



# **An Autosomal Recessive form of Cornelia de Lange Syndrome Due to Mutations in TRMT61A Gene: A Case Report**

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### **Author's contribution**

*The sole author designed, analyzed, interpreted and prepared the manuscript.*

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**Case Study**

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## **ABSTRACT**

**Background:** Cornelia de Lange syndrome is a rare genetic disorder presenting with craniofacial dysmorphism, developmental delay, intellectual disabilities, upper limb abnormalities and gastrointestinal problems. The disease is genetically heterogenous and the underlying genetic cause is unknown in around 30% of the cases.

**Case Presentation:** A Palestinian family visited our department for genetic counseling. The non-consanguineous parents have only two (4 and 2 years old) daughters, both presented with features consistent with Cornelia de Lange syndrome. Whole exome sequencing revealed two previously unknown coding variants in the autosomal *TRMT61A*, a gene that has not been linked to any human disease before. *TRMT61A* codes for the catalytic subunit of tRNA (adenine-N1-)-methyltransferase. The mother harbored an in-frame deletion variant c.478\_483delCGCACC (p.R160\_T161del), whereas the father carried a missense variant c.323G>T (p.C108F). Both variants are novel and were predicted as "damaging". So far, no autosomal recessive forms of the disease has been described in the literature. The carrier states of the parents and the existence of the two variants in compound heterozygous forms in both girls was further confirmed by allele-specific PCR. In their next pregnancy, prenatal diagnosis indicated that the fetus is a carrier of the mother's variant and the pregnancy culminated in the birth of a healthy baby girl.

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**Conclusion:** It can be concluded that the *TRMT61A* mutations are the cause of the disease features observed in this family and supports the proposal that RNA modification defects are involved in the molecular pathogenesis of Cornelia de Lange syndrome.

*Keywords:* *TRMT61A*; *Cornelia de Lange syndrome*; *gene variants*.

## 1. BACKGROUND

Cornelia de Lange syndrome; CdLS (OMIM: 122470) is a panethnic genetic disorder with an estimated incidence of about 1 per 50,000 in live births [1]. Individuals with CdLS present with characteristic craniofacial features, developmental delay, intellectual disabilities, upper limb abnormalities and gastrointestinal problems [2]. CdLS is genetically heterogenous; it comes about in an autosomal dominant (due to mutations in *NIPBL*, *SMC2*, or *RAD21* genes) or X-linked recessive (mutated *SMC1A* or *HDAC8* genes) form. The majority of the cases (~50%) possess mutation in the *NIPBL* gene. In around 30% of CdLS patients, the responsible genetic cause is unknown [3-7].

*TRMT61A* (GenBank NM\_152307) is a three-exon gene located on chromosome 14q32. The gene codes for the catalytic subunit of tRNA (adenine-N1-)-methyltransferase which catalyzes the formation of N1-methyladenine at position 58 (m1A58) in the initiator methionyl-tRNA (tRNA<sup>iMet</sup>). m1A58 methylation has been shown to be essential for tRNA<sup>iMet</sup> stability and subsequent protein synthesis rate [8]. The enzyme also methylates adenosine residues at the N1 position (m1A) of a small subset of mRNAs. m1A methylation might be important for promoting translation [9].

Studies on the molecular pathogenesis of CdLS in *NIPBL* and *HDAC8* mutant human cells have shown that the disease occurs due to RNA biogenesis defects and small changes in the transcriptional program of a large number of genes [10,11]. Indeed, *TRMT61A* is among the misregulated genes in *NIPBL*- and *SMC1A*-mutated CdLS cells [12].

The aim of this study was to investigate whether mutations in *TRMT61A* are associated with an autosomal recessive form of CdLS.

