A novel RAD21 p.(Gln592del) variant expands the clinical description of Cornelia de Lange syndrome type 4 – Review of the literature

Sanna Gudmundsson, Göran Annéren, Íñigo Marcos-Alcalde, Maria Wilbe, Malin Melin, Paulino Gómez-Puertas, Marie-Louise Bondeson

PII: S1769-7212(18)30189-7
DOI: 10.1016/j.ejmg.2018.08.007
Reference: EJMG 3526

To appear in: European Journal of Medical Genetics

Received Date: 6 March 2018
Revised Date: 14 August 2018
Accepted Date: 15 August 2018


This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.
A novel RAD21 p.(Gln592del) variant expands the clinical description of Cornelia de Lange syndrome type 4 – review of the literature

Sanna Gudmundsson¹, Göran Annéren¹, Íñigo Marcos-Alcalde²³, Maria Wilbe¹, Malin Melin¹, Paulino Gómez-Puertas², Marie-Louise Bondeson¹

¹Department of Immunology, Genetics and Pathology, Uppsala University, Science for Life Laboratory, 75108 Uppsala, Sweden
²Centro de Biología Molecular "Severo Ochoa" (CSIC-UAM). 28049 Madrid, Spain
³Faculty of Experimental Sciences, Francisco de Vitoria University, Pozuelo de Alarcón, 28223 Madrid, Spain

Corresponding authors: Sanna Gudmundsson, e-mail: sanna.gudmundsson@igp.uu.se. +4618-4714806. Marie-Louise Bondeson, e-mail: marie-louise.bondeson@igp.uu.se. +4618-611 5939. Address: Biomedical Center, Uppsala University, Box 815, 75108, Uppsala, Sweden.

CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.
ABSTRACT

Cornelia de Lange syndrome (CdLS) is a heterogeneous developmental disorder where 70% of clinically diagnosed patients harbor a mutation in one of five CdLS associated cohesin proteins. Around 500 mutations have been identified to cause CdLS, however only eight different alterations are identified in RAD21, encoding the RAD21 cohesin complex component protein that constitute the link between SMC1A and SMC3 within the cohesin ring. We report a 15-month-old boy presenting with developmental delay, distinct CdLS facial features, gastrointestinal reflux in early infancy, testis retention, prominent digit pads and diaphragmatic hernia. Exome sequencing revealed a novel RAD21 variant, c.1774_1776del, p.(Gln592del), suggestive of CdLS type 4. Segregation analysis of the two healthy parents confirmed the variant as de novo and bioinformatic analysis predicted the variant as disease-causing. Assessment by in silico structural model predicted that the p.Gln592del variant results in a discontinued contact between RAD21-Lys591 and the SMC1A residues Glu1191 and Glu1192, causing changes in the RAD21-SMC1A interface. In conclusion, we report a patient with a novel RAD21 p.(Glu592del) variant that expands the clinical description of CdLS type 4 and we validate the pathogenicity of the variant by in silico structural modeling that displayed disturbed RAD21-SMC1A interface.

KEYWORDS

RAD21 cohesin complex component; Cornelia de Lange syndrome type 4; cohesin protein; cohesin complex; cohesinopathies
INTRODUCTION

Cornelia de Lange syndrome (CdLS) is characterized by cognitive impairment, growth deficiency, skeletal malformations, distinct facial features such as long eyelashes and thick highly arched eyebrows, and other major system deficiencies like gastrointestinal reflux. The patient group is heterogeneous with great variety in clinical manifestations and severity, primarily depending on which of the five CdLS associated cohesin proteins that are affected and the type of variant. Around 60% of clinically diagnosed CdLS patients harbor a Nipped B-like (NIPBL) variant, which results in a severe CdLS phenotype. Approximately 5% are diagnosed with a Structural maintenance of chromosomes 1a (SMC1A) variant, 5% with a Histone deacetylase 8 (HDAC8) variant and less than 1% harbor a mutation in Structural maintenance of chromosome 3 (SMC3) or RAD21 cohesin complex component (RAD21). About 30% of CdLS patients are without a genetic diagnosis. The cohesin complex is involved in chromosome segregation, where RAD21, SMC1A and SMC3 constitute the cohesin ring that is responsible for sister chromatid adhesion during cell division (Figure 1A) (Nasmyth and Haering, 2009). The cohesin complex is also important for DNA repair and transcriptional control and CdLS is thought to arise due to dysregulated transcription rather than deficient chromosome segregation (Dorsett, 2007). In total, around 500 mutations affecting the cohesin complex have been associated to CdLS. However, only eight different alterations in RAD21 have been identified to give rise to CdLS, referred to as CdLS type 4 (MIM #614701) (Boyle et al., 2015).

