


Report of Two Novel Mutations in Indian Patients with Rothmund–Thomson Syndrome

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Abstract

Rothmund–Thomson syndrome (RTS) is a rare autosomal recessive disorder caused by mutations in *RECQL4* and has characteristic clinical features. We report two unrelated phenotypically diverse patients (cases 1 and 2) with RTS having novel variants in *RECQL4* gene. Case-1 was evaluated for poor growth and recurrent fractures and skin lesions. Case-2 presented at 4 months with failure to thrive and radial ray defect and developed poikilodermatous skin lesions after infancy. Both cases were confirmed to have homozygous pathogenic variants in *RECQL4*. Both patients have normal intellect and are on supportive therapy. The presence of characteristic poikiloderma lesions with specific distribution and skeletal anomalies in a patient with proportionate short stature is a clue toward the diagnosis of RTS.

Keywords

- ▶ Rothmund–Thomson syndrome
- ▶ *RECQL4*
- ▶ poikiloderma

Introduction

Rothmund–Thomson syndrome (RTS; OMIM 268400) is a rare genetic disorder characterized by distinctive skin lesions of poikiloderma, skeletal abnormalities, failure to thrive and high risk for developing cancers.¹ Two-thirds of RTS cases are caused by pathogenic variants in *RECQL4* on 8q24, which encodes for ATP-dependent DNA-helicase Q4 that bears homology to RecQ helicases and has a pivotal role in maintaining chromosomal integrity. Two other disorders with overlapping clinical features associated with mutation in *RECQL4* gene are RAPADILINO, that is, “radial ray defect, patellar aplasia, diarrhea, little size and limb anomalies, slender nose and normal intelligence” (OMIM-266280) and Baller–Gerold syndrome (OMIM-218600). Early diagnosis needs a high index of suspicion and close follow-up is necessary. Here we report two Indian cases with RTS with novel pathogenic variants in *RECQL4*.

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Patient 1

This 8-year-old male child was born to a nonconsanguineous couple. He presented with poor growth and recurrent fractures of right lower and left upper limb from the age of 4 years. He had history of multiple hyperpigmented skin lesions involving his face and bilateral upper and lower limbs associated with photosensitivity. There was no history of preceding erythema with blister formation. He achieved developmental milestones at the appropriate age and was intellectually normal. He was born at 38 weeks of gestation with a low birth weight of 1.5 kg. At 8 years, his weight was 16 kg (<3rd centile), height 116 cm (3–10th centile) according to Indian Academy of Pediatrics (IAP), 2015 standards² and upper to lower segment ratio was 1.05. Examination revealed a dysmorphic face with pointed nose, malocclusion of teeth, and receding chin (▶ Fig. 1A and B). Skin examination showed diffuse hyperpigmentation present in a reticular pattern, with superimposed discrete hypopigmented atrophic macules of 3 to 5 mm. Telangiectasia was noted over the cheeks and

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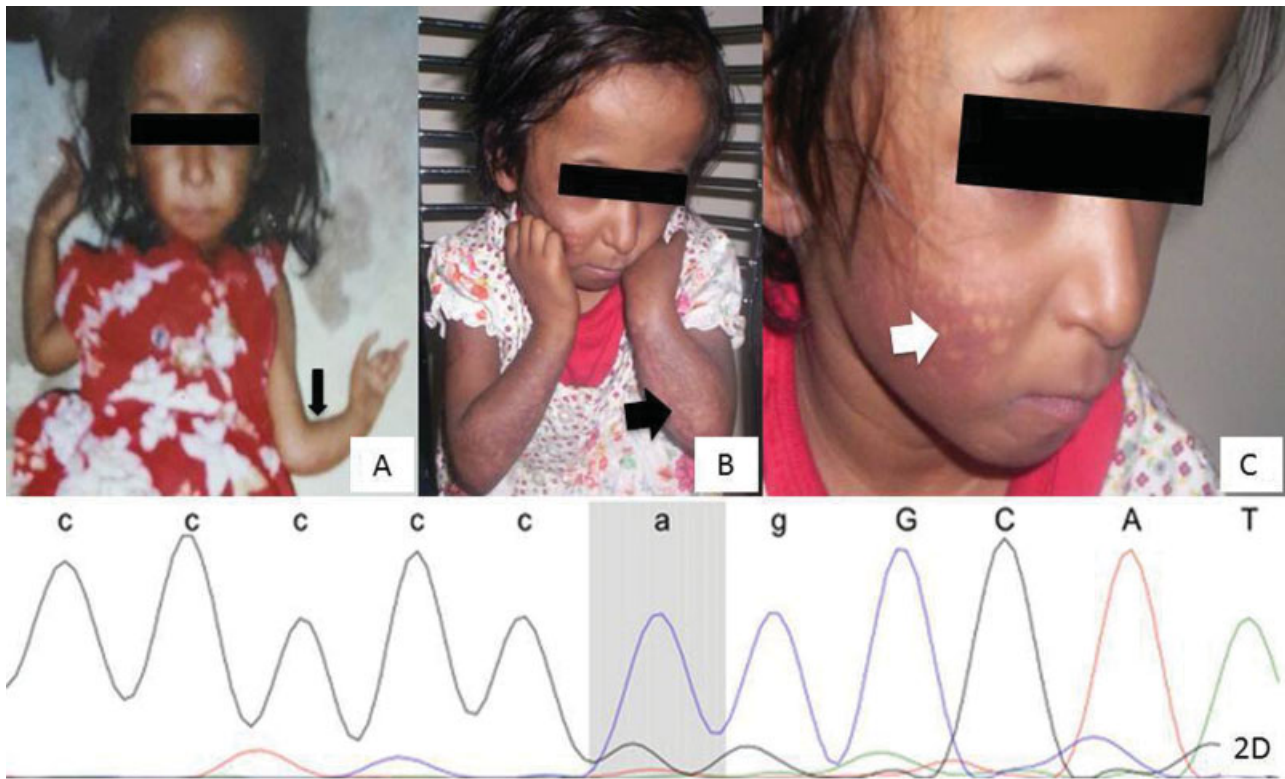


