**Title: Exome sequencing in Crisponi/CISS-like individuals reveals unpredicted alternative diagnoses.**

**Running title: Crisponi/CISS alternative diagnoses.**

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**Conflict of interest statement**

The authors declare that they have no conflict of interest.

**Abstract**

Crisponi/Cold-induced sweating syndrome (CS/CISS) is a rare autosomal recessive disorder characterized by a complex phenotype (hyperthermia and feeding difficulties in the neonatal period, followed by scoliosis and paradoxical sweating induced by cold since early childhood) and a high neonatal lethality. CS/CISS is a genetically heterogeneous disorder caused by mutations in *CRLF1* (CS/CISS1), in *CLCF1* (CS/CISS2) and in *KLHL7* (CS/CISS-like). Here, a whole exome sequencing approach in individuals with CS/CISS-like phenotype with unknown molecular defect revealed unpredicted alternative diagnoses. This approach identified putative pathogenic variations in *NALCN, MAGEL2* and *SCN2A*. They were already found implicated in the pathogenesis of other syndromes, respectively the congenital contractures of the limbs and face, hypotonia, and developmental delay syndrome (CLIFAHDD), the Schaaf-Yang syndrome (SHFYNG), and the early infantile epileptic encephalopathy-11 syndrome (EIEE11). These results suggest a high neonatal phenotypic overlap among these disorders and will be very helpful for clinicians. Genetic analysis of these genes should be considered for those cases with a suspected CS/CISS during neonatal period who were tested as mutation negative in the known CS/CISS genes, since an expedited and corrected diagnosis can improve patient management and can provide a specific clinical follow up.

**Keywords:** Crisponi/Cold induced sweating syndrome; *CRLF1; MAGEL2; NALCN; SCN2A*; whole exome sequencing.

**Introduction**

Crisponi syndrome (CS), the infantile presentation of cold-induced sweating syndrome (CISS), is characterized by dysmorphic features (distinctive facies, lower facial weakness, flexion deformity at the elbows, camptodactyly with fisted hands, misshapen feet, and overriding toes), poor suck reflex, severely impaired swallowing, and temperature spikes associated with an increased risk for seizures and sudden death.

CISS can present to the clinician from age 3 years onward as cold-induced sweating. Starting the first decade of life, children with CS/CISS develop profuse sweating of the face, arms and chest with ambient temperatures below 18°C to 22°C, and with other stimuli including apprehension or ingestion of sweets. Affected individuals sweat very little in hot environments and may feel overheated. In the second decade, progressive thoracolumbar kyphoscoliosis requiring intervention is common.[1]

The diagnosis of CS/CISS syndrome (MIM#272430) is established by clinical findings and by identification of pathogenic variants in *CRLF1* or *CLCF1*. So far more than 95% of cases are caused by mutations in *CRLF1* (CISS1), while the remaining by mutations in *CLCF1* (CISS2).[1] Alterations in *CRLF1* and *CLCF1* can be found in more than 60% of individuals with clinical diagnosis of CS/CISS.[2-4] Therefore, a subset of CS/CISS cases (about 40%) remains yet genetically unexplained. In 2016, *KLHL7* has been found mutated in five individuals with a CS/CISS-like phenotype associated to retinitis pigmentosa.[5]

We report here the results from a trio-based whole exome sequencing (WES) study in five unsolved individuals with an initial clinical diagnosis of CS/CISS. The aim of this study was to detect novel variants, and novel CS/CISS-like genes. We provide further evidence of locus heterogeneity within the CS/CISS-like phenotype spectrum and highlight overlaps with different syndromes requiring careful differential diagnoses.

**Methods: case presentations**

Five individuals with the initial diagnosis of CS/CISS but negative for mutations in *CRLF1* and *CLCF1* were clinically assessed by the respective clinical geneticists and selected for WES analysis. Clinical

findings of these cases are listed in Table 1. Our inclusion criteria comprised features related to the neonatal phenotype and especially to the four main typical CS criteria: hyperthermia in the first months of life, feeding difficulties, contraction of oropharyngeal muscles and camptodactyly.

