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Classic Cornelia de Lange syndrome with variant of unknown significance detected in *NIPBL* gene mutation: a case report

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Abstract

Background: Cornelia de Lange syndrome is a relatively uncommon disorder associated with multiple congenital anomalies/mental retardation of unknown etiology with its incidence varying from 1:10,000 to 1:50,000 live births in different population groups without any known racial predilections. Main clinical features of this syndrome consist of distinctive dysmorphic facial appearance, growth retardation, developmental delay, mental retardation, hirsutism, and skeletal formation anomaly.

Case presentation: This case presents a variation of unknown significance in the *NIPBL* gene-exon 39, chr5: 37048649T>A c.6635T>A (p.Val2212Glu) with clinical phenotype of Cornelia de Lange syndrome. Our patient belonged to South Indian origin with clinical features of synophrys, micrognathia, long smooth philtrum, and clinodactyly with bilateral simian crease.

Conclusion: Cornelia de Lange syndrome is a rare but well-characterized disorder, in which multiple systems of the body are affected. It is important that the treating physician ensures coordination of the diversiform aspects of care in both childhood and adulthood. Proper and timely diagnosis using next generation sequencing helps in management and possibility of prenatal diagnosis.

Keywords: Cornelia de Lange syndrome, Developmental delay, Dysmorphism, *NIPBL*, Missense mutation

Background

In 1916, Dr. W. Brachmann described the first case of this syndrome but in 1933 Cornelia de Lange, a Dutch pediatrician from Amsterdam was the first to report about two cases of this syndrome [1]. It is also called Brachmann de Lange syndrome, Amsterdam dwarfism, Bushy syndrome. There is variable incidence of this syndrome ranging from 1:10,000 to 1:50,000 live births with no racial predilection [2]. It is slightly more commonly seen in females when compared to males (F/M: 1.3/1). Most children with this syndrome cannot survive beyond 2 years. Pneumonia along with cardiac, respiratory, and gastrointestinal abnormalities is the main cause of death in these patients [2].

Cornelia de Lange syndrome (CDLS) is a multisystem developmental disorder with dysmorphic faces being the most diagnostic feature characterized by microcephaly, bushy eyebrows with synophrys, long eyelashes, hirsutism, smooth long philtrum, low set ears, thin lips, carp shaped mouth, and flared nostrils. Other clinical features are low birth weight, failure to thrive, prenatal and postnatal growth, mental and developmental retardation, learning difficulties, and failure to thrive [3, 4].

The overall CDLS phenotype involves a spectrum which includes the classic CDLS, non-classic CDLS as well as other syndromes phenotype (Coffin-Siris syndrome, Rubinstein-Taybi syndrome, Nicolaides–Baraitser syndrome) with almost identical clinical

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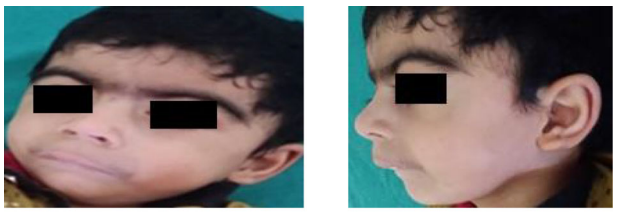
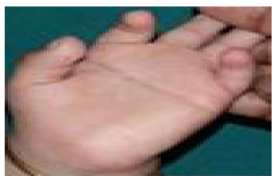


A B

Images A-B showing synophrys, thick eyebrows, long smooth philtrum, thin upper lip vermilion and micrognathia.



C

Image C showing simian crease, clinodactyly and brachydactyly.