Fig. 2 Patient 2: (A–C) patient 2 at 4 months (A) and 18 months of age (B and C) with a small pointed nose, smooth philtrum, and thin upper lip. Note absent thumbs and short bent left forearm (thin black arrowhead). Note the poikilodermatous skin lesions over the face (thick white arrowhead) and extensor aspects of the forearms (thick black arrowhead). (D) Sanger sequencing of the variant, c.1132–2A > G, IVS5–2A > G, detected in intron 5 in a homozygous state. The forward reference sequence is shown on the top line, with lower-case letters belonging to intronic and upper-case letters to exonic sequence. The peaks were obtained from sequencing of the reverse strand.

of the upper limb showed the presence of hypoplastic middle phalanges of the first and fourth fingers, bilateral curved ulnae, and hypoplastic radii. Her cardiac and renal evaluations were normal. Her initial workup including karyotype, neuroimaging, echocardiography, abdominal ultrasound, and testing for gastro esophageal reflux disease were normal. Chromosomal studies on peripheral blood lymphocytes were also performed using Mitomycin C to induce chromosomal breakage. The number of chromosomal breaks was counted in 100 metaphases in both this child and a control individual. This test is considered positive when the number of breaks is approximately 10 times higher in the patient compared with the control. In this patient, the results were negative. A growth hormone stimulation test using clonidine was indicated an insufficient response. In view of the dysmorphic features, absent thumbs and typical rash (poikiloderma), mutation analysis of *RECQL4* was instigated. All coding exons and flanking intronic sequences of *RECQL4* (Genbank NM_004260.3, NC_000008.10) were amplified by PCR and sequenced directly (Sanger’s sequencing) with internal or flanking primers as described by Kitao et al.⁵ *RECQL4* deletion testing was performed by quantitative real-time PCR with six intragenic amplicons as described by Borozdin et al.⁶ *RECQL4* sequencing showed the presence of a likely pathogenic homozygous variant c.1132–2A > G in intron 5 which is not reported in 1,000 genomes and ExAC databases and is reported once in a heterozygous state (1 of 246,158 alleles) in

gnomAD (>Fig. 2D). This mutation disrupts the canonical splice acceptor site of intron 5, thereby likely resulting in use of a cryptic splice site or exon skipping with the possible consequence of mRNA degradation or formation of a truncated protein. No deletion within or including *RECQL4* was detected. The parents could not be tested for this variant. Currently, the patient is 11.5 years old and has normal intellect.

Both the children were advised to follow strict photo protection and to use a broad-spectrum sunscreen with UV (ultraviolet) A and B protection. They were also advised to have regular follow-up to monitor their growth and skin lesions to look for any change in color or texture. Annual eye examination was also suggested. The parents were given the option of prenatal testing in future pregnancies.

Discussion

RECQL4 has an important role in DNA stability, DNA replication initiation, and double strand break repair.^{7,8} Homozygous/compound heterozygous mutations in *RECQL4* cause three allelic disorders. RAPADILINO syndrome is characterized by skeletal and palatal defects along with gastrointestinal abnormalities.⁹ Baller–Gerold syndrome includes radial ray defects, skeletal dysplasia, short stature, and craniosynostosis. The diagnostic features of RTS and RAPADILINO overlap due to common skeletal abnormalities and gastrointestinal symptoms,

Table 1 Comparison of clinical features of the affected individuals and *RECQL4* related disorders

Features	Patient-1	Patient-2	RTS	RAPADILINO syndrome	Baller–Gerold syndrome
Low birth weight	+	+	+	+	+
Poor growth	+	+	+	+	+
Facial features, palatal defects	Pointed nose, fine eyebrows, receding chin, no palatal defects	Small pointed nose, fine eyebrows, smooth philtrum, thin upper lip, no palatal defect.	Pinched nose, sparse hair, fine eyebrow, palatal defects reported in few patients.	Palatal defects present	Saddle nose, small mouth with thin vermilion border, high arched palate.
Radial defects	–	+	+	+	+
Osteopenia and fractures	+	–	+	+	–
Poikiloderma	+	+	+	–	+
Café au lait spot	–	–	–	+	
Craniosynostosis	–	–	–	–	+
Patellar abnormalities	–	–	+	+	+
Alopecia and sparse eye brows/eyelashes	+	+	+	–	–
Eye abnormality	–	–	Cataract	Blepharophimosis	Proptosis (secondary to craniosynostosis)
Gastro intestinal abnormalities (recurrent diarrhea)	–	+	+	+	–

