Neuropsychiatric Functioning in CDLS: A Detailed Phenotype and Genotype Correlation

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Research

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Abstract

Background: Cornelia de Lange Syndrome (CdLS) is a rare genetic disorder and its phenotype clinical expression is widely variable. Despite the growing interest on genotype-phenotype correlations and on behaviour phenotype of genetic syndromes, specific studies in CdLS cohorts evaluating the correlations between genotype and neurodevelopmental characteristics, Behaviour and communicative aspects are limited, most of these studies are descriptive and there is a lack of specific assessment protocols.

Methods: Neurodevelopmental and Behavioural phenotype of all the patients (N = 38) was assessed using a specific neuropsychiatric protocol, concerning Intellectual Quotient (IQ), General Quotient of Development (GQ), communicative skills, behavioural aspects and adaptive behaviour based on direct and indirect evaluation. Subsequently, we searched for possible genotype–phenotype correlations comparing individuals with NIPBL variants (CdLS NIPBL mutated group) and patients with negative molecular results (CdLS clinical diagnosis group).

Results: The first part of the study showed a higher percentage of subjects with normal Intellectual Quotient (IQ) and Borderline Intellectual Functioning if compared with previous data; adaptive skills were lower than expected for age in all participants and the weakest areas were Socialization, Motor Skills, and Communication. Expressive language was more compromised than receptive language, nevertheless receptive abilities were also impaired. 39.5% of the sample presented with Autism Spectrum Disorder (ASD). By stratifying CdLS phenotypes by genetic, NIPBL mutated individuals demonstrated a worse trend in cognitive functioning, in communication expressive skills, in motor skills and in ASD symptoms in comparison with the Clinical Diagnosis Group. Individuals with non-Truncating mutation (mostly missense) displayed better abilities in Communication and in relational aspects with no ASD while Truncating individuals presented with worse abilities in Daily Living Skills, Socialization, Motor Skills, and Communicative abilities.

Conclusions: These findings should increase our awareness of the strengths and weaknesses points in CdLS individuals to guide appropriate targeted management; interventions addressing communicative impairments represent a clinical priority in CdLS patients.

Background

Cornelia de Lange syndrome (CdLS, OMIM, #122470, #300590, #610759, #614701, #300882) is a rare genetic disorder characterized by peculiar facial dysmorphisms, major malformations, growth retardation, and developmental delay/intellectual disability with an estimated prevalence between 1 in 10,000 and 1 in 30,000 live births [Kline et al., 2007]. Classic (or typical) CdLS phenotype is easily recognized, but clinical expression is widely variable. A recent International Consensus Statement defined the whole CdLS phenotype as a spectrum and conceived a diagnostic score based on a combination of cardinal and suggestive features to uniform the definition of classic and non-classic CdLS [Kline et al., 2018]. CdLS spectrum (CdLSp) belongs to Coesinopathies because a mutation in one cohesin function-relevant gene (NIPBL, SMC1A, SMC3, RAD21, HDAC8, ANKRD11, BRD4) is identified in most patients, although the causal variant remains unknown in 15–20% of patients [Huisman et al., 2013; Kline et al., 2018].

In the past, different studies searched for genotype-phenotype correlations in CdLS; the general consensus suggests that NIPBL mutations are likely to be associated with a more “classic” clinical presentation and a more

severe impaired cognitive function [Gillis et al., 2004; Mannini et al., 2013]. According to the diagnostic score proposed by the International Consensus Statement, most patients with NIPBL mutation have a high score and meet the criteria for the definition of classic CdLS [Kline et al., 2018]. A kind of genetic gradient of severity for the other genes associated with CdLS was presumed with HDAC8 gene at the top with a more classic and severe clinical presentation, and SMC3 gene at the bottom with a mild presentation. None of these correlations, however, are based on statistical analysis of a large cohort of patients.

The clinical variability in CdLS is also clearly expressed in cognitive and behavioural aspects. CdLS Behavioural phenotypes, defined as physiological and Behavioural manifestations with distinctive social, linguistic, cognitive and motor profiles [O'Brien, 2006], is characterized by neurodevelopmental delay, intellectual disability, autistic traits, hyperactivity, Self Injurious Behaviours (SIB), sleep disorders, compulsive Behaviours and social anxiety disorders during adolescence [Ajmone et al., 2014; Basile et al., 2007; Cameron and Kelly, 1988; Grados et al., 2017; Kline et al., 1993; Kline et al., 2018; Mariani et al., 2016; Moss et al., 2008; Oliver et al., 2008; Saal et al., 1993; Sloneem, 2009; Zambrelli et al., 2016].

