Cornelia DE Lange Syndrome: A Case Report of a New Genetic Variant

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Abstract: We describe a clinical case of Cornelia de Lange syndrome (CDLs) diagnosed prenatally and present the pathological and cytogenetic findings.

Our observations from the pathological analysis, including upper limb hemimelia, short upper limbs, microretrognathia, and hypospadias, were compatible with CDLs. The cytogenetic study revealed a normal karyotype with a series of polymorphisms without clinical relevance according to current studies and a heterozygous duplication encoding a nonsense mutation in the *NIPBL* gene that resulted in a truncated protein lacking 38% of its amino acids. This duplication has not been previously described in any database or literature available to date.

Keywords: Cornelia de Lange syndrome, NIPBL gene, Congenital disorder, Multiple malformation.

INTRODUCTION

Cornelia de Lange syndrome (CDLs) is a multiple malformation congenital disorder [1] that affects the cognitive and physical development of afflicted infants. CDLs is characterised by facial alterations associated with late pre- and postnatal development, varying degrees of intellectual disability, and in some cases abnormal upper limbs [2]. Many of the clinical features are apparent at birth or at an early age. The exact incidence of this syndrome is unknown, but it is estimated to occur with a frequency of 1:10,000-30,000 with no differences between genders.

In 1933, two unrelated cases of infants bearing similar traits were reported by the Dutch paediatrician Cornelia de Lange who is recognised as having formally described the features comprising this syndrome for the first time. It is also known as Brachmann-de Lange syndrome because W. Brachmann [3] described similar findings in another patient in 1916. CDLs is a congenital syndrome. Although patients do not always show all of the features of this disorder, the presence of sufficient clinical characteristics usually enables a correct diagnosis.

Concerning the genetics of the syndrome, most cases are the result of spontaneous mutations. However, the affected genes causing the disease can be inherited from both progenitors and is what makes this syndrome an autosomal dominant disease.

Mutations in the *NIPBL*, *SMC1A*, and *SMC3* genes have been associated with CDLs.

A mutated *NIPBL* gene (Cohesin Loading Factor, locus 5p13-14) was discovered through research at the Children's Hospital of Philadelphia in 2004; with 47 exons, this gene presents isoforms with 2804 or 2697 amino acids and has been associated with approximately half of the CDLs cases. In 2006, Italian scientists reported a second gene (*SMC1A*, locus Xp11.2) linked with this syndrome. The heterogeneity of this syndrome is associated with mutations in other genes, including *SMC3*, *RAD21*, and *HDAC8* [4-7].

CDLs cases associated with *SMC1A* mutations exhibit an X-linked inheritance pattern [8]. Other studies concerning X-linked CDLs indicate that a single copy of the mutated gene per cell is sufficient to cause this disease. Unlike most X-linked conditions in which men are more frequently afflicted or experience more severe symptoms, X-linked CDLs seems to affect both genders equally.

Mutations in these genes related to the cohesin complex are responsible for compromised chromosome segregation during cell division [9, 10].

Most cases are the result of new genetic mutations occurring in individuals without a familial history of CDLs.

Currently, approximately 50% of patients with a compatible CDLs phenotype do not show mutations in any of the known associated genes.

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This study describes a case of CDLs associated with a mutation in the *NIPBL* gene that has not previously been described or linked with CDLs.

CASE PRESENTATION

A 31-year-old female with two previous pregnancies, the first of which was from a different partner, presented to the clinic for a routine first trimester pregnancy evaluation. No pathological signs were detected in the ultrasound, the serological test was normal, and the malformation screening was assessed as low risk. In the following visit, the 20-week ultrasound showed a 19-week-old male foetus with clear morphological alterations [*i.e*., affected distal upper limbs (right upper limb: short humerus below the 5th percentile with rudimentary forearm and hand; left upper limb: short humerus below the 5th percentile with forearm agenesis and rudimentary hand with ectrodactyly) and abnormal profile (microretrognathia, prominent upper lip and prefrontal oedema, discreet pericardial haemorrhage, and chordee suggesting hypospadias)] (Figure **1**). These observations were confirmed in subsequent examinations.

Once a diagnosis of skeletal abnormalities (bilateral hemimelia and microretrognathia) was made, the patient was informed of the neonatal prognosis. After a second opinion confirming the ultrasound findings was obtained, the patient opted for a legal termination of pregnancy four days after the initial diagnosis. A cytogenetic study on amniotic fluid samples obtained by amniocentesis was performed prior to pregnancy termination.

