



Disease anticipation is associated with progressive telomere shortening in families with dyskeratosis congenita due to mutations in *TERC*

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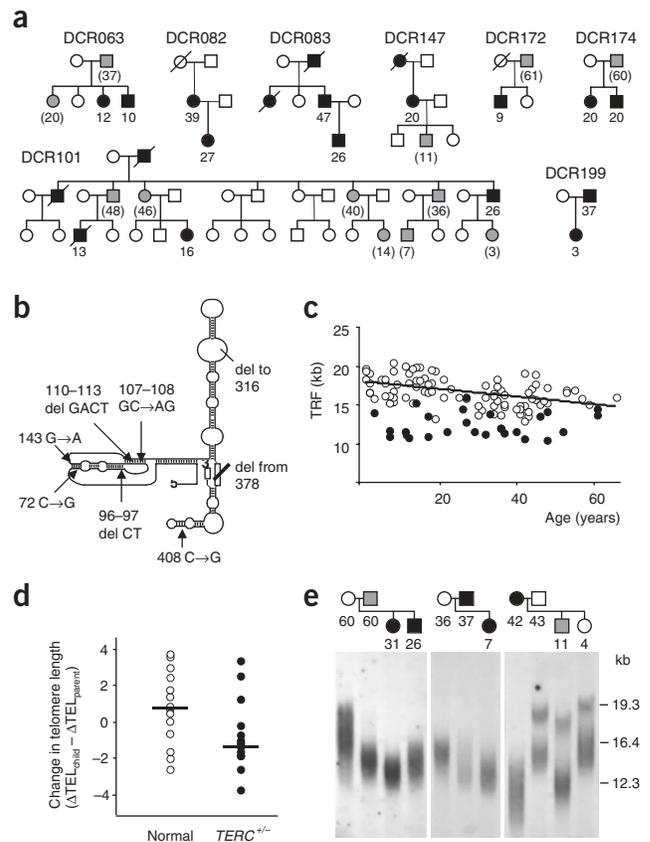
Telomerase is a ribonucleoprotein complex that is required to synthesize DNA repeats at the ends of each chromosome. The RNA component of this reverse transcriptase is mutated in the bone marrow failure syndrome autosomal dominant dyskeratosis congenita. Here we show that disease anticipation is observed in families with this disease and that this is associated with progressive telomere shortening.

In most somatic cells, the ends of each chromosome, the telomeres, shorten with each cell division owing to incomplete replication of the lagging strand of DNA. To compensate for this telomere loss, germ cells and some stem cells activate a reverse transcriptase called telomerase that synthesizes telomeric repeats onto the ends of each chromosome to maintain telomere lengths at an equilibrium¹. The possible relationship between telomere lengths, their rate of erosion and age-related disease has evoked considerable interest.

Figure 1 Families with AD-DC, mutations in *TERC* and their impact on telomere length. **(a)** Open circles and squares represent normal females and males. Black circles and squares represent affected females and males. Gray circles and squares represent asymptomatic females and males with *TERC* mutations. The age of disease onset for affected individuals or age of investigation (in brackets) for asymptomatic affected individuals is shown. **(b)** Model of *TERC* showing the locations of the mutations found in eight families with AD-DC. **(c)** Telomere length (measured as a TRF that includes ~8 kb subtelomeric DNA) is plotted against age for 87 unaffected individuals (open circles) and the 27 individuals with AD-DC (filled circles). A line of best fit is drawn through the normal points. **(d)** The change in age-adjusted telomere length measurement between parent-child combinations ($\Delta\text{TEL}_{\text{child}} - \Delta\text{TEL}_{\text{parent}}$) in normal and *TERC*^{-/-} families. Bars show median values. **(e)** Southern-blot analysis of telomere length. Family trees of families DCR174, DCR199 and DCR147 (from left to right) are drawn above the appropriate lanes. The age of each individual at the time of TRF analysis is shown. Note the bimodal distribution of telomere length in three members of family DCR147, including the unaffected father.

Dyskeratosis congenita is a multisystem disorder characterized by cutaneous abnormalities, bone marrow failure and an increased predisposition to cancer². Mutations in dyskerin, which is involved in ribosomal RNA processing³ and in the telomerase complex⁴, are responsible for the X-linked form of this disease⁵. Families with autosomal dominant inheritance of dyskeratosis congenita (AD-DC) have mutations in the gene encoding the RNA component of telomerase (*TERC*)⁶. These mutations seem to give rise to the disease through haploinsufficiency, through the absence of a 3' end, impaired RNA accumulation or a catalytic defect⁷.