RAD21 was first associated to CdLS type 4 in four unrelated CdLS patients (Deardorff et al., 2012). Two patients had de novo deletions spanning RAD21 (P1 and P4 in Figure 1D) and two patients had de novo RAD21 missense mutations (c.1127C>G, p.Pro376Arg and c.1753T>C, p.Cys585Arg). Two previously reported patients diagnosed with Langer-Giedion syndrome were also highlighted as their clinical features overlapped with CdLS type 4 and
they had deletions spanning RAD21 (McBrien et al., 2008; Wuyts et al., 2002). In 2014, Minor et al. reported two patients, one with a frameshift mutation (c.592_593dupAG, p.(Ser198Argfs*6)) of de novo or paternal origin, and one patient with a maternally inherited deletion spanning exon 13. The mother displayed very mild CdLS features (Minor et al., 2014). Ansari et al. also reported a familial case where an unaffected father had passed on a splice donor mutation (c.274+1G>A) to his affected daughter (Ansari et al., 2014). In 2017, Boyle et al. report a frameshift mutation, c.704delG, p.(Ser235Ilefs*19), in four female family members (Boyle et al., 2017) and Martínez et al. identified a de novo c.68G>A, p.(Trp23Ter) variant in a boy (Martínez et al., 2017) (Figure 1E).

Herein, we expand the clinical description of CdLS type 4 by reporting the clinical features of a 15-month-old boy with a novel mutation in RAD21. We also highlight the effect of the variant by in silico structural modeling.

CLINICAL REPORT

The patient was the fourth child of healthy non-related parents. He was born by caesarean section with a normal birthweight (3460 g). He presented with left sided congenital diaphragmatic hernia causing protruding of small intestine, stomach, spleen and part of colon to the thorax cavity, which was surgically treated two days post-delivery. He had gastroesophageal reflux disease during infancy and retention of the left testicle, which was surgically treated at 14 months of age. At 15 months he had the following features: distinct facial morphology, microcephaly (-3 SD), developmental delay and growth delay (length -1.5 SD, weight -3.5 SD) (Figure 1B, Table 1). He displayed prominent digit pads on all fingers and a single transvers palmar crease. No hearing impairment or malformations of distal limbs
were noted and there were no epileptic seizures. The patient occasionally took a few steps and could speak three words.

METHODS

Ethical consent
The study was performed according to the Declaration of Helsinki guidelines after approval by the local ethics committee, Uppsala (Dnr 2012/321) and collection of informed consent.

Whole-exome sequencing and segregation analysis
Whole-exome sequencing (WES) and analysis protocols, developed by the Clinical genomics facility in Uppsala, were adapted as a clinical WES test at the Department of Clinical Genetics, Uppsala University Hospital, Sweden. Briefly, genomic DNA from the trio was extracted from peripheral blood using automated systems (EZ1 and QIASymphony, QIAGEN) according to standard protocols. For library preparation with Clinical Research Exome and SureSelectQXT Target Enrichment System (Agilent Technologies, Santa Clara, CA, USA) 250 ng DNA was used. Sequencing was performed with 150 base pair long paired-end reads on a NextSeq500 sequencer (Illumina, San Diego, CA). Alignment of raw data to the human reference genome (GRCh37/hg19) was performed using BWA 0.7.10 and variant calling was performed with GATK haplotype caller (GATK framework 3.2.4, GenomeAnalysisTK 3.2.2) by using the Bcbio-Nextgen pipeline v 0.8.9 (https://github.com/chapmanb/bcbio-nextgen). Quality control parameters were calculated using FastQC 0.11.2, Picard HsMetrics 1.96 (http://broadinstitute.github.io/picard/) and GATK Depth of Coverage (GATK framework 3.2.4, GenomeAnalysisTK 3.2.2). For filtering of variants BenchLab NGS (Agilent Technologies, Inc.) was used, including population frequency, phenotype data from the Human Gene Mutation Database professional and
ClinVar databases. The allelic variants identified were classified according to the American College of Medical Genetics and Genomics and the Association for Molecular Pathology (Richards et al., 2015). The selected variant was confirmed by Sanger sequencing of the family trio according to standard protocols (available upon request).