DIFFERENTIAL DIAGNOSIS

The foetus and placenta were analysed by the Department of Anatomy and Pathology of our hospital. The samples obtained for the cytogenetic study were sent to our reference laboratory.

For the genetic study of CDLs, a comparative genomic hybridisation analysis (CGH) was conducted as follows. Genomic DNA was extracted from amniotic fluid samples using a MagNA Pure instrument (Roche), followed by specific amplification of 48 fragments in the coding and flanking intronic sequences of the *NIPBL* gene (NM_133433.2 GenBank); exons 21, 33, and 41 were not included in the analysis. The PCR amplicons were sequenced in an ABI 3130 Genetic Analyzer (Applied Biosystems). The sequences obtained were analysed using the SeqScape v2.5 software and compared against multiple databases.

The pathological study results showed a 284 g foetus with a crown-rump length of 16 cm and foot length of 3 cm. The foetus had bilateral palpebral oedema, severe retrognathia, phocomelia-type malformation of the upper limbs with rudimentary

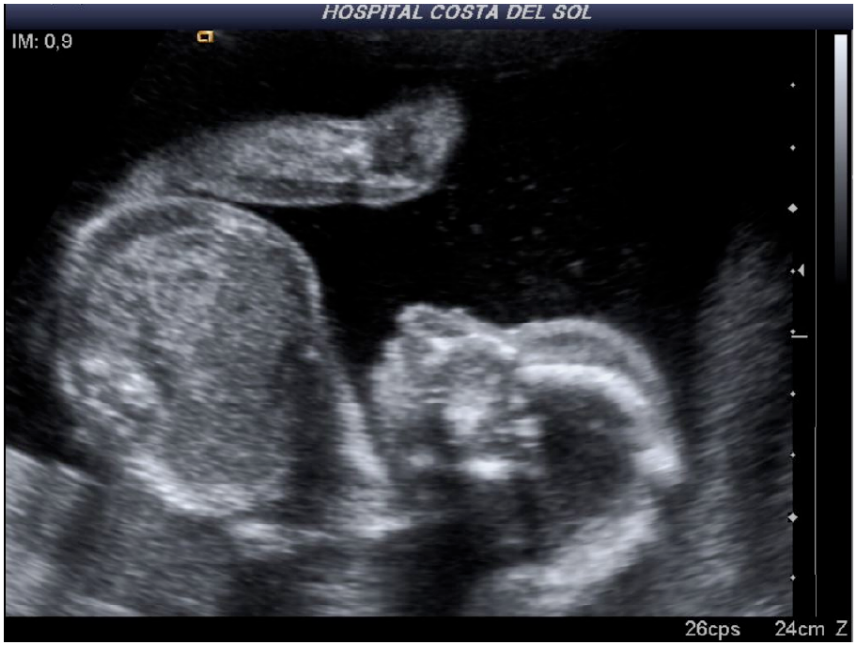

Figure 1: Facial alterations observed in the 20-week ultrasound.

forearms and hands, and hypospadias. No macroscopically evident internal malformations were observed. The trivascular umbilical cord was 4 cm in length. The placenta was normal. The final pathology report concluded that these foetal alterations could be grouped within cohesinopathies, which include CDLs.

The cytogenetic study at a 400-band resolution did not show any chromosomal anomaly. A male chromosome formula (46, XY) was observed.

A comparative genomic hybridisation analysis (Array CGH 60K, directed at the genomic analysis of *NIPBL*-related CDLs) (Figure **2**) was performed using amniotic fluid samples. The sequencing results revealed the heterozygous duplication c.5249dupA, which encoded a stop codon at p.Tyr1750Term (Y1750X). This duplication results in a truncated protein lacking 38% of the amino acids. At the time of writing this manuscript, this duplication has not been described elsewhere. These results do not exclude variations in the *NIPBL* gene, such as mutations outside the analysed region or mutations undetectable by DNA sequencing.

Gene *NIPBL* (OMIM: 608667) and locus 5p13.2 (OMIM phenotype: 122470) were analysed. The mutations exhibited autosomal dominant inheritance.

Additionally, a series of clinically irrelevant polymorphisms, according to our reference laboratory, were found.

DISCUSSION

We describe a new mutation in the *NIPBL* gene found during the study of a case of CDLs.

Genetic mutations in the *NIPBL* gene have been identified in more than half of the afflicted population, whereas mutations in other genes involved are less frequent [11]. The proteins produced by these genes play important roles in foetal development. Within the cells, these proteins assist in regulating the chromosome structure and organisation and are involved in DNA repair. These genes also regulate the activity of certain genes that participate in the development of the limbs, face, and other parts of the body.

