Aplastic anemia

TERC mutations in children with refractory cytopenia

Mutations in the human telomerase RNA gene (*TERC*) cause autosomal dominant dyskeratosis congenita and have been detected in individuals with bone marrow failure. Here, we screened for *TERC* mutations in a cohort of 80 children with hypocellular myelodysplastic syndrome and detected *TERC* alterations in two of them.

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The distinction between congenital disorders with bone marrow failure and acquired myelodysplastic syndrome (MDS) is challenging in the absence of an increased blast count. In children diagnosed with the hypocellular refractory cytopenia[5] subtype of MDS without a clonal marker, underlying congenital diseases may be overlooked. In 2001, Dokal and co-workers demonstrated that the gene coding for the RNA component of telomerase (*TERC*) is mutated in autosomal dominant dyskeratosis congenita,[1] a bone marrow failure disorder associated with hyperpigmentation of the skin, oral leukoplakia and nail dystrophy. Since the initial report, constitutional *TERC* mutations have been detected in a small number of adult individuals with bone marrow failure without physical stigmata of dyskeratosis congenita.[2-4] Here, we report on heterozygous *TERC* alterations identified in two unrelated patients by screening a cohort of 80 children with hypocellular refractory cytopenia.

Between 1 July 1998 and 31 December 2004, 106 German children were diagnosed with hypocellular refractory cytopenia after reference review of bone marrow biopsies and aspirates (*I. Baumann*). The diagnostic criteria for refractory cytopenia in children have been published elsewhere (Table 1).[5] Following informed consent, patients were enrolled in the diagnostic EWOG-MDS 98 study. Eleven children had an abnormal karyotype and were excluded from this analysis. Of the remaining 95 patients, we had DNA from 80 patients for mutational studies (Table 1). Genomic DNA was isolated from peripheral blood or bone marrow using a Ficoll gradient. Germline DNA was obtained from fibroblasts or mucosal cells. Descriptions of primers and denaturing high-performance liquid chromatography (DHPLC) procedures have been published elsewhere.[2] DHPLC-polymerase chain reaction products that gave rise to mutations were re-amplified from genomic DNA and sequenced.

The first mutation, 116C→T, detected in a 12-year old male, alters the helically paired P2b region of the pseudoknot domain believed to be relevant in the functional reconstitution of the human telomerase complex.[6] The boy presented with thrombocytopenia (100×10^9/L) at the age of 7 years. At the age of 12 years he developed fatigue and hemorrhages. At this time his white blood count was 3.5×10^9/L with 20% neutrophils, the hemoglobin 8.9 g/dL, the mean cell volume of red cells (MCV) 110 fL and the platelet count 23×10^9/L. The bone marrow biopsy was hypocellular, and the aspirate showed dysplastic features without blast forms. Subsequent screening of the family members detected the same mutation in the father and all three siblings. The father had an elevated MCV and reported an increased bleeding tendency and low platelet counts since childhood. The index patient's 13-year old brother showed isolated borderline macrocytosis; the 9-year old sister was affected by thrombocytopenia, mild macrocytic anemia and neutropenia and the 3-year old sister showed no hematologic abnormalities (Figure 1). The same mutation, 116C→T, was described by Young *et al.*[4] in several members of a large family. In this family, the index patient was a young adult who was initially diagnosed with acquired aplastic anemia. A consistent finding observed in our and other families with dyskeratosis congenita is an increased MCV in affected family members.[4]

The second variation, -99C→G, was found in a 12-year old female and is believed to modulate *TERC* promoter activity by disrupting a Sp1 transcription factor binding site.[7] The girl presented at the age of 12 years with fever, menorrhagia and severe anemia (hemoglobin 3.6 g/dL) and thrombocytopenia (14×10^9/L). The white blood count was 3.1×10^9/L with 32% neutrophils, the MCV 94.6 fL (after transfusion). The bone marrow aspirate showed refractory cytopenia with no increase of blast forms. Neither the Turkish parents nor the 9-month old half-brother were affected by hematologic abnormalities (*TERC* status unknown). As the patient remained transfusion-dependent, a stem cell transplantation from an unrelated donor was performed 6 months after presentation; the child died 3 months later from reactivation of cytomegalovirus infection. The -99C→G lesion was recently described by Dokal *et al.* in an 18-year old Turkish patient with the diagnosis of paroxysmal nocturnal hemoglobinuria.[7] The low frequency of *TERC* mutations found in our cohort is consistent with data published by other investigators, who identified *TERC* mutations in patients with MDS, aplastic anemia or paroxysmal nocturnal hemoglobinuria.[2,3,8-10] Our findings suggest