Three-dimensional structure modeling

The template structure was a stabilized model of human RAD21-Cterminal domain linked to the head domains of human SMC1A/SMC3 heterodimer, which was based on the structure of the C-terminal domain of yeast Scc1 protein (RAD21 in human) bound to yeast Smc1 homodimer (Protein Data Bank ID: 1W1W) (Haering et al., 2004), as previously described by Marcos-Alcalde et al. (Marcos-Alcalde et al., 2017). Model coordinates were built using the SWISS-MODEL server (http://swissmodel.expasy.org) and their structural quality was within the range of those accepted for homology-based structure (Anolea/Gromos/QMEAN4) (Benkert et al., 2011). To optimize geometries, the model was energy minimized using the GROMOS 43B1 force field implemented in DeepView (http://spdbv.vital-it.ch/), using 500 steps of steepest descent minimization followed by 500 steps of conjugate-gradient minimization. Figures were generated using the Pymol Molecular Graphics System (Schrödinger, LLC). Multiple sequence alignment of proteins from the RAD21 family was generated using TCOFFEE (http://www.tcoffee.org/) (Notredame et al., 2000).

RESULTS

Whole-exome sequencing revealed a novel RAD21 c. 1774_1776del, p.(Gln592del) variant

Whole-exome sequencing was performed on the family trio with 93% of the reads mapping to the reference genome, at an average read depth of 159x and >10x for 97% of the exome in the
index patient. Filtering of trio variants revealed heterozygosity for a novel \textit{RAD21} variant, c.1774_1776del, p.(Gln592del), chr8:117859859_117859861delTTG (NM_006265) that was confirmed \textit{de novo} in the index patient (Figure 1C). The variant is not reported in the population of ExAC, GnomAD or SweGen databases (Ameur et al., 2017; Lek et al., 2016). ExAC database revealed that the level of observed missense variants in \textit{RAD21} is lower than expected (ExAC: $z=2.76$). Further, there is only one homozygous missense variant reported (p.Asp414Glu, rs75160167, n=3), and no homozygous loss of function variants reported in the population databases (GnomAD, ExAC and SweGen). The patient phenotype and variant data has been submitted to ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/), accession number SCV000747141.

\textbf{In silico} modelling displayed changes in the p.Gln592del \textit{RAD21}-SMC1A interface

The p.Gln592del variant is located in the C-terminal of the last exon (14/14) within the SMC1A binding domain of RAD21 (p.558-628; Figure 1E). The three-nucleotide position of the variant is conserved (PhyloP score 4.2; Figure 2A) and the deletion is predicted to be deleterious (PROVEAN: -11.124) and disease causing (MutationTaster). Amino acids Gln592, Arg590 and Lys591 are located in a positively charged environment in close contact to the negatively charged residues Glu1191 and Glu1192 from the head domain of SMC1A (Figure 2B, right). Deletion of Gln592 results in rearrangement of the surrounding residues. In particular, the structural model predicts a significant positional change of Lys591, now located in the space previously occupied by Gln592. As a result, the previous contact between Lys591 and the SMC1A residues Glu1191 and Glu1192 is discontinued, causing significant changes in the RAD21-SMC1A interface (Figure 2B, left).

Residues Arg590, Lys591 and Gln592 are located in the same alpha helix as Lys605 (Figure 2C, left). Lys605 is a key residue for the ATPase activity of the active site 1 of the cohesin ring, as it stabilizes the position of the SMC1A residues Asn35 and Gly35 in contact
to ATP and the catalytic water molecule (Marcos-Alcalde et al., 2017). The change in the contact area between positive and negative patches on the interaction surface of RAD21 and SMC1A is expected to generate a local disorganization of the interface, thus affecting p.Lys605 and subsequently the ATPase-dependent functionality of the cohesin head (Figure 2C, right).

DISCUSSION

We report clinical and genetic findings of a patient with CdLS type 4, a syndrome of which clinical features of only 13 patients have been described in the literature before (Table 1). The index patient presented with clinical characteristics previously associated to CdLS type 4 as well as diaphragmatic hernia, which has been reported in about 1% of CdLS patients (Cunniff et al., 1993; Fryns, 1987; Jelsema et al., 1993; Marino et al., 2002; Pankau and Janig, 1993) but not in CdLS type 4 patients. The index patient also presented with prominent digit pads that has not been reported in CdLS patients before but has been reported in a patient with a deletion spanning RAD21 (McBrien et al., 2008) that shared clinical features with CdLS type 4 but was diagnosed with Langer-Giedion syndrome (Deardorff et al., 2012). Of note, exostoses, reported in three CdLS type 4 patients with micro deletions spanning EXT1 (Table 1; Figure 1D) (Deardorff et al., 2012; Pereza et al., 2015), are most likely not associated to CdLS type 4 and RAD21 mutations but caused by EXT1 deletions as described before (MIM #133700).