The *NIPBL* gene, which contains 47 exons and is 188 kb in size, has at least 43 polymorphisms that are not associated with pathological phenotypes. This gene is located in 5p13-14 and exhibits high allelic heterogeneity (199 allelic variants in 246 patients associated with 65% of CDLs cases).

To date, 144 different mutations affecting ~45% of patients have been reported. In our patient, we found a

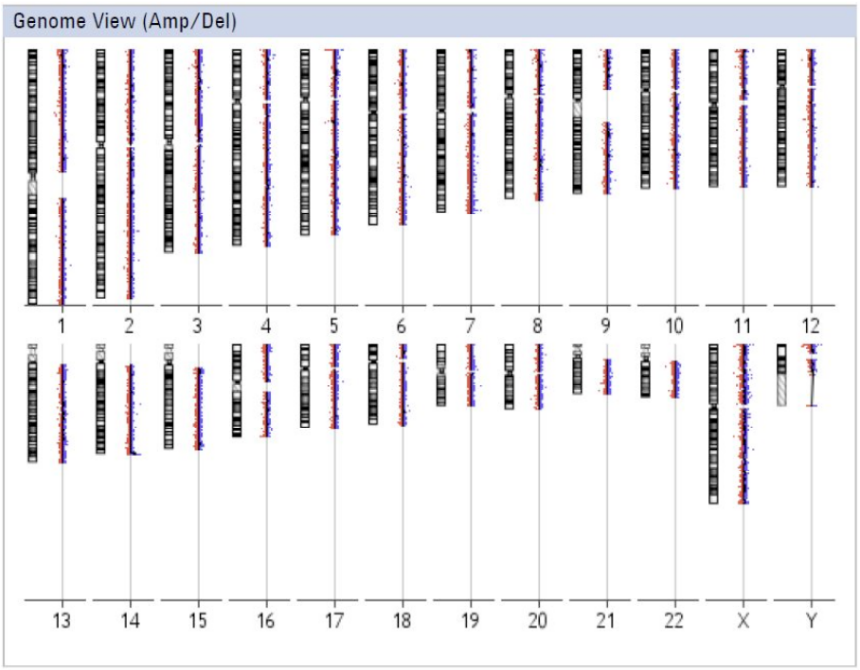

Figure 2: Karyogram using an array CGH. Blue represents gains (**right side**), and red represents losses (**left side**).

heterozygous duplication c.5249dupA encoding stop codon p.Tyr1750Term (Y1750X). These mutations result in a frameshift in 34.5% of cases, a missense mutation in 23.6% of cases, nonsense mutations (such as our clinical case) in 18.8% of cases, effects on splicing sequences in 16% of cases, and other effects in 6.2% of cases.

The positions of these mutations within the gene showed a peculiar distribution. Point and missense mutations were concentrated towards the HEAT (helix-turn-helix) repeat region in the C-terminus (43.3%), whereas the more severe nonsense mutations were more frequent towards the N-terminus. Curiously, the same phenotype is not always observed in patients with the same mutation, and occasionally, large phenotypic differences are found. Therefore, the existence of additional influencing factors (whether genetic or not) in the aetiology of this syndrome has been suggested.

The *NIPBL* gene encodes a protein known as Nipped-B-Like, which has a long isoform composed of 2804 amino acids and a short form composed of only 2697 amino acids. The more severe phenotypes of this disease are linked with nonsense mutations. Nipped-B-Like plays an important role in growth and development processes. During the prenatal stage, the protein is located in the developing limbs, cranial and facial bones, spinal column, heart, and other tissues [12]. This protein is also involved in chromosome migration during cell division and controls the interaction of the cohesin-DNA complex [13]. CDLs is characterised by a precocious separation of sister chromatids. In our case, the foetus showed severe retrognathia, phocomelia-type malformation of the upper limbs with rudimentary forearms and hands, and hypospadias. Anatomic abnormalities in the brain and central nervous system specific to CDLs have been observed and were correlated with changes in MRI images [14].

The precise cause of CDLs is unknown in approximately 35% of cases. Researchers are seeking additional modifications in the *NIPBL*, *SMC1A*, and *SMC3* genes as well as mutations in other genes that may be responsible for this condition.

Therefore, CDLs is a heterogeneous genetic disorder, which explains the diversity in the expression of its clinical phenotype.

