High Rate of Autonomic Neuropathy in Cornelia De Lange Syndrome

Maria Jesus Pablo
Hospital General San Jorge
Pilar Pamplona
Hospital Clinico Universitario Lozano Blesa Zaragoza
Maria Haddad
Hospital Universitario Miguel Servet
Isabel Benavente
Hospital General San Jorge
Ana Latorre-Pellicer
Universidad de Zaragoza
Maria Arnedo
Universidad de Zaragoza
Laura Trujillano
Hospital Clinico Universitario Lozano Blesa
Gloria Bueno-Lozano
Hospital Clinico Universitario Lozano Blesa
Lynne M Kerr
University of Utah Health Sciences Center
Sylvia A Huisman
Amsterdam UMC - Locatie AMC: Amsterdam UMC Locatie AMC
Frank J Kaiser
Universitätsklinikum Essen: Universitatsklinikum Essen
Feliciano J Ramos
Hospital Clinico Universitario Lozano Blesa
Antonie D Kline
Greater Baltimore Medical Center
Juan Pie (juanpie@unizar.es)
Universidad de Zaragoza Facultad de Medicina https://orcid.org/0000-0003-3203-6254
Beatriz Puisac
Universidad de Zaragoza Facultad de Medicina

Research Article

Keywords: Cornelia de Lange Syndrome, CdLS, small fiber nerve, peripheral neuropathy, autonomic neuropathy, sudomotor test, sweat gland density, NIPBL gene

Posted Date: June 7th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-534763/v1

Abstract

Background

Cornelia de Lange Syndrome (CdLS) is a rare congenital disorder characterized by typical facial features, growth failure, limb abnormalities, and gastroesophageal dysfunction that may be caused by mutations in several genes that disrupt gene regulation early in development. Symptoms in individuals with CdLS suggest that the peripheral nervous system (PNS) is involved, yet there is little direct evidence.

Method

Somatic nervous system was evaluated by conventional motor and sensory nerve conduction studies and autonomic nervous system by heart rate variability, sympathetic skin response and sudomotor testing. CdLS Clinical Score and genetic studies were also obtained.

Results

Sympathetic skin response and sudomotor test were pathological in 35% and 34% of the individuals with CdLS, respectively. Nevertheless, normal values in large fiber nerve function studies.

Conclusions

Autonomic nervous system (ANS) dysfunction is found in many individuals with Cornelia de Lange syndrome, and could be related to premature aging.

Background

Cornelia de Lange Syndrome (CdLS) is a genetic disease due to spontaneous mutations in genes of the cohesin protein complex, mainly *NIPBL*, in 70% of the cases [1–4] and *SMC1A*, *SMC3*, *RAD21*, *BRD4*, *HDAC8, ANKRD11* and *MAU2* [5–9]. Manifestations of the syndrome differ with mutated gene type, with variants in *NIPBL* often associated to more severe clinical phenotype. The syndrome is characterized by typical facial features, growth failure, limb abnormalities and the involment of many organs and systems including central nervous system. Sweating abnormalities, abnormal reactions to cold and heat, and severe gastrointestinal reflux are also prevalent and suggest a compromised peripheral nervous system [1]. More than 80% of individuals with CdLS have some autonomic nervous system dysfunction, while 26% of those have moderate to severe dysfunction as measured by the Compass-31 questionnaire, a validated survey tool for autonomic dysfunction [10]. The aim of this study was to get new insights into neuronal dysfunction in CdLS by analyzing large and small fiber nerves with different techniques.

Patients And Methods

All the peripheral nervous system studies, except the sudomotor test, were made in a group of 20 individuals with CdLS (7 male, 13 female, aged 3–37 years). In the sudomotor test the population was broaden to 47 individuals with CdLS (18 male, 29 female, aged 1.5–42 years) and 50 slightly older healthy controls (18 male, 32 female, aged 7–48 years). The protocol study was approved by the Ethics Committee of Clinical Research from the Government of Aragón (CEICA;PI16/225). All the individuals with CdLS and controls gave informed consent for their participation.

To evaluate the somatic peripheral nervous system, conventional motor and sensory nerve conduction studies [11–15] were carried out in upper and lower limbs (large fiber nerves).