Fig. 1 Clinical findings of the patient

features [5]. CDLS is caused by pathogenic variants in genes involved in cohesion functioning. As per Kline et al. [5], till date, there is no individual with a classic CDLS phenotype in whom a variant gene without cohesion function has been definitely shown to be causative. All recognized, established, and obvious causes of CDLS can thus be labeled as cohesinopathies but not all cohesinopathies result in CDLS [5]. Most of the cases with CDLS are usually sporadic and 10% of total cases have chromosomal alterations like unbalanced chromosomal rearrangements [6], duplications, or partial trisomy of chromosome 3q26-27 [7]. Mutation in the Nipped-B-Like (*NIPBL*) gene is the most common and has been diagnosed in 26 to 56% cases as an etiological factor [8]. However, as per the first international consensus statement, first-line molecular diagnostic approach should be next-generation sequencing (NGS)-based screening—either gene panel, whole-exome sequencing (WES) or whole-genome sequencing (WGS)—including currently known CDLS genes (*NIPBL*, structural maintenance of chromosomes 1A (*SMC1A*), structural maintenance of chromosomes 3 (*SMC3*), *RAD21* cohesin complex component (*RAD21*), Bromodomain-containing protein 4, *(BRD4*), histone deacetylase 8 (*HDAC8*), and ankyrin repeat domain 11 (*ANKRD11*) [5].

Here, we present a case of sporadic CDLS and its genetic workup.

Case presentation

A 2-year-old male child of South Indian origin (Kerala) was brought to the Department of Medical Genetics by the parents (Father—32 years/ Mother—28 years) for having progressive growth deficiency,

Table 1 Clinical features of Cornelia de Lange syndrome and our case based on First International Consensus Statement, 2018 [5]

	Our case
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	
• ≥ 11 points, of which at least 3 are cardinal: classic CdLs	**15**
• 9 or 10 points, of which at least 2 are cardinal: non- classic CdLs	
• 4–8 points, of which at least 1 is cardinal: molecular testing for CdLs indicated	
• < 4 points: insufficient to indicate molecular testing for CdLs	

