Phenotypes and Genotypes of Individuals with RAD21 Variants


Lianne C. Krab [1,2,3]


Sylvia A. Huisman [1,4]

Author affiliations:
1. *Dept of Pediatrics and Amsterdam Expertise Center for Developmental Disorders, Amsterdam UMC, University of Amsterdam, the Netherlands*
2. *Cordaan, Outpatient Clinic for ID Medicine, Amsterdam*
3. *Odion, Outpatient Clinic for ID Medicine, Wormer, the Netherlands*
4. *Zodiak, Prinsenstichting, Purmerend, the Netherlands*

Adapted from:
Lianne C. Krab, ……., Sylvia A. Huisman, ……., Raoul C. Hennekam. Delineation of phenotypes and genotypes related to cohesin structural protein RAD21. Hum Genet. 2020 [Epub ahead of print]. https://doi.org/10.1007/s00439-020-02138-2

Abstract

Background: Variants in the cohesion subunit RAD21 have been associated with Cornelia de Lange Syndrome (CdLS). To date, limited information on phenotypes attributable to RAD21 variants and genotype-phenotype relationships is available.
We gathered a series of 49 individuals from 33 families with RAD21 alterations, including 24 hitherto unpublished cases. In this report, we focus on the phenotypes of the 29 individuals for whom detailed clinical information was available.

Phenotypes and genotypes: The phenotype of the 29 individuals with RAD21 variants is an attenuated CdLS phenotype compared to that caused by variants in NIPBL or SMC1A for facial morphology, limb anomalies, and especially for cognition and behavior. In the other 20 patients, phenotypes were diverse, with or without CdLS features. Variants (four microdeletions and sixteen intragenic sequence variants) were frequently familial, and genotype-phenotype analyses demonstrated striking variability even within families.

Impact for Intellectual Disability physicians: Careful phenotyping is essential in interpreting consequences of RAD21 variants. The current study should be helpful when counseling families with a RAD21 variation. Although this study reports the largest RAD21 cohort worldwide, RAD21 variants are still ultra-rare. The CdLS expertise center at the Amsterdam Expertise Center for Developmental Disorders at Amsterdam UMC can support ID physicians in the care for individuals with RAD21 variants.

Introduction

RAD21 (ENSG00000164754; OMIM *606462, chromosome 8q24.11) is a key component of the cohesion complex. It forms a tri-partite ring together with SMC1A and SMC3. The cohesin complex is a major modulator of chromosome structure. It is important in pivotal processes including mitosis, DNA repair, chromatin condensation, gene transcription and epigenetic silencing.[1, 2] Variants in genes encoding various structural or functional components of the cohesin complex have been implicated in Cornelia de Lange Syndrome (CdLS), most commonly NIPBL and SMC1A.[3] CdLS is characterized by distinct facial features, growth delay, microcephaly, limb reduction defects, usually moderate to severe intellectual disability (ID) and behavioral problems, especially self-injurious behavior (SIB) and autism spectrum disorder (ASD).[3]

To date, only nine sequence variants and five microdeletions in RAD21 have been reported in CdLS patients.[3] Based on this small number of cases, their phenotype seems less pronounced compared to individuals with variants in the other cohesin complex genes.[3-4] Several of the reported microdeletions in RAD21

overlap with genes next to RAD21 (contiguous gene syndrome), complicating attribution of the phenotype to RAD21.[5]

In addition to CdLS, RAD21 variants have been found in single families with in Mungan syndrome (Chronic Idiopatic Intestinal Pseudoobstruction; OMIM #611376)[6-7] and sclerocornea[8], and incidentally in cases with holoprosencephaly[9] However, relationship of the phenotypes with the RAD21 variant is not always clear.[8-9] Using a combination of literature search, database search and international network enquiry, we identified 219 cases with RAD21 variants, of which 49 patients from 33 families were included in the original study.[10] The 49 patients were divided into two cohorts based on whether sufficient clinical data was available to calculate CdLS score.(Kline et al., 2018) In this report, we will focus on the cohort of 29 patients (22 families, age range 0-61 years, 15 males) with sufficient clinical data, including 13 hitherto unpublished cases.

For these 29 patients with RAD21 variations, we provide information on clinical phenotype, including cognitive and behavioral functioning, genotype and interfamilial and intrafamilial variability. We compare the RAD21 phenotype to that of patients with NIPBL and SMC1A variants.[11-13] The original article[10] contains detailed genotypic and phenotypic data on all patients, structural and functional analysis of missense variants, and genotype-phenotype analysis, which will not be elaborated upon here.