Literature reports intellectual disability (ID) ranging from mild to profound; most patients have a moderate-severe ID, but some individuals have a normal IQ. Adaptive behaviour is compromised and communication abilities vary widely but, typically, major difficulties are present in expressive language skills which are more compromised than receptive ones [Ajmone et al., 2014; Goodban, 1993; Sarimski, 1997]. Subsequent studies demonstrated that CdLS individuals showed difficulties in verbal comprehension and in explanation of concepts [Mulder et al., 2019].

The prevalence of ASD symptomatology in CdLS ranges between 27% and 82% [Basile, 2007; Mulder et al., 2016; Richards et al., 2015]. Moreover, restricted and repetitive Behaviours occur in patients with more marked ID and with ASD and usually they are associated with increased risk of self-injurious Behaviours [Arron et al., 2011].

It is important to point out that many researches have focused their attention on particular features of the Behavioural phenotype [Bruserudu et al., 2016; Cochran et al., 2015; Fisher, 2016; Moss et al., 2017] but few of them have used complete evaluations' protocols, characterized by the mix of direct and indirect tools, specifically chosen in order to obtain a broad and more appropriate assessment in these children. A direct assessment made by clinicians with experience in genetic syndromes and neurodevelopmental disorders combined with an indirect evaluation which takes into consideration information given by the parents reduces misdiagnosis and allows for a better intervention plan.

Despite the growing interest on genotype-phenotype correlations and on behaviour phenotype of genetic syndromes, specific studies in CdLS cohorts evaluating the correlations between genotype and neurodevelopmental characteristics, Behaviour and communicative aspects are limited [Ajmone et al., 2014; Bhuiyan et al., 2006; Huisman et al., 2017; Moss et al., 2017; Mudler et al., 2019]; most of these studies are descriptive and there is a lack of specific assessment protocols.

These studies suggest that CdLS individuals with NIPBL mutations show lower levels of cognitive functioning and adaptive Behaviour skills, as well as more self-injurious Behaviour (SIB) compared with patients with the SMC1A variant [Huisman et al., 2017; Mulder et al., 2019]. Furthermore, NIPBL truncating mutations usually cause more pronounced Behaviour problems, including autism, but similar problems also occur in missense mutations [Bhuiyan et al., 2006]. Finally, no studies have shown a clear association between the type of NIPBL variant and

level of ID [Selicornì et al., 2007; Wulffaert et al., 2009; Yan et al., 2006], only one study indicated that missense and in frame deletions of *NIPBL* were associated with mild cognitive phenotype [Ajmone et al., 2014].

In this study, we aim to outline the Behavioural phenotype of CdLS based on a broad functional description of a cohort of 38 patients evaluated using a tailored neuropsychiatric assessment. Moreover, we try to bring out some genotype-phenotype correlations, supported by statistical analysis. The results emerged from this study should increase our knowledge on specific neuroBehavioural strengths and weakness points of CdLS individuals to guide appropriate targeted clinical interventions and management for these individuals and their families in order to improve their quality of life.

Methods

Participants

38 CdLS Italian patients (19 males and 19 females), with an age at evaluation ranging between 7 and 184 months (mean: 77.18 ± 48.76 with a median of 69.5 months), referred to our clinic for assessment and follow-up, were enrolled in the study. All patients had a clinical diagnosis of CdLS according to diagnostic criteria proposed by Kline et al. [2018].

Molecular tests included *NIPBL* sequencing on blood and buccal smear and *NIPBL* MLPA analysis, *SMC1A*, *HDAC8*, *RAD21* and *SMC3* sequencing analyses were performed. Before the introduction of next generation sequencing-based cohesin gene panels in 2015, the molecular workflow included the sequence of single gene Sanger sequencing started with *NIPBL*, based on their frequencies, completed with *NIPBL* MLPA analysis.

Among 38 patients in the study cohort, 17 individuals were part of a previous study evaluating communication, cognitive level, development and Behaviour between 2009 and 2011 [Ajmone et al., 2014]; the other 21 were evaluated between 2011 and 2020.

All patients were assessed at Child and Adolescent Neuropsychiatric Service, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy. The assessment protocol was administered to all subjects during outpatient visits.

