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Case report

Endocrine Evaluation and Homeostatic Model Assessment in Patients with Cornelia de Lange Syndrome

Ascaso A et al. Endocrine Evaluation and Insuline Resistance in CdLS

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What is already known on this topic?
Cornelia de Lange Syndrome (CdLS) is a rare developmental genetic disorder associated with short stature and delayed puberty. However, research on other hormonal assessments in this condition, such as high homeostatic model assessments of insulin resistance (HOMA-IR), is scarce.

What this study adds?
Three of seven prepubertal patients had high HOMA-IR values but no metabolic risk factors, suggesting insulin resistance in this population. Two of the 17 postpubescent patients had altered HOMA-IR values associated with increased BMI, which to our knowledge has not been published before. These findings underscore the importance of endocrine follow-up in these patients.

Abstract
Objective: The aim of this study was to expand knowledge about endocrine disorders in individuals with Cornelia de Lange syndrome (CdLS), a rare developmental genetic disorder with anomalies in multiple organs and systems.
Methods: Hormone levels, clinical scores, anthropometric measurements, and molecular analysis were assessed in 24 individuals with CdLS.
Results: Hyperprolactinemia was the most common endocrine disorder. Three patients showed subclinical hypothyroidism. In the gonadotropic axis, mildly delayed puberty was observed, as well as genital anomalies, such as cryptorchidism. Despite short stature, levels of insulin-like growth factor 1 and insulin-like growth factor-binding protein 3 were normal, on average. Three prepubertal individuals without risk factors had higher than normal values for the homeostatic model assessment of insulin resistance (HOMA-IR) and for insulinemia, suggesting insulin resistance. Furthermore, two adults had elevated BMIs associated with HOMA-IR values over the cut-off values.
Conclusion: CdLS can lead to dysregulation of the endocrine system, particularly in patients with high HOMA-IR values and insulinemia who are at risk of insulin resistance. Therefore, clinical follow-ups with hormonal assessments are proposed for individuals with CdLS.
Keywords: Cornelia de Lange syndrome, HOMA-index, insulin resistance, endocrine evaluation and hypothalamic-pituitary axis.

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Introduction
Cornelia de Lange (CdLS) syndrome [(OMIM) #122470, 300590, 300882, 610759 and 614701)] is a congenital malformation syndrome characterized by distinctive facial features, microcephaly, growth retardation, and anomalies in multiple organ and systems. CdLS prevalence is estimated to be below 1:30000 live births (1). The onset of this syndrome has been linked to mutations involving proteins associated with the cohesin complex, which is a basic regulator of chromosomal biology. Eight causal genes (*NIPBL, SMC1A, SMC3, RAD21, BRD4, HDAC8, ANKRD11*, and *MAU2* (2–5) and several candidates have been identified. Mosaicism and splicing mutations are relatively frequent (6–8). Thus, a broad phenotypic spectrum (9) has been described, and a clinical score (2) has been developed.
Recently, small-fibre neuropathy and changes in body composition have been associated with CdLS (10,11). Abraham and Schlesinger (12,13) performed the first endocrine studies, Kline and colleagues reported CdLS-specific growth charts (14), and Schwartz et al. published a pituitary study of five patients (15). In 2007, an extensive study including comprehensive endocrinological work-ups on 49 patients with CdLS reported mildly delayed puberty (1), and these findings were included in a CdLS consensus paper published in 2018 (2). However, a thorough endocrine evaluation in these patients has been rarely reported. This study thus aimed to expand knowledge of endocrine evaluation for patients with CdLS.

Materials and Methods
Patients
A descriptive study of 24 Spanish individuals (7 prepubescent and 17 postpubescent patients, aged 2–37 years) with CdLS was performed. All subjects were evaluated by a clinical paediatrician or a clinical geneticist with experience in CdLS. After comprehensive and detailed clinical and auxologic evaluations, patients were classified based on recently published CdLS consensus criteria (2). Pubertal development in children was assessed by an expert endocrinologist using Tanner Staging (16).