The autonomic nervous system (small fibre nerves) was studied by mean of heart rate variability at rest, sympathetic skin response and sudomotor test. Heart rate variability (HRV) at rest was evaluated recording the heart rate for 5 minutes [16]. Sympathetic skin response (SSR) was studied with electric stimuli over the Median and Posterior Tibial nerves, recording the responses over the palm of both hands (Median) and the sole of both feet (Tibial) [17–18]. Nerve conduction studies, HRV and SSR were performed by the same group of neurophysiologists with a 5-channel Natus® Electromyography equipment. The sudomotor test, which gives the number of functioning sweat glands per cm^2 (sweat gland density, SGD) was obtained on a silicone mold after pilocarpine iontophoresis stimulation over the foot dorsum [19].

Genetic studies were realized by standard Sanger sequencing and Next Generation Sequencing (NGS) panels. Clinical severity score according to the first international consensus statement [1] was also studied (Table 2). Statistical studies were achieved with the SPSS program version 25.

Table 2
Genetics, Clinical Score and Sweat Gland Density (SGD) in individuals with CdLS in diff

INDIVIDUALS	Control group	1	2	3	4	5	6	7	8
Age (years)	1–10 years	1.5		1.5	2	3	3	3	3
Gender	Mean of 13	F		M	M	F	M	M	M
Gene	individuals	?		*SMC1A*	*HDAC8*	*NIPBL*		*NIPBL*	*NIPBL*
Mutation					c.305G > A p.Cys102Tyr	c.6549_6552delCTCA p.His218Glnfs*13			c.3021delA fibrob (mosaicism)
Clinical Score		13		5	11	15	15	17	14
GERD		++		-		+	-	+++	+
SGD	236.76 ± 33.45	229		159	300	287	322	322	200
INDIVIDUALS	11	12		13	14	15	16	17	18
Age (years)	4	5		5	5	5	6	6	7
Gender	F	F		F	M	F	F	M	M
Gene	*HDAC8*	*RAD21*		*SMC1A*	*NIPBL*	*NIPBL*	*NIPBL*	*NIPBL*	*NIPBL*
Mutation		c.1382C > T heterozygous		NM_006306:c2096 > T		C7736C > T p.Ala2579Val missense (exon45)			c.5329-15A > G
Clinical Score	14	8		5	15	9	6	11	6
GERD	++	+		+	+	-	-	++	+
SGD	235	307		243	218	91	339	280	174
INDIVIDUALS	22	23		24	Control group	25	26	27	28
Age (years)	8	9		10	11–20 years	11	11	11	11
Gender	F	F		F	Mean of 11	F	F	F	M
Gene	*NIPBL*	*NIPBL*		*SMC1A*	individuals	*SMC1A*	*NIPBL*	*NIPBL*	
Mutation	c.6860T->C p.L2287P exon40	c.5483G > A exon29				c.2369G > A p.R790Q(exon15)	Chr 5p 13.1	c.6272G > A exon 36	
Clinical Score	13			6		14	11	14	15
GERD	+	+++		++		+	+	+	++
SGD	166	254		167	217.18 ± 29.99	162	144	232	174
INDIVIDUALS	32	33		34	35	36	37	Control group	38
Age (years)	15	16		16	16	17	20	21–30 years	21
Gender	M	F		F	M	M	F	Mean of 10	M
Gene	*NIPBL*	*?*		*NIPBL*	*NIPBL*	*NIPBL*	*NIPBL*	individuals	*NIPBL?*
Mutation	Mosaicism			c.6964_6965insATTTA exon41		c.6242G->C p.G2081A Exon35			

Individuals are differentiated in decades of life by different shading colours from: white: 1st decade of life; light grey: 2nd decade of life; medium grey: 3rd de
PNS: Peripheral Nervous System, GERD: Gastroesophageal reflux disease (- no, + mild, ++ moderate, +++ severe), SGD: Sweat Gland Density: gland number/c

INDIVIDUALS	Control group	1	2	3	4	5	6	7	8
Clinical Score	8	9		15	9	14	6		16
GERD	+++	+		+++	+	+	-		++
SGD	76	171		125	126	188	209	206.40 ± 22.9	196
INDIVIDUALS	42	43		Control group	44	45	46	Control group	47
Age (years)	25	26		31–40 years	32	34	37	41–50 years	42
Gender	F	F		Mean of 8	F	M	F	Mean of 8	M
Gene	*NIPBL*	*?*		individuals	*NIPBL*	*NIPBL mosaicism*	*NIPBL*	individuals	*NIPBL*
Mutation							c.5471C > T p.S1824L Exon29		
Clinical Score	10	11			15	16	13		13
GERD	+++	-			+	-	-		+++
SGD	74	273		215.28 ± 31.40	127	174	159	202.50 ± 22.16	94