We assigned the five cases to rank 1 (very likely) and 2 (questionable) based on their phenotype similarity to CS/CISS. Rank 1 individuals (CS_239 and CS_306) presented in the neonatal period the four main typical CS criteria, while rank 2 (CS_125, CS_141 and CS_207) individuals fulfilled two or three of them (See clinical details and WES analysis, Supporting Information)

**Molecular results**

Here we identified two *de novo* missense variants in *NALCN* NM_052867.3: c.1800C>A: p.(Asp600Glu) and c.1571G>A: p.(Ser524Asn) in two unrelated CS patients (Family A and C). These novel variants, predicted as pathogenic by LRT, MutationTaster, Polyphen2 and FATHMM, occurred at highly conserved amino acids within NALCN from different organisms, even those very distinct from vertebrates. NALCN forms a voltage-independent, nonselective, non-inactivating cation channel permeable to Na+, K+, and Ca (2+). It is responsible for the neuronal background sodium leak conductance.[11] Functional testing of some human variants in *C. elegans* demonstrated that CLIFAHDD can be caused by dominant loss or gain of function mutations in ion channel function. In 2016, Bend et al.[12] conducted functional studies on *C. elegans* NCA-1 mutants carrying the p.(Asp647Glu) mutation, which is the orthologous position of p.(Asp600Glu) in human NALCN. They described this *de novo* variant as a gain of function, which gives very severe effects. In animal model, this mutation displays dramatically reduced locomotion, small body size and curly posture, consistent with neuronal dysfunction.

In family B and E, we identified two *MAGEL2* mutations of paternal origin: a *de novo* NM_019066.4: c.2056_2066del: p.(Trp686Alafs*23), already reported, and an insertion c.1996dupC: p.(Gln666Profs*47): rs770374710 which represents a mutational hotspot.[13]

In family D, we identified a *de novo* mutation in the *SCN2A* gene, NM_001040143.1: c.2567G>A: p.(Arg856Gln): rs797045942, which has been previously reported as associated to Ohthara syndrome.[18] This substitution results in the conversion of an arginine to glutamine. This arginine is highly conserved within SCN2A from different organisms, even those very distinct from vertebrates. It is predicted to be pathogenic by five popular algorithms, SIFT, Polyphen2, LRT, Mutation Assessor, and FATHMM.

Table 2 provides a summary of the patients' features and of the ACMG criteria satisfying the pathogenic nature of the identified variants in *NALCN, MAGEL2* and *SCN2A*.

**Discussion**

A trio-based WES in five individuals with an initial clinical diagnosis of CS/CISS disclosed alternative diagnoses not originally considered in the differential diagnosis in these children, and emphasizes the wide clinical spectrum overlap among different syndromes (Figure 1). These diagnoses include the CLIFAHDD,[6] the Schaaf-Yang (SHFYNG),[7] and the early infantile epileptic encephalopathy type 11 (EIEE11)[8] syndromes caused by heterozygous mutations respectively in *NALCN, MAGEL2* or *SCN2A*. An accurate revaluation of the clinical phenotype of each case reported so far, based on what found by WES, helped us to reassess it within the previous unpredicted diagnosis.

*CLIFAHDD syndrome – NALCN gene*

CLIFAHDD (MIM#616266) is an autosomal dominant disorder firstly described in 2015 by Chong et al.,[6] in five individuals initially diagnosed as having Freeman-Sheldon syndrome, or distal arthrogryposis type 2A (DA2A). Clinical features of CLIFAHDD are congenital contractures of the limbs and face, resulting in characteristic facial features, hypotonia, neonatal respiratory distress and variable degrees of developmental delay. Interestingly, CS/CISS was considered in the differential diagnosis for CLIFAHDD syndrome in two reported cases, of whom one actually had a heterozygous mutation in *NALCN*.[6,9] In the past, DA2A has been considered in the differential diagnosis of CS/CISS, particularly for the intermittent facial muscle contraction, the puckering of the lips of young children and for the camptodactyly although the clinical course is completely different.[10]