We investigated the telomere lengths and the disease status of 27 affected individuals from eight families with AD-DC (**Fig. 1a**). In these families, affected individuals are heterozygous with respect to mutations in *TERC*. Three of the eight families and the mutations they carry were previously described⁶: family DCR063 (408C→G), family DCR082 (107–108GC→AG) and family DCR101 (3' deletion,



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from nucleotide 378 onward). In two of the eight families, the index case presented with aplastic anemia: family DCR172 (72C→G) and family DCR174 (110–113 deletion GACT)⁸. Of the three mutations described here for the first time, two were detected by denaturing HPLC and direct sequencing of *TERC*: a CT deletion at nucleotides 96 and 97 (Supplementary Fig. 1 online) and the nucleotide substitution 143G→A (Supplementary Fig. 1 online) in families DCR147 and DCR199, respectively. In family DCR083, a 2,980-bp deletion extends from nucleotide 316 of *TERC*, 5' to nucleotide 835 in the 3' untranslated region of the neighboring gene, which encodes the actin-related protein M1 (Supplementary Fig. 1 and Supplementary Note online). This lesion would abolish *TERC* expression on one allele in affected individuals. Figure 1b shows the location of the *TERC* mutations found in the eight families.

In these eight families, the disease becomes more severe in succeeding generations (Table 1). We have information for 12 affected parents and 15 affected children. Of the affected parents, 7 of 12 are asymptomatic, ranging in age from 36 to 61 y. In these cases, AD-DC

was diagnosed only by the identification of a *TERC* mutation; subtle signs of the disease were often detected subsequently (Table 1). For the five remaining affected parents, the median age at which disease features were first identified was 37 y. Of the affected children, only 5 of 15 remain asymptomatic: they are aged 3, 7, 11, 14 and 20 y and were diagnosed only through mutation analysis. For the remaining ten affected children, symptoms of dyskeratosis congenita presented at a median age of 14.5 y. These data indicate that there is anticipation in the clinical expression of AD-DC, with onset of disease features being, on average, two decades earlier in the children than in their parents.

We next investigated whether the telomere length has a role in the process of disease anticipation. We measured the length of the telomere as a terminal restriction fragment (TRF) by Southern-blot analysis using a subtelomeric probe from the long arm of chromosome 7 (refs. 9,10; Supplementary Methods online). The value obtained was age-adjusted by comparing it with the line of best fit through TRF measurements of 87 normal individuals (Fig. 1c). In this way, we calculated

Table 1 Disease features and telomere lengths in AD-DC families

Parent				Child				Difference in telomere length between parent and child
Individual (gender)	Age at onset (y)	Disease symptoms and features	ΔTEL	Individual (gender)	Age at onset (y)	Disease symptoms and features	ΔTEL	
063 I.2 (M)	NA (37)	Asymptomatic, high MCV	-4.7	063 II.1 (F)	NA (20)	Asymptomatic, high MCV	-6.1	-1.4
				063 II.3 (F)	12	Leucopenia, thrombocytopenia, hypocellular BM, liver disease	-6.6	-1.9
				063 II.4 (M)	10	Abnormal skin pigmentation, dysplastic nails, thrombocytopenia, hypocellular BM	-6.0	-1.3
082 II.1 (F)	39	Skin hyperpigmentation, anemia	-3.9	082 III.1 (F)	27	Skin hyperpigmentation, leukoplakia	-0.6	3.3
083 II.3 (M)	47	Absent fingerprint, pancytopenia	-1.4	083 III.1 (M)	26	Thrombocytopenia, myelodysplasia	-2.2	-0.8
101 II.4 (M)	NA (48)	Asymptomatic	-5.2	101 III.3 (M)	13	Thrombocytopenia, anemia, hypocellular BM	-5.4	-0.2
101 II.5 (F)	NA (46)	Asymptomatic, premature gray	-2.2	101 III.7 (F)	16	Nail ridging, thrombocytopenia	-3.6	-1.4
101 II.11 (F)	NA (40)	Asymptomatic, nail ridging	-4.4	101 III.14 (F)	NA (14)	Asymptomatic	-2.0	2.4
101 II.14 (M)	NA (36)	Asymptomatic, mild leucopenia	-4.3	101 III.15 (M)	NA (7)	Asymptomatic	-6.8	-2.5
101 II.16 (M)	26	Thrombocytopenia, high MCV	-5.0	101 III.18 (F)	NA (3)	Asymptomatic	-3.8	1.2
147 II.1 (F)	20	Abnormal skin pigmentation, nail dystrophy, thrombocytopenia, carcinoma	-5.3	147 III.2 (M)	NA (11)	Asymptomatic	-6.4	-1.1
172 I.2 (M)	NA (61)	Asymptomatic, high MCV	-0.6	172 II.1 (M)	9	Thrombocytopenia	-2.5	-1.9
174 I.2 (M)	NA (60)	Asymptomatic, high MCV and HbF	-1.3	174 II.1 (F)	20	Pancytopenia, elfic appearance	-5.1	-3.8
				174 II.2 (M)	20	Pancytopenia	-3.9	-2.6
199 I.2 (M)	37	Myocardial infarction, hypoplastic-myelodysplasia	-4.2	199 II.1 (F)	3	Hypoplastic-myelodysplasia	-5.9	-1.7