Adult participants completed a questionnaire reporting the time of onset of main secondary sexual characteristics. All data were confirmed by checking levels of gonadotropic and sex steroid hormones. Informed consent from participants, their parents, or legal guardians was obtained before entry into the study.

Hormonal study

Venous blood samples were drawn and centrifuged, and plasma or serum was separated. Levels of insulin, thyrotropin, thyroxine, prolactin, adrenocorticotropic hormone, cortisol, luteinizing hormone, follicle-stimulating hormone, 17-beta-estradiol, and total testosterone were measured using specific electrochemiluminescence assays in a Cobas e601 autoanalyzer (Roche Diagnostic, Mannheim, Germany). Serum glucose was analysed using an enzymatic spectrophotometric method with the Cobas 8000 autoanalyzer (Roche Diagnostic). Insulin-like growth factor-1 (IGF-1) and insulin-like growth factor-binding protein-3 (IGFBP-3) levels were measured using chemiluminescent immunometric assays (Immulite 2000Xpi, Siemens Healthcare Diagnostics, Los Angeles, CA, USA). After ruling out comorbidities analyses were performed while participants were fasting and in a resting state for 20–30 minutes.

Weight was measured in kilograms (kg) using an AMGI-IMSA model and height in centimetres (cm) using a Harpenden Tallimeter. BMI was calculated as weight (kg) divided by height squared (m^2). The obtained value was expressed as a Z-score, according to the reference graphs in a Carrascosa's (2010) Spanish Growth Study (17). Insulin resistance was calculated by means of the homeostatic model assessment of insulin resistance (HOMA-IR), defined according to the following equation: HOMA-IR = fasting glucose (mmol/l) × fasting insulin (μU/ml)/22.5 (18). All molecular analyses for each individual were performed using a custom, targeted gene panel including *NIPBL, SMC1A, SMC3, RAD21, HDAC8, BRD4, ANKRD11*, and *MAU2* (19). **Chromosomal studies were performed using standard methods.**

Consent

The ethical guidelines for human research outlined by the Declaration of Helsinki and revised by Fortaleza (2013) were followed. The Ethics Committee of Clinical Research from the Government of Aragón (CEICA; PI15/00707) and the subjects approved the study protocol.

Results

Study participants totalled 24 (9 male and 15 female) Spanish patients aged 2–37 years (7 prepubescent and 17 postpubescent) with CdLS. Most had mild involvement of most endocrine axes. In the thyroid axis, three participants had **normal or low thyrotropin level and low thyroxine level.** Hyperprolactinemia was the most common endocrine disorder, affecting half of the participants (P1, P2, P4, P6, P9, P13, P14, P15, P16, P18, P20, and P22). Regarding pubertal and gonadal function, two male and one female patients had mildly delayed puberty, and four of the nine males had bilateral cryptorchidism. Four females (P15, P16, P19, and P21) reported having irregular menstrual cycles; a 16-year-old participant (P17) showed absence of menarche and no breast development. **Chromosomal studies were normal in all cases.** Among all 24 participants, 63% had prenatal growth retardation, and 80% had postnatal growth retardation. Finally, the HOMA values in three prepubertal (P5, P7, and P8) and two adult participants (P21 and P22) exceeded the cut-off points. Tables 1 and 2 summarize these results.

Discussion

Although individuals with CdLS rarely develop severe endocrine disorders, the endocrinological work-ups reported here suggest a mild involvement of most axes. **Decreased thyroxine values and normal thyrotropin levels might suggest a central subclinical hypothyroidism; however, no abnormalities were detected on brain imaging**. (P15, P19, and P22. Hyperprolactinemia was the most frequent endocrine disorder, occurring in 12 participants, among whom P9, P13, P14, P18, P20, and P22 were taking antipsychotic drugs that could explain this increase but P1, P2, P4, P6, P15, and P16 were not undergoing any treatment. All but six had normal adrenocorticotropic hormone and cortisol levels, thus ruling out **acute** stress as a cause of hyperprolactinemia.