Individuals are differentiated in decades of life by different shading colours from: white: 1st decade of life; light grey: 2nd decade of life; medium grey: 3rd de
PNS: Peripheral Nervous System, GERD: Gastroesophageal reflux disease (- no, + mild, ++ moderate, +++ severe), SGD: Sweat Gland Density: gland number/c

Results

Conventional motor and sensory nerve conduction studies (large fiber nerves) were normal in all 20 individuals with CdLS analyzed (Tables 1 to 3 of supplementary material). The study of the autonomic nervous system (small fiber nerves) in HRV at rest was normal as well (Table 1). Nevertheless, SSR revealed mild alterations in lower limbs in 7 of the 20 individuals, with asymmetrical responses (Table 1, Fig. 1). Sudomotor tests evinced reduced SGD in 16 of the 47 individuals with CdLS regarding the control group by decades of life (Table 2). The regression analysis showed that, in spite of dispersion, there were two different populations, with statistically significant differences between the control group and individuals with CdLS ($p < 0.05$ and $p < 0.01$) (Fig. 2A). The linear regression showed that the slope of the SGD reduction by age is much pronounced in individuals with CdLS than in controls (Fig. 2A). Independence samples T test showed the results of the mean differences of the sweat gland density (SGD) by age group, with reduction in the SGD more evident in the individuals with variants in *NIPBL* than in the controls ($p < 0.01$). These differences were found as in the whole *NIPBL* group as in all the decades of life, except the first one (Fig. 2B).

Table 1
Sympathetic Skin Response and Heart Rate Variability in CdLS.

Individuals		SSR hand			SSR Foot			HRV (RMSSD)
Gender/Age/Gene	Side	Lat. (ms)	Amp. (µV) ((µV)		Lat. (ms)	Amp. (µV)		(ms)
Normal values[7,13,14]		1.3±0.1	800±300		1.9±0.1	600±300		≥ 16,39ms (< 10years)
3	R	0,94	968,7		1,27	1589,3		54,10
M/2y/*HDAC8*	L	0,96	942,71		1,09	1874,8		
4	R	0,89	3352,7		1,11	1019,8	Assymetrical	64,64
F/3y/*NIPBL*	L	0,88	3198,5		1,26	329,8		
7	R	1,02	758,8		0,94	1285,5	Assymetrical	48,55
M/3y/*NIPBL*	L	1,18	1062,6		0,93	525,2		
8	R	1,16	696,2		1,39	398,5	Assymetrical	79,76
M/3y/*NIPBL*	L	1,22	1026,0		1,08	138,9		
12	R	1,04	306,9		1,61	545,0		148,46
F/5y/*RAD21*	L	1,02	396,5		1,69	413,7		
13	R	1,09	2464,1		1,91	745,0		21,68
F/5y/*SMC1A*	L	1,23	3151,1		1,65	868,7		
14	R	1,10	264,1		0,99	764,9		89,16
F/5y/*NIPBL*	L	1,16	236,6		1,28	876,3		
15	R	1,17	3580,2		0,94	5027,5	Assymetrical	66,49
M/5y/*NIPBL*	L	1,07	4200,0		1,55	1630,5		
18	R	0,88	1016,8		0,85	668,7		74,82
M/7y/*NIPBL*	L	0,99	1050,4		1,27	508,4		
22	R	1,20	658,0		1,36	893,6		58,94
F/8y/*NIPBL*	L	1,20	743,5		0,93	607,6		
23	R	1,05	1022,9	Assymetrical	1,97	7255,0	Assymetrical	54,95
F/9y/*NIPBL*	L	1,19	2062,6		1,79	3396,9		
Normal values[7,13,14]		1.3±0.1	800±300		1.9±0.1	600±300		≥ 16,39ms (< 20years)
25	R	1,26	366,4		1,49	706,9		180,41
F/11y/*SMC1A*	L	1,26	355,7		1,61	573,3		
27	R	0,76	1328,2		1,32	404,6		424,88
F/11y/*NIPBL*	L	0,93	1720,6		1,51	371,0		
30	R	0,65	748,1		0,89	543,5		138,36
F/15y/*NIPBL*	L	0,79	578,6		0,94	415,3		
31	R	0,92	957,3		1,42	1019,8		362,04
F/15y/*NIPBL*	L	1,05	879,4		1,14	1305,3		
34	R	1,01	1221,4		1,35	600,0		66,93
F/16y/*NIPBL*	L	1,13	1665,6		1,22	401,5		
36	R	0,88	1016,8		1,39	408,4		126,06
M/17y/*NIPBL*	L	0,99	1050,4		0,85	668,7		
Normal values[7,13,14]		1.3±0.1	800±300		1.9±0.1	600±300		≤ 14,54ms (≤ 25years)
40	R	0,93	363,4		1,80	361,8	Assymetrical	254,66
M/23y/*NIPBL*								

SSR: Sympathetic Skin Response. HRV: Heart Rate Variability. RMSSD: Root Mean Square of Successive Differences. Lat: latency, Amp: Amplitude, ms: milliseconds, µV: microvolts. NE: Not examined. P40 left arm not studied. P41 only cooperated for the SSR study in one hand.

