The versatile RECQL4

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The human DNA helicase RECQL4 interacts in an array of intracellular regulatory pathways from the initiation of DNA replication, through maintaining genomic stability, to the N-end rule pathway. Interestingly, mutations in *RECQL4* have recently been revealed not only in Rothmund-Thomson-, but RAPADILINO-, and cases of Baller-Gerold syndrome also. Although these disorders represent distinct genetic entities, clinical observations have delineated highly variable expressivity and significant overlaps in the associated phenotypic manifestations. Consequently, it is especially difficult to draw precise genotype-phenotype correlations in RECQL4 related syndromes. This is likely due to the complex and multiple cellular networks RECQL4 is associated with. **Genet Med** 2006:8(4):213–216.

Key Words: RECQL4, Rothmund-Thomson syndrome, RAPADILINO, Baller-Gerold syndrome, helicase

INTRODUCTION

Helicases are a highly conserved group of enzymes that unwind RNA or DNA in an ATP-dependent manner. These proteins function in processes where access to single-stranded nucleic acid is required, including DNA replication, DNA repair and recombination, and transcription of RNA.[1,2] Humans and other mammals contain at least five different helicases of the RecQ family, named after the single *RecQ* gene of *Escherichia coli*.[3] Deficiencies of these proteins have been associated with genomic instability disorders of man with several features of premature aging and/or cancer predisposition.[4–6] Mutations in the *RECQ*-like genes *BLM*, *WRN*, and *RECQL4* can result in Bloom syndrome (MIM #210900), Werner syndrome (MIM #277700), and Rothmund-Thomson syndrome/RTS/(MIM #268400), respectively.[4] However, *RECQL4* stands unique among these genes in several respects, among which the most apparent to a medical geneticist is the fact that it has been related not only to RTS, but to RAPADILINO syndrome (MIM #266280), and to Baller-Gerold syndrome (MIM #218600) also.[7,8] Interestingly, significant overlaps may be present in the phenotypic manifestations of these *RECQL4*-related genetic disorders and phenotype-genotype correlations are hard to delineate.[8] This review intends to highlight the recent advancements in the molecular biology of RECQL4 in relationship to the observed significant phenotypic variability of the associated genetic syndromes.

RECQL4

The *RECQL4* gene in located on human chromosome 8q24.3, containing 21 exons in less than 6.5 kb of genomic sequence, with 13 introns that are <100 bp in length.[9] This peculiar gene structure predisposes mutated *RECQL4* mRNA to mis-splicing alterations resulting from the mutation in the primary gene transcript.[10] Consequently, intronic size constraint and point mutations in the introns of the *RECQL4* gene have been shown to play a role in the development of RTS in addition to exonic defects.[10–12]

Human RECQL4 encodes a 133 kDa protein of 1,208 amino acids that contains characteristic sequences of the RecQ-family's helicase domain. Namely, a central, highly conserved domain is present in these proteins that consists of 380 amino acids and includes a Walker A-box motif responsible for ATP hydrolysis. However, in contrast to the BLM and WRN helicases, the two C-terminal signature motifs characteristic for the RecQ family are absent from RECQL4. Similarly, the Nuclear Localization Signal residing near the C-terminus of BLM and WRN proteins has not yet been identified in RECQL4. On the other hand, the N-terminal region of RECQL4 uniquely bears homology to the yeast proteins Sld2/DRC1 that are essential for the establishment of replication forks. Yet, RECQL4 has not been shown thus far to actually possess an RNA- or DNA helicase activity.[13–16] At the cellular level, defects in *RECQL4* cause genome instability, particularly trisomy, aneuploidy and a high frequency of chromosomal rearrangements.[17] Additionally, somatic cells isolated from RTS individuals are sensitive to ionizing radiation.[18] Despite this knowledge, the molecular function of RECQL4 and the possible cellular pathways in which it is involved remained poorly understood until recently.