## 2. CASE PRESENTATION

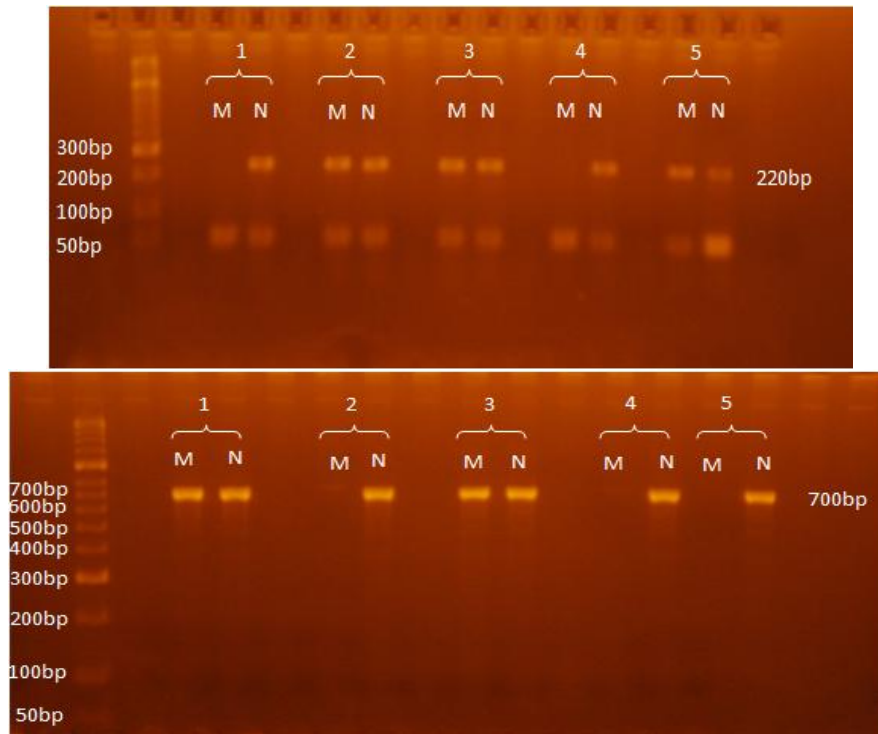
A non-consanguineous Palestinian couple visited our Medical Laboratory Sciences department at

the Islamic University of Gaza for genetic counseling. The healthy parents have only two (4- and 2-years old) daughters with symptoms consistent with CdLS. The features included craniofacial dysmorphism (e.g., microcephaly, synophrys, anteverted nares, and micrognathia), speech and hearing impairment, growth and cognitive retardation, hirsutism, upper limb abnormalities, and gastrointestinal problems. Given that the genetic cause is unidentified in around 30% of CdLS and that the available family information is not in line with an autosomal dominant inheritance pattern (of course the X-linked recessive form is out of question here), the family was advised to undertake whole exome sequencing (WES) testing. The DNA samples of the family were shipped via Hadassah hospital to Otogenetics, USA (CLIA lab) where WES was performed. WES analysis report did not show any remarkable variation in the known CdLS genes. Instead, the report noted that the elder daughter is a compound heterozygous for two previously unknown *TRMT61A* gene variants: the mother's indel variant c.478\_483delCGCACC (p.R160\_T161del) that causes deletion of two amino acids: arginine #160 and threonine #161. The father's missense variant c.323G>T (p.C108F) caused a cysteine (#108) residue mutation into phenylalanine. The potential phenotypic effect of the two variants was evaluated *in silico* using Sorting Intolerant from Tolerant (SIFT) ([http://sift.jcvi.org/www/SIFT\\_BLink\\_submit.html](http://sift.jcvi.org/www/SIFT_BLink_submit.html)) [13] and PROVEAN: Protein Variation Effect Analyzer; (<http://provean.jcvi.org/index.php>) [14] programs. Both programs predicted that the detected variants are functionally damaging. Moreover, both variants were also present in the younger affected daughter.

To discriminate wild-type and mutant alleles, allele specific PCR primers (Table 1) were designed and used to confirm the genotypes of the parents and their two daughters. Indeed, the PCR results validated the family genotypes mentioned above. Moreover, the primers affirmed the absence of the variants from 50 randomly selected healthy individuals from our population.