Individuals		SSR hand		SSR Foot		HRV (RMSSD)	
	L	NE	NE	1,69	167,9		
41	R	0,93	1485,5	NE	NE		57,96
F2/25y/*NIPBL*	L	NE	NE	NE	NE		
Normal values[7,13,14]		1.3±0.1	800±300	1.9±0.1	600±300	≤ 11,43ms (≤ 35years)	
46	R	1,16	693,9	1,28	164,9	Assymetrical	51,4
F/37y/*NIPBL*	L	1,15	708,4	1,45	247,3		

SSR: Sympathetic Skin Response. HRV: Heart Rate Variability. RMSSD: Root Mean Square of Successive Differences. Lat: latency, Amp: Amplitude, ms: milliseconds, µV: microvolts. NE: Not examined. P40 left arm not studied. P41 only cooperated for the SSR study in one hand.

Table 3
CdLS Clinical Score (Severity)

Individuals with CdLS	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	2
Cardinal features (2 points each if present)																								
Synophrys and/or thick eyebrows	+	+	+	+	+	+	+	+	-	+	+	-	-	+	+	+	+	+	-	+	+	+	+	+
Short nose, concave nasal ridge and/or upturned nasal tip	+	-	+	+	+	+	+	-	-	+	+	-	+	+	-	+	-	-	-	+	+	-	+	-
Long and/or smooth philtrum	+	-	-	+	+	+	+	-	-	+	+	+	-	+	+	-	+	-	-	+	+	+	+	-
Thin upper lip vermilion and/or downturned corners of mouth	+	+	-	+	+	+	+	-	-	+	+	+	-	+	+	-	+	-	-	+	+	+	+	-
Hand oligodactyly and/or adactyly	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-
Congenital diaphragmatic hernia	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Suggestive features (1 point each if present)																								
Global developmental delay and/or intellectual disability	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+
Prenatal growth retardation (< 2 SD)	+	-	+	+	+	+	+	-	+	-	+	-	-	+	-	-	+	-	+	+	+	+	+	+
Postnatal growth retardation (< 2 SD)	+	-	+	+	+	+	+	+	+	-	-	+	-	+	-	+	+	-	+	+	+	+	+	+
Microcephaly (prenatally and/or postnatally)	-	-	+	+	+	+	+	+	+	+	+	-	-	+	-	-	+	+	-	+	+	+	+	-
Small hands and/or feet	-	-	+	+	+	+	+	+	-	+	+	-	-	+	-	-	-	+	-	+	+	+	+	-
Short fifth finger	+	-	+	+	+	+	-	+	-	+	+	+	+	+	+	+	-	+	+	-	+	+	-	-
Hirsutism	+	-	+	+	+	+	+	-	-	+	+	+	+	+	+	-	+	-	-	+	+	+	+	+
Clinical Score	13	5	11	15	15	17	14	7	4	13	14	8	5	15	9	6	11	6	4	16	17	13	14	6
Individuals with CdLS	**25**	**26**	**27**	**28**	**29**	**30**	**31**	**32**	**33**	**34**	**35**	**36**	**37**	**38**	**39**	**40**	**41**	**42**	**43**	**44**	**45**	**46**	**47**	
Cardinal features (2 points each if present)																								