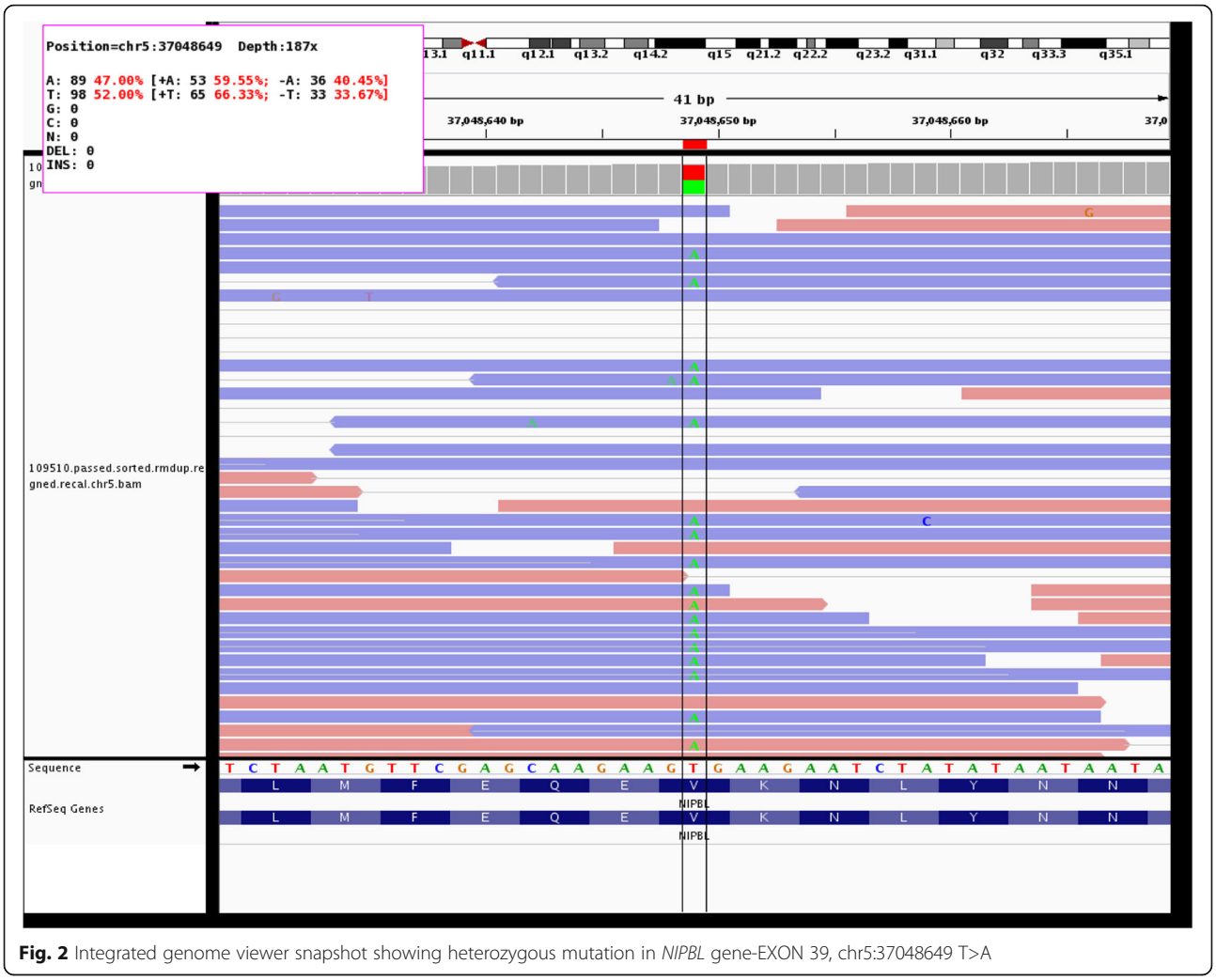


Fig. 2 Integrated genome viewer snapshot showing heterozygous mutation in *NIPBL* gene-EXON 39, chr5:37048649 T>A

childhood developmental delay, and mental retardation. He was the second male child born of a non-consanguineous marriage with uneventful antenatal period. He was born after pre-term caesarean section done at 33 weeks of gestation in view of previous caesarean section in labor (as per the hospital records and the discharge summary) with birth weight of 1.29 kg (small for gestational age) and cried immediately after birth. Their first child was a 5-years-old boy who appeared clinically normal. Both the parents also appeared clinically normal, and there three generations pedigree did not reveal any history of deformity/mental retardation. Anthropometric measurements of the child at presentation were as follows:

Weight: 7.6 kg (below 3rd percentile)
Height: 79 cm (below 3rd percentile)
Head circumference: 43 cm (below 3rd percentile)

Child had the following clinical features on examination:

Global developmental delay, mental retardation, growth retardation, dysmorphic face characterized by bushy eyebrows, dystichiasis, synophrys, long curly eyelashes, broad nasal root with flared nostrils, upturned nose, carp shaped mouth, thin bow-shaped lips with downturned corners, and long smooth philtrum was noted (Fig. 1a, b). The child also had short stubby fingers, bilateral simian crease, bilateral short 5th finger with clinodactyly (Fig. 1c), bilateral partial cutaneous syndactyly of 2nd and 3rd toe fingers, unilateral cryptorchidism, hirsutism, and craniosynostosis. Parents gave history of epilepsy and the child was on multiple antiepileptic medications. Child had not attained walking and sitting and milestones were delayed and mental retardation was seen.

As per the consensus, classic CDLS should have the score of 11 and above with presence of at least 3 cardinal features [5]. Our patient had cardinal features such