Abbreviations: RTS, Rothmund–Thomson syndrome; RAPADILINO, radial ray defect, patellar aplasia, diarrhea, little size and limb anomalies, slender nose and normal intelligence, “–” absent, “+” present.

but RAPADILINO lacks the characteristic poikiloderma rash, though late onset of the characteristic rash of RTS can further delay the specific clinical diagnosis (→ **Table 1**).

RTS is characterized by cutaneous rash, which presents as blisters and erythema, initially involving the face, and gradually spreads to the extremities and buttocks. With time, the rash develops into a chronic form known as poikiloderma that includes reticulated areas of depigmentation, hyperpigmentation, punctate atrophy, and telangiectasia.¹⁰ Although 90% of cases present in the first few years of life, cases have been reported with late onset poikiloderma.¹¹ Other features include café au lait spots, palmoplantar hyperkeratotic lesions, brittle nails, sparse and brittle scalp hair, and sparse eyebrows. Dental abnormalities including microdontia, oligodontia, delayed eruption, malocclusion, and crown malformations¹² may also be present.

Another major hallmark of RTS are various skeletal anomalies, seen in 68 to 75% of patients, including congenital radial ray defects, hypoplasia/agenesis of the patella and generalized osteopenia.¹³ A study by Cao et al¹⁴ demonstrated a positive correlation between the presence of pathogenic mutations in *RECQL4* and lumbar spine areal bone mineral density for age Z score ≤ -2.0 with number of fractures. Among patients with fractures and osteopenia, 50% had nonsense mutations resulting in a truncated protein. Through mouse models, it was postulated that loss *RECQL4* could affect osteoblast progenitor cells and reduce osteoid

formation and hence produce weak bones. Similarly, patient-1 had a truncating mutation and presented with subtle features of RTS with low bone mineral density and a history of fractures along with skin rash.

Patient-2 also had onset of skin lesions at 1 year and was initially misclassified as Holt–Oram syndrome in the absence of skin lesions and a molecular diagnosis. The development of facial lesions led to diagnosis to RTS which changed management advice. → **Table 1** shows comparison of clinical features of the two patients in this study and *RECQL4* related syndromes.

RECQL4 is one of the members of RecQ gene family of DNA helicases that have an important role in maintaining genomic stability and homeostasis. Loss of function mutations in *RECQL4* occurs in approximately two-thirds of RTS patients. Mutations in *RECQL4* lead to impaired response to oxidative stress induced DNA damage and a base excision repair defect. Mouse models have shown its differential expression in developing bones, skin, cartilage, and enterocytes which may be responsible for significant phenotypic variability of the associated genetic syndromes.¹⁵

Mutations in *RECQL4* are found in approximately 40 to 66% of RTS patients.¹⁶ Most of the mutations in *RECQL4* are truncating due to nonsense, splicing or frameshift mutations. In our study, we identified a frameshift (c.978_979delT-CinsG) and splice (c.1132–2A > G) variants in two individual cases. The diagnosis of RTS in both of our cases was confirmed by homozygous variants detected in *RECQL4*. One of

the common mutations in the Finnish population is c.1390 + 2delT (p.Ala420_Ala463del). More than 50% of these mutations are predicted to destroy the reading frame and thus disrupt the helicase domain which is essential for gene function. Truncating mutations are mostly associated with RTS and Baller–Gerold syndrome, although a few cases of RAPADILINO have been reported with truncating mutations. However, no clear genotype phenotype correlation is observed among the type of mutations and associated phenotype.¹⁷

Conclusion

To conclude, this article describes the need for careful evaluation of the dermatological lesions and subtle facial features associated with *RECQL4* related disorders. The presence of absent radii may act as a masquerader for allelic, as well as other common disorders of radial aplasia/hypoplasia such as Holt–Oram syndrome, and Thrombocytopenia Absent Radii (TAR) syndrome; however, careful follow-up in the clinical setting for the development of skin lesions and molecular evaluation is helpful for making a specific diagnosis.

Note

No disclaimers. Consent for publication of their details and photographs have been taken from the parents of both the children. There is no conflict of interest among all authors.

Author Contribution

S.Y. reviewed the literature and drafted the initial version of the manuscript. N.B. helped assess the dermatological manifestations. N.G. and S.T. helped in the acquisition of data. J.K. performed molecular testing. N.G., M.K., and S.T. contributed to literature review and critically revised the manuscript. All the authors contributed to drafting of the manuscript and approved the final version of the manuscript.

Conflict of Interest

None declared.

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