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Dyskeratosis Congenita Caused by a Novel TERT Point Mutation in Siblings With Pancytopenia and Exudative Retinopathy

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Abstract

Two siblings presenting with exudative retinopathy, thrombocytopenia, and macrocytosis were found to have markedly shortened telomeres and a previously unreported inherited mutation in TERT, c.2603A>G. Revesz syndrome, a subtype of dyskeratosis congenita (DC) caused by *TINF2* mutation, combines marrow failure with exudative retinopathy, intracranial calcifications, and neurocognitive impairment. As our patients manifested neither intracranial calcification nor significant neurocognitive impairment, we conclude that the c.2603A>G TERT mutation may define a subtype of DC manifesting first as exudative retinopathy without other signs of DC. Children with exudative retinopathy should be periodically screened for macrocytosis and cytopenias to evaluate for underlying DC.

Keywords

bone marrow failure; dyskeratosis congenita; retinopathy; telomerase

INTRODUCTION

Dyskeratosis congenita (DC) is an inherited marrow failure syndrome caused by defective telomere maintenance, caused by mutations in at least eight genes: *CTCI*, *DKC1*, *TERC*, *TERT*, *TINF2*, *NHP2*, *NOP10*, and *WRAP53* [1]. Inheritance can be X-linked recessive, autosomal dominant, or autosomal recessive depending on the molecular defect [2]. DC is characterized by marrow failure, pulmonary fibrosis and a variety of gene-specific defects including neurodevelopmental impairment, cancer predisposition, and ectodermal (hair, skin, nail) findings [3]. Revesz syndrome (RS) is a subtype of DC defined by marrow

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failure, bilateral exudative retinopathy, severe neurodevelopmental delay, and radiologic evidence of intracranial calcifications [4]. Herein, we report the cases of two siblings whose exudative retinopathy diagnosed as familial exudative vitreo-retinopathy (FEVR) was followed by marrow failure. Findings of markedly shortened telomere length and restrictive pulmonary disease led to the diagnosis of DC. Both patients carry a mutation in *TERT* (c.2603A>G(p.D868G)), which we posit may be associated with a phenotype of exudative retinopathy and progressive marrow failure without the severe neurocognitive phenotype more typical of *TINF2*-affected patients with RS.

CASE VIGNETTE

A 12-year-old male with a 9-year history of FEVR presented with scattered petechiae and purpura along with diminished visual acuity, esotropia of the left eye, and a faint hypopigmented rash on anterior chest. Height, weight, oral mucosa, hair, and nails were normal. School performance and development were appropriate, though he had mild verbal delay in early childhood (first word at 15 months) for which he underwent speech therapy. Family history was notable for three maternal female second cousins with *MUNC13-4*-associated familial hemophagocytic lymphohistiocytosis. Hematologic findings are shown (Table I). Anti-platelet antibody testing was negative and serum folate, red cell folate, and serum vitamin B₁₂ levels were normal. Coomb's testing was negative, and hemoglobin electrophoresis was notable for mildly increased fetal hemoglobin (>95% A₁, 2.5% A₂, 2% F). The patient's parents were both in good health, as was his 10-year-old sister aside from being diagnosed with FEVR 3 years prior. Both he and his sister had undergone multiple ocular procedures to treat retinopathy and to preserve vision.

Roughly 3 months after the onset of increased bruising, the patient's sister also developed petechiae. Her CBC results were similar to her brother's (Table I). Screening blood work of the parents (both healthy, without petechiae and without evidence of ocular disease by fluorescein angiography) revealed mild macrocytosis (100 fl) and borderline thrombocytopenia (167 platelets/ μ l) of the father (Table I). Each child had a diffusely hypocellular (10–30%) bone marrow without dysplasia, malignancy or fibrosis (Figs. 1A and B). Cytogenetic and FISH studies were negative for monosomy 5, monosomy 7, trisomy 8, or for deletions in 5q, 7q, or 20q. Fanconi anemia and paroxysmal nocturnal hemoglobinuria screening were negative. A telomeric length study found that each child had extremely shortened telomeres, well below the 1st percentile for age, consistent with the diagnosis of DC (Fig. 1C) [5]. Both siblings had mild restrictive defects by pulmonary function testing (Table I) and changes on high-resolution chest CT suggestive of early pulmonary fibrosis.

Genetic testing for known DC-causing genes (*DKC1*, *TERC*, *TERT*, *TINF2*, *NOLA2/NHP2*, *NOLA3/NOP10*, and *TCAB1/WRAP53*) revealed that each child had a previously unreported missense mutation (A $\Rightarrow$ G at position 2603 of the cDNA) in one allele of *TERT*, the gene that encodes the reverse transcriptase component of telomerase [6] (Supplemental Fig. 1) This point mutation predicted the incorporation of glycine (G) instead of aspartic acid (D) at amino acid position 868 of the mature telomerase protein. Although his *TERT* genotype was the same, their father's telomere length was not as severely affected (Fig. 1C)

and he had a milder pulmonary phenotype (Table I). Both children were managed with platelet transfusions given under a threshold of 40,000–50,000 platelets/ μ l blood in order to minimize hemorrhaging associated with exudative retinopathy followed by HLA-matched allogeneic stem cell transplantation with a fludarabine, melphalan, and alemtuzumab conditioning regimen.