Table 2 In-silico predictions (variant analysis report)*

S.No	Variant Annotation Tools	Categorical Description	Database	Result
1.	Sort Intolerated From Tolerated (SIFT)	D: Deleterious (SIFT score ≤ 0.05); T: Tolerated (SIFT score >0.05)	DbSNP	**Deleterious (**SIFT score = 0.01**)**
2.	Polymorphism Phenotyping v2 (Poly Phen v2)	D: Probably damaging (pp2_score ≥ 0.957), P: Possibly damaging (0.453 ≤ pp2_score ≤ 0.956) B: Benign (pp2_score ≤ 0.452)	PDB, DSSP, HumDiv, HumVar	**Possibly damaging** (pp2_score = 0.92)
3.	Mutation Taster	A: Disease_causing_automatic; D: Disease_causing; N: Polymorphism [probably harmless]; P: Polymorphism_automatic [known to be harmless]	dbSNP / TGP / ClinVar / HGMD	**Disease causing**
4.	Mutation Assessor	H: High; M: Medium; L: Low; N: Neutral.	OMIM, Uniprot, Refseq, Pfam, dbSNP	**Medium**
5.	Functional Analysis Through Hidden Markov Models (FATHMM V2.3)	D: Deleterious (FATHMM score > 0.5); T: Tolerated (FATHMM score < 0.5);	HGMD, SwissProt/TrEMBL	**Deleterious** (FATHMM_score = 0.99418)
6.	Combined Annotation Dependent Depletion (CADD)	Pathogenic ; (>20) Likely pathogenic; (>15) Likely benign; (<15) Benign (<10)	1000 Genomes, Ensembl	**Pathogenic** (CADD_Score = 28.2)
7.	Mendelian Clinically Applicable Pathogenicity (M-CAP)	Pathogenic; (>0.025), Benign (<0.025)	Ensembl	**Pathogenic** (M-CAP_score = 0.721)
8.	Likelihood Ratio Test (LRT)	D: Deleterious; N: Neutral; U: Unknown	HGMD	**Deleterious**
9.	**Pro**tein **V**ariation **E**ffect **An**alyzer (PROVEAN)	Deleterious < -2.5 Neutral > -2.5	Uniprot	**Deleterious** (Provean_score = -4.1)
10.	CONsensusDELeteriousness (Condel)	D: Deleterious (1.0) N: Neutral (0.0)	Ensembl	**Deleterious**
11.	Variant Effect Predictor (VEP)	HIGH; MODERATE; LOW	Ensembl	**HIGH**

Abbreviations: *dbSNP:* Database of Single Nucleotide Polymorphisms, *pp2_score:* PolyPhen2_score, *PDB:* Protein Data Bank, *DSSP:* Dictionary of Secondary Structure in Proteins, *TGP:* Thousand Genome Project, *HGMD:* Human Genome Mutation Database, *OMIM:* Online Mendelian Inheritance In Man, *Refseq:* Reference Sequence Database, *Pfam:* Database of Protein families, *TrEMBL:* Translated European Molecular Biology Laboratory

*Using ACMG Guidelines, 2015 [10]

as synophrys, long smooth philtrum, broad nasal root with upturned nostrils, bow-shaped lips with down turned corners with other suggestive features as mentioned above making the score of 15 (Table 1) which makes it classic CDLS.

In view of dysmorphism and mental retardation, genetic work up was done after obtaining a written informed consent from the parents. The karyotype was 46, XY normal at 500 bands resolution by GTG banding. The parents were offered genetic counseling and were conveyed about the possibility of CDLS based on the clinical features. Child's DNA was isolated from EDTA blood using commercial QIAGENE-QI Aamp-DNA mini kit and was subjected to targeted testing. The following genes were tested by Next generation sequencing: *NIPBL*, *SMC1A*, *SMC3*, *RAD21*, *HDAC8*, and *ANKRD11*. Next-generation sequencing-based screening of known CDLS gene showed a variant of unknown significance (VUS) in *NIPBL* gene. Molecular analysis report of the patient showed a VUS in Nipped-B-like protein (*NIPBL*) gene with heterozygous missense mutation (Fig. 2).

NIPBL gene-exon 39, chr5:37048649T>A

This missense mutation replaces valine at codon 2212 by glutamic acid during transcription.

