Genetic characterization of a patient with Cornelia de Lange syndrome with a novel *NIPBL* missense mutation

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Background
Cornelia de Lange syndrome (CdLS) is a rare clinically and genetically heterogeneous disease. Cardinal phenotypic manifestations include specific dysmorphic facial features, growth retardation, intellectual disability, and upper limb anomalies. Mutations in five genes including *NIPBL*, *SMC1A*, *SMC3*, *RAD21*, and *HDAC8* are known to be responsible for the syndrome, with the *NIPBL* gene mutation being the most prevalent (~80%). This study aimed to report the clinical, cytogenetic, and molecular characterization of a patient with CdLS with a heterozygous novel exonic missense mutation of the *NIPBL* gene.

Patients and methods
We have studied a male patient of 9 years and 4 months of age who presented with features suggestive of CdLS. Thorough clinical examination, conventional cytogenetic analysis, and molecular study using direct Sanger sequencing were performed.

Results
Clinical examination favored the diagnosis of CdLS. Conventional cytogenetic analysis revealed a normal 46, XY karyotype, with no evidence of premature sister chromatid separation. Molecular study showed a heterozygous novel exonic missense variant c. 2469G>T; p. (R657I) of the *NIPBL* gene.

Conclusion
A novel heterozygous exonic missense variant c. 2469G>T; p. (R657I) of the *NIPBL* gene was confirmed in our patient with CdLS. The phenotypic severity is probably correlated with the plausible effect of *NIPBL* gene mutation on the protein product rather than the variant type. The adverse effect of *NIPBL* gene mutation on the cohesion process mediated by cohesin complex is controversial.

Keywords:
cohesion, Cornelia de Lange syndrome, craniofacial dysmorphism, *NIPBL* gene, premature sister, premature sister chromatid separation (pscs)

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Introduction

Cohesin is a complex of multiple proteins contributing in various DNA-related processes including sister chromatid cohesion and chromosomal segregation during mitosis (Gelot *et al.*, 2016; Teresa-Rodrigo *et al.*, 2016). Mutations in either the cohesin complex subunits or cofactors are responsible for a syndromic category termed cohesinopathies (Barbero, 2013).

Cornelia de Lange syndrome (CdLs) (MIM; #122470, #300590, #610759, #614701, and #300882) is the most frequent cohesinopathy (Piché *et al.*, 2019), with an estimated prevalence of 1:10 000 live births (Cucco and Musio, 2016).

The condition is characterized clinically by distinctive craniofacial features including microcephaly, synophrys, arched eyebrows, flat nasal bridge, anteverted nares, long smooth philtrum, thin lips, micrognathia, and hirsutism. Growth retardation, intellectual disability, and upper limb anomalies are also cardinal features of this syndrome. Other manifestations, comprising gastroesophageal reflux, congenital heart diseases, and genitourinary problems, are also prevalent among patients with CdLs. (De Lange, 1933; Temtamy and Shoukry, 1975; Jackson *et al.*, 1993; El-Ruby and Temtamy, 1997; Kline *et al.*, 2007; Boyle *et al.*, 2015; Dowsett *et al.*, 2019).

Mutations in five genes encoding subunits of the cohesin complex [*SMC1A* (MIM*300040), *SMC3* (MIM*606062), and *RAD21* (MIM*606462)] and regulatory cofactors [*NIPBL* (MIM*608667) and *HDAC8* (MIM*300269)] are known to be accountable for the emergence of CdLs (Krantz *et al.*, 2004; Musio *et al.*, 2006; Deardorff *et al.*, 2012; Kaiser *et al.*, 2014).

Most molecularly diagnosed patients attain *NIPBL* gene mutation, which represent ~80% (Kaiser *et al.*, 2014; Boyle *et al.*, 2015).

NIPBL (nipped-B-like) gene is located on chromosome 5p13.2. It consists of 47 exons encoding a component of the cohesin complex and acts as a cohesion loader, which enables loading of cohesin ring onto chromatin (Deardorff *et al.*, 2012; Avagliano *et al.*, 2020).