Regarding gonadal function, cryptorchidism (14) was observed in two prepubertal participants (P1 and P6) and two pubertal ones (P14 and P18), suggesting that dysfunction of the pituitary-gonadal axis could be present in early gestation. In addition, P14 and P18 had delayed puberty, as diagnosed by the absence of testicular development at age 14 years and no progression of secondary sexual characteristics at more than two years after pubertal onset. Seven female participants reported irregular menstruation, and one **(P17)** reported delayed puberty and lack of breast development at age 13 (1,2,15). In these patients, gonadotropins were not elevated, suggesting a possible central origin of the disorder. **Regardless of LH levels, clinically there is a pubertal delay, and LH values may suggest that central hypogonadism could be transitory or permanent depending on the evolution, thus requiring close clinical follow-up**.

Prenatal and postnatal growth retardation is a common feature in individuals with CdLS (14,15,20). In this study, 20 of the 24 patients had heights >2 SD below the means for age and sex, and 15 were small for gestational age (>2 SD below means for birth weight or birth length), indicating a prenatal origin. In addition, 80% did not show catch-up growth at 4 years of age. However, levels of IGF-1 and IGFBP-3 were normal except in P5, whose low BMI could indicate malnutrition associated with secondary IGF-1 deficiency.

One research objective of this study was to evaluate hydrocarbon metabolism in these patients. Although the euglycemic-hyperinsulinemic clamp method is considered the gold standard technique to estimate insulin sensitivity, this approach can be invasive for patients with intellectual disabilities and behavioural disorders. Therefore, it is more appropriate to use a simple and indirect method, such as the HOMA-IR index (21), which estimates insulin resistance using a simple equation: fasting glucose (mmol/l) × fasting insulin (μU/ml)/22.5. The cut-off point for insulin resistance on this index remains a matter of controversy. In adults, more invasive techniques have confirmed a cut-off point of 3.8 (22). In children, such studies are more difficult and ethically controversial. However, a previous study in a Spanish cohort of 372 individuals established 3.42 as the cut-off point (23), and a recent meta-analysis of populations of various ethnicities indicated cut-off points between 2.30 and 3.54 (24).

Five participants, or approximately 21% of the study population, had HOMA-IR levels exceeding the respective 3.54 cut-off for insulin resistance in children (25) and the 3.8 cut-off for adults. Prepubertal participants P5, P7, and P8 had HOMA-IR values of 4.53, 5.54, and 7.2, respectively. Adults P21 and P22 had HOMA-IR values of 6.23 and 10.7, respectively. These results could be related to obesity and increased BMI, particularly in adults. However, the three prepubertal patients had normal BMIs and no family history or risk factors, such as hypertension or obesity, suggesting that CdLS could be associated with increased insulin resistance. Notably, a fasting insulin value above 16 μU/ml in children and adults is considered suggestive of hyperinsulinemia (22,25). Thus, all participants with elevated HOMA values also had high insulinemia.

To our knowledge, this is the first study to relate CdLS with elevated insulin and HOMA index values. It therefore seems reasonable to recommend follow-up assessments of carbohydrate metabolism in these patients. Close endocrinological follow-ups also are necessary to assess nutritional status, growth, and pubertal development in these patients so that severe endocrinological alterations of central origin (e.g., hypothyroidism and hypogonadism) can receive appropriate treatment as soon as possible.

Limitations of the Study

Limitations of this study include the low incidence of this rare disease and the absence of elderly participants. The method of measuring insulin resistance also may be considered a limitation. In most cases, hyperglycaemic/euglycemic clamp is the gold standard for quantifying in vivo insulin action, secretion, and disposal, but clamp studies are expensive to conduct and invasive, which raises ethical concerns for populations with physical, cognitive, and medical challenges, such as those with CdLS. Less invasive methods were used to evaluate axis integrity, including the luteinizing hormone-releasing hormone response test and thyrotropin-releasing hormone test, and the HOMA index was used as a surrogate marker of insulin resistance.

Conclusion

Individuals on the CdLS spectrum can have dysregulation of the endocrine system, including altered prolactin levels, mildly delayed puberty, cryptorchidism, and short stature. In addition, some of the HOMA-IR assessments of patients in this study suggest early development of insulin resistance. Therefore, clinical follow-ups with hormonal assessments are required in individuals with CdLS.

