

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

Running title: Crisponi/CISS alternative diagnoses.

Andrea Angius^{1}, Paolo Uva^{2*}, Manuela Oppo^{1,3}, Insa Buers^{4,5}, Ivana Persico¹, Stefano Onano^{1,3}, Gianmauro Cuccuru², Margot I. Van Allen^{6,7,8}, Gurdip Hulait⁷, Gudrun Aubertin⁸, Francesco Muntoni^{9,10}, Andrew E. Fry¹¹, Göran Annerén¹², Eva-Lena Stattin¹², María Palomares-Bralo¹³, Fernando Santos-Simarro¹³, Francesco Cucca^{1,3}, Giangiorgio Crisponi¹⁴, Frank Rutsch^{4,5§} and Laura Crisponi^{1,3§*}*

1 Istituto di Ricerca Genetica e Biomedica, Consiglio Nazionale delle Ricerche (CNR), Monserrato, Cagliari, Italy

2 Centre for Advanced Studies, Research and Development in Sardinia (CRS4), Science and Technology Park Polaris, Pula, Italy

3 Dipartimento di Scienze Biomediche, Università degli Studi di Sassari, Sassari, Italy

4 Cells in Motion Cluster of Excellence, Münster University, Münster, Germany

5 Department of General Pediatrics, Münster University Children's Hospital, Münster, Germany

6 Department of Medical Genetics, University of British Columbia, Vancouver, B.C.

7 Provincial Health Service Authority, B.C. Children's and Women's Health Centre, Vancouver, B.C.

8 Department of Medical Genetics, Victoria Island Health Authority, British Columbia, Vancouver, B.C.

9 Dubowitz Neuromuscular Centre, UCL Great Ormond Street Hospital, London, UK

10 NIHR Biomedical Research Centre at Great Ormond Street Hospital, London, UK

11 Institute of Medical Genetics, University Hospital of Wales, Heath Park, Cardiff, UK

12 Department of Immunology, Genetics and Pathology, Uppsala University, Science for Life Laboratory, Uppsala, Sweden

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/cge.13532

13 Institute of medical and molecular genetics, University Hospital La Paz, CIBERER, ISCiii, Madrid, Spain

14 Clinica Sant'Anna, Cagliari, Italy

**These authors contributed equally to this work. §These authors jointly supervised this work.*

Correspondence should be addressed to: Laura Crisponi, Istituto di Ricerca Genetica e Biomedica, Consiglio Nazionale delle Ricerche, c/o Cittadella Universitaria di Monserrato, SS554, bivio per Sestu, Km 4,500, 09042 Monserrato (Cagliari), Italy. Telephone: +390706754620, Fax: +390706754652, E-mail: laura.crisponi@irgb.cnr.it

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 fistled 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

Accepted Article

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.

Acknowledgments

We are grateful to all family members for their participation to the study, to the CRS4 HPC group for IT support. We would like to acknowledge the Associazione Sindrome di Crisponi e Malattie Rare for its constant support, encouragement and active participation. This work was supported by TELETHON exploratory grant GEP13093 to LC and by the fund “Innovative Medical Research” of the University of Münster Medical School (BU111506), Germany to IB and FR.