A L Hand – Two channel. **B** R Hand -Two channel

Individuals with CdLS	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	2
Synophrys and/or thick eyebrows	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Short nose, concave nasal ridge and/or upturned nasal tip	+	+	+	+	-	+	+	-	-	+	-	+	-	+	-	+	+	+	-	+	+	+	+	
Long and/or smooth philtrum	+	+	+	+	-	-	+	+	-	+	+	+	-	+	-	-	-	-	+	+	+	-	+	
Thin upper lip vermilion and/or downturned corners of mouth	+	+	+	+	-	-	-	-	-	+	-	+	-	+	-	+	-	-	+	+	+	+	+	
Hand oligodactyly and/or adactyly	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-	-	
Congenital diaphragmatic hernia	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Suggestive features (1 point each if present)																								
Global developmental delay and/or intellectual disability	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Prenatal growth retardation (< 2 sD)	+	-	-	+	+	-	-	-	+	+	+	+	-	+	+	+	+	+	+	+	+	+	-	
Postnatal growth retardation (< 2 sD)	+	-	+	+	+	-	+	-	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-	
Microcephaly (prenatally and/or postnatally)	+	-	+	+	+	-	+	-	+	+	-	+	-	+	-	+	-	+	+	+	+	+	+	
Small hands and/or feet	+	+	+	+	-	-	-	-	+	+	+	-	+	+	-	+	-	+	-	+	+	+	+	
Short fifth finger	-	-	+	+	+	+	+	+	+	+	+	+	+	-	-	+	-	+	-	+	-	+	+	
Hirsutism	+	+	+	+	+	+	+	-	+	+	-	+	-	+	-	+	-	-	+	+	+	+	+	
Clinical Score	14	11	14	15	8	7	11	8	9	15	9	14	6	16	4	13	7	10	11	15	16	13	13	
Clinical Score: ≥11 points, which at least 3 cardinal: classic CdLs; 9–10 points, which at least 2 cardinal: non-classic CdLs; 4–8 points, which at least 1 cardinal: molecular testing; <4 points: insufficient to indicate molecular testing CdLs. Dotted individuals : involved gene different than *NIPBL.*																								
A L Hand – Two channel. **B** R Hand -Two channel																								

Genetic studies of the 47 individuals with CdLS revealed 31 with variants in *NIPBL*, 4 in *SMC1A*, 2 in *RAD21*, 2 in *HDAC8* and 1 in *SMC3* and negative in 7 individuals (Table 2). In Table 3 there are the CdLS Clinical Scores [1]. No relationship between clinical score or gastroesophageal reflux disease (GERD) and findings of the sudomotor test was found. In Table 4 of the supplementary material is shown the SGD in the control group by decades of life.

Discussion

Though the clinical manifestations of CdLS suggest that the peripheral nervous system is affected, large fiber nerve studies (conventional motor and sensory nerve conduction studies) are within normal limits. However, we have shown evidence, for the first time, for autonomic nervous system dysfunction in

individuals with CdLS.

The sympathetic skin response reveals asymmetrical pathological responses in lower limbs in 7 of the 20 individuals (35%), with one of them affected in upper limbs as well. This could be consider a malformative manifestation of the syndrome. However, it is remarkable that the asymmetry is more frequent in lower than in upper limbs, which are often more affected [1–4]. This asymmetry does not seem not be related to GERD or the Clinical Severity Score (CSS), yet all the individuals had mutations in *NIPBL* gene. (Table 1).

Sudomotor testing shows a reduction in the sweat gland density (SGD) in 16 of 47 (34%) of the analyzed individuals with CdLS. These data are further supported by a reduction of the number of sweat droplets imprinted on the silicone after pilocarpine iontophoresis as indirect evidence of decreased postganglionic sudomotor nerve fibers, compared to an unaffected population. Though sweat gland density decreases physiologically with aging, individuals with CdLS show a reduction much greater than should be expected by their age. This decrease is evident from the second decade of life, and is more pronounced at older ages (Table 2, Fig. 2A). All of this seems to strengthen the hypothesis that these patients have premature aging. Nevertheless, no relationships were found between SGD reduction and clinical score or GERD.

The reduction in the SGD is evident in individuals with mutations in *NIPBL* (Table 2, Fig. 2B), and seems to be similar in individuals with variants in *SMC1A*. However, individuals with variants in *HDAC8* and *RAD 21* are in the first decade of life, so it is early to make an assessment. Surprisingly, there is a high value of sweat gland density in the only individual with an *SMC3* mutation, who is 39 years old. Further studies are warranted to look at autonomic nervous system dysfunction and relation to mutated gene and age in individuals with CdLS.

Conclusion

Individuals with CdLS have abnormal autonomic nervous system function, showing asymmetries in the sympathetic responses in lower limbs, and pathological results in the sudomotor test. The degree of dysfunction in postganglionic sudomotor nerve fibers might be related to premature aging. Even though, somatic nervous system function studies were normal.

Abbreviations

CdLS: Cornelia de Lange Syndrome

PNS: Peripheral Nervous System

SGD: Sweat Gland Density

GERD: Gastroesophageal Reflux disease

CSS: Clinical Severity Score

Declarations

Ethics approval and consent to participate

The protocol study was approved by the Ethics Committee of Clinical Research from the Government of

Aragón (CEICA; PI16/225). All the individuals with CdLS and controls gave informed consent for their

participation.