The index patient was diagnosed with a novel de novo RAD21 c.1774_1776, p.(Gln592del) variant. The affected p.Gln592 residue is conserved and the deletion is predicted to be deleterious and disease-causing. Further, the p.Gln592del variant is not reported in publically available databases and missense variants in RAD21 in the normal population are underrepresented, suggesting that variants in RAD21 might be disease-causing.
The lack of homozygous loss of function variants in the normal population suggests that complete loss of RAD21 might be lethal. Previously, eight unique heterozygous alterations of RAD21 variants have been reported in patients affected with CdLS type 4: three missense mutations (Deardorff et al., 2012; Martinez et al., 2017), two frameshift mutations (Boyle et al., 2017; Minor et al., 2014), one in-frame deletion including exon 13 of RAD21, one splice donor mutation (Ansari et al., 2014) and deletions spanning whole RAD21 (Deardorff et al., 2012; Pereza et al., 2015). Deardorff et al. has also highlighted two previously published patients, with deletions spanning RAD21, diagnosed with Langer-Giedion syndrome but with clinical symptoms overlapping CdLS type 4 (McBrien et al., 2008; Wuyts et al., 2002).

The RAD21 protein is a key unit of the cohesin complex, which is involved in chromosome segregation, DNA repair and transcriptional regulation (Dorsett, 2007; Nasmyth and Haering, 2009). Deletion of RAD21 has been shown to result in haploinsufficiency (reduced RAD21 RNA and RAD21 protein levels) but a disease-causing p.Pro376Arg variant did not affect the expression levels notably (Deardorff et al., 2012). Hence, different RAD21 disease-causing variants suggestively act through different pathogenic mechanisms. It is clear that RAD21 is sensitive to alterations and that variants can cause CdLS type 4, but there are also reports of heterozygous RAD21 missense variants associated with chronic intestinal pseudo-obstruction, p.Ala622Thr, (Bonora et al., 2015) and autism spectrum disorder, p.(Phe114Leu), (Yuen et al., 2015). The one-amino-acid deletion reported in this study is located in the C-terminal of RAD21 at the site responsible for SMC1A-head coupling (Haering et al., 2004). In silico modeling of RAD21 p.592del, display a clear structural change in residue Lys591 and, to a lesser extent, Arg590, which is predicted to affect the interface to SMC1A-head (Glu1191 and Glu1192). The p.592del variant is also suggested to influence RAD21 p.Lys605/Lys604 that facilitate a crucial function in ATP induced hydrolysis that is responsible for the opening of the cohesin molecule (Marcos-Alcalde et al.,
2017). Therefore, we suggest that the function of the cohesin ring, and specifically the binding to SMC1A is altered, causing the phenotype observed in the patient.

In summary, we present a novel RAD21 c.1774_1776del, p.(592del) variant, giving rise to CdLS type 4 in a boy. Segregation analysis, bioinformatic analysis, population data and in silico structural modeling vindicate the pathogenicity of the novel variant. This report summarizes previously reported clinical manifestations of CdLS type 4 but also highlights new clinical symptoms, which will aid correct counseling of future CdLS type 4.

ACKNOWLEDGMENTS

We would like to thank the family for participating in this study. The study was supported by grants from Uppsala University Hospital as well as grants from the Spanish Ministry of Economy, Industry and Competitiveness (contracts IPT2011-0964-900000 and SAF2011-13156-E to P.G-P). SG was supported by grants from the Sävstaholm foundation.
FIGURE TITLES AND LEGENDS

Figure 1: Overview of patient features and RAD21 properties. (A) The RAD21 protein (blue) serves as a link between SMC3 protein (green) and SMC1A protein (yellow) that form the cohesin ring, responsible for adhesion of the sister chromatids during cell division, DNA repair and transcriptional control. The position of the p.Gln592del variant is indicated by a red arrow. (B) The index patient presented with Cornelia de Lange syndrome phenotype. Parents and the three older siblings were healthy. (C) A novel de novo RAD21 p.(592del) variant was confirmed. (D) Three studies report deletions spanning RAD21 in patients with Cornelia de Lange syndrome type 4 (black bars). Two patients with deletions spanning RAD21 have been reported with Lager-Giedion syndrome (brown bars). (E) RAD21 is 631 amino acid long with three binding domains: SMC3 (green, p.1-103), STAG1/2 (purple, p.362-403) and SMC1A (yellow, p.558-628). Previously reported intragenic variants are marked as well as the novel p.(Gln592del) variant identified in the index patient (bold).

