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Audiological findings, genotype and clinical severity score in Cornelia de Lange syndrome

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ABSTRACT

Objective: Cornelia de Lange syndrome (CdLS) is a rare multisystem disorder in which hearing loss (HL) has been reported. However, no data are available concerning the association between audiological findings, clinical severity score and genotype.
Methods: The study involved 44 pediatric patients aged 1–18 years with a confirmed diagnosis of CdLS, all of whom underwent a full otolaryngological and audiological examination. The presence of *NIPBL* and *SMC1* mutations was also evaluated.
Results: According to the severity of clinical phenotypes, 12 (27.3%) children were mild, 15 (34.1%) were moderate and 17 (38.6%) were severe. Thirty-eight children (86%) had OME. Eight children had normal hearing, including one (12.5%) with a severe phenotype. Bilateral sensorineural hearing loss (SNHL) was diagnosed in 10 children (22.7%): the degree of HL was severe in 8 (80%), all with a severe phenotype. Conductive hearing loss (CHL) was present in 26 patients (59.1%), of whom 8 (30.8%) had a severe phenotype. A severe phenotype was more prevalent among the patients with moderate to severe HL (10/16, 62.5%) than among those with slight/mild HL or normal hearing (7/28, 25.0% $p = 0.013$). *NIPBL* mutations were detected in 22 patients (50%): 13 (59.1%) with truncating mutations, four (18.2%) with missense mutations, and five (22.7%) with splicing mutations. The frequency of *NIPBL* truncating mutations was similar in the children with SNHL and those with CHL, whereas this kind of mutation was not found in children with normal hearing.
Conclusion: Together with SNHL, CHL is an important cause of HL in children with CdLS, and can be associated with a severe phenotype. Moreover, CHL can be associated with *NIPBL* mutations, particularly truncating mutations. These results highlight the importance of the early identification of audiological problems in children with CdLS and their precise genetic characterization.

1. Introduction

Cornelia de Lange syndrome (CdLS) is a multiple congenital anomaly/mental retardation syndrome characterized by a distinctive facial appearance, pre- and post-natal growth retardation, hirsutism, small hands and feet or, more rarely, severe limb anomalies. Other findings include congenital heart defects, renal malformations, genital hypoplasia, orthopedic problems, cleft palate, gastrointestinal reflux, severe myopia worsened by eyelid ptosis, various degrees of language delay, and behavioral and psychiatric problems [1–9]. Most CdLS patients have hearing impairments due to sensorineural hearing loss (SNHL), conductive hearing loss (CHL) secondary to persistent otitis media with effusion (OME), or both [10]. The combination of all these clinical signs and symptoms varies significantly among affected individuals, and leads to various phenotypes ranging from relatively mild to severe disease [1].

CdLS is referred to as a cohesinopathy as it is caused by alterations in genes involved in the proper interaction of the cohesin complex, including *NIPBL*, *PDS5* (*i.e.* coding cohesion regulators) and *SMC1*, *SMC3*, *RAD21* (*i.e.* coding proteins of the

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core of the cohesin complex) [11]. Mutations in the *NIPBL* gene are the most frequent and found in about 50% of CdLS patients [12–15]. Most of these cases are severe [12], whereas CdLS patients with mutations in other genes usually have less severe phenotypes [16–18].

However, the prevalence of a number of features of CdLS in the different phenotypes and their associations with specific genetic variations are not precisely defined, although such information could not only lead to the better characterization of the phenotypes themselves, but also a more appropriate approach to treatment. This is the case of OME, which can be clinically relevant *per se* because it can cause significant CHL and worsen SNHL, defer language development, worsen neurological development, and hamper social relationships [19]. The diagnosis and appropriate treatment of OME could significantly reduce phenotype severity.

The aim of this study was to evaluate the association between audiological findings, genotype and clinical severity score in pediatric patients with CdLS.

2. Methods

Children regularly attending the pediatric outpatient clinic of Milan University's Department of Pediatrics because of CdLS diagnosed by means of clinical signs and symptoms and genetic tests during the period 2004–2009 were considered eligible for the study. The study protocol was approved by the Ethics Committee of the University of Milan, and written informed consent was obtained from a parent or legal guardian of the participants.

At the time of enrolment in 2010–2011, the children's demographic and clinical characteristics were collected by means of a questionnaire completed by trained residents in the presence of parents, and integrated with information taken from the children's medical records. Particular attention was given to the results of genetic and developmental tests undergone before enrolment: the enrolled children had to have undergone tests for the detection of the *NIPBL* and *SMC1A* genes, and had to have been tested using the Wechsler Preschool and Primary Scale of Intelligence [WPPSI] or Leiter's International Performance Scale (if 2–6 years old), or the Wechsler Intelligence Scale for Children-Revised [WISC-R] if older. In order to considered valid for the study, a developmental test should have been undergone within the previous six months in the case of children aged <7 years or within the previous 12 months in the case of older children.

