Published in final edited form as:

Pediatr Blood Cancer. 2012 August ; 59(2): 311–314. doi:10.1002/pbc.24193.

CTC1 Mutations in a Patient with Dyskeratosis Congenita

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Abstract

Dyskeratosis congenita (DC) is a rare inherited bone marrow failure syndrome caused by mutations in 7 genes involved in telomere biology, with approximately 50% of cases remaining genetically uncharacterized. We report a patient with classic DC carrying a compound heterozygous mutation in the *CTC1* (conserved telomere maintenance component 1) gene, which has recently implicated in the pleiotropic syndrome Coats plus. This report confirms a molecular link between DC and Coats plus and expands the genotype-phenotype complexity observed in telomere-related genetic disorders.

Keywords

aplastic anemia; bone marrow failure; telomerase; molecular genetics; non-malignant hematology

Introduction

Dyskeratosis congenita (DC) is an inherited multisystem disorder characterized by a classic clinical triad of skin pigmentation abnormalities, nail changes, and oral leukoplakia(1, 2). Patients exhibit premature degeneration of several tissues manifesting as bone marrow failure (BMF), pulmonary fibrosis, hematopoietic and epithelial malignancies, gray hair and other defects. DC is caused by mutations in at least 7 genes (*DKC1*, *TERC*, *TERT*, *TINF2*, *NHP2*, *NOP10*, *TCAB1*) involved in the maintenance of telomere integrity (3, 4), but in approximately 50% of cases a genetic cause has not been identified. DC is one of a wider phenotypic spectrum of “telomere diseases”, including the severe childhood disorders Hoyerall-Hreidarsson (HH) and Revesz syndromes, caused by *DKC1* and *TINF2* mutations(5, 6), as well as more tissue-restricted or later-onset disorders such as isolated aplastic anemia and familial idiopathic pulmonary fibrosis, caused by *TERC* and *TERT* mutations(7).

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Conflict of Interest Statement

The authors declare no conflicts of interest.

Coats plus is a pleiotropic, autosomal recessive disorder characterized by retinal telangiectasia and exudates, intracranial calcification with leukodystrophy and brain cysts, osteopenia with predisposition to fractures, and gastrointestinal bleeding and portal hypertension caused by vascular ectasias(8). A clinical link between Coats plus and telomere diseases is suggested by the observations that some patients with Coats plus show premature graying of hair, nail dystrophy and anemia; that Revesz syndrome patients have Coats retinopathy; and that both Revesz and HH syndrome patients can have intracranial calcifications(8, 9). Exome sequencing of patients with Coats plus and the related disorder cerebroretinal microangiopathy with calcifications and cysts (CRMCC) has revealed mutations in the *CTCI* gene, encoding “conserved telomere maintenance component 1”, leading to the hypothesis that *CTCI* mutations may also be found in patients with DC(9–11). Here we describe compound heterozygous *CTCI* mutations in a patient with classic DC, confirming the genetic link between Coats plus/CRMCC and DC.

Case report

The patient presented as a previously healthy 15 year-old female with fatigue and pancytopenia, with an absolute neutrophil count of 800/ μ L, hemoglobin 9.4 g/dL, mean corpuscular volume of 117 fL and platelets of 56,000/ μ L. Medical history was notable only for irregularities in skin pigmentation of unclear etiology and traumatic fractures of her digits. She had a normal neurocognitive, developmental and ophthalmological history. Family history was unremarkable. Physical examination was notable for height 5th percentile, graying hair, buccal leukoplakia, reticular hypo- and hyper-pigmented skin lesions over the face, trunk and extremities, and cracked fingernails and toenails (Fig. 1A). Bone marrow examination revealed marked hypocellularity (<5%) (Fig. 1B) and no evidence of myelodysplasia or malignancy. Telomere length testing showed very short age-adjusted telomere length in 5 of 6 peripheral blood cell subsets (Fig. 1C and Supplementary Fig. 1A)(12, 13). She was diagnosed with DC based on the classic triad of clinical manifestations, BMF and very short telomere length.

In the 3 years after initial presentation, the patient sustained fractures of her femur and metatarsal without significant trauma, and bone density scan showed osteopenia. Dental evaluation showed numerous caries and shortened incisor roots on radiography. Her pulmonary function tests showed decreased diffusion capacity of 67% predicted with normal lung volumes and spirometry. One year after her presentation with BMF the patient complained of blurry vision, and was found to have myopia corrected with eyeglasses. Fundoscopic exam was normal except for sheathed vessels and microaneurysm formation in the peripheral retina bilaterally (Fig. 1D). Visual acuity and fundoscopic exam were stable in follow up. The patient had worsening cytopenias and most recently has required red blood cell and platelet transfusions.

Molecular and cellular studies

Testing for mutations in *DKC1*, *TERC*, *TERT*, *NHP2*, *NOP10* and *TINF2* was negative. Informed consent was obtained for research studies. Based on Anderson *et al.*(9), we performed Sanger sequencing on the patient’s DNA to cover the 23 exons of the *CTCI* gene, and identified compound heterozygous mutations in exon 5 (het. c.724_727delAAAG; p.Lys242Leufs*41) and exon 18 (het. c.2954_2956delGTT; p.Cys985del) (Fig. 2A). Both alleles produced mRNA detectable by RT-PCR (Supplementary Fig. 1B and C). The patient’s mother carried only the exon 18 mutation (Supplementary Fig. 1D). No pathogenic *CTCI* mutations were found in 3 other patients with classic DC of unknown genetic basis.