Authorship Contributions

Conceptualization, J.P., F.J.R. and G.B.L.; molecular analyses, A.L.P., M.A., I.P., B.P., F.J.K. and J.P.; clinical studies, A.A., L.T.L., A.M.L, F.J.R. and G.B.L.; biochemical analyses, E.LL. and J.J.P.L.; writing—original draft preparation A.A and J.P.; writing—review, J.P., F.J.R. and G.B.L.; writing—editing A.A., A.L.P., B.P., L.T.L., M.A., I.P., E.LL., J.J.P.L., A.M.L., F.J.K., F.J.R., J.P., and G.B.L.; funding acquisition, J.P. and F.J.R. All authors have read and agreed to the published version of the manuscript.

(or their parents or guardians) have given their written informed consent.

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Table 1. Anthropometric values, clinical score and affected gene in individuals with CdLS Patients

Patient	***P1**	**P2**	**P3**	**P4**	**P5**	**P6**	**P7**	**P8**	**P9**	**P10**	**P11**	**P12**	**P13**	**P14**	**P15**	**P16**	**P17**	**P18**	**P19**	**P20**	**P21**	**P22**	**P23**	**P24**
Age	2	2	3	3	3	4	5	5	7	8	9	11	11	14	15	15	16	17	20	23	25	30	31	37
Gender	M	M	M	M	F	M	F	F	M	F	F	F	F	M	F	F	F	M	F	M	F	F	F	F
Gene*	*H*	*N*	*N*	*N*	*N*	*N*	*R*	*S*	*N*	*N*	*N*	*N*	*S*	*N*	*N*	-	*N*	*N*	*N*	*N*	*N*	*A*	*N*	*N*
Clinical Score*	11	14	7	14	15	13	8	5	6	13	-	14	14	14	7	11	15	14	16	13	-	9	9	13
Pubertal stage (Tanner)	I	I	I	I	I	I	I	I	I	I	II	II	III	I	V	V	III	III	V	V	V	V	V	V
Birth weight (SDS)	-1.24	-1.15	-1.08	-1.09	**-2.31**	**-2.17**	0.44	-1.04	-0.69	-1.9	-0.07	-1.15	-1.98	**-2.45**	0.34	-0.87	**-3.44**	**-2.03**	**-3.78**	**-2.91**	**-2.1**	**-3.61**	-0.83	**-2.06**
Birth length (SDS)	**-2.57**	**-2.39**	-0.97	**-2.18**	-1.27	**-3.45**	-0.28	0.57	1.69	-1.96	**-5.11**	-1.61	**-2.21**	**-4.48**	-0.77	-0.44	-1.7	**-2.04**	**-5.73**	**-3.84**	**-2.9**	**-5.73**	-1.25	**-5.2**
Weight (kg)	10.2	9.9	14	9.8	6.63	12.1	11.5	20	19.2	16.8	20.6	18.8	26.5	43	58.8	40.2	24	50.5	22.8	43.3	96.2	54.8	36	62.8
Weight (SDS)	**-2.12**	**-2.66**	-1.29	**-2.6**	**-4.02**	**-2.23**	**-2.47**	-0.11	-1.3	-2.09	-1.83	**-2.42**	-1.91	-1.23	0.24	-1.5	**-3.47**	-1.65	-	-	-	-	-	-
Height (cm)	78.2	81	95.2	87.2	78	93.3	98	108.8	115.2	111.1	114.8	114	135	134	155.8	148.8	118	166.1	107	153.6	140.3	138.4	142.8	148.3
Height (SDS)	**-3.93**	**-3.82**	**-2.09**	**-3.47**	**-5.49**	**-4.8**	**-2.98**	-0.98	-1.58	**-3.61**	**-3.75**	**-4.8**	**-2.28**	**-3.79**	-1.01	**-2.04**	**-6.98**	1.44	-	-	-	-	-	-
BMI (kg/m2)	16.6	15.09	15.45	12.89	10.8	13.9	11.9	16.9	14.47	13.6	15.6	14.4	14.54	23.95	24.22	18.16	17.2	18.3	19.9	18.31	**48.87**	**28.61**	17.65	**28.55**
BMI (SDS)	0.09	-1	-0.33	-1.75	**-2.88**	-1.04	-1.91	0.49	-0.89	-1.34	-0.82	-1.35	-1.47	0.74	0.87	-0.86	-1.39	-1.25	-	-	-	-	-	-
Waist circumference (cm)	-	-	-	39.7	-	-	43,2	57,7	-	45.4	56.4	49.8	-	76.8	73.8	63.5	-	70.4	-	67.2	-	76.6	57.8	86.5
Waist circumference (SDS)	-	-	-	**-2,66**	-	-	**-3,15**	0.39	-	-1,66	-1,19	**-2,14**	-	0,14	1,39	-0,66	-	-0,35	-	**-2,11**	-	0,73	-1,79	**3,92**