DISCUSSION

We report two cases of DC in siblings who presented with exudative retinopathy followed by marrow failure. Each was found to harbor a previously undescribed mutation in telomerase, the specialized polymerase enzyme that adds oligomeric repeats (TTAGGG) to 3' ends of lagging strands of DNA during replication to maintain proper telomeric structure [7]. Without telomerase, chromosomal DNA ends shorten with each replication until reaching a critical length that induces cellular senescence [8,9].

Telomerase is composed of two core components, TERC and TERT. TERC serves as the RNA template on which TERT, the enzymatic reverse transcriptase component catalyzes addition of nucleotides. Single amino acid substitutions in TERT, as is the case in this kindred, have been associated with DC and are known to cause telomere shortening through haploinsufficiency [10,11]. The mutation found in our kindred, *TERT*(c.2603A>G (p.D868G)) has not been described before, and results in the replacement of a phosphomimetic and acidic residue (D) with a non-polar residue (G) at position 868 of the telomerase protein. Coincidentally, residue 868 is predicted to reside in a putative active site of the catalytic reverse transcriptase domain of TERT (Supplemental Fig. 1). In silico analysis using two independent methods (PolyPhen v2 [12] and SIFT (Sorting Intolerant From Tolerant [13])) predicted that this mutation would be deleterious to telomerase structure/function with a high degree of confidence. Although functional studies have not yet been done on c.2603A>G (p.D868G) telomerase, we hypothesize that this mutation may lead to diminished telomerase protein activity, perhaps through interference with its reverse transcriptase enzymatic activity.

Interestingly, there appears to be genetic anticipation in the family, with worsening phenotype evident in the children when compared to their father. Thus, even though the father carried the same mutation, his phenotype (borderline thrombocytopenia, macrocytosis, premature hair graying, milder pulmonary disease, no retinopathy) was much milder than that of his children who had a much more severe phenotype and were roughly three decades younger than him at the time of their presentation. Genetic anticipation has been described in other cases of autosomal dominant cases of DC, presumably because of telomerase haploinsufficiency and decreased telomere length over successive generations [10,14].

Exudative retinopathy has been described in certain subtypes of DC [15–18].

RS is a rare variant of DC caused by mutations in *TINF2* that impact telomere maintenance [19]. Along with eventual marrow failure, RS patients typically present with bilateral exudative retinopathy, intrauterine growth retardation, intracranial calcifications, cerebellar

hypoplasia, and significant neurocognitive impairment [4]. Our patients presented with exudative retinopathy, at 2–3 years of age for the male and at 7–8 years of age for the female. Only after their ocular problems were longstanding and managed as FEVR did signs of marrow failure suggest the broader underlying diagnosis of DC. It is possible that their mutation, *TERT*(c.2603A>G (p.D868G)), may define a new subgroup of Revesz-like patients who share telomerase-associated retinopathy and marrow failure but are spared significant neurologic impairment characteristic of Revesz patients with *TINF2* mutations.

DC is a heterogeneous disease whose diagnosis can be challenging. Our patients' cases suggest that DC be considered in children affected by exudative retinopathy. Annual screening CBC's to detect macrocytosis or cytopenias and careful physical examination focusing on ectodermal tissues may facilitate early detection of DC to optimize care and provide timely genetic information to affected families.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.

Diagnostic marrow and telomere studies. **A,B:** Representative bone marrow core biopsy result from the 12-year-old boy (A) and his 10-year-old sister (B) revealing hypocellularity (10–30% for each) without evidence of cellular dysplasia, hematopoietic arrest, malignancy, fibrosis, or granulomas. **C:** Telomere length measurements in peripheral blood cell populations of both children and their parents. Circles represent females, squares represent males, and filled shapes represent individuals eventually found to have the c.2603A>G *TERT* mutation. M: 42-year-old mother, F: 42-year-old father, S: 12-year-old son, and D: 10-year-old daughter. Note the presence of markedly shortened telomere length in both children, the borderline-low telomere length in the father and normal telomere length in the unaffected mother. Telomere length measurements were performed by Flow-FISH procedure by Repeat Diagnostics, Inc. as published [20].

TABLE I**Presenting Hematologic and Pulmonary Function Testing Data**

Lab value	Son (12 y/o)	Daughter (10 y/o)	Father (44 y/o)	Mother (44 y/o)
Hematologic indices				
Total white cell count ($\times 10^3/\mu\text{l}$)	3.9	3.0	4.3	4.9
Hemoglobin (g/dl)	11.8	11.8	14.0	14.9
Hematocrit (%)	34.6	33.3	41.8	43.7
Platelet count ($\times 10^3/\mu\text{l}$)	62	58	183	234
Mean corpuscular volume (fl)	101	98	99	95
Absolute neutrophil count ($\times 10^3/\mu\text{l}$)	1.69	0.82	1.63	2.91
Pulmonary function testing (% of predicted values for age, gender, height, weight and ethnicity)				
FVC	75%	86%	94%	—
FEV1	75%	92%	88%	—
FEV1/FVC	85%	94%	74%	—
FEF 25–75%	68%	102%	76%	—
PEF	55%	90%	91%	—
TLC	78%	87%	99%	—
IC	78%	91%	120%	—
FRC	78%	89%	82%	—
DLCO	120%	68%	62%	—

FVC, forced vital capacity; FEV1, forced expiratory volume at 1 second; FEF 25–75%, forced expiratory flow 25–75%; PEF, peak expiratory flow; TLC, total lung capacity; IC, inspiratory capacity; FRC, functional residual capacity; DLCO, diffusing capacity.