Procedures

Neuropsychiatric phenotype of all the patients was assessed using a specific protocol, concerning Intellectual Quotient (IQ), General Quotient of Development (GQ), communicative skills, behavioural aspects and adaptive Behaviour based on direct and indirect evaluation.

Firstly, we analyzed and described each developmental area considering the whole cohort of patients in order to obtain a detailed and in-depth description of CdLS Behavioural phenotype. Moreover, we focused on identifying phenotype-genotype correlations comparing individuals with *NIPBL* variants (CdLS *NIPBL* mutated group) and patients with negative molecular results (CdLS clinical diagnosis group). Finally, a more specific comparison between two different types of *NIPBL* mutations (truncating versus missense) was performed. This comparison was possible because the sample was homogeneous in age and number.

Cognitive and developmental assessment

Two different scales were used to assess Intellectual Quotient (IQ) and General Quotient of Development (GQ): the Leiter International Performance Scales Revised – Leiter-R [Roid and Miller, 1997], and the Griffiths' Scale [Griffiths, 1986]. We used the Griffiths' Scale [Griffiths, 1986] to evaluate the general developmental quotient (GQ) in patients from 0 to 8 years old. The GQ is composed of six sub-quotients, one for each area investigated (locomotor, personal social, language, eye and hand coordination performance, and practical reasoning). The GQ identifies how children perform across developmental areas.

The Leiter International Performance Scales Revised – Leiter R (age range 2–21 years) [Roid and Miller, 1997], is a non-verbal cognitive test. It is useful because it has a short version that does not require high attention skills. Moreover, it does not require verbal communication abilities, and it can be administered when communication difficulties are present or to non-speaking children. We decided to consider the IQ obtained using Leiter scale because it may better highlight the real cognitive abilities of CdLS patients [Ajmone et al., 2014].

Although the two measures do not perfectly correspond, we considered both as indices of children's development. Both tests yielded a standardised quotient with $M = 100$ and $SD = 15$.

In literature, the use of a combined IQ/GQ index is quite common if participants are at significantly different stages with respect to the level of functioning [e.g., Noyola et al., 2001; Winterkorn et al., 2007; Van Schooneveld and Braun, 2013]. In addition, the Griffiths GQ appeared to be a good predictor of later IQ [e.g., Bowen et al., 1996].

Adaptive Behaviour assessment

The Vineland Adaptive Behaviour Scale (VABS) in its Italian adaptation and validation [Balboni and Pedrabissi, 2003] was used to assess adaptive Behaviour. The VABS is a semi-structured interview with caregivers and allows us to assess global adaptive behaviour skills (Adaptive Behaviour Composite) and ability on four specific domains (Communication, Daily Living Skills, Socialisation, Motor Skills).

Communication and Language Evaluation

For the assessment of expressive and receptive skills we used the Communication domains of VABS scale (expressive and receptive language). It provides a Language age-equivalent score.

Behavioural assessment and autism spectrum disorders

Behavioural characteristics of the participants were assessed using the Child Behaviour Checklist – CBCL [Achenbach and Rescorla LA, 2001], while ASD (autism spectrum disorders) symptomatology was evaluated with the Childhood Autism Rating Scale (CARS) [Schopler et al., 1988]. Child Behaviour Checklist (CBCL) (Achenbach and Rescorla, 2001) was used to assess children's Behavioural characteristics. The CBCL is a 100 item questionnaire completed by parents reflecting their point of view of the child's Behaviour at the time of administration and for the preceding 3 months. It provides a child Behaviour profile into 8 subscales: withdrawn behaviour, somatic complaints, anxiety/depressed behaviour, opposite behaviour, aggressive Behaviour, social problems, thought problems, and attention problems. Single sub-scales can also be scored in terms of two broad grouping of symptoms: internalizing (consisting of anxious/depressed, withdrawn, emotionally reactive, somatic complaints) and externalizing (consisting of attention problems, aggressive Behaviour, rule-breaking).

The Childhood Autism Rating Scale (CARS) is a Behaviour rating scale used as a screening measure of Autism Spectrum Disorder in clinical and research studies. This scale investigates the presence of Behavioural, cognitive, and communicative characteristics associated with autism and provides a final score, produced by the clinicians

at the end of the evaluation. Fifteen areas are considered, and for each area, the child is rated on a scale from 1 (*normal behaviour*) to 4 (*severely abnormal behaviour*). Thus, the total score ranges from 15 to 60. In compliance with the CARS manual, we adopted a cut-off score of 30. For the purposes of the present study, children were divided into two categories, namely, children without autistic traits, i.e., children with scores ranging between 0 and 30, and children with mild, moderate or severe autistic traits, i.e., children with scores ranging between 30 and 60.