Last year, molecular studies have revealed that at least in HeLa cells, a proportion of RECQL4 forms a stable complex with components of N-end rule pathway ubiquitin ligases, UBR1 and UBR2 in the cytosol.[19] This pathway has been shown to be essential for chromosome stability in yeast through affecting the degradation of a cohesin sub-unit, that mediates the conditional association of sister chromatids in mitosis and meiosis.[20] Yet, the precise role of "cytosolic" RECQL4 remains to be elucidated.

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Studies on the nuclear localization of RECQL4 have demonstrated it to be localized in distinct nuclear foci that coincide with proteins involved in the maintenance of genome stability. Namely, RECQL4 foci coincide with those formed by pro-myelocytic leukemia (PML) bodies and RECQL4 forms a complex and co-localizes with Rad51 and regions of single-stranded DNA (ssDNA) upon induction of DNA double-strand breaks (DSBs).[21] PML body and Rad51 foci have been shown to regulate the response to, and repair of DSBs.[22,23] These observations suggest that RECQL4 plays a role in the repair of DSBs by homologous recombination.[21]

Xenopus laevis complementation studies have shown that RECQL4 likely plays a role in DNA replication initiation by also promoting the loading of replication factors at initiation sites.[13]

These observations clearly implicate RECQL4 to function in several aspects of genomic stability maintenance and suggest that it plays an important role in fine-tuning such complex processes as DNA replication initiation and double-strand break repair.

It has been earlier suggested that the expression of *RECQL4* is tissue-specific, being most prominent in the thymus and testis.[24] Others have failed to detect such differences in human tissues, but found that *RECQL4* expression is most prominent in the chondrocytes of developing bones and cartilage, as well as in immature, proliferating intestinal enterocytes of embryonic mice older than 15 days post-conception.[7] *RECQL4*-deficient mice develop growth retardation and skin abnormalities (i.e., hair loss, colorless hair, brittle skin) in addition to bone dysplasias, intestinal villus atrophy, dental dystrophy and premature aging (death within two weeks).[25] These results indicate that RECQL4 expression is tissue-specific, especially during embryonic and early postnatal development and that this expression pattern likely accounts for the phenotypic manifestations of genetic syndromes resulting from RECQL4 deficiency.

Rothmund-Thomson syndrome

Auguste Rothmund and Sydney Thomson described two separate medical conditions which were thought to be part of the same entity and consequently designated Rothmund-Thomson Syndrome (RTS) by William Taylor (MIM #268400).[26–28] This is a rare, autosomal recessive disorder characterized by a poikilodermatous rash starting in infancy, cataracts, growth retardation, skeletal abnormalities (i.e., dysplasias, absent or malformed bones [i.e., absent radii], osteopenia, and delayed bone formation), sparse hair and eyebrows, dental anomalies, gastrointestinal disturbances, as well as a high incidence of malignancies, especially osteosarcomas.[18,29,30] Indeed, osteosarcoma develops in approximately 30% of the patients with a median age of 11.5 years.[30] RTS patients have also been reported to rarely develop cutaneous squamous cell carcinoma, non-cutaneous malignancies and myelodysplasia.[31,32] Regardless of other anomalies, the clinical diagnosis of RTS rests on the manifestation of the poikilodermatous rash. The skin is usually normal at birth and the rash of RTS develops between age three