REFERENCES

[1] Kline AD, Krantz ID, Sommer A, Kliewer M, Jackson LG, FitzPatrick DR, *et al*. Cornelia de Lange syndrome: clinical review, diagnostic and scoring systems, and anticipatory guidance. Am J Med Genet A 2007; 143A(12): 1287-96. https://doi.org/10.1002/ajmg.a.31757

[2] Clark DM, Sherer I, Deardorff MA, Byrne JL, Loomes KM, Nowaczyk MJ, *et al*. Identification of a prenatal profile of Cornelia de Lange syndrome (CdLS): a review of 53 CdLS pregnancies. Am J Med Genet A 2012; 158A(8): 1848-56. https://doi.org/10.1002/ajmg.a.35410

[3] Mei L, Liang D, Huang Y, Pan Q, Wu L. Two novel NIPBL gene mutations in Chinese patients with Cornelia de Lange syndrome. Gene 2015; 555(2): 476-80. https://doi.org/10.1016/j.gene.2014.11.033

[4] DeScipio C, Kaur M, Yaeger D, Innis JW, Spinner NB, Jackson LG, *et al*. Chromosome rearrangements in cornelia de Lange syndrome (CdLS): report of a der (3) t (3; 12) (p25.3; p13.3) in two half sibs with features of CdLS and review of reported CdLS cases with chromosome rearrangements. Am J Med Genet A 2005; 137A(3): 276-82. https://doi.org/10.1002/ajmg.a.30857

[5] Deardorff MA, Bando M, Nakato R, Watrin E, Itoh T, Minamino M, *et al*. HDAC8 mutations in Cornelia de Lange syndrome affect the cohesin acetylation cycle. Nature 2012; 489(7415): 313-7. https://doi.org/10.1038/nature11316

[6] Mannini L, Cucco F, Quarantotti V, Krantz ID, Musio A. Mutation spectrum and genotype-phenotype correlation in Cornelia de Lange syndrome. Hum Mutat 2013; 34(12): 1589-96. https://doi.org/10.1002/humu.22430

[7] Decroos C, Bowman CM, Moser JA, Christianson KE, Deardorff MA, Christianson DW. Compromised structure and function of HDAC8 mutants identified in Cornelia de Lange Syndrome spectrum disorders. ACS Chem Biol 2014; 9(9): 2157-64. https://doi.org/10.1021/cb5003762

[8] Kaiser FJ, Ansari M, Braunholz D, Concepción Gil-Rodríguez M, Decroos C, Wilde JJ, *et al*. Loss-of-function HDAC8 mutations cause a phenotypic spectrum of Cornelia de Lange syndrome-like features, ocular hypertelorism, large fontanelle and X-linked inheritance. Hum Mol Genet 2014; 23(11): 2888-900. https://doi.org/10.1093/hmg/ddu002

[9] Kline AD, Calof AL, Lander AD, Gerton JL, Krantz ID, Dorsett D, *et al*. Clinical, developmental and molecular update on Cornelia de Lange syndrome and the cohesin complex: abstracts from the 2014 Scientific and Educational Symposium. Am J Med Genet A 2015; 167(6): 1179-92. https://doi.org/10.1002/ajmg.a.37056

[10] Dorsett D. Cohesin: genomic insights into controlling gene transcription and development. Curr Opin Genet Dev 2011; 21(2): 199-206. https://doi.org/10.1016/j.gde.2011.01.018

[11] Pié J, Gil-Rodríguez MC, Ciero M, López-Vi-as E, Ribate MP, Arnedo M, *et al*. 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; 152A(4): 924-9. https://doi.org/10.1002/ajmg.a.33348

[12] Oliver C, Bedeschi MF, Blagowidow N, Carrico CS, Cereda A, Fitzpatrick DR, *et al*. Cornelia de Lange syndrome: extending the physical and psychological phenotype. Am J Med Genet A 2010; 152A(5): 1127-35. https://doi.org/10.1002/ajmg.a.33363

[13] Bose T, Gerton JL. Cohesinopathies, gene expression, and chromatin organization. J Cell Biol 2010; 189(2): 201-10. https://doi.org/10.1083/jcb.200912129

[14] Roshan Lal TR, Kliewer MA, Lopes T, Rebsamen SL, O'Connor J, Grados MA, *et al*. Cornelia de Lange syndrome: Correlation of brain MRI findings with behavioral assessment. Am J Med Genet C Semin Med Genet 2016; 172(2): 190-7. https://doi.org/10.1002/ajmg.c.31503

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