Consent for publication

All the individuals with CdLS and controls gave informed consent for the publication of this work.

Availability of data and material

The authors confirm that the data supporting the findings of this study are available within the article

and its supplementary materials.

Competing interests

Non-financial competing interests.

Funding

This work is supported by the FIS, Fundación de Investigación Sanitaria, Spain [Ref.# PI19/01860, to F.R. and J.P.] and the DGA (Diputación General de Aragón) – FEDER (Federación de Enfermedades Raras): European Social Fund (Group: B32_17R, to J.P.).

Authors' contributions

Conceptualization, M.J.P., F.R., J.P., and B.P.; nerve conduction studies, P.P., M.H.; autonomic nervous system studies, M.J.P., I.B., L.M.K.; clinical studies, F.R., G.B.L., L.T., F.J.K., S.A.H. and A.D.K.; genetics, A.L.P., M.A., S.A.H. and F.J.K.; writing—original draft preparation, M.J.P., J.P. and B.P.; writing—review, L.M.K., S.A.H., F.J.K., F.R., A.D.K., J.P. and B.P.; writing—editing, M.J.P., P.P., M.H., I.B., A.L.P., M.A., L.T., G.B.L., L.M.K., S.A.H., F.J.K., F.R., A.D.K., J.P. and B.P. All authors have read and agreed to the published version of the manuscript.

Acknowledgements

We thank the families who participated in this study.

Disclosure of conflicts of interest: none.

