European Journal of Medical Genetics xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

European Journal of Medical Genetics

journal homepage: www.elsevier.com/locate/ejmg

A novel *RAD21* mutation in a boy with mild Cornelia de Lange presentation: Further delineation of the phenotype

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ARTICLE INFO

Keywords:
CdLS4
RAD21
Speech delay
Microcephaly

ABSTRACT

Cornelia de Lange syndrome is a rare autosomal dominant or X-linked developmental disorder characterized by characteristic facial dysmorphism, intellectual disability, growth retardation, upper limb and multiorgan anomalies. Causative mutations have been identified in five genes coding for the cohesion complex structure components or regulatory elements. Among them, *RAD21* is associated with a milder phenotype. Very few *RAD21* intragenic mutations have been identified so far. Thus, any new patient is a valuable tool to delineate the associated phenotype. We discuss a new patient with *RAD21* confirmed molecular diagnosis and compare his clinical features to those of previously described patients carrying different *RAD21* intragenic mutations.

1. Introduction

Cornelia de Lange syndrome (CdLS) is a developmental/intellectual disability disorder (DD/ID) with broad phenotypic spectrum, mainly characterized by pre- and post-natal growth retardation, facial dysmorphism, upper limb and multiorgan anomalies, which is mirrored by high locus heterogeneity (for review see Deardorff MA and Krantz, 2016). Pathogenic variants of the seven genes encoding structural components (*SMC1A, SMC3, RAD21*) or regulators (*NIPBL, HDAC8, BRD4, ANKRD11*) of the cohesin complex underpin different CdLS entities, each endowed with pronounced clinical expressivity (Kline et al., 2018). Up to 70% of the clinically diagnosed CdLS cases are accounted for by the major *NIPBL* gene (CdLS1, MIM # 122470), while only a tiny fraction of them, with milder cognitive deficit and less physical abnormalities have been found to harbour *RAD21* pathogenic variants (CdLS4, MIM # 614701).

The *RAD21* gene (MIM 606462), encoding the kleisin subunit bridging the SMC1A/SMC3 heterodimer to the fourth STAG1/STAG2 cohesin subunit, is a key regulator of association/disassociation of functional cohesin to chromatin. In addition, it plays a central role in mediating DNA-damage response. Only eight germline intragenic pathogenic variations and five 8q24.1 deletions encompassing *RAD21* have been identified so far in CdLS4 patients (Deardorff et al., 2012; Ansari et al., 2014; Minor et al., 2014; Boyle et al., 2017; Martinez et al., 2017; Gudmundsson et al., 2018; Wuyts et al., 2002; McBrien et al., 2008; Pereza et al., 2015). Each new patient with confirmed molecular diagnosis is hence a valuable tool to delineate the CdLS4 phenotype and to assess its modulation, if any, depending on the impact of the sequence change on a defined protein domain.

2. Materials and methods

All scientific methods and ethics information can be found in the supplemental material.

2.1. Clinical report

The patient, a 5-year-old male, is the first child of healthy non-consanguineous parents of Belgian origin. Family history points out a maternal half-sister with dyslexia and dyscalculia and two paternal uncles with epilepsy. The pregnancy was uneventful and prenatal ultrasounds were normal with a regular growth. He was born at term by vaginal delivery. At birth his weight was 3610 g (50^{th} centile), his length 51 cm (50^{th}-75th centile) and head circumference 34 cm (10^{th}-25th centile). At the age of 2 months, he was hospitalised for an apparently life-threatening event due to gastro-oesophageal reflux. He

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https://doi.org/10.1016/j.ejmg.2019.01.010
Received 23 May 2018; Received in revised form 8 December 2018; Accepted 19 January 2019