*SHFYNG syndrome - MAGEL2 gene*

SHFYNG syndrome (MIM#615547) is an autosomal dominant multisystem disorder characterized by delayed psychomotor development, intellectual disability, neonatal hypotonia with poor sucking, feeding difficulties in infancy and behavioral abnormalities. Additional features include contractures and variable dysmorphic facial features. It is caused by mutations in *MAGEL2*, and individuals are affected only if the mutation occurs on the paternal allele, since *MAGEL2* is an imprinted, maternally silenced, gene located at 15q11-13, within the Prader-Willi region.[7]

A *de novo* nonsense mutation in *MAGEL2* was identified in one individual presenting with severe congenital contractures initially diagnosed as having Opitz-C syndrome (OTCS; MIM#211750),[14] indicating that there is an overlap between OTCS and SHFYNG syndromes. In addition, *MAGEL2* mutations were reported in individuals initially diagnosed as having Chitayat-Hall syndrome (MIM#208080) leading to the conclusion that Chitayat-Hall and SHFYNG syndromes are likely the same disorder.[15] *MAGEL2* is highly expressed in the hypothalamus and it is part of a multi-subunit protein complex consisting of MAGEL2, the TRIM27 E3 ubiquitin ligase, and the USP7 deubiquitinating enzyme.

*EIEE11 - SCN2A gene*

Infantile/childhood onset epileptic encephalopathies or early infantile epileptic encephalopathies (EIEE) represent a collection of serious seizure disorders. Particularly, early infantile epileptic encephalopathy-11 (EIEE11; MIM#613721) is caused by heterozygous mutations in the *SCN2A* gene and is typically characterized by severe, very early onset intractable seizures with subsequent developmental delay occasionally including autistic type symptoms. Variants in *SCN2A* are associated with a wide range of phenotypic heterogeneity (i.e. Ohtahara syndrome, epilepsy of infancy with migrating focal seizures, infantile spasms, West syndrome or unclassified severe epilepsy phenotypes and ASD/ID, characterized by global developmental delay, particularly of social and language milestones) and the mechanisms underlying this are poorly understood.[16]

The *SCN2A* gene encodes the voltage-gated sodium channel Nav1.2, one of the major neuronal sodium channels that play a role in the initiation and conduction of action potentials, chiefly in nerve and muscle.

**Conclusions**

In summary, heterozygous mutations in *NALCN, MAGEL2* and *SCN2A* can result in a CS/CISS-overlapping phenotype, which seems to be most similar in the neonatal period. Although CS/CISS, CLIFAHDD, SHFYNG and EIEE11 syndromes show overlapping features such as feeding problems in infancy, contractures and temperature instability, they must be considered as distinct entities. These observations suggest that similar pathophysiological mechanisms may lead to such clinically overlapping phenotypes.

Based on the considerable phenotypic overlap between CS/CISS and other syndromes described in this article, the clinical geneticist should employ the current clinical diagnostic criteria for CS/CISS with caution, especially in the neonatal period. Adequate counseling should only be possible after the diagnosis has been confirmed by molecular genetic testing. Therefore, sequencing analysis of these genes has to be considered for those cases with a suspected CS/CISS during neonatal period who were tested as mutation negative in the known genes, since an expedited and corrected diagnosis can improve patient management and can provide a specific clinical follow up.

