Expanding the clinical phenotype of autosomal dominant dyskeratosis congenita caused by *TERT* mutations

Dyskeratosis congenita (DC) is a hereditary disorder characterized by mucocutaneous manifestations, bone marrow failure, predisposition to malignancy, pulmonary and liver fibrosis and additional features.[1] X-linked recessive, autosomal recessive and autosomal dominant (AD) forms of inheritance have been recognized.[1] Families with AD inheritance may have mutations in genes that encode either the RNA component (*TERC*) or reverse transcriptase component (*TERT*) of telomerase[2,3] and they often lack the mucocutaneous features. Disease anticipation associated with progressive telomere shortening has been observed in these families.[3,4] Mutations in *TERT* have also been identified in patients with isolated aplastic anemia[5] and idiopathic pulmonary fibrosis.[6]

We identified a family of Iraqi Jewish origin with clinical features compatible with AD DC (Figure 1A, Table 1). In all the affected males, isolated thrombocytopenia was the presenting sign, followed by decreased RBC and/or WBC counts. Anticipation for aplastic anemia, premature greying of hair, pulmonary fibrosis and hepatic fibrosis was observed. The onset of the disease in males was earlier in each subsequent generation by 20-30 years. In 2 patients, cardiac abnormalities were detected: cardiac fibrosis in patient III-4 and cardiomegaly in patient III-7. The clinical features are summarized in Table 1.

While all 6 males in the family were severely affected, neither of the 2 females showed overt clinical manifestations of the disease except for premature grey hair in both and borderline RBC count in one of them. Death was due to respiratory failure or hepatic failure; one patient died as a result of myocardial infarction, a complication that has been noted once previously in AD DC in a TERC heterozygote.[4] No malignancies were observed. Height was normal.

Analysis of the *TERC* gene showed no abnormality. *TERT* gene analysis revealed a novel heterozygous missense mutation (c.1892 G>A; p.Arg631Gln), which creates a Pst I site that is not present in the normal gene (Figure 1B). The mutation affects a highly conserved residue in motif 2 of the RT domain of the protein and is very likely to disrupt the function of the enzyme. It has not been observed in 282 healthy controls from various ethnic groups[5] and in 200 chromosomes of Iraqi Jewish origin. Telomere length measurement using the T/S ratio obtained by real-time PCR[7] showed that the four available heterozygous individuals had significantly short telomeres ($p<0.01$), when compared with 16 age and sex-matched controls; this was not the case for the normal family members (Figure 1c). Heterozygotes with and without clinical symptoms did not show any difference in telomere length, by T/S ratio measurement or by Southern blot analysis (Figure 1D). While it has been shown that shortening of telomeres underlies the pathogenesis of DC and that the severity of the disease correlates with the length of the telomeres, this family demonstrates that telomere length alone cannot serve as a predictor of the disease severity.

We observed several clinical features that have not been described previously in DC and that are of importance in the clinical management of the patients and