Genotypes

The 22 families harbored twenty different RAD21 variants: four unique copy number variants (CNV's) including RAD21 and sixteen intragenic sequence variants (of which two were recurrent). The CNV's were all microdeletions, selected not to overlap with potentially obscuring morbid genes. Of the sixteen sequence variants, nine were predicted to be truncating (two nonsense, six frameshift and one splice site), four were missense and three in-frame deletions. Overall, the truncating variants were scattered over the RAD21 gene whereas the missense variants tended to locate at the protein binding domains. In the original study, pathogenicity of twelve intragenic RAD21 variants was evaluated by a combination of phenotype, protein modelling, and molecular dynamic studies, which supported pathogenicity for at least nine of these.[10] Seven out of seventeen variants for which inheritance was specified were familial.

Phenotypes

Physical phenotype in individuals with RAD21 variants

In this patient cohort, the majority of RAD21 variants lead to a CdLS phenotype (RAD21-CdLS) and if not, at least to a sufficient CdLS score to warrant molecular testing for CdLS.

Comparison to phenotypes of NIPBL and SMC1A variants

In patients with sufficient clinical data available, most features associated with CdLS are present. However, the prevalence of features is lower compared to those in the SMC1A and NIPBL cohort, and the degree of severity is typically less. Severe visual impairment and diaphragmatic hernias are rare in RAD21 patients, and feeding difficulties, present in the vast majority of CdLS neonates, are uncommon. RAD21 patients less frequently have increased body hair (hirsutism, bushy eyebrows, low scalp hair lines), major limb malformations are not reported, and hands and feet are generally of normal size. Still, minor anomalies of hands and feet are common, such as fetal pads, abnormal flexion crease patterns, and camptodactyly. Patients with RAD21 variants have generally less impaired growth at birth, and short stature and microcephaly often only develop postnatally. Prenatal microcephaly was not a predictor of more severe cognitive impairment in RAD21 patients. Frequency and severity of congenital heart defects is similar to those in the NIPBL and SMC1A cohorts. Gastro-esophageal reflux is similar in frequency but in RAD21 it is typically mild and restricted to early childhood. No RAD21 patients exhibit a Rett-like phenotype as can occur in a subgroup of patients with SMC1A variants.[11]

Unusual anomalies in the RAD21 cases are vertebral anomalies (clefts and hemivertebrae). Malformations of structures derived from the embryonic foregut are relatively frequent in RAD21 patients. Although one case in this cohort had holoprosencephaly, the prevalence of holoprosencephaly spectrum in RAD21-CdLS must remain uncertain as brain MRIs are typically not indicated in individuals with CdLS due to the burden of the procedure and lack of consequences of findings for care.[3]

Neurocognitive and behavioral phenotype

Most data on cognition and behavior in the present cohort are based on subjective information provided by physicians and not on formal testing. Therefore, reliability remains uncertain. Still, all data point to a lower prevalence and decreased severity of ID in RAD21 patients compared to NIPBL and SMC1A groups: developmental milestones are more frequently attained and cognitive level is estimated

higher (normal to only mildly impaired in over half of the cases; in contrast to about a third of those with SMC1A variants and a small minority of those with NIPBL variants). Over half of the patients show behavioral problems, mainly features of anxiety, ADHD, ASD and obsessive-compulsive behavior. However, aggression and ASD are less frequent, and SIB, a hallmark of CdLS in general [3], was reported in only one individual.

Even if an IQ is normal, subtle difficulties in neuropsychological domains known to be affected in CdLS[3] may influence cognitive performance. Periodic formal screening for neuropsychological and behavioral problems is still warranted in all individuals with RAD21 variants, to allow for early recognition of problems and access to relevant support systems. In addition, formal (in-person) assessments can prevent misdiagnoses, such as autism, by putting behavioral characteristics into perspective of the developmental level of patients.[12]

Natural history

The natural history data from the present study indicate that pregnancies and birth tend to progress normally, prenatal growth retardation being present in a small minority. About half of the patients have congenital anomalies (cleft palate; cardiac anomalies). Major limb defects have not been found; diaphragmatic hernia, anal atresia or choanal atresia occur occasionally. Patients typically have mild facial dysmorphisms, no small hands or feet, and increased body hair is less apparent compared to SMC1A and NIPBL patients. The clinical diagnosis of CdLS may therefore be difficult.