developed progressive microcephaly, which stabilised at about – 3SD. He walked at 12 months and pronounced the first words at 2 years. He manifested moderate fine motor delay and speech delay, requiring physiotherapy since the age of 2 years and speech therapy since the age of 3 years. He had frequent upper and lower respiratory tract infections. Ophthalmological investigation revealed myopia and astigmatism. Cerebral magnetic resonance imaging (MRI) and extensive blood, urine and cerebrospinal fluid metabolic work-up were normal. At the age of 3 years, neurocognitive assessment by the Leiter International Performance Scales Revised – Leiter R-indicated an Intellectual Quotient (IQ) of 112. At the age of 5 years, cognitive profile assessment using WPPSI-III (Wechsler Preschool and Primary Scale of Intelligence-Third Edition) showed a heterogeneous intellectual profile: verbal IQ in the low-average for age (89), performance IQ in the average (101) and an extremely low processing speed, especially for the coding subtest (69). Insufficient pragmatic language skills contributed to communication problems. The patient could present temper tantrum from time to time. The Autism Diagnostic Observation Schedule (ADOS) testing revealed expressive language difficulties, but no echolalia and no stereotypes. Regarding the reciprocal social interaction, good eye contact during a conversation, but less good to request certain objects was observed as well as a certain variability of facial expressions. The patient had no stereotyped behaviours, no restricted interests and no hyperactivity. He attended an adapted education school for children with mild learning disabilities. The last clinical evaluation at age of 5 pointed out the following features: weight 17,1 kg (−0.6SD), height 111 cm (+0.5SD), head circumference 46.2 cm (−3.1SD), synophrys with arched eyebrows, long eyelashes, long philtrum, thin upper lip with down-turned corners and bilateral 5th finger clinodactyly (Fig. 1 and Table 1). Neurological examination showed brisk reflexes and global motor difficulties.

2.2. Molecular analysis

As CdLS was suspected by clinical scoring of the patient according to the published diagnostic criteria (Kline et al., 2007), next generation sequencing targeted to a multi-gene panel, including the five known CdLS genes, was performed on genomic DNA from peripheral blood. The proband genomic DNA was processed on Illumina MiSeq using Illumina kit (Nextera Rapid Capture Enrichment). A *RAD21* (NM_006265: exon 9: c.943_946del (p.(Glu315Glnfs*9)) unreported mutation was identified in heterozygous state and then confirmed by conventional Sanger sequencing. The four-base deletion results in frameshift of the coding sequence from codon 315 to 322 converting 323 in a premature stop codon. This is expected to be associated with mRNA non-sense mediated decay (NMD). If the aberrant mRNA escapes NMD, it will be translated in a truncated protein which maintains only a small portion (aa 287-314) of the 287-403 RAD21 domain (Suppl Figure 1). This domain interacts with the WAPAL/PDS5B complex and is needed for cohesin release in interphase cells (Shintomi and Hirano, 2009). The mutation occurred *de novo* and is not listed in ExAC and GnomAD databases, thus featuring responsible for the CdLS phenotype displayed by the boy. The new likely pathogenic variant has been uploaded on the LOVD database (https://databases.lovd.nl/shared/individuals/00207752).

3. Discussion

The description of a novel CDLS4 patient with an unreported *RAD21* variant expands the restricted *RAD21* mutational spectrum and confirms the association of *RAD21* gene defects with the comparatively mildest phenotype out of those associated to the different CdLS entities. According to clinical records, our case places at the mild end of the phenotypic spectrum of Cornelia de Lange syndrome, including the small set of *RAD21*-mutated patients, as shown in Table 1. He showed typical facial features, gastro-oesophageal reflux, and myopia, but no growth delay and no malformation. At age of 5 years he displayed normal performance IQ, but speech delay and communication difficulties.

Thirteen patients with *RAD21* mutations have been reported until now in CDLS4 patients: 8 with an intragenic mutation and 5 with a 8q24.1 deletion (Deardorff et al., 2012; Ansari et al., 2014; Minor et al., 2014; Boyle et al., 2017; Martinez et al., 2017; Gudmundsson et al., 2018; Wuyts et al., 2002; McBrien et al., 2008; Pereza et al., 2015). We