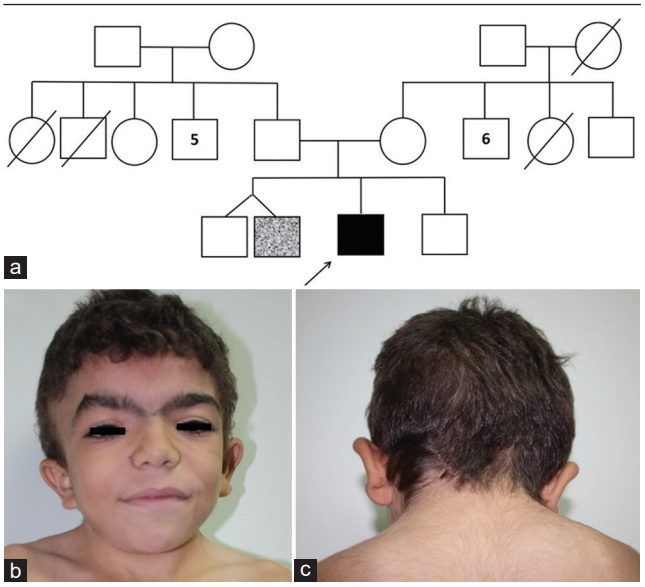
Heterozygous variants in the *NIPBL* gene mostly causes CdLS1 (MIM#122470) (Musio *et al.*, 2006; Rohatgi *et al.*, 2010), which is inherited in an autosomal dominant pattern (Russell *et al.*, 2001; McConnell *et al.*, 2003).

Herein, we report the clinical, cytogenetic, and molecular characterization of a patient with CdLS with a heterozygous novel exonic missense variant c. 2469G>T; p. (R657I) of the *NIPBL* gene. Direct Sanger sequencing has been done for the coding hot spot exons [2, 3, 7, 9 (A, B) 10 (A-F), 22, 35, 42, and 43] according to registered *NIPBL* variants in OMIM database (Online Mendelian Inheritance in Man, ■)

Clinical report

A male patient of 9 years and 4 months of age, presented to the General Genetics Clinic, National Research Centre, Cairo, Egypt, with dysmorphic features, short stature, and failure to thrive. He was the third child of nonconsanguineous parents, with older twin male siblings, one of them had hypospadias and a younger normal male (Figure 1a). Pregnancy history was uneventful, and he was delivered at term via normal vaginal delivery. At birth, the age of his mother and father was 26 and 33 years old, respectively. The infant's birth weight was normal, and the clinical examination revealed hypospadias. Cystourethrogram showed hypospadias with narrow distal urethral opening, which was surgically corrected at the age of 2 years. He had normal motor milestones of development but showed delayed speech. At the time of presentation, the patient showed characteristic craniofacial features that were highly suggestive of CdLS, including microcephaly, low anterior and posterior hair lines, arched eyebrows, synophrys, long eyelashes, low-set posteriorly rotated ears, short broad nose, anteverted nares, long flat philtrum, thin lips, down-turned corners of the mouth, and short neck (Figure 1b and c). Upper limb examination showed bilateral clinodactyly of the little finger, limited elbow extension, dropped shoulders, and winging of scapulae. Lower extremities were normal, and examination of the trunk revealed loss of subcutaneous fat and wrinkled skin, and hirsutism over the back. Cardiac, respiratory, abdominal, and neurological examinations were normal. Genital examination showed a bilaterally palpable gonad, normal penile length of 5 cm, and surgically corrected urethral opening at the penile tip. Anthropometric measurements revealed short stature (−3.8 SD), microcephaly (−2.9 SD), and normal weight (−1.34 SD). Radiography for the left hand and wrist was performed to assess bone age using Greulich and Pyle method and showed a delayed bone age (6 years). Abdominopelvic ultrasound revealed ectopic pelvic right kidney. Echocardiogram was normal. intelligence quotient assessment using Wechsler test was 57, consistent with mild intellectual disability.

Figure 1



The pedigree and dysmorphic features of the studied case. (a) Family pedigree with negative consanguinity, the affected proband, and older brother with hypospadias. (b) Face showing synophrys, arched eyebrows, long eyelashes, broad nose with anteverted nares, long smooth philtrum, thin lips, and low-set ears. (c) Back of head showing low posterior hair line, short neck, and hirsutism over the back.

The study was conducted according to the guidelines of the Medical Research Ethics Committee of the National Research Centre and an informed consent has been taken from the patient's guardians.

