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Two novel *RAD21* mutations in patients with mild Cornelia de Lange syndrome-like presentation and report of the first familial case

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ABSTRACT

Cornelia de Lange syndrome (CdLS) is a developmental disorder characterized by limb reduction defects, characteristic facial features and impaired cognitive development. Mutations in the *NIPBL* gene predominate; however, mutations in other cohesin complex genes have also been implicated, particularly in atypical and mild CdLS cases. Missense mutations and whole gene deletions in *RAD21* have been identified in children with growth retardation, minor skeletal anomalies and facial features that overlap findings in individuals with CdLS. We report the first intragenic deletion and frameshift mutations identified in *RAD21* in two patients presenting with atypical CdLS. One patient had an in-frame deletion of exon 13, while the second patient had a c.592_593dup frameshift mutation. The first patient presented with developmental delay, hypospadias, inguinal hernia and dysmorphic features while, the second patient presented with developmental delay, characteristic facial features, hirsutism, and hand and feet anomalies, with the first patient being milder than the second. The in-frame deletion mutation was found to be inherited from the mother who had a history of melanoma and other unspecified medical problems.

This study expands the spectrum of *RAD21* mutations and emphasizes the clinical utility of performing *RAD21* mutation analysis in patients presenting with atypical forms of CdLS. Moreover, the variability of clinical presentation within families and low penetrance of mutations as well as the significance of performing molecular genetic testing in mildly affected patients are discussed.

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1. Introduction

The cohesin complex is essential for the proper segregation of sister chromatids during cell division. It is a multi-protein structure composed of two SMC proteins, SMC1 and SMC3, RAD21 and a STAG subunit (Schüle et al., 2005). The cohesin complex forms a ring-like structure around the sister chromatids and holds them together along their length and at the centromere until segregation occurs (Haering et al., 2004). Regulatory proteins NIPBL and the acetyl transferase ESCO2 are required for the association of the cohesin complex with chromosomes during S phase, while the acetyl transferase HDAC8 is required for the disassociation of the cohesin complex from chromosomes during S phase (Rolef Ben-Shahar et al., 2008). Abnormalities of the components of the cohesin complex have been associated with cohesinopathies in humans, including Roberts syndrome and Cornelia de Lange Syndrome (Schüle et al., 2005; Selicorni et al., 2007).

Cornelia de Lange Syndrome (CdLS) is a multisystem developmental disorder characterized by limb reduction defects, characteristic facial features, impaired cognitive function ranging from moderate to severe, and poor growth (Rohatgi et al., 2010). CdLS is a genetically heterogeneous condition with mutations in *NIPBL* (MIM#608667) being the most common genetic cause of classical CdLS (Bhuiyan et al., 2006; Selicorni et al., 2007). In addition to the classical CdLS phenotype, patients with CdLS-variant phenotypes have been reported. Mutations in the X-linked *SMC1A* (MIM# 300040) and the autosomal *SMC3* (MIM# 606062) genes have been identified in ~5% of individuals with mild facial features of CdLS, moderate neurocognitive impairment and a paucity of major structural defects (Deardorff et al., 2007; Musio et al., 2006). More recently, mutations in the X-linked *HDAC8* gene (MIM# 300269) were identified in six probands with clinical features of CdLS but without significant limb involvement and with some additional features that may help to differentiate this group from individuals with CdLS caused by mutations in other genes (Deardorff et al., 2012a). Finally, missense mutations in *RAD21* (MIM# 606462), and contiguous gene deletions including this gene, have been identified in probands with

Abbreviations: CdLS, Cornelia de Lange Syndrome; ddCt, comparative threshold cycle method; OFC, occipitofrontal circumference.

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growth retardation, minor skeletal anomalies and facial features that overlap findings in individuals with CdLS but overall have milder cognitive impairment (Deardorff et al., 2012b). Although, the majority of CdLS associated mutations are *de novo* in origin, familial cases of CdLS have been described in the literature (Borck et al., 2006; Musio et al., 2006; Russell et al., 2001).

In humans, *RAD21* localizes to chromosome 8q24.11 (McKay et al., 1996). RAD21 is a nuclear phosphoprotein that shows highly regulated expression during the cell cycle and plays an important role in the formation and the dissociation of the cohesin complex during cell division (McKay et al., 1996). The interaction of RAD21 with SMC1 is essential for its association with chromatin during S phase (Michaelis et al., 1997). During the metaphase-to-anaphase transition, cleavage of RAD21 by a separase allows the dissociation of the cohesin complex from the sister chromatids and segregation to occur (Michaelis et al., 1997; Uhlmann et al., 1999, 2000). Premature separation of sister chromatids, improper chromosome segregation, failure of chromosome alignment at the metaphase plate, and a reduced ability to repair DNA damage have been associated with the loss of RAD21, indicating that sister chromatid cohesion is essential for these processes to occur correctly and that RAD21 plays an important role in facilitating these functions (McKay et al., 1996; Sonoda et al., 2001).

We present the first reported intragenic deletion and frameshift mutation in *RAD21* in two patients with an atypical form of CdLS. The patients presented with relatively mild features and mild developmental delay. Our findings support the association of *RAD21* gene defects with a congenital phenotype consistent with a 'cohesinopathy' and emphasize the utility of *RAD21* mutation analysis in patients presenting with CdLS-variant phenotypes. Furthermore, this is the first report of a parent-to-child transmission of CdLS caused by a mutation in *RAD21*.