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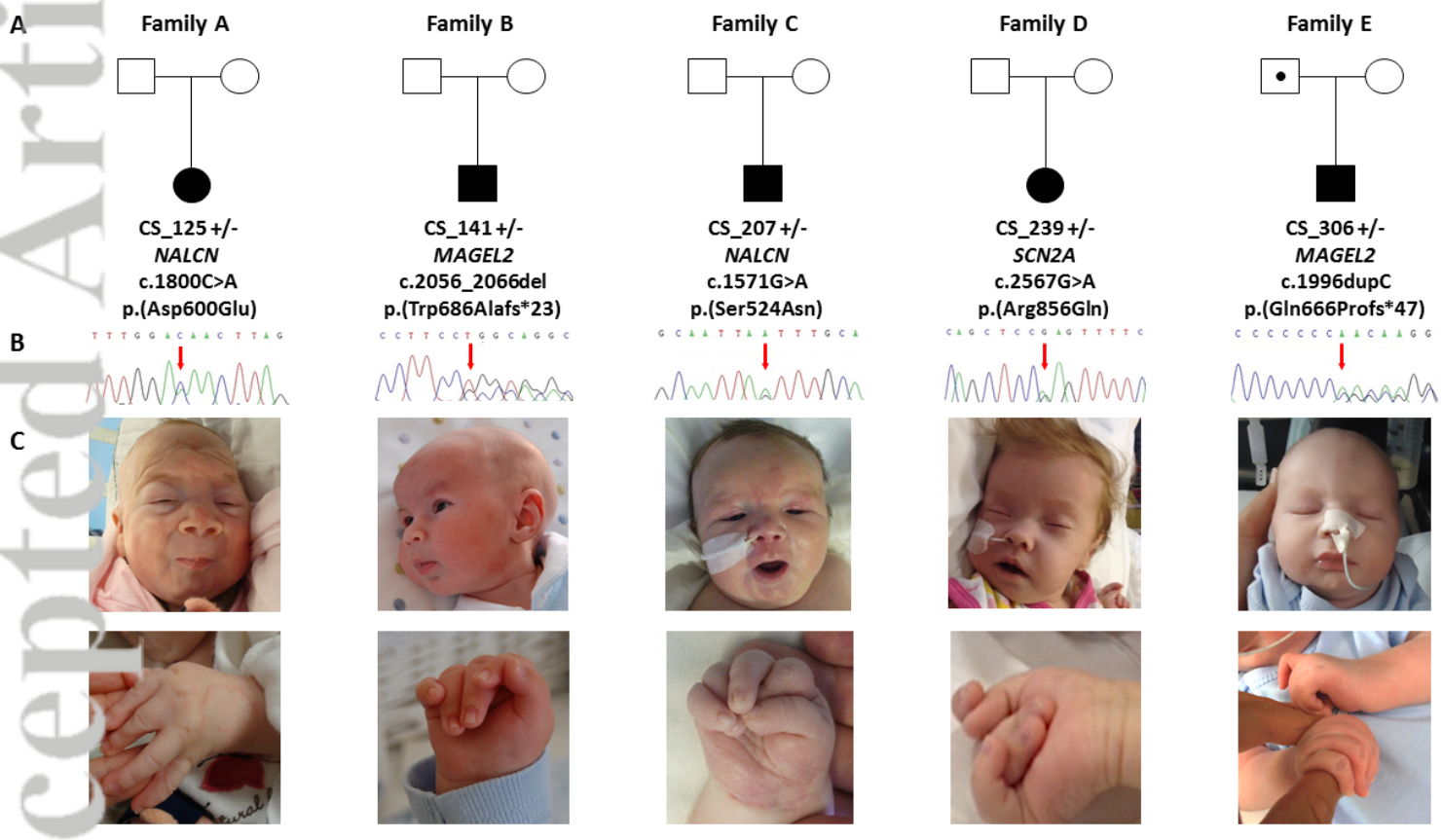
**Figure legend**

**Figure 1:** CS/CISS-like families analyzed by WES.

(A) Family pedigrees of individuals with mutations found by WES. Symbols and colors are defined as follows: square, male; circle, female; white, unaffected; dot, unaffected carrier; black, affected. Mutation status is indicated beneath symbols for each subject. +/- heterozygous carriers. (B) Sanger sequencing of the five mutations found by WES. Panels shows heterozygous status for CS_125 [*NALCN* c.1800C>A: p.(Asp600Glu)], CS_141 [*MAGEL2* c.2056_2066del: p.(Trp686Alafs*23)], CS_207 [*NALCN* c.1571G>A: p.(Ser524Asn)], CS_239 [*SCN2A* c.2567G>A: p.(Arg856Gln)] and CS_306 [*MAGEL2* c.1996dupC: p.(Gln666Profs*47)]. (C) Photographs of all the cases analyzed at the neonatal age. Written informed consent for publication of their clinical images was obtained from their parents.