Cytogenetic study

Conventional cytogenetic analysis was performed to detect any associated numerical or structural chromosomal aberrations as well as any evidence for premature sister chromatid separation (PSCS), using GTG banding technique (Verma and Babu, 1995). Approximately 50 metaphases have been analyzed, and cytogenetic nomenclature was described following the International System for Human Cytogenomic Nomenclature recommendations (ISCN, 2016). Karyotype revealed a normal 46, XY male karyotype,

and PSCS has not been detected in any of the studied metaphases (Figure 2).

Molecular study

Variant genotyping

The whole coding hot spot exon sequences of *NIPBL* gene, including exons 2, 3, 7, 9 [A, B], 10 (A-F), 22, 35, 42, and 43, were genotyped by direct Sanger sequencing. Sequencing was performed using the BigDye Terminator Cycle Sequencing kit (Perkin-Elmer) on the ABI3730XL sequencer in Macrogen Inc. (Seoul, South Korea) (Supplementary 1).

Direct Sanger sequencing of the screened hot spot sequence showed two distinct variants (Figures 3a, b and 4):

(1) Heterozygous reported intronic variant GRCh37. p13 chr5; NC_000005.9: g. 37052821A>T. It was reported as variant of unknown clinical significance but is not reported in ClinVar. Prediction of its probable pathogenicity was done using MutationTaster; TFBS analysis was done with regulation spotter, SNPnexus tools; and Variant Effect Predictor failed to predict pathogenic effect, and so it is considered to be a polymorphism.

(2) Heterozygous novel exonic missense variant c. 2469G>T; p. (R657I). Prediction of its probable pathogenicity was carried out using NNSPLICE (Reese *et al.*, 1997). Human Splicing Finder (Desmet *et al.*, 2009) showed that this variant is predicted to create a new splicing enhancer region linked to SRp55 protein. Therefore, the R657I variant might produce abnormal spliced mRNA causing the related phenotype. Molecular testing has been requested for the parents and siblings, but unfortunately, they did not show up again.

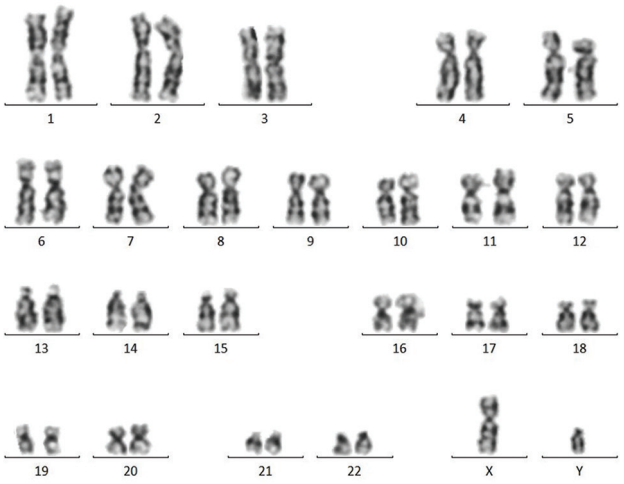
Discussion

CdLS is a rare clinically and genetically heterogeneous disease with a wide range of manifestations and multiorgan involvement; however, the characteristic facial dysmorphism is crucial for making the diagnosis (Van Allen *et al.*, 1993; Gillis *et al.*, 2004; Rohatgi *et al.*, 2010).

In this study, we have encountered a male patient of 9 years and 4 months of age, who presented with distinguished craniofacial features that were highly suggestive of CdLS.

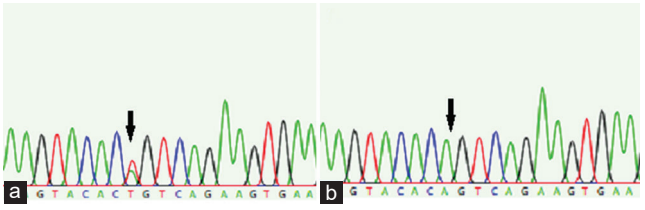
Our patient also showed growth retardation in the form of short stature, microcephaly, and delayed bone age.

Figure 2



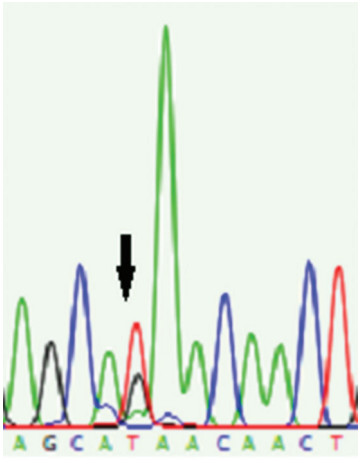
Normal male karyotype (46,XY), with no sister chromatid separation

Figure 3



(a) Partial sequence chromatogram showing heterozygous variant [rs300059, A>T]. (b) Partial sequence chromatogram showing the corresponding wild type.