2. Materials and methods

2.1. DNA isolation

Peripheral blood samples from patients were referred to molecular diagnostic laboratory at the University of Chicago for CdLS comprehensive mutation analysis. Genomic DNA was isolated from blood leukocytes on the AutoGenFlex STAR robotic workstation (Autogen, Holliston, MA) following the manufacturer's instructions.

2.2. DNA sequence analysis

PCR amplification of the *RAD21* (NM_006265) coding sequence and flanking intronic sequence was performed using HotStar Taq polymerase (Qiagen, Germantown, MD). Sequence analysis was performed in both forward and reverse directions on an ABI 3730 DNA Analyzer (Life Technologies, Grand Island, NY). Sequences were compared to the *RAD21* reference sequence using Mutation Surveyor software version 3.01 (SoftGenetics, State College, PA).

2.3. Array comparative genomic hybridization (Array-CGH)

Deletion and duplication analysis of the *RAD21* gene was performed using a high resolution, custom-designed, exon targeted 8X60K array-CGH platform (Agilent Technologies, Wood Dale, IL). A total of 463 probes spanning the *RAD21* gene and flanking regions were present on this design with an average resolution of approximately 1 probe/11 bp across the *RAD21* coding region. Genomic DNA samples of the patient and gender-matched control were processed and co-hybridized onto microarray slides according to the manufacture recommended procedures (Agilent Technologies, Wood Dale, IL). Microarray images were scanned at 2 micron resolution and the data was extracted using ImaGene (9.0) and analyzed using the Nexus software (6.0) (BioDiscovery, Hawthorne, CA). The genomic copy number was defined by analysis of the normalized $\log_2$ (Cy5/Cy3) ratio average of the CGH signal. Regions that reached a threshold of at least −0.32 were considered suspicious copy number losses consistent with deletions.

2.4. Quantitative Real-Time PCR

Quantitative Real-Time PCR analysis of *RAD21* exons 13 and 14 was performed using 20 ng of genomic DNA. Reactions were done in triplicate, wherein each target region was co-amplified with an internal control (*PMP22*: NM_153321) using the SYBR-Green detection chemistry and the ABI PRISM 7500 sequence detection system (Applied Biosystems, Grand Island, NY). Relative gene copy number was determined by the comparative threshold cycle method (ddCt) (Livak and Schmittgen, 2001). A melting curve analysis was performed subsequent to each amplification run to verify PCR product specificity.

2.5. Breakpoint junction sequence analysis

Breakpoint analysis of the deletion was performed by PCR primer walking using HotStar Taq polymerase (Qiagen, Germantown, MD). PCR primers were designed from the *RAD21* reference sequence, GenBank Accession number NM_006265, across the deleted region derived from the array-CGH results assuming the most likely rearrangement. The junction fragment in Patient 1 was amplified using primers (Forward) 5′-ATGGCTGGTATATAGTTTCACCATAGCCTG-3′ and (Reverse) 5′-AACGTCTGATGCTACACAATGACCCAATAA-3′. PCR products were sequenced in both forward and reverse directions on an ABI 3730 DNA Analyzer (Life Technologies, Grand Island, NY). Sequences were compared to the *RAD21* reference sequence using Mutation Surveyor software version 3.01 (SoftGenetics, State College, PA).

3. Results

3.1. Clinical findings

3.1.1. Patient 1

Patient 1 was referred to genetics clinic at age 2 1/2 years for evaluation of developmental delay, severe hypospadias, inguinal hernia and some dysmorphic features. His birth weight was 2.9 kg (35th centile) and his birth length was 52 cm (90th centile). After birth, the patient was noted having hypospadias, bifid scrotum, undescended testes and bilateral inguinal hernia; all were surgically repaired at 15 months of age. The patient had congenital motor nystagmus, strabismus and right ptosis. The patient underwent surgical repair of strabismus and ptosis. He had normal hearing.

The patient exhibited some motor and language delays and received speech and developmental therapies. He started rolling over and sitting without support at 10 months, crawling at 12 months, and walking at 14 months. Occasionally, he walks on tiptoes. At age 3 years, the patient was able to speak 4-5-word phrases. He exhibited some autistic features including repetitive behaviors and interests, hand wringing, echolalia, aversion to touch and lining toys up. He received physical and speech therapy.

On physical examination at 3 years of age, the patient's weight was at the 19th centile, height at 31st centile, and occipitofrontal circumference (OFC) at 13th centile. He had scaphocephaly, coarse facial features, frontal bossing, mild synophrys, right ptosis, depressed nasal bridge, short nose, micrognathia, and posteriorly rotated ears (Figs. 1a, b). He had bitemporal sparse hair. He had shawl scrotum and status post hypospadias repair. There was mild 5th finger clinodactyly and overlapping of toes two over three. His neurological exam was normal except for tiptoe walking. His diagnostic workup included normal brain MRI, renal ultrasound, and echocardiography. Chromosomal microarray analysis and urine mucopolysaccharides and oligosaccharides were normal. Table 1 summarizes the clinical features of this patient.

The patient's mother was 42 years old. She has a history of myopia and back and hip pain that is controlled by medication. She has