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| Category | Feature | CS/CISS | SHFYNG | CLIFAHDD | EIEE11 | Family A | Family B | Family C | Family D | Family E |
|---|---|---|---|---|---|---|---|---|---|---|
| **General** | Autism spectrum disorder | | + | | | | | | | |
| | Cognitive delay | | | + | | | + | | | + |
| | Cold induced sweating > 3years | + | | | | Increased sweating without fever | | | Cold induced sweating < 3 years | Profuse sweating |
| | Dehydration | + | | | | | | | Low serum sodium | Central diabetes insipidous during neonatal period |
| | Decreased fetal movements | | + | | | + | | | + | |
| | Developmental delay | | + | | | + | + | + | | + |
| | Excessive weight gain | | + | | | | | | | |
| | Hypotonia (at time of exam) | | + | | | | | | + | + |
| | Hyperphagia | | + | | | | | | | |
| | Hyperthermia | + | | | | | | | + | + |
| | Intellectual Disability | | + | | | + | + | + | | + |
| | Neonatal hypotonia, poor suck | + | + | | | GJ tube feeding, hypotonia at rest, hypertonia when handled or agitated | NG tube feeding, hypotonia at rest and hypertonia when handled or agitated | NG tube feeding | NG tube feeding, reduced movements and startling movements when handled | NG tube feeding, hypotonia at rest, hypertonia when handled or agitated |
| | Short stature | | + | | | | + | | | |
| | Temperature instability | + | + | | | | | | + | + |
| **Gastrointestinal** | Chronic constipation | | + | | | | + | | | + |
| | Feeding difficulties | + | + | | | + | + | Mostly nasogastric feeding | + | + |
| | Gastroesophageal reflux | + | + | + | | + | | + | | |
| | Swallowing difficulties | + | | | | + | + | + | + | + |
| **CNS** | EEG shows abundant slow waves and fast spike activity | | | | + | | | | | |
| | EEG shows suppression with ictal burst activities | | | | + | | | | + | |
| | Febrile seizures | + | | | + | | | | | |
| | Seizures, tonic-clonic | | | | + | + | | | + | |
| | Spastic quadriplegia | | | | + | | | | | |
| | Status epilepticus | | | | + | | | | Severe encephalopahy | |
| **Craniofacial** | Anteverted nasal tip | + | | + | | + | | + | | + |
| | Broad nasal bridge | | | + | | + | + | + | | - |
| | Chubby cheeks | + | | + | | + | + | + | ? | + |
| | Deep nasolabial folds | | | + | | + | + | + | | |
| | Depressed nasal bridge | + | | | | + | + | + | + | |
| | Downslanting palpebral fissures | | | + | | | | + | Upslanting palpebral fissures | |
| | H-shaped dimple chin | | | + | | | | + | + | |
| | High arcade palate | + | | | | + | | | | + |
| | Full cheeks | + | | + | | + | + | + | | + |
| | Large nares | + | | + | | + | | | | |
| | Long philtrum | + | | + | | + | + | + | | |
| | Micrognathia | + | | + | | + | | + | + | + |
| | Pursed lips | + | | + | | + | | Small mouth | + | |
| | Short columnella | | | + | | + | + | | | |
| **Ophtalmologic** | Esotropia | | + | + | | | | | | |
| | Strabismus | | + | | | | | | | + |
| **Musculoskeletal** | Adducted thumb | + | | + | | + | | + | | |
| | Calcaneovalgus deformity | | | + | | + | | + | | |
| | Camptodactyly | + | + | + | | + | + | + | + | + |
| | Clubfoot | + | | + | | + | | + | + | |
| | Contractures of finger joints | + | + | | | + | + | + | + | + |
| | Contraction of oropharyngeal muscles | + | | | | + | | + | + | + |
| | Elbow contractures | + | | + | | + | + | | + | + |
| | Foot anomalies | + | | + | | + | | Bilateral talipes | Unilateral clubfoot, overriding toes | |
| | Hip contractures | | | + | | | | | | + |
| | Joint contractures | + | | | | + | + | Clenched hands, difficult to fully extend knees | + | + |
| | Knee contractures | | | + | | + | + | + | + | + |
| | Muscle contractions, episodic | + | | | | + | + | Abnormal movement | Startling movements when touched | |
| | Scoliosis | + | + | + | | | Kyphoscoliosis | | | |
| | Short neck | | | + | | + | | + | | |
| | Small hands | | + | | | + | + | | | |
| | Small feet | | + | | | + | + | | | |
| | Ulnar deviation | | | + | | | + | + | + | + |
| **Behaviour** | Behavioral abnormaliities (impulsivity, compulsivity, stubbornness) | | + | | | | | | | |
| | Skin picking, automutilation | | + | | | | | | | |
| **Genitourinary** | Hypogonadism | | + | | | | Hypoplastic scrotum | | | |
| **Cardiopulmonary** | Cyanosis | + | | | | + | | + | Sectio, meconium, respiratory treatment and CPAP, APGAR 3-3-7 | |
| | Dyspnea/apneic spells | + | | | | | + | + | | |
| | Respiratory distress | + | | | | + | | + | | |
| | Sleep apnea | | + | | | + | + | + | | + |