Statistical analysis

Independent sample t-tests were used to evaluate differences in: IQ/GQ indexes, CARS scores and CBCL internalizing, externalizing and total scores between CdLS NIPBL mutated group and CdLS clinical diagnosis group. The same type of analysis was performed between truncating NIPBL mutations and non-truncating NIPBL mutation groups.

Age equivalent VABS scores were compared using ANCOVAs controlling for age. This practice is debated [Glass, Peckham and Sanders, 1972; Miller and Chapman, 2001] even though it is common in different research fields involving human participants [see, for example, recent papers from Couture et al., 2020; Flesher et al., 2020; Gaubatz et al., 2020].

A correlation between IQ/GQ indexes and CARS scores was performed to investigate a general relationship between developmental/cognitive levels and the characteristics associated with autism. A similar analysis was conducted using dichotomized variables of intellectual functioning (average or borderline functioning vs. mild, moderate, severe or profound disability) and autistic traits (with or without).

All data analyses were conducted using R 4.0.2 [R Core Team, 2020] and RStudio 1.3.1056 [RStudio Team, 2020] with packages effsize [Torchiano, 2020], emmeans [Lenth, 2020], janitor [Firke, 2020], knitr [Xie, 2020], rstatix [Kassambara, 2020] and tidyverse [Wickham et al., 2019].

Results

Molecular analysis

Participants were divided into two groups according to clinical and molecular diagnosis: 31 participants (81.6%) carried one heterozygous pathogenetic or likely pathogenetic variant in NIPBL gene (CdLS NIPBL mutated group) while 7 (18.4%), with a clinical diagnosis of CdLS, were negative for all CdLS associated genes analyzed (CdLS clinical diagnosis group).

Among individuals with NIPBL mutations, we divided patients on the basis of the truncating effect of NIPBL mutation in order to find some correlations with the behavioural and cognitive data. Most patients had a mutation with truncating effect (n: 17; 44.7%), while 10 individuals (26.3%) had a non-truncating, missense mutation. In 4 patients (3 or 7.9% with splicing mutation and 1 or 2.6% with in-frame deletion) it was not possible to define the mutation effect without functional studies. No significant age difference was present between the truncating NIPBL group and the non-truncating NIPBL group (mean age: 61.53 months; SD: 44.81; mean age: 67.60 months; SD: 40.67).

Clinical Assessment: Cognitive and developmental evaluation

Considering the whole sample and according to ICD-10 Classification, 5 participants fell into the Average Intellectual Functioning classification (n = 5, 13.2%) while others had a Borderline Intellectual Functioning (n = 6, 15.8%), Mild Intellectual Disability (n = 9, 23.7%), Moderate Intellectual Disability (n = 2, 5.3%), Severe Intellectual Disability (n = 11, 28.9%) or Profound Intellectual Disability (n = 5, 13.2%).

Subsequently, a between-groups comparison was performed on the participants' cognitive characteristics and their genotype.

The CdLS NIPBL mutated group showed a worse trend in cognitive level despite the fact that a significant difference was not found (IQ/GQ Mean index in NIPBL positive: 56.14, SD: 28.97 ; IQ/GQ Mean index in NIPBL negative: 42.81, SD: 29.71). A between-group comparison between the Truncating and missense NIPBL mutations group did not show any significant difference either.

Adaptive behaviour evaluation

Participants were evaluated for Adaptive Behaviour (VABS Adaptive Behaviour composite) (n = 36; M: 28.42; SD: 23.53) and an adjusted developmental age was obtained (age equivalent score). Adaptive skills were lower than expected for age in all participants (mean adaptive age: 28.42 months range from 2 to 116 months; SD: 23.57)

We obtained this profile in the main areas composing adaptive Behaviour: Communication (n = 32; M: 31.97; SD: 30.85), with Expressive Communication (n = 30; M: 33.10; SD: 28.75) and Receptive Communication subscales (n = 30; M: 33.60; SD: 51.85), Motor Skills (n = 30; M: 27.18; SD: 11.77), Daily Living skills (n = 32; M: 34.31; SD: 24.76), and Socialization (n = 32; M: 26.34; SD: 16.60). From parents' point of view, children had the lowest abilities in the Socialization area and in the Motor Skills Scale.