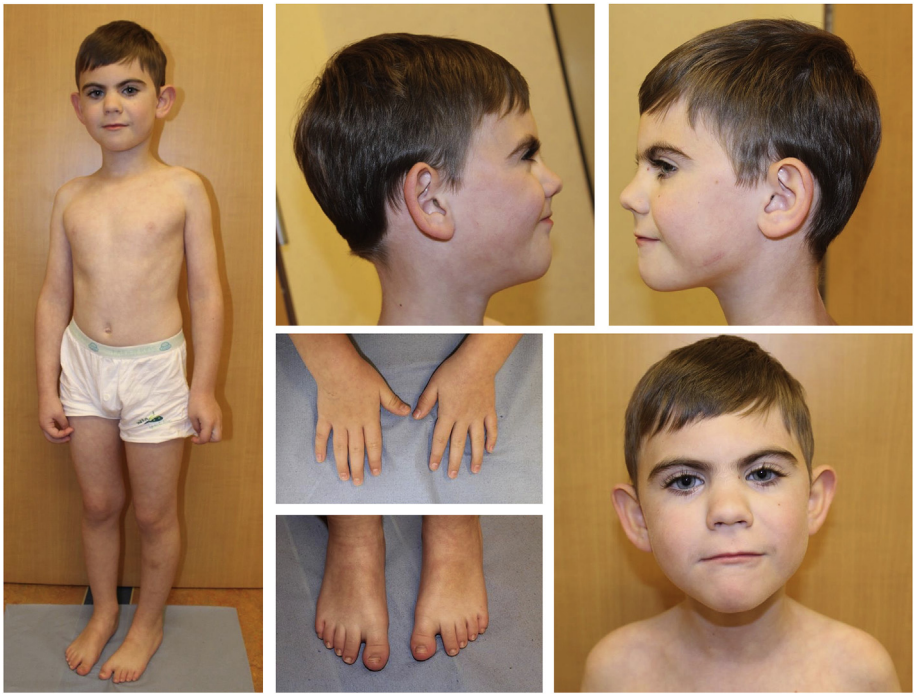

Fig. 1. Clinical features of the patient at the age of 5 years. Note the arched eyebrows with synophris, long eyelashes, broad nasal bridge, short nose with anteverted nares, long and smooth philtrum, thin upper lip.

Table 1
Lists the clinical features of our patient as compared to those of seven previously described CdLS index patients carrying different *RAD21* intragenic mutations. We excluded one patient with intragenic mutation due to lack of clinical data (Ansari et al., 2014). As can be seen our patient's phenotype places at the mild end of CdLS4 spectrum.

Reference	This study	Deardoff et al., 2012 (patient 5)	Deardoff et al., 2012 (patient 6)	Minor et al., 2014 (patient 1)[a]	Minor et al., 2014 (patient 2)	Boyle et al., 2017 [a]	Martinez et al., 2017
Gender/age at last evaluation	male 5y	male child	female child	male 3y	male 12y	female 26y	male
Mutation							
site	exon 9	exon 9	exon 14	exon 13	exon 6	exon 7	exon 2
type	frameshift	missense	missense	in-frame del	frameshift	frameshift	nonsense
cDNA	c.943_946del	c.1127C > G	c.1753T > C	c.1621-388_1704 + 193del	c.592_593dupAG	c.704delG	c.68G > A
protein	p.Glu315Glnfs[a]9	p.Pro376Arg	p.Cys585Arg	p.Asp541_Gln568del	p.Ser198Argfs[a]6	p.Ser235Ilefs[a]19	p.Trp23[a]
Craniofacial features							
arched and/or thick eyebrows	+	+	+	+	+	+	long eyebrows
synophrys	+	+	+	mild	+	mild	nd
long eyelashes	+	+	+	–	+	+	nd
ptosis	–	bilateral	–	+	–	–	nd
anteverted nostrils	+	+	+	–	+	+	nd
broad or depressed nasal bridge	+	+	+	+	+	–	nd
long philtrum	+	+	+	–	+	+	+
thin upper lip vermilion	+	+	+	–		+	nd
low-set/posteriorly rotated ears	–	nd	nd	+	+	–	+
high or cleft palate	–	submucous cleft palate	–	–	broad uvula	–	nd
low anterior or posterior hairline	–	nd	nd	–	+	+	nd
Growth, cognitive development							
low birth weight	–	nd	nd	–	+	nd	+
short stature	–	+	nd	–	–	+	nd
low OFC/microcephaly	+	+	+	–	+	+	+
DD/ID	speech delay	severe delay, verbal > motor	nd	verbal > motor	+	+	+
learning disabilities	+	ADHD	–	nd	+/ADHD	+	+
behavior problems	occasional temper tantrum	–	–	autistic features	–	–	nd
Neurosensory/skin symptoms							
hearing loss	–	+	–	–	–	–	nd
vision	myopia/astigmatism			nystagmus/strabismus	mild hyperopia	–	myopia
hirsutism	–	–	–	–	+	–	nd
cutis marmorata	+	nd	+	nd	nd	nd	nd
major systems manifestations							
gastroesophageal reflux disease	+	+	–	–	+	+	nd
congenital heart defect	–	tetralogy of Fallot	nd	–	–	–	nd
genito-urinary	–	–	–	hypospadias, bifid scrotum, undescended testes, bilateral inguinal hernia	–	–	abnormality of the genitalia
skeleton							
pectus excavatum/ carinatum	–	+	–	–	+	+	nd
limb abnormaties (upper)	5th finger clinodactyly	thin fingers, transverse palmar crease	short fingers	5th finger clinodactyly	5th finger clinodactyly, 2-3 finger syndactyly, bilateral transverse palmar crease, absent distal flexion crease of fingers 3 to 5	5th finger clinodactyly	abnormality of the hand
elbow limitation	–	radioulnar synostosis	–	–	–	+	nd