and six months as erythema, swelling, and blistering on the face spreading to the buttocks and extremities. The rash evolves over months to years into the chronic pattern of reticulated hypo- and hyperpigmentation, punctate atrophy, and telangiectases, collectively known as poikiloderma. Even patients with atypical rash or onset have been considered as RTS cases if they have additional features such as radial ray defect, loss of hair, cataracts, osteosarcoma or skeletal dysplasia (Fig. 1).[30] In 1999, Kitao et al. linked a subset of RTS cases to mutations in the human helicase *RECQL4*.[33] As earlier emphasized, observations in *RECQL4*-deficient mice supported the pathogenic role for this molecular defect in RTS.[25] A cohort study of 33 patients showed that in 60% of RTS cases, mutations of both *RECQL4* alleles can be found, and the overall *RECQL4* mutation detection rate was estimated to be 66% in RTS. Only one allele was found to carry a mis-sense or a deleterious mutation in another 30% of the individuals with this disorder. Additionally, linkage studies were negative in one family supporting genetic heterogeneity in this disorder and leaving the possibility of other RTS gene loci open.[29] Nevertheless, *RECQL4* analysis in the same cohort has revealed that all patients with RTS (and without cataracts) who developed osteosarcoma carried at least one deleterious (non-sense or frameshift) *RECQL4* mutation. Consequently, it was proposed that two types of RTS exist. Type I associated with poikiloderma and cataracts, but not with osteosarcoma (the form of RTS described by Rothmund); and Type II associated with osteosarcoma and deleterious mutations in *RECQL4*.[14,29] However, at least one RTS patient has been reported earlier in whom both juvenile cataracts and osteosarcoma developed, but unfortunately, *RECQL4* analysis for the proband was not described.[30] Nevertheless, these find-
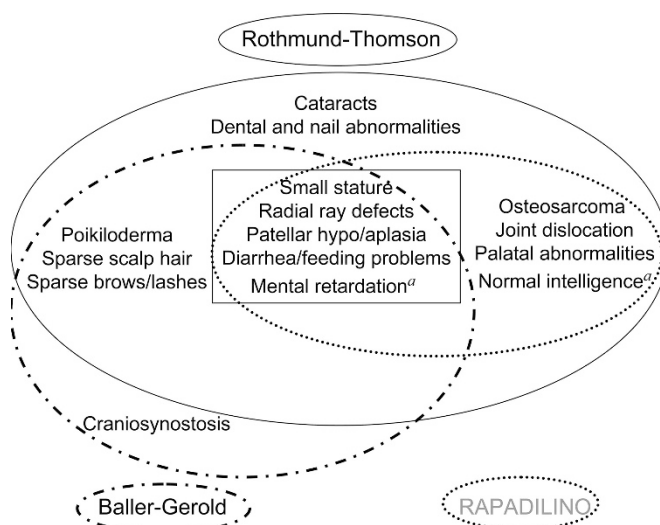

Fig. 1 The phenotypic spectrum of RECQL4 related genetic disorders. The clinical signs specific to each syndrome are circled (RTS: continuous line; BGS: dashed line; RAPADILINO: dotted line). The shared signs of the syndromes are framed in the center of the figure. *a*Mental retardation has only been incidentally noted in RTS and RAPADILINO, whereas affected patients usually have normal intelligence. While all features of RAPADILINO can be present in RTS, the specific absence of ectodermal symptoms and cataracts delineates RAPADILINO from RTS. The presence of craniosynostosis and the absence of cataracts appear to be the specific features of BGS.[8,14,37]

ings indicate that *RECQL4* analysis in RTS cases is warranted, and delineating genotype-phenotype correlations is of major importance in respect of prognosis and screening for osteosarcoma. Yet, such correlations have to be handled cautiously due to limited specificity.

RAPADILINO syndrome

A recent study described *RECQL4* mutations in patients with RAPADILINO syndrome (MIM #266280) also.[7] The acronym stands for the specific features of this rare autosomal recessive disorder: RAdial hypo-/aplasia, PAtellar hypo-/aplasia, cleft or highly arched PAlate, DIarrhea and DIslocated joints, LIttle size (<-2 SD in height) and LImb malformation, slender NOse and NOrmal intelligence.[34] This entity is most prevalent in Finland (with fourteen patients described), where in all of the observed cases, a specific splice site mutation of the *RECQL4* intron 7 (IVS7 + 2delT) was found in either homozygous or compound heterozygous form. This defect possibly leaves the helicase domain of the polypeptide intact and might only partially affect RECQL4 function. The preserved helicase activity may possibly lead to the phenotypic differences and to the lower tumor prevalence when comparing to the RTS patients.[7] Although several hallmarks of RAPADILINO have been described in RTS cases, their collective appearance and the peculiar absence of ectodermal symptoms (poikiloderma, sparse scalp hair, sparse brows and lashes) in RAPADILINO syndrome have distinguished it as a separate disorder (Fig. 1.).[7] Yet, a non-Finnish patient with RAPADILINO syndrome has been described earlier in whom poikiloderma developed, but the case was revisited later and classified as a severe form of RTS or a new syndrome.[35,36] Indeed, despite the differences, there are significant phenotypic overlaps between RTS and RAPADILINO. This observation was further supported by a recent report where a novel compound heterozygous *RECQL4* defect (g.2886delT/g.5435C>T) resulted in the phenotypic appearance of all RAPADILINO features in addition to poikiloderma.[37]