Table 1
Descriptive statistics of VABS

Scale	n valid	Mean	SD	Median	min	max
IQ/GQ index	38	45.27	29.66	50.0	10.0	93.0
VABS Composite	36	28.42	23.57	19.0	2.0	116.0
VABS Communication	32	31.97	30.85	18.5	18.0	158.0
VABS Com. expressive	30	33.10	28.75	18.0	18.0	110.0
VABS Com. receptive	30	33.60	51.85	18.0	18.0	288.0
VABS Daily Living Skills	32	34.31	24.76	28.5	18.0	116.0
VABS Socialization	32	26.34	16.60	19.5	18.0	84.0
VABS Motor Skills	28	27.18	11.77	23.5	18.0	58.0

Considering genotype comparison, significant differences between NIPBL mutated group and CdLS clinical diagnosis group were found in the Communication Expressive subscale (with a generalized eta squared of 0.336) and in the Motor Skills Scale (ges = 0.159). CdLS clinical diagnosis group had higher adjusted age equivalent means in Expressive communication (64.558 ± 9.384 vs. 23.526 ± 4.731) and in Motor skills (39.113 ± 5.866 vs. 25.189 ± 2.248) (Table 2).

Table 2
ANCOVA NIPBL mutated group Vs. Clinical diagnosis including age as covariate

VABS score	Effect	DFn	DFd	F	p	p < .05	Ges
Composite	NIPBL: Positive Vs. Negative	1	33	3.479	.071		.095
Composite	Age (months)	1	33	4.466	.042	*	.119
Communication	NIPBL: Positive vs. Negative	1	29	3.826	.060		.117
Communication	Age (months)	1	29	2.205	.148		.071
Comm. Expressive subscale	NIPBL: Positive Vs. Negative	1	27	13.673	.001	*	.336
Comm. Expressive subscale	Age (months)	1	27	.522	.476		.019
Comm. Receptive subscale	NIPBL: Positive Vs. Negative	1	27	3.690	.065		.120
Comm. Receptive subscale	Age (months)	1	27	1.524	.228		.053
Daily Living Skills	NIPBL: Positive Vs. Negative	1	29	4.028	.054		.122
Daily Living Skills	Age (months)	1	29	3.764	.062		.115
Socialisation	NIPBL: Mutated Vs. Clinical diagnosis	1	29	2.868	.101		.090
Socialisation	Age (months)	1	29	1.489	.232		.049
Motor Skills	NIPBL: Positive Vs. Negative	1	25	4.718	.040	*	.159
Motor Skills	Age (months)	1	25	.161	.692		.006

Within the NIPBL mutated group participants with Missense mutations had higher age-adjusted equivalent means in Composite score (34.395 ± 5.103 vs. 17.991 ± 3.5), Communication scale (36.333 ± 6.111 vs. 18.026 ± 4.778) and Expressive subscale (32.933 ± 4.767 vs. 19.108 ± 3.216), Daily Living Skills scale (37.461 ± 5.42 vs. 20.793 ± 4.238), Socialization scale (30.263 ± 3.731 vs. 18.684 ± 2.917) and Motor Skills scale (33.637 ± 3.511 vs. 21.158 ± 2.863) (Table 3).

Table 3
ANCOVA Non Truncating (Missense) Vs. Truncating including age as covariate

VABS Score	Effect	Dfn	Dfd	F	p	p < .05	Ges
Composite	Mutation: Missense vs. Truncating	1	22	7.024	.015	*	.242
Composite	Age (months)	1	22	3.997	.058		.154
Communication	Mutation: Missense vs. Truncating	1	18	5.499	.031	*	.234
Communication	Age (months)	1	18	1.868	.189		.094
Comm. Expressive subscale	Mutation: Missense vs. Truncating	1	16	5.687	.030	*	.262
Comm. Expressive subscale	Age (months)	1	16	0.017	.897		.001
Comm. Receptive subscale	Mutation: Missense vs. Truncating	1	16	1.517	.236		.087
Comm. Receptive subscale	Age (months)	1	16	.147	.706		.009
Daily Living Skills	Mutation: Missense vs. Truncating	1	18	5.795	.027	*	.244
Daily Living Skills	Age (months)	1	18	1.870	.188		.094
Socialisation	Mutation: Missense vs. Truncating	1	18	5.901	.026	*	.247
Socialisation	Age (months)	1	18	1.738	.204		.088
Motor Skills	Mutation: Missense vs. Truncating	1	17	7.533	.014	*	.307
Motor Skills	Age (months)	1	17	1.356	.260		0.074

Communication and Language Evaluation

Participants were evaluated with the VABS Communication scale (n = 32; M: 31.97; SD: 30.85) and the Communication Expressive and Receptive subscales were considered.