The CdLS clinical severity score of each patient was calculated using the system of Kline et al. [1], which considers seven features that are frequently abnormal in CdLS patients (birth weight; age at the time of first sitting alone, walking alone, and saying first words; the presence of upper limb malformations; the presence and number of other major malformations; and SNHL), and assigns a score of 1–5 to each: the higher the score, the more severe the abnormality. The children with a total score of <15 were considered as having mild disease; those with a score of 15–22 were considered as having moderate disease; and those with a score of >22 were considered as having severe disease.

After enrolment, all of the children underwent a physical examination of the nose and throat, and pneumatic otoscopy of both ears performed by a validated otoscopist (PM) using an airtight lens assembly (Model 20200, Welch Allyn Inc., Skaneateles Falls, NY). Cerumen was removed as necessary. Middle ear immittance (*i.e.* admittance or impedance) was evaluated by means of a set immittance measurements made using a middle ear analyser (Amplaid 724, Amplifon), the calibration of which was checked on a regular basis and always before an appointment. Immittance tympanometry at 226 Hz was used to measure tympanometry peak pressure (in daPA), static admittance (in mmho), gradient, and estimated ear canal volume. We used Jerger's classification in which middle ear pressure is >100 daPa in the A-type curve, <100 daPa in the C-type curve, and flat in the B-type curve. OME was defined as middle ear effusion (impaired mobility, opacification, fullness or retraction of the eardrum) associated with a tympanogram with a flat tracing (type B), and the absence of signs and symptoms of acute infection. Ipsilateral acoustic stapedius reflex (ASR) thresholds were elicited using a broadband noise stimulus going from 85 dB to 115 dB. At family conferences, the stapedial reflex was used as an indirect sign of hearing level: as 90% of normal healthy children of the same age have a positive stapedial reflex at 90 dB, the absence of a stapedial reflex at 115 dB correlates with a HL of at least 25 dB [20]. In cooperative children aged >5 years without severe mental retardation or aggressive behavior, air conduction pure-tone thresholds at 0.5, 1, 2 and 4 kHz were measured in each ear using a conventional clinical audiometer (Amplaid 171, Amplifon, Italy), and average thresholds were calculated. Bone conduction was tested when the air conduction thresholds exceeded a HL of 15 dB.

The degree of HL was categorized as slight (21–25 dB), mild (26–40 dB), moderate (41–65 dB) or severe (65–90 dB HL). All of the audiometric tests were conducted and interpreted by two of us (SB and EB), who were blinded to the previous otoscopic and tympanometric findings.

The data were recorded on pre-coded forms and analyzed using the SPSS program (SPSS Inc., Chicago, IL). The quantitative variables were compared using Student's test. The categorical data were expressed as numbers and percentages, and compared using the chi-square test with Yates' correction for 2×2 or 2×3 contigency tables; if the sample was too small, Fisher's exact *t* test was used. All of the *p* values are two-sided, and a *p* value of <0.05 was considered statistically significant.

3. Results

The study enrolled 44 children (50% males) aged 1–17 years (median age 7.8 years) with documented CdLS: 12 (27.3%) with mild, 15 (34.1%) with moderate, and 17 (38.6%) with severe disease. Genetic tests were positive for *NIPBL* gene mutations in 22 children (50.0%): 13 (59.1%) with truncating mutations, four (18.2%) with missense mutations, and five (22.7%) with slicing mutations. No mutations in *SMC1L1* gene were found. Thirty-eight children (86%) had OME, which was bilateral in all but one.

Table 1 shows the type and degree of HL by clinical phenotype severity. Eight children had normal hearing, including one (12.5%) with a severe phenotype; two of these children had OME. Bilateral SNHL was diagnosed in 10 children (22.7%): the degree of HL was severe in 8 (80%) and in these cases, all with a severe phenotype, OME was present therefore causing mixed hearing loss. CHL was identified in 26 patients (59.1%), of whom 8 (30.8%) had a severe clinical phenotype; all of them had also OME. A severe phenotype

Table 1
Type and degree of hearing loss (HL) and severity of clinical phenotype in children with Cornelia de Lange syndrome.

Results	Number of patients ($n=44$)	Mild phenotype ($n=12$)	Moderate phenotype ($n=15$)	Severe phenotype ($n=17$)
Normal hearing	8 (18.2%)	5 (62.5%)	2 (25.0%)	1 (5.9%)
Moderate/severe HL	16 (36.4%)	3 (18.7%)	3 (18.7%)	10 (62.5%)
SNHL	8 (18.2%)	0	0	8 (100%)
CHL	8 (18.2%)	3 (37.5%)	3 (37.5%)	2 (25.0%)
Slight/mild HL	20 (45.4%)	4 (20%)	10 (50.0%)	6 (30.0%)
SNHL	2 (4.5%)	0	2 (100%)	0
CHL	18 (40.9%)	4 (22.3%)	8 (44.4%)	6 (33.3%)

SNHL: sensorineural hearing loss; CHL: conductive hearing loss. $p=0.013$ *vs.* mild/moderate hearing loss in patients with a severe phenotype.