A Patient With Rothmund-Thomson Syndrome and All Features of RAPADILINO

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Background: Mutations of the human helicase gene RECQL4 have been identified in a subset of patients with Rothmund-Thomson syndrome (RTS) and in children with the diagnosis of RAPADILINO syndrome (RADial hypoplasia/aplasia, PAtellar hypoplasia/aplasia, cleft or highly arched PAlate, Dfarrhea and DIslocated joints, Little size [>2 SDs below the mean in height] and LImb malformation, and slender NOse and NOrmal intelligence). While many features of the 2 genetic disorders overlap, poikiloderma—a hallmark of RTS—has been described as generally absent in RAPADILINO syndrome.

Observations: We report herein a patient with RTS who carries a truncating mutation and a newly identified missense mutation of RECQL4. The proband uniquely developed all criteria of RAPADILINO in addition to his prominent skin findings.

Conclusions: Patients with RTS may possess all features of RAPADILINO. Consequently, a genetic approach to RTS and RAPADILINO could be beneficial. This approach may provide a better understanding of the wide variety of related phenotypic findings and improve prognostics.

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Auguste Rothmund1 and Sydney Thomson2 described 2 separate medical conditions that were thought to be part of the same entity and consequently designated Rothmund-Thomson syndrome (RTS) by William Taylor3 (Online Mendelian Inheritance in Man [OMIM] 268400). This is a rare, autosomal recessive disorder characterized by a poikilodermatous rash starting in infancy, cataracts, growth retardation, skeletal abnormalities, hair loss, and a high incidence of malignancies, especially osteosarcomas.4,5 In 1999, Kitao and coworkers6 linked a subset of RTS cases to mutations in the human helicase gene RECQL4. Recent observations in Recql4-deficient mice support a pathogenic role for this molecular defect in RTS.7

RECQL4 is a member of the human RECQ helicase family. DNA helicases are a highly conserved group of enzymes that unwind DNA. These proteins function in all processes in which access to single-stranded DNA is required, including DNA replication, DNA repair and recombination, and transcription of RNA. Deficiencies of these proteins have been associated with genomic instability disorders in humans and with several features of premature aging and/or cancer predisposition.8-12 Mutations in the RECQ-like genes BLM, WRN, and RECQL4 can result in Bloom syndrome, Werner syndrome, and RTS, respectively.8

The RECQL4 gene contains 21 exons in less than 6.5 kilobases of genomic sequence with 13 introns that are less than 100 base pairs in length.11 In 1999, Kitao and coworkers6 linked a subset of RTS cases to mutations in the human helicase gene RECQL4. Recent observations in Recql4-deficient mice support a pathogenic role for this molecular defect in RTS.7

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The RECQL4 gene contains 21 exons in less than 6.5 kilobases of genomic sequence with 13 introns that are less than 100 base pairs in length.11 This peculiar gene structure predisposes mutated RECQL4 messenger RNA to diffuse mis-splicing alterations.12 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.12-14 A cohort study of 33 patients showed that in about 57% of RTS cases, mutations of both RECQL4 alleles can be detected. Only 1 allele was found to carry a missense or a deleterious mutation in another 27% of the individuals with this disorder.15 In addition, linkage findings were negative in 1 family.15 These observations reveal genetic heterogeneity in this disorder and leave open the possibility of other RTS gene loci.

Apart from RTS cases, a recent study found RECQL4 mutations in patients with...
RAPADILINO syndrome (OMIM 266280). The acronym stands for the specific features of this autosomal recessive disorder: RADial hypoplasia/aplasia, PAtellar hypoplasia/aplasia, cleft or highly arched PAlete, Diarrhea and DIstorted joints, Little size (>2 SDs below the mean in height) and Limb malformation, and slender NOse and NONormal intelligence. This entity is most prevalent in Finland, where in all observed cases a specific splice site mutation of the RECQL4 intron 7 was found in either homozygous or compound heterozygous form. Although several hallmarks of RAPADILINO have been reported in RTS cases, their collective appearance and the peculiar absence of ectodermal symptoms (poikiloderma, sparse scalp hair, and sparse brows and lashes) that are present in RAPADILINO syndrome have distinguished it as a separate disorder.

The prevalence of osteosarcoma for patients with RAPADILINO (7%) is apparently lower than for a cohort of patients with RTS and RECQL4 mutations. However, no study has clearly documented the risk of osteosarcoma over time in patients with RAPADILINO.

Patients with RTS have also been reported to develop cutaneous squamous cell carcinoma, noncutaneous malignancies, and myelodysplasia on rare occasions. In addition, there are more female than male patients with RAPADILINO syndrome, and they seem to be more severely affected, while patients with RTS are more commonly male. However, a non-Finnish patient with RAPADILINO syndrome has been described who developed poikiloderma, but the case was later revisited and reclassified as either a severe form of RTS or a new syndrome. Indeed, there are significant phenotypic overlaps between RTS and RAPADILINO. Consequently, the possibility that these diseases are subtypes of a single disorder has been considered.