In general, both expressive (n = 30; M: 33.10; SD: 28.75) and receptive (n = 30; M: 33.60; SD: 51.85) communicative abilities were compromised.

Moreover, differences between NIPBL mutated group and CdLS clinical diagnosis group were found in the Communication Expressive subscale (with a generalized eta squared of 0.336) while no significant differences were found in receptive abilities; this finding confirms the expressive ability as a typical weakness in NIPBL mutated individuals.

Finally, inside NIPBL mutated group, participants with Missense mutation showed higher age-adjusted equivalent means in Communication scale (36.333 ± 6.111 vs. 18.026 ± 4.778) and in Expressive subscale (32.933 ± 4.767 vs.

19.108 ± 3.216) than Truncating mutations group (Table 3).

Autism spectrum disorders

Children were divided into two categories by CARS cut-off score of 30: 18 (47.5%) were classified having ASD, while 15 (39.5%) were found to be without autistic traits (5, 13,2%, were not evaluated because they were too young to be evaluated with this tool). Independent sample T-tests were conducted to test differences in Behavioural assessment scores between CdLS NIPBL mutated group and CdLS clinical diagnosis group. A difference (± 2.512 ; $p < .05$) was found in CARS scores where the NIPBL mutated group showed higher scores for ASD symptomatology (36.615 ± 12.878) than clinical diagnosis group (27 ± 7.61). A significant difference was found also between the Truncating group and Missense at CARS assessment (41.429 ± 12.113 vs. 29.312 ± 11.622).

The correlation between IQ/GQ indexes and CARS scores was strong and highly significant ($r = -0.83$, $p < .001$) and the chi-squared test confirmed an association between the dichotomized variables of intellectual functioning and autistic traits ($\chi^2 = 7.16$, $p < .01$).

Behavioural assessment

In this study we considered the three broad scales of CBCL: Internalizing (M: 61.03 ± 10.58), Externalizing (M: 57.19 ± 9.89) and Total Problems (M: 61.47 ± 10.76). CBCL was used according to the chronological age of participants ($n = 32$). Differences in CBCL scores had medium (for externalizing symptoms and total score) and large (for externalizing symptoms) effect sizes. T-tests comparison did not show significant group difference both between clinical diagnosis group and NIPBL mutated group and between Truncating and Missense group. This may be due to missing one CBCL evaluation of a participant with clinical diagnosis (Table 4, 5).

Table 4
Behavioural assessment: NIPBL Mutated Group Vs. Clinical Diagnosis Group

Behavioural Assessment	Clin.Diag. Group M	NIPBL Mut. M	Diff	T	Df	p	Conf. Int. 95%	Cohen's D	Mag.
CARS Tot	27.000	36.615	-9.615	-2.512	16.468	.023	-17.711/-1.519	-.799	M
CBCL EXT	52.833	58.192	-5.359	-1.033	6.504	.339	-17.822/7.104	-.546	M
CBCL INT	53.167	62.846	-9.679	-1.696	6.167	.139	-23.554/4.195	-.966	L
CBCL Tot	55.667	62.808	-7.141	-1.348	6.779	.221	-19.754/5.472	-.677	M

Note. Clin. Diag. Group M = Clinical diagnosis group mean; NIPBL. Mut. M = NIPBL mutated group mean; Mag.= Magnitude; CARS Tot = Childhood Autism Rating Scale total score; CBCL EXT = Child Behaviour Checklist externalizing symptoms; CBCL INT = Child Behaviour Checklist internalizing symptoms; CBCL Tot = Child Behaviour Checklist total score; L = Large; M = Medium.