Baller-Gerold syndrome

The similarly rare autosomal recessive Baller-Gerold syndrome (BGS) (MIM #218600) was delineated following the description of patients with bilateral radial aplasia and craniosynostosis by Baller and Gerold.[38,39] Other possible phenotypic manifestations of this nosologic entity are short stature, epicanthal folds, hypertelorism, micrognathia, anus malformations (fistula, anteposition, imperforation) and renal anomalies. However, the hallmark of the disorder is lambdoid and coronal craniosynostosis in association with radial hypoplasia. Consequently, only a limited number of additional patients have been reported since the first descriptions, while several atypical forms of the syndrome have been reassigned to other disorders such as Fanconi anemia and Roberts SC phocomelia.[8] Even patients with characteristic features of Baller-Gerold syndrome can have mutations in the TWIST gene, which is typically associated with Saethre-Chotzen syndrome.[40] Additionally, *FGFR2* mutations may also mimic

BGS.[41] Such reports led to a narrowed re-definition of BGS based on the exclusion of cytogenetic and hematopoietic abnormalities, as well as the absence of TWIST and FGFR mutations in patients with craniosynostosis and pre-axial upper limb abnormalities.[8]

The development of poikiloderma in two unrelated patients with Baller-Gerold syndrome has prompted investigators to evaluate for *RECQL4* defects in these cases recently. *RECQL4* mutations were present in both patients, one of whom interestingly possessed the identical compound heterozygous defect (g.2886delT/g.5435C>T)[8] as the earlier reported proband mentioned above, in whom all features of RAPADILINO developed in addition to poikiloderma. It appears currently, that the presence of craniosynostosis is the only selective manifestation that delineates BGS from RTS (Fig. 1.).

DISCUSSION

Recent observations have expanded the number of clinical syndromes related to *RECQL4* mutations. Rothmund-Thomson, RAPADILINO and Baller-Gerold syndromes have all been linked to RECQL4 defects.[7,8,33] This fact in itself emphasizes the highly variable expressivity of *RECQL4* mutations. Most alterations of *RECQL4* found in RTS patients represent non-sense or frameshift mutations, but the exception to this rule is frequent.[29,33] Consequently, precise phenotype-genotype correlations are hard to delineate in the RECQL4 associated genetic disorders. Observations where identical mutations of *RECQL4* resulted in either RTS with all features of RAPADILINO[37] or Baller-Gerold syndrome[8] underscore this statement. Interestingly, even significant intra-familial variability was noted in the latter report.

The reason for the highly versatile expressivity may lie in the diverse molecular functioning of RECQL4, in addition to potential redundancy with variation in function of the five RECQ proteins, as well as possible proband specific variability in the expression pattern of *RECQL4*. The intricate networks of associated proteins (which regulate DNA replication initiation and genome stability[13,19,21]) are possibly fine-tuned differentially at the cellular, tissue, organ and organism levels. This flexibility may provide room for the variable manifestation of RECQL4 related defects. In this sense, the delineation of RT-, RAPADILINO-, and Baller-Gerold syndromes could be considered as solely semantics once *RECQL4* mutations are identified in the affected patients. Yet, the example of the IVS7 + 2delT mutation in RAPADILINO syndrome may indicate that specific *RECQL4* mutations can lead to specific phenotypic manifestations, such as the peculiar absence of poikiloderma. This is supported by the observation that despite of some exceptions, osteosarcoma in RTS is mostly associated with deleterious *RECQL4* mutations in a complex heterozygous form.[29] Consequently, further efforts should be made in drawing specific phenotype-genotype correlations to support the prognostics and tumor screening of patients with *RECQL4*-related syndromes.

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