**METHODS**

Verbal and written informed consent was obtained from the parents, who also authorized the scientific presentation of the data. RECQL4 polymerase chain reaction and direct sequencing were performed according to Siitonen et al. All of the exons of RECQL4 were sequenced. In addition, each intron except intron 12 was fully sequenced. However, exon-intron boundaries of intron 12 were analyzed. No splice site mutations or intronic deletions were found in the RECQL4 gene. Primers for mutation detection were ex7-9-F GTGGCCATGTGTTGTTCTTG and ex7-9-R TTAQQGACAGCATGCTT for exons 7 through 9 and ex17-19-F GTGGAAGACGAGTTGGAAGA and ex17-19-R CACTGATCGCACAGGAAGAAG for exons 17 through 19.

The NetGene2 program (http://www.cbs.dtu.dk/services/NetGene2/) was used to predict creation of a possible new splice site by the R1021W amino acid change. For alterations in exonic splicing enhancer (ESE) elements, the ESEfinder program (http://exon.cshl.edu/ESE/) was used. The mutation did not cause a new splice site recognition sequence or a significant effect on ESEs. Predictions for 4 splicing proteins, SF2/ASF, SC35, SRp40, and SRp55 were made. Only SRp55 had a putative binding site in the wild-type sequence from the mutated area. The score for this motif was 5.322 in the wild-type (5′TGCCGCGC3′), the threshold for the high-score motif being 2.676. As a consequence of the mutation, the score was 3.770 (5′TGCGCGC3′) for the same area. These results suggest the mutation does not have an effect on the splicing.

The male patient was born at 37 weeks’ gestation from the second pregnancy of a mother with multiple sclerosis. His birth weight was 1550 g (<third percentile); length, 38 cm (<third percentile); and head circumference, 31 cm (third to tenth percentile). Bilateral radial aplasia and absence of the thumbs was noted immediately after birth along with hypoplasias, bilateral inguinal hernia, prominent anterior fontanelle, slender nose, and micrognathia. At age 10 weeks, findings from his cardiac, ophthalmologic, and spinal radiographs and head ultrasound evaluations were negative. This workup was prompted by failure to thrive, which was accompanied by loose voluminous stools. His loose stools and poor growth persisted. At age 2 to 3 months, he developed a progressive, light-sensitive skin rash.

At age 22 months (length, 67 cm [<third percentile]; weight, 5620 g [<third percentile]), he was rehospitalized for failure to thrive and persistent diarrhea. His extensive workup revealed bilateral absence of the patellae, subluxation of the femoral heads, and prominent osteoporosis in addition to the earlier radiographic findings. An abdominal ultrasound showed the spleen to be localized at the upper pole of the left kidney in a circumferential position. Both lactose intolerance and fat malabsorption were detected. Through a genetic evaluation, he was diagnosed as having RAPADILINO syndrome, and the dermatologist’s opinion was that the patient had xeroderma pigmentosum. His chromosomal evaluation revealed a normal 46,XY karyotype with no signs of instability.

The patient’s diarrhea resolved by age 4 years. He underwent corrective surgical procedures to correct the deformities of his upper forelimbs, hypoplasias, and inguinal hernias. During a repeated dermatology evaluation, he was found to have poikiloderma, not xeroderma pigmentosum, and he was rediagnosed as having RTS.

On a follow-up genetic examination at age 9 years, the patient was a bright little boy who attended fourth grade with no difficulties and had a mildly hoarse voice. He was proportionately small: weight, 13 kg (<third percentile); height, 106 cm (<third percentile); and head circumference, 46.5 cm (>2 SDs below the mean). He had sparse hair with areas of alopecia, sparse eyebrows, a prominent forehead, slender nose, highly arched palate, and micrognathia. A striking upper limb abnormality was observed, as described herein. A prominent, diffuse dermatosis affected all parts of the body, characterized by variegated cutaneous pigmentation, atrophy, and telangiectasia. The dermatosis appeared more erythematosus on sun-exposed areas such as the face, and the parents reported extreme sun sensitivity.

**RECORDING A CASE**

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g.2886delT) segregated maternally and 1 (g.5435C→T) paternally. The mutations in the maternal allele have been found previously in 2 different RTS probands.12,19 The g.2886delT mutation causes a frameshift resulting in an early stop codon 97 codons downstream, while the g.2881G→C transversion is likely an associated single-nucleotide polymorphism being part of the founder haplotype. The paternal allele carries the g.5435C→T transition, which leads to a R1021W missense mutation.