Neonatal feeding is usually not problematic. Reflux is common but not severe. Typical development is somewhat slow, mainly in speech development, and physical therapy or speech therapy may be indicated. As they grow up, children only occasionally develop new medical problems. Half of the children show a progressive but still mild growth delay in head circumference and height. Vision is mostly normal; hearing loss is found in a third of individuals and may require hearing devices. Most of the patients are able to attend regular education or education for children with mild cognitive disabilities. Most have some behavioral problems of limited severity, and aggression and SIB are uncommon. Not uncommonly, RAD21 patients are able to start a family, and some are only diagnosed when more severely affected offspring is recognized. This indicates that careful family analysis is paramount in each family in which someone is diagnosed with a RAD21 variant.

Explanation for inter- and intrafamilial variability

In the original article, attention is given to potential determinants of inter- and intrafamilial variability, including exogenous influences such as support and education, and epigenetic factors such as DNA methylation and gene silencing.10 Epigenetic factors are of particular interest, as genome-wide methylation patterns (epi-signatures) have been shown to be altered in CdLS.[14]

Counseling

In counseling of families with RAD21 variants, the relatively high frequency of familial occurrence and marked intrafamilial and interfamilial variability should be mentioned. Parental testing is warranted, even if signs or symptoms are apparently absent in parents, and standard testing of parents may further broaden the phenotype of RAD21 variants. We suggest a cautious use of data on variants in molecular databases, as due to the extremely variable and sometimes very mild phenotype wrong conclusions may be drawn in classifying the variants. In case of a CdLS phenotype and detection of a variant of unknown significance in RAD21 in which pathogenicity cannot be determined using clinical and molecular data of the parents, we recommend testing for variants in other CdLS associated genes and eventually carry out 'open' exome/genome sequencing in order to rule out variants in other genes.

Limitations

Although we used a broad search strategy and the present RAD21 cohort is the largest reported thus far, numbers are still small, and these preclude further statistical analyses. We may have an acquisition bias due to involvement of specialists in CdLS. This, in combination with selecting patients with sufficient clinical data only, can cause an overrepresentation of individuals with a CdLS phenotype. We expect that more widespread use of next generation sequencing based technologies will lead to identification of many additional patients with pathogenic RAD21 variants and milder phenotypes as clinical recognition may be difficult.

It should be noted that the original article contains several cases with different phenotypes including Mungan syndrome (biallelic variants), sclerocornea and schizophrenia, with or without CdLS features. Attribution to RAD21 is not always clear.[10] However, when we were able to retrieve more clinical data several patients did turn out to show CdLS features, suggesting the CdLS phenotype can be overlooked without careful phenotyping.

Recommendations for future research

The present results demonstrate that more information on larger groups of individuals with RAD21 variants is needed to determine the complete phenotypic spectrum. The frequency of CdLS characteristics such as sleep

disturbances and autonomic dysfunctions in individuals with RAD21 variants is still largely unknown. A specific issue that needs attention is the risk to develop cancer (incidentally reported to date in RAD21 patients).[4, 15] We also call for more detailed study of cognitive, behavioral and psychiatric phenotypes, as these are of utmost importance in clinical care.

Molecular and cellular mechanisms underlying cognitive problems are unclear, although cohesin-mediated 3D-organization of the genome is suggested to play a role in neuronal plasticity.[16] Studying RAD21 and other cohesin components in this process could contribute to the search for targeted influencing of cognition and especially behavior in CdLS. Effects of RAD21 variants on cellular functioning and relationships between genotype and phenotype may be elucidated further by studying epi-signatures. This may explain presently unexpected discrepancies between genotype and phenotype, and even allow for establishing pathogenicity in individuals with uncertain molecular findings.

Impact for ID physicians

CdLS is a very rare disorder (estimated incidence between 1 in 10,000 and 1 in 30,000 live births).[3] and this article focusses on a small subgroup of these patients. Despite the prediction that the incidence of CdLS is in fact higher due to as yet undiagnosed mild cases, RAD21 variants remain ultra-rare. Therefore, detailed physical, neurocognitive and behavioral phenotyping of patients as well as their family members is of utmost importance to understand genotype-genotype relationships and to offer the best possible care. Furthermore, detailed phenotypical data can help researchers to refine targets for translational studies, ultimately leading to targeted treatments. ID physicians have access to a wealth of information that can help further knowledge of a growing number of rare disorders. Researching these demands international collaboration, and this study with patients from thirteen different countries proved a very successful initiative and effective worldwide consortium. In the Netherlands, the Amsterdam Expertise Center for Developmental disorders at Amsterdam UMC includes a CdLS expertise center, which can support ID physicians in the care for individuals with RAD21 variants.