Patient bone marrow cells failed to proliferate for metaphase karyotype analysis. Stromal cultures from patient bone marrow yielded only single colonies of senescent fibroblasts after

5 weeks (n=2), whereas normal cultures typically produce $>10^6$ replicating cells after 3–4 weeks. Patient skin biopsy explant cultures yielded approximately 10^3 – 10^4 cells which showed signs of senescence (n=2) (Fig. 2B), in contrast to normal samples which routinely give $>10^6$ replicating cells after 5 weeks.

Follow-up

Because of the association of *CTCI* mutations with Coats plus/CRMCC, neuroimaging was performed and revealed a prominent thalamic calcification and a large septated syrinx extending from the cervical to mid-thoracic spinal cord (Fig. 2C). The patient has no neurological complaints except mild, intermittent headaches, and no deficits on examination.

Discussion

DC is a rare disease with an estimated annual incidence of less than 1 in 1 million, with multiple inheritance forms and myriad clinical manifestations(14). Genotype-phenotype correlations are complex, due to several factors including a variety of underlying hypomorphic gene mutations, disease anticipation, and genetic and environmental modifier effects(7). Our patient presented with typical skin, nail and oral mucosal changes, BMF and very short telomeres, thus fulfilling criteria for DC. She manifested other DC-associated abnormalities including gray hair, osteopenia/fractures, dental disorders, and reduced pulmonary diffusion capacity. Her fundoscopic exam showed vascular changes which may represent early signs of retinopathy, but not the exudate, hemorrhage, or retinal detachment characteristic of Coats disease. The patient had no significant neurological symptoms, but imaging prompted by the genetic findings revealed significant CNS abnormalities. There was no history indicative of gastrointestinal pathology. Therefore, while our patient presented clinically with classic DC and without cardinal symptoms of Coats plus/CRMCC, recognition of the common genetic mutation in *CTCI* led to the detection of unexpected, overlapping abnormalities.

The mutations found in *CTCI* in our patient are identical to alleles recurrently mutated in patients with Coats plus/CRMCC, and are not present in dbSNP or in the exome variant server database, establishing their pathogenicity(9, 10). The specific combination of alleles in our patient was not described in the two published series, but compound heterozygosity of a nonsense lesion with a missense variant was observed frequently. These data suggest that the phenotypic differences between our patient with DC and Coats plus/CRMCC patients are due to genetic and/or environmental modifying factors rather than allelic differences in *CTCI*.

We observed a pronounced defect in outgrowth and rapid senescence of *CTCI* mutant fibroblasts, which correlates with the marked degree of aplasia and indicates depletion of stromal as well as hematopoietic precursors in the patient's bone marrow. CTC1 is a conserved subunit of α -accessory factor, which stimulates the activity of DNA polymerase α -primase and may have a role beyond telomere biology in maintaining general genomic stability (15, 16). Based on these observations, we speculate that the cellular defects caused by *CTCI* mutations will be more extensive than those caused by DC-related mutations in telomerase genes.

In conclusion, we report a patient presenting with classic DC and BMF with *CTCI* mutations identical to those recently described in Coats plus/CRMCC. This report demonstrates that *CTCI* is a gene mutated in DC, and expands the range of phenotypic variation observed in genetic diseases of telomere biology.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank the patients and families for their participation in research. This work was supported by NIH grants K08HL089150 to S.A. and by 5RC1DK086861 to D.A.W and I.H.

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Fig. 1.

A: Skin and oral mucosal manifestations in our patient with DC and *CTC1* mutations. **B:** Bone marrow biopsy showing a markedly hypocellular marrow with rare hematopoietic elements. **C:** Measurement of telomere length by flow-cytometry/fluorescence *in situ* hybridization (flow-FISH) in lymphocytes (left) and granulocytes (right), plotted as percentile of age-adjusted normal values. **D:** Retinal photograph showing a normal posterior pole (left panel), but obliterated (black arrows) and sheathed (blue arrow) vessels in the temporal fundus periphery (right panel). Findings were similar bilaterally.

Fig. 2.

A: Sanger sequencing of *CTC1* exons 5 (upper panel) and exon 18 (lower panel) on DNA from peripheral blood cells. The 5' site of each deletion junction is indicated (red arrow), and the residues deleted on one allele are shown (red box). The normal sequence and resulting mutant allele sequence are depicted. **B:** Compared to normal fibroblasts (left panel), patient fibroblasts showed several features of senescence including frequent polygonal forms, ballooning, dendritic-like projections and degeneration(17, 18) (middle and right panels). **C:** Neuroimaging. Left panel: Cranial axial computed tomography (CT) scan showing right-sided thalamic calcification (red arrow). Right panel: cervical spine

sagittal T2 magnetic resonance imaging (MRI) showing extensive septated syrinx in cervical to thoracic portions of spinal cord (demarcated by blue line).