*Genes: *H (HDC8). N (NIPBL). R (RAD21). S (SMC1A). A (ANKRD11)* CH: Carbohydrate. IUGR: Intrauterine growth restriction. BMI: body mass index. Reference levels have been used according to age. sex and pubertal stage. Abnormal values are highlighted in bold. *HDC8, RAD21, SMC1A* and *ANKRD11* genes are highlighted in gray. * Clinical Score: According to Kline AD, Moss JF, Selicorni A, et al. (2018) Diagnosis and management of Cornelia de Lange syndrome: first international consensus statement. Nat Rev Genet; 19:649-666. Anthropometric values have been expressed in Z-scores according to the reference graphs (Spanish Growth Study 2010, Carrascosa). Abnormal values are highlighted in bold. *P: patient abbreviation.

Table 2. Hormonal study in individuals with CdLS

Patient		*P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12	P13	P14	P15	P16	P17	P18	P19	P20	P21	P22	P23	P24
Age		2	2	3	3	3	4	5	5	7	8	9	11	11	14	15	15	16	17	20	23	25	30	31	37
Gender		M	M	M	M	F	M	F	F	M	F	F	F	F	M	F	F	F	M	F	M	F	F	F	F
Thyroid axis/ prolactin	Thyrotropin	**5.94**	2.04	1.1	1.83	**0.26**	1.4	1.74	2.76	2.89	1.57	2.23	2.39	0.94	1.18	2.09	2.07	0.78	1.57	1.33	0.74	0.69	1.57	1.29	1.04
	Free thyroxine	1.23	1.41	0.99	1.28	1.28	1.24	1.6	1.07	1.22	1.25	1.08	1.44	1.2	1.2	**0.92**	1.21	1.13	1.21	**0.84**	1.18	1.59	**0.94**	1.27	0.98
	Prolactin	**38**	**90.6**	13.7	27	4.37	**28.9**	15.4	12.3	**37.6**	16.7	20.8	5.37	**33.6**	**40.4**	**26.5**	**29.6**	9.06	**34.6**	12.4	**60**	12.5	**29.6**	14	11.3
	Neuroleptic tx	-	-	-	-	-	-	-	-	+	-	-	-	+	+	-	-	-	+	-	+	+	+	-	-
Adrenal axis	Adreno-corticotropic hormone	**50.4**	-	17	32.9	19.3	46.9	17.4	30.7	21	16.8	**182**	13.3	10.6	**60**	13.7	35.7	10.9	28.9	-	11	16.9	9.6	6.33	7.61
	Cortisol	7.47	-	-	13.7	11.9	<0.3	7.53	12.3	-	10.2	13	7.66	9.16	16.6	8.2	9.89	7.35	14.7	-	7.28	9.27	3.46	6.44	9.15
Gonadal axis	Luteinizing hormone	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3	1.82	**0.58**	13.1	4.74	11.3	3.05	3.68	5.52	9.82	4.31	10.2	4.78	12.6	2.66
	Follicle-stimulating hormone	1.35	2.1	0.87	0.56	0.75	0.48	1.97	1.58	0.4	1.48	3.87	6.95	4.08	8.76	5.3	2.14	4.65	7.48	4.44	5.58	6.45	5.71	6.96	4.25
	17-beta-estradiol	-	-	-	-	<20	-	<20	<20	-	-	61.4	41.6	73.7	-	112	143	32.1	-	-	-	54.5	35.5	108	73.9
	Total testosterone	<0.2	<0.2	<0.2	<0.2	-	<0.2	-	-	<0.2	-	-	0.58	-	0.4	-	-	-	3.22	-	3.98	-	-	-	-
Growth axis	IGF-1	87.8	94.8	53.4	66.6	**<15**	151	78.1	116	145	119	245	164	266	213	279	282	194	259	-	157	214	118	124	164
	Insulin-like growth factor-binding protein 3	2.71	3.2	2.32	2.15	1.85	4.61	3.08	3.6	3.95	4.67	6.34	4.6	4.81	5.89	6.62	6.36	5.56	5.6	-	4.81	4.77	3.68	3.5	5.94
Carbohydrate metabolism	Glucose	71	87	**105**	84	90	82	**107**	90	93	72	94	86	91	90	82	79	75	87	86	**102**	85	**115**	80	87
	Insulin	**15.2**	2	7.5	**14.3**	**20.4**	2.1	**17.2**	**32.4**	7.48	2	9.32	8.57	7.85	8.36	9.38	14.6	14.4	9.3	-	14.7	**29.7**	**37.8**	16.6	9.36
	HOMA-IR	2.66	0.42	1.95	2.96	**4.53**	0.42	**4.54**	7.2	1.71	0.36	2.16	1,82	1.76	1.85	1.89	2.84	2.66	1.99	-	3.7	**6.23**	**10.7**	3.27	2.01