Table 1. Characteristics of a German cohort of 80 children diagnosed as having hypocellular refractory cytopenia.*

Male: Female (n.)	47 : 33
Karyotype	normal n = 63 no data n=17
Median age at diagnosis (years) (range)	10.5 (0.2-17.5)
Median MCV at diagnosis (fL) (range) n=69	97 (73-121)
Age dependent MCV at diagnosis n=69	microcytic n=2 normocytic n=26 macrocytic n=41
Median hemoglobin at diagnosis (g/dL) (range)	8.6 (3.0-13.1)
Median platelets at diagnosis (per μL) (range)	24,000 (2,000-424,000)
Median ANC at diagnosis (per μL) (range)	660 (68-4959)
Median HbF at diagnosis (%) (range)	4 (0-23)
Age dependent HbF at diagnosis n=29	normal n=3 high n=26

***Minimal diagnostic criteria for MDS in children.*[5]** *At least two of the following: (a) sustained unexplained cytopenia; (b) at least bilineage morphologic myelodysplasia; (c) acquired clonal cytogenetic abnormality in hematopoietic cells and (d) increased blasts (≥5%).*
***Diagnostic categories of MDS in children.*[5]** *Refractory cytopenia (peripheral blood blasts <2% and bone marrow blasts <5%), refractory anemia with excess blasts (RAEB) (peripheral blood blasts 2-19%, bone marrow blasts 5-19%), and RAEB in transformation (peripheral blood or bone marrow blasts 20-29%).*

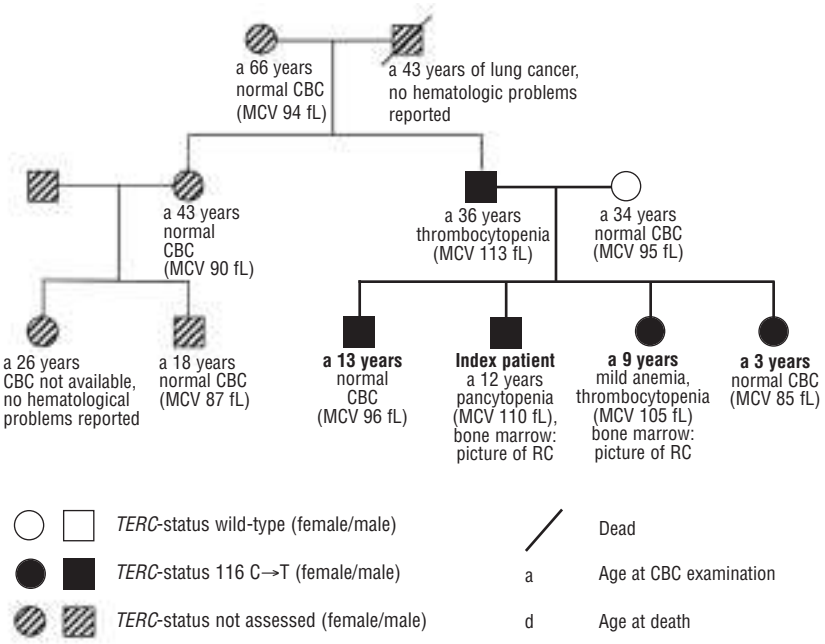

Figure 1. **Pedigree of a family with autosomal dominant dyskeratosis congenita (*TERC* mutation 116C→T). The index patient was initially diagnosed with hypoplastic refractory anemia with normal karyotype. No family member showed physical stigmata of dyskeratosis congenita. CBC: complete blood count; MCV: mean corpuscular volume; RC: refractory cytopenia.**

that a small percentage of children diagnosed with refractory cytopenia have autosomal dominant dyskeratosis congenita.

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