**Table 1**. Phenotypic comparison among Crisponi/CISS-like individuals harboring pathogenic or likely pathogenic variants detected by WES. Clinical symptoms specific for a given syndrome as described at clinical synopsis available at the Online Mendelian Inheritance in Man website are coloured accordingly (CS/CISS, blue; SHFYNG, green; CLIFAHDD, pink; EIE11, yellow). "+" in family columns denotes that the patient presented the given feature, and "blank" denotes that the patient did not presented the given feature or the feature could not be evaluated due to the early age. GJ, gastro-jejunal; NG, nasogastric; CNS, Central nervous system; EEG, electroencephalogram.

| | Family A | Family B | Family C | Family D | Family E |
|---|---|---|---|---|---|
| **Case ID** | CS_125 | CS_141 | CS_207 | CS_239 | CS_306 |
| **Sex** | Female | Male | Male | Female | Male |
| **Year of molecular diagnosis** | 2017 | 2017 | 2017 | 2017 | 2017 |
| **Nationality** | Canadian | Spanish | British | Swedish | Spanish |
| **Year of birth** | 2008 | 2008 | 2010 | 2012 | 2014 |
| **Age of death** | 3-1/2 months | | 10 months | 3-1/2 months | |
| **Origin** | Scottish/Northern European | Spanish | Wales | Caucasian | Romanian |
| **Consanguinity** | - | - | - | - | - |
| **Gene** | *NALCN* | *MAGEL2* | *NALCN* | *SCN2A* | *MAGEL2* |
| **HGVS variant nomenclature** | NM_052867.3: c.1800C>A: p.(Asp600Glu) | NM_019066.4: c.2056_2066del: p.(Trp686Alafs*23) | NM_052867.3: c.1571G>A: p.(Ser524Asn) | NM_001040143.1: c.2567G>A: p.(Arg856Gln) | NM_019066.4: c.1996dupC: p.(Gln666Profs*47) |
| **Mutation type** | Missense | Frameshift | Missense | Missense | Frameshift |
| **Zygosity** | Het (*de novo*) | Het (*de novo*) | Het (*de novo*) | Het (*de novo*) | Het (paternal) |
| **ACMG classification** | Pathogenic (ii) | Pathogenic (i) | Likely pathogenic (ii) | Likely pathogenic (ii) | Pathogenic (i) |
| **ACMG: very strong** | - | PVS1 | - | - | PVS1 |
| **ACMG: strong** | PS2, PS3 | PS2 | PS2 | PS1, PS2 | PS2 |
| **ACMG: moderate** | PM2 | PM2 | PM2 | PM2, PM5 | PM1, PM2 |
| **ACMG: supporting** | PP3 | - | PP3, PP4 | - | - |

**Table 2.** Summary of the patients' features and of the American College of Medical Genetics and Genomics (ACMG) criteria satisfying the pathogenic nature of the identified variants in *NALCN, MAGEL2* and *SCN2A*.