References

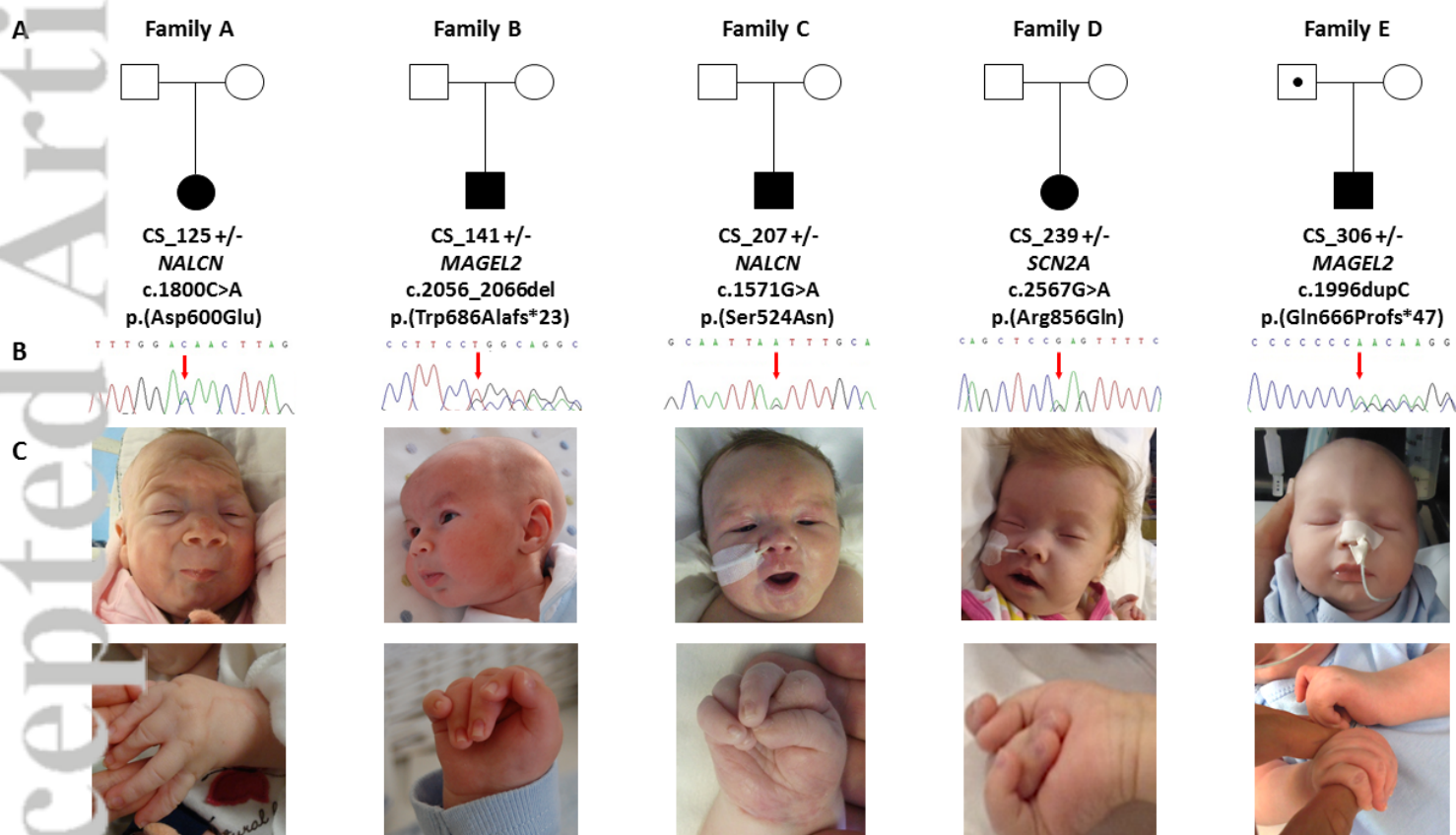
1. Hahn AF, Boman H. Cold-Induced Sweating Syndrome Including Crisponi Syndrome. In: Adam MP, Ardinger HH, Pagon RA, Wallace SE, Bean LJH, Stephens K, Amemiya A, editors. *GeneReviews® [Internet]. Seattle (WA): University of Washington*, 2016;Seattle 1993-2018.
2. Crisponi L., Crisponi G., Meloni A., et al. Crisponi syndrome is caused by mutations in the CRLF1 gene and is allelic to cold-induced sweating syndrome type 1. *Am J Hum Genet.* 2007;80:971-81.
3. Dagoneau N., Bellais S., Blanchet P., et al. Mutations in cytokine receptor-like factor 1 (CRLF1) account for both Crisponi and cold-induced sweating syndromes. *Am J Hum Genet.* 2007;80:966-70.
4. Piras R., Chiappe F., Torraca I.L., et al. Expanding the mutational spectrum of CRLF1 in Crisponi/CISS1 syndrome. *Hum Mutat.* 2014;35:424-33.
5. Angius A., Uva P., Buers I., et al. Bi-allelic Mutations in KLHL7 Cause a Crisponi/CISS1-like Phenotype Associated with Early-Onset Retinitis Pigmentosa. *Am J Hum Genet.* 2016;99:236-45.
6. Chong J.X., McMillin M.J., Shively K.M., et al. De novo mutations in NALCN cause a syndrome characterized by congenital contractures of the limbs and face, hypotonia, and developmental delay. *Am J Hum Genet.* 2015;96:462-73.
7. Schaaf C.P., Gonzalez-Garay M.L., Xia F., et al. Truncating mutations of MAGEL2 cause Prader-Willi phenotypes and autism. *Nat Genet.* 2013;45:1405-8.
8. Ogiwara, I., Ito, K., Sawaishi, Y., et al. De novo mutations of voltage-gated sodium channel alpha-II gene SCN2A in intractable epilepsies. *Neurology* 2009;73: 1046-1053.
9. Lozic B., Johansson S., Lovric Kojundzic S., Markic J., Knappskog P.M., Hahn A.F., Boman H. (2016) Novel NALCN variant: altered respiratory and circadian rhythm, anesthetic sensitivity. *Ann Clin Transl Neurol.* 3:876-883.
10. Accorsi P, Giordano L, Faravelli F. Crisponi syndrome: report of a further patient. *Am J Med Genet A.* 2003;123A:183-5.