Normal values: Glucose (60-100 mg/dL); insulin in children/adolescents (Tanner I: 0.62-11.57 µU/mL. Tanner II: 0.69-13.75 µU/mL. Tanner III: 3.42-16.28 µU/mL. Tanner IV-V: 2.02-20.76 µU/ml). Insulin < 15 µU/mL; thyrotropin (0.6- 4.84 mU/L); thyroxine libre (0.97- 1.67 ng/dL); prolactin: Male (4.04- 15.2 ng/mL). Female (4.79-23.2 ng/mL); IGF-1 Tanner I (53-332 ng/mL). Tanner II (84-431 ng/mL). Tanner III (114-773 ng/mL.) Tanner IV (217-843 ng/mL). Tanner V (147-842 ng/mL). adults 20-30 years (116-358 ng/mL). 30-40 years (109-307 ng/mL); IGF-BP3: Tanner I (1.3-6.3 microgr/mL). Tanner II (2.4-6.7 µg/ml). Tanner III (3.3-9.1 µg/mL). Tanner IV (3.5-8.6 µg/mL). Tanner V (2.7-8.9 µg/mL). Adults 20-30 years (3.4-7.8 µg/mL). 30-40 years (3.5-7 µg/mL); adrenocorticotropic hormone (0-46 pg/mL); Cortisol (5-25 mcg/dL); luteinizing hormone prepuberal < 0.3 UI/L. puberal: Male (1.7-8.6) UI/L. Female: follicular phase (2.4-12.6 UI/L). ovulation phase (14.0-95.6 UI/L). luteal phase (1-11.4 UI/L); follicle-stimulating hormone prepuberal: Male (<0.3-3) UI/L. Female (<0.3-4) UI/L; puberal: Male (15-12.4) UI/L. Female: follicular phase (3.5-12.5 UI/L). ovulation phase (4.7-21.5 UI/L). luteal phase (1.7-7.7 UI/L); 17-beta-estradiol: Male (0-56) pg/mL. Female: prepuberal < 20 pg/mL puberal >20 pg/mL; total testosterone: prepuberal < 0.2 ng/mL. puberal > 0.2 ng/mL. Abnormal values are highlighted in bold. Tx: treatment abbreviation. *P: patient abbreviation.