The disease symptoms and features are those observed at age of onset (or at time of first investigation for asymptomatic individuals). M, male; F, female; BM, bone marrow; ΔTEL, age adjusted value of telomere length in kb; HbF, fetal hemoglobin; MCV, mean cell volume (high MCV and HbF are early features of bone marrow failure); NA, not applicable (current age in brackets).



an age-adjusted value of telomere length, called Δ TEL (the difference between the actual value and the predicted value), for each individual¹¹. For all parents and children with *TERC* mutations, the Δ TEL was a negative value (Table 1), highlighting the fact that their telomeres are significantly shorter than normal ($P < 0.0001$ for both parents and children versus normal controls, Mann-Whitney). Looking at the difference in telomere length between parents and children (Δ TEL_{child} – Δ TEL_{parent}) in 15 transmissions of *TERC* mutations (Table 1 and Fig. 1d), we found that telomeres were significantly shorter in the second generation of affected families compared with normal families (14 parent-child measurements in normal families; $P = 0.036$, Mann-Whitney). This decrease in telomere length in successive generations may be responsible for the clinical anticipation in AD-DC.

During the course of this study, we noticed that a number of individuals had a bimodal distribution of telomere length (Fig. 1e). We observed this in 8 of 87 (9%) of the normal DNA samples. In the AD-DC families, 6 of 27 (22%) affected individuals (from three of the eight families) and 7 of 13 (54%) children who did not inherit the mutation from an affected parent (from the same three families) had a bimodal pattern of telomere length. A bimodal distribution was previously observed in human fibroblast cell lines where the short and long telomeres are linked to maternal and paternal alleles. The difference in telomere length between the two alleles seems to be maintained from the zygote throughout development¹².

The only convincing mechanism of disease anticipation in humans described so far involves a genetic change, namely the expansion of triplet repeats observed in several neurodegenerative disorders¹³. In the families described here, the genetic lesion has remained the same. We propose that its impact on the inherited telomere length has led to presentation of disease at a younger age in succeeding generations. There are clear analogies here with *Terc* knockout mice¹⁴. Parental mice have very long telomeres, and in the first generation, *Terc*^{-/-} mice are asymptomatic. Features of telomere shortening, which overlap the clinical features seen in dyskeratosis congenita, develop only in the

fourth generation. By the sixth generation these mice become infertile. Heterozygous *Terc*^{+/-} mice are asymptomatic but have a defect in their ability to elongate telomeres in interspecies crosses¹⁵. Our study shows that people who are heterozygous with respect to *TERC* mutations can remain asymptomatic well into adulthood. But this haploinsufficiency also causes dyskeratosis congenita, the severity of which seems to increase as telomeres shorten through the generations.

All samples used in this study were obtained with informed consent and with the approval of the Research Ethics Committee of the Hammersmith Hospitals NHS Trust.

Note: Supplementary information is available on the Nature Genetics website.

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COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

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Mutations in the mitochondrial GTPase mitofusin 2 cause Charcot-Marie-Tooth neuropathy type 2A

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We report missense mutations in the mitochondrial fusion protein mitofusin 2 (MFN2) in seven large pedigrees affected with Charcot-Marie-Tooth neuropathy type 2A (CMT2A). Although a mutation in kinesin family member 1B- β (*KIF1B*) was associated with CMT2A in a single Japanese family, we found no mutations in *KIF1B* in these seven families. Because these families include all published pedigrees with CMT2A and are ethnically diverse, we conclude that the primary gene mutated in CMT2A is *MFN2*.

Charcot-Marie-Tooth disease (CMT) comprises a frequently occurring, genetically heterogeneous group of peripheral neuropathies, although the clinical picture is rather uniform¹. Following electrophysiological criteria, CMT falls into two main forms: the demyelinating CMT type 1 with decreased nerve conduction velocities and

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