References

1. Kline AD, Moss JF, Selicorni A, et al. Diagnosis and management of Cornelia de Lange syndrome: first international consensus statement. *Nat Rev Genet* 2018 Oct;19(10):649-666.
2. Pié J, Gil-Rodríguez MC, Ciero M, López-Viñas E, Ribate MP, Arnedo M, Deardorff MA, Puisac B, Legarreta J, de Karam JC, Rubio E, Bueno I, Baldellou A, Calvo MT, Casals N, Olivares JL, Losada A, Hegardt FG, Krantz ID, Gómez-Puertas P, Ramos FJ. Mutations and variants in the cohesion factor genes *NIPBL*, *SMC1A*, and *SMC3* in a cohort of 30 unrelated patients with Cornelia de Lange syndrome. *Am J Med Genet A.* 2010 Apr;152A(4):924-9. doi: 10.1002/ajmg.a.33348. PMID: 20358602; PMCID: PMC2923429.
3. Teresa-Rodrigo ME, Eckhold J, Puisac B, et al. Functional characterization of *NIPBL* physiological splice variants and eight splicing mutations in patients with Cornelia de Lange syndrome. *Int J Mol Sci* 2014;15:10350-10364.
4. Ramos FJ, Puisac B, Baquero-Montoya C, et al. Clinical utility gene card for: Cornelia de Lange syndrome. *Eur J Hum Genet* 2015;23, doi:10.1038/ejhg.2014.270.
5. Gil-Rodríguez MC, Deardorff MA, Ansari M, Tan CA, Parenti I, Baquero-Montoya C, Ousager LB, Puisac B, Hernández-Marcos M, Teresa-Rodrigo ME, Marcos-Alcalde I, Wesselink JJ, Lusa-Bernal S, Bijlsma EK, Braunholz D, Bueno-Martinez I, Clark D, Cooper NS, Curry CJ, Fisher R, Fryer A, Ganesh J, Gervasini C, Gillessen-Kaesbach G, Guo Y, Hakonarson H, Hopkin RJ, Kaur M, Keating BJ, Kibaek M, Kinning E, Kleefstra T, Kline AD, Kuchinskaya E, Larizza L, Li YR, Liu X, Mariani M, Picker JD, Pié Á, Pozojevic J, Queralt E, Richer J, Roeder E, Sinha A, Scott RH, So J, Wusik KA, Wilson L, Zhang J, Gómez-Puertas P, Casale CH, Ström L, Selicorni A, Ramos FJ, Jackson LG, Krantz ID, Das S, Hennekam RC, Kaiser FJ, FitzPatrick DR, Pié J. De novo heterozygous mutations in SMC3 cause a range of Cornelia de Lange syndrome-overlapping phenotypes. *Hum Mutat.* 2015 Apr;36(4):454-62. doi: 10.1002/humu.22761. Epub 2015 Mar 17. PMID: 25655089.
6. Huisman S, Mulder PA, Redeker E, et al. Phenotypes and genotypes in individuals with *SMC1A*Am J Med Genet A 2017;173(8):2108-2125.
7. Parenti I, Gervasini C, Pozojevic J, et al. Expanding the clinical spectrum of the *'HDAC8*-phenotype' - implications for molecular diagnostics, counseling and risk prediction.*Clin Genet* 2016;89(5):564-573.
8. Cucco F, Sarogni P, Rossato S, Alpa M, Patimo A, Latorre A, Magnani C, Puisac B, Ramos FJ, Pié J, Musio A. Pathogenic variants in EP300 and ANKRD11 in patients with phenotypes overlapping Cornelia de Lange syndrome. *Am J Med Genet A.* 2020 Jul;182(7):1690-1696. doi: 10.1002/ajmg.a.61611. Epub 2020 May 31. PMID: 32476269.
9. Parenti I, Diab F, Gil SR, Mulugeta E, Casa V, Berutti R, Brouwer RWW, Dupé V, Eckhold J, Graf E, Puisac B, Ramos F, Schwarzmayr T, Gines MM, van Staveren T, van IJcken WFJ, Strom TM, Pié J, Watrin E, Kaiser FJ, Wendt KS. MAU2 and NIPBL Variants Impair the Heterodimerization of the Cohesin Loader Subunits and Cause Cornelia de Lange Syndrome. *Cell Rep.* 2020 May 19;31(7):107647. doi: 10.1016/j.celrep.2020.107647. PMID: 32433956.
10. Kerr, LM, Jones, A, Kline, AD, Fischer, PR. Compass-31 questionnaire screening in individuals with Cornelia de Lange syndrome. *Am J Med Genet A.* 2017 May; 173(5): 1172–1185.
11. Recommendations for the Practice of Clinical Neurophysiology. Guidelines of the International Federation of Clinical Neurophysiology. New York, Elsevier, 1999.
12. Kimura, Jun. Electrodiagnosis in diseases of nerve and muscle. Principles and Practice. Fourth Edition. New York. Oxford University Press, 2013.
13. Ryan CS, Conlee EM, Sharma R, Sorenson EJ, Boon AJ, Laughlin RS. Nerve conduction normal values for electrodiagnosis in pediatric patients. *Muscle & Nerve 2019, 60 (2): 155-160.*
14. Hylienmark L, Ludvigsson J, Brismar T. Normal values of nerve conduction studies in children and adolescents. *Electroencephalogr Clin Neurophysiol* 1995 Oct;97(5):208-14.
15. Tekgül H, Polat M, Tosun A, Serdaroğlu G, Gökben S. Electrophysiologic assessment of spasticity in children using H-reflex. *Turk JPediatr* 2013; 55: 519-523.
16. Ziegler D, Laux G, Dannehl K, et al. Assessment of cardiovascular autonomic function: age-related normal ranges and reproducibility of spectral analysis, vector analysis, and standard tests of heart rate variation and blood pressure responses. *Diabet Med*1992; 9:166–175.
17. Akyuz G, Turkdogan-Sozuer D, Turan B, et al: Normative data sympathetic skin responseand RR interval variation in Turkish children. *Brain Dev* 21:99-102.
18. Uncini A, Pullman SL, Lovelace RE, Gambi D. The sympathetic skin response normal values, elucidation of afferent components. *J Neurol Sci* 1988, 87:299-306.
19. Ferrer T, Ramos MJ, Pérez-Jiménez A, Pérez-Sales P, Álvarez E. Sympathetic sudomotor function and aging. *Muscle Nerve* 1995 Apr;18(4):395-401.