Although the mutation has been labeled as VUS, our in silico analysis showed it to be deleterious. The in silico predictions of this variant in our laboratory by ACMG (American College of Medical Genetics) guidelines [9, 10] shows it to be damaging by LRT (Likelihood Ratio Test), SIFT (Sort Intolerated From Tolerated), Mutation Taster and probably damaging by Polymorphism Phenotyping v2-Polyphen v2 (Table 2). Our search for the variant in databases like Clinvar, 1000Genome, and ExAC (Exome Aggregation Consortium) did not show any similar mutation. The parents were counseled about the disease condition, the genetic mutation, possible outcome, and prognosis. They were also offered mutation testing to rule out carrier status, and they were found to be negative for the same. Hence, the parents were explained that the mutation was probably a de novo/sporadic mutation with less than 1% recurrence risk although gonadal mosaicism cannot be ruled out.

CDLS is a relatively a rare entity associated with multiple congenital anomalies. Its cause and recurrent risk are unknown. This syndrome could occur due to inherited error of metabolism with no known environmental cause. Though autosomal dominant, autosomal recessive, and chromosomal abnormality have been suggested, most cases are sporadic in nature [1]. The distinct facial characteristics along with the physical features such as pre- and post-natal growth retardation, microcephaly, limb defects, hirsutism, and undescended testis helped in diagnosing CDLS easily in this patient.

Gupta and Goyal in 2005 and Sopori et al. in 2020 have each described a case report of CDLS from India, which was diagnosed based on striking characteristic phenotype and radiological features alone [4, 11]. With the advances in genetic testing, the association of *NIPBL* gene on chromosome 5 as the causative factor for CDLS was discovered followed by addition of other genes like *SMC1A*, *SMC3*, *RAD21*, *HDAC8*, and *ANKRD11* in the list. *NIPBL* gene mutations have been found to be the most common causes of CDLS worldwide and contribute to approximately 50% of all CDLS cases [12]. The genetic knowledge helped in understanding CDLS as a spectrum of disorders involving classic CDLS, non-classic CDLS, and other phenotypes sharing limited signs of CDLS [5].

Conclusion

This case report delineates the importance of genetic testing and correlation of clinical phenotype and molecular genotype. Although this mutation has been reported VUS, the strong clinical features were suggestive of classical CDLS and the re-analysis of VUS mutation *NIPBL* gene-exon 39, chr5:37048649T>A suggests it to be disease causing. This emphasizes the advantage of reporting more number of population specific mutations in database like 1000genome, Clinvar, and ExAC for better understanding of genotype and phenotype.

Hence, it is necessary to have a genetic work-up of the disease by molecular analysis using next-generation sequencing (NGS) which helps in correct identification of mutation and thereby providing accurate results even in rare diseases. This further helps in providing genetic counseling and offering pre-natal diagnosis to anxious couples for future pregnancies.

Abbreviations

CDLS: Cornelia de Lange syndrome; chr: Chromosome; *NIPBL*: Nipped-B-like protein; *SMC1A*: Structural maintenance of chromosomes 1A; *SMC3*: Structural maintenance of chromosomes 3; *BRD4*: Bromodomain-containing protein 4; *RAD21*: *RAD21* cohesin complex component; *HDAC8*: Histone deacetylase 8; *ANKRD11*: Ankyrin repeat domain 11; NGS: Next-generation sequencing; WES: Whole-exome sequencing; VUS: Variant of unknown significance; SD: Standard deviation; ACMG: American College of Medical Genetics; LRT: Likelihood ratio test; SIFT: Sort Intolerated From Tolerated; Polyphen v2: Polymorphism phenotyping v2; ExAC: Exome Aggregation Consortium

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Authors' contributions

J.J.D conceived and designed the study. N.S.B and J.J.D performed clinical assessments, performed the experiments, contributed to data acquisition, analysis, and interpretation. N.S.B and P.S drafted the manuscript. All authors contributed to critical revision of the manuscript for intellectual content and final approval of the manuscript. All authors have read and approved final the manuscript.

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Availability of data and materials
Data can be found in the archives of the Department of Medical Genetics, Lifeline Super Specialty Hospital.

Ethics approval and consent to participate
The present study was approved by the hospital ethical committee and consented for publication (LLH/EC/017/SEP 2020).

Consent for publication
Written informed consent was obtained from the parents of the patient.

Competing interests
The authors declare that they have no competing interests.

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