Figure 4



Partial sequence chromatogram displaying the exon 10 of NIPBL gene for the proband presented R657I variant [G>T substitution].

Retarded growth is considered a cardinal manifestation in this syndrome, (Boog *et al.*, 1999; Boyle *et al.*, 2015). Kline *et al.* (2007) reported that patients with CdLS usually have prenatal as well as enduring postnatal growth retardation. However, our patient had a normal birth weight.

The patient of the current study showed normal motor developmental milestones; however, his speech was delayed. Previous reports concluded that speech and language are the most severely affected fields of development. Approximately 35 and 25% of the patients present with delayed and limited speech, respectively (Sarimski, 1997; Kline *et al.*, 2007).

Intellectual disability is common finding, which has various presentations ranging from mild to severe impairment (Boyle *et al.*, 2015). Assessment of our patient revealed an intelligence quotient of 57 and rated as having mild intellectual disability.

Skeletal abnormalities are prevailing among these patients with extremity involvement in almost all of them, where upper limb is more commonly affected than lower limb. Upper limb anomalies include fifth finger clinodactyly (74% of the patients), limited elbow extension, and dislocation of radial head (64% of the patients). Limb reduction defects have been also described ranging from oligodactyly, ulnar deficiency to absent forearm (Jackson *et al.*, 1993; Kline *et al.*, 2007). The patient of the current study showed bilateral clinodactyly of little finger, limited elbow extension, dropped shoulders, and winging of scapulae, without any affection of lower extremities.

Renal malformations and urinary tract anomalies are frequent, reaching 40%. Approximately 57% of male patients usually experience hypoplastic genitalia and/or cryptorchidism (Borck *et al.*, 2006; Minor *et al.*, 2013). This was consistent with the findings in our patient, who showed ectopic right kidney and hypospadias with a narrow distal urethral opening that was surgically corrected at the age of 2 years.

Although patients with CdLS have numerous phenotypic features in common, patients' presentations usually vary from mild to severe, and detection of the causative gene mutation can give a clue for the genotype-phenotype correlation (Mannini *et al.*, 2013).

Prevalence of *NIPBL* gene mutation among patients diagnosed by molecular techniques is estimated to be ~ 80% (Kaiser *et al.*, 2014; Boyle *et al.*, 2015) and is inherited in an autosomal dominant pattern (Russell *et al.*, 2001; McConnell *et al.*, 2003).

Various studies on human embryonic tissue showed expression of *NIPBL* gene in hand bones as well as the ulnar primordial cartilage. Subsequently, expression of this gene in the craniofacial tissue and spinal column was unveiled, which could rationalize the CdLS phenotype in correspondence to *NIPBL* gene mutation (Barbero, 2013; Zuin *et al.*, 2014).

As open reading frame of *NIPBL* gene starts in exon 2, and continues to exon 47 (Tonkin *et al.*, 2004), our study included hot mutant spot exons and detected a heterozygous reported intronic variant GRCh37.p13 chr5; NC_000005.9: g. 37052821A>T and identified a heterozygous novel exonic missense variant c. 2469G>T; p. (R657I).

Nucleotide alterations of *NIPBL* gene can cause changes in mRNA sequence transcripts and *NIPBL* protein structure and function. RNA sequencing approach could detect abnormal splicing and pathogenic variants among cases suspected to have CdLS (Krawczynska *et al.*, 2019; Rentas *et al.*, 2020). In correspondence to these conclusions and according to Human Splicing Finder (Desmet *et al.*, 2009), pathogenicity of the novel p. (R657I) in our patient possibly resulted via producing abnormal spliced mRNA owing to creation of a new exonic splicing enhancer region linked to SRp55 protein.

The genotype reports of CdLS detected several variants including splice site, frameshift, small in frame deletion, and missense variants (Gillis *et al.*, 2004; Oliveira *et al.*, 2010; Park *et al.*, 2010; Wang *et al.*, 2017).