Figures

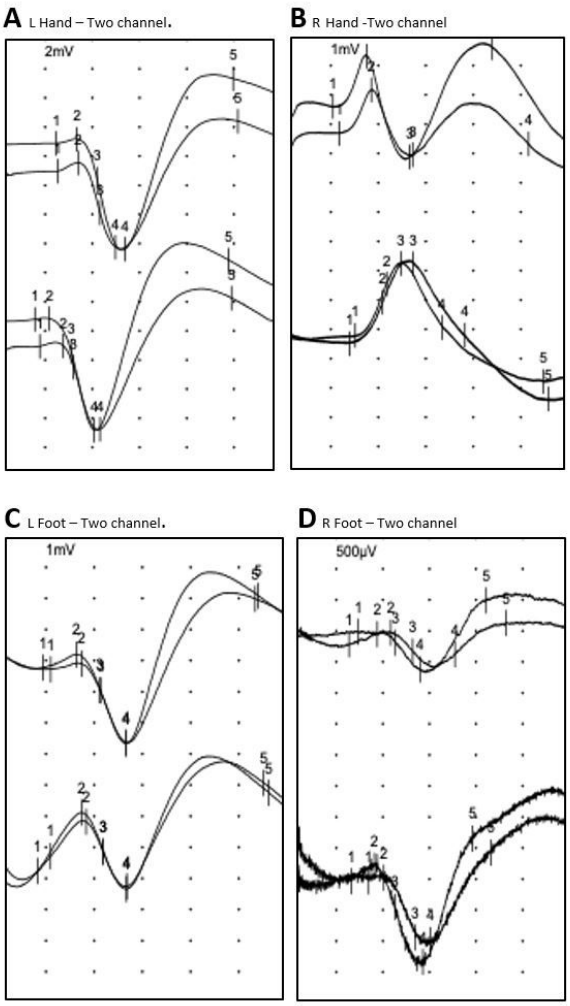
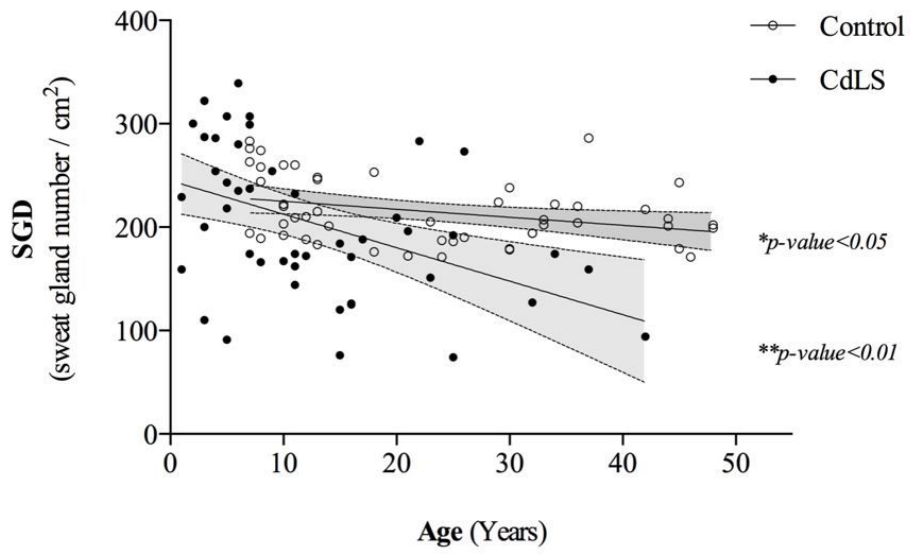


Figure 1

Sympathetic Skin response in upper and lower limbs. A: Normal symmetrical sympathetic skin response (SSR) in upper limbs in individual 30. B: Pathological asymmetrical in amplitude and morphology SSR in upper limbs in individual 23. C: Normal symmetrical normal SSR in lower limbs in individual 30. D: Pathological symmetrical in amplitude SSR in lower limbs in individual 40.

A)



B)

	Controls	N	CdLS NIPBL	N	p[a]
Mean SGD (g/cm²)±SD	217.60±30.61	50	178.03±72.53	27	0.001

Mean SGD (g/cm²)±SD by decades of life	**Controls**	**N**	**CdLS NIPBL**	**N**	**p[a]**
≤10 years	236.76±34.81	13	226.00±86.60	10	0.686
11-20 years	217.18±29.99	11	159.09±45.35	11	0.002
21-30 years	206.40±22.9	10	139.00±59.90	3	0.010
31-40 years	215.28±32.40	8	143.00±22.62	2	0.013
41-50 years	202.50±22.16	8	94.0	1	0.002

Figure 2

A: Analysis of SGD. (SGD: sweat gland density: gland number/cm2): Each dot corresponds to a different individual at the indicated age. Filled dots are CdLS individuals (n=47) and empty dots correspond to control individuals (n=50). Lines show mean linear fit and 95% confidence intervals (shadowed areas). Significant non-zero slope, linear regression, *p-value<0.05, **p-value<0.01, ***p-value<0.001. B: SGD by decades of life. Values for sweat gland density in CdLS individuals with variants in NIPBL and controls by groups of age. aIndependent samples t test. There are statistically significative differences (p<0.05) in the SGD global mean (control group compared to the global NIPBL group) and by decades of life, in all the decades except in the first one.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Tables1to4Supplementarymaterial.docx