Lianne C. Krab, Cordaan, Outpatient Clinic for ID Medicine, Klinkerweg 81, 1033 PK Amsterdam, The Netherlands. lkrab@cordaan.nl. Phone: +31-6-24733226.

References

1. *Watrin E, Kaiser FJ, Wendt KS. Gene regulation and chromatin organization: relevance of cohesin mutations to human disease. Curr Opin Genet Dev. 2016;37:59-66.*
2. *Fisher JB, Peterson J, Reimer M, Stelloh C, Pulakanti K, Gerbec ZJ, et al. The cohesin subunit Rad21 is a negative regulator of hematopoietic self-renewal through epigenetic repression of Hoxa7 and Hoxa9. Leukemia. 2017;31(3):712-9.*
3. *Kline AD, Moss JF, Selicorni A, Bisgaard AM, Deardorff MA, Gillett PM, et al. Diagnosis and management of Cornelia de Lange syndrome: first international consensus statement. Nat Rev Genet. 2018;19(10):649-66.*
4. *Minor A, Shinawi M, Hogue JS, Vineyard M, Hamlin DR, Tan C, et al. Two novel RAD21 mutations in patients with mild Cornelia de Lange syndrome-like presentation and report of the first familial case. Gene. 2014;537(2):279-84.*
5. *Maas SM, Shaw AC, Bikker H, Ludecke HJ, van der Tuin K, Badura-Stronka M, et al. Phenotype and genotype in 103 patients with tricho-rhino-phalangeal syndrome. Eur J Med Genet. 2015;58(5):279-92.*
6. *Bonora E, Bianco F, Cordeddu L, Bamshad M, Francescatto L, Dowless D, et al. Mutations in RAD21 disrupt regulation of APOB in patients with chronic intestinal pseudo-obstruction. Gastroenterology. 2015;148(4):771-82 e11.*
7. *Mungan Z, Akyuz F, Bugra Z, Yonall O, Ozturk S, Acar A, et al. Familial visceral myopathy with pseudo-obstruction, megaduodenum, Barrett's esophagus, and cardiac abnormalities. Am J Gastroenterol. 2003;98(11):2556-60.*
8. *Zhang BN, Chan TCY, Tam POS, Liu Y, Pang CP, Jhanji V, et al. A Cohesin Subunit Variant Identified from a Peripheral Sclerocornea Pedigree. Dis Markers. 2019;2019:8781524.*
9. *Kruszka P, Berger SI, Casa V, Dekker MR, Gaesser J, Weiss K, et al. Cohesin complex-associated holoprosencephaly. Brain. 2019;142(9):2631-43.*
10. *Krab LC, Marcos-Alcalde I, Assaf M, Balasubramanian M, Andersen JB, Bisgaard AM, et al. Delineation of phenotypes and genotypes related to cohesin structural protein RAD21. Hum Genet. 2020 [Epub ahead of print].*
11. *Huisman S, Mulder PA, Redeker E, Bader I, Bisgaard AM, Brooks A, et al. Phenotypes and genotypes in individuals with SMC1A variants. Am J Med Genet A. 2017;173(8):2108-25.*
12. *Mulder PA, Huisman S, Landlust AM, Moss J, Consortium SA, Piening S, et al. Development, behaviour and autism in individuals with SMC1A variants. J Child Psychol Psychiatry. 2019;60(3):305-13.*
13. *Moss J, Penhallow J, Ansari M, Barton S, Bourn D, FitzPatrick DR, et al. Genotype-phenotype correlations in Cornelia de Lange syndrome: Behavioral characteristics and changes with age. Am J Med Genet A. 2017;173(6):1566-74.*
14. *Aref-Eshghi E, Kerkhof J, Pedro VP, Barat-Houari M, Ruiz- Pallares N, Andrau JC, et al. Evaluation of DNA methylation EpiSigns for diagnosis and phenotype correlations in 42 Mendelian neurodevelopmental disorders. Am J Hum Genet (in press). 2020.*
15. *Deardorff MA, Wilde JJ, Albrecht M, Dickinson E, Tennstedt S, Braunholz D, et al. RAD21 mutations cause a human cohesinopathy. Am J Hum Genet. 2012;90(6):1014-27.*
16. *Fujita Y, Yamashita T. Spatial organization of genome architecture in neuronal development and disease. Neurochem Int. 2018;119:49-56.*