Figure 2: In silico modeling of Gln592del mutant. (A) Multiple sequence alignment display conservation of the C-terminal domain of RAD21 (colored according to BLOSUM62 score). Dots indicate RAD21 residues connecting with SMC1A (blue) and the position of the deletion, p.Gln592 (red). (B) Surface of the structure model for wild-type RAD21-Cterminal domain (left) and for RAD21-Cterminal domain with p.Gln592del (right) colored according to electrostatic characteristics (red: negative, blue: positive, white: neutral). Positively charged amino acid Arg590, interacting with SMC1A residue Glu1198, is affected by the p.Gln592 deletion. Lys591 has lost its connection to SMC1A residues Glu1191 and Glu1192. (C) Structure model for wild-type RAD21-Cterminal domain (left) and for RAD21-Cterminal domain with p.Gln592del (right) with residues Arg590, Lys591, Gln592 and Lys605/Lys604* labeled. Lys591 residue have repositioned to the space previously occupied by the deleted Gln592 residue.
### TABLES

**Table 1:** Clinical features reported in the index patient and/or >2 previously described

<table>
<thead>
<tr>
<th>Clinical anomalies reported in &gt;2 patients with distinct variants, or in the index patient</th>
<th>Index patient</th>
<th>Previously reported patients (tot 13)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CdLS-like facial features</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Synophrys</td>
<td>-</td>
<td>9</td>
</tr>
<tr>
<td>Thick and highly arched eyebrow</td>
<td>+</td>
<td>11</td>
</tr>
<tr>
<td>Long and prominent eyelashes</td>
<td>+</td>
<td>6</td>
</tr>
<tr>
<td>Short nose and nares anteverted</td>
<td>+</td>
<td>9</td>
</tr>
<tr>
<td>Wide or depressed nasal bridge</td>
<td>-</td>
<td>6</td>
</tr>
<tr>
<td>Long philtrum</td>
<td>-</td>
<td>9</td>
</tr>
<tr>
<td>Upper and lower lip thin vermillion</td>
<td>-</td>
<td>8</td>
</tr>
<tr>
<td>Macrotia</td>
<td>+</td>
<td>6 (4)</td>
</tr>
<tr>
<td>Ptosis</td>
<td>+</td>
<td>3</td>
</tr>
<tr>
<td>High or submucous cleft palate</td>
<td>+</td>
<td>3</td>
</tr>
<tr>
<td>Low-set or/and posterior angulation increased ear</td>
<td>+</td>
<td>3</td>
</tr>
<tr>
<td>Micrognathia</td>
<td>+</td>
<td>2</td>
</tr>
<tr>
<td>Developmental delay or Intellectual disability</td>
<td>+</td>
<td>12 (1)</td>
</tr>
<tr>
<td>Microcephaly</td>
<td>+</td>
<td>10</td>
</tr>
<tr>
<td>Gastroesophageal reflux disease</td>
<td>+</td>
<td>7</td>
</tr>
<tr>
<td>Short stature</td>
<td>+</td>
<td>3 (2)</td>
</tr>
<tr>
<td>Abnormality of the genital system</td>
<td>+</td>
<td>2</td>
</tr>
<tr>
<td>Bridged or single transverse palmar crease</td>
<td>+</td>
<td>3</td>
</tr>
<tr>
<td>Prominent digit pad</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>Diaphragmatic hernia</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>5th finger clinodactyly</td>
<td>-</td>
<td>8 (1)</td>
</tr>
<tr>
<td>Low birth weight/ Decreased body weight</td>
<td>-</td>
<td>5</td>
</tr>
<tr>
<td>Dislocated elbow/ Abnormal extension</td>
<td>-</td>
<td>5</td>
</tr>
<tr>
<td>Sparse scalp hair</td>
<td>-</td>
<td>3</td>
</tr>
<tr>
<td>Toe cutaneous syndactyly</td>
<td>-</td>
<td>3 (1)</td>
</tr>
<tr>
<td>Exostoses $^+$</td>
<td>-</td>
<td>3</td>
</tr>
</tbody>
</table>

Cornelia de Lange type 4 patients.

$^+$ Observed in the index patient, II:1. $^-$ Not observed in the index patient. $^*$Clinical features reported in >2 patients with different mutations: Number of affected patients (patients reported as normal). $^+$Suggestively associated to *EXT1* deletions and not *RAD21* variants.
REFERENCES