11. Lu B, Su Y, Das S, et al. The neuronal channel NALCN contributes resting sodium permeability and is required for normal respiratory rhythm. *Cell*. 2007;129:371-83.
12. Bend E.G., Si Y., Stevenson D.A., Bayrak-Toydemir P., et al. NALCN channelopathies: Distinguishing gain-of-function and loss-of-function mutations. *Neurology*. 2016;87:1131-9.
13. McCarthy J, Lupo PJ, Kovar E, et al. Schaaf-Yang syndrome overview: Report of 78 individuals. *Am J Med Genet A*. 2018;1-11.
14. Urreizti R., Cueto-Gonzalez A.M., Franco-Valls H., et al. De Novo Nonsense Mutation in MAGEL2 in a Patient Initially Diagnosed as Opitz-C: Similarities Between Schaaf-Yang and Opitz-C Syndromes. *Sci Rep*. 2017;7:44138.
15. Jobling R., Stavropoulos D.J., Marshall C.R., et al. Chitayat D. Chitayat-Hall and Schaaf-Yang syndromes:a common aetiology: expanding the phenotype of MAGEL2-related disorders. *J Med Genet*. 2018;55:316-321.
16. Wolff M., Johannesen K.M., Hedrich U.B., et al. Genetic and phenotypic heterogeneity suggest therapeutic implications in SCN2A-related disorders. *Brain*. 2017;140:1316-1336.
17. [dataset] Angius et al.; 2018; ClinVar (<https://www.ncbi.nlm.nih.gov/clinvar/>), submission ID: SUB4691372; Organization ID: 505792

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.



CGE_13532_Figure 1 Angius et al. 2018.tif

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				+	+			+	
Craniofacial	Spastic quadriplegia				+					
	Status epilepticus				+				Severe encephalopathy	
	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 columella			+		+	+			
Ophthalmologic	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 abnormalities (impulsivity, compulsivity, stubbornness)		+							
	Skin picking, aut mutilation		+							
Genitourinary	Hypogonadism		+				Hypoplastic scrotum			
Cardiopulmonary	Cyanosis	+				+		+	Stool, 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	<i>NALCN</i>	<i>MAGEL2</i>	<i>NALCN</i>	<i>SCN2A</i>	<i>MAGEL2</i>
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
Zygoty	Het (<i>de novo</i>)	Het (<i>de novo</i>)	Het (<i>de novo</i>)	Het (<i>de novo</i>)	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*.