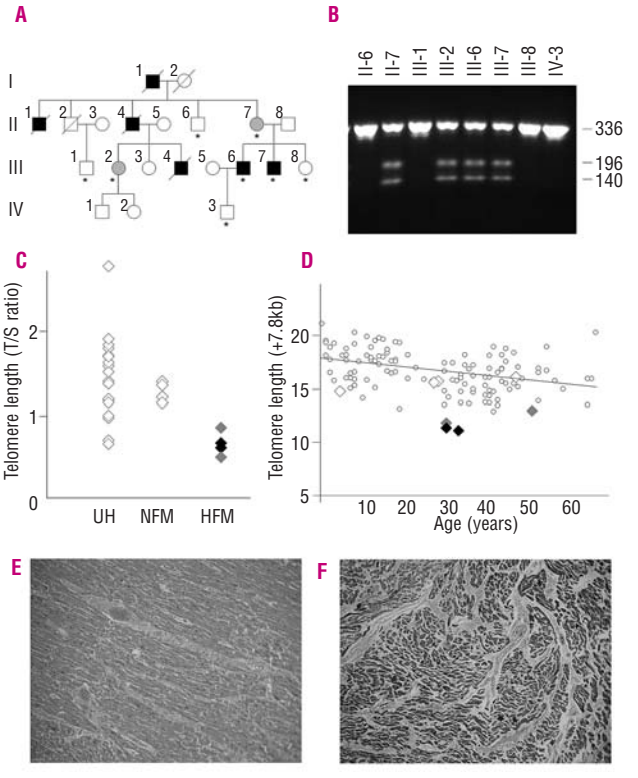

Figure 1. (A) Pedigree of the family. Affected individuals are indicated in black. Asymptomatic female carriers are indicated in grey. Genetically tested individuals are indicated by an asterisk. (B) Agarose gel electrophoresis of Pst I digestion products of exon 4 of the TERT gene from family members, as indicated. The two smaller fragments are seen when the mutation is present. (C) Telomere length distribution in the normal and heterozygous family members compared with healthy control individuals. UH: unrelated healthy individuals; NFM: normal family members; HFM: heterozygous family members. The grey diamonds are the asymptomatic individuals, while the black diamonds are the affected individuals. (D) Telomere length measurement by Southern blot analysis. Healthy controls are shown as open circles, with a line of best fit drawn through these points. The family members are shown as diamonds shaded as in panel. (C) (E) Thin fibrous septa in myocardial interstitium (H&E x 100). (F). Masson-trichrome stain highlights interstitial fibrosis (blue-green) (magnification x 100).

genetic counseling. In patient III-4, cardiac fibrosis was observed at *post-mortem* examination, although clinically it was asymptomatic. Gross examination of the heart of patient III-4 confirmed weight was 272 grams. No macroscopic pathological changes were seen. On microscopic examination, diffuse mild interstitial fibrosis was observed, highlighted by Masson-trichrome stain (Figure 1E,F). Mild focal myocyte hypertrophy was also noted. In patient III-7, dilated cardiomyopathy was detected by echocardiography. Although cardiac abnormalities have not been described in DC patients, the role of telomerase in cardiac muscle cell growth and survival has been shown to be important in mice.[8] TERC null mice display premature aging symptoms including heart dysfunction.[9] The frequency of cardiac fibrosis in patients with DC caused by *TERT* mutations has still to be determined in a larger cohort of patients.

Another unusual clinical observation was different disease expressivity in females compared with males. Severely affected females have been previously reported in families with *TERT* mutations[3,4,10] with no differ-

ences in disease severity between males and females. In our family, severe disease was observed in males, while the heterozygous females were healthy at the time of examination; individual II-7 was healthy at the age of 52 years, while her two sons showed the first signs of the disease at the age of 16-17 years. In generation III, affected males (III-6 and III-7) had abnormal blood counts from the age of 16-17 years, while the 33-year-old female in the same generation (III-2) showed only borderline low RBC counts with no additional clinical symptoms. Interestingly, the frequency of *TERT* mutations in sporadic patients with aplastic anemia was found to be higher in males than in females; the mutation was identified in 10 males and only 4 females.[9,11] This finding suggests lower penetrance in females, although only a small number of patients have been studied here.

Splenomegaly is not considered an important clinical feature of DC and has only rarely been described.[12] In our family, 3 of the patients demonstrated splenomegaly from a very young age. There was no evidence for portal vein thrombosis, Epstein-Barr virus or cytomegalovirus infection or hemolytic disease. Therefore, the reason for splenomegaly in DC remains obscure.

To summarize, a consistent and recognizable pattern of clinical features, including restrictive pulmonary disease caused by pulmonary fibrosis, hepatic dysfunction or failure caused by hepatic fibrosis, thrombocytopenia or aplastic anemia and splenomegaly, combined with a positive history of premature greying of hair should facilitate early diagnosis of DC. The absence of skin and nail changes and an AD inheritance pattern should raise suspicion of the presence of *TERT* mutations. In addition to evaluation of the pulmonary, hepatic and hematopoetic systems, examination for detection of cardiac abnormalities should be carried out. Since several inheritance patterns are possible in DC, it is important to be aware that asymptomatic female carriers can escape clinical detection and, therefore, their risk of having affected offspring might be underestimated.

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