm.yyyy)

Treatment

Administered Drugs:

Drug	dose / kg body-weight	Duration of treatment (from...to...)	comment

SupportiveTreatment:

	yes	no	Duration of treatment (from...to...)	comment
Artificial ventilation				
O2 supplementation				
Nasogastric feeding				
PEG tube				
Others				

Outcome

If still alive, please specify age: _____
If deceased, please specify age and cause of death: _____
_____

Autopsy Performed: yes □ no □
(if yes, please attach autopsy report)