**Table 1. Allele-specific primers designed for detecting the identified variants**

<i>TRMT61A</i> variant	Allele-specific primers	Product size (bp)	PCR protocol
c.323G>T	Common F: AGCTCCTGGAGAAGGGTGAT G-allele R: CTGAGGAACTCACCAGACTCAC T-allele R: CTGAGGAACTCACCAGACTCAA	701	• 95°C, 2 min • 30 cycles: 95°C, 30 sec 62°C, 30 sec 72°C, 80.0 sec • 72°C, 5 min
c.478_483delCGCACC	Normal F: CTGGGTGACTGTGCGCAC Mutant F: CTGGGTGACTGTGCAGGA Common R: TCTCTGGACCCCTCTGAGAT	226 (normal) 220 (mutant)	• 95°C, 2 min • 30 cycles: 95°C, 30 sec 59°C, 30 sec 72°C, 80.0 sec • 72°C, 5 min



**Fig. 1. Photos illustrating the allele-specific PCR results for the mother (top photo) and father (bottom photo) mutations. The PCR products were electrophoresed on 3% agarose and directly visualized with ethidium bromide under UV light. The bands correspond to 1: Father; 2: Mother; 3: Affected daughter; 4: Normal control; 5: the newborn. Left lane: DNA size markers. M and N denote mutant and normal alleles, respectively**

The parents chose to proceed with prenatal diagnosis for their next pregnancy. DNA extracted from amniocentesis sample indicated that the fetus is a carrier for the mother's mutation and after an uneventful pregnancy a full term healthy baby girl was born. Allele-specific PCR validated the genotype of the newborn daughter. Fig. 1 illustrates the allele-specific PCR results for the parents, an affected daughter, a

normal control and the newborn. Unfortunately, the parents did not permit publishing the photos of their affected daughters.

### 3. DISCUSSION AND CONCLUSIONS

CdLS is a relatively rare genetic disorder that is present worldwide [1]. Dominant deleterious mutations in *NIPBL* gene have been shown to be

responsible for most of the cases reported so far [3]. Around 20% of the reported cases are due to either autosomal dominant (*SMC2*, or *RAD21*) or X-linked recessive (*SMC1A* or *HDAC8* genes) forms [4-7]. The case presented here is the first autosomal recessive form caused by novel mutations in *TRMT61A* gene. *TRMT61A* codes for the catalytic subunit of tRNA (adenine-N1)-methyltransferase and has never been linked to any human genetic disorder. The enzyme installs methylation of adenosine residues at the N1 position in tRNA<sup>Met</sup> and in a small subset of mRNAs. This form of post-transcriptional modification is essential for tRNA stability and promotion of protein synthesis [8,9].

The fact that RNA modification defects are an important component of the molecular mechanism of CdLS have been the focus of several studies. The study of Zuin et al.[15] reported that NIPBL acts as a transcription factor and that genes involved in RNA post-transcriptional modification are among the target downstream genes in this process. In fact, inspection of the promoter (-1000 to + 100 to the TSS) region of *TRMT61A* showed that it contains two CpG islands (-431 to -133 and -123 to +46) and two prominent (-369 to -360 and -105 to -96) SP1 binding sites. These characteristics are consistent with the NIPBL binding sites reported by Zuin et al. [15]. Therefore, it is expected that *TRMT61A* is among the accessory genes regulated directly by *NIPBL*.

On the other hand, Boudaoud [12] work on profiling gene expression in NIPBL-deficient CdLS cells has shown that *TRMT61A* is one of the misregulated genes in *NIPBL*- and *SMC1A*-mutated CdLS cells. Moreover, Yuen et al. revealed lower expression of genes involved in RNA processing and modification in CdLS [10].

Furthermore, this work provides several lines of evidence that can consolidate the participation of *TRMT61A* in the molecular pathogenesis of this autosomal recessive CdLS form: (i) both affected daughters are compound heterozygotes for the identified mutations, (ii) each of the healthy parents is a carrier of one of the mutations, (iii) the healthy newborn female is a carrier for the mother's mutation, (iv) the absence of the variants in 50 normal individuals, and finally (v) *in silico* predicting tools confirmed the damaging nature of the identified *TRMT61A* variants.

On the whole, this case study links pathogenic mutations in *TRMT61A* to a new autosomal recessive form of CdLS and recommends

considering mutations in this gene in CdLS cases of unknown genetic cause.

## CONSENT AND ETHICS APPROVAL

Institute permission was obtained to collect the family's necessary information and their medical reports.

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## COMPETING INTERESTS

Author has declared that no competing interests exist.

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