This defect of RECQL4 has not been described previously, to our knowledge. A missense mutation affecting the same triplet but leading to another amino acid substitution (R1021Q) has been found in a patient with RTS who carried only 1 allelic mutation of RECQL4.15 This mutation does not affect a conserved amino acid or the helicase domain of RECQL4. Computer-assisted analysis of the mutation suggested that it does not affect splicing mechanisms either. However, it leads to the loss of the positively charged arginine, which is substituted by the hydrophobic neutral tryptophan in our case. Similarly, the mutation reported by Wang et al15 leads to the net loss of a positive charge at the same location (arginine to glutamine) of the polypeptide. This alteration could potentially affect the normal folding of the helicase or lead to the destruction of the misfolded protein or mislocalization of the protein in the cell. Unfortunately, a functional assay to determine the RECQL4 activity is not yet available.15

Despite significant phenotypic overlaps between RAPADILINO and RTS, distinct findings have separated the 2 disorders. The present case is unusual in that all criteria of RAPADILINO developed in addition to the ectodermal symptoms (Table). This observation shows that patients with RTS may develop significant extracutaneous findings that may lead to a diagnostic dilemma between RTS and RAPADILINO. The presence of poikiloderma, alopecia, and sparse eyebrows, which are characteristic of RTS, may be useful to distinguish the 2 disorders. Patients whose rash or its onset has been atypical have been considered to represent RTS cases if they have additional features such as radial ray defect, loss of hair, cataracts, osteosarcoma, or skeletal dysplasia.5 However, based on our findings and those of others,23,24 we believe that genetic evaluation is important in probands who phenotypically possess features of RAPADILINO and/or RTS.

A recent cohort showed that all patients with RTS who developed osteosarcoma had at least 1 truncating RECQL4 mutation.19 On the contrary, patients with RAPADILINO have been shown to carry the IVS7 + 2delT mutation leading to inframe skipping of exon 7, either in a 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 lead to the phenotypic differences and to the lower tumor prevalence than is found in RTS.16 Consequently, designating these disorders as RECQL4 diseases should prompt a careful molecular genetic evaluation of this helicase in each new case. This approach may be more useful for prognostics and determining the need for careful tumor surveillance in the affected patients than distinguishing the probands solely by phenotypic features.

It is interesting to note that the patient of the present report has 1 truncating RECQL4 mutation and 1 missense mutation of a nonconserved amino acid outside the helicase domain. However, we cannot exclude the possibility that our sequence analysis may have missed another pathogenic mutation affecting the same allele as the R1021W mutation. Nevertheless, a similar defect has been reported in 1 earlier RTS case, but its significance has been questioned.15 The detection of the second type of base substitution affecting the charge of the same amino acid argues for a pathogenic role for this type of mutation in RECQL4 disease. Yet the real effect of this amino acid change on the protein is not known. Whether it only partially affects RECQL4 function, which has been proposed for the IVS7 + 2delT mutation found in the Finnish RAPADILINO probands or leads to a more severe defect of RECQL4 remains to be solved. Further genetic evaluation of the patients with mutated RECQL4 and better understanding of RECQL4 function will elucidate these questions.

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REFERENCES


Table. Comparison of the Clinical Features of RTS, RAPADILINO, and the Present Case

<table>
<thead>
<tr>
<th>Clinical Finding</th>
<th>RTS12 (n = 42)</th>
<th>RAPADILINO15 (n = 14)</th>
<th>Present Case</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male sex</td>
<td>69</td>
<td>29</td>
<td>+</td>
</tr>
<tr>
<td>Small stature</td>
<td>66</td>
<td>93</td>
<td>+</td>
</tr>
<tr>
<td>Poikiloderma</td>
<td>100</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td>Sparse scalp hair</td>
<td>51</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td>Sparse eyebrows/eyelashes</td>
<td>74</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td>Radial ray defects</td>
<td>22</td>
<td>100</td>
<td>+</td>
</tr>
<tr>
<td>Cataracts</td>
<td>6</td>
<td>0</td>
<td>−</td>
</tr>
<tr>
<td>Osteosarcoma</td>
<td>31</td>
<td>7</td>
<td>−†</td>
</tr>
<tr>
<td>Patellar hypoplasia/aplasia</td>
<td>2</td>
<td>86</td>
<td>+</td>
</tr>
<tr>
<td>Joint dislocation</td>
<td>In some</td>
<td>57</td>
<td>+</td>
</tr>
<tr>
<td>Diarrhea/feeding problems</td>
<td>17</td>
<td>86</td>
<td>+</td>
</tr>
<tr>
<td>Normal intelligence</td>
<td>95</td>
<td>85</td>
<td>+</td>
</tr>
</tbody>
</table>

Abbreviations: RTS, RAdial hypoplasia/aplasia, PATellar hypoplasia/aplasia, clef or highly arched PAtelle, DiarRhea and DiRtlocated joints, Little size (>2 SDs below the mean in height) and Limb malformation, and slender NOse and NOrmal intelligence; RTS, Rothmund-Thomson syndrome; +, clinical finding present; −, clinical finding absent. *Unless otherwise noted, data are reported as percentage of patients with the noted characteristic in the study population. †The patient was only 9 years old at the time of the evaluation.

COMMENT