Table 5
Behavioural assessment: Non Truncating (Missense) vs. Truncating

Behavioural Assessment	Miss. M	Trunc. M	Diff	T	Df	p	Conf. Int. 95%	Cohen's D	Mag
CARS Tot	29.312	41.429	-12.116	-2.316	15.228	.035	-23.251/-9.981	-1.014	L
CBCL EXT	57.778	57.231	.547	.126	14.489	.902	-8.748/9.842	.057	N
CBCL INT	62.667	63.692	-1.026	-.251	12.923	.805	-9.846/7.794	-.117	N
CBCL Tot	62.222	62.846	-.624	-.128	12.532	.900	-11.204/9.956	-.060	N

Note. Miss. M = Missense mean; Trunc. M = Truncating mean; Mag.= Magnitude; CARS Tot = Childhood Autism Rating Scale total score; CBCL EXT = Child Behaviour Checklist externalizing symptoms; CBCL INT = Child Behaviour Checklist internalizing symptoms; CBCL Tot = Child Behaviour Checklist total score; L = Large; N = Negligible.

Discussion

The aim of the study was twofold. First of all, we delineated in detail the neurodevelopmental and Behavioural phenotype of individuals with CdLS using a specific direct and indirect neuropsychiatric evaluation protocol; subsequently, we searched for possible genotype-phenotype correlations within the collected data. The first part of the study, describing the general CdLS behavioural phenotype in the sample, confirms the preliminary results of our previous data [Ajmone et al., 2014] and those found in literature [Basile et al., 2007; Kline et al., 2018; O'Brien, 2006; Oliver et al., 2008; Moss et al., 2008; Sarimsky, 2002] showing typical points of strengths and weaknesses of these children. Results show a higher percentage of subjects with normal IQ (n = 5, 13.2%) and Borderline Intellectual Functioning (n = 6, 15.8%), as compared with previous literature [Basile et al., 2007; Kline et al., 2018]; this confirms the notion that using more appropriate instruments for cognitive evaluation (short and nonverbal tools like the Leiter Scale) may better highlight the real cognitive abilities of these individuals. In accordance with the literature, adaptive skills were lower than expected for age in all participants (mean adaptive age: 28.42 months range from 2 to 116 months; SD: 23.57) [Basile et al., 2007; Kline et al., 2018]; the weakest areas were Socialization, Motor skills, and Communication. Moreover, previous researches showed that Adaptive Behaviour skills in CdLS can decrease with age [Buyhan et al., 2006; Kline et al., 2018; Sivrastava et al., 2014], therefore early support for children and family in this area is needed.

With regards to language and communication, expressive language is more compromised than receptive language, in line with previous reports [Ajmone et al., 2014; Goodban, 1993; Sarimski, 2002; Mudler 2019; Kline et al., 2018]; nevertheless receptive abilities are also impaired, showing difficulties in verbal comprehension as recently described in a research too [Mudler, 2019].

Direct evaluation showed the presence of ASD in 39,5% of the sample, which is consistent with previous studies that showed a range between 27% and 82% [Basile, 2007; Mulder et al., 2016; Richards et al., 2015]. Furthermore, consistent with previous studies, our data confirm a strong correlation between ID and ASD [Basile et al., 2007].

The second aim of the study was to define a specific genotype-phenotype correlation in CdLS patients. According to the consensus conference [Kline et al., 2018] we focused on the NIBPL variant and its different mutations (truncating and missense). The literature describes a more severe phenotype in patients with a truncating mutation

than in those with a missense mutation [Buyhan et al., 2006], but to the best of our knowledge, the literature lacks specific and standardized neuropsychiatric assessment studies to confirm it. By stratifying CdLS phenotypes by genetic cause, significant differences in neurodevelopmental levels, and Behavioural phenotypes were observed. NIPBL mutated individuals demonstrated a worse trend in cognitive functioning, in communication expressive skills, in motor skills, and in ASD symptoms compared to the Clinical Diagnosis Group. Due to the heterogeneity of our sample for age and number, our considerations are not to be considered absolute.

In agreement with previous research [Ajmone et al., 2014; Buyman et al., 2006], the severity of the behavioural phenotype in patients with truncating mutation was significantly higher if compared with patients with missense mutation. The Missense group showed higher adjusted age equivalent means in Communication scale (36.333 ± 6.111 vs. 18.026 ± 4.778) and in Expressive subscale (32.933 ± 4.767 vs. 19.108 ± 3.216) and fewer scores in ASD symptomatology (29.312 ± 11.622 vs. 41.429 ± 12.113) than Truncating mutation group regardless of cognitive level (no ID significant difference was found between the different groups). The homogeneity of number, age and cognitive level of this sample allowed us to clearly define two different neuropsychiatric phenotypes: truncating and Missense phenotype. Patients with missense mutation showed better abilities in Communication (especially in expressive language) and in relational aspects with no ASD while truncating patients showed worse abilities in Adaptive behaviour, Daily Living Skills, Socialization, Motor Skills, and Communicative abilities. The absence of a significant difference in IQ level between the two groups underlines that the ASD seems to be a peculiar characteristic of Truncating phenotype. This is one of the first genotype-phenotype studies in a relatively large cohort of CdLS individuals evaluated with a tailored neuropsychiatric assessment. Our purpose was to investigate different molecular mutations and their neurodevelopmental correlates, while taking prognostic and rehabilitative aspects into accounts.