(*continued on next page*)

Table 1 (*continued*)

Reference	This study	Deardoff et al., 2012 (patient 5)	Deardoff et al., 2012 (patient 6)	Minor et al., 2014 (patient 1)[a]	Minor et al., 2014 (patient 2)	Boyle et al., 2017 [a]	Martinez et al., 2017
Gender/age at last evaluation	male 5y	male child	female child	male 3y	male 12y	female 26y	male
limb abnormalities (distal)	–	mild 2-3 syndactyly	mild 2-3 syndactyly	toes 2-3 overlapping	2-3 syndactyly	–	nd

[a] Familial case nd = not determined ADHD = Attention Deficit Hyperactivity Disorder DD developmental delay ID intellectual disability.

excluded from the clinical comparison in this study (Table 1) one patient with intragenic mutation due to lack of clinical data (Ansari et al., 2014) and the 5 patients with large deletions, as the phenotype could be influenced by the concomitantly deleted genes (Wuyts et al., 2002; McBrien et al., 2008; Deardorff et al., 2012; Pereza et al., 2015).

The mild developmental and cognitive deficits of CdLS4 patients are consistent with the familial transmission in three previously reported cases with *RAD21* intragenic mutations, underlining the relevance of molecular diagnosis and genetic counselling to probands and their families. In the first case, the mutation was inherited from the mother with classical CdLS facial features and microcephaly, but the diagnosis had not been suspected prior the study (Boyle et al., 2017). The mutation was also present in other relatives with milder phenotype, suggesting variable clinical expressivity. In a second family, the mutation was inherited from the mother, who presented mild phenotype and facial features (Minor et al., 2014). Finally, in the third case, the mutation was inherited from an apparently unaffected father, but clinical data were not reported (Ansari et al., 2014). It is unknown if the mutation in the two latter cases might have been present in a mosaic state in the pauci -/asymptomatic parent.

The mutation identified in our patient (c.943_946del; p.Glu315Glnfs*9) predicts in case of stability and translation of the aberrant mRNA a truncated protein devoid of most of the domain interacting with the cohesin unloading complex WAPAL/PSD5 and all the downstream domains. Such protein might be potentially deleterious due to gain-of-function or dominant-negative activity. More likely, as previously suggested for one patient carrying an earlier *RAD21* frameshift mutation (Minor et al., 2014), the mutant RNA might be targeted by non-sense mediated decay leading to *RAD21* haploinsufficiency.

Contrary to loss-of-function, two dominant *RAD21* missense mutations have been shown by functional studies to result in severe structural and cognitive clinical findings (Deardorff et al., 2012). That *RAD21* is not so dosage-sensitive as other cohesin genes, in particular *NIPBL*, can be also inferred by the mild phenotype shared by patients with 8q24.1 microdeletions including *RAD21* (Deardorff et al., 2012, Pereza et al., 2015, McBrien et al., 2008, Wuyts et al., 2002) and the occurrence of *RAD21* homozygous mutation in patients with Chronic Intestinal Pseudo-Obstruction (CIPO) (Bonora et al., 2015). Although behavioural problems are reported in one of the five described CdLS4 patients (Minor et al., 2014), it is unclear whether the occurrence of the *de novo RAD21* missense variant [p.(Phe114Leu)] in an individual manifesting only autism spectrum disorder is causative or coincidental (Yuen et al., 2015). Long-term clinical follow up and further CdLS4 patients should be diagnosed on clinical and molecular ground and mutations impacting on key protein domains modelled and functionally investigated to highlight the phenotypic features associated to *RAD21* mutational spectrum.

Conflicts of interest

None.

Acknowledgement(s)

The Authors would like to thank the patient's family for their cooperation. This work was supported by Italian Ministry of Health to Istituto Auxologico Italiano (LL- RC 08C625).

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ejmg.2019.01.010.

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