Splice site, frameshift, and nonsense variants are usually associated with a moderate to severe form of the syndrome. Phenotypic presentation usually includes severe dysmorphic facial features, moderate to severe developmental delay and growth retardation, moderate to profound intellectual disability, major organs anomalies, as well as limb involvement (Gillis *et al.*, 2004; Boyle *et al.*, 2015). Missense variants are generally associated with the milder phenotype, as it is usually characterized by mild intellectual disability, less severe developmental and growth affection, absent major organ anomaly, in addition to intact extremities (Mannini *et al.*, 2013; Krawczynska *et al.*, 2019).

Regarding our patient, apart from ectopic pelvic kidney, the presented phenotype is correlated with the mild phenotype previously described with the missense variant (Table 1). Boyle *et al.* (2015) reported

Table 1 Phenotypic manifestations of our patient compared to common features of patients with missense variants of NIPBL gene as reported by Boyle *et al.* (2015)

Frequent presentations of patients with missense variant of NIPBL gene	Findings in our patient
Facial dysmorphic features	Facial dysmorphic features
Mild cognitive delay	Mild cognitive delay
Mild growth retardation	Mild growth retardation
No limb anomalies	Mild affection in the form of bilateral clinodactyly of little finger and limited elbow extension
No major organ anomalies	Ectopic pelvic right kidney

that phenotypic severity could be adjusted by other factors rather than the variant type, including loss of specific and larger coding sequences in *NIPBL* gene that affect critical domains of the protein as well as other genetic and environmental modifying factors. Moreover, point mutations at consensus 'cis' sequences that determine exon-intron boundaries or at other splicing regulatory sequences can lead to formation of an aberrant transcript and may result in faulty intron removal leading to alterations of the open reading frame (Anna and Monika 2018). These assumptions could justify the increased severity of phenotypic presentation in our patient despite having missense variant.

At the chromosomal level, *NIPBL* gene is required for loading of cohesin complex on chromatin during S-phase, G1, and G2 (Kajii and Ikeuchi, 2004; Deardorff *et al.*, 2012). Cohesin by its turn is involved in chromatid cohesion and precise distribution of chromosomes during cell division (Peters *et al.*, 2008; Jeppsson *et al.*, 2014; De Gabory *et al.*, 2018).

NIPBL gene is the human homolog of the Drosophila Nipped-B gene, which has been detected to be fundamental for sister chromatid cohesion during mitosis. This raised the suggestion that mutation of *NIPBL* gene in human could have a role in defective cohesion, and this defect will consequently lead to PSCS (Rollins *et al.*, 2004).

Kaur *et al.* (2005) studied 90 patients with CdLS and 90 controls. They recognized a statistically significant increase in PSCS among the CdLS patients (41%) compared with the controls (9%). They concluded that perceiving PSCS could support the diagnosis in patients with CdLS when they are either tested negative for *NIPBL* gene mutation or molecular diagnosis is not undertaken.

However, Castronovo *et al.* (2009) in their study found no difference in the frequency of PSCS between the patients and controls and excluded its probable use as an additional diagnostic tool. In conformity to this study, we did not depict PSCS during performing cytogenetic analysis for our patient, and the karyotype showed a normal result (46, XY).

Subsequently, it was suggested that certain level of reduction in *NIPBL* transcription and dysregulation in the function of cohesin complex is required for the evolving of phenotypic abnormalities of CdLS owing to gene expression alteration; however, additional reduction is imperative for the affection of chromosomal segregation and the appearance of defective cohesion (Mannini *et al.*, 2013).

Schwarzer *et al.* (2017) and Avagliano *et al.* (2020) accentuated that the biological mechanism of CdLS may not be directly associated with the disruption of sister chromatid cohesion but to the defective ability of the cohesin complex to regulate other cellular mechanisms including the expression of developmental genes.

Further researches are recommended to clarify the mechanisms of gene expression performed by the cohesin complex and how its dysfunction could contribute in the emerging of CdLS.

Conclusion

In this study, we report a patient with CdLS with heterozygous novel exonic missense variant c. 2469G>T; p. (R657I) of the *NIPBL* gene. The phenotypic severity is probably correlated with the plausible effect of *NIPBL* gene mutation on the protein product rather than the variant type. Genotypic–phenotypic correlation could not be maintained at the cytogenetic level as the role of *NIPBL* gene mutation on the sister chromatid cohesion mediated by cohesin complex is still debatable.

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Conflicts of interest

There are no conflicts of interest.

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