This study showed that NIPBL mutated individuals demonstrated a worse trend in cognitive level, expressive communication, motor area, and ASD symptoms if compared with the Clinical diagnostic group. Literature and everyday clinical practice show how in CdLS individuals impaired communication ability is prominent and impacts both on socialization and Behaviour [Goodban, 1993; Kline et al., 2018; Moss et al., 2013; Sarimski et al., 2002], but this is the first work that defines expressive language as a typical point of weakness in individuals with NIPBL pathogenetic variant and specifically in patients with truncating mutation. Furthermore, receptive language seems to be a typical fragility of all CdLS individuals regardless of the genotype: these data are consistent with other previous findings [Ajmone et al., 2014; Mulder et al., 2019] and confirm the need for an early rehabilitation and augmentative communication approach in all communicative areas. Moreover, it is well known that communication disorders play a very significant role in the occurrence of behavioural disorders such as oppositional and withdrawal traits, in line with our results showing a significant ASD symptomatology in NIPBL mutated group and in truncating mutations group. These findings guide us to consider the communicative aspect as one of the main intervention priorities in these patients. As in the case of any child with developmental delay, early intervention on communication and language is highly recommended to produce favorable outcomes on development and reduce the risk of Behavioural disorders. Early Augmentative and Alternative Communication (AAC) interventions and speech therapy are therefore of paramount importance from the outset to prevent the onset and aggravation of challenging Behaviours [Kline et al., 2018]. Finally, particular attention is paid to motor skills, which are more compromised in patients with NIPBL Truncating mutation involving motor delay, clumsiness and coordination impairment. These data are consistent with literature that describes major limb defects in individuals with truncating NIPBL variants [Selicorni et al., 2007; Gills et al., 2004; Mehtha, 2016]. These

malformations have a great impact on motor and adaptive skills. Early neuromotor rehabilitation is a priority for all CdLS children, and above all for patients with NIPBL truncating variants.

Conclusion

In this study we delineated in detail the neurodevelopmental and behavioural phenotype of individuals with CdLS with a standardized and tailored assessment. Furthermore, although truncating mutations were generally found in literature to cause a more severe phenotype, our findings allow us to define two different neuropsychiatric phenotypes: truncating and Missense phenotype with specific strengths and weaknesses. The results allow the clinicians to develop a tailored and effective management with a preventive approach and rehabilitative priorities.

Abbreviations

CdLS: Cornelia de Lange syndrome; IQ: Intelligence quotient; ID: intellectual disability; GMDS II: Griffiths' Mental Developmental Scale II/III); VABS II: Vineland Adaptive Behaviour Scale II; CBCL: Child Behaviour Checklist 1,5-5 anni; CARS: Childhood Autism Rating Scale.

Declarations

Ethical approval and Consent to participate

The present study was approved by the Ethics Committee of Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy and was performed in accordance with the Declaration of Helsinki (1964). Written Informed Consent was obtained from parents/legal tutors, following a full explanation of the procedures undertaken.

Consent for publication

The manuscript does not contain data from any individual person. Consequently, consent for publication is not applicable.

Availability of data and materials

At present, the datasets generated and analyzed during the current study are not publicly available due to confidentiality constraints within our ethical approvals.

Competing interests

The authors declare that they have no competing interest.

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The authors have not received specific funding for this work.

Authors' contributions

PA provided substantial contributions to the design and drafted the work, BA, GM, AC, FD, CR, MM, PV provided substantial contributions to the conception and design of the work, provided substantial contributions to the analysis and interpretation of data, and revised the work critically for important intellectual content. AS and AC

revised the work critically for important intellectual content and provided final approval of the version to be published.

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Figures

Figure 1

CARS scores between CdLS NIPBL mutated group and CdLS clinical diagnosis group